STEREOCHEMISTRY OF ENZYMIC HYDROGEN TRANSFER TO PYRIDINE NUCLEOTIDES

J. W. Cornforth, G. Ryback

(National Institute for Medical Research, London, England)

G. Popják, C. Donninger* and G. Schroepfer, Jr.**

(Medical Research Council, Experimental Radiopathology Research Unit, Hammersmith Hospital, London, England)

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The pyridine nucleotides, nicotinamide adenine dinucleotide (NAD^+) and its 3'-phosphoric acid derivative ($NADP^+$), with their reduced forms (NADH, NADPH), are coenzymes in a very large number of enzymic oxidations and reductions.

Westheimer, Vennesland and collaborators (1,2) first showed that when ethanol is oxidized by NAD^+ on the enzyme alcohol dehydrogenase (yeast), a hydrogen atom from the α-carbon of ethanol is transferred directly to give NADH, and that the same hydrogen atom is given back when the NADH reduces acetaldehyde on the same enzyme.

The chemical structure (I; R = adenosinediphosphoribosyl) of NADH has been deduced by Colowick (3) and others. The hydrogen atom transferred to and from substrates is one of the two attached to C-4 of the dihydronicotinamide ring. The two hydrogen atoms, which are stereochemically distinct, may be called H_A and H_B.

During the past few years it has been possible to divide a large number of pyridine-nucleotide-linked enzymes into two classes according to stereochemical specificity. Enzymes of class A transfer H_A to a substrate;

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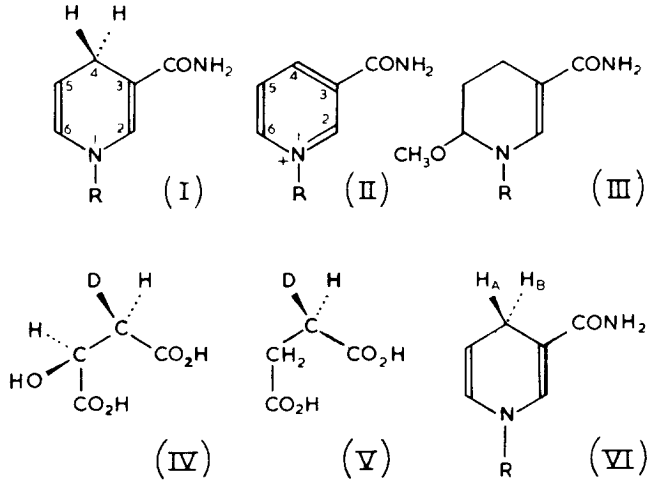

(I) (II) (III)

(IV) (V) (VI)

enzymes of class B transfer H_B. Since the reactions are reversible one can express this in another way: enzymes of class A add hydrogen to one side of the pyridine ring in NAD^+ (II; R = adenosinediphosphoribosyl); enzymes of class B add hydrogen to the other side. The two classes are tabulated in a recent review (4).

We demonstrate in this note which of the two hydrogen atoms at C-4 of NADH is H_A and which is H_B.

Two specimens of 4-deuterio-NADH were prepared. One of these (A) was made by enzymic transfer of deuterium from 3-methyl-2-butenol-1-D_2 to NAD^+ on crystalline liver alcohol dehydrogenase. Since this enzyme is of class A, the deuterium occupies the position of H_A. The second specimen was made by reducing 4-deuterio-NAD^+ (prepared by the exchange method of San Pietro (5)) by means of ethanol and alcohol dehydrogenase. The deuterium in this specimen (B) therefore occupies the position of H_B.

1,4-Dihydro-1-methylnicotinamide (I; R = CH_3) was prepared (6) by chemical reduction of nicotinamide methiodide. In dry methanol containing acetic acid this substance lost the characteristic absorption peak at 350 mμ, which was replaced during 12 hours at room temperature by a new peak at 286 mμ (ε = 15,500). This change is attributable to

formation of the adduct (III; R = CH_3) and is characteristic of 1, 4-dihydronicotinamides, including NADH, with nucleophilic agents in the presence of weak acids (6,7, 8). The product in acetic acid was treated with ozone, the peak at 286 mμ disappearing. Immediate further oxidation with peroxyacetic acid gave succinic acid, which must originate from positions 3, 4, 5, 6 of the nicotinamide ring; no other interpretation of the course of degradation appears tenable.

The same procedure was now applied to NADH. Analogous changes in light absorption were observed except that the purine absorption, which contributed to a broad composite peak in the acidic-methanol product, remained after ozonolysis. Succinic acid was isolated from the ether-extractable acidic products by distillation of the methyl ester, hydrolysis with acid, and two recrystallizations from water.

The two specimens A and B of 4-deuterio-NADH similarly gave deuterated succinic acids A and B which were assayed as methyl esters by mass spectrometry (9). Specimen A was approximately 88% and specimen B approximately 50% monodeuteriosuccinic acid. The lower deuterium content of specimen B is ascribed to incomplete exchange during preparation of 4-deuterio-NAD^+.

A third specimen (C) of monodeuteriosuccinic acid was prepared otherwise. Fumaric acid with crystalline fumarase in 99.8% deuterium oxide gave 3-deuteriomalic acid (IV). The absolute configuration of the acid prepared in this way has been demonstrated (10,11) to be 2S: 3R, as shown (IV). The dimethyl ester with thionyl chloride and pyridine gave dimethyl 2-chloro-3-deuteriosuccinate, which was reduced in acetic acid by the zinc-copper couple. Hydrolysis by acid and recrystallization from water gave C, which mass-spectrometric assay showed to be ca. 93% monodeuteriosuccinic acid. It follows from the mode of preparation that this is 2R-monodeuteriosuccinic acid (V).

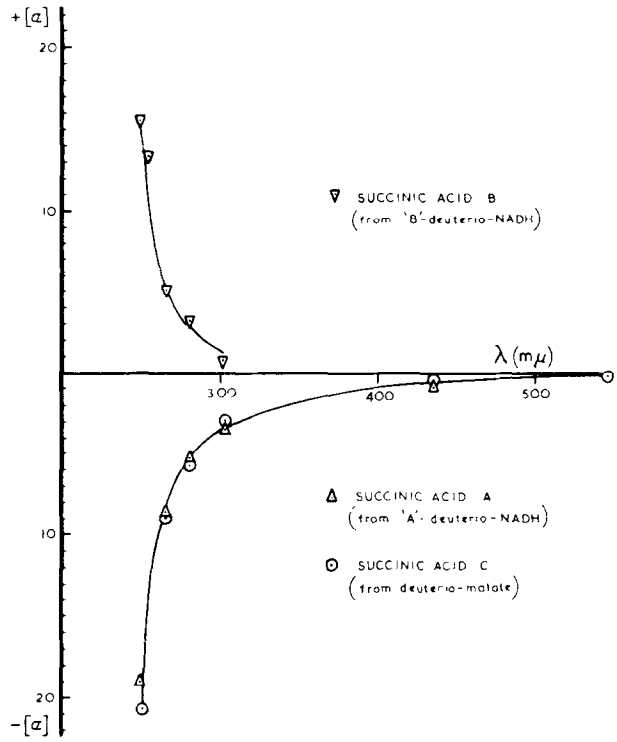

Fig.1 Optical rotatory dispersion of deuterio-succinic acids.

The optical rotatory dispersion curves of the three monodeuterio-succinic acids A, B and C were measured on a new spectropolarimeter developed at the National Physical Laboratory, Teddington and built by Bellingham and Stanley Ltd., London. The curves shown (Fig. 1) have been corrected for the actual content of monodeuteriosuccinic acid found by mass spectrometry. The ORD curve of sample C was the same for material recrystallized once and five times.

We consider that the close agreement in the ORD curves of A and C, and the opposite signs of rotation of the specimens A and B prepared by identical procedures from 4-deuterio-NADH, make it unlikely that traces of optically active impurity could have contributed significantly to the observed rotations.

It follows, therefore, that succinic acid A is R-monodeuterio-succinic acid, and thence that H_A and H_B in NADH have the stereochemistry

shown (VI); a recent correlation (12) proves that this is also true of NADPH. The conclusion is expressed in the following rule:

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 anti-clockwise order.

Acknowledgments

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