STEREOSPECIFICITY FOR NICOTINAMIDE NUCLEOTIDES IN ENZYMATIC AND CHEMICAL HYDRIDE TRANSFER REACTIONS

Author: **Kwan-sa You**
W. Alton Jones Cell Science Center
Lake Placid, New York

Referee: Norman J. Oppenheimer
Department of Pharmaceutical Chemistry
University of California
San Francisco, California

I. INTRODUCTION

The pyridine nucleotide coenzymes NAD and NADP (see Section II) are ubiquitous in all living systems. They are required for the reactions of more than 370 different kinds of enzymes according to the most recent edition of *Enzyme Nomenclature*.[1] This number represents 17% of all classified enzymes.[2] Many more exist but the Enzyme Commission numbers have not been assigned to them yet. Undoubtedly, these coenzymes are responsible for more enzymatic reactions than any other coenzyme. Figure 1 represents the molecular structures of NAD(P).

In the vast majority of cases, the role of these coenzymes in enzyme-catalyzed reactions is to serve as the acceptor of what is equivalent to a hydride ion (H^-) from the substrate in a reversible manner. Substrates can be organic compounds or inorganic molecules such as H_2 (hydrogen dehydrogenase) (EC.1.12.1.2.) and NO_3^- (nitrate reductase) (EC.1.6.6.2.). In short, these coenzymes serve as cellular electron carriers. The site where this hydride transfer reaction takes place on the coenzyme molecule is C-4 of the nicotinamide/dihydronicotinamide ring (Figure 1).[3]

In most of these reactions, the coenzyme and the substrate have a stoichiometric relationship and the coenzyme does not return to its original form at the end of the reaction. In this sense, pyridine nucleotide coenzyme is different from other coenzymes such as thiamine pyrophosphate, biotin, and pyridoxal phosphate, which act catalytically and return to their original forms upon completion of the reaction. For this reason, pyridine nucleotide is sometimes called cosubstrate.

Some hydrolases [e.g., adenosylhomocysteinase (EC.3.3.1.1.)], lyases [e.g., dTDPglucose 4,6-dehydratase (EC.4.2.1.46.) and urocanate hydratase (EC.4.2.1.49.)], isomerases [e.g., UDPglucose 4-epimerases (EC.5.1.3.2.) and *myo*-inositol-1-phosphate synthase (EC.5.5.1.4.)], and decarboxylases [e.g., UDP-glucuronate decarboxylase (EC.4.1.1.35.)] contain very tightly bound NAD(P), which undergo no net change at the termination of reaction. In these cases, the bound dinucleotide acts coenzymatically in the true sense by serving catalytically as the hydrogen acceptor as well as donor for the intermediates in the reaction sequence of a given catalysis.[4,5] (It is of some interest to note that there is no known case in which the pyridine nucleotide is covalently bound to its host enzyme; however, flavin nucleotide coenzymes, another group of major cellular electron carriers, have been found to be covalently bound to a small number of enzymes.)

According to the definition by Hanson,[6] the methylene carbon (C-4) of the dihydronicotinamide ring of NAD(P)H is a prochiral center. If one of the two *prochiral hydrogens* at the C-4 is replaced with an isotope (deuterium or tritium), a new chiral center is introduced to the ring and as a result two diastereomers are produced depending on the position of the isotope. Figure 1 also illustrates the absolute configuration of this prochiral center as well as the spatial position of the isotope (deuterium) in the two diastereomers $NADD_A$[(4R-

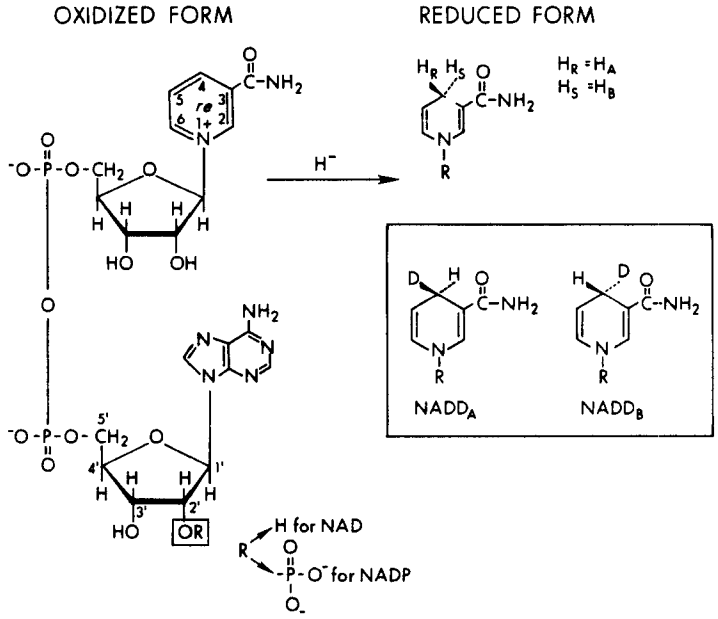


FIGURE 1. Structure of NAD(P) and the absolute configuration of C-4 prochiral center of the dihydronicotinamide ring. As it is drawn, the *re* face of the nicotinamide ring of $NAD(P)^+$ faces the reader and the *si* face the back of the page. The *pro-R* hydrogen (H_R) and the *pro-S* hydrogen (H_S) of the reduced coenzyme or analogs are also widely known as the A hydrogen (H_A) and the B hydrogen (H_B), respectively. The inset presents the chiral center of the deuterium-labeled diasteromers, $NADD_A$ and $NADD_B$. Unless otherwise indicated, in this and other figures, R represents the ADPR moiety of the coenzyme.

^{2}H)NADH] and $NADD_B$ [(4S-^{2}H)NADH]. (See Section II for the use of the A and B notations.)

It is a well-established theorem that asymmetric agents like enzymes can distinguish the two apparently identical groups on a prochiral center including *prochiral hydrogens*, and usually exhibit absolute discrimination between the two. This remarkable stereospecificity of enzymes has been demonstrated, perhaps most dramatically, with the enzymatic redox reactions involving NAD(P).

As far as we know, all of the reactions catalyzed by NAD(P)-linked enzymes exhibit absolute stereospecificity with respect to the coenzymes. [The only possible exception is CDP-4-keto-6-deoxy-D-glucose reductase (EC.1.17.1.1.). See the Appendix and Section IV. A.] Hence, in the case of the oxidation of NAD(P)H, one group of the oxidoreductases transfers only the *pro-R* hydrogen at the prochiral center of the dihydronicotinamide to their substrates, while the other group removes only the *pro-S* hydrogen. In the reverse reaction, when a hydrogen is added to C-4 of the nicotinamide ring to form NAD(P)H, the enzymes which remove the *pro-R* hydrogen from the reduced form transfer the hydrogen on the *re* face of the trigonal C-4 (Figure 1), and those acting upon the *pro-S* hydrogen, to the *si* face.

Ever since this striking stereochemical orderliness in enzyme-catalyzed reactions was first discovered by Fisher et al[7] in 1952, it has become increasingly evident that this aspect of stereospecificity is one of the most stringently conserved properties of NAD(P)-linked oxidoreductases during the evolutionary course. Furthermore, the information about this stereospecificity of a given enzyme is critically important in the elucidation of its reaction mechanism as well as active site structure.

For these reasons, immense effort has been directed to the establishment of the stereospecificities of as many enzymes as possible during the ensuing 3 decades since the discovery. As the consequence, the stereospecific hydrogen transfer by oxidoreductases has been the best-studied example of the power of enzyme in making stereochemical choices. In this article, I will present the compilation of the stereospecificity of the enzymes that have been established to date (Appendix), and will attempt to rationalize our current understanding about the stereospecificity emphasizing the conformational implication, and discuss other issues that are relevant to the present topic. It should be pointed out that, in great majority of cases, NAD(P)-linked enzymes are also stereospecific with respect to the substrate in regard to hydrogen transfer. For instance, in alcohol dehydrogenase-catalyzed oxidation of ethanol, the enzyme removes only the *pro-R* hydrogen of the substrate; likewise, in the reverse reaction, the hydrogen is transferred to the *re*-face of the carbonyl group of acetaldehyde.[8,9]

In formulating the compilation (Appendix), if the stereospecificity of a given enzyme with respect to its substrate is also known, that specific position is marked on the structure of the substrate. At the outset, however, I should point out clearly that this review does not discuss the stereospecificity involving the substrate and product. Covering the coenzyme portion alone is already immensely voluminous work; if the stereospecificity of oxidoreductases on substrate is deemed worth discussing, it should be dealt with in a separate treatise.

Previous reviews on the present subject can be found in References 10 to 17. A monograph, which exclusively deals with the biochemistry of the pyridine nucleotide coenzyme, has recently been made available.[18]

II. NOMENCLATURE AND ABBREVIATION

Because the stereospecificity of NAD(P)-linked enzymes was discovered before the absolute configuration of the redox site of the coenzymes was established, various nomenclatural designations have been provisionally applied to differentiate the two methylene hydrogens of the dihydronicotinamide ring of NAD(P)H. The most widely accepted nomenclature, at least among biochemists, is the A/B designation.

Conventionally, the face of the nicotinamide ring, which is involved in alcohol dehydrogenase reaction, illustrated in Figure 2, has been arbitrarily named the A side, and the opposite side of the ring plane the B side. The hydrogen located on the A-side of the dihydronicotinamide ring is called the A side hydrogen (H_A) and that on the opposite side the B-side hydrogen (H_B).

In the alcohol dehydrogenase-catalyzed reaction shown in Figure 2, the deuterium originated from [1,1-dideutero]-ethanol occupies the A side of the dihydronicotinamide of the product ($NADD_A$).[19] The enzymes having the same stereospecificity as alcohol dehydrogenase and those with the opposite stereospecificity are called the A and the B stereospecific enzymes, respectively.

Originally, the A and B hydrogens were called the α and β hydrogens, respectively, by Vennesland in 1956.[20] However, this notation was abandoned several years later because it invoked confusion with the α- and β-configurational terms for the nicotinamide-ribosyl glycosidic linkage. The confusion was compounded in case the substrates were carbohydrates or steroids.

In order to alleviate this confusion, Jaraback and Talalay[21] introduced the I/II system in place of the α/β in 1960. But this system, too, was unsatisfactory as they could be mistaken as coenzyme I (CoI) and coenzyme II (CoII), the old names for NAD and NADP, respectively. Kaplan[22] suggested the use of 1/2 designation but it received limited acceptance.

Finally in 1962, Vennesland's group[10] introduced the A/B system and the majority of researchers have preferred it to other systems. Unfortunately, however, the course of this

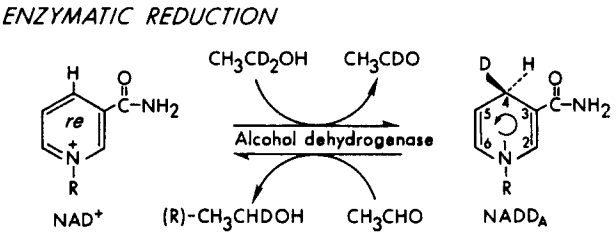


FIGURE 2. Stereospecificity of alcohol dehydrogenase (EC.1.1.1.1.)-catalyzed reaction. The deuteride from [1,1-D]-ethanol is added to the *re* face of the nicotinamide ring of NAD^+ in the reaction: the deuterium is added to "that side of the nicotinamide ring on which the ring carbon atoms 1 to 6 appear in *anticlockwise* order". The chiral center of the dihydronicotinamide ring thus created exhibits the *R* configuration. Furthermore, this enzymatic hydrogen transfer is stereospecific with regard to the substrates as well: only the *pro-R* hydrogen of ethanol and the *re* face of acetaldehyde participate in the reaction. (Adopted from Bentley, R., *Stereochemistry*, Tamm, Ch., Ed., Elsevier, Amsterdam, 1982, 113. With permission).

nomenclature intricacy has introduced substantial confusion in the literature concerning NAD(P).

Organic chemists favor the use of the *R/S* and the *pro-R/pro-S* systems for the methylene hydrogens of dihydronicotinamide ring and the *re/si* designation for designation of the face of the trigonal C-4 of the nicotinamide ring. Undoubtedly, these systems are superior to the conventional ones since they designate the absolute configuration without requiring additional information. In the case of dihydronicotinamide ring, the A and the B hydrogens correspond to the *pro-R* and the *pro-S* hydrogens and, in the case of nicotinamide, the A and the B sides are the same as the *re* and the *si*-faces, respectively (Figure 1).

For the sake of brevity, many abbreviations are employed in this article in order to describe various forms of the coenzymes, their fragments and analogs, and isotopic labels: NAD and NADP, abbreviations for nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate, respectively, signifying the coenzymes irrespective of their redox state; NAD(P), both NAD and NADP; NAD^+ and $NADP^+$, oxidized forms of NAD and NADP; NADH and NADPH, reduced forms of NAD and NADP; $NAD(D)^+$ and $NADP(D)^+$, oxidized coenzymes labeled with deuterium (D) at C-4 of the nicotinamide (in case the isotope is tritium, T is used instead of D); $NADD_A$ and $NADD_B$ ($NADPD_A$ and $NADPD_B$), reduced coenzymes labeled with deuterium at the A and B positions of the dihydronicotinamide, respectively; (3AcPy)AD, 3-acetylpyridine adenine dinucleotide; (3IPy)AD, 3-iodopyridine adenine dinucleotide; (3PyA1)AD, 3-pyridine aldehyde adenine dinucleotide; (3CNPy)AD, 3-cyanopyridine adenine dinucleotide; (3-AminoPy)AD, 3-aminopyridine adenine dinucleotide; (TN)AD, thionicotinamide adenine dinucleotide; NMN^+ and NMNH, oxidized and reduced forms of nicotinamide mononucleotide, respectively; ADPR, adenosine diphosphate ribose; $NMNH(OAc)_2$, NMNH with both C-2′ and C-3′ hydroxyl groups acetylated; $NADH(OAc)_4$, NADH with all ribosyl hydroxyl groups acetylated; ϵ-NADH, the 1,N^6-ethenoadenosine analog of NADH; DHNR, dihydronicotinamide riboside; N(Hyp)DH, nicotinamide hypoxanthine dinucleotide; (*N*-Met)NAD, *N*-methylnicotinamide adenine dinucleotide; $N(CH_3NH\text{-}A)D$, nicotinamide 8-methylaminoadenine dinucleotide; $N((CH_3)_2N\text{-}A)DH$, nicotinamide 8-dimethylaminoadenine dinucleotide; $N(CH_3CH_2NH\text{-}A)DH$, nicotinamide 8-ethylaminoadenine dinucleotide; and N(Br-A)DH, nicotinamide 8-bromoadenine dinucleotide. Unless otherwise indicated, all nucleotides have the β-glycosidic linkage between the base and the ribose.

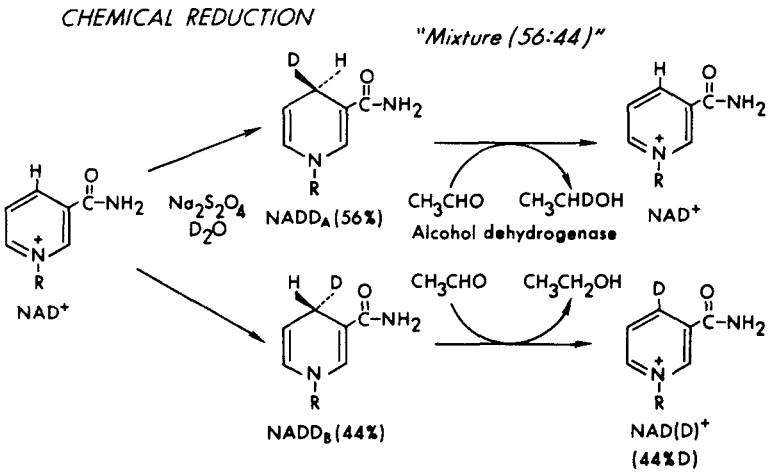


FIGURE 3. Chemical reduction of NAD^+ with sodium dithionite in D_2O. This particular instance shows that the population ratio of formed $NADD_A$ to $NADD_B$ is 56:44%; usually, however, higher values for $NADD_A$ formation are observed (Table 1). Reoxidation of the mixture of $NADD_A$ and $NADD_B$ by alcohol dehydrogenase in the presence of acetaldehyde produces NAD^+ and $NAD(D)^+$ at an overall deuterium content of 44%. (Adapted from Bentley, R., *Stereochemistry*, Tamm, Ch., Ed., Elsevier, Amsterdam, 1982, 113. With permission.)

III. CHRONOLOGY OF MAJOR DEVELOPMENTS

As noted earlier, Fisher et al.[7] were the first to report in 1952 that yeast alcohol dehydrogenase catalyzed the transfer of hydride between the substrate and NAD in a reversibly stereospecific manner with respect to the redox site of the coenzyme. In the preceding year, this group of workers had already discovered that this hydrogen transfer occurred in a direct manner without the mediation of either the protein or the medium.[23] [In the case where the hydrogen from NAD(P)H is transferred to N, S, or O atom of the acceptor, it may be difficult to prove the direct transfer because the transferred hydrogen is subject to exchange with the one in the medium. Examples of such cases are dihydropteridine reductase (EC.1.6.99.7.)(N), dihydrolipoamide dehydrogenase (EC.1.8.1.4.) (S on the enzyme), and carbonyl reductase (EC.1.1.1.184.) (O with quinone substrate). Nonetheless, in these cases, too, the enzyme-catalyzed steps of the overall reactions involve the direct hydrogen transfer. It is the subsequent nonenzymatic exchange reaction which causes the loss of the original hydrogen from the coenzymes. This exchange does not always occur, however; in a case such as *Pseudomonas* transhydrogenase, a flavoprotein, the hydrogen transferred from NADPH to FAD does not exchange with medium hydrogen unless the enzyme structure is disrupted with, e.g., 1 *M* urea.[24] Cytochrome b_5 reductase (EC.1.6.2.2) is another flavoprotein catalyzing the direct hydrogen transfer from NADH to $(3AcPy)AD^+$.[25]]

Fisher et al.[19] prepared NADD from NAD^+ by two procedures: first, enzymatically by employing yeast alcohol dehydrogenase and CH_3CD_2OH (Figure 2), and second, chemically with sodium dithionite in D_2O (Figure 3). When the alcohol dehydrogenase-catalyzed reaction was carried out in the reverse reaction in the presence of the enzymatically prepared NADD and acetaldehyde, the product NAD^+ contained no isotope (Figure 2). In the same reaction, but with the chemically prepared NADD, however, the resulting NAD^+ contained 0.44 atoms of deuterium per molecule. Clearly, the chemically prepared NADD contained a mixture of two diastereomers, $NADD_A$ and $NADD_B$, whereas the enzymatic preparation contained pure $NADD_A$. The result also demonstrates that the chemical reduction slightly

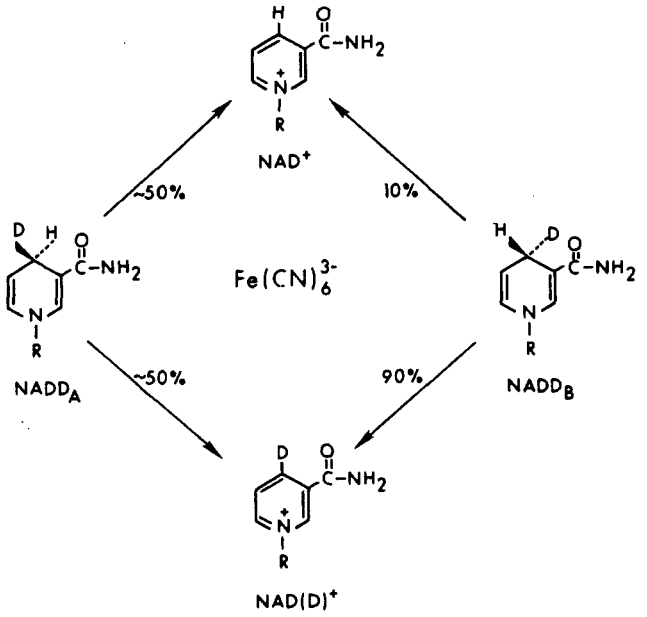


FIGURE 4. The oxidation of $NADD_A$ and $NADD_B$ by neutral fericyanide. In the oxidation of $NADD_A$, about equal amounts of deuterium and hydrogen are removed from the reduced coenzyme, producing approximately equal amounts of NAD^+ and $NAD(D)^+$. When $NADD_B$ is oxidized by the same procedure, on the other hand, only 10% of deuterium is removed and, as the result, 90% of the product is $NAD(D)^+$. It is certainly a steric effect, and not a deuterium isotope effect, that discriminates the two diastereomers in removing the deuterium. (From Colowick, S. P., van Eys, J., and Park, J. H., *Comprehensive Biochemistry*, Vol. 14, Florikin, M. and Stotz, E. H., Eds., Elsevier, Amsterdam, 1966, 1. With permission.)

favors the formation of $NADD_A$ to $NADD_B$. This unequal formation of $NADD_A$ and $NADD_B$ in the chemical procedure, thought to be within the experimental error at that time,[19] later was proved to be caused by the nature of the molecular folding of the free coenzyme.

Pullman et al.[3] found in 1954 that the degree of the A-side preference in this chemical reduction was much higher than originally reported. In addition, Pullman et al.[3] as well as San Pietro et al.[26] demonstrated that chemical oxidation of the reduced form of the coenzyme by ferrycyanide, too, favors the A side as illustrated in Figure 4. The steric preference in chemical redox reactions involving the coenzyme has played a significant role in elucidating the conformation of free coenzyme in solution. This aspect will be discussed later in detail.

It is a matter of considerable interest to note that when this enzyme stereospecificity was discovered even the redox site of the coenzyme was not clearly delineated. In fact, the position was erroneously assumed to be one of the two *ortho*-positions (C-2 and C-6) of the pyridine ring. [It was 1954 when Pullman et al.[3] established that the *para* position (C-4) was the reaction site.] The thesis that the enzyme stereospecificity for the coenzyme was not a unique property of alcohol dehydrogenase-catalyzed reaction but a common characteristic of other NAD-linked enzymes was proved when the reactions of lactate dehydrogenase (EC.1.1.1.27.)[27] and malate dehydrogenase (EC.1.1.1.37.)[28] were found to be likewisely stereospecific.

The stereospecificity of all of these three enzymes are the same, the A side. However, that the enzymatic reactions are not limited to the A side of the coenzyme was demonstrated in 1955, when Talalay et al.[29] and San Pietro et al.,[26] respectively, discovered that the

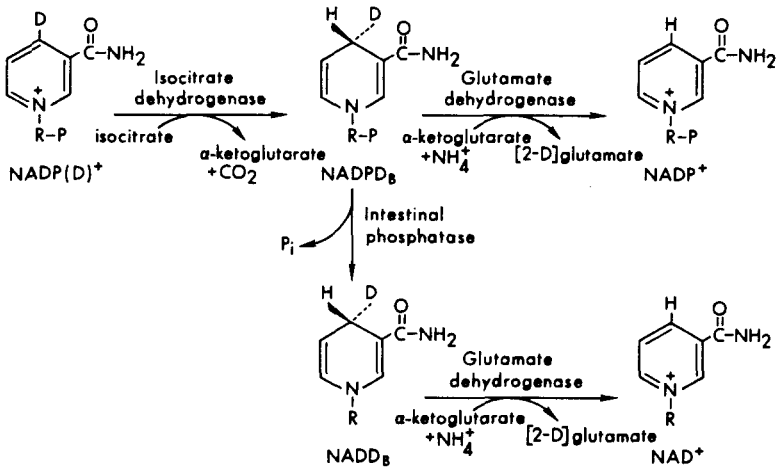


FIGURE 5. The identical configurational relationship of C-4 hydrogens of the dihydronicotinamide rings of NADH and NADPH. The opposite stereospecificity between isocitrate dehydrogenase (EC.1.1.1.42.)-catalyzed reaction and glutamate dehydrogenase (NAD(P)$^+$) (EC.1.4.1.3.)-catalyzed reaction with respect to NADP is demonstrated first by reducing NADP(D)$^+$ with isocitrate dehydrogenase, followed by reoxidation with glutamate dehydrogenase. The NADP$^+$ produced by the action of glutamate dehydrogenase contains, unlike the original dinucleotide, no deuterium (deuterium was recovered in the product as [2-D-glutamate), proving that these two enzymes possess the opposite stereospecificity. The NADPD generated by the action of isocitrate dehydrogenase is also converted to NADD by removing the C-2′ phosphate group of NADPD with intestine phosphatase. The NADD thus produced is oxidized with the same glutamate dehydrogenase and the resulting NAD$^+$ is also found to be devoid of the isotope. These results demonstrate that the hydrogens at the C-4 prochiral centers of the dihydronicotinamide rings of NADH and NADPH have the same spatial relationship. (From Bentley, R., *Stereochemistry*, Tamm, Ch., Ed., Elsevier, Amsterdam, 1982, 113. With permission.)

reactions catalyzed by β-hydroxysteroid dehydrogenase (EC.1.1.1.51.) and transhydrogenase involve the opposite side — the B side — of NAD.

In 1960, Nakamoto and Vennesland[30] experimentally proved for the first time that the reactions of NADP-linked enzymes such as isocitrate dehydrogenase (EC.1.1.1.42.) and glutamate dehydrogenase (EC.1.4.1.3.) are also stereospecific in the same manner as that of NAD-linked enzymes. Because their experiment may be considered as a classical example for an elegant but simple case of enzymological work, it is illustratively reproduced in Figure 5. In their experiment, a sample of NADPD was prepared from NADP(D)$^+$ with isocitrate dehydrogenase (pig heart) in the presence of isocitrate. The product NADPD was then reoxidized with glutamate dehydrogenase (beef liver) in the presence of α-ketoglutarate and NH_4^+. Glutamate dehydrogenase had been known to utilize both NAD and NADP as coenzyme and was previously shown to possess the B stereospecificity with NAD.[31] The deuterium presented in the NADP was not retained in the NADP$^+$ produced by the action of the glutamate dehydrogenase, but was found in the product glutamate. This finding unambiguously proved that isocitrate dehydrogenase and glutamate dehydrogenase have the opposite stereospecificity. However, an ambiguity had remained as to whether the configurational positions of the H_A and H_B in NADH were the same as those in NADPH.

In order to solve this problem, these workers converted the NADPD produced by the action of isocitrate dehydrogenase to NADD by removing the C-2′ phosphate group of the adenosine moiety with intestinal phosphatase. When the NADD thus produced was oxidized with the same glutamate dehydrogenase, the resulting NAD$^+$, like NADP$^+$ described above, did not contain the isotope. On the basis of this result, they unequivocally concluded that the H_A and H_B in NADH have the same spatial relationship as those in NADPH and, furthermore, that isocitrate dehydrogenase is A stereospecific.

Through 1960, the stereospecificity research had been carried out by employing exclusively deuterium-labeled coenzymes and substrates. The quantitation of the deuterium content in the final products (i.e., H_2 gas) by mass spectrometry was not only an extremely laborious endeavor, but also involved a large margin of experimental errors. When radioisotopes began to be increasingly available for biochemical research, Jaraback and Talay[21] were the first to take the advantage of tritium-labeled compounds to determine the stereospecificity of 3α-hydroxysteroid dehydrogenase (EC.1.1.1.50), β-hydroxysteroid dehydrogenase, and estradiol 17β-dehydrogenase (EC.1.1.1.62.). Since that time tritium has virtually replaced the use of deuterium in the study of the stereospecificity.

In 1961, Velick[32] offered a logical explanation for the steric preference in the chemical redox reaction of the dinucleotide. According to him, NADH molecule in aqueous medium was folded in such a way that the faces of the adenine and the dihydronicotinamide rings were stacked in parallel formation, the B side of the dihydronicotinamide facing the plane of the adenine ring. This conformation of the free coenzyme molecule introduces steric hindrance against the removal of the H_B in the chemical oxidation with, e.g., ferricyanide; as a result, the H_A had to be more easily removed than the H_B as observed experimentally (Figure 4).[3,25] Because the chemical reduction of NAD^+ exhibited the same steric preference,[3,19] Velick concluded that the oxidized form should be similarly folded as the reduced form. Velick's conclusion was heavily based upon the energy transfer property from the adenine ring to the dihydronicotinamide, which was originally demonstrated fluorometrically by Weber[33] followed by Shifrin and Kaplan.[34,35] By applying the same energy transfer principle to the enzyme-bound reduced coenzyme, Velick[32] suggested that the coenzyme might assume open conformation when bound to the active site of its host enzyme, on the contrary to the free state.

However, Jardetzky and Wade-Jardetzky[120] first formulated the currently accepted dynamic "folded-open" conformation theory in 1966, on the basis of proton magnetic resonance (PMR) study on the dinucleotide.

For 10 years since the stereospecific hydrogen transfer was discovered, the absolute configuration of the methylene prochiral center of the dihyronicotinamide ring of the reduced coenzyme had not been established. It was, therefore, impossible to assign the positions of the two diastereotopic hydrogens — H_A and H_B — spatially on the C-4. All that could be said about this was the relative positions in reference to the position of the hydrogen transferred by the action of alcohol dehydrogenase to the coenzyme.

In 1962, however, a landmark investigation was carried out by Cornforth et al.[36] to determine the absolute configuration of the redox site of the coenzyme. As shown in Figure 6, they first converted the dihydronicotinamide moiety of $NADD_A$ to a 6-methoxy derivative by treating the deuterium-labeled coenzyme with methanolic acetic acid. Succinic acid was subsequently obtained by degrading the 6-methoxy derivative with ozone followed by peroxyacetic acid. The deuterium-labeled succinic acid thus produced from the degradation of $NADD_A$ produced a levorotatory ORD curve, which overlapped with the curve obtained with a standard (-)(2R)-monodeuterosuccinic acid. As expected, the succinic acid obtained from $NADD_B$ by the same procedure gave the ORD curve that had the opposite sign of rotation. Based upon this result, they concluded that the chiral center of the dihydronicotinamide ring of $NADD_A$ and $NADD_B$ has the *R*- and *S*-configurations, respectively. In the words of Cornforth et al.:[36] "When an enzyme of class A transfers hydrogen from a substrate to a pyridine nucleotide, the hydrogen is added to that side of the nicotinamide ring on which the ring atoms 1 to 6 appear in *anticlockwise* order" (Figure 2).

Meanwhile, the number of enzymes whose stereospecificity became known increased steadily and some common features had emerged. By 1966, Colowick et al.[11] were able to draw several systematic generalizations about the stereospecificity (often referred to as Bentley's generalizations[12]) and many other minor generalizations followed soon. Because

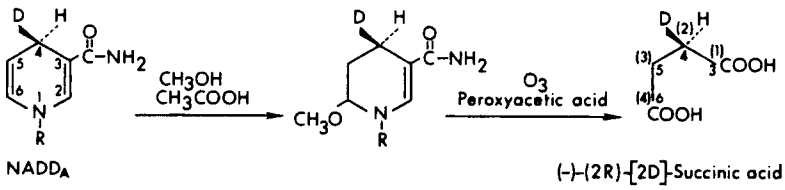


FIGURE 6. Determination of the absolute configuration of the chiral center of the dihydronicotinamide ring of NADD$_A$. By sequentially degrading NADD$_A$ with methanolic acetic acid, ozone, and peroxyacetic acid, a deuterium-labeled succinic acid, which is comprised of C-3, C-4, C-5, and C-6 of the original dihydronicotinamide ring of the coenzyme, is prepared. The ORD curve of this product coincides with that of standard (-)-(2R)-[2-D]-succinic acid; the monodeuterosuccinic acid obtained from NADD$_B$ by the same procedure, on the other hand, yields the ORD curve that is identical to the curve for (+)-(2S)-[2-D]-succinic acid. From these findings, it is concluded that the chirality of C-4 of the dihydronicotinamide of NADD$_A$ is the *R* configuration. The numbers in parentheses on succinic acid in accordance with the numbering system of succinic acid. (From Bentley, R., *Stereochemistry*, Tamm, Ch., Ed., Elsevier, Amsterdam, 1982, 113. With permission.)

enough body of data about the stereospecificity has been accumulated since these generalizations were proposed, we have now reached a good time to examine their validity comprehensively (see Section V.A.).

In the 1960s, the conformation of the coenzyme was also investigated by spectrophotometry, circular dichroism, and nuclear magnetic resonance (NMR) techniques. The results obtained by these techniques generally confirmed the earlier conclusion reached by the fluorescence work. Among these, the technique that has contributed the most to our current understanding about the conformation of the coenzyme is NMR spectrometry. With the advancement of instrumentation, the study primarily by high resolution PRM has corrected some of the early erroneous conclusions. This technique still serves as one of the most powerful tools for conformational work.

The stereospecificity of the enzymes containing tightly bound, catalytically acting coenzyme is difficult to study. In 1970, the stereospecificity of one of the enzymes belonging to this category, dTDPglucose 4,6-dehydratase,[37] was established and since then the stereospecificity of several more of such enzymes has been determined.

During 1971 to 1974, Oppenheimer et al.[38] and Arnold and Kaplan[39] discovered that while the formation of the adduct betwen NAD^+ and pyruvate in the presence of lactate dehydrogenase is totally stereospecific in accordance with the physiological stereospecificity of that enzyme, the nonenzymatic formation of the same adduct, as well as the cyanide adduct, favors the A side over the B side, just like the chemical oxidation-reduction of the coenzyme.

Recently, deuterium has been increasingly replacing tritium in the stereospecificity work as the use of more facile instrumentations such as high resolution PMR,[40,41] gas-mass spectrometry,[42-44] or electron impact as well as field desorption mass spectrometry[45] have been gaining widespread popularity.

Beginning with lactate dehydrogenase in 1970,[46] X-ray crystallography has been applied extensively for the study of the conformation of enzyme-bound coenzymes. In every case studied thus far, the coenzymes bind to their host enzymes in the open conformation without exception. Available X-ray data demonstrate that the nicotinamide/dihydronicotinamide ring of the coenzyme bound to the A-stereospecific enzymes (*anti* orientation) shows 180° opposite orientation from that bound to the B-stereospecific enzymes (*syn* or orientation), and this difference in the torisonal conformation is apparently the basis for the enzyme stereospecificity.

Even at the time when the elucidation of the overall conformation of enzyme-bound coenzyme was well underway by X-ray crystallography, the crystal conformation of free coenzyme had been elusive due to the unavailability of suitable crystal. In 1977, however,

Saenger et al.[47] were able to obtain the crystal of the coenzyme in the form of Li^+-NAD^+ complex and establish by X-ray crystallography that it is in the open form but in a different manner from that of the enzyme-bound form.

In more recent years, several groups of investigators studied the conformation of protein-bound coenzyme and other nucleotides by employing the transferred proton-proton nuclear Overhauser effect (TRNOE). The results obtained by this approach are generally in good agreement with those obtained by X-ray crystallography.

IV. SURVEY OF AVAILABLE DATA (APPENDIX)

A. Overview

The Appendix lists the stereospecificity of 158 pyridine nucleotide-linked enzymes (not all of them are classified yet). It also includes the enzyme sources, the reactions that were carried out for the actual stereospecificity determination, and whenever possible, the location of the transferred hydrogen on the molecular formulas of the substrates or products. Certain enzymes included [e.g., xanthine oxidase (EC.1.2.3.2.) and 2-nitropropane dioxygenase (EC.—)] are not NAD(P) linked, but are stereospecifically active with the coenzymes.

The enzymes in the Appendix are remarkably evenly divided between the A-stereospecific group (77 enzymes) and the B-stereospecific group (80 enzymes). One enzyme, Enzyme E_3 of CDP-4-keto-6-deoxy-D-glucose reductase (EC.1.17.1.1.) was described as having no stereospecificity. It has been also reported that a hydrogen from the medium incorporates into the product CDP-4-keto-3,6-dideoxy-glucose (thus, indicating an indirect hydrogen transfer) in the multistep reactions catalyzed by this enzyme. This enzyme is also capable of acting as an NAD(P)H oxidase.[48]

Historically, several enzymes had been claimed for lacking the stereospecificity, but such data have been proven to be repeatedly incorrect. Some of the examples of such cases are milk xanthine oxidase, which was reportedly sterically nonspecific,[20] but later was found to be a B-stereospecific enzyme;[49] dihydrolipoamide dehydrogenase (dihydrolipoyl dehydrogenase), which was described as having a mixed stereospecificity,[50,51] but actually was a strictly B-stereospecific enzyme;[40,52,53] and, as pointed out elsewhere,[54] orcinol 2-monooxygenase (EC.1.14.13.6.), which was initially reported as exhibiting a mixed stereospecificity when resorcinol or *m*-cresol (so called "uncouplers" of the monooxygenation) served as the substrate,[55] but afterward was proved to have the A stereospecificity.[56]

Because of these precedences, it may be worthwhile to investigate the stereospecificity of Enzyme E_3 at each step very carefully in order to make it certain that the reaction is truly nonstereospecific. It seems that the key work is establishing the stereospecificity of the NAD(P)H oxidase reaction of the enzyme.

One enzyme listed in the Appendix flavopapain, is semisynthetic. Levine and Kaiser[57] synthesized this product by covalently linking 7α-bromoacetyl-10-methylisoalloxazine to the thiol group of Cys-25 of papain, and showed that it acted like an oxidoreductase in the oxidation of NAD(P)H. Although it is listed as an A-stereospecific enzyme according to these workers, it should be pointed out that this artificial enzyme lacks the absolute stereospecificity seen with natural enzymes. In fact, the reported stereospecificity of this enzyme, i.e., the removal of 68 and 12% of deuterium from $NADD_A$ and $NADD_B$, respectively,[57] represents no more stereospecificity than the chemical oxidation of these diastereomers with ferricyanide (Table 1).

That the enzymes which can employ both NAD and NADP as coenzyme exhibit the same stereospecificity toward both of them is well confirmed in numerous cases without exception (Appendix). In addition, the substitution of NAD with analogs such as (3AcPy)AD, (3CNPy)AD, and (TN)AD does not change the stereospecificity of alcohol dehydrogenase,[58] glutamate dehydrogenase,[58] lactate dehydrogenase,[53] and melilotate 3-monooxygenase

Table 1
THE STEREOPREFERENCE IN CHEMICAL OXIDATION-REDUCTION OF NAD(P)

Reaction		Product (%)		Ref.
Reduction		$NADD_A$	$NADD_B$	
		56	44	19
		58	42	27
NAD^+		72	28	3
		63	37	26
	$S_2O_4^{2-}$ in D_2O	$NADPD_A$	$NADPD_B$	
		64	36	26
$NADP^+$		70	30	26
Oxidation		NAD^+	$NAD(D)^+$	
		35	65	3
$NADD_A$		51	49	26
		54	46	26
	$Fe(CN)_6^{3-}$			
$NADD_B$		10	90	26

(EC.1.14.13.4.).[59] It is also worth noting that yeast alcohol dehydrogenase oxidizes α-NADH with the same stereospecificity as for NADH (α-NADH is oxidized at about three orders of magnitude slower rate than is NADH).[60]

B. Application to Enzyme Classification: A Proposal

Alcohol dehydrogenase (NADP) (EC.1.1.1.2.), better known as aldehyde reductase, and carbonyl reductase (ketone reductase) are two confusing groups of NADP-linked enzymes with largely unclear physiological function.[61] The extremely broad as well as often overlapping substrate specificity, presence of multiple forms, and the similarities in molecular properties (i.e., the monomeric structure with molecular weights of 30,000 to 40,000) among these enzymes have made their distinction very confusing.[61-69] In general, alcohol dehydrogenases (NADP) act upon aldehydes, but some of them can also reduce a limited number of ketones.[62,67] Carbonyl reductases, on the other hand, appear to reduce both aldehydes and ketones equally well.[63,67]

There are now a number of reports which make it clear that previously individually classified enzymes such as glucuronate reductase (L-hexonate dehydrogenase) (EC.1.1.1.19.), mevaldate reductase (NADP) (EC.1.1.1.33.), and lactaldehyde reductase (EC.1.1.1.55.) are in fact alcohol dehydrogenases (NADP).[61,68,69] All of these enzymes are A stereospecific (Appendix).

By employing electrofocusing, Felsted et al.[65,67] separated a number of xenobiotic alcohol dehydrogenases (NADP) and carbonyl reductases from rabbit liver. Among these enzymes, naloxone reductase and naltrexone reductase (these are also called dihydromorphinone reductase) showed the A stereospecificity, whereas oxisuran reductase and metyrapone reductase were B stereospecific. They also found that *p*-nitrobenzaldehyde reductase, *p*-nitroacetophenone reductase, daunorubicin reductase, and 3,7-dimethyl-1-(5-oxohexyl)-xanthine reductase were present in more than two forms, which could be distinguished by their pIs: the low pI forms of these enzymes were B stereospecific, while the high pI forms showed the A stereospecificity. Recently, Wermuth[63] presented the evidence that carbonyl reductase isolated from human brain is B stereospecific.

These data clearly demonstrate that aldo-keto reductases can be separated into two groups depending on their stereospecificity for NADP. Obviously, the enzyme that acts upon one diastereomer or enantiomer catalyzes a different reaction from the one that employs the opposite stereoisomer as substrate; consequently, therefore, these two enzymes must be classified separately. A good example for such case is L-lactate dehydrogenase (EC.1.1.1.27.) and D-lactate dehydrogenase (EC.1.1.1.28.) (both are A stereospecific), which act upon the opposite enantiomers of lactate. The same classification treatment should apply to enzymes which share the same substrate with the same stereospecificity but exhibit the opposite stereospecificity with respect to the coenzyme.

In accord with this view, the Nomenclature Committee (IUPAC-IUB) very recently properly assigned a new EC number (1.1.1.184.) to carbonyl reductase, separate from alcohol dehydrogenase (NADP) (EC.1.1.1.2.).[1c] In the same recommendation,[1c] however, the committee deleted EC.1.1.1.149. (20α-hydroxysteroid dehydrogenase) and combined it with EC.1.1.1.62. (estradiol 17β-dehydrogenase). In my opinion, this is a mistake because although estradiol 17β-dehydrogenase, a B-stereospecific enzyme, may also have 20α-hydroxysteroid dehydrogenase activity in addition to estradiol 17β-dehydrogenase activity,[70] there is another 20α-hydroxysteroid dehydrogenase which shows the A stereospecificity (Appendix). It thus appears that it is more proper to separate estradiol 17β-dehydrogenase and 20α-hydroxysteroid dehydrogenase under two independent EC numbers.

Transhydrogenase is another case which requires some consideration for reclassification. Although the enzyme from *P. aeruginosa* and that from beef heart mitochondria are classified under the same EC.1.6.1.1., they are two completely distinct enzymes: while the former is a soluble flavoprotein (FAD) showing the B stereospecificity for NAD as well as NADP (the BB type), the latter is an insoluble protein devoid of any prothetic group and, more critically, exhibits the A stereospecificity for NAD, but the B stereospecificity with respect to NADP (the AB type).[71] Consequently, these two types of enzyme do not catalyze the same reaction and, therefore, it is reasonable to propose that separate EC numbers should be assigned to these two types of transhydrogenase.

By the same reasoning, 3α-hydroxysteroid dehydrogenase from rat liver,[72] 2-oxopantoyl lactone reductase from yeast,[73] and enoyl-(acyl-carrier-protein) reductase from pigeon (or rat) liver[74] (all A stereospecific) are different from the identically named enzymes, respectively, from *P. testosteroni*[21] (also that from rooster comb[75] or from rat seminal vescicle[76]) (EC.1.3.1.50.), *Escherichia coli* (EC.1.1.1.168.),[73] and yeast[78] (or *E. coli*[77]) (1.3.1.10.) (all have the B stereospecificity). In the appendix the first two enzymes are listed under E.C.1.1.1.?. and the third one, under E.C.1.3.1.?. [Nitrate reductase (EC.1.6.6.3.) from yeast was reported[79] to have the opposite stereospecificity from nitrate reductases (EC.1.6.6.1. and 1.6.6.2.) from other sources.[48,80] However, Davies and Kenworthy[81] have corrected the erroneous result initially obtained with the crude yeast enzyme and now report that this enzyme is, like the enzyme from other sources, A stereospecific.]

V. PROPOSED GENERALIZATIONS, EVOLUTIONARY IMPLICATIONS, AND PHYSIOLOGICAL SIGNIFICANCE

A. Review of Generalizations

Concurrent with the increase in the volume of the enzyme stereospecificity data, various researchers have proposed a number of empirical generalizations in order to assort the enzymes on the basis of their stereospecificity and at the same time to predict the stereospecificity of a given enzyme in case it is unknown. Unquestionably, some of the generalizations were proposed in haste when only very limited information was available. Because the stereospecificity of quite extensive numbers of enzymes is now known, this is an ideal opportunity to review their validity.

Colowick et al.[11] were the first to present three systematic major generalizations in 1966:

1. Stereospecificity for a given hydrogen transfer reaction is always the same, regardless of the source of the enzyme.
2. Dehydrogenation of a given substrate shows the same stereospecificity whether the nucleotide involved is NAD or NADP (proposed originally by Krakow et al.[82]).
3. Enzyme which is nonspecific with respect to substrate will show the same stereospecificity when different substrates are used.

This set of generalizations has been widely reiterated over the ensuing years, albeit there may be some variations in wording.[12-14,83,84]

It was Vennesland[83] who pointed out the self-evident nature of generalization No. 1. The expression "a given hydrogen transfer reaction" already implies the same stereospecificity. If two enzymes from different sources act upon the same substrate with different stereospecificity with respect to the coenzyme, these two enzymes catalyze two different reactions, not a given (or the same) reaction. For this reason, this generalization will not be considered as a valid generalization in this article (see Section IV.B). [Furthermore, the untenability of this generalization should have been overwhelmingly evident from the fact that many enzymes have overlapping substrate and product specificity. For instance, glutamate dehydrogenase, a B-stereospecific enzyme, can catalyze the reversible oxidative deamination of L-alanine,[85] the physiological substrate of alanine dehydrogenase (EC.1.4.1.1.), which is an A-stereospecific enzyme. Should this generalization hold, glutamate dehydrogenase must show the A stereospecificity with L-alanine, a highly unlikely, but not impossible prospect. For the sake of literature survey, however, it should be mentioned that the oxidation of ribitol to D-ribulose by ribitol dehydrogenase (EC.1.1.1.56.) (B stereospecific) and by L-iditol dehydrogenase (EC.1.1.1.14.) (A stereospecific) was considered to be another exception to this generalization,[86] in addition to the reactions of the three enzymes (i.e., 3α-hydroxysteroid dehydrogenase, 2-oxopantoyl lactone reductase, and enoyl-(acyl-carrier-protein) reductase) previously described in Section IV.B.]

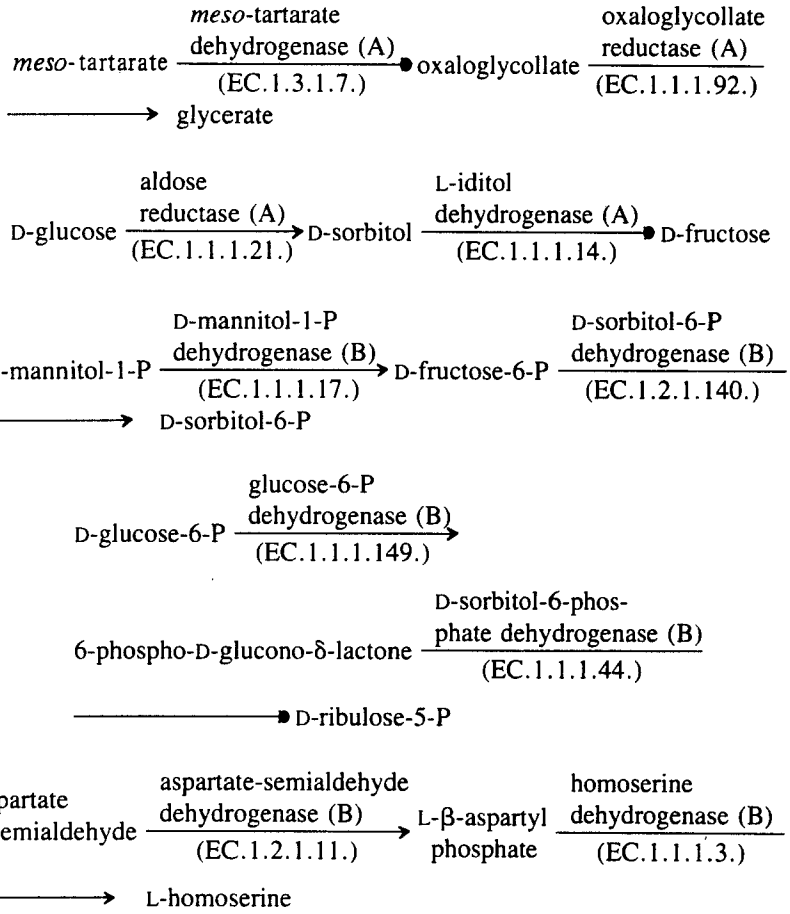
In a vast majority of cases, the same enzymes from different sources nevertheless show the same stereospecificity. Hence, Arnold et al.[40] examined the stereospecificity of L-lactate dehydrogenase and malate dehydrogenase, typical A-stereospecific enzymes, and glyceraldehyde-phosphate dehydrogenase (EC.1.2.1.12.), a typical B-stereospecific enzyme, from a wide variety of evolutionarily divergent sources and confirmed that they exhibit the same stereospecificity in all cases.

Generalization Nos. 2 and 3 are holding without exception in all reactions examined so far, and, in all probability, they will remain so since the coenzyme must bind to the host enzyme in a specifically juxtaposed way in relation to the substrate in order for the reaction of a given enzyme to proceed.

In 1972, Davies et al.[79] proposed the fourth rule: when a metabolic sequence involves consecutive NAD(P)-dependent reactions, the dehydrogenases involved in the sequence have the same stereospecificity. The enzymes involved in the following sequences of various metabolic pathways are examples of this rule.

$$\text{Ethanol} \xrightarrow[(\text{EC.1.1.1.1.})]{\text{alcohol dehydrogenase (A)}} \text{acetaldehyde} \xrightarrow[(\text{EC.1.2.1.3.})]{\text{aldehyde dehydrogenase (A)}} \text{acetate}$$

$$\text{D-xylulose} \xrightarrow[(\text{EC.1.1.1.9.})]{\text{D-xylulose reductase (B)}} \text{xylitol} \xrightarrow[(\text{EC.1.2.1.10.})]{\text{L-xylulose reductase (B)}} \text{L-xylulose}$$



It should be pointed out, however, that nitrate reductases and nitrite reductase (EC.1.6.6.4.) are involved in sequential reactions, however, these two classes of enzymes show the opposite stereospecificity: The former is A stereospecific, while the latter is a B-stereospecific enzyme.[81] Mansell et al.[87] also pointed out that although cinnamoyl alcohol dehydrogenase (EC.1.1.1.?.) and cinnamoyl-CoA reductase (EC.1.2.1.44.) are involved in a consecutive metabolic sequence in plant, they possess the opposite stereospecificity.

Reviewing the hydrogen transfer between NADPH and steroids, catalyzed by various steroid oxidoreductases from mammalian sources, Akhtar et al.[88] noted the fifth generalization: NADPH-dependent mammalian steroid dehydrogenases and reductases catalyze transfer of hydrogen from the B-side of the coenzyme to the α-face of the steroids and the A-side hydrogen to the β face. The examples of the enzymes consistent with this rule are estradiol 17β-dehydrogenase (human placenta), testosterone 17β-dehydrogenase (porcine testicular microsome) (EC.1.1.1.64.), 20α-hydroxysteroid dehydrogenase (rat ovary) (EC.1.1.1.149.), 3α-hydroxysteroid dehydrogenase (rat liver soluble fraction) (EC.1.1.1.?.), cortisone β-reductase (rat liver soluble fraction) (EC.1.3.1.3.), cortisone α-reductase (rat liver microsome)(EC.1.3.1.4.), 7-dehydrocholesterol reductase (rat liver microsome) (EC.1.3.1.21.), and $\Delta^{14(15)}$ sterol dehydrogenase (rat liver microsome) (EC.1.3.1.?.). The 3α-hydroxysteroid dehydrogenase (EC.1.1.1.50.) from rat seminal vesicles is, on the other hand, a B-stereospecific enzyme and, therefore, is an exception to this generalization.

Alizade and Brendel[14] summarized some of the minor generalizations (such as generalization No. 6) proposed through 1975: enzymes with A specificity have smaller substrates than those with B specificity (originally proposed by Krakow et al.[82]). Colowick et al.[1]

challenged this statement by citing the fact that hydrogen dehydrogenase takes the smallest possible molecule, H_2, as substrate, and that the substrate for methylenetetrahydrofolate dehydrogenase (EC.1.5.1.5.) is large, yet these enzymes showed the B and A stereospecificity, respectively.

Nevertheless, You et al.[16] confirmed that this rule held in an overwhelming number of cases, but modified it as in the following: those enzymes whose physiological substrates are *nonphosphorylated organic compounds having three or less carbon atoms* show the A stereospecificity. Hence, as You et al.[16] pointed out, it is worth noting that although alanine dehydrogenase, glutamate dehydrogenase, and leucine dehydrogenase (EC.1.4.1.9.) catalyze similar type of oxidative deaminations reactions, alanine dehydrogenase is A stereospecific, while the other two show the B stereospecificity. [The only possible exception to this rule is dihydroxyacetone reductase (EC.1.1.1.156.) from *Mucor javanicus*. However, it should be noted that this particular enzyme possesses unusually broad substrate specificity, exhibiting higher activity with alicyclic compounds such as (9S)-*trans*-1,4-decalinedione than dihydroxyacetone;[89,90] in many ways, it resembles carbonyl reductase.]

7. Reactions involving the oxidation of secondary OH groups adjacent to carboxyl groups always have the A stereospecificity regardless of the stereochemistry of the alcohol (originally proposed by Krakow et al.[82]). It should be noted, however, that 2-oxopantoate reductase (EC.1.1.1.169.) catalyzes such reaction, but has the B stereospecificity.
8. All constitutive NAD- or NADP-linked dehydrogenases of phosphorylated substrates (originally proposed by Vennesland[83]) or substrates substituted with CoA or nucleotides are of the B type. You et al.,[16] however, pointed out that phosphoglycerate dehydrogenase (EC.1.1.1.95.), whose substrate contains a phosphoryl group, is an A-stereospecific enzyme. So far as is known, all of the dehydrogenases which act on nucleotide-containing substrates are B stereospecific. However, many exceptions exist as regard to the enzymes acting on the CoA derivatives: hydroxymethylglutaryl-CoA reductase (EC.1.1.1.54.), acetaldehyde dehydrogenase (acylating) (EC.1.2.1.10.), and acyl-CoA dehydrogenase (EC.1.3.1.8.) are examples of the exception.
9. All inducible NAD-linked dehydrogenases from the *Pseudomonas* genus are of the B type. There are only four enzymes which belong to this category: 3α-hydroxysteroid dehydrogenase; β-hydroxysteroid dehydrogenase; D-galactose dehydrogenase (EC.1.1.1.48.); and carnitine dehydrogenase (EC.1.1.1.108.). Exceptions are, on the other hand, more numerous: *meso*-tartrate dehydrogenase (EC.1.3.1.7.) and all of the six external monooxygenase (EC.1.14.13.—.) listed in the Appendix all of which are inducible in *Pseudomonas*.
10. Most dehydrogenases which are not specific for either NAD or NADP but can transfer hydrogen to both coenzymes are of the B type. Examples of the enzymes which have this property but show A stereospecificity are salicylate 1-monooxygenase (EC.1.14.13.1.), NAD(P)H dehydrogenase (EC.1.6.8.1.), and ferredoxin-$NADP^+$ reductase (EC.1.18.1.2.).
11. The overwhelming majority of all constitutive NAD- or NADP-linked dehydrogenase utilizing primary or secondary alcohols or amines which are not phosphorylated or substituted with similar ligands are of the A type. There are, however, too many exceptions to this rule to make it valid. The following enzymes act on the primary or secondary alcohol groups but show the B stereospecificity: homoserine dehydrogenase (EC.1.1.1.3.), aryl-alcohol dehydrogenase (EC.1.1.1.91.), 21-hydroxysteroid dehydrogenase (EC.1.1.1.150.) (these three enzymes act upon the primary alcohol groups and those listed below, upon the secondary alcohol groups), *myo*-inositol 2-dehydrogenase (EC.1.1.1.18.), 3-hydroxybutyrate dehydrogenase (EC.1.1.1.30.), 15-hydrox-

yprostaglandin dehydrogenase (EC.1.1.1.141.), glycerol 2-dehydrogenase ($NADP^+$) (EC.1.1.1.156.), 2-oxopantoyl lactone reductase (EC.1.1.1.168.), and various steroid dehydrogenases such as 3α-hydroxysteroid dehydrogenase from other sources than rat liver soluble fraction, 20β-hydroxysteroid dehydrogenase (EC.1.1.1.53.), estradiol 17β-dehydrogenase, testosterone 17β-dehydrogenase, and estradiol 17α-dehydrogenase (EC.1.1.1.148.). Like alanine dehydrogenase, glutamate dehydrogenase and leucine dehydrogenase act on the primary amines, but the latter two have the B stereospecificity.

A twelfth generalization can also be raised: the enzymes which reduce disulfide are B stereospecific. The enzymes belonging to this category are glutathione reductase (EC.1.6.4.2.), dihydrolipoamide dehydrogenase (EC.1.8.1.4.), and thioredoxin reductase (EC.1.6.4.5.).

You et al.[91] screened the stereospecificity of a special group of enzymes called external monooxygenases (flavoenzymes) and found that every enzyme examined to date (one half dozen) showed the A stereospecificity; thus, a new generalization, No. 13, was introduced: all external flavoprotein monooxygenases have the A stereospecificity. No exception to this rule has been noted so far.

Finally, it now appears feasible to propose an additional generalization, No. 14, concerning another special group of enzymes — those with tightly bound NAD (which acts catalytically): the enzymes with catalytically acting, tightly bound NAD possess the B stereospecificity. All of such enzymes examined to date — dTDPglucose 4,6-dehydratase, UDPglucose 4-epimerase, and L-*myo*-inositol-1-phosphate synthase — are uniformly B stereospecific. (Glyceraldehyde-phosphate dehydrogenase is another enzyme having tightly bound NAD. Although the bound NAD does not act catalytically in this case, this enzyme is also B stereospecific.)

Review of the generalization pertaining to the stereospecificity clearly indicates that there are too many of them. It is reasonable to eliminate meaningless generalization No. 1 and those with too many exceptions such as Nos. 9, 10, and 11.

B. Evolutionary Implications

In the previous section, it was possible to see that the members of several distinct clusters of enzymes share a uniform stereospecificity. Since a theoretical elucidation for these empirical findings is apparently lacking, explanations for this uniformity have been sought from the evolutionary point of view.[92]

Hence, the generalization of Davies et al. (No. 4)[79] can be explained with the theory that the second and third enzymes in metabolic sequences were evolutionarily derived from the first enzyme, the common ancestor. Due to evolutionary necessities, many enzymological properties among these enzymes have been changed, but the stereospecificity remained conserved. This "common ancestor" theory can be applied to other generalizations such as Nos. 7, 8, and 12 to 14.

According to Garavito et al.[93] the proper positioning of carboxamide group of the coenzyme on the enzyme allows for the close approach of the C-4 atom on the nicotinamide and the reactive carbon of the substrate. It follows, then, that once the conformation of the substrate has been established with respect to the functional groups of the enzyme at the active site, the stereospecificity for the coenzyme is predetermined. Hence, dehydrogenases which are divergently evolving from a common precursor must maintain the same stereospecificity as long as the protein fold of the catalytic domain is conserved for a given substrate.

From the observation that the enzymes whose substrates are small organic compounds are A stereospecific (generalization No. 6), You et al.[16] speculated that the A-stereospecific enzymes might have evolved before the B-stereospecific enzymes. This speculation was borne out from the notion that the prebiotic chemical process more likely produced simple small molecules before complex large compounds, which are in general the products of

biotic processes in already evolutionarily advanced organisms. When primitive organisms appeared, they must have lived on small compounds as energy source because not only were these the only ones present, but also the organisms were more likely to possess very rudimentary catabolic systems, which could not break down large molecules. Hence, the fact that there is no B-stereospecific enzyme that acts on small organic compounds (except the exception noted) suggests that the A-stereospecific enzymes appeared before the B-stereospecific enzymes.

C. Physiological Significance

The physiological purpose for the existence of the two groups of enzymes having the opposite stereospecificity for NAD(P) has not been convincingly elucidated. In all probability, it may be sound to conclude now that there is no apparent physiological purpose behind the two discrete stereospecificities.

Historically, there was a proposal which was discussed extensively but is no longer considered valid. In the 1950s it was found that in the presence of pyruvate, lactate dehydrogenase as well as alcohol dehydrogenase in the presence of acetaldehyde, could oxidize the NADH bound to glyceraldehyde-phosphate dehydrogenase.[94,95] On the basis of this finding, Velick[96] hypothesized that dehydrogenase-catalyzed reactions could be regulated in vivo by the simultaneous attachment of two enzymes through a coenzyme molecule. He pointed out that in such a case the two dehydrogenases were likely to have the opposite stereospecificity in order to avoid steric hindrance. (The stereospecificity of glyceraldehyde-phosphate dehydrogenase was not known at that time).

This speculation received substantial support when the stereospecificity of glyceraldehyde-phosphate dehydrogenase was subsequently found to be indeed the opposite (B stereospecific) from that of alcohol and lactate dehydrogenases (A stereospecific).[97] Supporting Velick's hypothesis, Levy and Vennesland[31] thus proposed circumspectively that this type of enzyme-enzyme interaction might be the physiological basis for the existence of the A and B stereospecificities.

This proposal was weakened considerably, however, when Astrachan et al.[98] reported that the bound NADH to glyceraldehyde-phosphate dehydrogenase can be oxidized with glutamate dehydrogenase, which is also a B-stereospecific enzyme. Furthermore, Hung and Hoberman[99] studied the influence of the stereospecificity on the rates of hydrogen exchange between substrates of NAD-coupled dehydrogenases and concluded that their study did not support Levy-Vennesland's proposal. Vennesland seemed to be content with this conclusion.[83]

VI. STEREOSPECIFICITY IN CHEMICAL OXIDATION-REDUCTION AND IN FORMATION OF ADDITION PRODUCTS

In addition to the reduction by H^-, the pyridinium ring of $NAD(P)^+$ can also be converted to the addition products having 1,4-dihydropyridine rings by the attack on the C-4 by various nucleophiles such as CN^-, HSO_3^-, and carbonyl compounds.[22] Figure 7, in which the nucleophiles are represented by $X:^-$, relates that the pyridinium ring can exist as a resonating system with the positive charge distributed between the ring nitrogen and the C-2, C-4, and C-6. The C-4 is particularly susceptible to nucleophilic attack since the inductive effect of the carboxamide moiety, an electron withdrawing group, enhances its carbonium ion character.

Not only the H^- transfer but also the addition reactions take place enzymatically as well as chemically, although the rates of enzyme-mediated reactions are uncomparably faster than those of nonenzymatic reactions. One possible reason for the great efficiency when an enzyme is present is that the nicotinamide ring of the enzyme-bound coenzyme in the transition state is probably puckered, simulating the boat form (Figure 8).

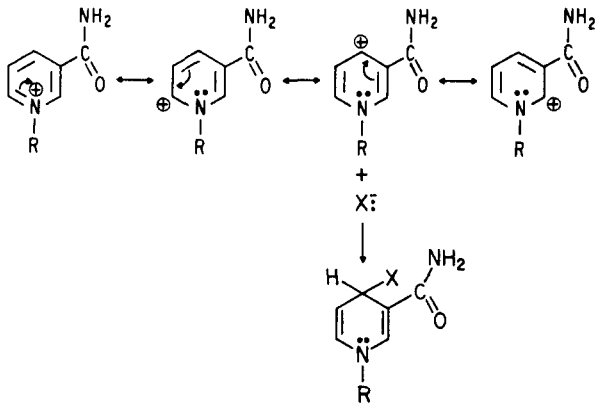


FIGURE 7. Possible positions of nucleophilic addition on the pyridinium ring of NAD^+. The resonating system of the pyridinium ring makes the three even-numbered ring carbons share partial carbonium ion character. However, the C-4 atom is particularly susceptible to the attack by a nucleophile $X:^-$ since its positive charge is more enhanced due to the presence of an electron-withdrawing carboxamide group at C-3.

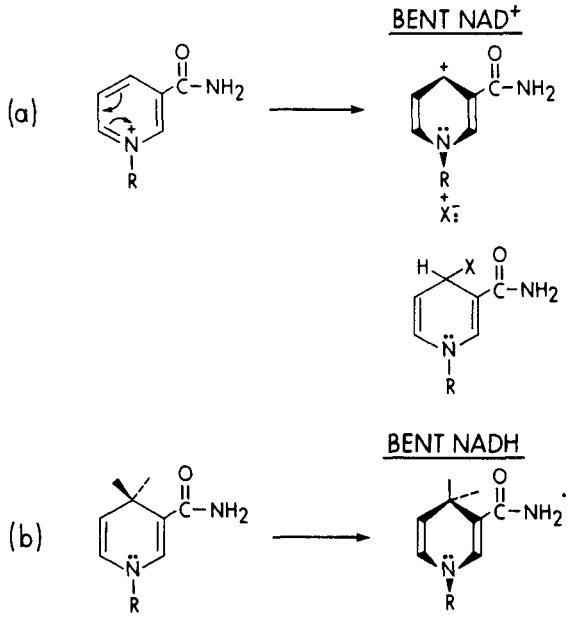


FIGURE 8. Puckered forms of NAD^+ (a) and NADH (b). In the transition state, enzyme distorts the nicotinamide ring (a) and dihydronicotinamide ring (b) of enzyme-bound NAD^+ and NADH, respectively, into the boat form causing the N-1 of both rings to be pyramidal. The N-1–C-1′ bond is no longer in the plane of nicotinamide/dihydronicotinamide ring, but is bent far enough out of the plane to destroy any resonance between a lone pair of electrons on the N-1 and the remaining double bonds in the ring. In this boat form, the carbonium ion character of the nicotinamide ring of NAD^+ is localized at C-4, which becomes susceptible to nucleophilic attack by $X:^-$. There is some evidence that the nicotinamide/dihydronicotinamide ring of the NAD(H) in the folded form is also puckered (free in solution, folded form to open form = 36:64%). However, the degree of folding in enzyme-bound coenzyme may be more profound and the enzyme may stabilize the transition state of the coenzyme. Hence the rate of the nucleophile addition reactions with enzyme-bound NAD^+ is often several orders of magnitude faster than that with free NAD^+. The puckered form of the ring in enzyme-bound coenzyme has never been detected by X-ray crystallography, however. (Adopted from Cook, P. F., Oppenheimer, N. J., and Cleland, W. W., *Biochemistry*, 20, 1817, 1981. With permission.)

A. Chemical Oxidation-Reduction

As noted previously, from the very article that described the enzyme stereospecificity originally, it has been repeatedly confirmed that the chemical reduction of $NAD(P)^+$ with reductants such as sodium dithionite in D_2O form a mixture of $NAD(P)D_A$ and $NAD(P)D_B$ at an *unequal proportion,* the amount of $NAD(P)D_A$ always exceeding $NAD(P)D_B$.

Likewise, the chemical oxidation of NADH with neutral ferricyanide also favors the removal of the A-side hydrogen of the dihydronicotinamide ring. Table 1 presents the summary of the stereopreference data in chemical oxidation-reduction from several reports.

It should be noted from Table 1 that 46 to 65% of the ferricyanide oxidation product of $NADD_A$ is $NAD(D)^+$, but much less deuterium is removed [90% of deuterium remains in $NAD(D)^+$] when $NADD_B$ is oxidized by the same procedure. This observation, which is also illustrated in Figure 4 for clarity, proves that it is the steric effect, not the deuterium isotope effect, that makes the removal of deuterium from $NADD_A$ easier than from $NADD_B$. This steric effect will be explained explicitly when the conformation of free coenzyme in solution is dealt with in Section VII.

B. Formation of Addition Products (Adducts)

Cyanide[100,101] and carbonyl compounds[102,103] are well-known nucleophiles which form addition products with $NAD(P)^+$ or with their analogs. In preparing the cyanide adducts with NAD^+ and $(3AcPy)AD^+$, Arnold and Kaplan[39] and Oppenheimer et al.,[38] respectively, found that the cyanide anion could, like H^-, attack either the A or the B side of the pyridinium ring resulting in two diastereomers, $NADCN_A$ and $NADCN_B$ [or $(3AcPy)ADCN_A$ and $(3AcPy)ADCN_B$], as shown in Figure 9. In these addition reactions, too, the yield of the A-side stereoisomer is invariably higher than the B-side addition product, i.e., $NADCN_A/NADCN_B = 1.5$,[39] and $(3AcPy)ADCN_A/(3AcPy)ADCN_B = 1.8$ (in D_2O).[38] Such unequal proportion of the diastereomeric adduct formation does not occur with NMN^+.[104]

The adducts formed between NAD^+ and various carbonyl compounds have been studied extensively because there is some experimental evidence that various dehydrogenases catalyze the formation of these adducts under physiological condition (alkaline condition is preferred, however) for the physiological purpose of regulating the rate of metabolism.[105,106]

First, however, the nonenzymatic formation of the adducts with carbonyl compounds will be considered by employing pyruvate as the example of the carbonyl compounds (Figure 10). Under alkaline condition, the tautomeric enol form, not the keto form, of pyruvate forms a covalent bond with its α carbon (π electrons) to the enol group and C-4 of the pyridinium ring of NAD^+ as illustrated in Figure 10a. Understandably, this reaction requires the alkaline condition to enhance the formation of the enol form of pyruvate. Figure 10b shows that the addition reaction is followed by the second nucleophilic attack on the carbonyl carbon of the covalently bound pyruvate by the carboximide nitrogen to give reduced naphthyridone structure (Figure 10c). The carbonyl addition with NAD^+ is considerably faster in less polar medium such as alcohol than in aqueous solution.[22]

The addition product thus formed with the substrate of a given dehydrogenase usually serves as a specific inhibitor of that enzyme. For instance, the NAD-pyruvate adduct inhibits lactate dehydrogenase but exerts little effect on malate dehydrogenase, whereas NAD-oxaloacetate adduct is a strong inhibitor of malate dehydrogenase but shows poor inhibition on lactate dehydrogenase.[103] In recent years, these adducts have proved useful as biospecific eluants in affinity chromatography in the purification of various dehydrogenases.[107]

Figure 11 presents the mechanism of enzyme-catalyzed adduct formation employing lactate dehydrogenase as an example of the dehydrogenases and pyruvate, the carbonyl compounds as before. The extraction of the hydroxyl proton of the tautomeric enol form of pyruvate by a base on the enzyme (Figure 11a) facilitates the nucleophilic attack on C-4 of the pyridinium ring of the enzyme-bound NAD^+ by the π electrons, forming an "abortive ternary complex" (true "abortive ternary complex").

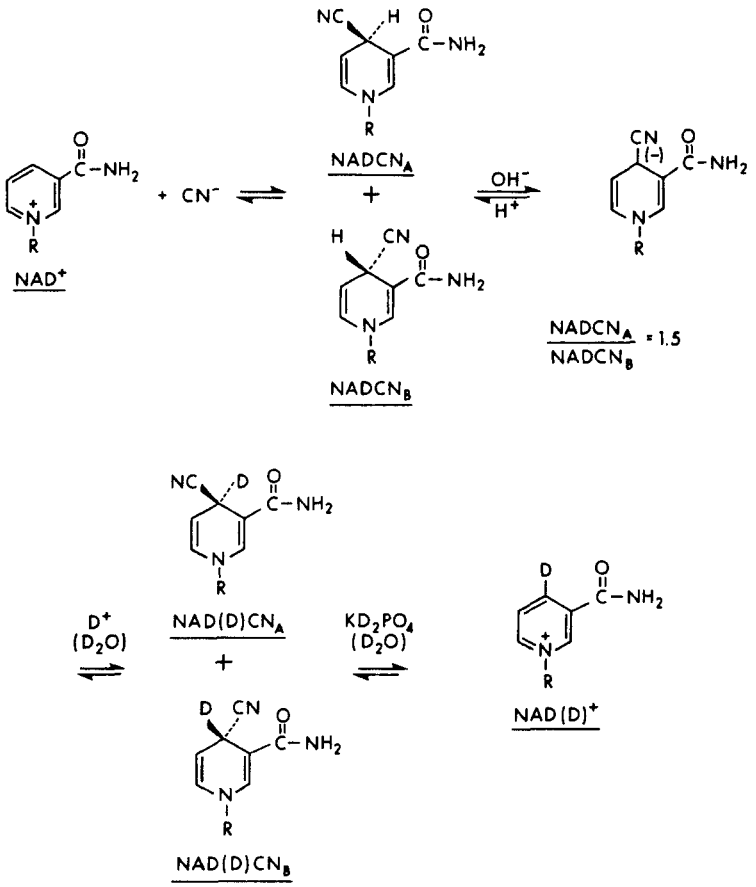


FIGURE 9. Reversible formation of NADCN adduct and preparation of NAD(D)$^+$ from NAD$^+$ via this adduct. Owing to the NAD$^+$ conformation in solution (the B face of nicotinamide folded against adenine), the addition of CN^- to the A side of the nicotinamide of NAD$^+$ is favored to the B side addition. The C-4 proton of NADCN is acidic enough to be exchanged freely with D^+ in D_2O medium. Upon the exchange, the dissociation of CN^- from the adduct is facilitated by dilution as well as lowering the pD of the medium. NADP(D)$^+$ can be obtained by the same procedure. If the reaction is carried out in THO medium, instead of D_2O, tritiated oxidized coenzyme is obtained.

[It should be noted, however, that unlike the ternary complex formed between the chemically synthesized NAD-pyruvate adduct and the enzyme, the true "abortive ternary complex" is acyclic, meaning that the second six-membered ring is not closed. This structural difference is the basis for the difference in fluorometric as well as spectral (UV and CD) properites between these two types of ternary complexes.[39,111] Nonetheless, the synthetic NAD-pyruvate adduct is structurally so similar to the acyclic NAD-pyruvate moiety of the abortive ternary complex that it can release the latter from the enzyme through competition.[111] The NAD-pyruvate moiety can also be dissociated from the abortive ternary complex by treating it with base (Figure 11) or urea.[39,111] Once that moiety is dissociated from the enzyme, the second six-membered ring closes and the product becomes indistinguishable from the chemically synthesized adduct (cf. Figures 10 and 11).[39,112] The formation of the nonproductive "abortive ternary complex" reduces the concentration of free enzyme thus resulting in inhibition, and that is generally regarded as the mechanism for the inhibition of dehydrogenase activity by high concentration of substrate. It is important to realize that

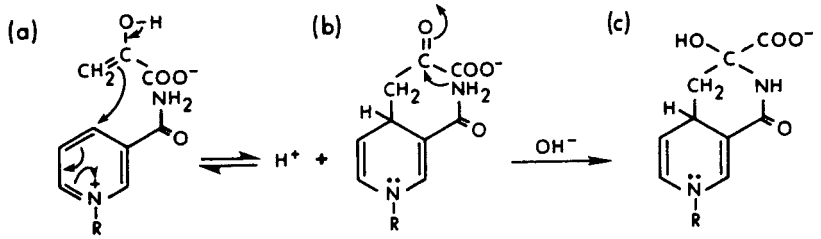


FIGURE 10. Base-catalyzed formation of the addition product between NAD^+ and pyruvate. The π electrons of the tautomeric enol form of pyruvate attack electrophilic C-4 of the nicotinamide (a) to form a covalent bond (b), followed by another nucleophilic attack from the carboxamide nitrogen to close the second six-membered ring (c). (Adopted from Arnold, L. J., Jr. and Kaplan, N. O., *J. Biol. Chem.*., 249, 652, 1974. With permission.)

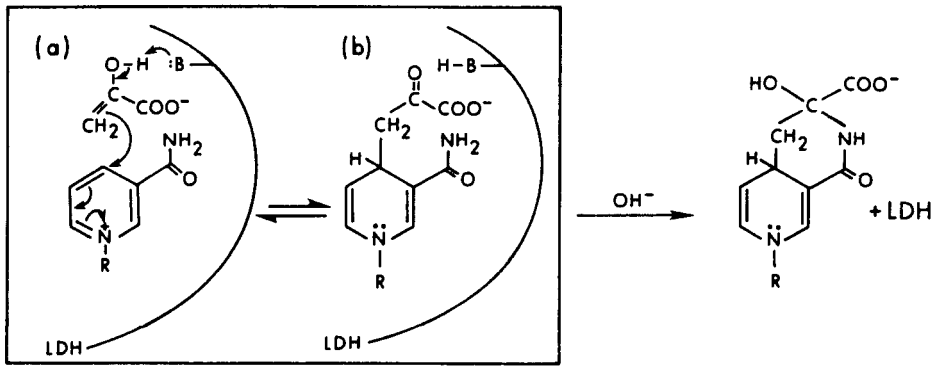


FIGURE 11. Enzyme-catalyzed formation of the "abortive ternary complex" among L-lactate dehydrogenase (EC.1.1.1.27.), NAD^+ and pyruvate. While the keto form of pyruvate serves as the physiological substrate for this enzyme, it is the enol form that is responsible for forming the "abortive ternary complex". The extraction of the hydroxyl proton by a base on the enzyme molecule facilitates the nucleophilic attack on the nicotinamide C-4 by the π electrons of the pyruvate (a), but the second six-membered ring remains unclosed in the ternary complex (b) (enclosed box). The structure of the abortive ternary complex is, therefore, different from the ternary complex formed between the chemically synthesized NAD-pyruvate binary adduct and the enzyme. Under alkaline condition (or in 6 *M* urea), however, the NAD-pyruvate adduct dissociates from the protein and only then the second ring closes. The formation of the ternary complex reduces the free enzyme level and, therefore, results in inhibition of the physiological reaction of the enzyme. (From Arnold, L. J., Jr. and Kaplan, N. O., *J. Biol. Chem.*, 249, 652, 1974. With permission.)

while the physiological substrate of lactate dehydrogenase is the keto form of pyruvate,[27,113] it is the tautomeric enol form that is responsible for the inhibition of that enzyme.[114] Everse et al.[105] and Everse and Kaplan[106] extensively discussed the differential regulation of the activities of lactate dehydrogenase isozymes by cellular pyruvate concentration in relation to their metabolic roles in vivo.]

Arnold and Kaplan[39] found that the NAD-pyruvate adduct was formed at a rate about an order of magnitude faster in the presence of lactate dehydrogenase compared to the nonenzymatic formation. There is also a long-established fact that the concentrations of the nucleophiles such as sulfite and cyanide required to form their respective adducts with free NAD^+ are several orders of magnitude higher than those required for the same reaction with lactate dehydrogenase-bound NAD^+.[108,109]

These observations may be explained, in part, by the probable hydrophobic nature of the environment on the enzyme where the addition reaction takes place. However, it is more

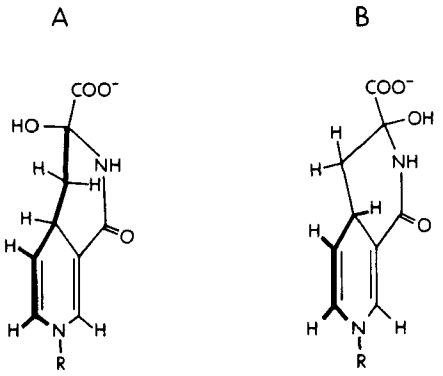


FIGURE 12. The structures of the A-side (A) and the B-side (B) addition products between NAD^+ and pyruvate. Chemical synthesis favors the formation of the A-side isomer to the B-side isomer at a ratio of 60:40%. The adduct formed by the action of lactate dehydrogenase is purely the A-side isomer. The pyruvate moiety of the adduct is equitorial which keeps the hydrogen on the opposite side in the fully axial position. (From Arnold, L. J., Jr., and Kaplan, N. O., *J. Biol. Chem.*, 249, 652, 1974. With permission).

likely that a greater role is played by the nature of the electronic structure of the nicotinamide ring in the transition state when the coenzyme is bound to a host enzyme. Cook et al.[110] reported some evidence which suggests that in the transition state the nicotinamide ring as well as the dihydronicotinamide of enzyme-bound coenzyme is distorted into the boat form (Figure 8), which in turn results in the localization of the positive charge of the pyridinium ring at the C-4. Therefore, the bent nicotinamide ring structure facilitates the attack on the carbonium ion, C-4, by the nucleophile X^- as illustrated in Figure 8a.

Since the carbonyl adducts with NAD^+ can also be formed enzymatically as well as nonenzymatically, the stereospecificity of these addition reactions is a matter of significant interest. By employing proton magnetic resonance, Arnold and Kaplan[39] and Arnold et al.[112] were able to establish that the base-catalyzed, nonenzymatic addition reaction also prefers the formation of the A-side diastereomer to the B-side product at a ratio of 60:40%. Figure 12 represents the structures of the A- and the B-side NAD-pyruvate adduct. In contrast to NAD^+, the pyruvate adduct formed with NMN^+ is in an equal population mixture of the A- and B-side isomers.[112] These data demonstrate that the AMP moiety of NAD^+ is responsible for the observed stereopreference in chemical formation of adduct.

Arnold and Kaplan,[39] however, demonstrated that the NAD-pyruvate addition product formed in the presence of lactate dehydrogenase (chicken heart) is the pure A-side isomer. This finding is consistent with the stereospecificity of the enzyme in the physiological reaction. To date, the stereochemical analysis of the addition product formed in the presence of a B-stereospecific enzyme has not been carried out.

It is critical to point out that the significant stereopreference in the nonenzymative reactions involving the dinucleotides, be it the redox reaction or the adduct formation, is always for the A side. Unlike enzyme-catalyzed reactions, there has been no known chemical reaction in which the B side of the coenzyme is favored. This one-sided steric preference in the reactions involving the entire coenzyme molecule, but not the half-molecule such as NMN^+, is ascribed entirely to the conformation of the free dinucleotide in solution.

The puckering of the nicotinamide/dihyronicotinamide ring and the conformation of the coenzyme, both in free and in enzyme-bound states, are discussed in some detail in the following sections.

VII. CONFORMATION OF FREE COENZYME

The study on the conformation of NAD(P) has been an intensive research subject for over 2 decades. Various techniques have been employed for this purpose and our understanding about this subject has been refined and misinterpretations corrected as there are improvements in instrumentations: fluorescence energy transfer and quenching;[32-35] hyperchromicity of the chromophores;[115,116] circular dichroism;[116,117] X-ray crystallography;[47,118] and NMR.[119-130]

Of these, fluorometric study and, more recently, NMR have made the most significant contribution to the elucidation of the conformation of the coenzyme in solution state. Since the data obtained by the former have been reviewed several times already, here the emphasis will be given to the NMR work. The X-ray crystallography of the coenzyme was carried out on the Li^+-NAD^+ complex crystallized from aqueous methanol as dihydrates.

It should be pointed out that extensive coverage of the conformation of nucleotides is beyond the scope of this article. Here, the coverage will be limited to the overall conformation of the coenzyme molecule and other conformational properties that are relevant to the stereopreference in chemical reactions and, in Section VIII, to the absolute stereospecificity in enzymatic reactions.

Detailed conformational parameters such as, among others, various bond angles, orientation of pyrophosphate linkage, and the mode of puckering of ribose rings (endo-exo forms) of the coenzyme, both in free state in solution, as well as in crystalline form, and in enzyme-bound state have been authoritatively reviewed very recently in the articles that deal exclusively with the conformational aspects.[104,131,172]

A. In Solution

1. Fluorescence Property: Evidence for Folded Nature

Because the oxidized form of the coenzyme does not fluoresce, the conformational work by fluorometry has been virtually limited to the reduced form. Weber[33] was the first to point out that there was intramolecular association between the adenine base and the dihydrodnicotinamide moiety of NADH. He convincingly showed that the excitation of the NADH molecules in an aqueous solution with the beam at 260 nm (80 to 90% of which is absorbed by the adenine ring), in addition to that at 340 nm (which is absorbed entirely by the dihydronicotinamide ring), caused the fluorescence emission of the dihydronicotinamide ring (λ_{max} = 462 nm).

This observation clearly indicates that there is excitation energy transfer from the adenine base to the dihydronicotinamide ring of NADH, which becomes possible only by the close, stacked juxtaposition of these two moieties. In order for them to be stacked closely, the backbone of the coenzyme molecule must be folded, because in open conformation, such as that in Figure 13, the two rings are far apart and, as a result, energy transfer between these two ring systems is unlikely.

The folded nature of NADH conformation in aqueous medium would be confirmed by studying the fluorescence characteristics as a function of solvent, temperature, and structural modification, for those factors are likely to perturb the molecular conformation and cause changes in the mode of folding.

In solvents having lower dielectric constants than water such as propylene glycol[33] and ethylene glycol monomethyl ether,[32] the fluorescence emission of NADH associated with the excitation at 340 nm is usually enhanced by several fold, while that with the 260 nm excitation virtually disappears, demonstrating clearly the lack of energy transfer due to unfolding of the molecule in these media.

Velick[32] also found that raising sample temperature stepwisely from 9 to 62° caused the loss of the 260 nm-excited emission more rapidly than the 340 nm-excited emission. The decrease in the emission associated with the 340 nm is ascribed to thermal dissipation of

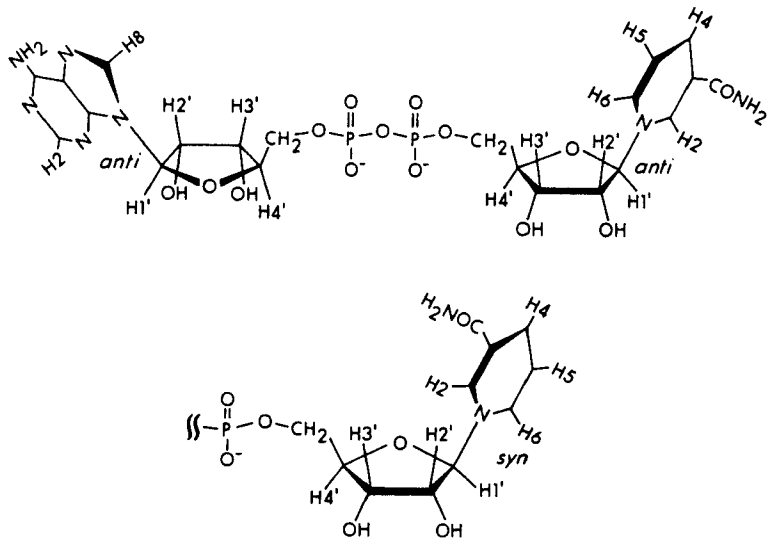


FIGURE 13. The NAD in the full open conformation. The top structure shows the *anti* orientation of both the adenine and the nicotinamide rings and the bottom, the NMN^+ portion of the coenzyme, presents the nicotinamide ring in the *syn* form. It can be seen clearly that when the nicotinamide is in the *anti* form, the nicotinamide C-2 proton is closer to the C-1′ proton than is the C-6 proton; in the *syn* orientation, on the other hand, the nicotinamide C-6 proton is in closer proximity to the C-1′ than is the C-2 proton. Also, in the *syn* form the C-2 proton is closer to the proton on the C-3′ than is the C-6 proton. (From Levy, H. R., Ejchart, A., and Levy, G. C., *Biochemistry*, 22, 2792, 1983. With permission.)

the excitation energy, however, in the case of the 260-nm excitation, both thermal dissipation and decreased energy transfer owing to molecular unfolding are responsible for the rapid loss of the fluorescence emission.

Shifrin and Kaplan[34] noted that in an aqueous medium the nonphysiological isomer α-NADH and N(HyP)DH analog show only a fraction of the 260-nm-excited emission seen with the physiological NADH, although the emission accompanied by the excitation at 340 nm remained relatively unchanged. Hence, they concluded that the two ring bases in the two nonphysiological molecules are, for steric reasons or otherwise, not close enough for efficient excitation energy transfer.

These observations led Velick[32] to propose that NADH in an aqueous solution is in the folded form, in which the adenine and the dihydronicotinamide rings are stacked nearly in parallel, the former lying above the B face of the latter. This conformational model is consistent with the low reactivity of the B side in the oxidation of NADH by ferricyanide since the hydrogen on this face is, unlike the H_A, sterically shielded by the adenine ring and as a result less accessible to the oxidant (Table 1 and Figure 4).

Velick[32] also deduced that NAD^+ should be similarly folded since its chemical reduction with dithionite attacks the B side less preferably than the A side (Table 1 and Figure 3). In support of this interpretation. Shifrin and Kaplan[35] showed that the fluorescent, oxidized form of the analog (3Amino)AD^+ exhibited over 11 times more intense fluorescence in propylene glycol than it did in water; however, 3-aminopyridine methiodide, which lacks, among others, the adenine ring did not show this variation in fluorescence intensity in these two media. This finding can be best explained by the fluorescence quenching by the adenine ring of the oxidized analog in water (folded conformation), but not in the organic solvent (open conformation). Furthermore, the preponderance of the A-side diastereomer in the formation of the addition products between NAD^+ and CN^- or carbonyl compounds strongly

supports the view that the oxidized form, too, is folded in such a way that the B side of the nicotinamide ring faces the adenine ring.

2. Nuclear Magnetic Resonance Studies

Our current knowledge about the conformation of NAD(P) and other nucleotides of biological importance is largely derived from NMR study, especially PMR investigation in D_2O medium. Attention should be drawn to the fact that many of the early investigations employing PMR contain widespread erroneous interpretations and misleading conclusions due to the unavailability of high resolution instruments as well as to the use of isotopically impure samples. Many of the serious errors are pointed out and corrected here and in Reference 38.

a. Evidence for the Dynamic "Folded-Open" Conformation

In any ring/ring-stacked conformation — caused by intramolecular folding — of a molecule containing more than one aromatic rings, it is expected that the anisotropic shielding by the ring current originating from the aromatic ring will profoundly influence the chemical shifts of the protons of the facing ring, whether it has an aromatic or nonaromatic structure.

Meyer et al.[119] were the first to note that the chemical shifts of the protons on the dihydronicotinamide ring of NADH appear in significantly higher fields when compared to those of *N*-benzyl-1,4-dihydronicotinamide. Likewise, Jardetzky and Wade-Jardetzky[120] found that the chemical shifts of the protons on both adenine and pyridinium or dihydropyridine rings of NAD(P) moved uniformly to a considerably higher field compared to their counterparts on the mononucleotides AMP (or ADPR[121]) and NMN (which were present in an equimolar mixture), respectively (see Table 2). Similarly, the chemical shifts of the protons on the dihydropyridine ring of NAD-pyruvate adduct (Figure 12) (as well as NAD-oxaloacetate and NAD-acetaldehyde adducts) are also shielded without exception compared with the resonances of the counterpart protons of the adducts formed with NMN^+.[112]

The fact that the chemical shifts of all of the protons on the carbons (and also the resonances for the carbons themselves, see below) of both pyridinium/dihydropyridine and adenine rings of the dinucleotide are affected, always shifting to the upper field, demonstrates that there is strong intramolecular interaction between the two rings and that it is magnetic, i.e., ring current effect, not electric effect, that is responsible for the shielding. In order for the ring current to take effect, the two rings must be in close proximity. As indicated in the preceding subsection, the same conclusion was reached by the fluorometric study.

Unlike the pyridinium ring of NAD^+, the dihyropyridine ring of NADH is not aromatic and, therefore, imparts only a small degree of shielding to the adenine protons compared to what the nicotinamide ring of NAD^+ does.[132] Also, the upfield shifts of the resonances of the pyridinium ring of α-NAD^+ is less prominent,[121] suggesting that this nonphysiological isomer has weaker intramolecular association than the physiological one. It is, however, also true that the difference in the direction of the nicotinamide ring in α-NAD^+ is responsible for at least a part of the change in the chemical shifts.[60]

Lowering the pH below the pK_a (3.8) of the N-1 of the adenine ring causes unfolding of NAD^+ due to the creation of the electrostatic repulsion between the pyridinium ring and now the protonated adenine ring.[120,123] Since the dihydronicotinamide ring is not charged, NADH molecule remains to be folded at low pH.[123] (Caution should be exercised for NAD(P)H decomposes rapidly under acidic condition.)

In accordance with the PMR work, Blumenstein and Raftery[130] reported that the ^{13}C chemical shifts of the nicotinamide ring of NAD^+ at neutrality (where the coenzyme assumes the folded conformation) were uniformly shifted upfield compared with those for the same carbons of NMN^+. (The comparison was made with the resonances of NMN^+ obtained at pH 4, where the phosphate group is singly ionized. Since NMN^+ phosphate carries two negative charges at neutral pH, these workers caution that the comparison should not be

Table 2
CHEMICAL SHIFTS OF THE PROTONS ON THE DIHYDROPYRIDINE RING[a]

	C-2	C-6	C-5	C-4		$\Delta\delta_{AB}$
Mononucleotides						
DHNR	7.157	6.118	5.009	3.075		<0.002
NMNH	7.166	6.239	5.036	3.075		<0.002
$NMNH(OAc)_2$	7.139	6.252	5.045	3.055		<0.002
				H_A	H_B	
Dinucleotides						
NADH	6.934	5.963	4.750	2.739	2.636	0.103
NADPH	6.923	5.957	4.784	2.809	2.723	0.086
$NADH(OAc)_4$	6.925	6.025	4.868	2.870	2.759	0.111
ϵ-NADH	6.720	5.870	4.655	2.600	2.525	0.080
N(Hyp)DH	6.986	6.057	4.882	2.898	2.830	0.059
(N-Met)NADH	6.868	6.007	4.741	2.661	2.525	0.136
(TN)ADH	7.736	6.007	4.741	2.661	2.525	0.136
(3AcPy)ADH	7.280	6.000	4.930	2.573	2.532	0.041
(3PyAl)ADH	7.093	6.002	4.984	2.648	2.602	0.045
$N(CH_3NH\text{-}A)DH$				2.83	2.56	0.27
$N(CH_3CH_2NH\text{-}A)DH$				2.87	2.67	0.20
N(Br-A)DH				2.90	2.78	0.12

[a] Chemical shifts are in ppm from TSP and are accurate to within 0.002 ppm. The samples were 50 m*M*, the pD was 8.5, and the temperature was 22°C, except for NADH and NADPH where the concentration was 5 m*M*.

From Oppenheimer, N. J., *The Pyridine Nucleotide Coenzymes*, Everse, J., Anderson, B. M., and You, K., Eds., Academic Press, New York, 1982, 51. With permission.

made with the values obtained at near neutrality, but should be with that obtained at pH 4). In contrast, at acidic pH, where the coenzyme molecule unfolds due to electric repulsion between the nicotinamide and protonated adenine, the ^{13}C resonances for the carbons of the pyridinium ring of NAD^+ are virtually identical with those in NMN^+. These results are consistent with those obtained by PMR work: the adenine and nicotinamide/dihyronicotinamide rings are stacked in parallel due to the intramolecular folding at the backbone of the coenzyme molecule.

The evidence for the folded nature of the coenzyme conformation can be more easily elucidated with the reduced form than with the oxidized form. In addition to the upfield shift of the chemical shifts of the dihydronicotinamide ring protons of NAD(P)H compared to the counterpart protons on NMNH,[120,124] Table 2 clearly demonstrates that the chemical shifts of the two geminal A and B protons of the dihydropyridine ring of various reduced dinucleotides are nonequivalent, the chemical shift for the *B-proton* always appearing in higher field than that for the A proton. The nonequivalence between these two protons exists regardless of the modification of the dihydropyridine ring, the purine base, and the ribose (bonded to the dihydropyridine ring) of the reduced dinucleotides.

It is important to note in Table 2 that in mononucleotides such as NMNH and DHNR, the chemical shifts of the A and B protons are coalesced to a single resonance, thus showing no indication of nonequivalence between them, and move downfield. Why are the two geminal protons — H_A and H_B — of the dinucleotides nonequivalent?

Since the two diastereotopic A and B protons of the dinucleotides as well as the mononucleotides cannot be related by any symmetry, their chemical as well as magnetic environment must be different. In order to observe discrete resonances, however, the two methylene

protons on the dihydropyridine ring require that the chemical or magnetic anisotropy generated by nearby chiral centers must be greater than the line width of the signals. If the chiral centers are too distant or nearly symmetrical with respect to the subject protons, the existing anisotropy may not be sufficient enough to result in resolution. In the case of NMNH and DHNR, the nonequivalence, if any, between the A and the B protons must be caused by the ribose chiral centers. The absence of resolved resonances in these mononucleotides indicates that the magnetic anisotropy generated by the sugar is too small to cause resolution, at least on 220-MHz scale.

Hence, the resolution of the A and B resonances of NAD(P)H and their analogs must be ascribed to the anisotropy generated by the aromatic adenine ring of the dinucleotide molecule. However, any magnetic influence on the prochiral A and B protons from the adenine ring is not possible if the coenzyme assumes a linearly extended, open conformation such as that seen in Figure 13, since these protons are located farthermost away from the adenine in such conformation. The only logical choice in explaining the PMR data is the folded model, in which the two bases of the dinucleotide are stacked face to face.

Since the A and B protons lie above and below the dihydropyridine ring plane of NAD(P)H (all other protons on this ring are coplanar) in the folded form, one proton should face the adenine ring and the other should be exposed to the medium. The proton which faces the adenine ring is expected to receive stronger shielding by the adenine ring current than the one exposed to the medium and, as a consequence, the former's chemical shift must appear in a higher field than the latter's. Experimentally, it has been proven that the chemical shift of the B proton appears in a higher field than that of the A proton (Table 2). It is, therefore, the B proton which faces the adenine ring.

Oppenheimer et al.[38] noted that the difference between the chemical shifts for the A and B protons of α-NADH ($\delta_A - \delta_B = 0.05$ ppm) was much smaller than that for the same protons in the physiological NADH ($\delta_A - \delta_B = 0.10$ ppm). This finding demonstrates that the reduced form of this nonphysiological isomer is also in the folded conformation, the B side likewisely facing the adenine ring; however, the degree of intramolecular interaction is probably much weaker than that of the physiological form, as Shifrin and Kaplan[39] originally inferred. This conclusion, nonetheless, does not imply that α-NADH and β-NADH have the same three-dimensional structure.

Dissolving NADH in a medium having lower polarity than D_2O (e.g., D_2O-CD_3OD mixture) causes the chemical shifts for the A and B protons to merge together and shift downfield to the limiting values of the chemical shifts for the same protons in NMNH.[128] This result confirms that in apolar solvent the reduced form of the coenzyme unfolds and as a result the A and B protons lose the nonequivalence.

Perturbation of NAD structure by dissolving it in apolar solvent[38,123] or by raising temperature[120,121,125] causes downfield shift of the chemical shifts for the protons on both bases rather uniformly, indicating again that it is the ring current effect that is affected by the unfolding caused under these environmental conditions. The same conclusion was drawn by the change in fluorescent property of NADH under the same conditions, as discussed previously.

Figure 14 schematically represents the coenzyme in the folded conformation at the backbone. For clarity, the two bases are shown separated, but in reality the B face of the nicotinamide/dihydronicotinamide ring should be right on top of the adenine ring. The difference between the two structures is the glycosidic torisonal orientations of the two bases, which is the subject of the next section.

The PMR property of NAD(P) described above can be best rationalized with a model in which the coenzyme molecules exist at a rapid, dynamic equilibrium state between the folded form (Figure 14) and the open form (Figures 13 and 20). The equilibrium is rapid on the PMR time scale; therefore, the observed chemical shifts (Table 2) represent a time-averaged weighted sum of the contributions from these two forms.

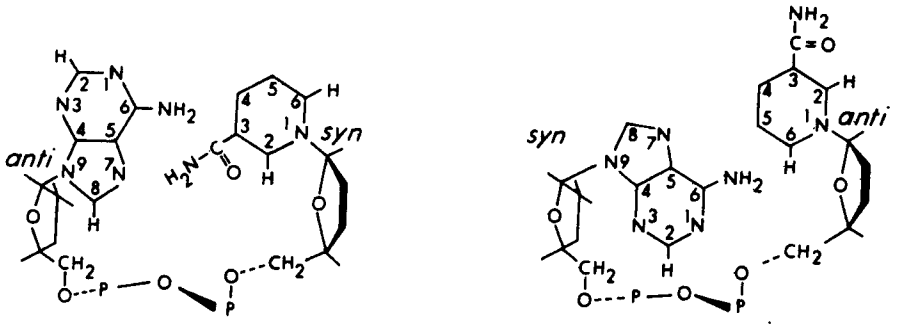


FIGURE 14. Schematic representation of NAD in folded conformation with the bases oriented in *syn* or *anti* angles. The structure on the left shows the *anti* orientation of the adenine-ribose linkage (the C-8 is closest to the C-2′ proton and relatively close to the protons on C-3′ and C-5′, but distant from the C-1′ proton; the C-2 proton is, on the other hand, distant from any of the ribose protons) and the *syn* conformation of the nicotinamide-ribose linkage. Shown on the right is the structure of the coenzyme in which the adenine- ribose linkage is in the *syn* form (the proton on the C-2 is closest to the C-2′ proton and relatively close to the proton on the C-5′) and the nicotinamide-ribose linkage in the *anti* orientation. (From Sarma, R. H. and Mynott, R. J., *J. Am. Chem. Soc.*, 95, 7470, 1973. With permission.)

On the basis of thermodynamic parameters derived from chemical shift-temperature relationship, Jardetzky and Wade-Jardetzky[120] estimated that the population of the NAD^+ molecule with folded conformation is 20 to 40% at pH 7.2 and 27°C. A significantly lower value, 15%, was reported by McDonald et al.[125] The estimations based upon the ionization constants of the adenine N-1 of NAD^+ and ADPR[133] and upon UV spectrophotometric[115,116] as well as circular dichroism[116] studies, however, indicate the population to be 44 and 40 to 50%, respectively. Based upon vicinal coupling constants, $^3J_{4\text{-}5}$, of the dihydronicotinamide ring, Oppenheimer et al.[128] estimated the folded population in an intriguing manner. The observed $^3J_{A\text{-}5}$ and $^3J_{B\text{-}5}$ are 3.1 and 3.9 Hz, respectively; in the fully open NADH (i.e., at 70°), as well as in NMNH, these two constants merge to a single, midpoint value of 3.5 Hz. They made the assumption that in the fully folded form, more than two constants should approach the limiting values of 2.2 and 4.6 Hz, respectively. (See Section VII.A.2.c for the rationale behind this assumption.) The mole fraction for the folded form X_F then should have the following relationshp with the coupling constants: $J_{obs} = X_F \cdot J_F + J_o(1 - X_F)$, where $J_{obs} = 3.1$ and 3.9, $J_F = 2.2$ and 4.6, and $J_o = 3.5$. From this equation these workers were able to conclude that 36% of the coenzyme population is in the folded form.

Clearly, the population proportion between the folded form and the open form is extremely sensitive to the environmental conditions, i.e., temperature, pH, ionic strength, and polarity of the medium. This is probably the reason for the widely variable estimate for the folded form population. Nevertheless, the reported values are in approximate agreement with the deuterium incorporation into the B side during chemical reduction with dithionite in D_2O(Table 1).

b. Base-Ribose Torisonal Orientations

The torisonal angle, χ, about the glycosidic linkages which bond the adenine (χ_A) and the nicotinamide (χ_N) to their adjacent ribose is another major conformational feature of the dinucleotide. According to the Nomenclature Commission of the IUPAC-IUB,[134] the torisonal angle χ_A is defined as the angle between the N-9–C-4 bond of the purine base and the C-1′–O-1′ bond of the adenosine ribose, and χ_N as the angle between the N-1–C-2 bond of the pyridinium/dihydropyridine ring and the C-1′–O-1′ bond of the ribose in NMN. Figure 15 presents these angles diagramatically.

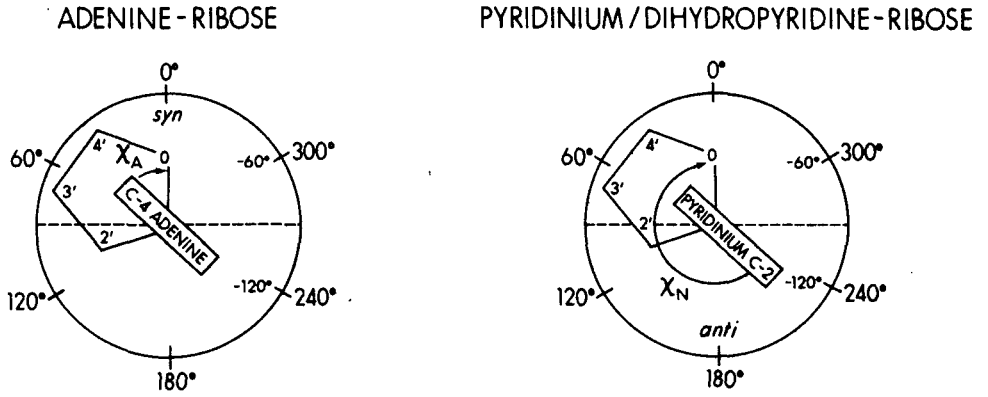


FIGURE 15. The *anti* and *syn* glycosidic torisonal angles of adenine (χ_A) and nicotinamide/dihydronucotinamide (χ_N) rings. The angle χ_A is defined as the clockwise angle between the C-1–O-1′ bond and the N-9–C-4 bond of the purine ring while facing the glycosidic bond from N-9; the angle χ_N is the clockwise angle between the C-1–O-1′ and the N-1–C-2 bond of the nicotinamide/dihydronicotinamide ring. There are two thermodynamically stable torisonal regions: *syn* and *anti*. The angle ranges for these regions are also indicated on the figure. (Adapted from IUPAC-IUB Joint Commission on Biochemical Nomenclature, *Eur. J. Biochem.*, 131, 9, 1983. With permission.)

In general, there are two energetically preferred regions, designated *syn* and *anti*, in the torisonal orientation of the glycosidic bond: the χ value in the *syn* region ranges 0 ± 90° and that for the *anti* region, 180 ± 90° (Figure 15).[134] Figure 13 (top) shows the *anti* orientations of both the adenine and nicotinamide/dihydronicotinamide rings of the coenzyme in the linearly open conformation. The NMN^+ half molecule shown in the bottom of Figure 13 represents the *syn* orientation of the nicotinamide/dihydronicotinamide ring.

The torisonal orientation of the two glycosidic linkages in the folded coenzyme molecules can be seen in Figure 14. The structure on the left shows the adenine ring in the *anti* form and the nicotinamide/dihydronicotinamide ring in the *syn* orientation; the structure on the right shows the 180° rotation of the two bases, thus, the adenine ring is in the *syn* form and the other, in the *anti* form.

Glycosidic orientation in AMP — Usually, β-nucleotides, including AMP, in aqueous solution are in a rapid equilibrium state between the *anti* and *syn* conformations, with the preference for the *anti* form to the *syn*.[122,135-137]

The preference for the *anti* form can be demonstrated by measuring the chemical shift of the C-2′ proton of AMP. In the *syn* form the C-2′ proton is in closer proximity to the adenine base than it is in the *anti* conformation (Figure 14). Consequently, the purine ring in the *anti* orientation is expected to exert less deshielding on the C-2′ proton than that in the *syn* orientation.

Evans and Kaplan[138] reported that the chemical shift of the C-2′ proton of AMP was very similar to the model compounds, 8-amino-AMP and 8-methylamino-AMP, which are known to have the *anti* orientation, but it moved to substantially higher field compared to that of the same proton on 8-dimethylamino-AMP, which is known to have the *syn* conformation (below). These data support that the adenine ring in AMP is *anti* oriented. (The intramolecular hydrogen bond between 8-NH- and 0-5′ of the ribose stabilizes the *anti* conformation of 8-amino-AMP and 8-methylamino-AMP. However, the 8-dimethylamino analog, which lacks such hydrogen bond, is predominantly in *syn* conformation due to the bulky dimethylamino substituent.)

Analogous to the case of AMP, Lappi et al.[139] observed that the chemical shift of the adenosine C-2′ proton of NADH shifted 0.69 ppm upfield compared to that of N[$(CH_3)_2$–N-A]DH, which is in the *syn* conformation (see above). However, the difference in chemical shifts of the same protons in NADH and in N(CH_3-NH-A)DH, and also in N(CH_3-CH_2-NH-A)DH (both analogs have the *anti* conformation) are only 0.09 and 0.05 ppm, respectively. From this finding, they concluded that the adenine ring in AMP moiety of NADH has, like that in free AMP, the *anti* orientation.

Glycosidic orientation in NMN — Eagan et al.[129] studied the torisonal conformation of the nicotinamide-ribose linkage in NMN^+ by measuring the intramolecular proton-proton nuclear Overhauser effect (NOE) enhancement. The NOE is the most sensitive NMR method for the conformation analysis of nucleotides in solution since it can determine the relative proximity of two intramolecular protons in space. Saturation irradiation at the frequency of the resonance of a given proton causes the largest NOE enhancement to its closest proton.

From Figure 13 it can be seen that in the *syn* form of the nicotinamide ring in NMN^+ the C-6 proton is in closer proximity to the C-1′ proton than is the C-2 proton; conversely, in the *anti* conformation the C-2 proton is situated closer to the C-1′ proton than is the proton on C-6. The actual measurement of the NOE enhancement for the protons on C-6 and C-2 at the saturation of C-1′ proton revealed that the *syn* and *anti* conformers of the mononucleotide were about equally populated in a pH and temperature-independent manner. In other words, these two conformational isomers are isoenergetic.

Zens et al.[127] employed proton T_1 (spin-lattice relaxation time) measurement to determine the *syn/anti* conformer population of NAD^+. If the predominant conformer of NAD^+ is *syn*, there would be a large change in T_1 for C-1′ proton in going from isotopically natural NAD^+ to monodeuterated NAD^+ at nicotinamide C-6, but little change with the dinucleotide labeled at the C-2. Conversely, if the *anti* form predominates, a large change should be expected in T_1 for C-1′ in going to deuterium-labeled NAD^+ at the C-2, but no change with that at the C-6. Experimentally observed data, however, were not consistent with any of these expectations, but it could be best explained with the model that the nicotinamide ring of NAD^+ is librating between the *syn* and the *anti* orientations. Hence, in agreement with the conclusion drawn from the NOE study with NMN^+, the populations of the *syn* and *anti* isomers of NAD^+, too, are about equal.

This conclusion does not support the view expressed by Sarma and Mynott,[126,140] who proposed that the nicotinamide ring in NMN^+, as well as in NAD^+, favored the *syn* orientation (while NMNH favored the *anti* form) on the basis of the 5′-phosphate ionization-dependent perturbation of the resonances for the protons on the C-6 and C-2. Eagan et al.[129] and Oppenheimer[104] discussed the ambiguity that is associated with the conclusion derived from the titration of the phosphate group.

Although Oppenheimer[104] regards the *anti* orientation of the nicotinamide ring in the folded form of the coenzyme more favorable, presently available data do not provide any information as to whether the two torisonal conformers are equally populated both in the open and folded conformations. It is quite possible that in the dynamic "folded-open" conformation of the dinucleotide the open form favors one conformer, whereas the folded form favors the opposite conformer in such way that the apparent conformer populations average out to be approximately equal.

The conformational features of the free coenzyme in aqueous medium can be summarized as follows:

1. The coenzyme molecule, regardless of the redox state, is at the dynamic equilibrium between the open and folded conformations.
2. In the folded form, the adenine and the nicotinamide/dihydronicotinamide rings are stacked in parallel, the B face of the nicotinamide/dihydronicotinamide ring facing the adenine ring, for the maximum intramolecular interaction.

3. While the adenine ring is *anti* oriented the nicotinamide ring prefers neither *syn* nor *anti* orientations, thereby making the population of these conformers equal.
4. It is not known if the two torisonal conformers are equally populated both in the open and the folded forms of the molecule.

c. Puckering of the Dihydronicotinamide Ring in the Folded Conformation

Albeit it is somewhat speculative, another possibly significant conformational feature which has become apparent from PMR studies is that when NADP(H) is in the folded form but not in the open form, the dihydronicotinamide ring may not be planar but puckered in the manner that the C-4 and N-1 ends are bent away (like the boat form) from the adenine ring. The evidence for this puckering has been derived from the unusual observation with NADH that the vicinal coupling constant $^3J_{B\text{-}5}$(3.9 Hz) is larger than the $^3J_{A\text{-}5}$(3.1 Hz).[128] As mentioned in Section VII.A.2.a, such difference is absent in NMNH, as well as in DHNR, in which both coupling constants have the same value of 3.5 Hz;[104,128] elevated temperature or apolar medium, under which condition the two bases destack, causes the two coupling constants of NADH to merge to the value obtained with the mononucleotides.[128]

According to the Karplus relation.[141] vicinal coupling constants are maximal when the bonds of the coupled protons are coplanar and minimal when they are orthogonal. The fact that $^3J_{A\text{-}5}$ is smaller and $^3J_{B\text{-}5}$ is larger than 3.5 Hz signifies that the A proton of NADH is more *perpendicular* to the dihydropyridine plane and the B proton more *horizontal* than their respective protons in NMNH. This interpretation was corroborated by the analysis of the long-range coupling between the C-6 proton and the methylene protons on the C-4, $^5J_{A\text{-}6}$(2.0 Hz) and $^5J_{B\text{-}6}$(1.5 Hz); such difference is again absent in NMNH, in which both constants are identical (1.7 Hz) at about midway between these two values.[128] Since vinylic coupling is maximal when the proton is orthogonal with the ring and minimal when it is coplanar, the long-range coupling constants obtained with the dinucleotide support the notion that the A hydrogen is displaced *axial* and the B hydrogen *equatorial*.[128]

Therefore, in the folded conformation of NADH, the dihydropyridine ring may not be planar but the two bonds connecting the C-3, C-4, and C-5 are puckered away from the bulky adenine ring. The fact that $^4J_{2\text{-}6}$ is also decreased from 1.7 Hz in NMNH[126] to 1.5 Hz in NADH[104] suggests that the N-1 end of the dihydropyridine ring is also puckered. The dihydropyridine of the coenzyme is thus very likely in the boat form when it is stacked against the adenine ring.

The limiting values of $^3J_{A\text{-}5}$ (= 2.2 Hz) and $^3J_{B\text{-}5}$ (= 4.6 Hz) (see Section VII.A.2.a) for the puckered dihydronicotinamide ring in fully folded NADH were derived by Oppenheimer et al.[128] from the PMR data of NAD-pyruvate and NADCN adducts, respectively. In the former adduct (Figure 12), the equatorially displaced pyruvate moiety keeps the proton on the C-4 in the fully axial position, whereas in the latter, the proton is fully in the equatorial position due to the axial addition of CN^-. These limiting values for the vicinal coupling constants correspond to a change in the dihedral angle between the C-4 methylene and the C-5 methine of at least 15°. The result, therefore, demonstrates a puckering of the C-4 position by about 30° from the plane consisting of the C-2, C-3, C-5, and C-6 atoms.[104] This estimate is in close agreement with the degree of puckering observed in the X-ray study on the model compound, the crown ether *N*-methyl-1,4-dihydropyridine crystal complexed with sodium perchlorate (Figure 16a). The X-ray study revealed that the N-1 and the C-4 atoms of the dihydropyridine ring of this compound are forced out of the ring plane by 27.7 and 36°, respectively.[142]

Rob et al.[143] also found by X-ray crystallography that the dihydropyridine ring of an achiral bridged 1,4-dihydro-3,5-biscarboxamido pyridine is in the pronounced boat conformation [Figure 16b (RED) the actual compound subjected to the X-ray study is devoid of the three methyl groups]. Consequently, the two methylene protons in the bridged dihydro-

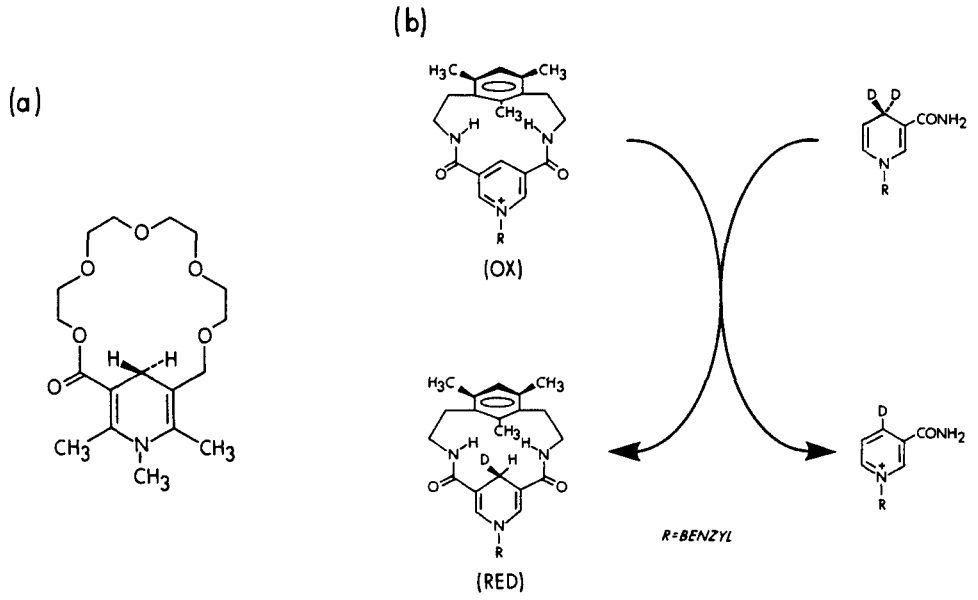


FIGURE 16. The dihydropyridine model compounds have the boat conformation: (a) crown ether *N*-methyl-1,4-dihydropyridine complexed with sodium perchlorate. X-ray structural determination reveals that the dihydropyridine is in a prominent boat conformation, tilted relative to the tetraethylene glycol chain; (b) stereoselective hydrogen transfer from *N*-benzyl-1,4-[4,4-D]-dihydronicotinamide to an achiral bridged 1,4-dihydro-3,5-biscarboxamide pyridine. The deuterium is transferred to the oxidized form of the model compound in a highly stereoselective manner, more than 90% of the deuterium ending up in magnetically more shielded diastereotopic C-4 position. X-ray investigation with the reduced model compound (but lacking the three methyl groups on the benzene ring) shows that the ring is considerably distorted into the boat form. (Diagram b is adapted from Rob, F., van Ramesdonk, H. J., Verhoeven, J. W., Pandit, U. K., and Boer, T. J., *Tetrahedron Lett.*, 21, 1549, 1980. With permission.)

pyridine ring shown in Figure 16b (RED) are present in different magnetic environment and, therefore, show different chemical shifts and coupling constants: $\Delta\delta_{AB} = 0.13$ ppm; $^2J_{AB} = 14$ Hz. Of more significant consequence is that *N*-benzyl-1,4-[4,4-dideutero]-dihydronicotinamide leads to highly *stereoselective* (≥90%) introduction of deuterium at the magnetically most shielded disastereotopic C-4 position of the model compound, as represented in the reaction shown in Figure 16b.

Only above two dihydropyridine model systems are known to have distorted ring structure. The dihydronicotinamide ring in a model compound like *N*-benzyl-1,4-dihydronicotinamide is perfectly planar (below).

B. In Crystal

Saenger et al.[47,118] elucidated the conformation of NAD^+ in the crystal of Li^+-NAD^+ complex, which is illustrated in Figure 17 by X-ray crystallography at 1.09 Å resolution. Two Li^+ are coordinated to the dinucleotide: one is attached to the adenine N-7 and an unesterified oxygen atom of the phosphate group in NMN^+ and the other to the unesterified oxygen of the phosphate group in AMP and the remaining unesterified oxygen on the NMN^+ phosphate.

The carboxamide group is nearly coplanar with the obviously planar pyridinium ring, the C-NH_2 bond being *cis*-planar with the C-3–C-4 bond of the pyridinium ring. (In the N-1 unsubstituted nicotinamide, however, the C-NH_2 in the carboxamide group is rotated by 180° to be *trans* oriented to the C-3–C-4 bond.[144] Wright and King[144] presented the bond angles and distances of the N-1 unsubstituted nicotinamide ring).

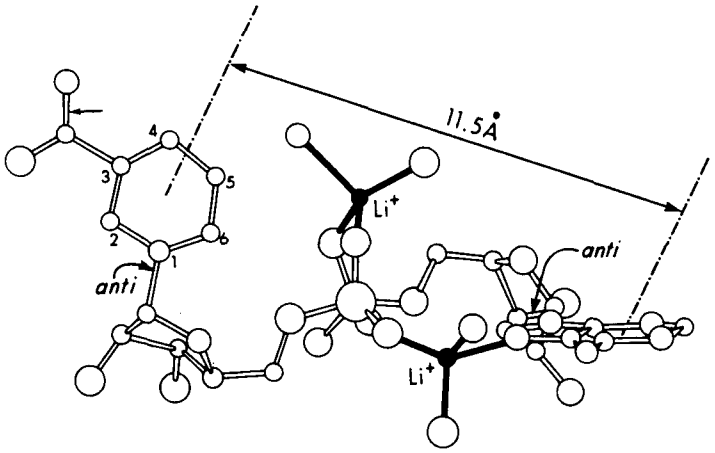


FIGURE 17. Conformation of Li^+-NAD^+ complex crystal. The coordination of the two Li^+ is marked by solid bonds: one Li^+ is attached to the adenine N-7 and an unesterified oxygen atom of the phosphate group in NMN^+ and the other to the unesterified oxygen of the phosphate group in AMP and the remaining unesterified oxygen on the NMN^+ phosphate. The carboxamide group is nearly coplanar with the pyridinium ring, the C–NH_2 bond (arrow) being *cis*-planar with the C-3–C-4 bond. The adenine and nicotinamide bases are nearly perpendicular to each other and are separated by 11.5 Å. The glycosidyl linkages of both bases are *anti* oriented: $\chi_A = 232°$; $\chi_N = 195°$. (From Saenger, W., Reddy, B. S., Muhlegger, K., and Welmann, G., *Nature* (London), 267, 225, 1977. With permission.)

Unlike the conformation in solution state, NAD^+ molecules in crystal exist in the open form, the two bases being separated by about 11.5 Å and nearly perpendicular to each other. The glycosidic linkages of both rings are in the *anti* orientation, i.e., $\chi_A = 232°$; $\chi = 195°$.[47,118]

It was inferred that the conformation of Li^+-$NADP^+$ is very similar to Li^+-NAD^+. Since the 2′-phosphate is fairly remote from the pyrophosphate group, these two groups cannot interact directly or by means of a chelating cation. It is, however, possible that the adenine N-3 may be liganded by a cation with the oxygen on the 2′-phosphate.

In order to examine the planarity of the dihydronicotinamide ring, Karle[145] determined the bond lengths and angles of *N*-benzyl-1,4-dihydronicotinamide by X-ray crystallography, and the data are presented in Figure 18.

The N-1–C-2 and N-1–C-6 distances are 1.38 and 1.43 Å, respectively, compared to the expected single bond length of 1.47 Å. The shorter N-1–C-2 distance relative to the N-1–C-6 is ascribed to the presence of an enhanced resonance form of the ring with the coplanar carboxamide group (Figure 18). This resonance form, which again requires a planar ring nitrogen, is presumably responsible for the 340 nm absorption band in the UV spectrum, usually a typical and unique property of reduced form of dinucleotide, since the 3-halopyridine nucleotide analogs which are coenzymatically active lack this absorption band.[146]

Hence, it now appears evident that the dihydronicotinamide ring in NADH in the open conformation and in *N*-benzyl-1,4-dihydronicotinamide (Figure 18) is planar, but in the folded NADH and in the crown ether-1,4-dihydronicotinamide as well as in the achiral bridged 1,4-dihydro-3,5-biscarboxamido pyridine (Figure 16), it is in the puckered, boat form.

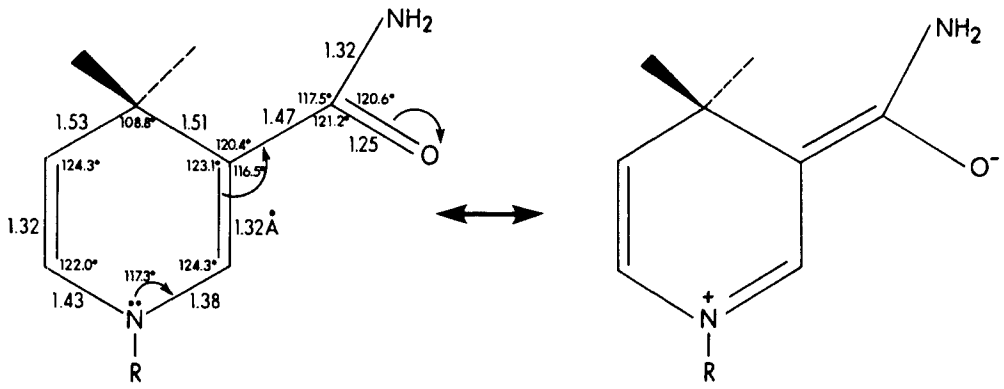


FIGURE 18. The planarity of the dihydronicotinamide ring in *N*-benzyl-1,4-dihydronicotinamide. The bond lengths and the dihedral angles between the atoms in the ring are consistent with those in a planar ring conformation. In addition, the resonating system between the dihydropyridine ring and carboxamide group requires that the carboxamide group be coplanar with the ring; it is this resonance form that is responsible for the light absorption at 340 nm, a unique and typical UV spectrum property of reduced form of the coenzyme and of many analogs.

VIII. CONFORMATION OF ENZYME-BOUND COENZYME

A. Overall Mode of Binding

Before a wealth of information about the conformation of NAD(P) bound to their host enzyme has been available through extensive X-ray crystallography, some fluorometric and PMR studies had suggested that the dinucleotide held, unlike its conformation in solution, base-destacked open conformation in most cases. For instance, based upon the persistence and disappearance of fluorescence emission at 260 nm excitation upon binding of NADH to various enzymes, Velick[32] deduced that the coenzyme was in the open conformation at the active sites of lactate dehydrogenase, alcohol dehydrogenase, glutamate dehydrogenase, and glycerol-3-phosphate dehydrogenase (EC.1.1.1.18.) because the excitation at 260 nm no longer imparted the fluorescence emission at 485 nm. The NADH bound to glyceraldehyde-phosphate dehydrogenase was, however, assumed to be in the folded conformation since the fluorescence emission (at 485 nm) upon the excitation at 260 nm was retained.[32]

Similarly, Lee et al.[132] reported downfield shift of the chemical shifts of protons on both nicotinamide and adenine rings of NAD^+ upon its binding to several dehydrogenases with the exception of glyceraldehyde-phosphate dehydrogenase. Since downfield shift represents base destacking (no ring current effect), these workers also concluded that NAD^+ held the open conformation when they were bound to enzymes with a possible exception of glyceraldehyde-phosphate dehydrogenase.

Extensive X-ray crystallographic investigations on the conformation of enzyme-bound NAD(P), initiated in Rossman's laboratory in 1970 with the complex with dog fish M_4 lactate dehydrogenase,[46] have proved, however, that the coenzyme, regardless of the redox state and of the stereospecificity of the host enzyme, holds the open conformation, stretched between the adenine binding crevice and the nicotinamide binding pocket at the coenzyme binding domain of enzyme.[131,147,172]

In order to fix spatially a flexible molecule like NAD(P) on a protein, a network of hydrophobic force, hydrogen bonding, and ionic interaction displays intricate association between the coenzyme and various amino acid residues in the coenzyme binding domain of a given enzyme. Some of these amino acids in each type of interaction appear to be strictly conserved both in character and relative positioning among dehydrogenases.

Figure 19A and B schematically show the overall conformations of the NAD bound to a

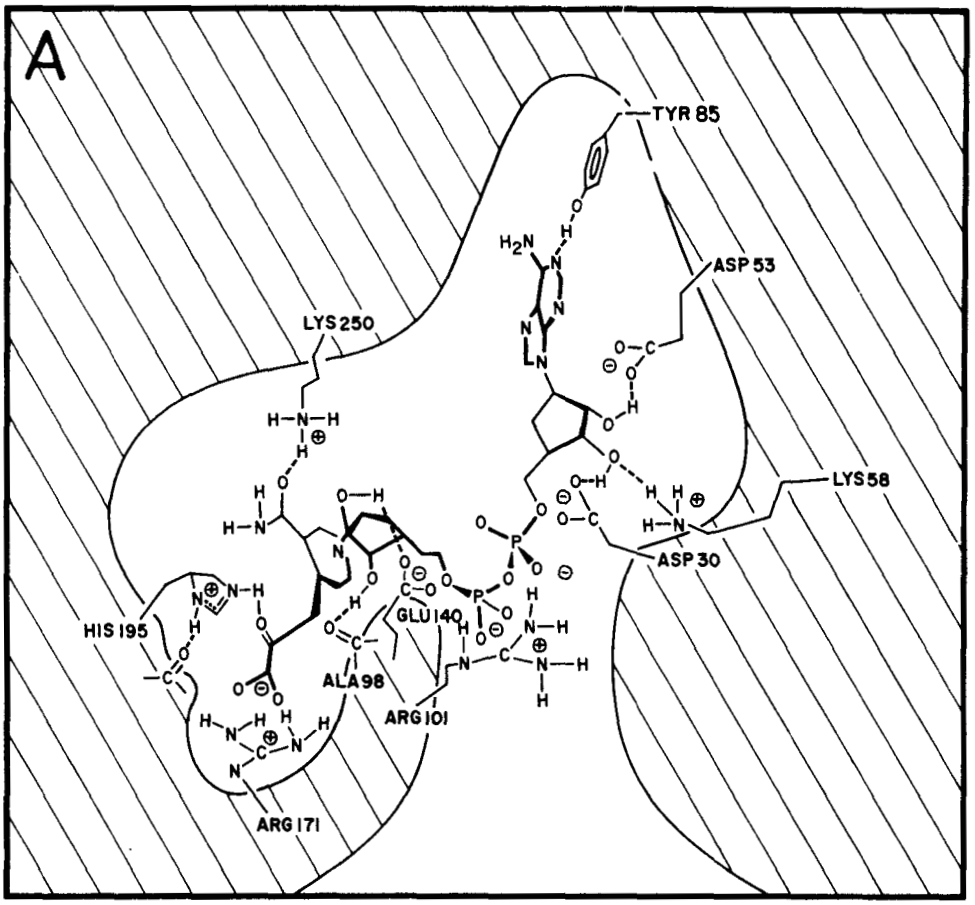


FIGURE 19. Schematic representation of the conformation of NAD in lactate dehydrogenase·NAD·pyruvate ternary complex (A) and in glyceraldehyde-phosphate dehydrogenase·NAD^+ binary complex (B). In (A), the adenine binds in a hydrophobic pocket. Tyr-85 provides an interaction during the initial stage of coenzyme binding. Asp-53 gives some specificity to the adenine orientation. The adenosine ribose is positioned by three hydrogen bonds with its hydroxyls: one between the 2′-OH and Asp-53, and two between the 3′-OH and Asp-30 as well as Lys-58. The negative charge of the pyrophosphate group is balanced by the interaction with Arg-101. The NMN ribose forms hydrogen bonds between the 2′-OH and Glu-140, and between 3′-OH and Ala-98. The nicotinamide ring itself is supported by hydrophobic residues (Val-32 and 247) and by a hydrogen bond between its carboxamide group and ϵ-amino group of Lys-250. When in the oxidized form, the pyridinium ring can interact with Glu-140. The glycosidic torisonal orientations of both the adenine and nicotinamide rings of the bound coenzymes are in the *anti* form. (From Holbrook, J. J., Liljas, A., Steindel, S. J., and Rossman, M. G., *The Enzyme*, Vol. 11, 3rd ed., Boyer, P. D., Ed., Academic Press, New York, 1975, 191. With permission). In (B), only the overall conformation of the bound coenzyme is shown. The most striking difference between the coenzyme conformation shown in (A) and (B) is that on B-sterospecific enzyme, the nicotinamide ring of the dinucleotide is *syn* oriented, a 180° opposite rotation from that bound to lactate dehydrogenase. (From Moras, D., Olsen, K. W., Sabesan, M. N., Buehner, M., Ford, G. C., and Rossman, M. G., *J. Biol. Chem.*, 250, 9137, 1975. With permission.)

typical A-stereospecific enzyme (lactate dehydrogenase) and a B-stereospecific enzyme (glyceraldehyde-phosphate dehydrogenase), respectively. The most remarkable conformational feature of the coenzyme in both complexes is that the dinucleotide molecule is in the base-base destacked open form, although it does not reach the full linearity due to a fold at the pyrophosphate bridge.

Figure 20 diagramatically demonstrates the fold at the pyrophosphate group and the glycosidic torisonal orientations of the two bases of the coenzyme bound to an A-stereo-

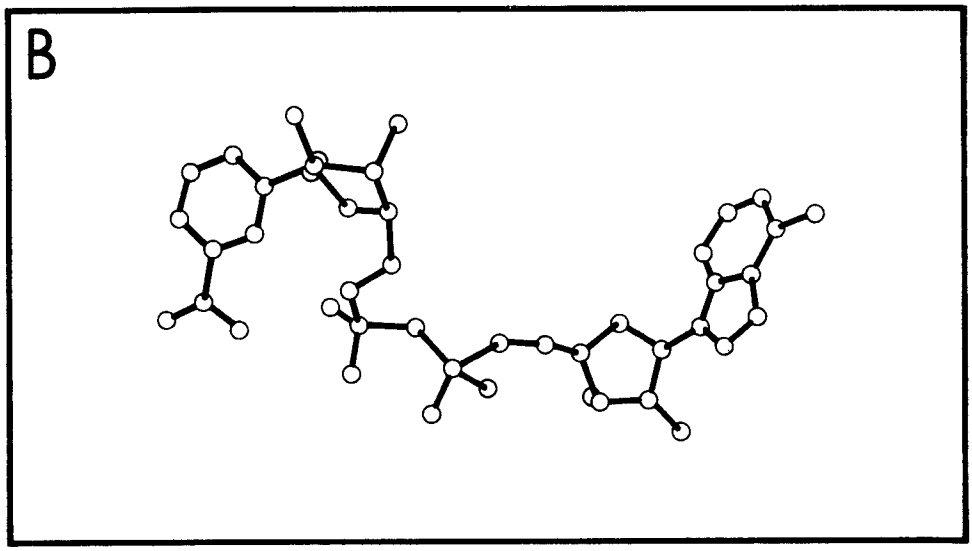


FIGURE 19B.

specific enzyme (lactate dehydrogenase). In this diagram, both the adenine and nicotinamide are *anti* oriented. The fold at the pyrophosphate bridge determines the degree of opening of the dinucleotide molecule, which is represented by the base-base distance, i.e., C-6_A · · · ·C-2_N for the nicotinamide in the *anti* form and C-6_A · · · ·C-6_N for that in *syn*. (The subscripts A and B represent adenine and nicotinamide, respectively.) The degree of opening of enzyme-bound coenzyme differs from one complex to another; for instance, the base-base distance in dihydrofolate reductase·NADPH·Methotrexate (EC.1.5.1.3.)-bound NADPH is a full 3 Å longer than that in lactate dehydrogenase-bound NAD^+ (Table 3).

Table 3 presents the summary of some of the important conformational parameters of the coenzyme bound to several dehydrogenases. In addition to the complexes in Table 3, Schulz et al.[148] and Pai and Schulz[149] reported that the NADPH in the ternary complex, glutathione reductase (human erythrocytes; EC.1.6.4.2.)·NADPH·oxidized glutathione (3 Å resolution) is in the open conformation. Likewise, Volz et al.[150] and Biesecker et al.,[151] respectively, reported that the coenzymes in dihydrofolate reductase (chicken liver) ·NADPH·phenyltriazine (2.9 Å) and glyceraldehyde-phosphate dehydrogenase (*Bacillus stearothermophilus*)·NAD^+ (2.7 Å) complexes are also in the open form.

Thus far, there has been no case in which enzyme-bound coenzyme exhibits the base-base stacked, folded conformation. The overall conformation of enzyme-bound coenzyme is, therefore, profoundly different from the dynamic "folded-open" conformation of free coenzyme in solution.

It now becomes difficult to reconcile the X-ray data about the conformation of the NAD^+ bound to glyceraldehyde-phosphate dehydrogenase (open conformation) with the conclusion reached by fluorometric[32] and PMR studies[132] (folded conformation). Obviously, an alternative explanation must be searched. A clearly known fact is that in a negative cooperative, tetrameric glyceraldehyde-phosphate dehydrogenase, the four coenzyme binding sites (one per subunit) are close to each other near the central molecular waist.[152,153] Because of this close proximity, it is possible that there is an intermolecular interaction among the NAD molecules on each subunit. Even though the enzyme-bound coenzyme is in the open form, the intermolecular interaction may cause the energy transfer[29] and the ring current effects to occur.[132] In the noncooperative, tetrameric enzyme like lactate dehydrogenase, this intermolecular interaction between the bound coenzyme molecules is unlikely because the

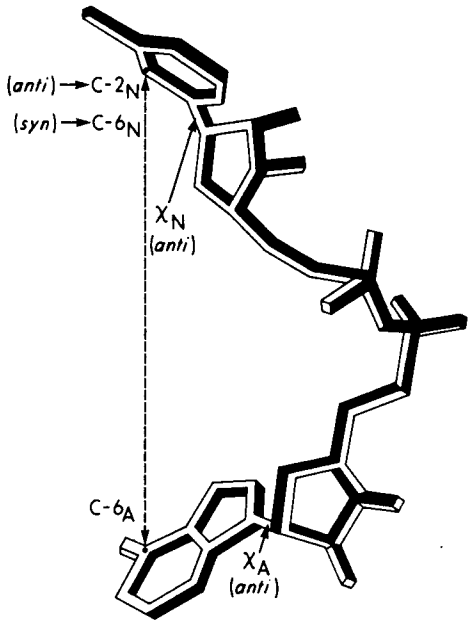


FIGURE 20. Schematic diagram of the overall conformation of NAD^+ bound to the A-stereospecific enzyme. The molecule is in ring-ring destacked, open conformation with the nicotinamide and the adenine rings *anti* oriented. The molecule is not, however, in the completely linear form (cf. Figure 13), due to a fold at pyrophosphate linkage, which determines the base-base distance. When the nicotinamide is *syn* oriented the distance $C\text{-}6_A \cdots C\text{-}6_N$ is used instead of $C\text{-}6_A \cdots C\text{-}2_N$ *anti*), in order to compensate the 180° rotational difference. Some important conformational parameters of NAD(P) bound to the A-stereospecific as well as B-stereospecific enzymes are given in Table 3. (Adapted from Brändén, C.-I., and Eklund, H., *Dehydrogenases Requiring Nicotinamide Coenzymes*, Jeffrey, J., Ed., Birkhauser Verlag, Basel, 1980, 41. With permission.)

dinucleotide is bound completely within each subunit giving little possibility for intermolecular association.[153]

Figure 21 compares the conformation of NAD^+ in the lactate dehydrogenase ternary complex and that in Li^+-NAD^+ crystal.[47,118] Owing to the different bond angles about the C-4′–C-5′ bonds, indicated by an arrow, the adenosine groups in the two complexes turn to the opposite directions although their distances to the nicotinamides are comparable. In addition, the adenosine glycosidic bond in Li^+-NAD^+ complex is nearly vertical to the plane of the paper, whereas it is in the same plane as the paper in the ternary complex.

B. Specific Interaction between Coenzyme and Enzyme

Because the mode of binding of the nicotinamide/dihydronicotinamide ring of the coenzyme to a given enzyme is critically important in eventuating the stereospecificity of that enzyme, its interaction with various amino acid residues on the protein is separately discussed in detail in a later section.

The specific features in binding of the ADPR moiety of the coenzyme to those enzymes listed in Table 3 are, however, discussed briefly here. Further information about the conformation of the coenzyme can be found in the recent references that are written specifically for that purpose.[131,172]

In every complex analyzed, the adenine ring binds to a hydrophobic crevice on the surface

Table 3
CONFORMATIONAL PARAMETERS OF NAD(P) BOUND TO VARIOUS DEHYDROGENASES

	L-Lactate dehydrogenase (dog fish M_4) (1.1.1.27.)	L-Lactate dehydrogenase (pig H_4) (1.1.1.27.)	Alcohol dehydrogenase (horse liver) EE isozyme) (1.1.1.1.)	Malate dehydrogenase (pig heart cytoplasmic) (1.1.1.37.)	Dihydrofolate reductase (*Lactobacillus casei*) (1.5.1.3.)	Glyceraldehyde-phosphate dehydrogenase (lobster tail) red subunit (1.2.1.12.)	6-Phosphogluconate dehydrogenase (sheep liver) (1.1.1.44.)
	3.0 Å E·NAD$^+$·Pyruvate	2.7 Å E·S-Lactate-NAD$^+$[a]	2.9 Å E·ADPR	2.5Å E·NAD$^+$	1.7 Å E·NADPH·Methotrexate	2.9 Å E·NAD$^+$	6 Å E·NADP$^+$
	(Ref. 281)	(Ref. 131)	(Ref. 282)	(Ref. 283)	(Ref. 161)	(Ref. 131)	(Ref. 284)
Specificity	A	A	A	A	A	B	B
χ_A^b	205.5°(*anti*)	225.5°(*anti*)	194.5°(*anti*)	226.5°(*anti*) (subunit 1) 231.5°(anti) (subunit 2)	233.5°(*anti*)	250.5° (*anti*)	216.5° (*anti*)
χ_N^b	222.5°(*anti*)	270.5°(*anti*)	—	248.5°(*anti*) (subunit 1) 249.5° (*anti*) (subunit 2)	221.5°(*anti*)	39.5°(*syn*)	35.5°(*syn*)
$C\text{-}6_A$–$C\text{-}2_N$ (for *anti*)	14.1 Å	14.8 Å	—	14.3 Å (subunit 1) 14.2 Å (subunit 2)	17.1 Å	—	—
$C\text{-}6_A$–$C\text{-}6_N^c$ (for *syn*)	—	—	—	—	—	15.8 Å	?

[a] (3S)-5-(3-Carboxy-4-hydroxypropyl)-NAD$^+$.

[b] The originally reported values are converted to the angles defined by the IUPAC-IUB[134] by employing the conversion equation described for Sundaralingam:[285] the subscripts A and N represent adenine ring and nicotinamide ring, respectively.

[c] Due to the 180° rotation of the nicotinamide ring in the *syn* conformation compared with the *anti* form, the distance $C\text{-}6_A$–$C\text{-}6_N$ is shown.

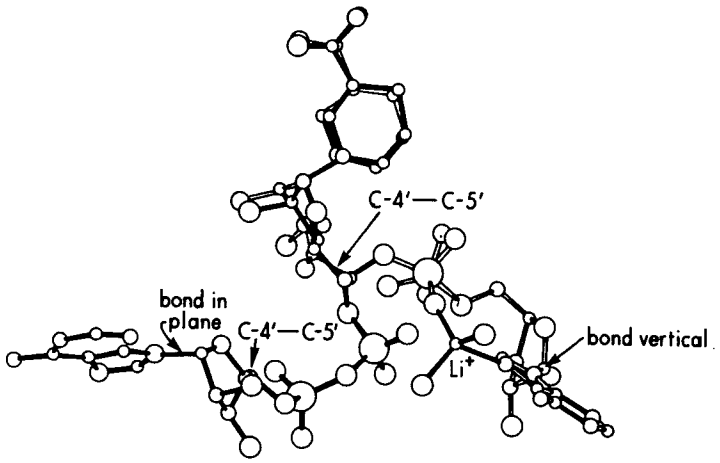


FIGURE 21. Comparison of the conformations of NAD^+ in lactate dehydrogenase·NAD^+·pyruvate ternary complex (solid bonds) and Li^+-NAD^+ complex (open bonds). Owing primarily to the different conformation about the C-4′–C-5′ bonds, indicated by arrows, the ADP moieties in the two complexes turn to opposite directions although the distances from the adenine rings to the nicotinamide rings are comparable. Also, the adenine rings in the two complexes are oriented differently; in Li^+-NAD^+ complex the adenosine glycosidic bond is nearly vertical to the plane of the paper, while it is in the same plane as the paper in the complex with the enzyme. (From Saenger, W., Reddy, B. S., Muhlegger, K., and Weinman, G., *Nature (London)*, 267, 225, 1977. With permission.)

of enzyme in a relatively unspecific manner, with its torisonal angle (χ_A) *anti* (Table 3). The crevice can also accommodate other aromatic rings as long as they are not too large. The adenine portion of the coenzyme, therefore, can be drastically modified without the total loss of coenzymatic function.[154] What is also true is that many aromatic molecules with negatively charged groups often serve as competitive inhibitors of dehydrogenases for the coenzyme. In some complexes, the binding of the adenine ring may be further facilitated by hydrogen bonding between the N-1 (lactate dehydrogenase) or the N-3 (lactate dehydrogenase and glyceraldehyde-phosphate dehydrogenase) with the protein. The 6–NH_2 group of the adenine ring is somewhat protruding out of the adenine crevice toward the medium. Consequently, the protein does not render steric hindrance to this group when the coenzyme is immobilized for affinity chromatography through a spacer chain attached to this amino group.[155]

In general, the adenosine ribose interacts with enzyme through the combination of weak hydrophobic force and hydrogen bonds with the 2′-OH and 3′-OH groups. In the case of bound NADPH, the 2′-phosphate group is also hydrogen bonded (dihydrofolate reductase). The degree of opening of the coenzyme molecule (i.e., the distance between the two bases) is governed by the bond angle of the pyrophosphate bridge, which is in turn determined by ionic interaction, hydrogen bonding, and hydrophobic interaction with various amino acid residues in the domain. The NMN ribose is, like the adenosine ribose, bound through hydrophobic force as well as hydrogen bonds with its hydroxyl groups.

Figure 19A, the ternary complex of lactate dehydrogenase, represents, with the exception of the orientation of the nicotinamide ring, the typical overall mode of interaction between the dinucleotide and enzymes.

1. Torisonal Orientation of Adenine Ring

In addition to the X-ray crystallographic evidence, there is clear NMR evidence that the

torisonal orientation of the adenine ring (χ_A) in protein-bound mononucleotide and dinucleotide is usually *anti*.

Clore and Gronenborn[156,288] measured the transferred proton-proton nuclear Overhauser effect (TRNOE) between the protons on the purine carbons and the ribose protons of AMP molecule bound to alcohol dehydrogenase and glutamate dehydrogenase. When the adenosine of AMP is in the *anti* form, the purine C-8 proton is closest to the C-2′ proton and also maintains relatively close in proximity to the protons on C-3′ and C-5′, but is distant from the C-1′ (as well as C-4′) proton. The proton on the purine C-2 is, on the other hand, remote from all of the ribose protons (Figure 13).

Upon irradiating at the resonance frequencies of various protons on the ribose, the above workers observed the following TRNOE:

1. Irradiation at the resonance of the C-2′ proton caused a large negative TRNOE (about 50%) on the observed resonance of the proton on the adenine C-8.
2. Irradiation at the resonances of the protons on C-3′ and C-5′ caused smaller but significant TRNOE on the resonance of the same proton.
3. Irradiation at the C-1′ proton resonance caused no change in the intensity of the C-8 proton resonance.
4. Irradiation at the resonance frequency of any of the above ribose protons did not cause any change in the intensity of the purine C-2 proton.

These results unequivocally demonstrate that the glycosidic bond in AMP bound to NAD(P)-linked enzymes is *anti*.

The torisonal conformation of adenine ring in enzyme-bound $NADP^+$ and $(TN)ADP^+$ was also determined by measuring the TRNOE. Irradiation at the resonance frequency of the protons at $C\text{-}2'_A$, $C\text{-}3'_A$, and $C\text{-}5'_A$, (the subscript A denotes the ribose in adenosine moiety) of the coenzyme and the analogs which are bound to dihydrofolate reductase gave large TRNOE on the adenine C-8 proton, whereas irradiation at $C\text{-}1'_A$ resonance caused no effect. Similarly, Levy et al.[157] observed that the irradiation at the resonance of $C\text{-}2'_A$ proton but not of $C\text{-}1'_A$ proton of $NAD(P)^+$ bound to both A-stereospecific enzyme (lactate dehydrogenase) and B-stereospecific enzymes [glucose-6-phosphate dehydrogenase (EC.1.1.1.149.) and glutamate dehydrogenase] gave large TRNOE on the purine C-8 proton, while that of $C\text{-}1'_A$ showed no effect.

These data demonstrate that the glycosidic linkages in the adenosine moiety of enzyme-bound AMP, $NAD(P)^+$, and $(TN)ADP^+$ are all *anti* oriented, just like those of free AMP in solution and in Li^+-NAD^+ crystal irrespective of the stereospecificity of the enzyme.

C. Protein-Bound Nicotinamide Ring and Torisonal Orientation

1. X-Ray Crystallographic Study

In contrast to the adenine ring of the dinucleotide which binds to enzyme in a relatively unspecific manner and can also be altered somewhat drastically without losing the coenzymatic activity, the mode of binding of nicotinamide/dihydronicotinamide ring is quite specific and only limited modification of the ring is permitted before the coenzyme loses its function in enzymatic reactions.

Although the conformation of the ADPR portion of all of the enzyme-bound coenzymes studied so far is quite similar, there is a striking 180° rotational difference in the orientation of the nicotinamide/dihydronicotinamide ring between the coenzyme complexed with the A-stereospecific enzymes and that with the B-stereospecific enzymes (cf. Figure 19A, B).

On the basis of limited X-ray crystallographic data, You et al.[16] speculated in 1978 that the torisonal angle (χ_N) of the glycosidic bond of the nicotinamide/dihydronicotinamide ring of enzyme-bound NAD(P) would be *anti* on the A-stereospecific enzymes and *syn* on the

B-stereospecific enzymes. The accumulated data, since that time (summarized in Table 3), confirm this proposal to be the case.

The orientation of the nicotinamide/dihydronicotinamide ring is determined mainly by two types of interaction with the protein: hydrophobic interaction with the pyridinium/dihydropyridine moiety, which is present always on the unreactive face of the ring away from the substrate; and one or more hydrogen bonds between the carboxamide atoms and the logistically located amino acid residues. Usually, the hydrogen bonding is primarily responsible for the proper orientation of the ring, thus resulting in the stereospecificity of a given enzyme. Table 4 presents the list of the amino acid residues involved in these two types of interaction with the nicotinamide ring in several enzyme-coenzyme complexes.

It should be pointed out that this torisonal orientation-stereospecificity relationship does not necessarily hold in those cases where the immediate hydrogen acceptor from the coenzyme is not the substrate, but the enzyme itself or a prothetic group that is already present on the enzyme. (In these cases the hydrogen transfer occurs in the so-called "indirect manner".) Under such circumstances, it is obvious that there is no need for spatial juxtaposition between the substrate and the coenzyme. For instance, on glutathione reductase (human erythrocytes), a B-stereospecific enzyme, the dihydronicotinamide ring of the bound NADPH is apparently *anti* oriented.[149] In glutathione reductase-catalyzed reaction, however, it is either the enzyme-bound FAD or, more likely, lysine-66, not the substrate (glutathione) that accepts the hydrogen originating from the B side of NADPH.[149,158]

Early studies with the coenzyme analogs provided that the pyridinium ring whose C-3 is bonded to a carbon (C-7), which is double bonded to an electronegative atom such as O, S, or N (which are obviously also typical hydrogen bond formers) can serve as the coenzyme. The coenzyme function of (3CNPy)AD[58] does not represent a major deviation from this concept. The importance of the hydrogen bond with the C-3 substitutent was demonstrated by Samama et al.,[159] who studied the binding of the coenzyme analogs pyridine adenine dinucleotide (which contains a hydrogen in place of the carboxamide) and (3IPy)AD^+ (in which C-3 is occupied by an iodine) to horse liver alcohol dehydrogenase. They found that the binding of the ADPR moiety of the true coenzyme and of these analogs is quite similar, but the binding of the pyridinium and the halopyridinium rings in the analogs is quite different from that of the nicotinamide ring in that the former two rings are oriented away from the active site and the catalytically active Zn atom by as much as 15 Å.

These two analogs are, thus, expected to form catalytically inactive complexes with the enzyme. The pyridine adenine dinucleotide indeed does not function as a coenzyme for alcohol dehydrogenase; however, the halo-analog is coenzymatically active. Samama et al.[159] ascribe this apparent inconsistency to the fact that this halo-analog is possibly in two conformations when complexed with the enzyme in solution, one of which is the active form and the other having the observed unproductive binding. The crystallization conditions would favor the formation of the inactive complex, whereas there would be an appreciable concentration of the active complex under the activity assay condition in aqueous medium. The iodine atom can form a hydrogen bond with the amino acids at the binding site of the protein and, at the same time, its electron withdrawing power can certainly enhance the carbonium ion character of the C-4, just as does the carboxamide group.

2. NMR(TRNOE) Study

Feeney et al.[160] confirmed the *anti* nature of the orientation of the nicotinamide ring of dihydrofolate reductase-bound $NADP^+$ by measuring the TRNOE of the protons on the nicotinamide C-6 and C-2 upon irradiation at the resonances of various ribose protons.

Recently, Levy et al.[157] studied the orientation of the nicotinamide ring of NAD^+ as well as of $NADP^+$ bound to glucose-6-phosphate dehydrogenase, glutamate dehydrogenase, and lactate dehydrogenase also by measuring the TRNOE. These workers observed significant

Table 4
INTERACTION OF NICOTINAMIDE/DIHYDRONICOTINAMIDE RING WITH ENZYMES

	Alcohol dehydrogenase (horse liver) E·NADH·Imidazole (Refs. 131, 159)	L-Lactate dehydrogenase (dog fish M_4) E·NAD^+·Pyruvate (Ref. 286)	Dihydrofolate reductase (*Lactobacillus casei*) E·NADPH·Methotrexate (Ref. 161)	Glyceraldehyde phosphate dehydrogenase (lobster) E·NAD^+ (Ref. 287)	Glutathione reductase (human erythrocytes) E·NADPH·GSSG (Ref. 149)
Stereospecificity	A	A	A	B	B
Hydrophobic force					
Pyridinium/	Val-203	Val-32	Ile-13	Ile-11	
Dihydropyridine ring	Val-292	Val-247	Leu-19 Tyr-45 Phe-103 Gly-98 Gly-99	Tyr-317	
Hydrogen bond					
0-7	Thr-178 (side chain) Phe-319 (peptide-NH)	Lys-250 (ϵ-NH_3^+)	Ala-6 (peptide-NH)	Asn-313 (side chain)	
NH_2-7	Val-292 (Carbonyl-O)		Ala-6 (Carbonyl-O) Ile-13 (Carbonyl-O)		Val-370 (Carbonyl-O) Glu-201 (Carboxyl)

TRNOE on the proton on the nicotinamide C-6, but very little on the C-2 proton upon irradiation at the resonance of C-1′ proton of both NAD^+ and $NADP^+$ bound to the B-stereospecific enzymes (glucose-6-phosphate dehydrogenase and glutamate dehydrogenase, which are active with both coenzymes). Conversely, irradiation at the resonance of the proton on C-3′ gave substantial TRNOE on the C-2 proton, but little or none on the proton on C-6.

These results demonstrate that while the nicotinamide C-6 proton is in close proximity to the C-1′ proton, the proton on C-2 is to the C-3′ proton. This conformational requisites can be satisfied only under the *syn* orientation of the nicotinamide ring (Figure 13b). As expected, precisely the opposite effect was observed with the NAD^+ bound to lactate dehydrogenase, which has the opposite stereospecificity from the preceding two enzymes. Thus, significant TRNOE values were obtained on the resonances for the nicotinamide C-2 proton but not on C-6 proton upon irradiation at the resonance of the proton on C-1′, and conversely, irradiation at the resonance for the C-3′ proton produces considerable TRNOE on the C-6 proton but not on the C-2 proton;[157] clearly, the nicotinamide ring in this case is *anti* oriented.

D. Distortion of Nicotinamide/Dihydronicotinamide in Transition State

The possibility of distortion of nicotinamide/dihydronicotinamide ring of enzyme-bound coenzyme was first raised by Levy and Vennesland.[31] It had been known that when the coenzyme was bound to a dehydrogenase, it could still serve as a coenzyme for a second dehydrogenase, often with greater efficiency.[94,95] Velick[96] thereupon speculated, but received no sustaining support, that coupling two dehydrogenases with sandwiched coenzyme might be a physiological way of regulating metabolic rate (see Section V.C.).

If the two coupled enzymes happened to have the opposite stereospecificity, the sandwiched coenzyme could accommodate the reactions of both enzymes without being dissociated by puckering the nicotinamide ring to one direction (which results in the boat conformation) for the reaction of the first enzyme; for the second enzyme reaction having the opposite stereospecificity, the ring simply puckers to the opposite direction to form the mirror-imaged boat form, thus exposing the opposite side of the ring to the second enzyme.[31]

Puckering of the dihydronicotinamide ring causes one of the C-4 methylene hydrogens to become axial and the other equitorial, as discussed in a previous subsection. Levy and Vennesland[31] pointed out that the axial hydrogen was more likely to be oxidized in the reaction because it was this hydrogen that caused the smallest shift of atoms from the positions they occupied in the oxidized, planar form of the coenzyme. As a matter of fact, their view has turned out to be correct by bond energy calculation: the C–H bond of the equitorially displaced hydrogen would approach an sp^2-*1s* hybrid orbital, which has a bond energy of approximately 109 kcal/mol, whereas that of the axially displaced hydrogen would approach a weaker *2p-1s* hybrid, whose energy would be approximately 80 kcal/mol.[128]

Although the hypothesis that two enzymes are coupled, bridged by the coenzyme, is no longer considered tenable, the evidence for the distorted nature of the nicotinamide/dihydronicotinamide ring has been steadily fortified in recent years. By employing the NAD in which N-1 of the nicotinamide/dihydronicotinamide ring was labeled with ^{15}N, Cook et al.[110] demonstrated normal secondary kinetic isotope effect in both forward and reverse directions of alcohol dehydrogenase-catalyzed reaction. (Cyclohexanol and cyclohexanone were serving as the substrates.) They ascribed this isotope effect result to the lower bond order (i.e., looser bonding) of the N-1 of enzyme-bound NAD^+ and NADH in the transition state. They proposed that, in the transition state, enzymes distort the nicotinamide of NAD^+ and dihydronicotinamide ring of NADH into the boat forms causing the N-1 of both rings to be pyramidal (Figure 8) at the step where an alkoxide ion is formed prior to the actual hydride transfer.

As the result, the N-1–C-1′ bond of NMN is no longer in the plane of the nicotinamide/

dihydronicotinamide ring, but is bent far enough out of plane to destroy any resonance between a lone pair of electrons on the N-1 and the remaining double bonds in the ring. Consequently, in the case of NAD^+, the nicotinamide ring develops a carbonium ion character localized exclusively at C-4. This is the very reason why the hydride transfer in enzymatic reactions is restricted to the C-4 and does not occur at other ring carbons which may well share a partial positive charge if the ring is in the planar form (Figure 7). Conversion of the distorted nicotinamide/dihydronicotinamide ring to planar form occurs in the subsequent fast steps.

Based upon X-ray crystallographic work on dihydrofolate reductase from *Lactobacillus casei*, however, Filman et al.[161] recently presented a somewhat different version of the coenzyme conformation in the transition state. They speculated that the trigonal N-1 of the nicotinamide ring does pyrimidalize, probably by forming a hydrogen bond between its lone pair orbital and a water molecule located nearby. This pyrimidalization does not, however, change the planar conformation of the ring, but it does make the C-1′ of the ribose out of plane. In other words, the nicotinamide ring remains to be planar, but is tilted relative to the glycosidic linkage.

This planar ring conformation allows not only the C-4 but also the other two even-numbered ring carbons to share carbonium ion character (Figure 7). The principal reason for them to prefer this model to that of Cook et al.[110] is that they found three oxygen atoms, from the amino acids located nearby, in the plane of, and quite close to the nicotinamide ring of the enzyme-bound NADPH. These oxygen atoms may interact with the three partially positively charged ring carbons (C-2, C-4, and C-6) through C–H–O type polar interaction (or actual hydrogen bond). In this case, it must be the spatial juxtaposition between the substrate and the coenzyme that prevents other partially carbonium carbons from serving as hydride acceptor. In any event, all of the X-ray crystallographic work on enzyme-bound coenzyme reveals absolutely no evidence which supports distorted nicotinamide/dihydronicotinamide ring structure. Transition state is supposed to be highly unstable, short lived, and often elusive.

IX. DOES STABILITY OF CARBONYL SUBSTRATE DICTATE THE STEREOSPECIFICITY?

The only mechanistic explanation that elucidates the discrete enzyme stereospecificity and at the same time is receiving growing acceptance, is the *anti-syn* nicotinamide orientation hypothesis proposed by You et al.[16] Based upon the correlation between equilibrium constants (K_{eq}) and stereospecificity of some simple, enzyme-catalyzed ketone-alcohol redox reactions, Benner and co-workers[162,163] recently postulated that reactive carbonyls are reduced with the A hydrogen of the reduced coenzyme, while thermodynamically more stable carbonyls are reduced with the B hydrogen.

The bases for this postulate are

1. The reduced coenzyme binds to A-stereospecific enzymes with the torisonal angle of the dihydronicotinamide ring *anti* and to B-stereospecific enzymes with *syn* orientation.
2. The K_{eq} of the A-stereospecific enzyme-catalyzed reactions are smaller ($pK_{eq} \geq 11.3$, i.e., the ketones are more reactive) than those of the B-stereospecific enzyme-catalyzed reactions ($pK_{eq} < 11.3$, i.e., the ketones are more stable), suggesting that *anti*-NADH is a weaker reducing agent than *syn*-NADH.
3. The dihydronicotinamide ring is distorted into a boat form causing the A hydrogen of *anti*-NADH and the B hydrogen of *syn*-NADH to move axial.
4. Principles of orbital overlap suggest that the axial hydrogen (i.e., A hydrogen in *anti*-NADH and B hydrogen in *syn*-NADH) is more labile and therefore, is subject to easier transfer than the equatorial one.

5. On the grounds of these considerations, the stereospecificity of lactaldehyde reductase (EC.1.1.1.55.) — the K_{eq} (= 12.6) of whose reaction belongs to the A category — was predicted and experimentally verified to be an A-stereospecific enzyme.

In order to validate this postulate, these workers imposed a number of restrictions such as: (1) the enzyme must catalyze the reduction of simple, unconjugated carbonyls to alcohol; (2) the natural substrate must be defined clearly; (3) in case the dehydrogenation accompanies other steps (such as decarboxylation), the K_{eq} for only the dehydrogenation step, not that for the overall reaction, must be applied; and (4) the substrate and product should not interconvert to other forms (such as hemiacetal formation of sugars) which obscure the true K_{eq}.

It should be pointed out that this postulate is mainly concerned about the thermodynamic properties of the A and B hydrogens and the carbonyl compounds; there is no mention about the role of the spatial juxtaposition between the coenzyme and substrate. In large part, this postulate is based obviously upon conjectures and is, as is the case with many of such situations, weakened inevitably by some serious factual inconsistencies.

First, the enzyme they selected to support their claim — lactaldehyde reductase — is, as pointed out previously (Section IV.B), the same enzyme as alcohol dehydrogenase (NADP), which can reduce diverse carbonyls; most importantly, the natural substrate of this enzyme has not been clearly defined.[69,164] This is not only exactly the type of enzyme they want to avoid in discussing their postulate,[162] but also its A stereospecificity is a mere confirmation of the data that have been known since 1966 through the mevaldate reduction reaction.

Second, many enzymes catalyze the reductions of a variety of ketones whose equilibrium constants differ by several orders of magnitude, thus, spreading well into both the A and the B categories. For example, lactate dehydrogenase reduces a variety of α-keto acid including α-ketobutyrate and α-keto-γ-hydroxybutyrate, in addition to pyruvate; the pK_{eq} of the reactions involving these substrates are 10.5, 9.9, and 11.3 to 11.6, respectively.[163,166] The pK_{eq} for pyruvate reduction belongs unquestionably to the A category, but it is difficult to explain how the enzyme can catalyze the reduction of the stable carbonyls in α-ketobutyrate and in α-keto-γ-hydroxybutyrate — which clearly require a stronger reducing agent, than *anti*-NADH — and still maintain the A stereospecificity.

Third, if *syn*-NADH is a stronger reducing agent than *anti*-NADH, it is difficult to accept the notion that *syn*-NADH cannot reduce the thermodynamically easier, more reactive carbonyls.

Fourth, the pK_{eq} (= 8.0) of 3α-hydroxysteroid dehydrogenase reaction belongs well into the B category.[163] However, this enzyme from rat liver (soluble fraction) shows the A stereospecificity (Appendix). A similar situation exists with 2-oxopantoyl lactone reductase (K_{eq} unknown) from *E. coli* (B stereospecific) and from yeast (A stereospecific). (See Section IV.B.)

Fifth, the following reactions are catalyzed by at least a pair of enzymes (thus, each reaction should exhibit the same K_{eq}, as an enzyme cannot alter the equilibrium position). However, the enzymes in each pair possess the opposite stereospecificity (Appendix): the reaction of dihydroxyacetone to glycerol (pK_{eq} = 11.3)[163] by glycerol dehydrogenase (EC.1.1.1.6). (A stereospecific) and by glycerol 2-dehydrogenase (NADP) (B stereospecific); and the conversion of D-ribulose to ribitol (pK_{eq} = 10.3)[167] by ribitol dehydrogenase (B stereospecific) and by L-iditol dehydrogenase (A stereospecific).

Due to the restriction imposed and unavailability of the equilibrium constants of many of the dehydrogenase-catalyzed reactions, Nambiar et al.[163] could test their postulate with only a small fraction of the enzymes with known stereospecificity (i.e., 15% or 24 out of 158 enzymes). The drawbacks against the postulate are overwhelming. Like many other postulates, whose generalization rules, physiological significance, and elucidations of mecha-

nism were proposed and then proven false, the validity of this new postulate should await the test of time.

X. METHODS IN STEREOSPECIFICITY DETERMINATION

In order to determine the stereospecificity of the hydrogen transfer reactions that involve methylene prochiral centers, it is imperative to carry out the reactions with deuterium- or tritium-labeled compounds. In the case of the reactions of the pyridine nucleotide-linked enzymes, C-4 hydrogen of nicotinamide of $NAD(P)^+$, one of the C-4 methylene hydrogens of dihydronicotinamide of NAD(P)H, or the substrate hydrogen that is going to be transferred to the coenzyme must be replaced with the isotope. The isotopically labeled substrates are usually purchased for they are generally difficult to prepare without having labeled coenzymes first. In this section, popular methods for the preparation of isotopically labeled $NAD(P)^+$ as well as NAD(P)H and for detection of the isotope contents in the products are described.

A. Chemical Preparation of Isotopically Labeled Oxidized Coenzyme

Figure 9 demonstrates the preparation of $NAD(D)^+$ from NAD^+ via the cyanide adduct in D_2O. Colowick et al.[100] and San Pietro[101] showed that the cyanide adduct is formed in a reversible manner by incubating NAD^+ with KCN under alkaline condition (pD $\simeq$ 12). The stereospecificity of this addition reaction has been discussed (see Section VI.B).

The dihydropyridine C-4 proton of the adduct is acidic enough to undergo exchange with a deuteron in D_2O medium, resulting in the formation of NAD(D)CN. Since the adduct formation is a reversible reaction, CN^- can be dissociated from the deuterated adduct by lowering the pD to about 7 and concurrently diluting the reaction mixture by adding KH_2PO_4 or H_3PO_4 dissolved in about 10 volumes of D_2O (Figure 9).

$NADP(D)^+$ can be prepared by the same procedure by carrying out the reaction with $NADP^+$ instead of NAD^+.[30] It is also possible to obtain $NAD(T)^+$ and $NADP(T)^+$ by replacing D_2O with THO.[21,82]

Once the isotope-labeled $NAD(P)^+$ is prepared, the stereospecificity of a given enzyme can be determined by carrying out that the reaction of the enzyme with the reduced substrate in the presence of the enzyme and the isotopically labeled, oxidized coenzyme. The position of the isotope in the resulting reduced coenzyme can be found by oxidizing it by an enzyme with known stereospecificity in the presence of the oxidized substrate of the enzyme. It should be pointed out that the stereospecificity of the enzyme under investigation is opposite to the location of the isotope in the reduced form because the isotope is already present in the oxidized coenzyme prior to the reduction.

B. Enzymatic Preparation of Stereospecifically Labeled NAD(P)H with Isotope

If substrates labeled with deuterium or tritium at appropriate positions are available, preparation of stereospecifically labeled NADH or NADPH is simple. By carrying out the enzymatic reaction of the substrate with NAD^+ or $NADP^+$, the reduced coenzyme containing the isotope at the A or B position, depending on the stereospecificity of the enzyme, can be obtained.

Several enzymes and their labeled substrates are notably popular for this purpose: alcohol dehydrogenase is the most widely employed enzyme for the preparation of the A side-labeled coenzyme. For this purpose, the reaction is carried out with [1,1-D/T]-ethanol or less costly [U-D/T]-ethanol. Since the equilibrium of this enzymatic reaction lies overwhelmingly in the direction of the oxidation of NADH, it is necessary to pull the reaction toward the reduction of NAD^+ by converting the acetaldehyde produced into other compounds. The inclusion of either aldehyde reductase (EC.1.2.1.3.) or hydrazine or semicarbazide in the reaction mixture satisfies this goal. Providing that properly labeled substrates are available,

L-lactate dehydrogenase, malate dehydrogenase, and decarboxylating malate dehydrogenase (EC.1.1.1.39.) are other examples of enzymes that are useful for preparing the A side-labeled reduced NAD.

Because alcohol dehydrogenase from horse liver is also active with NADP, it can be used for the preparation of the A side-labeled reduced NADP.[168] Isocitrate dehydrogenase (NADP) (EC.1.1.1.42.) with *threo*-D,L- [2-D/T]-isocitrate is, however, a more popular system for the A-side labeling.[169]

For the preparation of the B-side-labeled reduced NAD(P), glutamate dehydrogenase [NAD(P)], accompanied by DL-[2-D/T]-glutamate, is mostly widely employed. Another popular enzyme system for this purpose is glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides*, which is capable of utilizing both NAD and NADP with the substrate D-[1-D/T]-glucose-6-phosphate (which can be synthesized by the action of hexokinase in the presence of D-[1-D/T]-glucose and ATP). Viola et al.[170] took the advantage of glucose dehydrogenase activity of glucose-6-phosphate dehydrogenase in organic solvent (i.e., 40% dimethylsulfoxide) and used only D-[1-D]-glucose, without ATP and hexokinase, to prepare $NADPD_B$.

Glucose dehydrogenase (EC.1.1.1.47.), galactose dehydrogenase (EC.1.1.1.48.), and glyceraldehyde-phosphate dehydrogenase are other enzymes which are sometimes used to prepare the B side-labeled reduced NAD. Labeled reduced NAD can also be obtained if labeled reduced NADP is available by hydrolyzing off the 2'-phosphate group from the reduced coenzyme with alkaline phosphatase.[280]

Once stereospecifically labeled reduced coenzyme is available, the stereospecificity of an enzyme with unknown stereospecificity can be determined by oxidizing the coenzyme with the enzyme under study in the presence of oxidized substrate. The analysis for the presence or absence of the isotope in the resultant oxidized coenzyme directly reveals the stereospecificity of that enzyme.

In 1971, Oppenheimer et al.[38] introduced an ingenious way of preparing $NADD_B$. They carried out dihydrolipoamide dehydrogenase (from pig heart) reaction in the presence of NAD^+, a catalytic amount of lipoamide, and an excess amount (over NAD^+) of dithiothreitol in highly pure, buffered D_2O medium. In this mixture, practically all of the hydrogens of the sulfhydro groups in both dithiothreitol and reduced lipoamide are exchanged with the deuterium in the medium. At the same time, dithiothreitol keeps the lipoamide substrate in the reduced form at all times. Under this reaction condition, the reduced coenzyme formed by the action of dihydrolipoamide dehydrogenase (a B-stereospecific enzyme) contains a deuterium on the B side. The reduced coenzyme thus prepared can be separated from other components in the reaction mixture by ion-exchange chromatography. The use of volatile buffer, such as ammonium bicarbonate, for the chromatography as well as for the reaction is particularly convenient since the product coenzyme without salts can be obtained by simple lyophilization of the eluate. The reduced coenzyme labeled with deuterium on the A side can be obtained by carrying out above reaction in H_2O with $NAD(D)^+$. Stereospecifically labeled reduced (3AcPy)AD can be also prepared by this procedure.[59] Arnold et al.[40] and Arnold and You[171] described these procedures in detail.

Arnold et al.[40] also pointed out that it should be possible to prepare stereospecifically labeled reduced NADP by replacing dihydrolipoamide dehydrogenase, lipoamide, and NAD^+ with glutathione reductase, glutathione, and $NADP^+$, respectively. Recently, Wong and Wong[41] described a facile proton magnetic resonance technique for the determination of the stereospecificity of several enzymes with the $NADPD_B$ produced according to this very suggestion.

C. Techniques for Quantitation of the Isotope in Coenzyme

As mentioned earlier (Section III), deuterium was used exclusively as the tracer of the

stereospecificity determination until 1960. The content of deuterium in the product coenzymes was then analyzed by means of mass spectrometry. Since 1960, however, tritium has replaced deuterium because scintillation counting of the radioisotope is much easier than the old mass spectrometric quantitation of the stable isotope.

Because scintillation counting is rather straightforward and most researchers are familiar with it, it will not be discussed here. Due to the improvement of instrumentation in proton magnetic resonance and mass spectrometry, recent trend is, however, reversal to the use of deuterium. Of these, PMR is unique in that it makes possible direct monitoring of the deuterium content at the exact reaction site of the coenzymes.[40,171]

1. Proton Magnetic Resonance (PMR)

For the analysis of the oxidized coenzyme by PMR, deuterium-labeled reduced coenzyme is oxidized with the enzyme under study. The product of this reaction is either NAD^+ or $NAD(D)^+$ depending on the stereospecificity of the test enzyme. NAD^+ and $NAD(D)^+$ can be easily distinguished from their spectra in the aromatic region since the former must show the resonance for the C-4 proton of the nicotinamide at 8.95 ppm (pD 3, 23°C) without interference from the resonances for other protons of the nucleotides, designated PC_4H in Figure 22a; as expected, however, the spectrum of the latter is devoid of this PC_4H peak (Figure 22b). (P denotes pyridinium ring.)

In order to examine the presence or absence of the resonance for the C-4 proton, the reaction mixture is lyophilized upon the completion of the reaction (monitored by absorbance change at 340 nm) followed by dissolving in D_2O. Since no other resonance is likely to appear in this aromatic position, the product coenzyme usually need not be purified. (Unreacted NADH does not interfere with the spectrum of the nicotinamide because the resonances for the protons on the dihydronicotinamide ring appear in the aliphatic region.)

In case a reaction does not show an absolute stereospecificity, like the occasion of chemical oxidation, the degree of stereopreference can be quantitated by integrating the PC_4H peak area and comparing it with the adjacent PC_6H peak area, which should be taken as the value for 100% proton. If the PC_4H peak area appears to be smaller than that for PC_6H, the sample contains a mixture of deuterium-labeled and -unlabeled NAD^+.

An example of stereospecificity determination by this approach is illustrated in Figure 23, which presents the spectra of the oxidized coenzyme produced from $NADD_B$ (Figure 23a) and $NADD_A$ (Figure 23b) by the action of salicylate 1-monooxygenase.[91] When $NADD_B$ is oxidized, the spectrum of the resulting oxidized coenzyme lacks the resonance for the PC_4H proton, whereas the oxidation product of $NADD_A$ retains this peak. It must be, therefore, concluded that the hydrogen is removed from $NADD_B$ and the deuterium from $NADD_A$, an unambiguous demonstration of the A-stereospecific reaction.

In case the equilibrium of a given enzymatic reaction lies dominantly toward the formation of NADH, or the oxidized form of the substrate is not available, the stereospecificity must be determined by analyzing the spectrum of the resulting reduced coenzyme. The dihydronicotinamide ring of NADH is no longer aromatic; hence, the resonances for the A and B geminal protons appear very close to each other in the aliphatic region, as Figure 24 displays: the resonance for the A proton is located at 2.77 ppm with an unresolved coupling constant ($J_{A\text{-}5}$) of 3.1 Hz (Figure 24a), while that for the B proton is at 2.67 ppm with a resolved coupling constant ($J_{B\text{-}5}$), of 3.9 Hz (Figure 24b) (at 20 m*M* and 23°C).

Glucose-6-phosphate dehydrogenase from *L. mesenteroides* is an enzyme whose stereospecificity must be determined by analyzing NADH since its reaction is irreversible due to the rapid hydrolysis of the product, D-glucono-δ-lactone-6-phosphate, in aqueous medium. Figure 24c demonstrates that the reduction product of $NAD(D)^+$ by this enzyme is $NADD_A$, indicating that a hydrogen was transferred to the B side of $NAD(D)^+$; the enzyme is, therefore, B stereospecific.

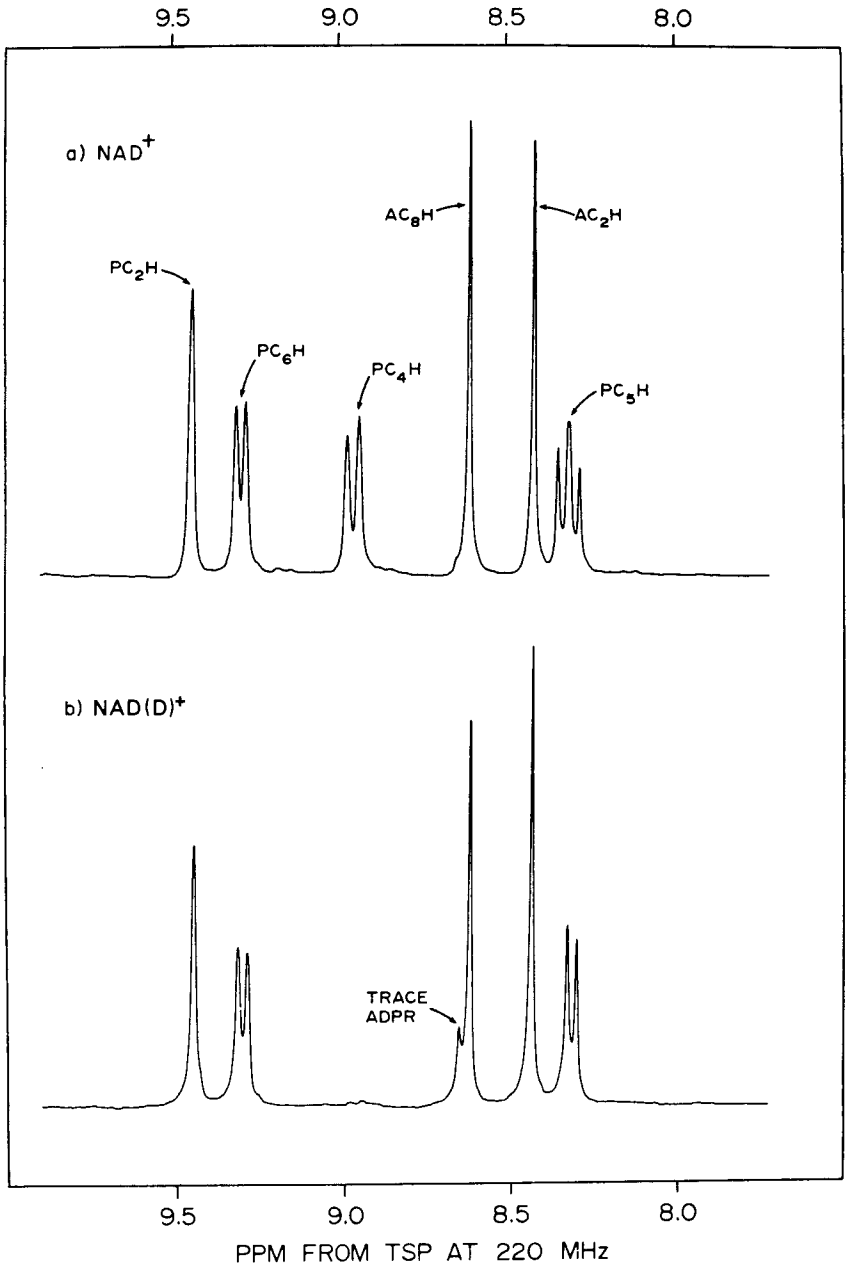


FIGURE 22. The aromatic region of the PMR spectrum for NAD^+ (a) and $NAD(D)^+$ (b). The letters PC and AC denote the carbon of the pyridinium ring and the adenine ring, respectively. The chemical shift of the proton at the redox site, PC_4H, appears as a doublet between PC_6H and AC_xH at 8.95 ppm from trimethylsilyl[U-D]priopionate (TSP); $NAD(D)^+$ lacks this resonance. The small resonance on the left side of the AC_xH signal in (b) is caused by contaminating trace amounts of ADPR. (From Arnold, L. J., Jr., You, K., Alison, W. S., and Kaplan, N. O., *Biochemistry*, 15, 4844, 1976. With permission.)

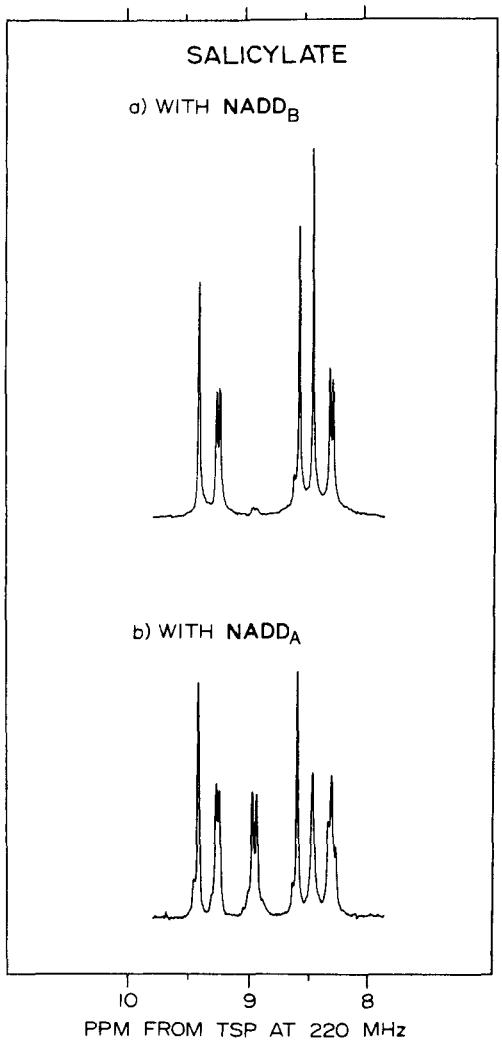


FIGURE 23. The aromatic region PMR spectra of the products from the oxidation of $NADD_B$ (a) and $NADD_A$ (b) with salicylate 1-monooxygenase (EC.1.14.13.1.) in the presence of salicylate and O_2. While the product from $NADD_A$ shows the resonance at 8.95 ppm intact (b), that from $NADD_B$ is devoid of it (a), indicating the presence of proton at the redox site of the former but deuterium in the latter. The enzyme is, therefore, clearly A stereospecific. (From You, K., Arnold, L. J., Jr., and Kaplan, N. O., *Arch. Biochem. Biophys.*, 180, 550, 1977. With permission.)

Obviously, working with NAD^+ is much easier and requires a simpler instrument (having a magnetic field of as low as 40 MHz) than NADH, which requires at least a 100-MHz instrument equipped with Fourier transformation capability in order to resolve the two closely situated chemical shifts for the C-4 methylene protons. The handling of NADH can be avoided if it is reoxidized with a standard enzyme such as alcohol dehydrogenase, whose stereospecificity is well established. It should be pointed out that Wong and Wong[41] employed essentially the same procedure to determine the stereospecificity of the enzymatic reactions which involve NADP.

The proton magnetic resonance technique has many advantages over the radioisotopic procedures: (1) monitoring of the exact redox site rather than the entire or a fragment of the

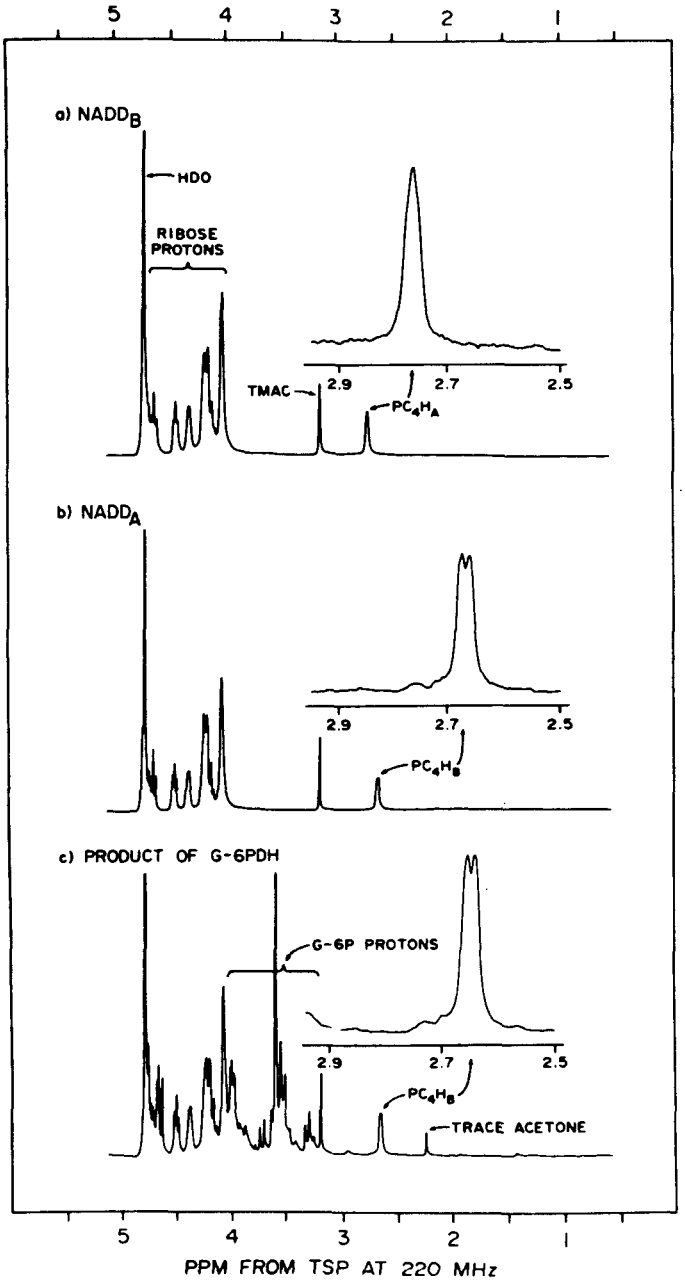


FIGURE 24. The PMR (aliphatic region) spectra of $NADD_B$, $NADD_A$, and the reduced coenzyme produced from NAD(D)$^+$ by the action of glucose-6-phosphate dehydrogenase (EC.1.1.1.49.) (G-6PDH) from *L. mesenteroides* in the presence of glucose-6-phosphate. The spectrum for $NADD_B$ shows the unresolved resonance for the A proton of the dihydropyridine ring at 2.77 ppm with the coupling constant, $J_{A\text{-}5}$, of 3.1 Hz, whereas $NADD_A$ presents the resolved chemical shift of the B-proton at 2.67 ppm with the coupling constant, $J_{B\text{-}5}$, of 3.9 Hz. The product of glucose-6-phosphate dehydrogenase reaction shows the chemical shift of the B proton, indicating that the enzyme is B stereospecific. TMAC represents the internal standard, tetramethylammonium chloride. (From Arnold, L. J., Jr., You, K., Allison, W. S., and Kaplan, N. O., *Biochemistry*, 15, 4844, 1976. With permission.)

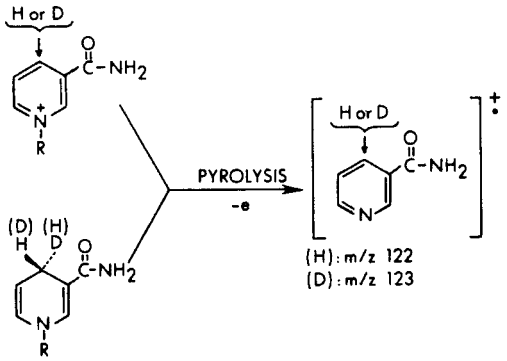


FIGURE 25. Thermolytic formation of nicotinamide from NAD^+ (or $NAD(D)^+$) and NADH during mass spectrometric detection of the presence of deuterium at the redox site of the oxidized or reduced coenzymes; the peak at *m/z* 122 indicates the presence of hydrogen and *m/z* 123 deuterium.

coenzyme molecule; (2) elimination of purification steps; (3) ability of using crude extract as the enzyme (in this case, the substrate must be pure); (4) alleviation of the hazards associated with radioactivity; and (5) requirement of a micomole quantity of the test coenzyme.

2. Mass Spectrometry

In the early days of stereospecificity study (through 1960), the reaction products were completely degraded to hydrogen gas, whose deuterium content was then analyzed by mass spectrometry. Obviously, this approach is now obsolete. In recent years, gas-liquid chromatography/mass spectrometry is employed for the analysis of deuterium-containing, volatile reaction products, thus alleviating cumbersome sample preparation for analysis.[42-44]

In 1980, Ehmke et al.[45] introduced electron impact as well as field desorption mass spectrometry for the detection of deuterium in the nicotinamide moiety. Because of high polarity, thus low volatility, NAD^+ and NADH molecules must be degraded to nicotinamide by pyrolysis as shown in Figure 25.

The base peak of the electron impact mass spectrum is the molecular ion of nicotinamide at *m/z* 122 formed by the thermal degradation of the intact molecules. Further degradation of nicotinamide also gives signals at *m/z* 106, 78, and 51 according to the steps

$$122 \xrightarrow{-NH_2} 106 \xrightarrow{-CO} 78 \xrightarrow{-HCN} 51$$

If the molecular ions are derived from NAD^+ containing a deuterium at a position (not necessarily at the redox site, however), the base peak will be at *m/z* 123, rather than 122, and other peaks will shift likewise one mass unit higher.

Sensitivity of this technique is so great that 1 μg of the coenzyme is all it takes for a successful determination; like the PMR technique, this one does not require purification of the coenzyme from the reaction mixture.

It should be pointed out that this technique is limited to the oxidized coenzyme. In case the product is NADH, it should be oxidized completely to NAD^+ for the analysis. If the reaction mixture contains the reduced coenzyme, partially or wholly, ambiguity arises since the label is removed from the reduced form during the enzymatically unrelated pyrolysis step (Figure 25).

In field desorption spectrometry, too, the coenzyme must be degraded thermolytically.

The unlabeled NAD^+ shows the desorption peak at *m/z* 122 with little background and $NAD(D)^+$ at *m/z* 123. By employing this approach, it is possible to differentiate the deuterium-labeled reduced substrates (e.g., L-[2-D]-glutamate in glutamate dehydrogenase-catalyzed reaction) from the unlabeled ones, which cannot be achieved by electron impact mass spectrometry due to their low volatility.[45]

XI. CONCLUSIONS

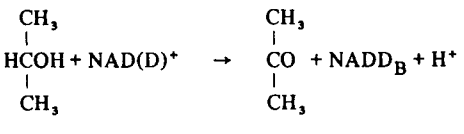
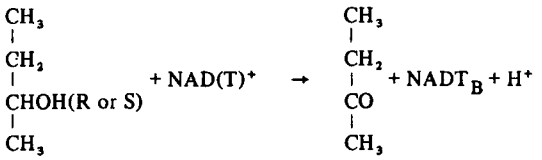
The pyridine nucleotide-linked enzymes — more than 150 of them tested to date — exhibit absolute stereospecificity in the hydrogen transfer reactions, with respect to the redox site of the dinucleotide (with the possible exception of one enzyme). The number of A- and B-stereospecific enzymes are almost evenly distributed. There is apparently no germane physiological logic that can explain this enzyme stereospecificity. The enzyme stereospecificity is discussed in part in relation with the generalizations, evolutionary implication, and application to enzyme classification. Also included is the discussion of some of the recent proposals on the mechanistic basis for the stereospecificity. The most arduous emphasis is given to the conformational interpretation of the stereospecificity. Free coenzyme in aqueous medium is at rapid equilibrium between the folded and open forms. Due to this dynamic "folded-open" conformation, nonenzymatic reactions, hydride transfer or adduct formation, in aqueous solution show one-sided steric preference, always partially favoring the A side. The nicotinamide/dihydronicotinamide ring in the folded form of the coenzyme may be puckered in the boat form. In holoenzyme complexes, all of the cases studied to date, the dinucleotide holds the base-base destacked open conformation, which is effectively maintained by an intricate network of hydrophobic force, ionic interaction, and hydrogen bond between the coenzyme and enzyme. The most critical determinant in governing the stereospecificity of a given enzyme is the torisonal orientation of the nicotinamide/dihydronicotinamide ring of protein-bound coenzyme: on the A-stereospecific enzymes, the orientation is *anti* and on the B-stereospecific enzymes, it is *syn*. This relationship apparently holds only in the case where the hydrogen transfer takes place in a direct manner between the substrate and the coenzyme. The carboxamide group, which is anchored by one or more hydrogen bonds, plays an especially crucial role in making the designated orientation of the nicotinamide/dihydronicotinamide ring. In the transition state, these rings of protein-bound coenzyme may also be puckered in the boat form. X-ray crystallography and NMR spectrometry have made invaluable contributions in expanding our understanding about the relationship between the dinucleotide conformation and the enzyme stereospecificity.

ACKNOWLEDGMENT

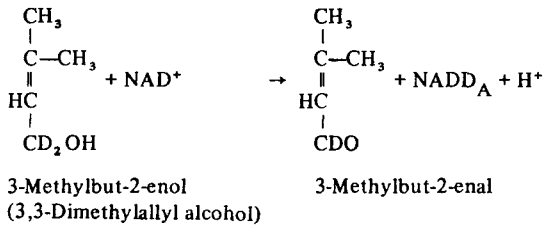
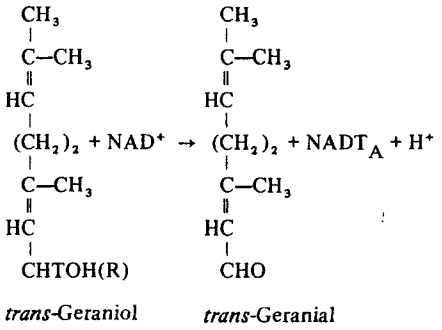
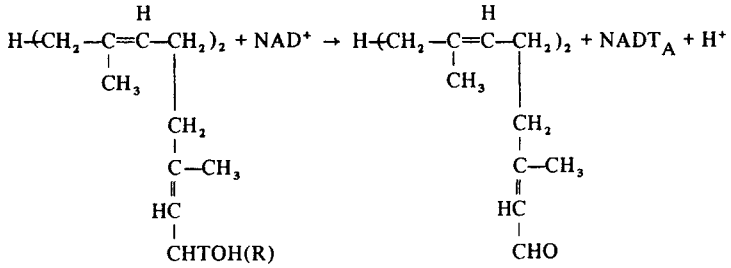
I wrote a major part of this article when I was a member of the faculty at Department of Pediatrics, Duke University Medical Center, Durham, N.C.

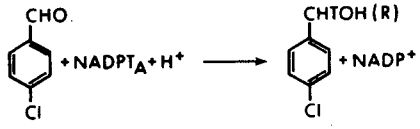
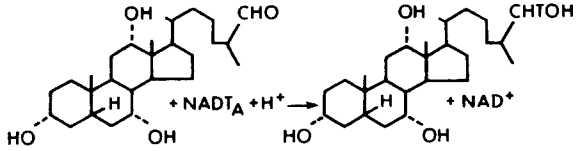
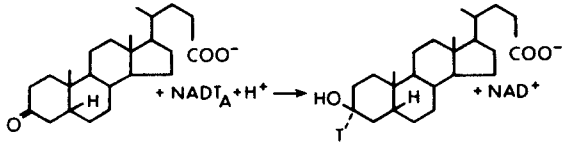
Appendix
SURVEY OF THE STEREOSPECIFICITY OF NICOTINAMIDE ADENINE DINUCLEOTIDE-LINKED ENZYMES

Enzyme	E.C.	Source	Reaction (as the experiment was performed)	Coenzyme	Stereo-specificity	Ref.
Alcohol dehydrogenase	1.1.1.1	*Pseudomonas testosteroni*	$CH_3CD_2OH + NAD^+ \rightarrow CH_3CDO + NADD_A + H^+$ Ethanol → Acetaldehyde	NAD	A	20
		Aerobacter aerogenes	$CH_3CHO + NADD_A + H^+ \rightarrow CH_3CHDOH(R) + NAD^+$ Acetaldehyde → Ethanol	NAD	A	16
		Yeast	$Ethanol + NAD^+ \rightarrow Acetaldehyde + NADD_A + H^+$ (See *P. testosteroni* alcohol dehydrogenase 1.1.1.1.)	NAD	A	19
			$Acetaldehyde + (3AcPy)ADT_A + H^+ \rightleftarrows Ethanol + (3AcPy)AD^+$	(3AcPy)AD	A	58
			$Acetaldehyde + (3CNPy)ADT_A + H^+ \rightarrow Ethanol + (3CNPy)AD^+$ (See *A. aerogenes* alcohol dehydrogenase 1.1.1.1 for the location of T in ethanol)	(3CNPy)AD	A	58
			$Acetaldehyde + (3CNPy)ADT_B + H^+ \rightarrow Ethanol + (3CNPy)AD(T)^+$	(3CNPy)AD	A	58
			$CH_3CHTOH(R) + (TN)AD^+ \rightarrow CH_3CHO + (TN)ADT_A + H^+$	(TN)AD	A	58
			$Acetaldehyde + \alpha\text{-}NADD_B + H^+ \rightarrow Ethanol + \alpha\text{-}NAD(D)^+$	α-NAD	A	60
			$Acetaldehyde + NADPT_A + H^+ \rightarrow Ethanol + NADP^+$ (See *A. aerogenes* alcohol dehydrogenase 1.1.1.1)	NADP	A	67

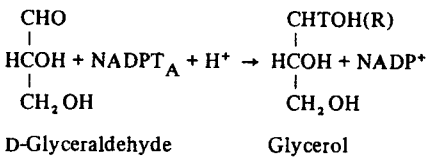
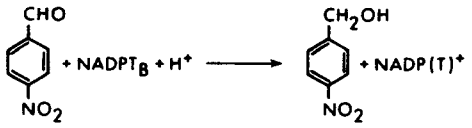
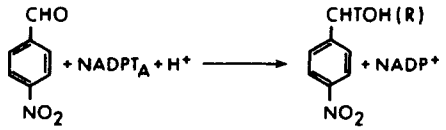
	 Propan-2-ol (Isopropyl alcohol)	NAD	A	31
Wheat germ	$Ethanol + NAD^+ \rightarrow Acetaldehyde + NADD_A + H^+$ (See *Pseudomonas* alcohol dehydrogenase 1.1.1.1.)	NAD	A	20
Horse liver	Same as above	NAD	A	31
	$Acetaldehyde + (3AcPy)ADT_A + H^+ \rightarrow Ethanol + (3AcPy)AD^+$	(3AcPy)AD	A	58
	$Acetaldehyde + (3CNPy)ADT_A + H^+ \rightarrow Ethanol + (3CNPy)AD^+$	(3CNPy)AD	A	58
	$Acetaldehyde + (TN)ADT_A + H^+ \rightarrow Ethanol + (TN)AD^+$	(TN)AD	A	58
		(TN)AD	A	58
	$Ethanol + (TN)AD(T)^+ \rightarrow Acetaldehyde + (TN)ADT_B + H^+$	(TN)AD	A	58
	$Propan-2-ol + NAD(D)^+ \rightarrow Acetone + NADD_B + H^+$ (See yeast alcohol dehydrogenase 1.1.1.1.)	NAD	A	173
	 D(or L)-Butan-2-ol Butan-2-one (Methylethyl ketone)	NAD	A	173

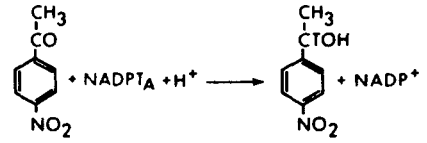
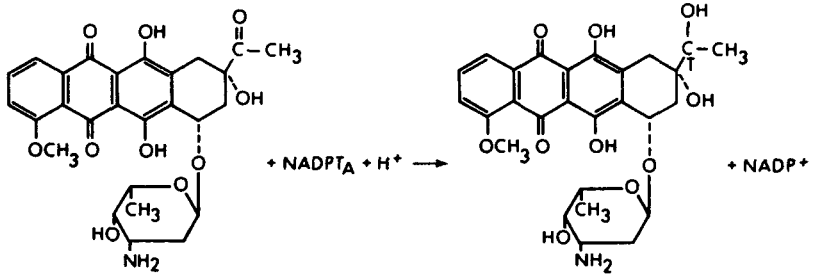
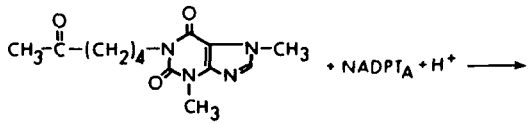
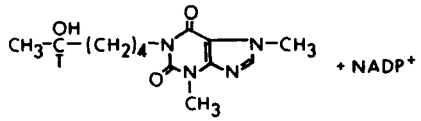
Appendix (continued)
SURVEY OF THE STEREOSPECIFICITY OF NICOTINAMIDE ADENINE DINUCLEOTIDE-LINKED ENZYMES

Enzyme	E.C.	Source	Reaction (as the experiment was performed)	Coenzyme	Stereo-specificity	Ref.
		Horse liver	 3-Methylbut-2-enol (3,3-Dimethylallyl alcohol) → 3-Methylbut-2-enal	NAD	A	174
			 *trans*-Geraniol → *trans*-Geranial	NAD	A	175
				NAD	A	165

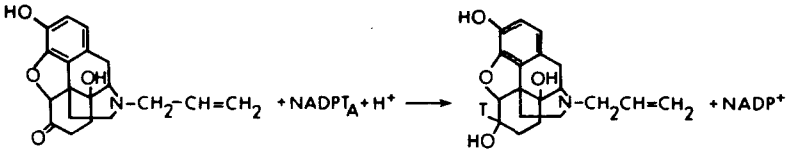
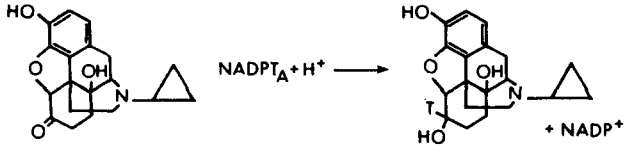
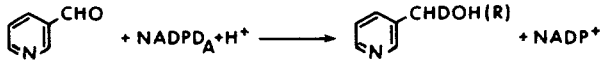
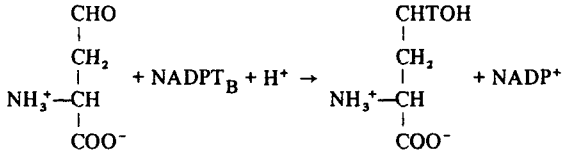
		Horse liver	CH_3-$CHOH$-CHO + $NADD_A$ + H^+ → CH_3-$CHOH$-$CHDOH(R)$ + NAD^+ D(or L)-Lactaldehyde → D(or L)-1,2-Propanediol	NAD	A	176
			 *p*-Chlorobenzaldehyde → *p*-Chlorobenzyl alcohol	NADP	A	177
		Rat liver	 5β-Cholestan-3α,7α,12α-triol-26-al → 5β-Cholestan-3α,7α,12α,26-tetrol	NAD	A	178
			 5β-Cholan-3-one-24-oate → 5β-Cholan-3β-ol-24-oate	NAD	A	178
Alcohol dehydrogenase (NADP+) (Aldehyde reductase)	1.1.1.2	*Lactobacillus mesenteroides*	CD_3CD_2OD + $NADP^+$ → CD_3CDO + $NADPD_A$ + D^+	NADP	A	41

Appendix (continued)
SURVEY OF THE STEREOSPECIFICITY OF NICOTINAMIDE ADENINE DINUCLEOTIDE-LINKED ENZYMES

Enzyme	E.C.	Source	Reaction (as the experiment was performed)	Coenzyme	Stereo-specificity	Ref.
[Glucuronate reductase (1.1.1.19), mevaldate reductase (NADP) (1.1.1.33), and lactaldehyde reductase (1.1.1.55) are the same enzymes as this one]		Yeast	 D-Glyceraldehyde Glycerol	NADP	A	179
		Fruit fly	Same as above	NADP	A	179
		Rat kidney	Same as above	NADP	A	179
		Chicken kidney	Same as above	NADP	A	179
		Rabbit kidney	Same as above	NADP	A	179
		Human liver	 *p*-Nitrobenzaldehyde *p*-Nitrobenzyl alcohol	NADP	A	180
		Rat liver	 *p*-Nitrobenzaldehyde *p*-Nitrobenzyl alcohol	NADP	A	67

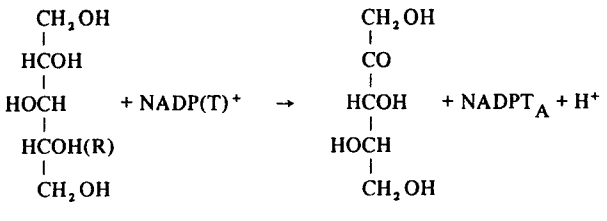
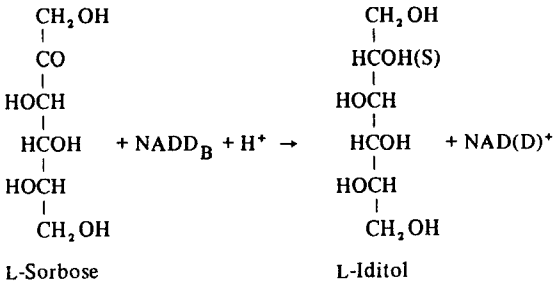
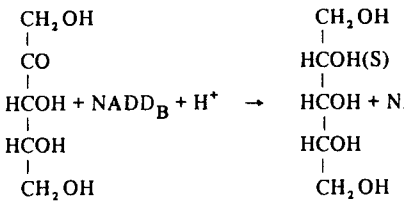
(High pI *p*-nitrobenzaldehyde reductase)	Rabbit liver	Same as above	NADP	A	67
(High pI *p*-nitroacetophenone reductase)	Rabbit liver	 *p*-Nitroacetophenone → 2-Hydroxyethyl-*p*-nitrobenzene	NADP	A	67
(High pI daunorubicin reductase)	Rabbit liver	 Daunorubicin → Daunorubicinol	NADP	A	67
	Rat liver	Same as above	NADP	A	67
(High pI 3,7-dimethyl-1-(5-oxohexyl)-xanthine reductase)	Rabbit liver	 3,7-Dimethyl-1-(5-oxohexyl)-xanthine  3,7-Dimethyl-1-(5-hydroxyhexyl)-xanthine	NADP	A	67

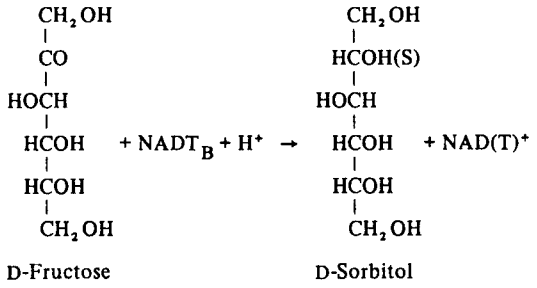
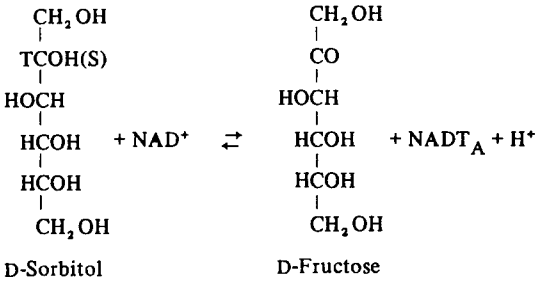
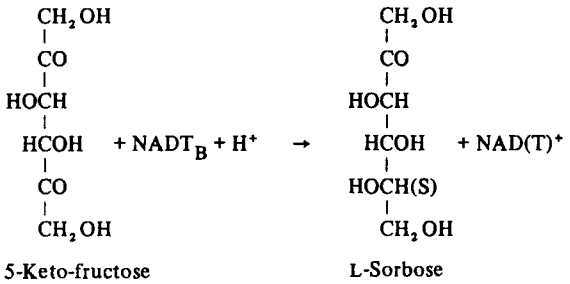
Appendix (continued)
SURVEY OF THE STEREOSPECIFICITY OF NICOTINAMIDE ADENINE DINUCLEOTIDE-LINKED ENZYMES

Enzyme	E.C.	Source	Reaction (as the experiment was performed)	Coenzyme	Stereo-specificity	Ref.
[Dihydromorphinone reductase] (Naloxone reductase)		Rabbit liver	 Naloxone → Naloxol	NADP	A	67
(Naltrexone reductase)		Rabbit liver	 Naltrexone → Naltrexol	NADP	A	67
(Aldehyde reductase I and II)		Pig liver	 Pyridine 3-aldehyde → 3-Hydroxymethylpyridine (Result confirmed with $NADPD_B$)	NADP	A	62
Homoserine dehydrogenase	1.1.1.3	Pea	 L-Aspartic β-semialdehyde → L-Homoserine	NADP	B	79

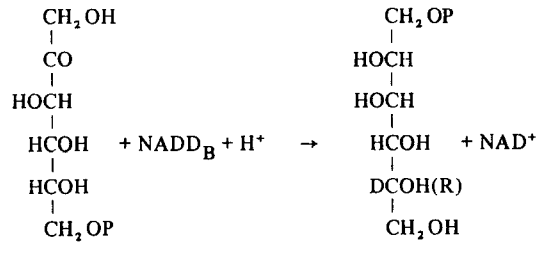
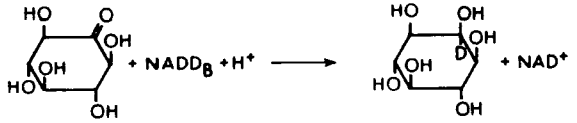
Glycerol dehydrogenase	1.1.1.6	*Aerobacter aerogenes*	CH_2OH / CO / CH_2OH (Dihydroxyacetone) + $NADD_B$ + H^+ → CH_2OH / $DCOH$ / CH_2OH (Glycerol) + $NAD(D)^+$	NAD	A	16
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*Escherichia coli*	CH_2OH / CO / CH_2OP (Dihydroxyacetone-phosphate) + $NADPT_B$ + H^+ → CH_2OH / $HOCT(R)$ / CH_2OP ((*sn*)-Glycerol-3-phosphate) + $NADP^+$	NADP	B	181
		Rabbit skeletal muscle	CH_2OH / CO / CH_2OP (Dihydroxyacetone-phosphate) + $NADD_A$ + H^+ → CH_2OH / $HOCH(R)$ / CH_2OP ((*sn*)-Glycerol-3-phosphate) + $NAD(D)^+$	NAD	B	31
			Dihydroxyacetone-phosphate + $NADPD_A$ + H^+ → (*sn*)Glycerol-3-phosphate + $NADP(D)^+$	NADP	B	41
D-Xylulose reductase (Xylitol dehydrogenase)	1.1.1.9	Yeast	CH_2OH / $HCOH(S)$ / $HOCH$ / $HCOH$ / CH_2OH (Xylitol) + $NAD(D)^+$ → CH_2OH / CO / $HOCH$ / $HCOH$ / CH_2OH (D-Xylulose) + $NADD_A$ + H^+	NAD	B	16
		Guinea pig liver	Xylitol + $NAD(T)^+$ → Xylulose + $NADT_A$ + H^+ (See yeast D-xylulose reductase 1.1.1.9)	NAD	B	182

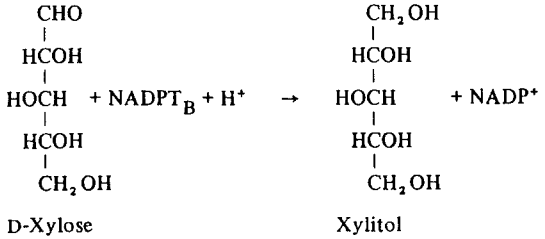
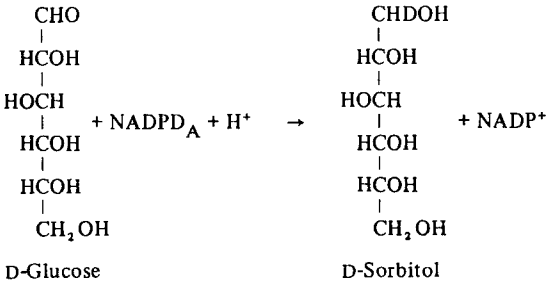
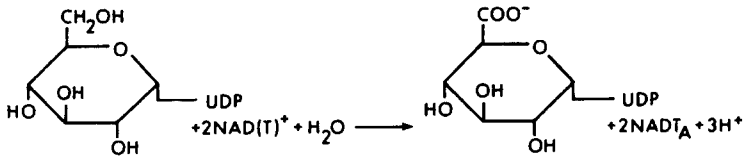
Appendix (continued)
SURVEY OF THE STEREOSPECIFICITY OF NICOTINAMIDE ADENINE DINUCLEOTIDE-LINKED ENZYMES

Enzyme	E.C.	Source	Reaction (as the experiment was performed)	Coenzyme	Stereo-specificity	Ref.
L-Xylulose reductase (Xylitol dehydrogenase)	1.1.1.10	Pigeon liver	 Xylitol → L-Xylulose	NADP	B	182
L-Iditol dehydrogenase	1.1.1.14	Sheep liver	 L-Sorbose → L-Iditol	NAD	A	16
			Xylitol + NAD(D)$^+$ → D-Xylulose + NADD$_B$ + H$^+$ (See yeast D-Xylulose reductase 1.1.1.9)	NAD	A	16
			 D-Ribulose → Ribitol	NAD	A	16

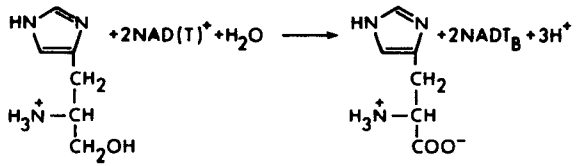
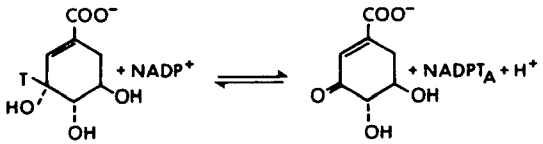
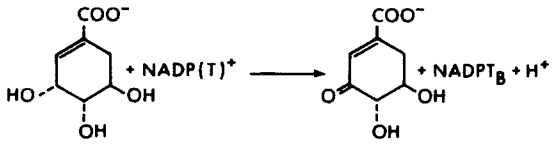
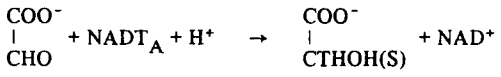
Ribitol + NAD(T)$^+$ → D-Ribulose + NADT$_B$ + H$^+$	NAD	A	86
 D-Fructose → D-Sorbitol	NAD	A	183
 D-Sorbitol ⇄ D-Fructose	NAD	A	184
 5-Keto-fructose → L-Sorbose	NAD	A	183

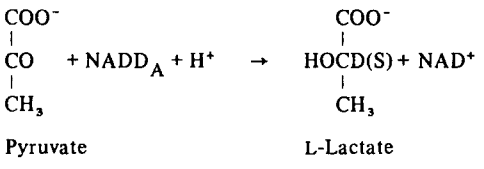
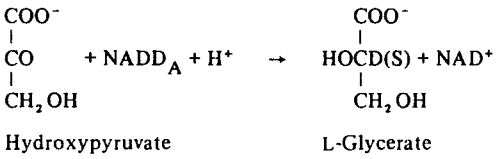
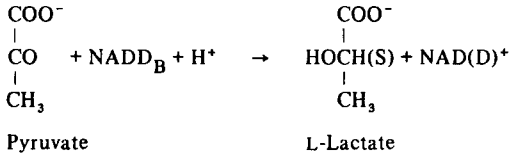
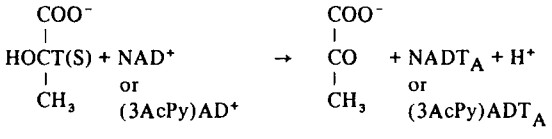
Appendix (continued)
SURVEY OF THE STEREOSPECIFICITY OF NICOTINAMIDE ADENINE DINUCLEOTIDE-LINKED ENZYMES

Enzyme	E.C.	Source	Reaction (as the experiment was performed)	Coenzyme	Stereo-specificity	Ref.
Mannitol-1-phosphate dehydrogenase	1.1.1.17	*Escherichia coli*	 D-Fructose-6-phosphate → D-Mannitol-1-phosphate	NAD	B	16
			D-Mannitol-1-phosphate + NAD(T)$^+$ → D-Fructose-6-phosphate + $NADT_A$ + H^+	NAD	B	86
		Aerobacter aerogenes (Reclassified as *Klebsiella pneumoniae*[156]	Same as above	NAD	B	182
myo-Inositol 2-dehydrogenase	1.1.1.18	*Aerobacter aerogenes*	 *myo*-Inosose-2 → *myo*-Inositol	NAD	B	16

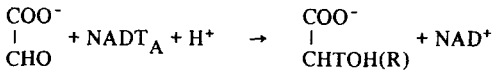
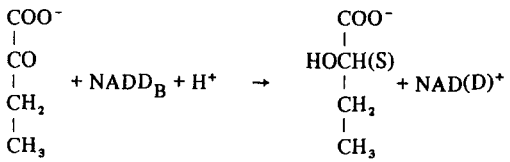
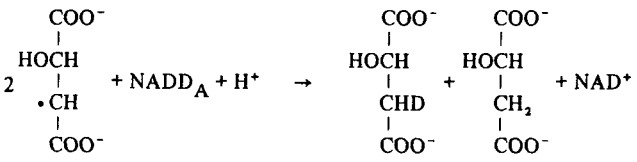
Glucuronate reductase [The same enzyme as alcohol dehydrogenase (NADP) (1.1.1.2.)]	1.1.1.19	Pig kidney	D-Glyceraldehyde + $NADPT_A$ + H^+ → Glycerol + $NADP^+$ (See alcohol dehydrogenase (NADP) 1.1.1.2.)	NADP	A	185
Aldose reductase	1.1.1.21	Yeast	 D-Xylose → Xylitol	NADP	A	183
		Human placenta	 D-Glucose → D-Sorbitol	NADP	A	43
UPD-glucose dehydrogenase	1.1.1.22	Beef liver	 UDP-α-D-glucose → UDP-α-D-glucuronate	NAD	B	82

Appendix (continued)
SURVEY OF THE STEREOSPECIFICITY OF NICOTINAMIDE ADENINE DINUCLEOTIDE-LINKED ENZYMES

Enzyme	E.C.	Source	Reaction (as the experiment was performed)	Coenzyme	Stereo-specificity	Ref.
Histidinol dehydrogenase	1.1.1.23	*Neurospora*	 L-Histidinol → L-Histidine	NAD	A	79
Shikimate dehydrogenase	1.1.1.25	*Escherichia coli*	 (−)Shikimate → 5-Dehydroshikimate	NADP	A	186
		Pea	 (−)Shikimate → 5-Dehydroshikimate	NADP	A	79
Glyoxylate reductase	1.1.1.26	Spinach	 Glyoxylate → Glycolate	NAD	A	82, 187

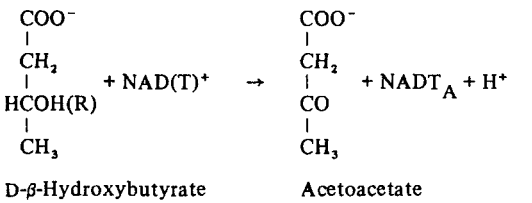
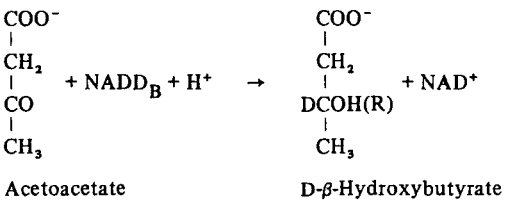
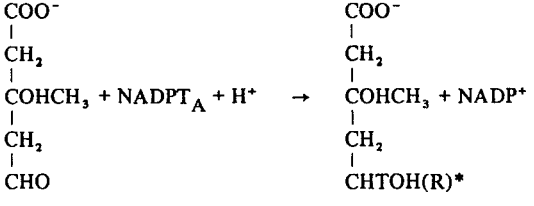
L-Lactate dehydrogenase	1.1.1.27	*Lactobacillus arabinosus*		NAD	A	188
		Potato tuber	Same as above	NAD	A	52
				NAD	A	52
		Bull frog		NAD	A	40
		Halibut	Same as above	NAD	A	40
		Dog fish		NAD (3AcPy)AD	A A	53 53
		Turkey	Pyruvate + NADD$_B$ + H⁺ → L-Lactate + NAD(D)⁺ (See bull frog L-lactate dehydrogenase 1.1.1.27)	NAD	A	40
		Beef heart	Pyruvate + NADD$_A$ + H⁺ → L-Lactate + NAD⁺ (See *L. arabinosus* L-lactate dehydrogenase 1.1.1.27)	NAD	A	27

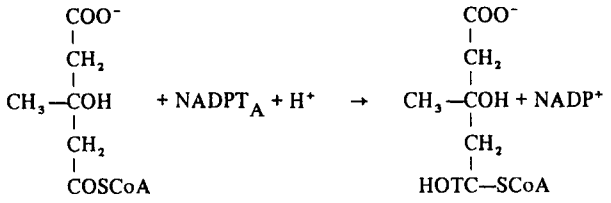
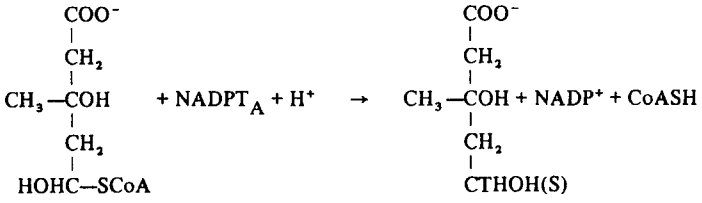
Appendix (continued)
SURVEY OF THE STEREOSPECIFICITY OF NICOTINAMIDE ADENINE DINUCLEOTIDE-LINKED ENZYMES

Enzyme	E.C.	Source	Reaction (as the experiment was performed)	Coenzyme	Stereo-specificity	Ref.
		Beef heart	 Glyoxylate → Glycolate	NAD	A	189
		Rabbit skeletal muscle	Same as above	NAD	A	52
			Pyruvate + $NADPD_A$ → L-Lactate + $NADP^+$ (See *L. arabinosus* L-lactate dehydrogenase 1.1.1.27)	NADP	A	41
			Pyruvate + $NADPD_B$ → L-Lactate + $NADP(D)^+$	NADP	A	41
			Hydroxypyruvate + $NADD_A$ + H^+ → L-Glycerate + NAD^+ (See potato tuber L-lactate dehydrogenase 1.1.1.27)	NAD	A	52
		Pig heart	 α-Ketobutyrate → L-α-Hydroxybutyrate	NAD	A	40
			 L-Malate radical → L-Malate	NAD	A	190

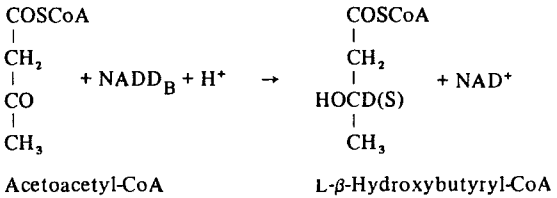
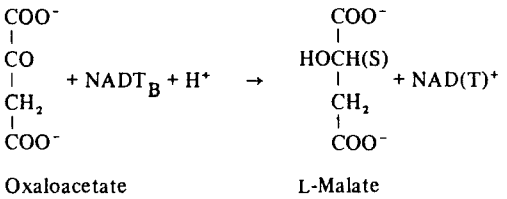
D-Lactate dehydrogenase	1.1.1.28	*Lactobacillus arabinosus*	$\begin{matrix}COO^-\\ \vert\\ CO\\ \vert\\ CH_3\end{matrix}$ + $NADD_A$ + H^+ → $\begin{matrix}COO^-\\ \vert\\ DCOH(R)\\ \vert\\ CH_3\end{matrix}$ + NAD^+ Pyruvate → D-Lactate	NAD	A	188
		Horse shoe crab (*Limulus polyphemus*)	$\begin{matrix}COO^-\\ \vert\\ CO\\ \vert\\ CH_3\end{matrix}$ + $NADT_A$ + H^+ → $\begin{matrix}COO^-\\ \vert\\ TCOH(R)\\ \vert\\ CH_3\end{matrix}$ + NAD^+ Pyruvate → D-Lactate	NAD	A	191
		Sea worm *(Nereis virens)*	Same as above	NAD	A	191
		Abalone	$\begin{matrix}COO^-\\ \vert\\ CO\\ \vert\\ CH_3\end{matrix}$ + $NADD_B$ + H^+ → $\begin{matrix}COO^-\\ \vert\\ HCOH(R)\\ \vert\\ CH_3\end{matrix}$ + $NAD(D)^+$ Pyruvate → D-Lactate	NAD	A	40
		Escherichia coli	$\begin{matrix}COO^-\\ \vert\\ CHO\end{matrix}$ + $NADT_A$ + H^+ → $\begin{matrix}COO^-\\ \vert\\ CTHOH(S)\end{matrix}$ + NAD^+ Glyoxylate → Glycolate	NAD	A	187
Glycerate dehydrogenase	1.1.1.29	Spinach	$\begin{matrix}COO^-\\ \vert\\ CO\\ \vert\\ CH_2OH\end{matrix}$ + $NADD_A$ + H^+ → $\begin{matrix}COO^-\\ \vert\\ DCOH(R)\\ \vert\\ CH_2OH\end{matrix}$ + NAD^+ Hydroxypyruvate → D-Glycerate	NAD	A	31
			Hydroxypyruvate + $NADPD_A$ + H^+ → D-Glycerate + $NADP^+$	NADP	A	41
			Hydroxypyruvate + $NADPD_B$ + H^+ → D-Glycerate + $NADP(D)^+$	NADP	A	41

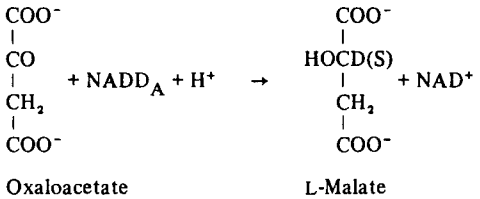
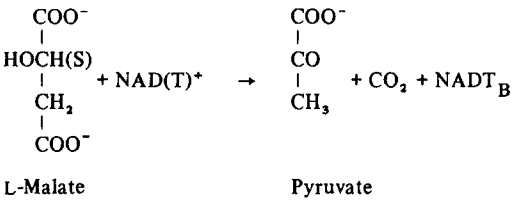
Appendix (continued)
SURVEY OF THE STEREOSPECIFICITY OF NICOTINAMIDE ADENINE DINUCLEOTIDE-LINKED ENZYMES

Enzyme	E.C.	Source	Reaction (as the experiment was performed)	Coenzyme	Stereo-specificity	Ref.
		Parsley	Hydroxypyruvate + $NADD_A$ + H^+ → D-Glycerate + NAD^+	NAD	A	52
3-Hyroxybutyrate dehydrogenase	1.1.1.30	*Rhodopseudomonas spheroides*	 D-β-Hydroxybutyrate Acetoacetate	NAD	B	192
			 Acetoacetate D-β-Hydroxybutyrate	NAD	B	40
		Beef heart mitochondria	Same as above	NAD	B	40
Mevaldate reductase	1.1.1.32	Pig liver	 D(or L)-Mevaldate D(or L)-Mevalonate (*For D-mevalonate only; unknown for L-mevalonate)	NADP	A	165

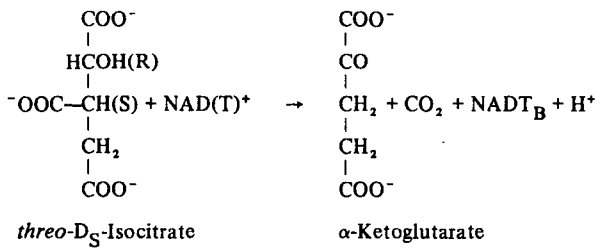
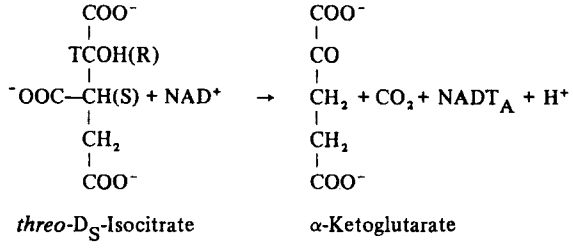
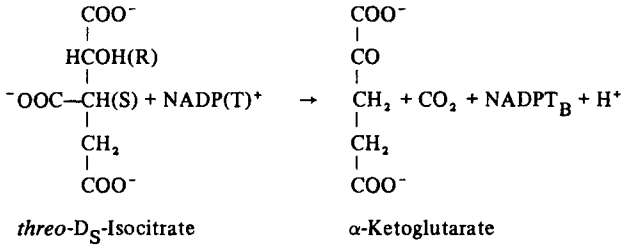
Mevaldate reductase (NADPH) [The same enzyme as alcohol dehydrogenase (NADP) (1.1.1.2.)]	1.1.1.33	Rat liver	Same as above	NADP	A	165
			D(or L)-Mevaldate + $NADT_A$ + H^+ → D(or L)-Mevalonate + NAD^+ (See pig liver mevaldate reductase 1.1.1.32)	NAD	A	165
Hydroxymethylglutaryl CoA reductase (NADPH)	1.1.1.34	Yeast	 (3S)-3-Hydroxy-3-methyl-glutaryl-CoA + $NADPT_A$ + H^+ → D(or 3S)-Mevaldate-CoA-hemithioacetal + $NADP^+$	NADP	A	193
			 D(or 3S)-Mevaldate-CoA-hemithioacetal + $NADPT_A$ + H^+ → D(or 3R)-Mevalonate + $NADP^+$ + CoASH (Results confirmed with $NADPT_B$)	NADP	A	193
		Rat liver microsome	Same as above	NADP	A	194

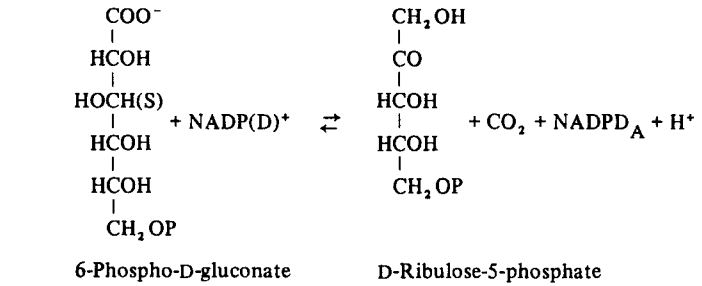
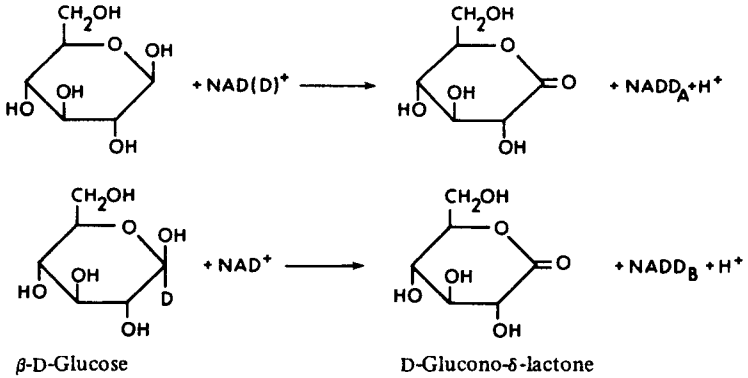
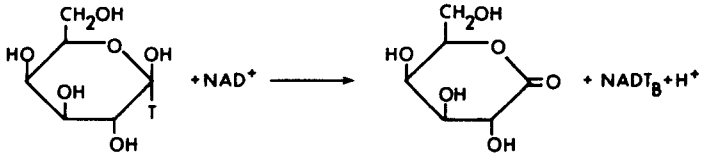
Appendix (continued)
SURVEY OF THE STEREOSPECIFICITY OF NICOTINAMIDE ADENINE DINUCLEOTIDE-LINKED ENZYMES

Enzyme	E.C.	Source	Reaction (as the experiment was performed)	Coenzyme	Stereo-specificity	Ref.
3-Hydroxyacyl-CoA dehydrogenase	1.1.1.35	Pig heart	 Acetoacetyl-CoA → L-β-Hydroxybutyryl-CoA	NAD	B	195
Malate dehydrogenase	1.1.1.37	*Pseudomonas testosteroni*	 Oxaloacetate → L-Malate	NAD	A	196
		Pseudomonas putida	Same as above	NAD	A	182
	1.1.1.37	*Bacillus subtilis*	Oxaloacetate + $NADD_B$ + H^+ → L-Malate + $NAD(D)^+$ (See *P. testosteroni* malate dehydrogenase 1.1.1.37)	NAD	A	40
		Neurospora crassa	Same as above	NAD	A	40
		Potato tuber mitochondria	Oxaloacetate + $NADT_B$ + H^+ → L-Malate + $NAD(T)^+$ (See *P. testosteroni* malate dehydrogenase 1.1.1.37)	NAD	A	79
		Potato tuber cytoplasm	Same as above	NAD	A	79

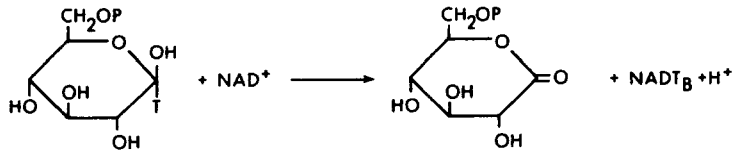
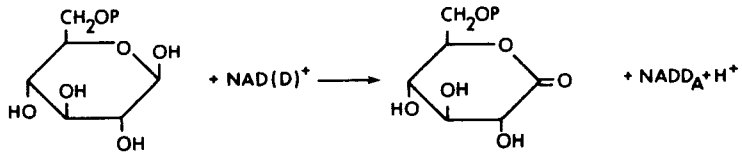
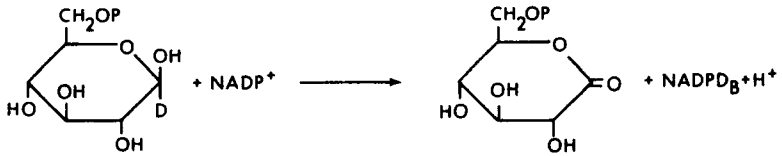
		Wheat germ	 Oxaloacetate L-Malate	NAD	A	28
		Chicken heart mitochondria	Oxaloacetate + $NADT_B$ + H^+ → L-Malate + $NAD(T)^+$ (See *P. testosteroni* malate dehydrogenase 1.1.1.37)	NAD	A	197
		Pig heart mitochondria	Same as above	NAD	A	79
		Pig heart cytoplasm	Oxaloacetate + $NADD_A$ + H^+ → L-Malate + NAD^+ (See wheat germ malate dehydrogenase 1.1.1.37)	NAD	A	198
		Pig heart	Oxaloacetate + $NADPD_A$ + H^+ → L-Malate + $NADP^+$	NADP	A	41
			Oxaloacetate + $NADPD_B$ + H^+ → L-Malate + $NADP(D)^+$	NADP	A	41
Malate dehydrogenase (decarboxylating)	1.1.1.39	Cauliflower	 L-Malate Pyruvate	NAD	A	79
Malate dehydrogenase (Oxaloacetate decarboxylating) ($NADP^+$) (Malic enzyme)	1.1.1.40	Pigeon liver	L-Malate + $NADP(T)^+$ → Pyruvate + CO_2 + $NADPT_B$ (See cauliflower malate dehydrogenase 1.1.1.39)	NADP	A	82

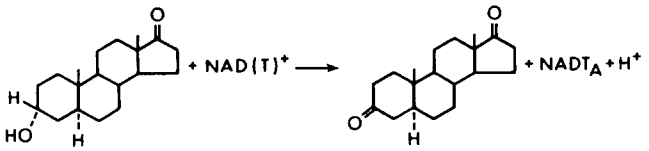
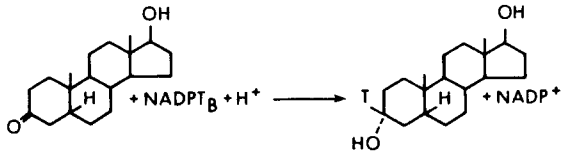
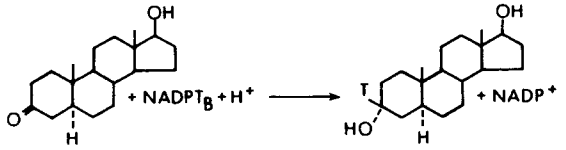
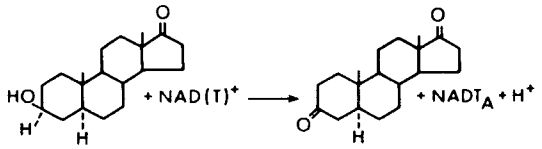
Appendix (continued)
SURVEY OF THE STEREOSPECIFICITY OF NICOTINAMIDE ADENINE DINUCLEOTIDE-LINKED ENZYMES

Enzyme	E.C.	Source	Reaction (as the experiment was performed)	Coenzyme	Stereo-specificity	Ref.
Isocitrate dehydrogenase (NAD^+)	1.1.1.41	Pea		NAD	A	79
		Beef heart		NAD	A	199
Isocitrate dehydrogenase ($NADP^+$)	1.1.1.42	Pea		NADP	A	79
		Pig heart	*threo*-D_S-Isocitrate + $NADP(D)^+$ → α-Ketoglutarate + CO_2 + $NADPD_B$ + H^+ (See pea isocitrate dehydrogenase 1.1.1.42)	NADP	A	30

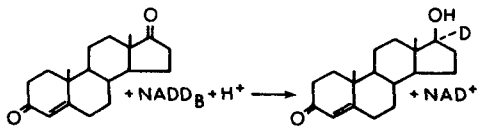
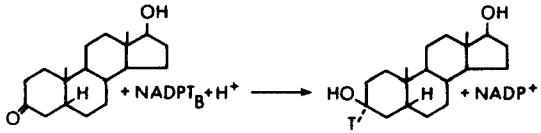
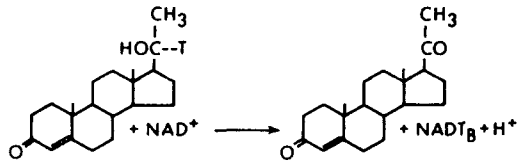
Phosphogluconate dehydrogenase (decarboxylating)	1.1.1.44	Yeast	 6-Phospho-D-gluconate → D-Ribulose-5-phosphate	NADP	B	200
Glucose dehydrogenase	1.1.1.47	Beef liver	 β-D-Glucose → D-Glucono-δ-lactone	NAD	B	201, 202
Galactose dehydrogenase	1.1.1.48	*Pseudomonas fluorescens*	 D-Galactose → D-Galactono-δ-lactone	NAD	B	203, 204
			D-[1-T]-Galactose + $NADP^+$ → D-Galactono-δ-lactone + $NADPT_B$ + H^+	NADP	B	205

Appendix (continued)
SURVEY OF THE STEREOSPECIFICITY OF NICOTINAMIDE ADENINE DINUCLEOTIDE-LINKED ENZYMES

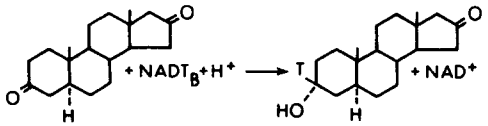
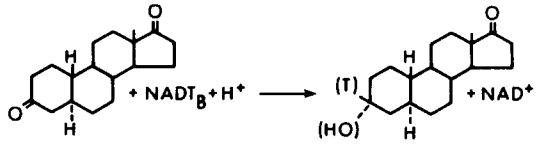
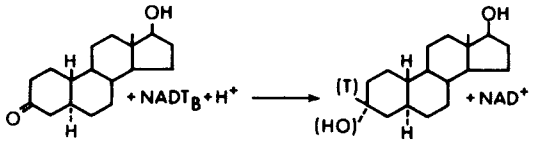
Enzyme	E.C.	Source	Reaction (as the experiment was performed)	Coenzyme	Stereo-specificity	Ref.
		Pseudomonas saccharophila	Same as above	NAD	B	204
Glucose-6-phosphate dehydrogenase	1.1.1.49	*Pseudomonas fluorescens*	 β-D-Glucose-6-phosphate D-Glucono-δ-lactone-6-phosphate	NAD	B	206
		Leuconostoc mesenteroides	 β-D-Glucose-6-phosphate D-Glucono-δ-lactone-6-phosphate	NAD	B	40
		Yeast	 β-D-Glucose-6-phosphate D-Glucono-δ-lactone-6-phosphate	NADP	B	200

3α-Hydroxysteroid dehydrogenase	1.1.1.50	*Pseudomonas testosteroni*	 5α-Androstan-3α-ol-17-one(5α-Androsterone) → 5α-Androstan-3,17-dione (5α-Androstanedione)	NAD	B	21
		Rooster comb (microsome + mitochondria)	 5β-Androstan-17β-ol-3-one (5β-Dihydrotestosterone) → 5β-Androstan-3α, 17β-diol	NADP	B	75
		Rooster comb (microsome + cytosol)	 5α-Androstan-17β-ol-3-one (5α-Dihydrotestosterone) → 5α-Androstan-3α, 17β-diol	NADP	B	75
		Rat seminal vesicles	Same as above	NADP	B	76
β-Hydroxysteroid dehydrogenase (3(or 17)β-Hydroxy-steroid dehydrogenase)	1.1.1.51	*Pseudomonas testosteroni*	 5α-Androstan-3β-ol-17-one (Epiandrosterone) → 5α-Androstan-3,17-dione (Androstanedione)	NAD	B	21

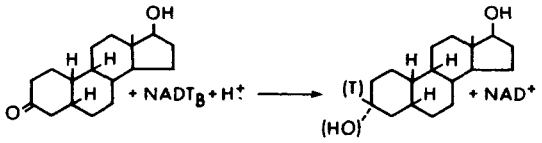
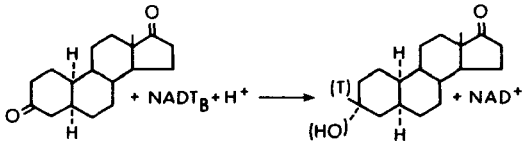
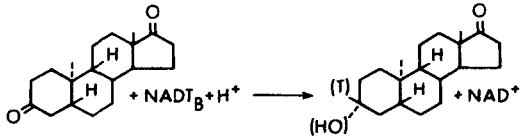
Appendix (continued)
SURVEY OF THE STEREOSPECIFICITY OF NICOTINAMIDE ADENINE DINUCLEOTIDE-LINKED ENZYMES

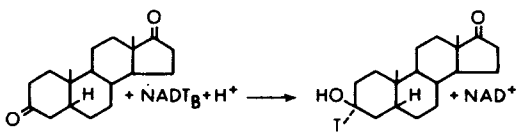
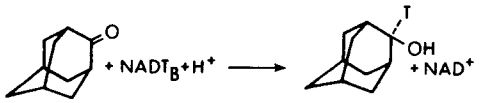
Enzyme	E.C.	Source	Reaction (as the experiment was performed)	Coenzyme	Stereo-specificity	Ref.
			 4-Androsten-3,17-dione → 4-Androsten-17β-ol-3-one (Testosterone)	NAD	B	29
		Rooster comb (cytosol)	 5β-Androstan-17β-ol-3-one (5β-Dihydrotestosterone) → 5β-Androstan-3β,17β-diol	NADP	B	75
20β-Hydroxysteroid dehydrogenase (Cortisone reductase)	1.1.1.53	*Streptomyces hydrogenans*	 4-Pregnen-20β-ol-3-one → 4-Pregnen-3,20-dione	NAD	B	207
			5β-Androstan-3,17-dione (5β-Androstanedione) + $NADT_B$ + H^+ → 5β-Androstan-3α-ol-17-one(5β-Androsterone) + NAD^+	NAD	B	208

(See rat liver 3α-hydroxysteroid dehydrogenase 1.1.1.50)

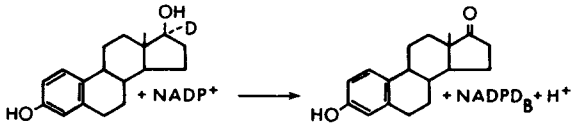
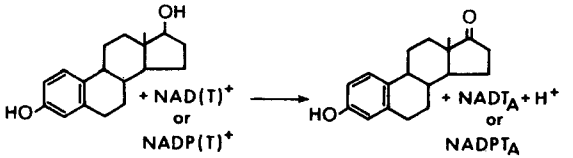
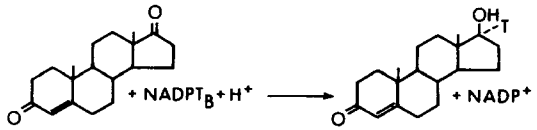
5α-Androstan-3,17-dione (5α-Androstanedione) + $NADT_B$ + H^+ → 5α-Androstan-3α-ol-17-one(5α-Androsterone) + NAD^+	NAD	B	208
(See rat liver 3α-hydroxysteroid dehydrogenase 1.1.1.50)			
 5α-Androstan-3,16-dione 5α-Androstan-3α-ol-16-one	NAD	B	208
5β-Androstan-17β-ol-3-one + $NADT_B$ + H^+ → 5β-Androstan-3α,17β-diol + NAD^+	NAD	B	208
(See rooster comb 3α-hydroxysteroid dehydrogenase 1.1.1.50)			
 5α,10β-Estran-3,17-dione 5α,10β-Estran-3α(orβ)-ol-17-one	NAD	B	208
 5α,10β-Estran-17β-ol-3-one 5α,10β-Estran-3α(orβ), 17β-diol	NAD	B	208

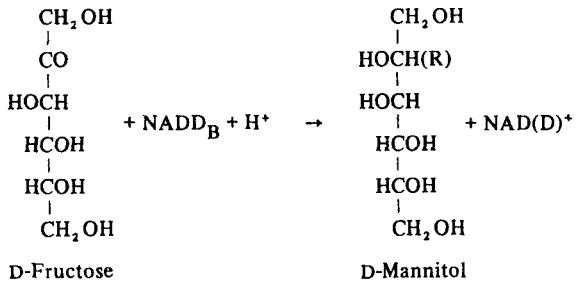
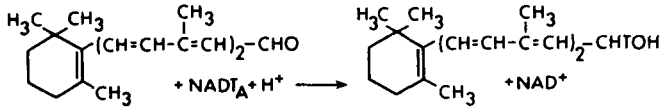
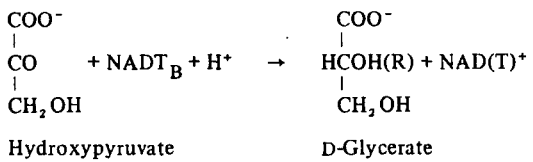
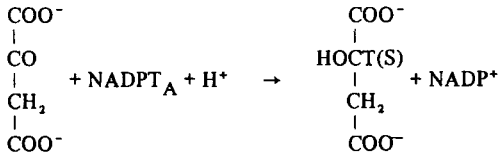
Appendix (continued)
SURVEY OF THE STEREOSPECIFICITY OF NICOTINAMIDE ADENINE DINUCLEOTIDE-LINKED ENZYMES

Enzyme	E.C.	Source	Reaction (as the experiment was performed)	Coenzyme	Stereo-specificity	Ref.
			 5β,9β,10β-Estran-17β-ol-3-one → 5β,9β,10β-Estran-3α(orβ), 17β-diol	NAD	B	208
			 5α,10α-Estran-3,17-dione → 5α,10α-Estran-3α(orβ)-ol-17-one	NAD	B	208
			 5β,9β,10α-Androstan-3-17-dione → 5β,9β,10α-Androstan-3α(orβ)-ol-17-one	NAD	B	208

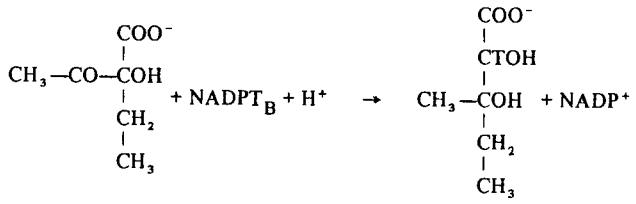
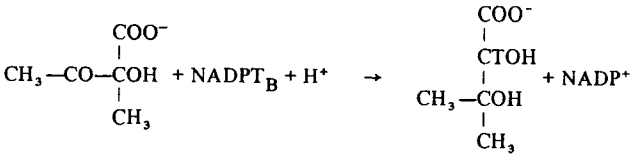
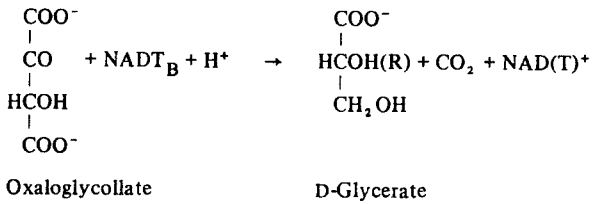
			 5β-Androstan-3,17-dione (5β-Androstanedione) → 5β-Androstan-3β-ol-17-one(5β-Androsterone)	NAD	B	208
			 2-Adamantanone → Adamantan-2-ol	NAD	B	208
Lactaldehyde reductase [The same enzyme as alcohol dehydrogenase (NADP) (1.1.1.2)]	1.1.1.55	Pig kidney	CH_3-HCOH-CHO + $NADPT_A$ + H^+ → CH_3-HCOH-CHTOH + $NADP^+$ L-Lactaldehyde → 1,2-Propanediol (Result confirmed with $NADPT_B$)	NADP	A	163
Ribitol dehydrogenase	1.1.1.56	*Aerobacter aerogenes*	D-Ribulose + $NADD_A$ + H^+ → Ribitol + $NAD(D)^+$ (See sheep liver L-iditol dehydrogenase 1.1.1.14)	NAD	B	16
		Klebsiella pneumoniae	Ribitol + $NAD(T)^+$ → D-Ribulose + $NADT_A$ + H^+ (See sheep liver L-iditol dehydrogenase 1.1.1.14)	NAD	B	86
Tartronate-semialdehyde dehydrogenase	1.1.1.60	*Pseudomonas putida*	COO^--HCOH-CHO + $NADT_B$ + H^+ → COO^--HCOH-CH_2OH + $NAD(T)^+$ Tartronate-semialdehyde → D-Glycerate	NAD	A	182

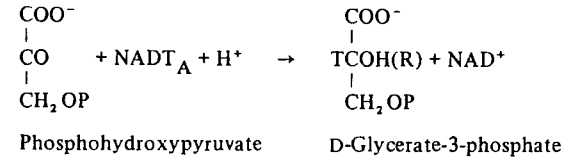
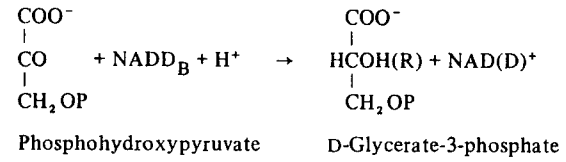
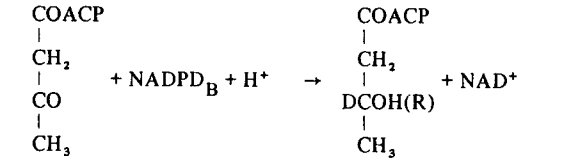
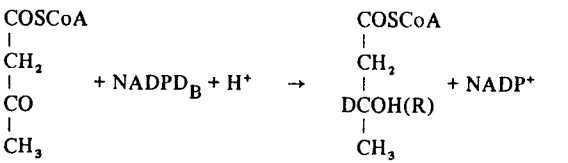
Appendix (continued)
SURVEY OF THE STEREOSPECIFICITY OF NICOTINAMIDE ADENINE DINUCLEOTIDE-LINKED ENZYMES

Enzyme	E.C.	Source	Reaction (as the experiment was performed)	Coenzyme	Stereo-specificity	Ref.
Estradiol 17β-dehydrogenase	1.1.1.62	Chicken liver	 1,3,5(10)-Estratrien-3,17β-diol(Estradiol-17β) → 1,3,5(10)-Estratrien-3-ol-17-one(Estrone)	NADP	B	44
		Human placenta	 1,3,5(10)-Estratrien-3,17β diol(Estradiol-17β) → 1,3,5(10)-Estratrien-3-ol-17-one(Estrone)	NAD NADP	B B	21 21
			$NADT_A + (3AcPy)AD^+ \rightarrow NAD(T)^+ + (3AcPy)ADH$	NAD	B	21
Testosterone 17β-dehydrogenase	1.1.1.64	Porcine testicular microsome	 4-Androsten-3,17-dione → 4-Androsten-17β-ol-3-one	NADP	B	209

Mannitol dehydrogenase	1.1.1.67	*Leuconostoc mesenteroides*	 D-Fructose → D-Mannitol	NAD	A	16
Alcohol dehydrogenase (NAD(P)$^+$) (Retinal reductase)	1.1.1.71	Rat intestinal mucosa	 Retinal → Retinol	NAD	A	210
Glycerol dehydrogenase (NADP$^+$)	1.1.1.72	Rabbit muscle	D-Glyceraldehyde + $NADPT_A$ + H^+ → Glycerol + $NADP^+$ (See yeast alcohol dehydrogenase (NADP) 1.1.1.2)	NADP	A	211
Glyoxylate reductase (NADP$^+$)	1.1.1.79	Pea	Glyoxylate + $NADPT_A$ + H^+ → Glycolate + $NADP^+$ (See spinach glyoxylate reductase 1.1.1.26)	NADP	A	82
Hydroxypyruvate reductase	1.1.1.81	*Pseudomonas putida*	 Hydroxypyruvate → D-Glycerate	NAD	A	182
Malate dehydrogenase (NADP$^+$)	1.1.1.82	Maize leaf	 Oxaloacetate → L-Malate	NADP	A	79

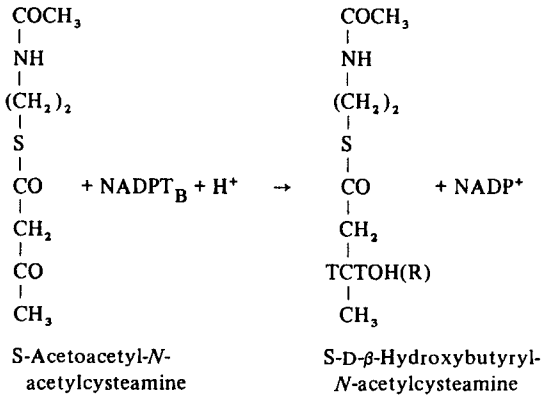
Appendix (continued)
SURVEY OF THE STEREOSPECIFICITY OF NICOTINAMIDE ADENINE DINUCLEOTIDE-LINKED ENZYMES

Enzyme	E.C.	Source	Reaction (as the experiment was performed)	Coenzyme	Stereo-specificity	Ref.
Ketol-acid reducto-isomerase (α-Aceto-hydroxy acid isomeroreductase)	1.1.1.86	*Salmonella typhimurium*	 α-Aceto-α-hydroxybutyrate → α,β-Dihydroxy-β-methylvalerate	NADP	B	212
			 α-Acetolactate → α,β-Dihydroxyiso-valerate	NADP	B	212
Aryl-alcohol dehydro-genase (Aromatic aldehydeketone reductase)	1.1.1.91	Rabbit kidney cortex	*p*-Chlorobenzaldehyde + $NADPT_B$ + H^+ → *p*-Chlorobenzyl alcohol + $NADP^+$ (See horse liver alcohol dehydrogenase 1.1.1.1)	NADP	B	177
Oxaloglycollate reduc-tase (decarboxylating)	1.1.1.92	*Pseudomonas putida*	 Oxaloglycollate → D-Glycerate	NAD	A	182

Phosphoglycerate dehydrogenase	1.1.1.95	*Escherichia coli*	 Phosphohydroxypyruvate D-Glycerate-3-phosphate	NAD	A	213
		Pea	Same as above	NAD	A	214
		Chicken liver	 Phosphohydroxypyruvate D-Glycerate-3-phosphate	NAD	A	16
3-Oxoacyl-(acyl-carrier-protein) reductase	1.1.1.100	*Escherichia coli*	 Acetoacetyl-acyl-carrier-protein D-β-Hydroxybutyryl-acyl-carrier-protein	NADP	B	77
			 Acetoacetyl-CoA D-β-Hydroxybutyryl-CoA	NADP	B	77

Appendix (continued)
SURVEY OF THE STEREOSPECIFICITY OF NICOTINAMIDE ADENINE DINUCLEOTIDE-LINKED ENZYMES

Enzyme	E.C.	Source	Reaction (as the experiment was performed)	Coenzyme	Stereo-specificity	Ref.
		Brevibacterium ammoniagenes	$CH_3\text{-}COSCoA + 8\ CH_2(COO^-)\text{-}COSCoA + 8\ NADPD_B + 8\ NADH + 16H^+ \rightarrow$ (Acetyl-CoA; Malonyl-CoA) $CH_3\text{-}(CDH\text{-}CH_2)_8COO^- + 8\ CO_2 + 9\ CoASH + 7\ H_2O + 8\ NADP^+ + 8\ NAD^+$ (Stearate)	NADP	B	42
		Yeast	$CH_3\text{-}COSCoA + 8\ CH_2(COO^-)\text{-}COSCoA + 16\ NADPD_B + 16\ H^+ \rightarrow$ (Acetyl-CoA; Malonyl-CoA) $CH_3\text{-}(CD_2\text{-}CH_2)_8COO^- + 8\ CO_2 + 9\ CoASH + 7\ H_2O + 16\ NADP^+$ (Stearate)	NADP	B	42
		Pigeon liver	$CH_3\text{-}CO\text{-}CH_2\text{-}COSCoA + NADPT_B + H^+ \rightarrow CH_3\text{-}CH(OH)(R)\text{-}CH_2\text{-}COSCoA + NADP^+$ (Acetoacetyl-CoA; D-β-Hydroxybutyryl-CoA)	NADP	B	74



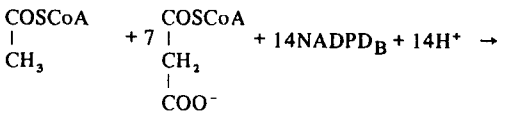
S-Acetoacetyl-*N*-acetylcysteamine | S-D-β-Hydroxybutyryl-*N*-acetylcysteamine | NADP | B | 74

Rat liver — Same as above (Results confirmed with $NADPD_B$ and $NADPD_A$) | NADP | B | 74
NADP | B | 215

$$\text{Acetoacetyl-CoA} + NADPT_B + H^+ \rightarrow \beta\text{-Hydroxybutyryl-CoA} + NADP^+$$

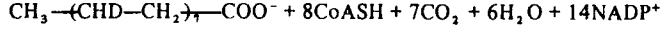
NADP | B | 74

(See pigeon liver 3-oxoacyl-(acyl-carrier-protein) reductase 1.1.1.100)



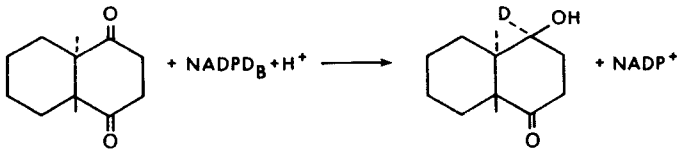
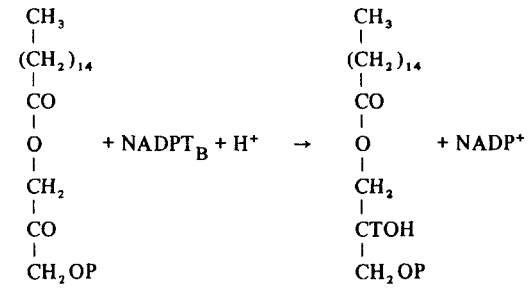
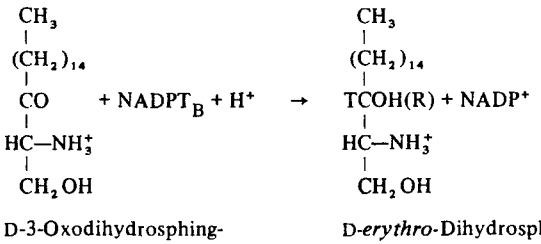
NADP | B | 215

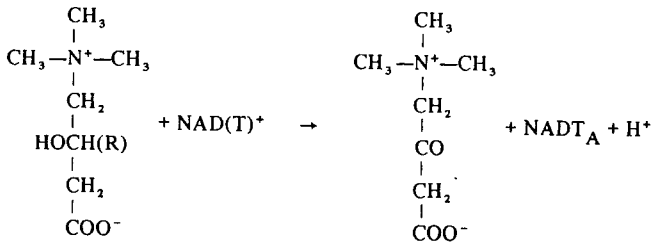
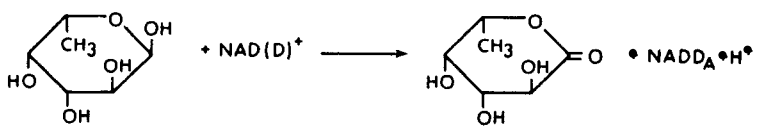
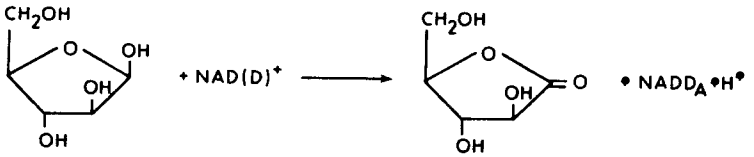
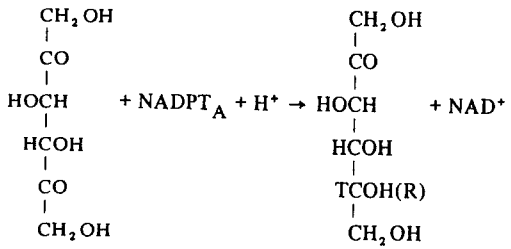
Acetyl-CoA | Malonyl-CoA



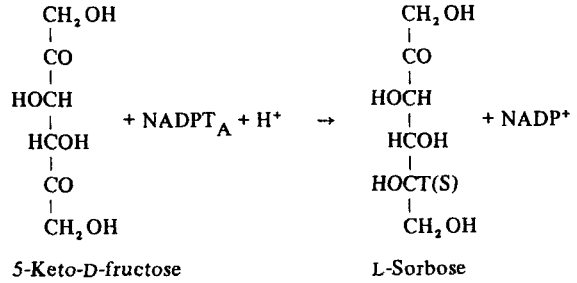
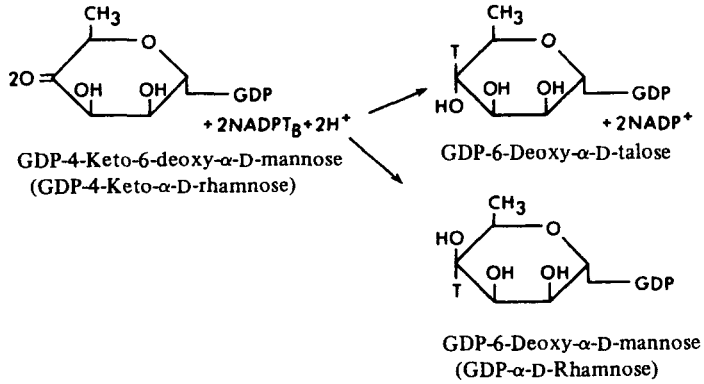
Palmitic acid

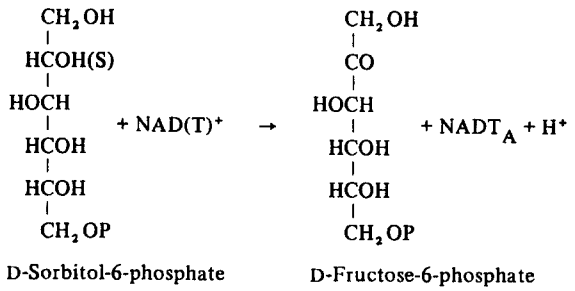
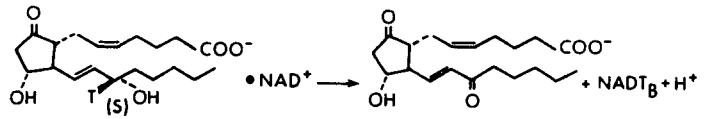
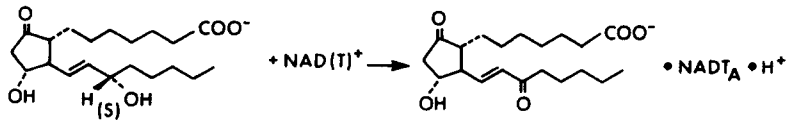
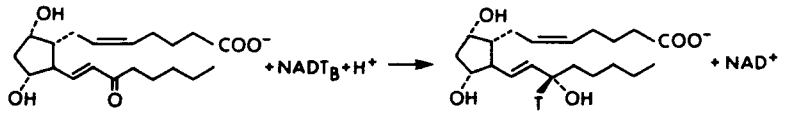
Appendix (continued)
SURVEY OF THE STEREOSPECIFICITY OF NICOTINAMIDE ADENINE DINUCLEOTIDE-LINKED ENZYMES

Enzyme	E.C.	Source	Reaction (as the experiment was performed)	Coenzyme	Stereo-specificity	Ref.
		Pig liver	 (9R)-*trans*-1,4-Decalindione → (4S,9R)-*trans*-4-Hydroxy-1-decalone	NADP	B	216
Palmitoyldihydroxy-acetone-phosphate reductase (Acyl/aryl dihydroxyacetone phosphate NADPH-oxidoreductase)	1.1.1.101	Ascites cell microsome	 Palmitoyldihydroxy-acetone-phosphate → Palmitoylglycerol-3-phosphate	NADP	B	168
3-Dehydrosphinganine reductase (D-3-Oxosphinganine reductase)	1.1.1.102	Beef liver microsome	 D-3-Oxodihydrosphing- → D-*erythro*-Dihydrosphingosine	NADP	B	217

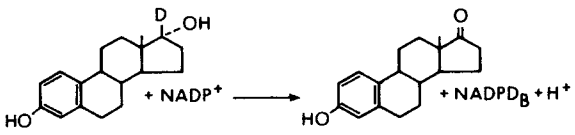
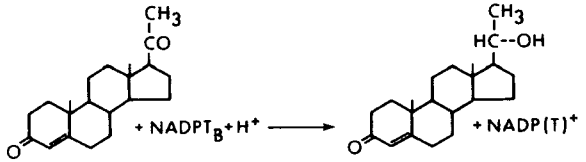
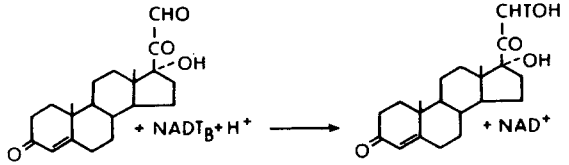
Carnitine dehydrogenase	1.1.1.108	*Pseudomonas aeruginosa*	 L-Carnitine → 3-Dehydrocarnitine	NAD	B	218
L-Fucose dehydrogenase	1.1.1.122	Sheep liver	 α-L-Fucose → L-Fucono-δ-lactone	NAD	B	16
			 β-D-Arabinose → D-Arabino-δ-lactone	NAD	B	16
Sorbose dehydrogenase (NADP⁺) (NADPH-Dicarbonyl reductase)	1.1.1.123	*Gluconobacter cerinus*	 5-Keto-D-fructose → D-Fructose	NADP	A	219

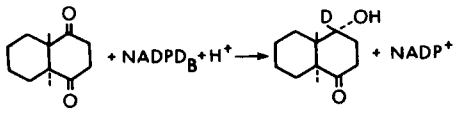
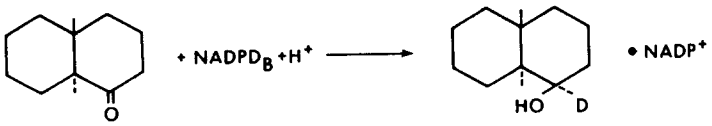
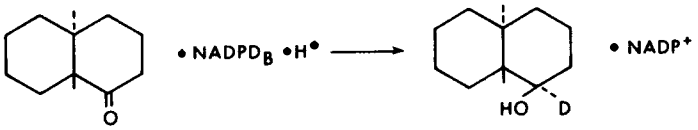
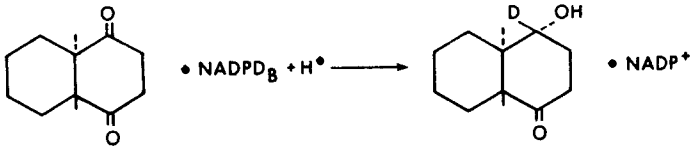
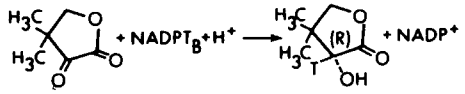
Appendix (continued)
SURVEY OF THE STEREOSPECIFICITY OF NICOTINAMIDE ADENINE DINUCLEOTIDE-LINKED ENZYMES

Enzyme	E.C.	Source	Reaction (as the experiment was performed)	Coenzyme	Stereo-specificity	Ref.
		Yeast	 5-Keto-D-fructose → L-Sorbose	NADP	A	183
GDP-6-Deoxy-D-talose dehydrogenase	1.1.1.135	Soil bacterium	 GDP-4-Keto-6-deoxy-α-D-mannose (GDP-4-Keto-α-D-rhamnose) → GDP-6-Deoxy-α-D-talose; GDP-6-Deoxy-α-D-mannose (GDP-α-D-Rhamnose)	NADP	B	220

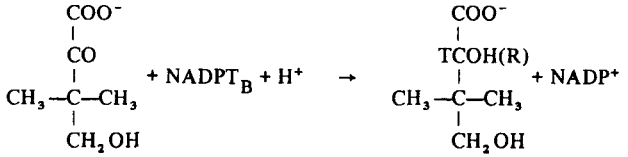
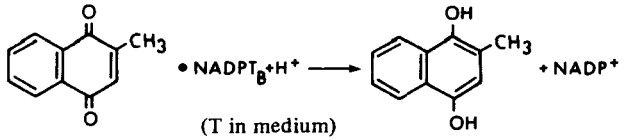
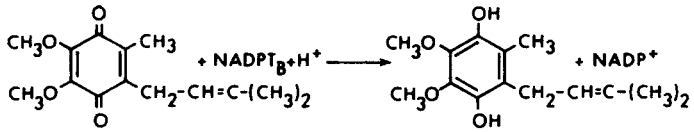
D-Sorbitol-6-phosphate dehydrogenase	1.1.1.140	*Aerobacter aerogenes* (Reclassified as *Klebsiella pneumoniae*[156])	 D-Sorbitol-6-phosphate → D-Fructose-6-phosphate	NAD	B	182
15-Hydroxyprostaglandin dehydrogenase	1.1.1.141	Human placenta	 PGE_2 → 15-Keto-PGE_2	NAD	B	221
		Pig kidney	 PGE_1 → 15-Keto-PGE_1	NAD	B	222
			 15-Keto-$PGF_{2\alpha}$ → $PGF_{2\alpha}$	NAD	B	222
			$[15\text{-T}]PGE_2 + NAD^+ \rightarrow$ 15-Keto-$PGE_2 + NADT_B + H^+$ (See above human placenta enzyme)	NAD	B	222

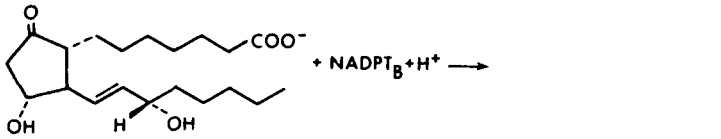
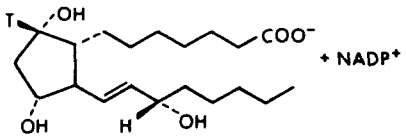
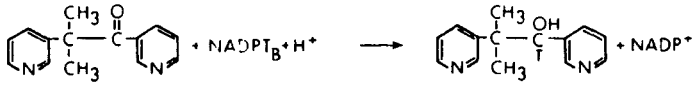
Appendix (continued)
SURVEY OF THE STEREOSPECIFICITY OF NICOTINAMIDE ADENINE DINUCLEOTIDE-LINKED ENZYMES

Enzyme	E.C.	Source	Reaction (as the experiment was performed)	Coenzyme	Stereo-specificity	Ref.
Estradiol 17α-dehydrogenase	1.1.1.148	Chicken liver	 1,3,5(10)-Estratrien-3,17α-diol (Estradiol-17α) → 1,3,5(10)-Estratrien-3-ol-17-one (Estrone)	NADP	B	208
20α-Hydroxyseroid dehydrogenase	1.1.1.149	Rat ovary	 4-Pregnen-3,20-dione (Progesterone) → 4-Pregnen-20α-ol-3-one	NADP	A	223
21-Hydroxysteroid dehydrogenase	1.1.1.150	Bovine adrenal gland	 4-Pregnen-17α-ol-3,20-dione-21-al → 4-Pregnen-17α,21-diol 3,20-dione	NAD	B	224

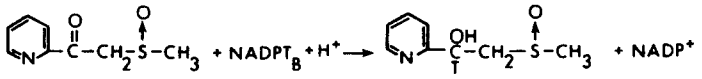
Glycerol 2-dehydrogenase ($NADP^+$) (Dihydroxyacetone reductase)	1.1.1.156	*Mucor javanicus*	 (9S)-*trans*-1,4-Decalindione (4R,9S)-*trans*-4-Hydroxyl-1-decalone	NADP	B	89
			 (9S)-*trans*-1-Decalone (1R,9S)-*trans*-1-Decanol	NADP	B	89
			 (9R)-*trans*-1-Decalone (1R,9R)-*trans*-1-Decanol	NADP	B	89
			 (9R)-*trans*-1,4-Decalindione (4R,9R)-*trans*-4-Hydroxy-1-decalone	NADP	B	89
2-Oxopantoyl lactone reductase	1.1.1.168	*Escherichia coli*	 2-Ketopantoyl lactone Pantoyl lactone	NADP	B	73

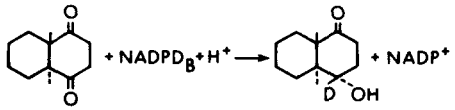
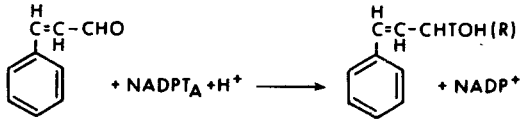
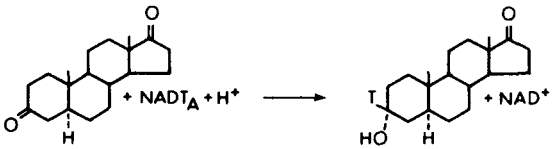
Appendix (continued)
SURVEY OF THE STEREOSPECIFICITY OF NICOTINAMIDE ADENINE DINUCLEOTIDE-LINKED ENZYMES

Enzyme	E.C.	Source	Reaction (as the experiment was performed)	Coenzyme	Stereo-specificity	Ref.
2-Oxopantoate reductase	1.1.1.169	*Escherichia coli*	 Ketopantoate → Pantoate	NADP	B	73
		Yeast	Same as above	NADP	B	73
Carbonyl reductase (Aldehyde reductase 1)	1.1.1.184	Human brain	 Menadione → Menadiol (T in medium)	NADP	B	63
			 Ubiquinone-1 → Ubiquinol-1 (T in medium)	NADP	B	63

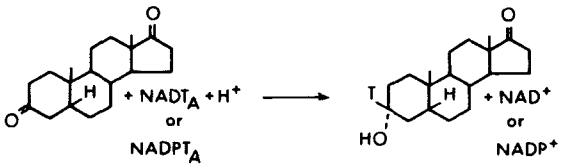
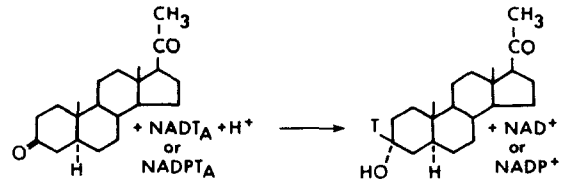
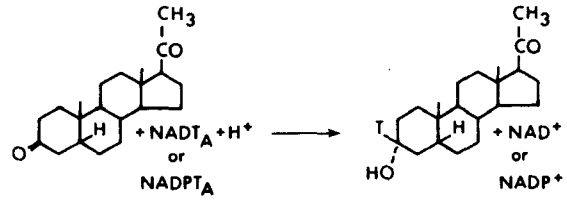
		 PGE$_1$  PGF$_{1\alpha}$	NADP	B	63
		p-Nitrobenzaldehyde + NADPT$_B$ + H$^+$ → *p*-Nitrobenzyl alcohol + NADP$^+$ See alcohol dehydrogenase (NADP) 1.1.1.2) (All the above results with carbonyl reductase have been confirmed with NADPT$_A$)	NADP	B	63
(Low pI *p*-nitrobenzaldehyde reductase)	Rabbit liver	*p*-Nitrobenzaldehyde + NADPT$_B$ + H$^+$ → *p*-Nitrobenzyl alcohol + NADP$^+$ (See rat liver alcohol dehydrogenase (NADP) 1.1.1.2)	NADP	B	67
(Low pI *p*-nitroacetophenone reductase)		*p*-Nitroacetophenone + NADPT$_B$ + H$^+$ → 2-Hydroxyethyl-*p*-nitrobenzene + NADP$^+$ [See alcohol dehydrogenase (NADP) 1.1.1.2. (High pI *p*-nitroacetophenone reductase)]	NADP	B	67
(Metyrapone reductase)		 2-Methyl-1,2-di-3-pyridyl-1-propanone (Metyrapone) 2-Methyl-1,2-di-3-pyridyl-1-propanol	NADP	B	67
(Low pI daunorubicin reductase)		Daunorubicin + NADPT$_B$ + H$^+$ → Daunorubicinol + NADP$^+$ (See rabbit liver alcohol dehydrogenase (NADP) 1.1.1.2)	NADP	B	67

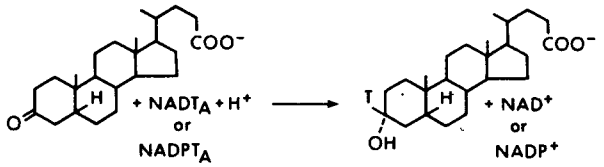
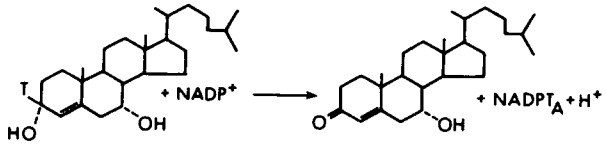
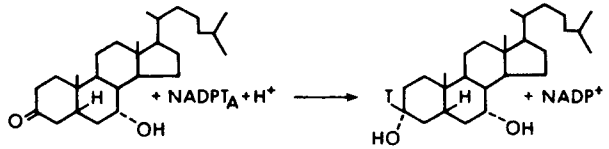
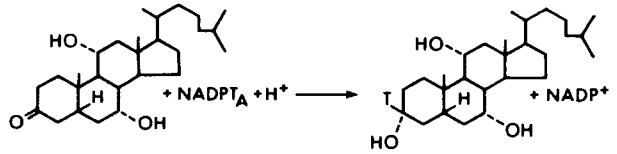
Appendix (continued)
SURVEY OF THE STEREOSPECIFICITY OF NICOTINAMIDE ADENINE DINUCLEOTIDE-LINKED ENZYMES

Enzyme	E.C.	Source	Reaction (as the experiment was performed)	Coenzyme	Stereo-specificity	Ref.
(Low pI 3,7-dimethyl-1-(5-oxohexyl)-xanthine reductase)			3,7-Dimethyl-1-(5-oxohexyl)-xanthine + $NADPT_B$ + H^+ → 3,7-Dimethyl-1-(5-hydroxyhexyl)-xanthine + $NADP^+$ [See alcohol dehydrogenase (NADP) 1.1.1.2. (High pI 3,7-dimethyl-1-(5-oxohexyl)-xanthine reductase)]	NADP	B	67
(Oxisuran reductase)			 Oxisuran → Oxisuranol	NADP	B	67
1,2-Propanediol dehydrogenase	1.1.1.?	*Gonococcus*	$CH_3CH(OH)CH_2OH$ + $NAD(D)^+$ → CH_3COCH_2OH + $NADD_B$ + H^+ (1,2-Propanediol → Hydroxyacetone)	NAD	A	16
17-Ketostearate reducing enzyme (to D-17-hydroxystearate)	1.1.1.?	Rat liver microsome	$CH_3CO(CH_2)_{15}COO^-$ + $NADT_B$ + H^+ → $CH_3CH(R)OH(CH_2)_{15}COO^-$ + NAD^+ (17-Ketostearate → D-17-Hydroxystearate)	NAD	B	225
17-Ketostearate reducing enzyme (to L-17-hydroxystearate)	1.1.1.?	Rat liver microsome	$CH_3CO(CH_2)_{15}COO^-$ + $NADPT_A$ + H^+ → $CH_3CH(S)OH(CH_2)_{15}COO^-$ + $NADP^+$ (17-Ketostearate → L-17-Hydroxystearate)	NADP	A	225

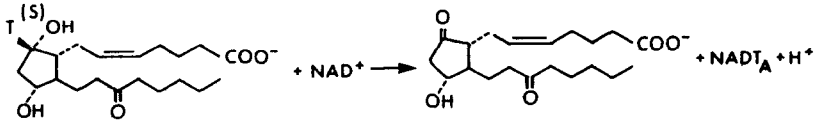
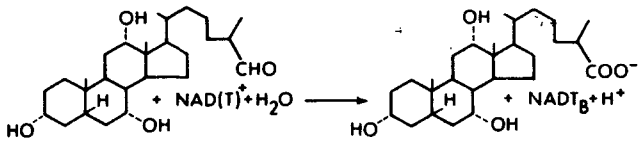
		Guinea pig liver microsome	Same as above	NADP	A	225
Carbonyl reductase (Ketone reductase)	1.1.1.?	*Curvularia falcata*	 (9S)-*trans*-1,4-Decalindione → (1S,9S)-*trans*-1-Hydroxy-4-decalone	NADP	B	89
Multifunctional oxidoreductase	1.1.1.?	*Penicillium charlesii*	Acetaldehyde + NADD (or T)$_A$ + H^+ → Ethanol + NAD^+ (See *A. aerogenes* alcohol dehydrogenase 1.1.1.1.)	NAD	A	226
Cinnamoyl alcohol dehydrogenase	1.1.1.?	Soybean	 Coniferyl aldehyde → Coniferyl alcohol (Result confirmed with $NADPT_B$)	NADP	A	227
		Forsythia suspensa	Same as above	NADP	A	87
3α-Hydroxysteroid dehydrogenase	1.1.1.?	Rat liver (soluble fraction)	 5α-Androstan-3,17-dione (5α-Androstanedione) → 5α-Androstan-3α-ol-17-one (5α-Androsterone)	NAD	A	72

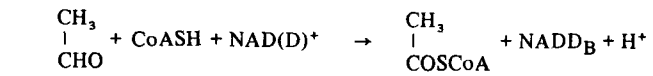
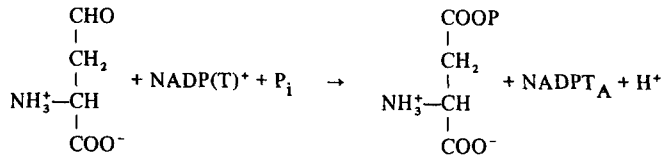
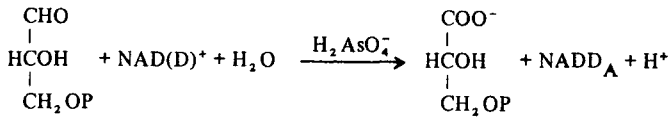
Appendix (continued)
SURVEY OF THE STEREOSPECIFICITY OF NICOTINAMIDE ADENINE DINUCLEOTIDE-LINKED ENZYMES

Enzyme	E.C.	Source	Reaction (as the experiment was performed)	Coenzyme	Stereo-specificity	Ref.
				NAD	A	72
				NADP	A	72
			5β-Androstan-3,17-dione (5β-Androstanedione) → 5β-Androstan-3α-ol-17-one (5β-Androsterone)			
				NAD	A	72
				NADP	A	72
			5α-Pregnan-3,20-dione → 5α-Pregnan-3α-ol-20-one			
				NAD	A	72
				NADP	A	72
			5β-Pregnan-3,20-dione → 5β-Pregnan-3α-ol-20-one			

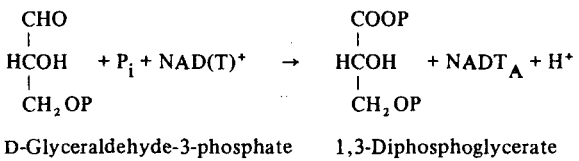
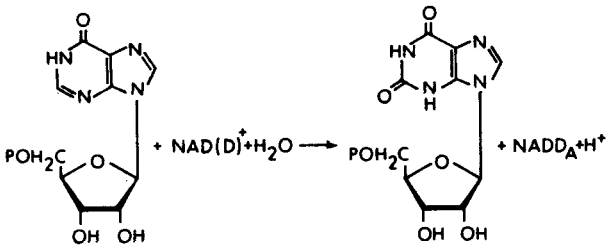
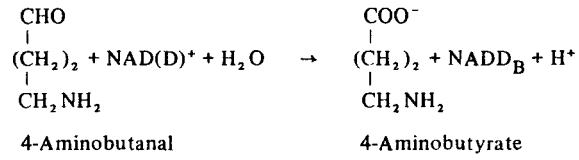
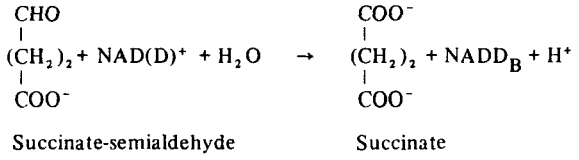
			 5β-Cholanate-3-one → 5β-Cholanate-3α-ol	NAD	A	72
				NADP	A	72
			 4-Cholesten-3α,7α-diol → 4-Cholesten-7α-ol-3-one	NADP	A	72
			 5β-Cholestan-7α-ol-3-one → 5β-Cholestan-3α,7α-diol	NADP	A	72
			 5β-Cholestan-7α,12α-diol-3-one → 5β-Cholestan-3α,7α,12α-triol	NADP	A	228
2-Oxopantoyl lactone reductase	1.1.1.?	Yeast	2-Ketopantoyl lactone + $NADPT_A$ + H^+ → Pantoyl lactone + $NADP^+$ (See *E. coli* 2-oxopantoyl lactone reductase 1.1.1.168)	NADP	A	73

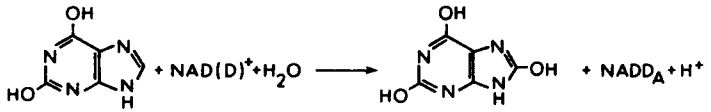
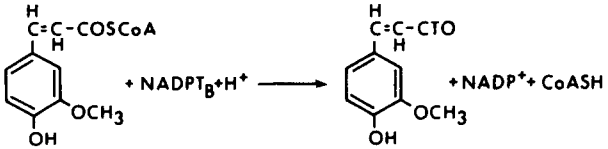
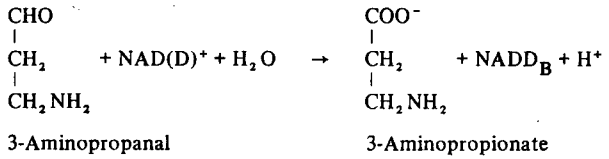
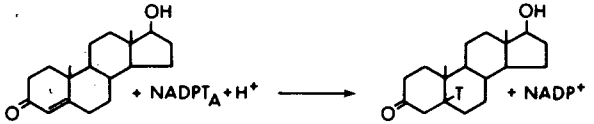
Appendix (continued)
SURVEY OF THE STEREOSPECIFICITY OF NICOTINAMIDE ADENINE DINUCLEOTIDE-LINKED ENZYMES

Enzyme	E.C.	Source	Reaction (as the experiment was performed)	Coenzyme	Stereo-specificity	Ref.
9-Hydroxyprostaglandin dehydrogenase	1.1.1.?.	Rat kidney	 15-Keto-13,14-dihydro-$PGF_{2\alpha}$ 15-Keto-13,14-dihydro-PGE_2	NAD	A	229
Formate dehydrogenase	1.2.1.2	Pea	$HCOO^- + NAD(T)^+ \rightarrow CO_2 + NADT_B$ Formate	NAD	A	82
		Achromobacter paruvulus	$HCOO^- + NAD(D)^+ \rightarrow CO_2 + NADD_B$	NAD	A	230
Aldehyde dehydrogenase	1.2.1.3.	Yeast	$CH_3CHO + NAD(D)^+ + H_2O \rightarrow CH_3COO^- + NADD_B + H^+$ Acetaldehyde Acetate	NAD	A	16
		Beef liver	Same as above	NAD	A	31
		Horse liver	Acetaldehyde + $NAD(T)^+ + H_2O \rightarrow$ Acetate + $NADT_B + H^+$ (See yeast aldehyde dehydrogenase 1.2.1.3)	NAD	A	178
			 5β-Cholestan-3α,7α,12α-triol-26-al 5β-Cholestan-3α,7α,12α-triol-16-oate	NAD	A	178

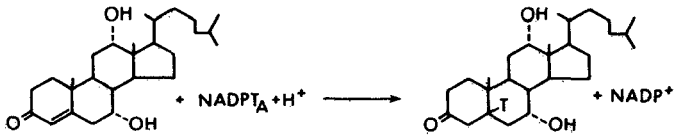
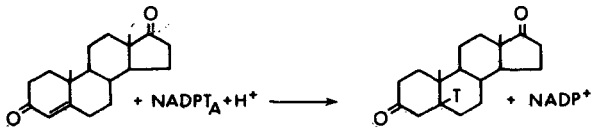
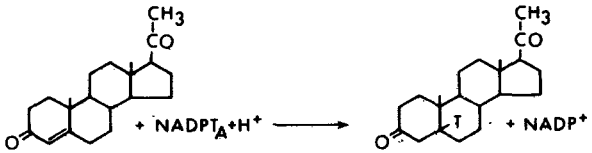
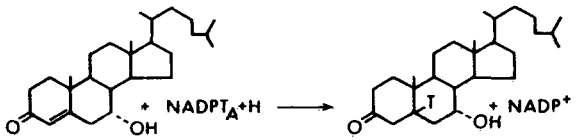
Acetaldehyde dehydrogenase (acylating)	1.2.1.10	*Clostridium kluyveri*	 Acetaldehyde → Acetyl-CoA	NAD	A	16
Aspartate-semialdehyde dehydrogenase	1.2.1.11	*Escherichia coli*	 L-Aspartate-β-semialdehyde → L-β-Aspartylphosphate	NADP	B	231
		Pea	Same as above	NADP	B	79
Glyceraldehyde-phosphate dehydrogenase	1.2.1.12	*Escherichia coli*	 D-Glyceraldehyde-3-phosphate → D-3-Phosphoglycerate	NAD	B	40
		Yeast	Same as above	NAD	B	97
		Horse shoe crab	Same as above	NAD	B	40
		Sturgeon	Same as above	NAD	B	40
		Bee	Same as above	NAD	B	40
		Turkey	Same as above	NAD	B	40
		Rabbit skeletal muscle	Same as above	NAD	B	97

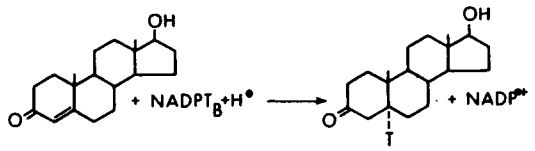
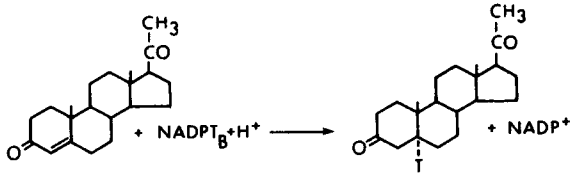
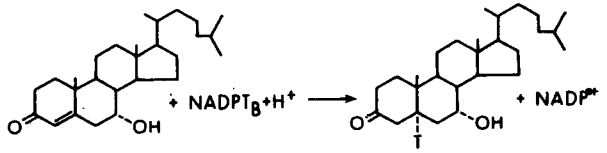
Appendix (continued)
SURVEY OF THE STEREOSPECIFICITY OF NICOTINAMIDE ADENINE DINUCLEOTIDE-LINKED ENZYMES

Enzyme	E.C.	Source	Reaction (as the experiment was performed)	Coenzyme	Stereo-specificity	Ref.
		Pea	 D-Glyceraldehyde-3-phosphate → 1,3-Diphosphoglycerate	NAD	B	79
IMP dehydrogenase	1.2.1.14	*Aerobacter aerogenes*	 Inosine-5-phosphate (IMP) → Xanthosine-5-phosphate (XMP)	NAD	B	16
Amniobutyraldehyde dehydrogenase	1.2.1.19	*Pseudomonas*	 4-Aminobutanal → 4-Aminobutyrate	NAD	A	40
Succinate-semialdehyde dehydrogenase	1.2.1.24	*Pseudomonas*	 Succinate-semialdehyde → Succinate	NAD	A	16

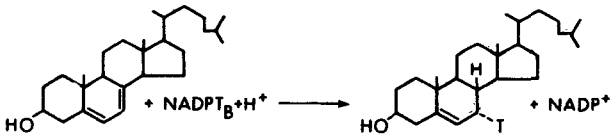
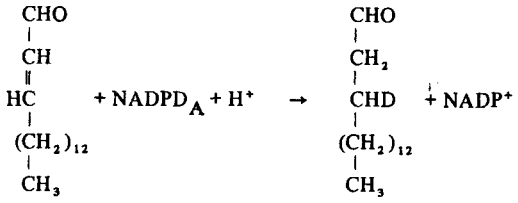
Xanthine dehydrogenase	1.2.1.37	Chicken liver	 Xanthine → Urate	NAD	B	16
Cinnamoyl-CoA reductase	1.2.1.44	*Forsythia suspensa*	 Feruloyl-CoA → Coniferyl aldehyde (Result confirmed with $NADPT_A$)	NADP	B	232
2-Aminopropylaldehyde dehydrogenase	1.2.1.?	Chicken liver	 3-Aminopropanal → 3-Aminopropionate	NAD	A	40
Xanthine oxidase	1.2.3.2	Cow milk	$NO_3^- + NADT_B + H^+ \rightarrow NO_2^- + HTO + NAD^+$	NAD	B	49
			$\frac{1}{2}O_2 + NADT_B + H^+ \rightarrow HTO + NAD^+$	NAD	B	49
Cortisone β-reductase (Δ^4-5β-Reductase)	1.3.1.3	Rat liver (soluble fraction)	 4-Androsten-17β-ol-3-one (Testosterone) → 5β-Androstan-17β-ol-3-one (5β-Dihydrotestosterone)	NADP	A	233

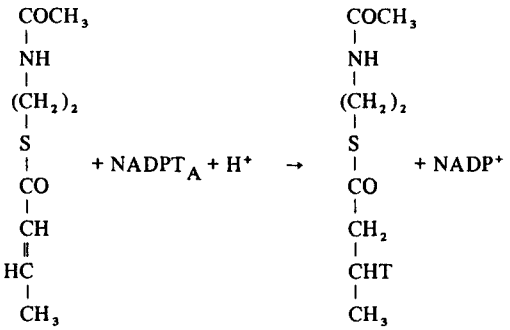
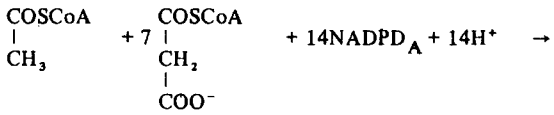
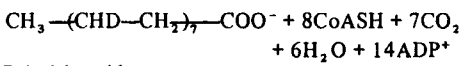
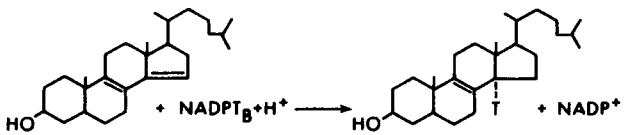
Appendix (continued)
SURVEY OF THE STEREOSPECIFICITY OF NICOTINAMIDE ADENINE DINUCLEOTIDE-LINKED ENZYMES

Enzyme	E.C.	Source	Reaction (as the experiment was performed)	Coenzyme	Stereo-specificity	Ref.
			 4-Cholesten-7α,12α-diol-3-one → 5β-Cholestan-7α,12α-diol-3-one	NADP	A	228
		Rat liver microsome	 4-Androsten-3,17-dione → 5β-Androstan-3,17-dione	NADP	A	72
			 4-Pregnen-3,20-dione (Progesterone) → 5β-Pregnan-3,20-dione	NADP	A	72
			 4-Cholesten-7α-ol-3-one → 5β-Cholestan-7α-ol-3-one	NADP	A	72

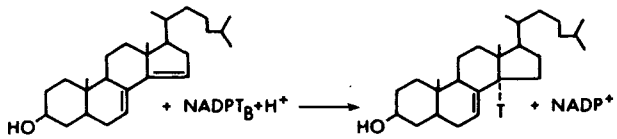
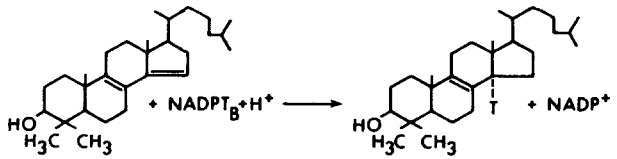
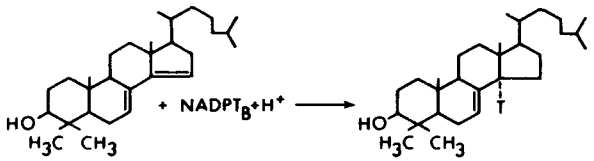
Cortisone α-reductase (Δ^4-5α-Reductase)	1.3.1.4	Rat liver microsome	 4-Androsten-17β-ol-3-one (Testosterone) → 5α-Androstan-17β-ol-3-one (5α-Dihydrotestosterone)	NADP	B	233
			 4-Pregnen-3,20-dione (Progesterone) → 5α-Pregnan-3,20-dione	NADP	B	234
			 4-Cholesten-7α-ol-3-one → 5α-Cholestan-7α-ol-3-one	NADP	B	235
		Rat prostate nuclear fraction	4-Androsten-17β-ol-3-one (Testosterone) + $NADPT_B$ + H^+ → 5α-Androstan-17β-ol-3-one (5α-Dihydrostestosterone) + $NADP^+$ (See rat liver microsome cortisone α-reductase 1.3.1.4)	NADP	B	236
		Rat prostate microsome	Same as above	NADP	B	236

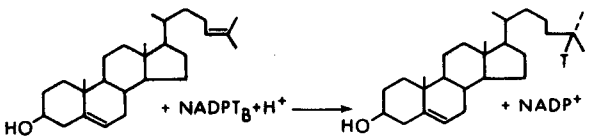
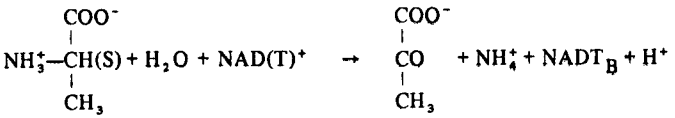
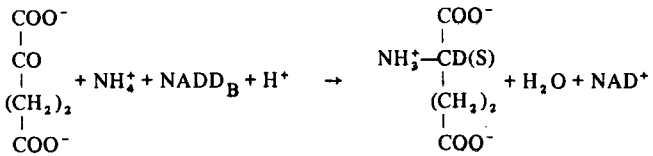
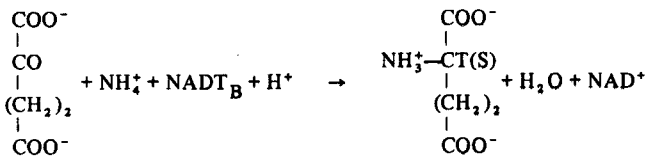
Appendix (continued)
SURVEY OF THE STEREOSPECIFICITY OF NICOTINAMIDE ADENINE DINUCLEOTIDE-LINKED ENZYMES

Enzyme	E.C.	Source	Reaction (as the experiment was performed)	Coenzyme	Stereo-specificity	Ref.
Orotate reductase (NADPH)	1.3.1.15	Aerobic bacterium	Orotate + $NADPT_A$ + H^+ → Dihydroorotate + $NADP^+$ (T in medium) (See *Z. oroticum* orotate reductase 1.3.1.14)	NADP	A	82
7-Dehydrocholesterol reductase (Δ^7(8)Sterol dehydrogenase)	1.3.1.21	Rat liver microsome	 5(6),7-Cholestdien-3β-ol → 8β-5(6)-Cholesten-3β-ol (Cholesterol)	NADP	B	239
2-Hexadecenal reductase (2-Alkenal reductase)	1.3.1.27	Rat liver	 2-*trans*-Hexadecenal → Palmityl aldehyde (Hexadecanal)	NADP	A	240

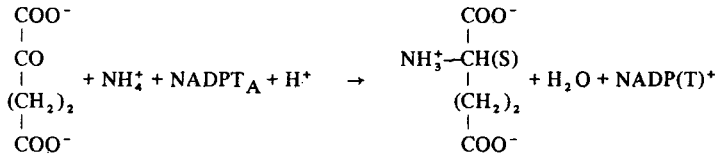
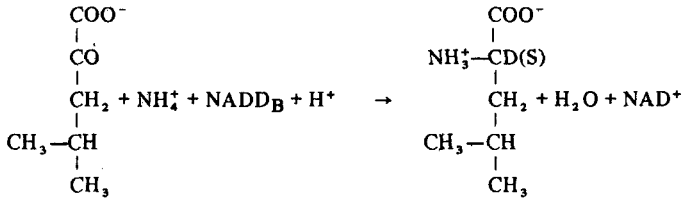
Enoyl-(acyl-carrier-protein) reductase (NADPH)	1.3.1.?	Pigeon liver	 S-Crotonyl-*N*-acetyl-cysteamine → S-Butyryl-*N*-acetyl-cysteamine	NADP	A	74
		Rat liver	Same as above (Result confirmed with $NADPD_B$)	NADP	A	74 215
			 Acetyl-CoA Malonyl-CoA	NADP	A	215
			 Palmitic acid (Result confirmed with $NADPD_B$)			
$\Delta^{14(15)}$ Sterol dehydrogenase	1.3.1.?	Rat liver microsome	 8(9),14(15)-Cholestdien-3β-ol 14α-8(9)-Cholesten-3β-ol	NADP	B	241

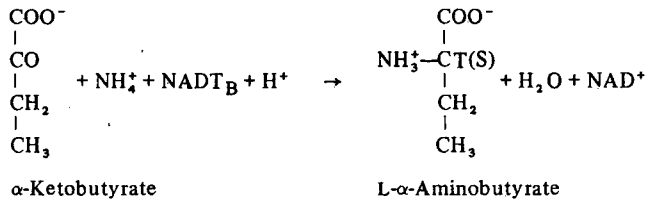
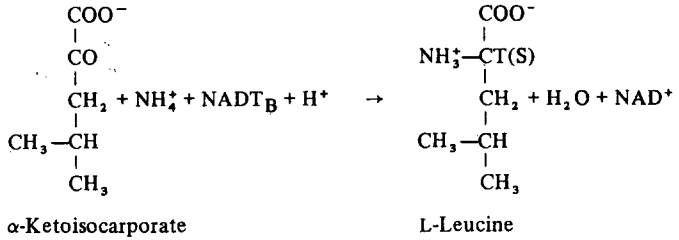
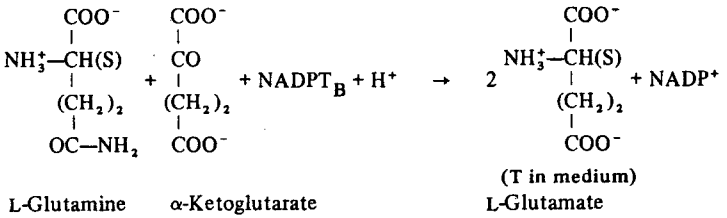
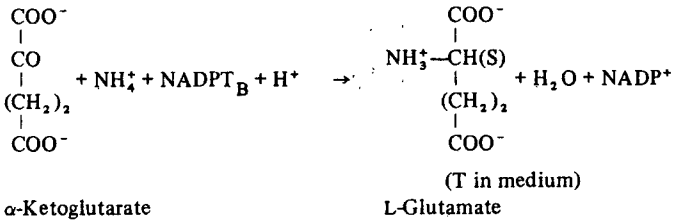
Appendix (continued)
SURVEY OF THE STEREOSPECIFICITY OF NICOTINAMIDE ADENINE DINUCLEOTIDE-LINKED ENZYMES

Enzyme	E.C.	Source	Reaction (as the experiment was performed)	Coenzyme	Stereo-specificity	Ref.
			 7,14(15)-Cholestdien-3β-ol → 14α-7-Cholesten-3β-ol	NADP	B	241
			 8(9),14(15)-Cholestdien-4,4′-dimethyl-3β-ol → 14α-8(9)-Choesten-4,4′-dimethyl-3β-ol	NADP	B	241
			 7,14(15)-Cholestdien-4,4′-dimethyl-3β-ol → 14α-7-Cholesten-4,4′-dimethyl-3β-ol	NADP	B	241

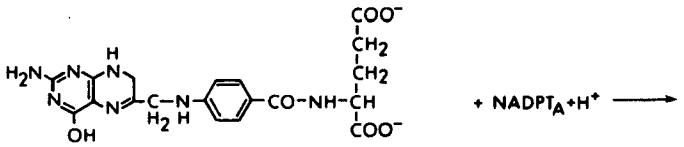
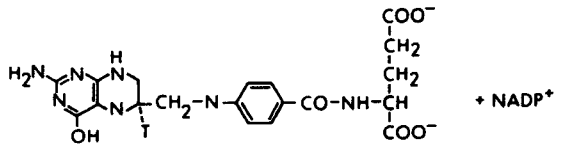
$\Delta^{24(25)}$ Sterol dehydrogenase	1.3.1.?	Rat liver microsome	 5(6),24-Cholestdien-3β-ol (Demosterol) → 5(6)-Cholesten-3β-ol	NADP	B	241
Alanine dehydrogenase	1.4.1.1	*Bacillus subtilus*	 L-Alanine → Pyruvate	NAD	A	242
Glutamate dehydrogenase	1.4.1.2	*Lemna minor*	 α-Ketoglutarate → L-Glutamate	NAD	B	45
		Pea mitochondria	 α-Ketoglutarate → L-Glutamate	NAD	B	79
Glutamate dehydrogenase (NAD(P)$^+$)	1.4.1.3	Beef liver	α-Ketoglutarate + NH_4^+ + $NADPD_B$ + H^+ → L-Glutamate + H_2O + $NADP^+$ (See *L. minor* glutamate dehydrogenase 1.4.1.2.)	NADP	B	30
			α-Ketoglutarate + NH_4^+ + $NADD_B$ + H^+ → L-Glutamate + H_2O + NAD^+ (See *L. minor* glutamate dehydrogenase 1.4.1.2.)	NAD	B	31

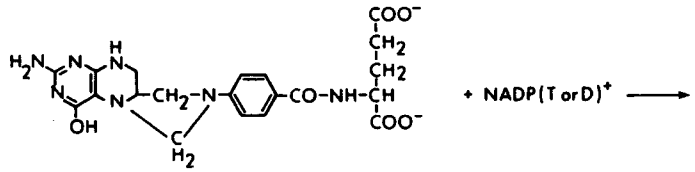
Appendix (continued)
SURVEY OF THE STEREOSPECIFICITY OF NICOTINAMIDE ADENINE DINUCLEOTIDE-LINKED ENZYMES

Enzyme	E.C.	Source	Reaction (as the experiment was performed)	Coenzyme	Stereo-specificity	Ref.
		Pig liver	α-Ketoglutarate + NH_4^+ + (3AcPy)ADT_A + H^+ → L-Glutamate + (3AcPy)AD(T)$^+$	(3AcPy)AD	B	58
			α-Ketoglutarate + NH_4^+ + (3CNPy)ADT_B + H^+ → L-Glutamate + (3CNPy)AD^+ (See pea mitochondria glutamate dehydrogenase 1.4.1.2.)	(3CNPy)AD	B	58
			α-Ketoglutarate + NH_4^+ + (TN)ADT_A + H^+ → L-Glutamate + (TN)AD(T)$^+$	(TN)AD	B	58
Glutamate dehydrogenase ($NADP^+$)	1.4.1.4	Yeast	 α-Ketoglutarate → L-Glutamate	NADP	B	79
		Aerobacter aerogenes	α-Ketoglutarate + NH_4^+ + $NADPT_B$ + H^+ → L-Glutamate + H_2O + $NADP^+$ (See pea mitochondria glutamate dehydrogenase 1.4.1.2)	NADP	B	243
Leucine dehydrogenase	1.4.1.9	*Bacillus subtilis*	 α-Ketoisocarporate → L-Leucine	NAD	B	16

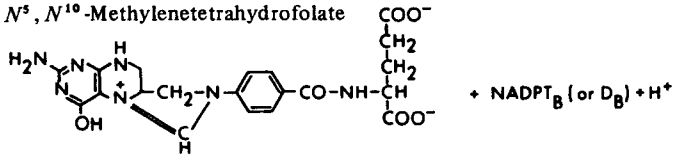
			 α-Ketobutyrate → L-α-Aminobutyrate	NAD	B	244
		Bacillus Sphaerius	 α-Ketoisocarporate → L-Leucine	NAD	B	244
Glutamate synthase (NADPH)	1.4.1.13	*Escherichia coli*	 L-Glutamine + α-Ketoglutarate → 2 L-Glutamate (T in medium)	NADP	B	243
			 α-Ketoglutarate → L-Glutamate (T in medium)	NADP	B	243

Appendix (continued)
SURVEY OF THE STEREOSPECIFICITY OF NICOTINAMIDE ADENINE DINUCLEOTIDE-LINKED ENZYMES

Enzyme	E.C.	Source	Reaction (as the experiment was performed)	Coenzyme	Stereospecificity	Ref.
		Aerobacter aerogenes	L-Glutamine + α-Ketoglutarate + $NADPT_B$ + H^+ → 2 L-Glutamate + $NADP^+$ (T in medium) (See *E. coli* glutamate synthase 1.4.1.13)	NADP	B	243
		Aerobacter aerogenes	α-Ketoglutarate + NH_4^+ + $NADPT_B$ + H^+ → L-Glutamate + H_2O + $NADP^+$ (T in medium) (See *E. coli* glutamate synthase 1.4.1.13)	NADP	B	243
			$NADPT_B + H_2O \rightarrow NADPH + HTO$	NADP	B	243
			$NADPT_B$ + (TN)$NADP^+$ → $NADP^+$ + (TN)NADPT	NADP	B	243
Glutamate synthase (NADH)	1.4.1.14	*Lupinus angustifolius*	α-Ketoglutarate + L-Glutamine + $NADT_B$ + H^+ → 2 L-Glutamate + NAD^+ (See *E. coli* glutamate synthase 1.4.1.3)	NAD	B	245
			$NADT_B + H_2O \rightarrow NADH + HTO$	NAD	B	245
Tetrahydrofolate dehydrogenase	1.5.1.3	*Streptococcus faecalis*	 Dihydrofolate  Tetrahydrofolate	NADP	A	246

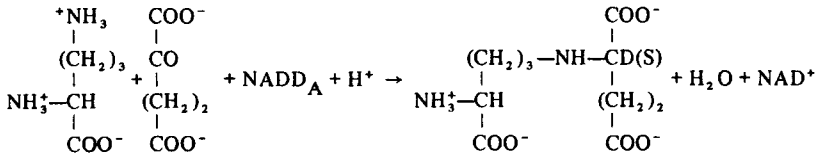
		leukemic cell				
		Chicken liver	Same as above	NADP	A	247
Methylenetetrahydrofolate dehydrogenase ($NADP^+$)	1.5.1.5	Yeast	 + NADP(T or D)$^+$ ⟶	NADP	A	248

N^5, N^{10}-Methylenetetrahydrofolate

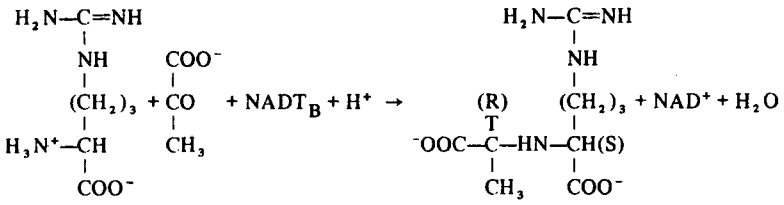


+ $NADPT_B$ (or D_B) + H^+

N^5, N^{10}-Methenyltetrahydrofolate

Saccharopine dehydrogenase	1.5.1.7	Yeast		NAD	A	249

L-Lysine α-Ketoglutarate Saccharopine

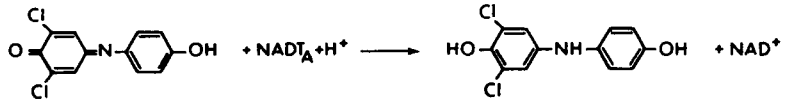
Octopine dehydrogenase	1.5.1.11	Scallop Crown gall tumor		NAD	B	250 251 251

L-Arginine Pyruvate D-Octopine

(Most T was recovered from the solvent as the enzyme catalyzed the exchange of hydrogen between NADH and solvent)

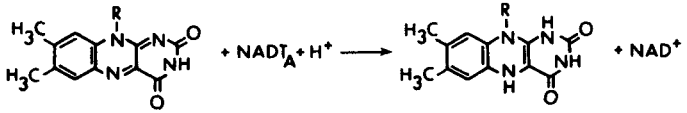
Appendix (continued)
SURVEY OF THE STEREOSPECIFICITY OF NICOTINAMIDE ADENINE DINUCLEOTIDE-LINKED ENZYMES

Enzyme	E.C.	Source	Reaction (as the experiment was performed)	Coenzyme	Stereo-specificity	Ref.
		Scallop	L-Arginine + Pyruvate + (3CNPy)ADT_A + H^+ → D-Octopine + (3CNPy)$AD(T)^+$ + H_2O	(3CNPy)AD	B	250
$NAD(P)^+$ Transhydrogenase	1.6.1.1	*Escherichia coli*	$NADPH + NAD(T)^+ \rightarrow NADP^+ + NADT_B$	NAD	A	252
		Rhodosprillum rubrum	$NADP^+ + NADT_A \rightarrow NADPT_B + NAD^+$	NAD	A	253
				NADP	B	253
			$NADT_A$ + (3AcPy)$AD^+ \rightarrow NAD^+$ + (3AcPy)ADT_A	(3AcPy)AD	A	254
		Bovine heart mitochondria	$NADPT_B + NAD^+ \rightarrow NADP^+ + NADT_A$	NAD	A	255,256, 257
				NADP	B	255,256, 257
$NAD(P)^+$ Transhydrogenase (Note: This enzyme shows different stereospecificity from above transhydrogenase)		*Pseudomonas aeruginosa*	$NADP^+ + NADT_B \rightarrow NADPT_B + NAD^+$	NAD	B	24
				NADP	B	24
		Pseudomonas fluorescens	$(NADPD_A + NADPD_B) + NAD^+ \rightarrow NADP^+ + NADD_B$	NAD	B	26
		Azotobacter vinelandii	$NADPT_A + NAD^+ \rightarrow NADP(T)^+ + NADH$	NADP	B	258
			$NADT_A + NADP^+ \rightarrow NAD(T)^+ + NADPH$	NAD	B	258
			$NADPT_A + NADP^+ \rightarrow NADP(T)^+ + NADPH$	NADP	B	258
			$NADT_A + NADH \rightarrow NAD(T)^+ + NADH$	NAD	B	258

Cytochrome b_5 reductase	1.6.2.2	Rat liver microsome	$2Fe^{+++} + NADD_A \rightarrow 2Fe^{++} + NAD^+ + D^+$	NAD	A	25
			$NADD_A + (3AcPy)AD^+ \rightarrow NAD^+ + (3AcPy)ADD_A$	NAD	A	25
				(3AcPy)AD	A	25
Glutathione reductase (NAD(P)H)	1.6.4.2	*Escherichia coli*	$GS\text{-}SG + NADPD_B + H^+ \rightarrow GSD + GSH + NADP^+$	NADP	B	258
		Yeast	$2GSD + NADP^+ \rightarrow GS\text{-}SG + NADPD_B + D^+$	NADP	B	41
			$NADPH + D_2O \rightleftarrows NADPD_B + HDO$	NADP	B	260
			$GS\text{-}SG + NADPD_B + H^+ \rightarrow GSD + GSH + NADP^+$	NADP	B	260
			$GS\text{-}SG + NADD_A + H^+ \rightarrow 2GSH + NAD(D)^+$	NAD	B	260
Thioredoxin reductase (NADPH)	1.6.4.5	*Escherichia coli*	$Insulin\text{—}(S)_2 + NADPT_B + H^+ \xrightarrow{\text{Thioredoxin}} Insulin\text{—}(SH)_2 + NADP^+$ (T in medium)	NADP	B	261
NADH dehydrogenase (solubilized)	1.6.5.3.	Beef heart mitochondria	$2Fe^{+++} + NADT_B \rightarrow 2Fe^{++} + NAD^+ + T^+$	NAD	B	262
		Escherichia coli	$Ubiquinone\text{-}1 + NADD_B + H^+ \rightarrow Ubiquinol\text{-}1 + NAD^+$ (See carbonyl reductase 1.1.1.84)	NAD	B	290
Nitrate reductase (NADH)	1.6.6.1	*Chlorella vulgaris*	$NO_3^- + NADT_A + H^+ \rightarrow NO_2^- + HTO + NAD^+$	NAD	A	49
				NAD	A	49

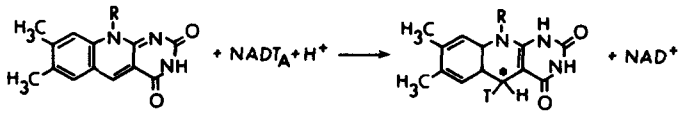
2,6-Dichlorophenolindophenol(ox) 2,6-Dichlorophenolindophenol(red) (T in medium)

Appendix (continued)
SURVEY OF THE STEREOSPECIFICITY OF NICOTINAMIDE ADENINE DINUCLEOTIDE-LINKED ENZYMES

Enzyme	E.C.	Source	Reaction (as the experiment was performed)	Coenzyme	Stereo-specificity	Ref.
			$2\text{Cytochrome } c(\text{ox}) + \text{NADT}_A \rightarrow 2\text{Cytochrome } c(\text{red}) + \text{NAD}^+ + \text{T}^+$	NAD	A	49
		Chlorella fusca	$NO_3^- + \text{NADT}_A + H^+ \rightarrow NO_2^- + \text{HTO} + \text{NAD}^+$	NAD	A	80
		Spinach	Same as above	NAD	A	80
Nitrate reductase (NAD(P)H)	1.6.6.2	*Neurospora crasa*	$NO_3^- + \text{NADPT}_A + H^+ \rightarrow NO_2^- + \text{HTO} + \text{NADP}^+$	NADP	A	80
		Rhodotorsula glutinus	Same as above	NADP	A	80
Nitrate reductase (NADPH)	1.6.6.3	*Candida utilis*	$NO_3^- + \text{NADPT}_A + H^+ \rightarrow NO_2^- + \text{HTO} + \text{NADP}^+$	NADP	A	81
			$NO_3^- + \text{NADT}_A + H^+ \rightarrow NO_2^- + \text{HTO} + \text{NAD}^+$ (Confirmed with NADPT_B and NADT_B)	NAD	A	81
		Spinach	$NO_3^- + \text{NADPT}_B + H^+ \rightarrow NO_2^- + H_2O + \text{NADP(T)}^+$	NADP	A	81
Nitrite reductase	1.6.6.4	*Candida utilis*	$NO_2^- + 3\text{NADPT}_A + 4H^+ \rightarrow NH_4OH + H_2O + 3\text{NADP(T)}^+$	NADP	B	79
			$NO_2^- + 3\text{NADPT}_B + 4H^+ \rightarrow NH_4OH + \text{HTO} + 3\text{NADP}^+$	NADP	B	81
Hydroxylamine reductase	1.6.6.11	Yeast	$NH_2OH + \text{NADPT}_A + H^+ \rightarrow NH_3 + H_2O + \text{NADP(T)}^+$	NADP	B	79
NAD(P)H dehydrogenase	1.6.8.1	*Vibrio harveyi* (Formerly *Beneckea harveyi*)		NAD	A	263

Riboflavin(ox) (T in medium) Riboflavin(red)

R= CH_2—$(CHOH)_3$—CH_2OH

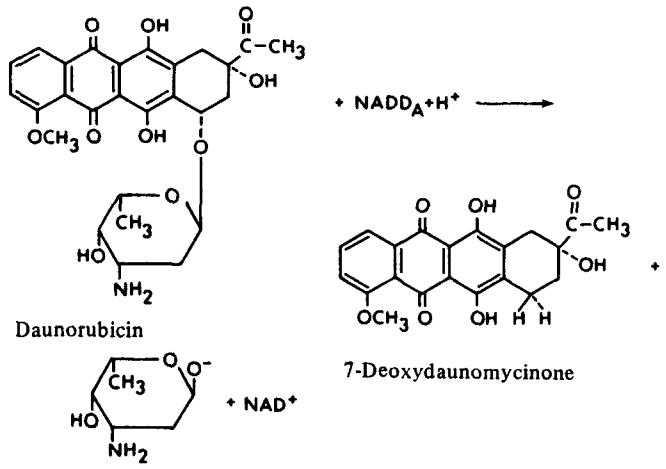


5-Deazariboflavin(ox) 5-Deazariboflavin(red)

(R = CH_2—$(CHOH)_3$—CH_2OH

NAD A 263

*The absolute configuration is unknown. The hydrogen, which is removed in this enzymatic reaction is designated the B-side hydrogen.[217] (See also 8-hydroxy-5-deazaflavin-dependent NADP$^+$ reductase below).



Daunorubicin

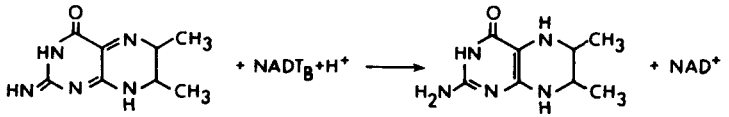
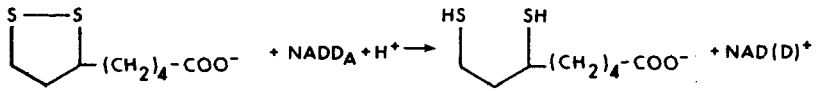
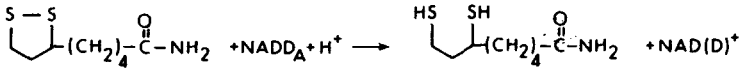
7-Deoxydaunomycinone

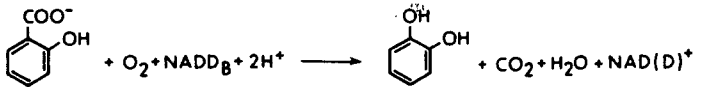
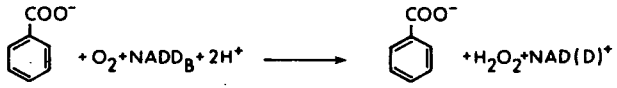
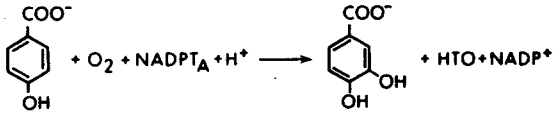
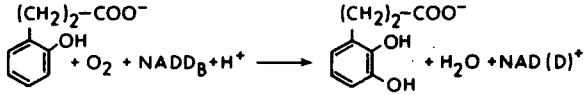
Daunosamine
(D in medium)

NAD A 264

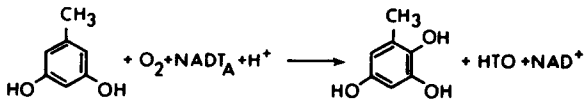
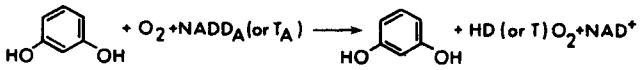
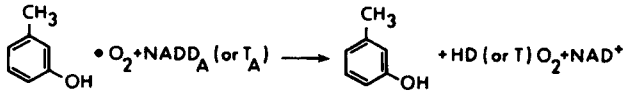
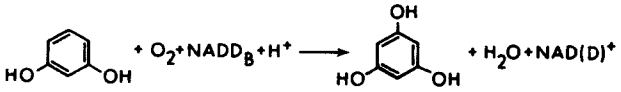
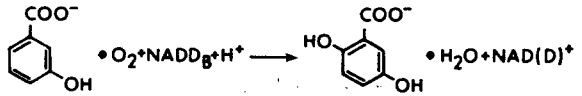
NADPH dehydrogenase (Old yellow enzyme)	1.6.99.1	Yeast	Acceptor(ox) + NADPT$_A$	→	Acceptor(red) + NADP$^+$	NADP	A	265

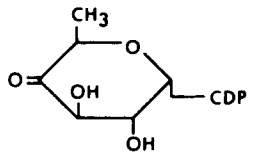
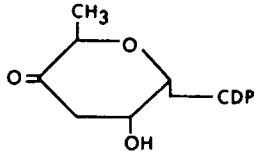
Appendix (continued)
SURVEY OF THE STEREOSPECIFICITY OF NICOTINAMIDE ADENINE DINUCLEOTIDE-LINKED ENZYMES

Enzyme	E.C.	Source	Reaction (as the experiment was performed)	Coenzyme	Stereo-specificity	Ref.
NAD(P)H dehydrogenase (Quinone reductase, DT-diaphorase)	1.6.99.2	Rat liver	2,6-Dichlorophenolindophenol(ox) + $NADT_A$(or $NADPT_A$) + H^+ → 2,6-Dichlorophenolindophenol(red) + NAD^+(or $NADP^+$) (T in medium) (See *C. vulgaris* nitrate reductase 1.6.6.1.)	NAD	A	257
				NADP	A	257
NADH dehydrogenase (external)	1.6.99.3	Rat liver mitochondria	2Cytochrome *c*(ox) + $NADT_A$ → 2Cytochrome *c*(red) + NAD^+ + T^+	NAD	A	262
Dihydropteridine reductase	1.6.99.7	Human liver	 d,1-*cis*-6,7-Dimethyl-6,7(8H)-dihydropterin → d,1-*cis*-6,7-Dimethyl-5,6,7,8-tetrahydropterin (T in medium)	NAD	B	266
		Sheep liver	Same as above	NAD	B	266
Dihydrolipoamide reductase (NADH) (Diaphorase, lipoyl dehydrogenase, lipoamide dehydrogenase, lipoamide reductase)	1.8.1.4 (Formerly 1.6.4.3.)	Spinach	 α-(+)-Lipoic acid → α-(+)-Dihydroliopoic acid	NAD	B	267
			 α-(+)-Lipoamide → α-(+)-Dihydrolipoamide	NAD	B	267

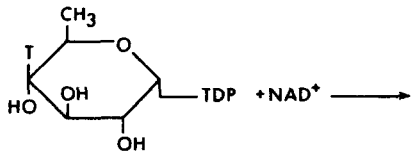
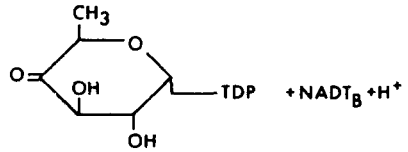
		Beef heart	α-(+)-Lipoamide + $NADT_B$ + H^+ → α-(+)-Dihydrolipoamide + NAD^+ (T in medium) (See spinach dihydrolipoamide reductase 1.8.1.4.)	NAD	B	262
Hydrogen dehydrogenase	1.12.1.2	*Hydrogenomonas ruhlandii*	$T_2 + NAD^+ \rightarrow NADT_B + T^+$	NAD	B	268
Salicylate 1-monooxygenase (Salicylate hydroxylase)	1.14.13.1	*Pseudomonas cepacia*	 Salicylate → Catechol	NAD	A	91
			 Benzoate → Benzoate	NAD	A	91
4-Hydroxybenzoate 3-monooxygenase (*p*-Hydroxybenzoate hydroxylase)	1.14.13.2	*Pseudomonas fluorescens*	 4-Hydroxybenzoate → 3,4-Dihydroxybenzoate (Protocatechuate)	NADP	A	265
Melilotate 3-monooxygenase (Melilotate hydroxylase)	1.14.13.4	*Pseudomonas sp.*	 2-Hydroxyphenylpropionate (Melilotate) → 2,3-Dihydroxyphenylpropionate	NAD	A	91
			2-Hydroxyphenylpropionate + O_2 + (3AcPyd)ADD_A + H^+ → 2,3-Dihydroxyphenylpropionate + HDO + (3AcPy)AD^+ (Melilotate)	(3AcPy)AD	A	59

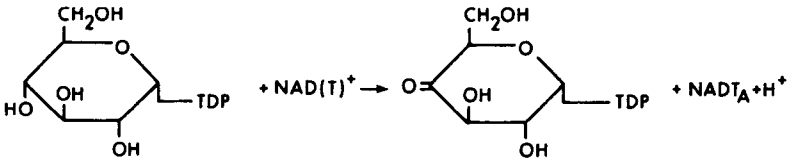
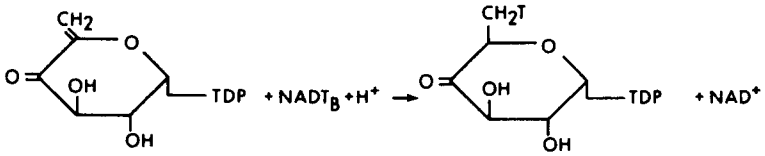
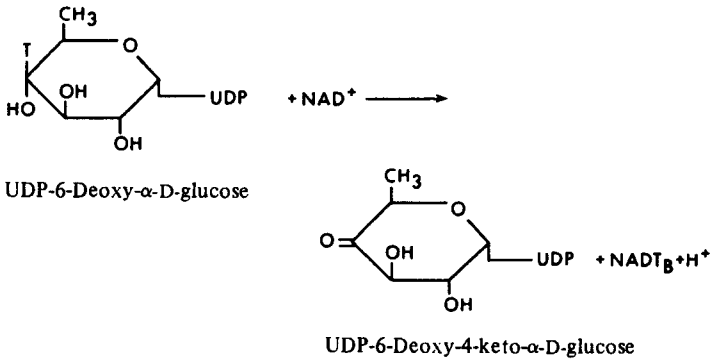
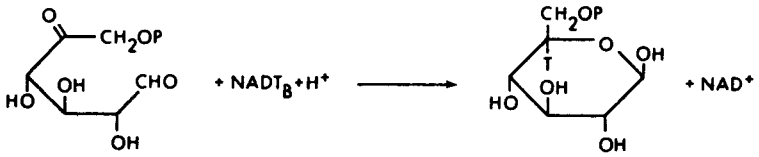
Appendix (continued)
SURVEY OF THE STEREOSPECIFICITY OF NICOTINAMIDE ADENINE DINUCLEOTIDE-LINKED ENZYMES

Enzyme	E.C.	Source	Reaction (as the experiment was performed)	Coenzyme	Stereo-specificity	Ref.
Orcinol 2-monooxygenase (Orcinol hydroxylase)	1.14.13.6	*Pseudomonas putida*	 Orcinol → 2,3,5-Trihydroxytoluene	NAD	A	55
			 Resorcinol → Resorcinol	NAD	A	56
			 Cresol → Cresol	NAD	A	56
Resorcinol 6-monooxygenase (Resorcinol hydroxylase)	1.14.13.?	*Pseudomonas putida*	 Resorcinol → 3,5-Dihydroxyphenol	NAD	A	91
3-Hydroxybenzoate 6-monooxygenase (3-Hydroxybenzoate hydroxylase)	1.14.13.?	*Pseudomonas aeruginosa*	 3-Hydroxybenzoate → 3,6-Dihydroxybenzoate	NAD	A	91

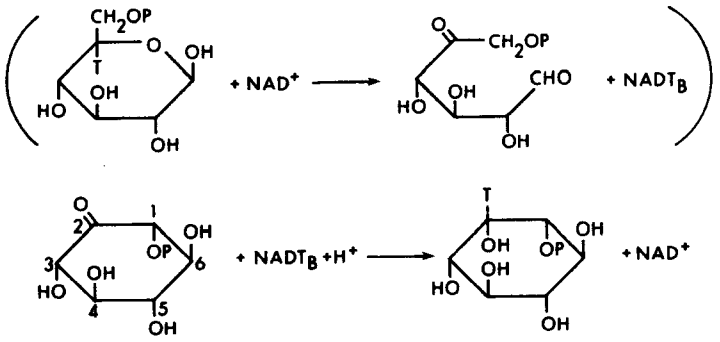
CDP-4-Keto-6-deoxy-D-glucose reductase (E_3 Enzyme)	1.17.1.1	*Pasteurella pseudo-tuberclosis*	 CDP-4-Keto-6-deoxy-α-D-glucose $\bullet\ NADPT_{AorB} + H^+ \longrightarrow$  $+ H_2O + NADP(T)^+$ CDP-4-Keto-3,6-dideoxy-α-D-glucose (No T transfer from either $NADPT_A$ or $NADPT_B$)	NADP	No specificity	48
Ferredoxin-$NADP^+$ reductase	1.18.1.2	Spinach chloroplast	$2\ \text{Ferredoxin(ox)} + NADPT_A \rightarrow 2\ \text{Ferredoxin(red)} + NADP^+ + T^+$	NADP	A	269
			$O_2 + NADPT_A + H^+ \xrightarrow{\text{Ferredoxin FMN}} HTO_2 + NADP^+$	NADP	A	269
			$H_2O + NADP(T)^+ \xrightarrow[h\nu]{\text{Ferredoxin FMN}} \tfrac{1}{2}O_2 + NADPT_B + H^+$	NADP	A	269
			$H_2O + NAD(T)^+ \xrightarrow[h\nu]{\text{Ferredoxin FMN}} \tfrac{1}{2}O_2 + NADT_B + H^+$	NAD	A	267
			$NAD(T)^+ + NADPH \rightarrow NADT_B + NADP^+$	NAD	A	267
(Adrenodoxin reductase)	1.18.1.2	Bovine adrenal cortex mitochondria	$\text{Adrenodoxin(ox)} + NADPT_B \rightarrow \text{Adrenodoxin(red)} + NADP^+ + T^+$	NADP	B	270
			$2Fe^{+++} + NADPD_B \rightarrow 2Fe^{++} + NADP^+ + D^+$	NADP	B	271

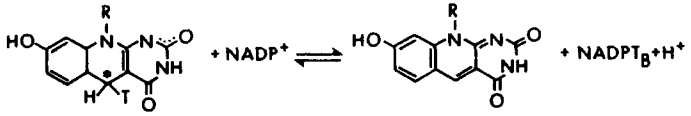
Appendix (continued)
SURVEY OF THE STEREOSPECIFICITY OF NICOTINAMIDE ADENINE DINUCLEOTIDE-LINKED ENZYMES

Enzyme	E.C.	Source	Reaction (as the experiment was performed)	Coenzyme	Stereo-specificity	Ref.
			2,6-Dichlorophenolindophenol(ox) + $NADPT_B$ + H^+ → 2,6-Dichlorophenolindophenol(red) + $NADP^+$ (T in medium) (See *C. vulgaris* nitrate reductase 1.6.6.1)	NADP	B	271
			2Cytochrome *c*(ox) + $NADPD_B$ —Adrenodoxin→ 2Cytochrome *c*(red) + $NADP^+$ + D^+	NADP	B	271
			2Cytochrome P-450(ox) + $NADPD_B$ —Adrenodoxin→ 2Cytochrome P-450(red) + $NADP^+$ + D^+	NADP	B	271
dTDPglucose 4,6-dehydratase (TDPglucose oxidoreductase)	4.2.1.46	*Escherichia coli*	 TDP-6-Deoxy-α-D-glucose  TDP-4-Keto-6-deoxy-α-D-glucose	NAD	B	37

			 TDP-α-D-Glucose → TDP-4-Keto-α-D-glucose	NAD	B	37
			 TDP-4-Keto-α-D-5,6-glucoseen → TDP-4-Keto-6-deoxy-α-D-glucose	NAD	B	37
UPDglucose 4-epimerase	5.1.3.2	*Escherichia coli*	 UDP-6-Deoxy-α-D-glucose → UDP-6-Deoxy-4-keto-α-D-glucose	NAD	B	272
L-*myo*-Inositol-1-phosphate synthase	5.5.1.4	Rat testis	 5-Keto-glucose-6-phosphate → β-D-Glucose-6-phosphate	NAD	B	273

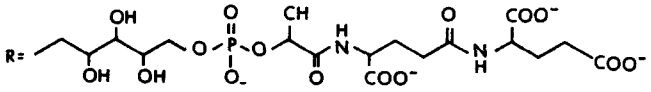
Appendix (continued)
SURVEY OF THE STEREOSPECIFICITY OF NICOTINAMIDE ADENINE DINUCLEOTIDE-LINKED ENZYMES

Enzyme	E.C.	Source	Reaction (as the experiment was performed)	Coenzyme	Stereo-specificity	Ref.
		Rat mammary gland	Same as above	NAD	B	273
		Rat testis		NAD	B	273
			myo-Inosose-2-1-phosphate 1L-Inositol-1-phosphate			
		Rat mammary gland	Same as above	NAD	B	207
NADH-Cytochrome *c* reductase	–	Pig heart mitochondria	2Cytochrome *c*(ox) + $NADD_B$ → 2Cytochrome *c*(red) + NAD^+ + D^+	NAD	B	274
		Rat liver mitochondria	D_2O + NADH → HDO + $NADD_B$	NAD	B	274
NADH Oxidase	–	Rat liver mitochondria	D_2O + NADH → HDO + $NADD_B$	NAD	B	274

		Beef heart submitochondrial particle	$\frac{1}{2}O_2 + NADT_B + H^+ \rightarrow HTO + NAD^+$	NAD	B	257
			$\frac{1}{2}O_2 + NADPT_B + H^+ \rightarrow HTO + NADP^+$	NADP	B	275
8-Hydroxy-5-deazaflavin-dependent $NADP^+$ reductase	–	*Methanococcus vannielii*		NADP	B	276

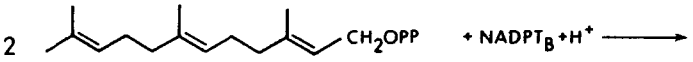
1,5-Dihydro-8-hydroxy-5-deazaflavin anion

8-Hydroxy-5-deazaflavin

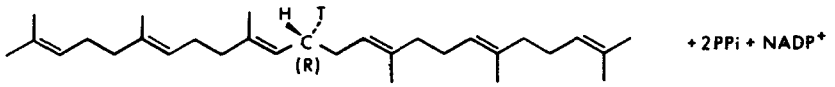


*The hydrogen removed in this enzymatic reaction is designated as the A-side hydrogen.

(See NAD(P)H dehydrogenase 1.6.8.1.)

NADPH oxidase (superoxide generating)	–	Human polymorphonuclear leukocytes	$2O_2 + NADPT_A \rightarrow 2O_2^- + NADP^+ + T^+$	NADP	A	277
Squalene synthase	–	Rat liver microsome		NADP	B	278

Farnesyl pyrophosphate



Squalene

Appendix (continued)
SURVEY OF THE STEREOSPECIFICITY OF NICOTINAMIDE ADENINE DINUCLEOTIDE-LINKED ENZYMES

Enzyme	E.C.	Source	Reaction (as the experiment was performed)	Coenzyme	Stereo-specificity	Ref.
2-Nitropropane dioxygenase	–	*Hansenula mrakii* (Yeast)	$NADT_A + O_2 + H^+ \rightarrow NAD^+ + HTO_2$ (85.4%) [$NAD(T)^+$ (14.6%)] (Confirmed with $NADT_B$)	NAD	A	279
Flavopapain	–	Papain	$O_2 + NADD_A + H^+ \rightarrow HDO_2 + NAD^+$ (68%) [$NAD(D)^+$ (32%)]	NAD	A	56
			$O_2 + NADD_B + H^+ \rightarrow H_2O_2 + NAD(D)^+$ (88%) [NAD^+ (12%)]			
NADPH-Cytochrome P-450 reductase		Rabbit liver	$2Fe^{+++} + NADPD_A \rightarrow 2Fe^{++} + NADP^+ + D^+$	NADP	A	291
			2Cytochrome *c*(ox) + $NADPD_A + H^+ \rightarrow$ 2Cytochrome *c*(red) + $NADP^+ + D^+$ (Result confirmed with $NADD_B$)	NADP	A	291

REFERENCES

1a. Recommended by the Nomenclature Committee of the International Union of Biochemistry on the Nomenclature and Classification of Enzyme, *Enzyme Nomenclature*, Academic Press, New York, 1978.
1b. Supplement 1, *Eur. J. Biochem.*, 104, 1, 198.
1c. Supplement 2, *Eur. J. Biochem.*, 116, 423, 1981.
1d. Supplement 3, *Eur. J. Biochem.*, 125, 1, 1982.
1e. Supplement 4, *Eur. J. Biochem.*, 131, 461, 1983.
2. **White, H. B., III,** Evolution of coenzymes and the origin of pyridine nucleotides, in *The Pyridine Nucleotide Coenzymes*, Everse, J., Anderson, B. M., and You, K., Eds., Academic Press, New York, 1982, 1.
3. **Pullman, M. E., San Pietro, A., and Colowick, S. P.,** On the structure of reduced diphosphopyridine nucleotide, *J. Biol. Chem.*, 206, 129, 1954.
4. **Gabriel, O.,** Common mechanistic and structural properties of enzymes, *Trends Biochem. Sci.*, 3, 193, 1978.
5. **Gabriel, O. and van Lenten, L.,** The interconversion of monosaccharides, in *Biochemistry of Carbohydrates II*, Int. Rev. Biochem., Manners, D. J., Ed., University Park Press, Baltimore, 1978, 1.
6. **Hanson, K. R.,** Applications of the sequence rule. I. Naming the paired ligands g,g at a tetrahedral atom X_{ggij}. II. Naming the two faces of a trigonal atom Y_{ghi}, *J. Am. Chem. Soc.*, 88, 2731, 1966.
7. **Fisher, H. F., Ofner, P., Conn, E. E., Vennesland, B., and Westheimer, F. H.,** Direct enzymic transfer of hydrogen atoms between substrates and DPN, *Fed. Proc., 11, (Abstr.)*, 211, 1952.
8. **Loewus, F. A., Westheimer, F. H., and Vennesland, B.,** Enzymatic synthesis of the enantimorphs of ethanol-1-d, *J. Am. Chem. Soc.*, 75, 5018, 1953.
9. **Levy, H. R., Loewus, F. A., and Vennesland, B.,** Optical rotation and configuration of a pure enantiomorph of ethanol-1-d, *J. Am. Chem. Soc.*, 79, 2949, 1957.
10. **Levy, H. R., Talalay, P., and Vennesland, B.,** The steric course of enzymatic reactions at *meso* carbon atoms: Application of hydrogen isotopes, in *Progress in Stereochemistry*, Vol. 3, De La Mare, P. B. D. and Klyne, W., Eds., Butterworths, London, 1962, 299.
11. **Colowick, S. P., van Eys, J., and Park, J. H.,** Dehydrogenation, in *Comprehensive Biochemistry*, Vol. 14, Florikin, M. and Stotz, E. H., Eds., Elsevier, Amsterdam, 1966, 1.
12. **Bentley, R.,** Dehydrogenation and related processes, in *Molecular Asymmetry in Biology*, Vol. 2, Academic Press, New York, 1970, 1.
13. **Popjak, G.,** Stereospecificity of enzymic reactions, in *The Enzymes*, Vol. 2, 3rd ed., Boyer, P. D., Ed., Academic Press, New York, 1970, 115.
14. **Alizade, M. A. and Brendel, K.,** Tentative classification of NAD(P)-linked dehydrogenases in regard to their stereochemistry of hydrogen transer to the coenzyme, *Naturwissenschaften*, 62, 346, 1975.
15. **Simon, H. and Kraus, A.,** Hydrogen isotope transfer in biological process, in *Isotopes in Organic Chemistry*, Vol. 2, Buncel, E. and Lee, C. C., Eds., Elsevier, Amsterdam, 1976, 153.
16. **You, K., Arnold, L. J., Jr., Allison, W. S., and Kaplan, N. O.,** Enzyme stereospecificities for nicotinamide nucleotides, *Trends Biochem. Sci.*, 3, 265, 1978.
17. **You, K.,** Stereospecificities of the pyridine nucleotide-linked enzymes, *Methods Enzymol.*, 87, 101, 1982.
18. **Everse, J., Anderson, B. M., and You, K., Eds.,** *The Pyridine Nucleotide Coenzymes*, Academic Press, New York, 1982.
19. **Fisher, H. F., Conn, E. E., Vennesland, B., and Westheimer, F. H.,** The enzymic transfer of hydrogen. I. The reaction catalyzed by alcohol dehydrogenase, *J. Biol. Chem.*, 202, 687, 1953.
20. **Vennesland, B.,** Steric specificity of hydrogen transfer in pyridine nucleotide dehydrogenase reactions, *J. Cell. Comp. Physiol.*, 47, (Supp. 1), 201, 1956.
21. **Jarabak, J. and Talalay, P.,** Stereospecificity of hydrogen transfer by pyridine nucleotide-linked hydroxysteroid dehydrogenase, *J. Biol. Chem.*, 235, 2147, 1960.
22. **Kaplan, N. O.,** The pyridine coenzymes, in *The Enzymes*, Vol. 3, 2nd ed., Boyer, P. D., Lardy, H., and Myback, K., Eds., Academic Press, New York, 1960, 105.
23. **Westheimer, F. H., Fisher, H. F., Conn, E. E., and Vennesland, B.,** The enzymatic transfer of hydrogen from alcohol to DPN, *J. Am. Chem. Soc.*, 73, 2403, 1951.
24. **Louie, D. D. and Kaplan, N. O.,** Stereospecificity of hydrogen transfer reactions of the *Pseudomonas aeruginosa* pyridine nucleotide transhydrogenase, *J. Biol. Chem.*, 245, 5691, 1970.
25. **Drysdale, G. R., Spiegel, M. J., and Sprittmatter, P.,** Flavoprotein-catalyzed direct hydrogen transfer between pyridine nucleotides, *J. Biol. Chem.*, 236, 2323, 1961.
26. **San Pietro, A., Kaplan, N. O. and Colowick, S. P.,** Pyridine nucleotide transhydrogenase. IV. Mechanism and stereospecificity of the reaction in *Pseudomonas fluorescence*, *J. Biol. Chem.*, 212, 941, 1955.
27. **Loewus, F. A., Ofner, P., Fisher, H. F., Westheimer, F. H., and Vennesland, B.,** The enzymic transfer of hydrogen. II. The reaction catalyzed by lactate dehydrogenase, *J. Biol. Chem.*, 202, 699, 1953.
28. **Loewus, F. A., Tchen, T. T., and Vennesland, B.,** The enzymatic transfer of hydrogen. III. The reaction catalyzed by malic dehydrogenase, *J. Biol. Chem.*, 212, 787, 1955.

29. **Talalay, P., Loewus, F. A., and Vennesland, B.,** The enzymatic transfer of hydrogen. IV. The reaction catalyzed by a β-hydroxysteroid dehydrogenase, *J. Biol. Chem.*, 212, 801, 1955.
30. **Nakamoto, T. and Vennesland, B.,** The enzymatic transfer of hydrogen. VIII. The reactions catalyzed by glutamic and isocitric dehydrogenase, *J. Biol. Chem.*, 235, 202, 1960.
31. **Levy, H. R. and Vennesland, B.,** The stereospecificity of enzymatic hydrogen transfer from diphosphopyridine nucleotide, *J. Biol. Chem.*, 228, 85, 1957.
32. **Velick, S. F.,** Spectra and structure in enzyme complexes of pyridine and flavine nucleotides, in *Light and Life*, McElroy, W. D. and Glass, H. B., Eds., Johns Hopkins, Baltimore, 1961, 108.
33. **Weber, G.,** Intramolecular transfer of electronic energy in dihydrodiphosphopyridine nucleotide, *Nature (London)*, 180, 1409, 1958.
34. **Shifrin, S. and Kaplan, N. O.,** Structure of diphosphopyridine nucleotide, *Nature (London)*, 183, 1529, 1959.
35. **Shifrin, S. and Kaplan, N. O.,** Interactions in pyridine nucleotides, in *Light and Life*, McElroy, W. D. and Glass, H. B., Eds., Johns Hopkins, Baltimore, 1961, 144.
36. **Cornforth, J. W., Ryback, G., Popjack, G., Donninger, C., and Schroepfer, G., Jr.,** Stereochemistry of enzymic hydrogen transfer to pyridine nucleotides, *Biochem. Biophys. Res. Commun.*, 9, 371, 1962.
37. **Wang, S.-F. and Gabriel, O.,** Biological mechanusms involved in the formation of deoxysugars. VI. Role and function of enzyme-bound nicotinamide adenine dinucleotide thymidine diphosphate D-glucose oxidoreductase, *J. Biol. Chem.*, 245, 8, 1970.
38. **Oppenheimer, N. J., Arnold, L. J., Jr., and Kaplan, N. O.,** A structure of pyridine nucleotides in solution, *Proc. Natl. Acad. Sci. U.S.A.*, 68, 3200, 1971.
39. **Arnold, L. J., Jr. and Kaplan, N. O.,** The structure of the abortive diphosphopyridine nucleotide-pyruvate-lactate dehydrogenase ternary complex as determined by proton magnetic resonance analysis, *J. Biol. Chem.*, 249, 652, 1974.
40. **Arnold, L. J., Jr., You, K., Allison, W. S., and Kaplan, N. O.,** Determination of the hydride transfer stereospecificity of nicotinamide adenine dinucleotide-linked oxidoreductases by proton magnetic resonance, *Biochemistry*, 15, 4844, 1976.
41. **Wong, S. S. and Wong, L.-J. C.,** A one-step PMR determination of hydrogen transfer stereospecificity of $NADP^+$-linked oxidoreductases, *Int. J. Biochem.*, 15, 147, 1983.
42. **Seyama, Y., Kasama, T., Yamakawa, T., Kawaguchi, A., and Okuda, S.,** Stereochemical studies of hydrogen incorporation from nucleotides with fatty acid synthetase from *Brevibacterium ammoniagenes*, *J. Biochem.*, 81, 1167, 1977.
43. **Feldman, H. B., Szczepanik, P. A., Havre, P., Corrall, R. J. M., Yu, L. C., Rodman, H. M., Rosner, B. A., Klein, P. D., and Laundau, B. R.,** Stereospecificity of the hydrogen transfer catalyzed by human placental aldose reductase, *Biochim. Biophys. Acta*, 480, 14, 1977.
44. **George, J. M., Orr, J. C., Renwick, A. G. C., Carter, P., and Engel, L. L.,** The stereochemistry of hydrogen transfer to $NADP^+$ by enzymes acting upon stereoisomeric substrates, *Bioorg. Chem.*, 2, 140, 1973.
45. **Ehmke, A., Flossdorf, J., Habicht, W., Schiebel, H. M., and Schulten, H. R.,** A new technique for determining the hydride transfer stereospecificity of nicotinamide adenine dinucleotide-linked oxidoreductases: electron impact and field desorption mass spectrometry, *Anal. Biochem.*, 101, 413, 1980.
46. **Adams, M. J., McPherson, A., Jr., Rossman, M. G., Schevitz, R. W., and Wonacott, A. J.,** The structure of the nicotinamide-adenine dinucleotide coenzyme when bound to lactate dehydrogenase, *J. Mol. Biol.*, 51, 31, 1970.
47. **Saenger, W., Reddy, B. S., Muhlegger, K., and Weinmann, G.,** X-ray study of the lithium complex of NAD^+, *Nature (London)*, 267, 225, 1977.
48. **Rubenstein, P. A. and Strominger, J. L.,** Enzymatic synthesis of cytidine diphosphate 3,6-dideoxyhexoses. VIII. Studies of the properties of E3 and its role in the formation of cytidine diphosphate-4-keto-3,6-dideoxyglucose, *J. Biol. Chem.*, 249, 3782, 1974.
49. **Guerrero, M. G. and Vennesland, B.,** Stereospecificity of hydrogen removal from pyridine nucleotide: the reactions catalyzed by nitrate reductase and by xanthine oxidase, *FEBS Lett.*, 51, 284, 1975.
50. **Mahler, H. R. and Cordes, E. H.,** *Biological Chemistry*, Harper & Row, New York, 1971, 412.
51. **Lehninger, A. L.,** *Biochemistry*, 2nd ed., Worth Publishers, New York, 1975, 481.
52. **Loewus, F. A. and Stafford, H. A.,** The enzymatic transfer of hydrogen of glyceric and lactic dehydrogenase, *J. Biol. Chem.*, 235, 3317, 1960.
53. **Biellmann, J. F. and Rosenheimer, N.,** Dogfish lactate dehydrogenase. The stereochemistry of hydrogen transfer, *FEBS Lett.*, 34, 143, 1973.
54. **You, K. and Roe, C. R.,** Affinity chromatography of Pseudomonas salicylate hydroxylase, *Anal. Biochem.*, 114, 177, 1981.
55. **Higgins, H. J. and Ribbons, D. W.,** Stereospecificity of hydride transfer in the flavin mono-oxygenase orcinol hydroxylase, *Biochem. J.*, 127, 65p, 1972.

56. **Ryerson, C. C., and Walsh, C.,** The stereochemistry of NADH utilization by the flavoenzyme monooxygenase orcinol hydroxylase, *J. Biol. Chem.*, 254, 4349, 1979.
57. **Levine, H. L. and Kaiser, E. T.,** Stereospecificity in the oxidation of NADH by flavopapain, *J. Am. Chem. Soc.*, 102, 343, 1980.
58. **Biellmann, J.-F., Hirth, C. G., Jung, M. J., Rosenheimer, N., and Wrixon, A. D.,** Studies with analogues of nicotinamide adenine dinucleotide, *Eur. J. Biochem.*, 41, 517, 1974.
59. **Schopfer, L. M. and Massey, V.,** Kinetic and mechanistic studies on the reduction of melilotate hydroxylase by reduced pyridine nucleotide, *J. Biol. Chem.*, 254, 10634, 1979.
60. **Oppenheimer, N. J. and Kaplan, N. O.,** The alpha beta epimerization of reduced nicotinamide adenine dinucleotide, *Arch. Biochem. Biophys.*, 166, 526, 1975.
61. **Flynn, T. G.,** Aldehyde reductases: monomeric NADPH-dependent oxidoreductases with multifunctional potential, *Biochem. Pharmacol.*, 31, 2705, 1982.
62. **Branlant, G. and Biellmann, J.-F.,** Purification and some properties of aldehyde reductases from pig liver, *Eur. J. Biochem.*, 105, 611, 1980.
63. **Wermuth, B.,** Purification and properties of an NADPH-dependent carbonyl reductase from human brain, *J. Biol. Chem.*, 256, 1206, 1981.
64. **Ashmed, N. K., Felsted, R. L., and Bachur, N. R.,** Heterogeneity of anthracycline antibiotic carbonyl reductases in mammalian livers, *Biochem. Pharmacol.*, 27, 2713, 1978.
65. **Felsted, R. L. and Bachur, N. R.,** Ketone reductases, in *Enzymatic Basis of Detoxication*, Vol. 1, Jakoby, W. B., Ed, Academic Press, New York, 1980, 281.
66. **Ris, M. M. and von Wartburg, J-P.,** Heterogeneity of NADPH-dependent aldehyde reductase from human and rat brain, *J. Biochem.*, 37, 69, 1973.
67. **Felsted, R. L., Richter, D. R., Jones, D. M., and Bachur, N. R.,** Isolation and characterization of rabbit liver xenobiotic carbonyl reductases, *Biochem. Pharmacol.*, 29, 1503, 1980.
68. **Jeffrey, J.,** Stereochemistry of dehydrogenases, in *Stereochemistry*, Tamm, Ch., Ed., Elsevier, Amsterdam, 1982, 113.
69. **Von Wartburg, J.-P. and Wermuth, B.,** Aldehyde reductase, in *Enzymatic Basis of Detoxication*, Vol. 1, Jakoby, W. B., Ed., Academic Press, New York, 1980, 249.
70. **Strickler, R. C., Tobias, B., and Covey, D. F.,** Human placental 17β-estradiol dehydrogenase and 20α-hydroxysteroid dehydrogenase, *J. Biol. Chem.*, 256, 316, 1981.
71. **Fisher, R. R. and Earle, S. R.,** Membrane-bound pyridine dinucleotide transhydrogenase, in *The Pyridine Nucleotide Coenzymes*, Everse, J., Anderson, B. M., and You, K., Eds., Academic Press, New York, 1982, 279.
72. **Björkhem, I. and Danielsson, H.,** Stereochemistry of hydrogen transfer from pyridine nucleotides catalyzed by Δ^4-3-oxosteroid 5β-reductase and 3α-hydroxysteroid dehydrogenase from rat liver, *Eur. J. Biochem.*, 12, 80, 1970.
73. **Wilken, D. R., King, H. L., Jr., and Dyar, R. E.,** Ketopantoic acid and ketopantoyl lactone reductases stereospecificity of transfer of hydrogen from reduced nicotinamide adenine dinucleotide phosphate, *J. Biol. Chem.*, 250, 2311, 1975.
74. **Dugan, R. E., Slakey, L. L., and Porter, L. W.,** Stereospecificity of the transfer of hydrogen from reduced nicotinamide adenine dinucleotide phosphate to the acyl chain in the dehydrogenase-catalyzed reactions of fatty acid synthesis, *J. Biol. Chem.*, 245, 6312, 1970.
75. **Mori, M., Suzuki, K., and Tamaoki,** B.-I., Testosterone metabolism in rooster comb, *Biochim. Biophys. Acta.*, 337, 118, 1974.
76. **Suzuki, K. and Tamaoki, B.-I.,** *In vitro* metabolism of testosterone in seminal vesicles of rats, *J. Steroid Biochem.*, 5, 249, 1974.
77. **Saito, K., Kawaguchi, A., Okuda, S., Seyama, Y., and Yamakawa, T.,** Incorporation of hydrogen atoms from deuterated water and stereospecifically deuterium-labeled nicotinamide nucleotides into fatty acids with the *Escherichia coli* fatty acid synthetase system, *Biochim. Biophys. Acta*, 618, 202, 1980.
78. **Seyama, T., Kasama, T., Yamakawa, T., Kawaguchi, A., Saito, K., and Okuda, S.,** Origin of hydrogen atoms in the fatty acids synthesized with yeast fatty acid synthetase, *J. Biochem.*, 82, 1325, 1977.
79. **Davies, D. D., Teixeria, A., and Kenworthy, P.,** The stereospecificity of nicotinamide-adenine dinucleotide-dependent oxidoreductase from plants, *Biochem. J.*, 127, 335, 1972.
80. **Guerrero, M. G., Jetschmann, K., and Volker, W.,** The stereospecificity of nitrate reductase for hydrogen removal from reduced pyridine nucleotides, *Biochim. Biophys. Acta*, 482, 19, 1977.
81. **Davies, D. D. and Kenworthy, P.,** The stereospecificity of the reduction of nitrate by reduced nicotinamide-adenine dinucleotides catalysed by *Candida utilis* preparations, *Biochem. J.*, 205, 581, 1982.
82. **Krakow, G., Ludowieg, J., Mahler, J. H., Normore, W. M., Tosi, L., Ukada, S., and Vennesland, B.,** Some stereospecificity studies with tritiated pyridine nucleotides, *Biochemistry*, 2, 1009, 1963.
83. **Vennesland, B.,** Stereospecificity in biology in, *Top. Curr. Chem.*, 48, 39, 1974.
84. **Stryer, L.,** *Biochemistry*, 2nd ed., W. H. Freeman & Co., San Francisco, 1981, 297.

85. **Fisher, H. F. and McGregor, L. L.,** The mechanism of the glutamic dehydrogenase reaction. II. Substrate specificity of the enzyme, *J. Biol. Chem.*, 236, 791, 1961.
86. **Alizade, M. A., Gaede, K., and Brendel, K.,** Chirality of the hydrogen transfer to the coenzyme catalyzed by ribitol dehydrogenase from *Klebsiella pneumoniae* and D-mannitol 1-phosphate dehydrogenase from *Escherichia coli*, *Hoppe-Seyler's Z. Physiol. Chem.*, 357, 1163, 1976.
87. **Mansell, R. L., Gross, G. G., Stockigt, J., Franke, H., and Zenk, M., H.,** Purification and properties of cinnamyl alcohol dehydrogenase from higher plants involved in lignin biosynthesis, *Phytochemistry*, 13, 2427, 1974.
88. **Akhtar, M., Wilson, D. C., Watkinson, I. A., and Rahimtula, A. D.,** Substrate activation in pyridine nucleotide-linked reactions: illustrations from steroid field, *Proc. R. Soc. London B*, 180, 167, 1972.
89. **Dutler, H., Van Der Baan, J. L., Hochuli, E., Kis, Z., Taylor, K. E., and Prelog, V.,** Dihydroxyacetone reductase from *Mucor javanicus*. I. Isolation and properties, *Eur. J. Biochem.*, 75, 423, 1972.
90. **Hochuli, E., Taylor, K. E., and Dutler, H.,** Dihydroxyacetone reductase from *Mucor javanicus*. II. Identification of the physiological substrate and reactivity towards related compounds, *Eur. J. Biochem.*, 75, 433, 1977.
91. **You, K., Arnold, L. J., Jr., and Kaplan, N. O.,** The stereospecificity of bacterial external flavoprotein monooxygenases for nicotinamide adenine dinucleotide, *Arch. Biochem. Biophys.*, 180, 550, 1977.
92. **Jeffrey, J.,** The specificity of dehydrogenases, in *Dehydrogenases Requiring Nicotinamide Coenzymes*, Jeffrey, J., Ed., Birkhauser Verlag, Basel, 1980, 85.
93. **Garavito, M., Rossman, M. G., Argos, P., and Eventoff, W.,** Convergence of active center geometries, *Biochemistry*, 16, 5065, 1977.
94. **Cori, C. F., Velick, S. G., and Cori, G. T.,** The combination of diphosphopyridine nucleotide with glyceraldehyde phosphate dehydrogenase, *Biochim. Biophys. Acta*, 4, 160, 1950.
95. **Nygaard, A. P. and Rutter, W. J.,** Interaction of pyridine-nucleotide linked enzymes, *Acta Chem. Scand.*, 10, 37, 1956.
96. **Velick, S. F.,** Biological oxidations, *Annu. Rev. Biochem.*, 25, 257, 1956.
97. **Loewus, F. A., Levy, H. R., and Vennesland, B.,** The enzymatic transfer of hydrogen. VI. The reaction catalyzed by D-glyceraldehyde-3-phosphate dehydrogenase, *J. Biol. Chem.*, 223, 589, 1956.
98. **Astrachan, L., Colowick, S. P., and Kaplan, N. O.,** The reactivity of the bound DPN of triose phosphate dehydrogenase, *Biochim. Biophys. Acta*, 24, 141, 1957.
99. **Hung, H. C. and Hoberman, H. D.,** Influence of steric specificity on the rates of hydrogen exchange between substrates of NAD-coupled dehydrogenases, *Biochem. Biophys. Res. Commun.*, 46, 399, 1972.
100. **Colowick, S. P., Kaplan, N. O., and Ciotti, M. M.,** The reaction of pyridine nucleotide with cyanide and its analytical use, *J. Biol. Chem.*, 191, 447, 1951.
101. **San Pietro, A.,** On the structure of the diphosphopyridine nucleotide-cyanide complex, *J. Biol. Chem.*, 217, 579, 1955.
102. **Burton, R. M. and Kaplan, N. O.,** The reaction of pyridine nucleotides with carbonyl compounds, *J. Biol. Chem.*, 206, 283, 1954.
103. **Everse, J., Zoll, E. C., Kahan, L., and Kaplan, N. O.,** Addition products of diphosphopyridine nucleotides with substrates of pyridine nucleotide-linked dehydrogenases, *Bioorg. Chem.*, 1, 207, 1971.
104. **Oppenheimer, N. J.,** Chemistry and solution conformation of the pyridine coenzymes, in *The Pyridine Nucleotide Coenzymes*, Everse, J., Anderson, B. M., and You, K., Eds., Academic Press, New York, 1982, 51.
105. **Everse, J., Berger, R. L., and Kaplan, N. O.,** Complexes of pyridine nucleotides and their function, in *Structure and Function of Oxidation and Reduction Enzymes*, Akeson, A. and Ehrenberg, A., Eds., Pergamon Press, Oxford, 1972, 691.
106. **Everse, J. and Kaplan, N. O.,** Lactate dehydrogenases: structure and function, *Adv. Enzymol.*, 37, 61, 1973.
107. **Lee, C.-Y. and Chen, A. F.** Immobilized coenzymes and derivatives, in *The Pyridine Nucleotide Coenzymes*, Everse, J., Anderson, B. M., and You, K., Eds., Academic Press, New York, 1982, 189.
108. **Pfleiderer, G., Jeckel, D., and Wieland, T.,** Zum Wirkungsmechanismus der Milchsaure-Dehydrogenase. Die Schutzende von Coenzyme-Addukten Genenuber Denaturierenden Agentien, *Biochem. Z.*, 329, 104, 1957.
109. **Gelach, D., Pfleiderer, G., and Holbrook, J. J.,** Enzymatische Katalyse der Cyanid-Addition an Nicotinamid-Adenindinucleotid, *Biochem. Z.*, 343, 354, 1965.
110. **Cook, P. F., Oppenheimer, N. J., and Cleland, W. W.,** Secondary deuterium and nitrogen-15 isotope effects in enzyme-catalyzed reactions. Chemical mechanism of liver alcohol dehydrogenase, *Biochemistry*, 20, 1817, 1981.
111. **Torreilles, J., Marchand, J., Guerin, M.-C., Gabriel, M., and Larcher, D.,** The structure of the abortive nicotinamide adenine dinucleotide-pyruvate-lactate dehydrogenase complex as determined by circular dichroism, *Biochimie*, 65, 193, 1983.

112. **Arnold, L. J., Jr., Oppenheimer, N. J., Lee, C.-Y., and Kaplan, N. O.,** Proton nuclear magnetic resonance study of the conformation and configuration of the cyclized pyridine nucleotide adducts, *Biochemistry*, 18, 2787, 1978.
113. **Fromm, H. J.,** The nature of pyruvate involved in the enzymic formation of L-lactate in the rabbit-muscle lactate dehydrogenase reaction, *Biochim. Biophys. Acta*, 99, 540, 1965.
114. **Coulson, C. J. and Rabin, B. R.,** Inhibition of lactate dehydrogenase by high concentrations of pyruvate: the nature and removal of the inhibitor, *FEBS Lett.*, 3, 333, 1969.
115. **Cross, D. G. and Fisher, H. F.,** Conformation and conformational changes of reduced diphosphopyridine nucleotide in solution, *Biochemistry*, 8, 1147, 1969.
116. **Reisbig, R. R. and Woddy, R. W.,** Characterization of a long wavelength feature in the absorption and dichroism spectra of beta-nicotinamide adenine dinucleotide. Evidence for a charge transfer transition, *Biochemistry*, 17, 1974, 1978.
117. **Miles, D. W. and Urry, D. W.,** Reciprocal relations and proximity of bases in pyridine dinucleotides, *J. Biol. Chem.*, 243, 4181, 1968.
118. **Saenger, W., Reddy, B. S., Muhlegger, K., and Welmann, G.,** Conformation of NAD^+ in solution, in holoenzymes and in the crystalline Li^+-complex, in *Pyridine Nucleotide-Dependent Dehydrogenases*, Sund, H., Ed., Walter de Gruyter, Berlin, 1977, 222.
119. **Meyer, W. L., Mahler, R., and Baker, R. H., Jr.,** Nuclear magnetic resonance spectra and conformation of 1,4-dihydropyridines, *Biochim. Biophys. Acta*, 64, 353, 1962.
120. **Jardetzky, O. and Wade-Jardetzky, N. G.,** The conformation of pyridine dinucleotides in solution, *J. Biol. Chem.*, 241, 85, 1966.
121. **Sarma, R. H., Ross, V., and Kaplan, N. O.,** Investigation of the conformation of beta-diphosphopyridine nucleotide (beta-nicotinamide-adenine dinucleotide) and pyridine dinucleotide analogs by proton magnetic resonance, *Biochemistry*, 7, 3052, 1968.
122. **Danyluk, S. S. and Hruska, F. E.,** The effect of pH upon the nuclear magnetic resonance spectra of nucleosides and nucleotides, *Biochemistry*, 7, 1038, 1968.
123. **Catterall, W. A., Hollis, D. P., and Walter, C. F.,** Nuclear magnetic resonance study of the conformation of nicotinamide-adenine dinucleotide and reduced nicotinamide-adenine dinucleotide in solution, *Biochemistry*, 8, 4032, 1969.
124. **Sarma, R.H. and Kaplan, N. O.,** High frequency nuclear magnetic resonance study of the *M* and *P* helices of reduced pyridine dinucleotides, *Biochemistry*, 9, 539, 1970.
125. **McDonald, G., Brown, B., Hollis, D., and Walter, C.,** Some effects of environment on the folding of nicotinamide-adenine dinucleotides in aqueous solutions, *Biochemistry*, 11, 1920, 1972.
126. **Sarma, R. H. and Mynott, R. J.,** Conformation of pyridine nucleotides studied by phosphorous-31 and hydrogen-1 fast Fourier transform nuclear magnetic resonance spectroscopy. I. Oxidized and reduced mononucleotides, *J. Am. Chem. Soc.*, 95, 1641, 1973.
127. **Zens, A. P., Taffy, J. W., Wisowaty, J. C., Fisher, R. R., Dunlap, R., B., Bryson, T. A., and Ellis, P. D.,** Nuclear magnetic resonance studies on pyridine dinucleotides. II. Solution conformational dynamics of nicotinamide adenine dinucleotide and nicotinamide mononucleotides as viewed by proton T_1 measurements, *J. Am. Chem. Soc.*, 97, 2850, 1975.
128. **Oppenheimer, N. J., Arnold, L. J., Jr., and Kaplan, N. O.,** Stereospecificity of the intramolecular association of reduced pyridine coenzymes, *Biochemistry*, 17, 2613, 1978.
129. **Eagan, W., Forsen, S., and Jacobus, J.,** The solution conformation of nicotinamide mononucleotide: a quantitative application of the nuclear Overhauser effect, *Biochemistry*, 14, 735, 1975.
130. **Blumenstein, M. and Raftery, M. A.,** Natural abundance ^{13}C nuclear magnetic resonance spectra of nicotinamide adenine dinucleotide and related nucleotides, *Biochemistry*, 12, 3585, 1973.
131. **Grau, U. M.,** Structural interactions with enzymes, in *The Pyridine Nucleotide Coenzymes*, Everse, J., Anderson, B. M., and You, K., Eds., Academic Press, New York, 1982, 135.
132. **Lee, C.-Y., Eichner, R. D., and Kaplan, N. O.,** Conformations of diphosphopyridine coenzymes upon binding to dehydrogenases, *Proc. Natl. Acad. Sci. U.S.A.*, 70, 1593, 1973.
133. **Sovago, I. and Martin, R. B.,** Nicotinamide adenine dinucleotide is about 44% folded in neutral aqueous solutions, *FEBS Lett.*, 106, 132, 1979.
134. IUPAC-IUB Joint Commission on Biochemical Nomenclature, Abbreviations and symbols for the description of conformations of polynucleotide chains, *Eur. J. Biochem.*, 131, 9, 1983.
135. **Schweizer, M. P., Brown, A. D., Ts'O, P. O. P., and Hollis, D. P.,** Studies of inter- and intramolecular interaction in mononucleotides by proton magnetic resonance, *J. Am. Chem. Soc.*, 90, 1042, 1968.
136. **Chan, S. I. and Nelson, J. H.,** Proton magnetic resonance studies of ribose dinucleoside monophosphates in aqueous solutions. I. The nature of the base-stacking interaction in adenylyl (3'-5')- adenosine, *J. Am. Chem. Soc.*, 91, 168, 1969.
137. **Follman, H. and Gremels, G.,** Adenine nucleosides in solution: stabilization of the anti-conformation by C-5' substituents, *Eur. J. Biochem.*, 47, 187, 1974.

138. **Evans, F. E. and Kaplan, N. O.,** 8-Alkylaminoadenyl nucleotides as probes of dehydrogenase interactions with nucleotide analogs of different glycosyl conformation, *J. Biol. Chem.*, 251, 6791, 1976.
139. **Lappi, D. A., Evans, F. E., and Kaplan, N. O.,** Reduced nicotinamide 8-(alkylamino)adenine dinucleotides: enzyme-coenzyme interactions with different adenyl glycosyl bond conformations, *Biochemistry*, 19, 3841, 1980.
140. **Sarma, R. H. and Mynott, R. J.,** Conformation of pyridine nucleotides studied by phosphorus-31 and hydrogen-1 fast Fourier transform nuclear magnetic resonance spectroscopy. III. Oxidized and reduced dinucleotides, *J. Am. Chem. Soc.*, 95, 7470, 1973.
141. **Karplus, M.,** Vicinal proton coupling in nuclear magnetic resonance, *J. Am. Chem. Soc.*, 85, 2870, 1963.
142. **van der Veen, R. H., Kellog, R. M., and Vos, A.,** X-ray crystal structures of a 1,4-dihydropyridine-crown ether sodium perchlorate complex crystallyzed with a molecule of acetone, *J. Chem. Soc. Chem. Commun.*, 923, 1978.
143. **Rob, F., van Ramesdonk, H. J., Verhoeven, J. W., Pandit, U. K., and de Boer, T. J.,** Pyridine-dinucleotide models. IV. Stereoselective hydride transfer at a bridged pyridinium-ion, *Tetrahedron Lett.*, 21, 1549, 1980.
144. **Wright, W. B. and King, G. S. D.,** The crystal structure of nicotinamide, *Acta Cryst.*, 7, 283, 1954.
145. **Karle, I. L.,** The crystal structure of N-benzyl-1,4-dihydronicotinamide, *Acta Cryst.*, 14, 497, 1961.
146. **Abdallah, M. A., Biellmann, J.-F., Samama, J. P., and Wrixon, A. D.,** Preparation and properties of 3-halopyridine-adenine dinucleotides, NAD^+ analogues and of model compounds, *Eur. J. Biochem.*, 64, 351, 1976.
147. **Rossman, M. G., Liljas, A., Brändén, C.-I., and Banaszak, L.,** Evolutionary and structural relationships among dehydrogenases, in *The Enzymes*, 3rd ed., Boyer, P. D., Ed., Academic Press, New York, 1975, 61.
148. **Schulz, G. E., Schirmer, R. H., Sachsenheimer, W., and Pai, E. F.,** The structure of the flavoenzyme glutathione reductase, *Nature (London)*, 273, 120, 1978.
149. **Pai, E. F. and Schulz, G. E.,** The catalytic mechanism of glutathione reductase as derived from X-ray diffraction analyses of reaction intermediates, *J. Biol. Chem.*, 258, 1752, 1983.
150. **Volz, K. W., Matthews, D. A., Alden, R. A., Freer, S. T., Hansch, C., Kaufman, B. T., and Kraut, J.,** Crystal structure of avian dihydrofolate reductase containing phenyltriazine and NADPH, *J. Biol. Chem.*, 257, 2528, 1982.
151. **Biesecker, G., Harris, J. I., Thierry, J. C., Walker, J. E., and Wonacott, A. J.,** Sequence and structure of D-glyceraldehyde 3-phosphate dehydrogenase from *Bacillus stearothermophilus*, *Nature (London)*, 266, 328, 1977.
152. **Buehner, M., Ford, G. C., Moras, D., Olsen, K. W., and Rossman, M. G.,** Structure determination of crystalline lobster D-glyceraldehyde-3-phosphate dehydrogenase, *J. Mol. Biol.*, 82, 563, 1974.
153. **Buehner, M., Ford, G. C., Moras, D., Olsen, K. W., and Rossman, M. G.,** Three dimensional structure of D-glyceraldehyde-3-phosphate dehydrogenase, *J. Mol. Biol.*, 90, 25, 1974.
154. **Anderson, B. M.,** Analogs of pyridine nucleotide coenzymes, in *Pyridine Nucleotide Coenzymes*, Everse, J., Anderson, B. M., and You, K., Eds., Academic Press, New York, 1982, 91.
155. **Mosbach, K.,** Immobilized coenzymes in general ligand affinity chromatography and their use as active coenzymes, *Adv. Enzymol.*, 46, 205, 1978.
156. **Clore, G. M. and Gronenborn, A. M.,** Theory and applications of the transferred nuclear Overhauser effect to the study of the conformations of small ligands bound to proteins, *J. Magn. Resonance*, 48, 402, 1982.
157. **Levy, H. R., Ejchart, A., and Levy, G. C.,** Conformations of nicotinamide coenzymes bound to dehydrogenases determined by transferred nuclear Overhouser effects, *Biochemistry* 22, 2792, 1983.
158. **Holmgren, A.,** Pyridine nucleotide-disulfide oxidoreductase, in *Dehydrogenases Requiring Nicotinamide Coenzymes*, Jeffrey, J., Ed., Birkhauser Verlag, Basel, 1980, 149.
159. **Samama, J.-P., Zeppezauer, E., Biellmann, J.-F., and Brändén, C.-I.,** The crystal structure of complexes between horse liver alcohol dehydrogenase and the coenzyme analogues 3-iodopyridine-adenine dinucleotide and pyridine-adenine dinucleotide, *Eur. J. Biochem.*, 81, 403, 1977.
160. **Feeney, J., Birdsall, B., Roberts, G. C. K., and Burgen, A. S. V.,** Use of transferred nuclear Overhauser effect measurements to compare binding of coenzyme analogues to dihydrofolate reductase, *Biochemistry*, 22, 628, 1983.
161. **Filman, D. J., Bolin, J. T., Matthews, D. A., and Kraut, J.,** Crystal structures, of *Escherichia coli* and *Lactobacillus casei* dihydrofolate reductase refined at 1.7 Å resolution, *J. Biol. Chem.*, 257, 13663, 1982.
162. **Benner, S. A.,** The stereospecificity of alcool dehydrogenases: a stereochemical imperative?, *Experientia*, 38, 633, 1982.
163. **Nambiar, K. P., Stauffer, D. M., Kolodziej, P. A., and Benner, S. A.,** A mechanistic basis for the stereoselectivity of enzymatic transfer of hydrogen from nicotinamide cofactors, *J. Am. Chem. Soc.*, 105, 5886, 1983.

164. **von Wartburg, J.-P. and Wermuth, B.,** Aldehyde reductase from human tissues, *Methods Enzymol.*, 89, 506, 1982.
165. **Donninger, C. and Popjack, G.,** Studies on the biosynthesis of cholesterol. XVIII. The stereospecificity of mevaldate reductase and the biosynthesis of asymmetrically labelled farnesyl pyrophosphate, *Proc. R. Soc. London, B*, 163, 465, 1966.
166. **Lane, R. S. and Dekker, E. E.,** 2-Keto-4-hydroxybutyrate. Synthesis, chemical properties, and as a substrate for lactate dehydrogenase of rabbit muscle, *Biochemistry*, 8, 2958, 1969.
167. **Chakravorty, M., Veiga, L. A., Bacila, M., and Horecker, B. L.,** Pentose metabolism in *Candida*. II. The diphosphopyridine nucleotide-specific polyol dehydrogenase of *Candida utilis*, *J. Biol. Chem.*, 237, 1014, 1962.
168. **Labelle, E. F. and Hajra, A. K.,** Enzymatic reduction of alkyl and acyl derivatives of dihydroxyacetone phosphate by reduced pyridine nucleotides, *J. Biol. Chem.*, 247, 5825, 1972.
169. **Freudenthal, R. I., Kepler, J. A., and Cook, C. E.,** The preparation of stereospecific tritium-labeled reduced nicotinamide adenine dinucleotide phosphate, *Anal. Biochem.*, 51, 632, 1973.
170. **Viola, R. E., Cook, P. F., and Cleland, W. W.,** Stereoselective preparation of deuterated reduced nicotinamide adenine nucleotides and substrates by enzymatic synthesis, *Anal. Biochem.*, 96, 334, 1979.
171. **Arnold, L. J., Jr. and You, K.,** The hydride transfer stereospecificity of nicotinamide adenine dinucleotide linked enzymes: a proton magnetic resonance technique, *Methods Enzymol.*, 54, 223, 1978.
172. **Branden, C.-I. and Eklund, H.,** Structure and mechanism of liver alcohol dehydrogenase, lactate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase, in *Dehydrogenases Requiring Nicotinamide Coenzymes*, Jeffrey, J., Ed., Birkhauser Verlag, Basel, 1980, 41.
173. **Dickinson, F. M. and Dalziel, K.,** Substrate specificity and stereospecificity of alcohol dehydrogenases, *Nature (London)*, 214, 31, 1967.
174. **Cornforth, J. W., Cornforth, R. H., Donninger, C., Popjack, G., Rybak, G., and Schroepfer, J.,** Studies of the biosynthesis of cholesterol. XVII. The asymmetric synthesis of a symmetrical molecule, *Proc. R. Soc. London B.*, 163, 436, 1966.
175. **Donninger, C. and Ryback, G.,** The stereochemistry of hydrogen transfer catalyzed by liver alcohol dehydrogenase: 1R(1 − ^{3}H)geraniol as substrate, *J. Biochem.*, 91, 11, 1964.
176. **Karabatos, G., Fleming, J. S., Hsi, N., and Abeles, R. H.,** Comments on the factors controlling product stereospecificity in the reduction of carbonyl compounds with alcohol dehydrogenase and diphosphopyridine nucleotide, *J. Am. Chem. Soc.*, 88, 849, 1966.
177. **Culp, H. W. and McMahon, R. E.,** Reductase for aromatic aldehydes and ketones. The partial purification and properties of a reduced triphosphopyridine nucleotide-dependent reductase from rabbit kidney cortex, *J. Biol. Chem.*, 243, 848, 1968.
178. **Fukuba, R.,** Stereochemistry of hydrogen transfer between pyridine nucleotide and some intermediates of cholesterol catabolism catalyzed by liver alcohol and aldehyde dehydrogenase, *Biochim. Biophys. Acta*, 341, 48, 1974.
179. **Davidson, W. S., Walton, D. J., and Flynn, T. G.,** A comparative study of the tissue and species distribution of NADPH-dependent aldehyde reductase, *Comp. Biochem. Physiol.*, 60B, 309, 1978.
180. **Wermuth, B., Munch, J. D. B., and von Wartburg, J. P.,** Stereospecificity of hydrogen transfer of aldehyde reductase, *Experientia*, 35, 1288, 1979.
181. **Edgar, J. R. and Bell, R. M.,** Biosynthesis in *Escherichia coli* of *sn*-glycerol-3-phosphate, a precursor of phospholipid, *J. Biol. Chem.*, 255, 3492, 1980.
182. **Do Nascimento, K. H. and Davies, D. D.,** The stereospecificity of sequential nicotinamide-adenine dinucleotide-dependent oxidoreductases in relation to the evolution of metabolic sequences, *Biochem. J.*, 149, 553, 1975.
183. **Englard, S., Kaysen, G., and Avigard, G.,** 5-Keto-D-fructose. VI. A specific reduced nicotinamide adenine dinucleotide phosphate-linked reductase from yeast, *J. Biol. Chem.*, 245, 1311, 1970.
184. **Alizade, M. A., Bressler, R., and Brendel, K.,** Stereochemistry of the L-iditol dehydrogenase catalyzed hydrogen transfer to NAD, *Biochim. Biophys. Acta*, 370, 354, 1974.
185. **Flynn, T. G., Shires, J., and Walton, D. J.,** Properties of the nicotinamide adenine dinucleotide phosphate-dependent aldehyde reductase from pig kidney, *J. Biol. Chem.*, 250, 2933, 1975.
186. **Dansette, P. and Azerad, R.,** The shikimate pathway. II. Stereospecificity of hydrogen transfer catalyzed by NADPH-dehydroshikimate reductase of *E. coli*, *Biochimie*, 56, 751, 1974.
187. **Krakow, S. and Vennesland, B.,** The stereospecificity of glyoxylate reduction in leaves, *Biochem. Z.*, 338, 31, 1963.
188. **Dennis, D. and Kaplan, N. O.,** D- and L-lactic acid dehydrogenases in *Lactobacillus plantarium*, *J. Biol. Chem.*, 235, 810, 1960.
189. **Rose, I. A.,** The absolute configuration of dihydroxyacetone phosphate titrated by aldolase reaction, *J. Am. Chem. Soc.*, 80, 5835, 1958.
190. **Chan, P. C. and Bielski, B. H. J.,** Lactate dehydrogenase-catalyzed stereospecific hydrogen atom transfer from reduced nicotinamide adenine dinucleotide to dicarboxylate radicals, *J. Biol. Chem.*, 250, 7266, 1975.

191. **Long, G. L. and Kaplan, N. O.,** Diphosphopyridine nucleotide-linked D-lactate dehydrogenase from the horse shoe crab, *Limulus polyphemus*, and the sea worm, *Nereis virens*, *Arch. Biochem. Biophys.*, 154, 711, 1973.
192. **Savage, N. and Weight, M. B.,** The stereochemistry of hydrogen transfer catalyzed by D(−)-3-hydroxybutyrate dehydrogenase, *Biochim. Biophys. Acta*, 159, 401, 1968.
193. **Dugan, R. E. and Porter, J. W.,** Stereospecificity of the transfer of hydrogen from reduced nicotinamide adenine dinculeotide phosphate in each of the two reductive steps catalyzed by β-hydroxy-β-methylglutaryl coenzyme A reductase, *J. Biol. Chem.*, 246, 5361, 1971.
194. **Beedle, A. S., Munday, K. A, and Wilson, D. C.,** The stereochemistry of hydrogen transfer from NADPH catalysed by 3-hydroxy-3-methylglutaryl coenzyme A reductase from rat liver, *Eur. J. Biochem.*, 28, 151, 1972.
195. **Marcus, A., Vennesland, B., and Stern, J. R.,** The enzymic transfer of hydrogen. VIII. The reaction catalyzed by β-hydroxybutyryl-CoA dehydrogenase, *J. Biol. Chem.*, 233, 722, 1958.
196. **You, K. and Kaplan, N. O.,** Purification and properties of malate dehydrogenase from *Pseudomonas testosteroni*, *J. Bacteriol.*, 123, 704, 1975.
197. **Bernstein, L. H. and Everse, J.,** Studies on the mechanism of the malate dehydrogenase reaction, *J. Biol. Chem.*, 253, 8702, 1978.
198. **Graves, J. I., Vennesland, B., Utter, M. E., and Pennington, R. S.,** The mechanism of the reversible carboxylation of phosphoenol pyruvate, *J. Biol. Chem.*, 223, 551, 1956.
199. **Chen, R. F. and Plaut, G. W. E.,** Studies on the hydrogen transfer mediated by the DPN-linked isocitric dehydrogenase of heart, *Biochemistry*, 2, 752, 1963.
200. **Stern, B. K. and Vennesland, B.,** The stereospecificity of glutothione reductase for pyridine nucleotides, *J. Biol. Chem.*, 235, 209, 1960.
201. **Levy, H. R., Loewus, F. A., and Vennesland, B.,** The enzymic transfer of hydrogen. V. The reaction catalyzed by glucose dehydrogenase, *J. Biol. Chem.*, 222, 685, 1956.
202. **Levy, H. R., Graves, J. L., and Vennesland, B.,** Hydrogen transfer in DPN-linked enzyme reactions, *Fed. Proc.*, 15, (Abstr.), 300, 1956.
203. **Brendel, K., Bressler, R., and Alizade, M. A.,** Stereochemistry of the hydrogen transfer to NAD catalyzed by D-galactose dehydrogenase from *Pseudomonas fluorescens*, *Biochim. Biophys. Acta*, 397, 1, 1975.
204. **Ueberschär, K. H., Blachnitzky, E. O., Lehmann, J., and Kurtz, G.,** Stereospecificity of hydrogen transfer catalyzed by D-galactose dehydrogenase from *Pseudomonas saccharphila* and *Pseudomonas fluorescens*, *Biochem. Biophys. Acta*, 391, 15, 1975.
205. **Alizade, M. A., Bressler, R., and Brendel, K.,** Stereochemistry on the hydrogen transfer to NADP catalyzed by D-galactose dehydrogenase from *Pseudomonas fluorescens*, *Life Sci.*, 17, 787, 1975.
206. **Lessmann, D., Schimz, K. L., and Kurz, G.,** D-Glucose-6-phosphate dehydrogenase (Entner-Doudoroff enzyme) from *Pseudomonas fluorescens*, *Eur. J. Biochem.*, 59, 545, 1975.
207. **Betz, G. and Warren, J. C.,** Reaction mechanism and stereospecificity of 20β-hydroxysteroid dehydrogenase, *Arch. Biochem. Biophys.*, 128, 745, 1968.
208. **Gibb, W. and Jeffrey, J.,** The steric course with respect to the reduced nicotinamide-adenine dinucleotide of the reduction of 3-oxo-steroids catalyzed by cortisone reductase, *Eur. J. Biochem.*, 34, 395, 1973.
209. **Inano, H. and Tamaoki, B.-I.,** Relationship between steroids and pyridine nucleotides in the oxidoreduction catalyzed by the 17β-hydroxysteroid dehydrogenase purified from the porcine testicular microsomal fraction, *Eur. J. Biochem.*, 53, 319, 1975.
210. **Fidge, N. H. and Goodman, D. S.,** The enzymatic reduction of retinal to retinol in rat intestine, *J. Biol. Chem.*, 243, 4372, 1968.
211. **Walton, D. J.,** Stereochemistry of reduction of D-glyceraldehyde catalyzed by a nicotinamide adenine dinucleotide phosphate-dependent dehydrogenase from skeletal muscle, *Biochemistry*, 12, 3472, 1973.
212. **Arfin, S. M. and Umbarger, H. E.,** Some studies on the mechanism of action of α-acetohydroxy acid isomeroreductase, *Fed. Proc.*, 27, 454, 1968.
213. **Winicov, I.,** Stereospecificity of hydrogen transfer by phosphoglycerate dehydrogenase, *Biochim. Biophys. Acta.*, 397, 288, 1975.
214. **Slaughter, J. C. and Davies, D. D.,** The isolation and characterization of 3-phosphoglycerate dehydrogenase from peas, *Biochem. J.*, 109, 743, 1968.
215. **Seyama, Y., Kawaguchi, A., Kasama, T., Sasaki, K., Arai, K., Okuda, S., and Yamakawa, T.,** Identification of sources of hydrogen atoms in fatty acids synthesized using deuterated water and stereospecifically deuterium labelled NADPH by gas chromatographic mass spectrometric analysis, *Biomed. Mass Spectrom.*, 5, 357, 1978.
216. **Dutler, H., Coon, M. J., Kull, A., Vogel, H., Waldvogel, G., and Prelog, V.,** Fatty acid synthetase from pig liver. I. Isolation of the enzyme complex and characterization of the component with oxidoreductase activity for alicyclic ketones, *Eur. J. Biochem.*, 22, 203, 1971.

217. **Stoffel, W., LeKim, D., and Sticht, G.,** Stereospecificity of the NADPH-dependent reduction reaction of 3-oxodihydrosphingosine (2-amino-1-hydroxyoctadecane-3-one), *Hoppe-Seyler's Z. Physiol. Chem.*, 349, 1637, 1968.
218. **Alizade, M. A., Bressler, R., and Brendel, K.,** The stereospecificity of the hydrogen transfer from (R)-carnitine to NAD, catalyzed by (R)-carnitine dehydrogenase from *Psedomonas aeruginosa*, *Biochim. Biophys. Acta*, 364, 204, 1973.
219. **Englard, S., Avigad, G., and Profsky, L.,** 5-Keto-D-fructose. III. Proof of structure based on stereospecific patterns of enzymatic reduction, *J. Biol. Chem.*, 240, 2302, 1965.
220. **Winkler, N. W. and Markovitz, A.,** Guanosine diphosphate-4-keto-D-rhamnose reductase. A non-stereoselective enzyme, *J. Biol. Chem.*, 246, 5868, 1971.
221. **Saintot, M., Thaler-Dao, H., Descomps, B., and Crastes de Paulet, A.,** Stereospecificity of hydrogen transfer by the human placental 15-hydroxy prostaglandin dehydrogenase, *FEBS Lett.*, 72, 238, 1976.
222. **Tai, H.-H.,** Enzymatic synthesis of (15S)-[15-^{3}H]-prostaglandins and their use in the development of a simple and sensitive assay for 15-hydroxyprostaglandin dehydrogenase, *Biochemistry*, 15, 4586, 1976.
223. **Kersey, W. H. and Wilcox, R. B.,** Stereochemistry of hydrogen transfer by rat ovary 20α-hydroxysteroid dehydrogenase, *Biochemistry*, 9, 1284, 1970.
224. **Furfine, C. S.and White, A.,** Purification and properties of a bovine adrenal 21-dehydrosteroid reductase, *J. Biol. Chem.*, 243, 1190, 1968.
225. **Björkhem, I. and Hamberg, M.,** ω-Oxidation of fatty acids. II. Enzymatic oxido-reduction of 17-hydroxystearic acid, *J. Biol. Chem.*, 246, 7417, 1971.
226. **Herschlag, D. and Gander, J. E.,** Stereospecificity of ethanol oxidation by a multifunctional NAD-dependent oxidoreductase, *Fed. Proc.*, 42, 2177, 1983.
227. **Wyrambik, D. and Grisebach, H.,** Enzymic synthesis of lignin precursors. Further studies on cinnamyl-alcohol dehydrogenase from soybean-cell-suspension cultures, *Eur. J. Biochem.*, 97, 503, 1979.
228. **Berséus, O. and Björkem, I.,** Enzymatic conversion of a Δ^4-3-ketosteroid into a 3α-hydroxy-5β-steroid: mechanism and stereospecificity of hydrogen transfer from NADPH, *Eur. J. Biochem.*, 2, 503, 1967.
229. **Tai, H.-H. and Yuan, B.,** A simple and sensitive assay for 9-hydroxyprostaglandin dehydrogenase, *Anal. Biochem.*, 78, 410, 1977.
230. **Tishcov, V. I., Kulikov, N. S., and Egorov, A. M.,** Stereospecificity of hydride transfer to NAD-dependent enzyme bacterial formate dehydrogenase, *Biorg. Khim.*, 8, 80, 1982.
231. **Biellmann, J.-F., Eid, P., Hirth, C., and Jörnvall, H.,** Aspartate-β-semialdehyde dehydrogenase from *Escherichia coli*. Purification and general properties, *Eur. J. Biochem.*, 104, 53, 1980.
232. **Gross, G. G. and Kreiten, W.,** Reduction of coenzyme A thioesters of cinnamic acid with an enzyme preparation from lignifying tissue of *Forsythia*, *FEBS Lett.*, 54, 259, 1975.
233. **Abul-Hajj, Y. J.,** Stereospecificity of hydrogen transfer from NADPH by steroid Δ^4-5α- and Δ^4-5β-reductase, *Steroids*, 20, 215, 1972.
234. **Björkhem, I. and Holmberg, I.,** Mechanism of enzymatic reduction of steriod double bonds, *Eur. J. Biochem.*, 33, 364, 1973.
235. **Björkhem, I.,** Mechanism and stereochemistry of the enzymatic conversion of a Δ^4-3-oxosteroid into a 3-oxo-5α-steroid, *Eur. J. Biochem.*, 8, 345, 1969.
236. **Nozu, K. and Tamaoki, B.-I.,** Characteristics of the nuclear and microsomal steroid Δ^4-5α-hydrogenase of the rat prostate, *Acta Endocrinol.*, 76, 608, 1974.
237. **Mizugaki, M. and Uchiyama, M.,** Stereospecific hydrogen transfer by NADPH-enoyl coenzyme A reductase to *cis* and *trans*-isomers of 2-enoic acid, *J. Biochem. (Tokyo)*, 74, 691, 1973.
238. **Graves, J. L. and Vennesland, B.,** The stereospecific hydrogen exchange in the dihydroorotic dehydrogenase reaction, *J. Biol. Chem.*, 226, 307, 1957.
239. **Wilson, D. C., Munday, K. A., Skimmer, S. J. M., and Akhtar, M.,** The biological conversion of 7-dehydrocholesterol into cholesterol and comments on the reduction of double bonds, *Biochem. J.*, 106, 803, 1968.
240. **Stoffel, W. and Darr, W.,** 2-Alkenal reductase. Isolation, properties and specificities, *Hoppe-Seyler's Z. Physiol. Chem.*, 355, 54, 1974.
241. **Wilson, B. C., Watkinson, I. A., and Akhtar, M.,** The stereospecificity of hydrogen transfer from reduced nicotinamide adenine dinucleotide phosphate in the reduction of ethylenic linkages during cholesterol biosynthesis, *Biochem. J.*, 119, 673, 1970.
242. **Alizade, M. A., Bressler, R., and Brendel, K.,** Stereochemistry of the hydrogen transfer to NAD catalyzed by (S)alanine dehydrogenase from *Bacillus subtilis*, *Biochim. Biophys. Acta*, 397, 5, 1975.
243. **Geary, L. E. and Meister, A.,** On the mechanism of glutamine-dependent reductive amination of α-ketoglutarate catalyzed by glutamate synthase, *J. Biol. Chem.*, 252, 3501, 1977.
244. **Ohshima, T., Misono, H., and Soda, K.,** Properties of crystalline leucine dehydrogenase from *Bacillus sphaericus*, *J. Biol. Chem.*, 253, 5719, 1978.
245. **Boland, M. J. and Kennedy, L. D.,** Stereospecificity and NADH-H_2O hydrogen exchange of NADH-dependent glutamate synthase from *Lupin* nodules, *FEBS Lett.*, 108, 237, 1979.

246. **Blakely, R. L., Ramasastri, B. V., and McDougall, B. M.,** The biosynthesis of thymidylic acid. V. Hydrogen isotope studies with dihydrofolic reductase and thymidylate synthase, *J. Biol. Chem.*, 238, 3075, 1963.
247. **Pastore, E. J. and Friedkin, M.,** The enzymatic synthesis of thymidylate. II. Transfer of tritium from tetrahydrofolate to the methyl group of thymidylate, *J. Biol. Chem.*, 237, 3802, 1962.
248. **Ramasastri, B. V. and Blakely, R. L.,** 5,10-Methylenetetrahydrofolic dehydrogenase from Baker's yeast. III. Stereospecificity of hydrogen transfer in the reaction catalyzed by the enzyme, *J. Biol. Chem.*, 239, 112, 1964.
249. **Fujioka, M. and Takata, Y.,** Stereospecificity of hydrogen transfer in the sacchropine dehydrogenase reaction, *Biochim. Biophys. Acta*, 570, 210, 1979.
250. **Biellmann, J.-F., Branlant, G., and Olomucki, A.,** Stereochemistry of the hydrogen transfer to the coenzyme by octopine dehydrogenase, *FEBS Lett.*, 32(2), 254, 1973.
251. **Schrimsher, J. L. and Taylor, K. B.,** Octopine dehydrogenase from crown gall tumor and from *Pecten maximus*, *J. Biol. Chem.*, 257, 8953, 1982.
252. **Hoek, J. B., Rydström, J., and Höjeberg, B.,** Comparative studies on nicotinamide nucleotide transhydrogenase from different sources, *Biochim. Biophys. Acta*, 333, 237, 1974.
253. **Fisher, R. R. and Guillory, R. J.,** Resolution of enzymes catalyzing energy-linked transhydrogenation, *J. Biol. Chem.*, 246, 4687, 1971.
254. **Jacobs, E. and Fisher, R. R.,** Resolution and reconstitution of *Rhodosprillum rubrum* pyridine dinucleotide transhydrogenase: chemical modification with *N*-ethylmaleimide and 2,4-pentanedione, *Biochemistry* 18, 4315, 1979.
255. **Griffiths, D. E. and Robertson, A. M.,** Energy-linked reactions in mitochondria: studies on the mechanism of the energy-linked transhydrogenase reaction, *Biochim. Biophys. Acta*, 118, 453, 1966.
256. **Kawasaki, T., Satoh, K., and Kaplan, N. O.,** The involvement of pyridine nucleotide transhydrogenase in ATP-linked TPN reduction by DPNH, *Biochem. Biophys. Res. Commun.*, 17, 648, 1964.
257. **Lee, C. P., Simard-Duquesne, N., Ernster, L., and Hoberman, H. D.,** Stereochemistry of hydrogen transfer in the energy-linked pyridine nucleotide transhydrogenase and related reactions, *Biochim. Biophys. Acta*, 105, 397, 1965.
258. **Middleditch, L. E. and Chung, A. E.,** Pyridine nucleotide transhydrogenase from *Azotobacter vinelandii* cells: stereospecificity of hydrogen transfer, *Arch. Biochem. Biophys.*, 146, 449, 1971.
259. **Vennesland, B.,** Stereospecificity of hydrogen transfer in pyridine nucleotide dehydrogenase reactions, *Fed. Proc.*, 17, 1150, 1958.
260. **Stern, B. K. and Vennesland, B.,** The enzymatic transfer of hydrogen. IX. The reactions catalyzed by glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase, *J. Biol. Chem.*, 235, 205, 1960.
261. **Larsson, A. and Thelander, L.,** The stereospecificity of thioredoxin reductase for triphosphopyridine nucleotide, *J. Biol. Chem.*, 240, 2691, 1965.
262. **Ernster, L., Hoberman, H. D., Howard, R. L., King, T. E., Lee, C.-P., Mackler, B., and Scottacasa, G.,** Stereospecificity of certain soluble and particulate preparations of mitochondrial reduced nicotinamide-adenine dinucleotide dehydrogenase from beef heart, *Nature (London)*, 207, 940, 1965.
263. **Fisher, J. and Walsh, C.,** Enzymatic reduction of 5-deazariboflavine from reduced nicotinamide adenine dinucleotide by direct hydrogen transfer, *J. Am. Chem. Soc.*, 96, 4345, 1974.
264. **Fisher, J., Ramakrishman, K., and Becvar, J. E.,** Direct enzyme-catalyzed reaction of anthracyclines by reduced nicotinamide adenine dinucleotide, *Biochemistry*, 22, 1347, 1983.
265. **Spencer, R.,** Flavin Analogs and Mechanisms of Flavoenzyme Catalysis, Ph.D. thesis, Massachusetts Institute of Technology, Cambridge, 1978.
266. **Armarego, W. L.,** Hydrogen transfer from 4-R and 4-S (4-^{3}H) dihydropterin with dihydropteridine reductase from human liver and sheep liver, *Biochim. Biophys. Res. Commun.*, 89, 246, 1979.
267. **Ammeraal, R. N., Krakow, G., and Vennesland, B.,** The stereospecificity of the hill reaction with diphosphopyridine nucleotide, *J. Biol. Chem.*, 240, 1824, 1965.
268. **Bone, D. H.,** Inhibitor, isotopic and kinetic studies on hydrogen dehydrogenase, *Biochim. Biophys. Acta*, 67, 689, 1963.
269. **Krakow, G., Ammeraal, N., and Vennesland, B.,** The stereospecificity of the Hill reaction with triphosphopyridine nucleotide, *J. Biol. Chem.*, 240, 1820, 1965.
270. **Sih, C. J., Tsong, Y. Y., and Stein, B.,** The roles of reduced nicotinamide-adenine dinucleotide phosphate in steroid hydroxylation, *J. Am. Chem. Soc.*, 90, 5300, 1968.
271. **Light, D. and Walsh, C.,** Flavin analogs as mechanistic probes of adrenodoxin reductase-dependent electron transfer to the cholesterol side chain cleavage cytochrom P-450 of the adrenal cortex, *J. Biol. Chem.*, 255, 4264, 1980.
272. **Nelsestuen, G. L. and Kirkwood, S.,** The mechanism of action of the enzyme uridine diphosphoglucose-4-epimerase. Proof of an oxidation-reduction mechanism with direct transfer of hydrogen between substrate and the B-position of the enzyme bound pyridine nucleotide, *J. Biol. Chem.*, 246, 7533, 1971.

273. **Byun, S. M. and Jeness, R.,** Stereospecificity of L-*myo*-inositol-1-phosphate synthase for nicotinamide adenine dinucleotide, *Biochemistry*, 20, 5174, 1981.
274. **Drysdale, G. R.and Cohn, M.,** The stereospecificity of enzymatic interaction of diphosphopyridine nucleotide with water, *Biochim. Biophys. Acta.*, 21, 397, 1956.
275. **Hatefi, Y., Hanstein, W. G., Davis, K., and You, K.,** Structure of the mitochondrial electron transport system, *Ann. N.Y. Acad. Sci.*, 227, 504, 1974.
276. **Yamazaki, S., Tsai, L., Stadtman, T. C., Jacobson, F. E., and Walsh, C.,** Stereochemical studies of 8-hydroxy-5-deazaflavin-dependent $NADP^+$ reductase from *Methanococcus vannielii*, *J. Biol. Chem.*, 255, 9025, 1980.
277. **Light, D. R., Walsh, C., O'Callaghan, A. M., Goetzl, E. J., and Tauber, A. I.,** Characteristics of the cofactor requirements for the superoxide-generating NADPH oxidase of human polymorphonuclear leukocytes, *Biochemistry*, 20, 1468, 1981.
278. **Popjack, G., Schroepfer, G. L., Jr., and Cornforth, J. W.,** Stereospecificity of hydrogen transfer from reduced triphosphopyridine nucleotide to squalene during its synthesis from farnesyl pyrophosphate, *Biochem. Biophys. Res. Commun.*, 6, 438, 1962.
279. **Soda, K., Kido, T., and Asada, K.,** 2-Nitropropane dioxygenase from *Hansenula markii:* generation and participation of superoxide anion in the reaction, in *Biochemical and Medical Aspects of Active Oxygen*, Hayaishi, O. and Asada, K., Eds., University Park Press, Baltimore, 1976, 119.
280. **Little, J. W.,** Method for preparing NAD labelled with ^3H of high specific radioactivity in the 4-nicotinamide position, *Anal. Biochem.*, 48, 217, 1972.
281. **Chandrasekhar, K., McPherson, A., Jr., Adams, M. J., and Rossman, M. J.,** Conformation of coenzyme fragments when bound to lactate dehydrogenase, *J. Mol. Biol.*, 76, 503, 1973.
282. **Abdallah, M. A., Biellmann, J.-F., Nordström, B., and Brändén, C. I.,** The conformation of adenosine diphosphoribose and 8-bromoadenosine diphosphoribose when bound to liver alcohol dehydrogenase, *Eur. J. Biochem.*, 50, 475, 1975.
283. **Webb, L. E., Hill, E. J., and Banaszak, L. J.,** Conformation of nicotinamide adenine dinucleotide bound to cytoplasmic malate dehydrogenase, *Biochemistry*, 12, 5101, 1973.
284. **Abdallah, M. A., Adams, M. J., Archibald, I. G., Biellman, J.-F., Helliwell, J. R., and Jenkins, S. E.,** Binding of coenzyme and substrate and coenzyme analogues to 6-phosphogluconate dehydrogenase from sheep liver, *Eur. J. Biochem.*, 98, 121, 1979.
285. **Sundaralingam, M.,** The concept of a conformationally "rigid" nucleotide and its significance in polynucleotide conformational analysis, in *Conformation of Biological Molecules and Polymers*, Bergman, E. D. and Pullman, B., Eds., The Israel Academy of Sciences and Humanities, Jerusalem, 1973, 417.
286. **Holbrook, J. J., Liljas, A., Steindel, S. J., and Rossman, M. G.,** Lactate dehydrogenase, in *The Enzyme*, Vol. 11, 3rd ed., Boyer, P. D., Ed., Academic Press, New York, 1975, 191.
287. **Moras, D., Olsen, K. W., Sabesan, M. N., Buehner, M., Ford, G. C., and Rossman, M. G.,** Studies of asymmetry in the three-dimensional structure of lobster D-glyceraldehyde-3-phosphate dehydrogenase, *J. Biol. Chem.*, 250, 9137, 1975.
288. **Clore, G. M. and Gronenborn, A. M.,** Conformation of NAD^+ bound to yeast and horse liver alcohol dehydrogenase in solution. The use of proton-proton transferred nuclear Overhauser enhancement, *J. Mol. Biol.*, 157, 155, 1982.
289. **Bentley, R.,** Chemical methods for the investigation of stereochemical problems in biology, in *Stereochemistry*, Tamm, Ch., Ed., Elsevier, Amsterdan, 1982, 49.
290. **Campbell, H. D. and Young, I. G.,** Stereospecificity and requirements for activity of the respiratory NADH dehydrogenase of *Escherichia coli*, *Biochemistry*, 22, 2754, 1983.
291. **Sugiyama, T. and Mason, H. S.,** "Hydride" transfer from NADPH to NADPH-cytochrome P-450 reductase, *Fed. Proc.*, 43, 2061, 1984.