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BIOCHEMICAL STUDIES OF SOLUBLE AND IMMOBILIZED ALDEHYDE DEHYDROGENASE FROM YEAST

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Aldehyde dehydrogenase from baker's yeast was purified to homogeneity. The soluble and immobilized forms of this enzyme were characterized and compared biochemically. These included steady state kinetics, stability study, fluorescence, and NMR spectroscopy. Evidence of the coenzyme binding to this enzyme in the absence of aldehyde substrates was obtained by fluorescence and NMR studies. A significant quenching of protein fluorescence was observed upon the addition of coenzymes to the aldehyde-free enzyme solution. Significant shifts and broadening of coenzyme proton resonances in the presence of enzyme also indicate the enzyme–coenzyme interactions in the aldehyde-free solution. the enzyme was immobilized on glass beads by three different methods. The immobilized enzyme was found to exhibit physical and biochemical properties similar to those of the soluble enzyme. A system in which the immobilized alcohol, aldehyde, and steriod dehydrogenases are included in an enzyme reactor is described.

INTRODUCTION

Aldehyde dehydrogenase from baker's yeast is known to catalyze the irreversible oxidation of a broad range of aromatic and aliphatic aldehydes to the corresponding acids with the concomitant reduction of NAD^+ (1–4). Through investigation based on the kinetic evidence as well as fluorescence study, Bradbury and Jakoby showed that the reaction catalyzed by this enzyme follows a mechanism of ordered binding (3, 4); i.e. aldehyde binds to the enzyme first and then to the coenzyme. This is in contrast with the mechanism of many known dehydrogenases. Recently we have reexamined some of the biochemical properties of this enzyme and immobilized the enzyme on glass beads for comparisons. The application of immobilized aldehyde dehydrogenase in an enzyme reactor utilizing the concept of coenzyme recycling is also described in this communication.

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MATERIALS AND METHODS

Aldehyde dehydrogenase was purified from baker's yeast according to the procedures of Clark and Jakoby (1). To avoid proteolysis during the initial stages of purification, phenyl methyl sulfonyl fluoride[2] was included during the enzyme isolations. A homogeneous enzyme was obtained as judged from the specific activity, gel electrophoresis, and analytical centrifugations. The enzyme was assayed according to the previously described procedure (1). To remove aldehyde impurities in the prepared enzyme, the concentrated enzyme was dialyzed against 10 mM NAD^+ solution followed by extensive dialysis against 0.1 M Tris-HCl buffer, pH 8.0, or 0.1 M phosphate buffer containing 20% glycerol. Absence of aldehyde contamination in the dialyzed enzyme was demonstrated by the absence of 340–450 nm fluorescence after incubating the enzyme with NAD^+.

NAD^+ and NADH were purchased from P-L Biochemicals, Inc. (Milwaukee, Wisconsin). The coenzyme analogs, $(AcPy)AD^+$ and $(TN)AD^+$, were prepared in our laboratory. $(PyAd)AD^+$ and NHD^+ were purchased from P-L Biochemicals. Benzaldehyde, D,L-glyceraldehyde, and glutaraldehyde were purchased from Aldrich Chemical Company (Milwaukee, Wisconsin). Acetaldehyde was obtained from Mallinckrodt Chemical Works. *N*-Methyl pyridine aldehyde was prepared by methylation of pyridine 3-aldehyde with methyl iodide.

Steady state kinetics were studied using a Perkin-Elmer spectrophotometer, Model 126, equipped with an automatic recorder. Fluorescence study was performed with an Aminco fluorometer, Model SPF 125. A 2-mm slit width was used for routine measurements. To avoid concentration quenching the absorbance of the components in the cell was kept below 0.1 OD at the excitation wavelength.

Binding of coenzymes and aldehydes to the enzyme was studied with an HR-220 NMR spectrometer equipped with a Nicolet 1074 time-averaging computer. The measurements were made at 23 ± 1°C in solutions containing 10 mM β-mercaptoethanol, 10% glycerol, and 0.02 M Tris-HCl, pH 8.0, in 99.7% D_2O. Tetramethyl ammonium chloride was employed as an internal standard for chemical shift measurements.

Selected porous glass beads (550Å) were used for the immobilization of the enzyme and were obtained from the Corning Glass Works. Covalent coupling of the enzyme to glass beads followed the published procedures (5).

[2]Abbreviations used: PMSF, phenyl methyl sulfonyl fluoride; $(AcPy)AD^+$, 3-acetyl pyridine analog of NAD^+; $(TN)AD^+$, 3-thionicotinamide analog of NAD^+; $(PyAd)AD^+$, 3-pyridine aldehyde analog of NAD^+; NHD^+, hypoxanthine analog of NAD^+; ALDH, yeast aldehyde dehydrogenase; ADH, alcohol dehydrogenase; SDH, steroid dehydrogenase; NMR, nuclear magnetic resonance; DAA, reduced NAD^+-acetaldehyde adduct.

Three different types of immobilized enzymes were prepared: (a) by coupling in the presence of water-soluble carbodiimide to the alkylamine glass, (b) by diazotization on the arylamine glass, and (c) by cross-linking with glutaraldehyde on the alkylamine glass. The glutaraldehyde-linked enzyme on the glass beads was further treated with 0.1 M $NaBH_4$ solution for 30 min at neutral pH. The immobilized enzyme was assayed by using the apparatus previously designed in this laboratory (6).

RESULTS

General Kinetic Properties

This enzyme is known to catalyze the irreversible oxidation of various aldehydes to acids in the presence of K^+ or NH_4^+ (7). Acetaldehyde, glutaraldehyde, and benzaldehyde are the best substrates, whereas D,L-glyceraldehyde and pyridine aldehyde are relatively poor substrates. Substrate inhibitions were observed when greater than 1 mM of acetaldehyde or benzaldehyde was employed for assays.

NAD^+ can be replaced by a number of coenzyme analogs in the enzymatic reaction. Among these, $NADP^+$, NHD^+, and $(AcPy)AD^+$ exhibit 50%, 25%, and 15%, respectively, of the enzyme activity relative to NAD^+, whereas the rate of both $(TN)AD^+$ and $(PyAd)AD^+$ is less than 4%.

The relative rate of the dehydrogenase reaction was determined as a function of pH. It was found to increase with pH from 5.5 to 10, with denaturation at pH 10.5 occurring within the period of assays. A similar pH rate profile was observed for the immobilized enzymes on the glass beads. The results are presented in Fig. 1.

The apparent K_m for both aldehydes and coenzymes were measured for soluble and immobilized enzymes. Acetaldehyde exhibits lowest K_m among the aldehydes examined. K_m values for aldehydes vary significantly with the buffers and immobilization of the enzyme. K_m values for acetaldehyde in immobilized enzymes were one to two orders of magnitude higher than that obtained for the soluble enzyme. The results of this study are summarized in Table 1. In contrast, K_m values for the coenzyme NAD^+ do not vary significantly with the types of enzyme studied, i.e., soluble or immobilized. Among the coenzyme analogs, $(TN)AD^+$ exhibits the lowest K_m (7×10^{-6} M). Experimentally it was also observed that V_{max} for the dehydrogenase reaction using $(TN)AD^+$ as a coenzyme is only 6.7% that of NAD^+, and the reduction of $(TN)AD^+$ is stimulated by the presence of NAD^+ or NADH.

In agreement with the original observations by Bradbury and Jakoby (3), NADH is a competitive inhibitor of NAD^+ during the dehydrogenase

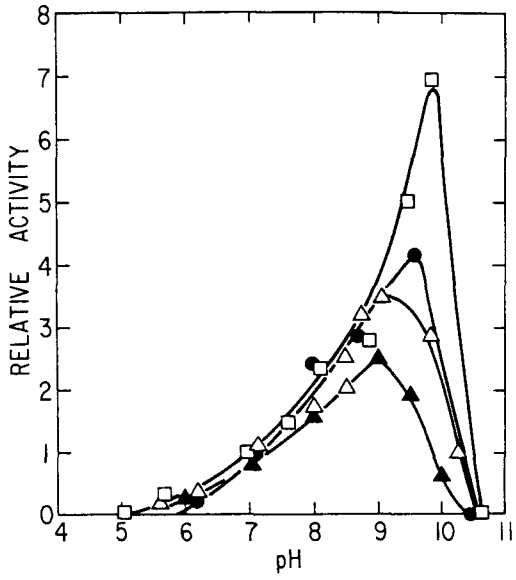

FIG. 1. pH-dependent activities of yeast aldehyde dehydrogenases. □, Benzaldehyde and the soluble enzyme; ●, acetaldehyde and soluble enzyme; △, acetaldehyde and immobilized enzyme (carbodiimide); ▲, acetaldehyde and immobilized enzyme (glutaraldehyde).

reaction. Although the K_i values obtained are approximately on the order of 10^{-5} M for both soluble and the immobilized enzymes, there is no apparent correlation between variations for K_m values of NAD^+ and K_i values of NADH. The condensation product of NAD^+ and acetaldehyde, reduced NAD-acetaldehyde adduct (DAA) inhibits the enzyme reaction with a K_i of 1×10^{-4} M. An esterase activity was detected in yeast enzyme with a K_m of 4.2×10^{-5} M, when *p*-nitrophenyl acetate was a substrate. It was inhibited by 1 mM acetaldehyde but stimulated by 10 μM NAD^+.

Binding Studies

Fluorescence. Evidence of the coenzyme binding to yeast aldehyde dehydrogenase in the absence of aldehyde was shown by the significant quenching of protein fluorescence upon the titration of the oxidized coenzymes. From the quenching of protein fluorescence (excitation at 280 nm and emission at 340 nm) as a function of the added coenzyme, the percentage of maximum quenching of protein fluorescence (ΔF) in the 100% complex and the dissociation constants (K_d) for the enzyme–coenzyme

TABLE 1. **Kinetic Constants for Soluble and Immobilized Aldehyde Dehydrogenase**

Enzyme	Substrates (buffer)	Coenzymes (inhibitors)	Reactions	K_m or K_i (M)
Soluble	Benzaldehyde (Tris)[a]	NAD^+	Dehydrogenase	$K_m 5.5\times10^{-5}$ (NAD^+)[b]
Soluble	Benzaldehyde (Tris)	(TN)AD^+	Dehydrogenase	$K_m 7\times10^{-6}$ [(TN)AD^+]
Soluble	Benzaldehyde (Tris)	NAD^+ [(TN)AD^+]	Dehydrogenase	$K_i 6\times10^{-6}$ [(TN)AD^+]
Soluble	Acetaldehyde (Tris)	NAD^+	Dehydrogenase	$K_m 1.5\times10^{-5}$ (Acetaldehyde)
Soluble	Acetaldehyde (Tris)	NAD^+	Dehydrogenase	$K_m 1.3\times10^{-4}$ (NAD^+)
Soluble	Acetaldehyde (K−P)[c]	NAD^+	Dehydrogenase	$K_m 3\times10^{-6}$ (Acetaldehyde)
Soluble	Acetaldehyde (Tris)	NAD^+ (NADH)	Dehydrogenase	$K_i 6.5\times10^{-5}$ (NADH)
Soluble	Acetaldehyde (Tris)	NAD^+(DAA)	Dehydrogenase	$K_i 1\times10^{-4}$ (DAA)
Immobilized A[d]	Acetaldehyde (Tris)	NAD^+	Dehydrogenase	$K_m 3.2\times10^{-4}$ (Acetaldehyde)
Immobilized A	Acetaldehyde (K−P)	NAD^+	Dehydrogenase	$K_m 2.2\times10^{-5}$ (Acetaldehyde)
Immobilized A	Acetaldehyde (K−P)	NAD^+	Dehydrogenase	$K_m 8\times10^{-5}$ (NAD^+)
Immobilized A	Acetaldehyde (K−P)	NAD^+(NADH)	Dehydrogenase	$K_i 1.5\times10^{-5}$ (NADH)
Immobilized B[e]	Acetaldehyde (K−P)	NAD^+	Dehydrogenase	$K_m 1.5\times10^{-4}$ (Acetaldehyde)
Immobilized B	Acetaldehyde (K−P)	NAD^+	Dehydrogenase	$K_m 2\times10^{-4}$ (NAD^+)
Immobilized B	Acetaldehyde (K−P)	NAD^+(NADH)	Dehydrogenase	$K_i 1\times10^{-4}$ (NADH)
Immobilized C[f]	Acetaldehyde (K−P)	NAD^+	Dehydrogenase	$K_m 5\times10^{-5}$ (Acetaldehyde)
Soluble	*P*-Nitrophenyl acetate (K−P)[g]		Esterase	$K_m 4.2\times10^{-5}$

[a] 0.1 M Tris buffer at pH 8.0, 5 mM mercaptoethanol, 0.2 M KCl.
[b] K_m of NAD^+ was determined under a constant aldehyde concentration of 0.5 mM. K_m of aldehyde was determined under a constant coenzyme concentration of 1 mM.
[c] 0.1 M potassium phosphate buffer at pH 8.0, 5 mM mercaptoethanol.
[d] Enzyme immobilized on the glass by carbodiimide-catalyzed coupling.
[e] Enzyme immobilized on the glass by diazo coupling.
[f] Enzyme immobilized on the glass by glutaraldehyde reaction, and then treated with 0.1 M $NaBH_4$ for 30 min.
[g] 0.1 M potassium phosphate buffer at pH 7.0.

TABLE 2. **Fluorescence Study**

Coenzyme	Substrate	K_d (M)	Maximum percentage of quenching of protein fluorescence
NADH (Tris)[a]		3×10^{-5}	60±10
NADH (Tris)	Acetaldehyde	1×10^{-5}	60±10
NAD^+(K−P)		$\sim6\times10^{-5}$	55±10
NADH (K−P)[b]		$\sim1\times10^{-5}$	45± 5
NADH (K−P)	Acetaldehyde	$\sim2\times10^{-6}$	35± 5
(TN)NAD^+		5×10^{-6}	50±10
NADH (Tris)No KCl		5×10^{-5}	50±10

[a] 0.1 M Tris buffer pH 8.0, 0.2 M KCl.
[b] 0.1 M potassium phosphate buffer at pH 8.0.

complex are given in Table 2. It can be seen that the K_d values for NAD^+–enzyme and (TN)AD^+–enzyme complexes are 6×10^{-5} and 5×10^{-6} M, respectively, which are close to the K_m values estimated from steady state kinetics. The K_d for NADH–enzyme complex in the absence of aldehyde was 3×10^{-5} M, which was also close to the K_i similarly determined.

In the absence of aldehydes, no enhancement of NADH fluorescence (340–450 nm) and little energy transfer (280–450 nm) were observed in the enzyme–coenzyme binary complex. The protein fluorescence was also quenched upon the addition of a high concentration of acetaldehyde or benzaldehyde (1 mM). In the presence of acetaldehyde (1.6 mM), the apparent K_d for the aldehyde–NADH–enzyme complex was lowered by one order of magnitude. However, the maximum percentage of quenching of protein fluorescence remained essentially unchanged. Besides protein quenching, significant enhancement of NADH fluorescence as well as the energy transfer from protein was observed upon the addition of enzyme to NADH solution in the presence of acetaldehyde. The results of this study are shown in Fig. 2.

A Scatchard plot was obtained from the data on quenching of protein fluorescence and the enhancement of NADH fluorescence upon the enzyme–coenzyme interactions. From these results, it is concluded that the tetrameric enzyme binds only 2 moles of coenzyme per mole of enzyme, which is consistent with the previous observations made by Bradbury and Jakoby (4). Further detailed studies together with stopped-flow experiment regarding this observation are published elsewhere (8).

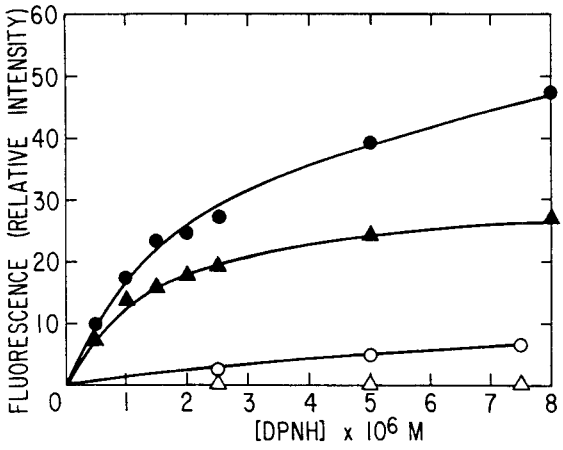

FIG. 2. Enhancement of NADH fluorescence (excited at 340 nm, emit at 450 nm) as well as protein energy transfer (excited at 280 nm, emit at 450 nm) as a function of NADH concentration in the presence and the absence of acetaldehyde. Protein concentration 60 μg/ml. ▲ and △ represent NADH fluorescence enhancement with and without 1.6 mM acetaldehyde, respectively. ● and ○ represent protein energy transfer in the presence and the absence of 1.6 mM acetaldehyde, respectively.

The quenching of protein fluorescence by NADH was found to be relatively pH independent in either the presence or absence of aldehyde. However, the enhancement of NADH fluorescence and the protein energy transfer were strongly pH dependent. The maxima of both fluorescence emissions occurred at pH 7.0.

Potassium ion was found to have no effect on protein fluorescence, although it increases the affinity of the coenzymes to the soluble enzyme.

Nuclear Magnetic Resonance. In order to show that coenzymes indeed bind strongly to aldehyde dehydrogenase in the absence of aldehyde, NMR spectroscopy was employed. Evidence for the binding of NAD^+ analog, $(AcPy)AD^+$, to yeast aldehyde dehydrogenase could be shown by a significant broadening and shift of coenzyme resonances upon the addition of coenzyme to a solution of aldehyde dehydrogenase (Fig. 3). The effect was observed to decrease with increasing coenzyme/enzyme concentration ratios. The extrapolated shifts of coenzyme resonances in the complex are similar to those for the binding of oxidized coenzymes to lactate dehydrogenase from chicken heart (9).

Benzaldehyde was also shown to bind the enzyme in the absence of reduced coenzyme. In a solution containing a benzaldehyde/enzyme

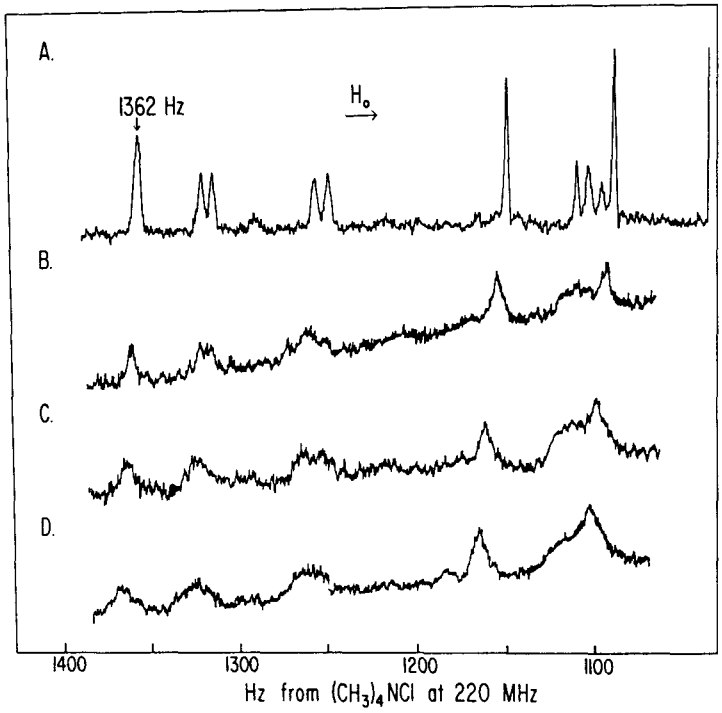

FIG. 3. 220 MHz proton NMR spectra of $(AcPy)AD^+$ in the absence (A) and the presence (B, C, D) of yeast ALDH. Experimental conditions: (A) $(AcPy)AD^+$ (5 mM) in 0.03 M Tris buffer at pH 8.0 containing 10% glycerol; (B) $(AcPy)AD^+$ (3.3 mM), yeast ALDH (0.4 mM), and KCl (0.16 M) in the same buffer as part A; (C) $(AcPy)AD^+$ (3.3 mM) and yeast ALDH (0.4 mM) in the same buffer as part A; and (D) $(AcPy)AD^+$ (2.2 mM) and yeast ALDH (0.4 mM) in the same buffer as part A. Resonances shown on the figure are those of acetylpyridine H_2, H_6, H_4, adenine H_8, acetylpyridine H_5, and adenine H_2, respectively, from left to right. (Subunit concentration of yeast ALDH was used.)

concentration ratio of 3 : 1, the aldehyde proton was observed to shift upfield 15 Hz. The ortho, para, and meta protons of the benzene ring were observed to shift upfield by 6, 12, and 2 Hz, respectively. The shift, as well as the line broadening of all the proton resonances of this substrate, also decreased with increasing substrate/enzyme binding ratios.

A significant broadening without an apparent shift in the resonance of NADH protons was observed in the presence of enzyme. Upon the addition of benzaldehyde to this binary solution, a gradual sharpening of proton resonance of the coenzyme was observed. The spectral behavior of the

binding between enzyme and coenzyme or aldehyde did not change significantly when a high concentration of potassium ion was added.

PHYSICAL CHEMICAL PROPERTIES OF SOLUBLE AND IMMOBILIZED ENZYMES

Both the soluble and immobilized enzymes are relatively heat stable. At 55°C and neutral pH, the enzyme was stable for at least 30 min without detectable loss of enzyme activity. Threefold increase of enzyme activity was observed at 55°C as compared to that at 25°C.

The enzyme was stable at pH 4.5 for at least 30 min, but denatured with time at pH 3.5 and 10.5. Both the soluble and the immobilized enzymes have the same pH stability.

In the presence of 5.3 M urea, 90% of the activity of the soluble enzyme was lost in the first 5 min of incubation. Under the same conditions, 30–50% enzyme activity remained for the immobilized enzymes. After 48 h of dialysis, 65% of the activity was recovered for the soluble enzyme whereas 90% of activity was recovered for the glutaraldehyde-linked immobilized enzyme.[3]

Coupled Enzyme Reactor

In a solution containing 1 mM NAD^+ and 0.1 M ethanol, the oxidation of ethanol was initiated by horse liver alcohol dehydrogenase. When 3×10^{-5} M of NADH (~0.2 OD at 340 nm) was produced, the addition of aldehyde dehydrogenase resulted in a two- to threefold burst of NADH formation before approaching a steady state. A similar phenomenon could also be demonstrated with immobilized enzymes. It was observed that in the coupled reactions, 90% of acetaldehyde produced from the oxidation of ethanol by alcohol dehydrogenase was further oxidized to acetic acid in the presence of aldehyde dehydrogenase.

A reaction mixture which initially contained alcohol, NAD^+, and 20-ketosteroid was monitored for the formation of 20-β-hydroxysteroid upon incubation with immobilized alcohol dehydrogenase, aldehyde dehydrogenase, and 20-β-hydroxysteroid dehydrogenase. The concentration 1×10^{-4} M NAD^+ is sufficient to catalyze the conversion of most of 20-ketosteroid to 20-β-hydroxysteroid. This conversion was monitored by a thin-layer chromatography (chloroform : acetone, 1 : 1). A general scheme for this coupled enzyme reactor is shown in Fig. 4.

[3]The enzyme immobilized by these three different methods was stable for months at 4°C.

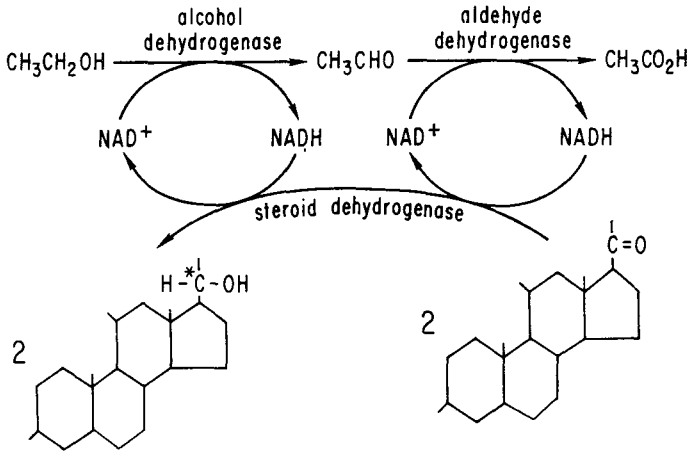

FIG. 4. Reaction schemes which describe the enzyme-catalyzed oxidation of ethanol to acetic acid with a concomitant enzymatic reduction of 2 equiv of 20-β-ketosteroid to 20-β-hydroxysteroid. In a typical experiment, 2 U each of the immobilized enzymes on glass beads were used, i.e., horse liver ADH (glutaraldehyde-linked), yeast ALDH (carbodiimide-linked), and SDH (glutaraldehyde-linked). In a shaken 20-ml vial, containing immobilized enzymes, 0.1 M ethanol, 1×10^{-4} M NAD^+, and 1 mg/ml of 20-β-ketosteroid (prednisolone) in 10 ml of 0.1 M potassium phosphate buffer at pH 7.5. Ten microliters of aliquot was removed every 30 min for monitoring the course of reaction by thin-layer chromatography.

DISCUSSION

Evidence of Coenzyme Binding to Aldehyde Dehydrogenase

Significant quenching of protein fluorescence was observed upon the addition of coenzymes to the enzyme solution, either in the presence or absence of the added aldehydes. Furthermore, the inability to reduce an oxidized coenzyme excludes the possibility of contamination by trace impurities of aldehyde in our enzyme preparation. Therefore it is evident that the quenching of protein fluorescence upon the titration of coenzymes is due to the formation of enzyme–coenzyme complexes in the absence of aldehydes. As shown in Table 2, the dissociation constants for the NAD^+–enzyme as well as the $(TN)AD^+$–enzyme complexes are very similar to the corresponding K_m values determined by the kinetic studies. In the presence of aldehyde the affinity of NADH to the enzyme is one order of magnitude higher than that in the absence of aldehyde.

Coenzyme fluorescence in the absence of aldehyde can be clearly distinguished from that in the presence of aldehyde: in the absence of aldehyde, no enhancement of coenzyme fluorescence and little energy

transfer from protein to coenzyme were observed, and in the presence of 1.5 mM of acetaldehyde, both of the phenomena were detectable. The fluorescent enhancement of the coenzyme, and protein energy transfer in the presence of aldehyde, is pH dependent. In contrast, the quenching of protein fluorescence by NADH is independent of both pH and aldehyde. These data indicate that the formation of a ternary complex consisting of enzyme, coenzyme, and aldehyde may induce conformational changes in the enzyme and coenzyme molecules. An amino acid residue near the active site with pK_a 7.0 may be responsible for the formation of ternary complex.

Binding studies using NMR provide independent evidence about the interaction of coenzyme and enzyme in the absence of aldehyde. The downfield shift and broadening of proton resonances of $(AcPy)AD^+$ in the presence of dehydrogenase indicate that the binding rate of oxidized coenzyme is fast on the NMR time scale. The estimated shifts of various coenzyme proton resonances also imply that the coenzyme appears to exist in an open conformation in the active site of this enzyme, similar to that observed for lactate dehydrogenase (9). The addition of potassium ion does not seem to change significantly the binding of coenzyme to dehydrogenase. The dissociation rate of enzyme–NADH complex is slow on the NMR time scale. The sharpening of the NADH resonances in the presence of benzaldehyde indicated that the dissociation rate for NADH is further decreased with the formation of the ternary complex.

Similar to aldehyde dehydrogenases from other sources (10, 11), esterase activity was also observed in the yeast enzyme. Much higher concentration of acetaldehyde was required to inhibit 50% of the esterase activity than that of coenzymes required for an equal enhancement of the esterase activity, indicating that the enzyme has a greater affinity to coenzymes than aldehydes.

Analogous to the case of lactate dehydrogenase from chicken heart which is inhibited by high concentrations of pyruvate (12), substrate inhibition in yeast aldehyde dehydrogenase could arise from the formation of the ternary complex among enzyme, NADH, and aldehyde.

Judging from the results of fluorescence titration and NMR studies, it is clearly shown that coenzymes can bind to yeast aldehyde dehydrogenase in the absence of aldehydes. Therefore the mechanism of the ordered binding of this enzyme originally proposed by Bradbury and Jakoby (3) is in question.

Comparisons between Soluble and Immobilized Enzyme

Generally, yeast aldehyde dehydrogenase immobilized by three different methods on glass beads exhibits physical as well as chemical properties similar to those of the soluble enzyme. The immobilized enzymes

exhibit slightly higher stability to heat, pH, as well as denaturation by salt or urea in comparison to the native enzyme. For example, after incubation at 70°C or in a solution of 5.3 M LiCl for 5 min, almost 90% of the activity of the native enzyme was lost. The immobilized enzyme still retained 30–50% of its original activity after the same treatment. The activity of the native enzyme after salt or urea treatment could be mostly recovered after 24 h of dialysis. The activity of the enzyme immobilized on glass beads by glutaraldehyde linkage was also recovered to a similar degree. However, the enzyme coupled by diazo linkage lost its activity after a similar treatment. This study may indicate that in some cases the immobilized enzyme is tightly linked to the solid support such that denaturation by salt or urea treatment does not permit reversible dissociation of the subunits.

With respect to its catalytic properties, the immobilized enzyme in general exhibits a K_m acetaldehyde that is one or two orders of magnitude higher than that of the native enzyme. However, the K_m for NAD^+ did not change significantly with the modification of enzyme. The inhibition by NADH (K_i) also varies with types of immobilized enzyme studied. The results of our study indicate that upon immobilization of the enzyme some of the catalytic properties of this enzyme remain unchanged.

Coupled Enzyme Reactors

Since the oxidation from aldehyde to acid catalyzed by yeast aldehyde dehydrogenase is essentially irreversible, the consecutive reactions using ethanol as a substrate can be oxidized to acetic acid with concomitant production of 2 mol NADH/mol ethanol. The burst increase in the rate of NADH formation upon the addition of yeast aldehyde dehydrogenase to the system of ethanol and alcohol dehydrogenase could be attributed to the relatively high affinity of yeast aldehyde dehydrogenase to acetaldehyde. In view of the low K_m observed for the oxidation of acetaldehyde catalyzed by yeast aldehyde dehydrogenase, the high reducing power can be obtained from such a coupled enzyme reactor using ethanol as a substrate.

As shown in Fig. 4, one of the potential applications of these coupled enzyme reactions is the generation of high reducing power (i.e., 2 mol NADH/mol ethanol) which can then be used to produce more valuable reduced substrates than alcohol. Our preliminary studies have demonstrated that these reactions can be further coupled with 20-β-hydroxysteroid dehydrogenase in a system of immobilized enzyme reactors. High reducing power of ethanol can be transmitted to 20-β-ketosteroid via a catalytic amount of NAD^+. With the consumption of only half of the equivalent of ethanol, quantitative amounts of the optically active 20-β-hydroxysteroid can be produced with a relatively high efficiency.

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