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ESTERASE ACTIVITY OF LIVER ALCOHOL DEHYDROGENASE

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SUMMARY

Liver alcohol dehydrogenase is found to possess, in addition to its dehydrogenase and dismutase activities, the ability to hydrolyze octanoate esters at a rate approximately 1/500 - 1/1000 of that of the dehydrogenase reaction. The esterase and dehydrogenase activities exhibit an identical isozyme pattern indicating that the same protein catalyzes both reactions. Inhibition studies suggest that the esterase activity presumably shares the catalytic domain with the dehydrogenase activity.

INTRODUCTION

Alcohol dehydrogenase (alcohol:NAD^+ oxidoreductase, EC 1.1.1.1) from horse liver (LADH[1]) is characterized by a broad substrate specificity. The enzyme catalyzes the oxidation of aliphatic, alicyclic and aromatic primary as well as secondary alcohols (1). In addition, the enzyme has been reported to mediate the dismutation of aldehydes to the corresponding acids (2,3). However, the physiological significance of the dismutase reaction is questionable (3). The physiological oxidation of aldehydes to acids is catalyzed by a group of aldehyde dehydrogenases (4) which also exhibit esterase activity (5,6). Using p-nitrophenyl acetate as a substrate at pH 7.4, Hinson and Neal (7) failed to demonstrate the esterolytic activity of LADH. In view of the fact that the dismutase activity of LADH increases with an increase in carbon chain of substrates to reach a maximum at octanal with a higher optimum pH, we tested the ability

[1]Abbreviations used are: LADH, liver alcohol dehydrogenase; NPA, p-nitrophenyl acetate; NPO, p-nitrophenyl octanoate; PCMB, p-chloromercuribenzoate.

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of LADH to hydrolyze octanoate esters at pH 8.2 and were able to demonstrate the esterolytic activity of LADH.

MATERIALS AND METHODS

Alcohol dehydrogenase, NAD^+, p-nitrophenyl acetate (PNA), p-nitrophenyl octanoate (NPO), p-chloromercuribenzoate (pCMB) and iodoacetate were obtained from Sigma Chemical Co. Ethyl octanoate was a product of K & K Laboratories. i-Butyramide and pyrazole were purchased from Eastman Organic Chemicals. p-Nitrophenol, o-phenanthroline, dioxane (spectrograde), sodium thiocyanide and cadmium acetate were obtained from Fisher Scientific Co. LADH was dialyzed against distilled water overnight prior to use. Isozymes were separated by CM-cellulose (CM-52, Whatman)column chromatography as described (8). The enzyme concentration was estimated from A_{280} taking $\varepsilon^{1\%}_{1cm} = 4.2$ and a subunit weight of 42,000 (9).

The dehydrogenase activity of LADH was assayed as described (10). The esterase activity of LADH was assayed spectrophotometrically by following the rate of p-nitrophenol release at 400 nm of a reaction mixture (2% dioxane) containing 0.10 mM NPO and 5.0 ± 2.5 μM LADH in 1.0 ml of 0.10 M borate buffer, pH 8.2 with a Beckman DB or Perkin-Elmer (model 124) spectrophotometer equipped with a variable output recorder. To correct the rate of spontaneous hydrolysis of NPO, an identical reaction was carried out in the absence of LADH. NPO was dissolved in dioxane due to its solubility and to minimize the spontaneous hydrolysis.

The titrimetric assay of the esterase activity was carried out by following the rate of acid liberation of a reaction mixture (6.0 ml) containing 0.10 M KCl, 0.20 mM ethyl octanoate and 5.0 μM LADH by means of a pH-stat (Radiometer TTT1a/SBR2c/SBU1) at pH 8.2. All assays were maintained at 30±2°C.

TABLE I. Spontaneous and LADH-catalyzed Hydrolysis of Ethyl Octanoate

Reaction	v(uncorrected rates) Acid Liberated ($\mu M\ min^{-1}$)	NADH formed ($\mu M\ min^{-1}$)
Et-octanoate alone	0.833	0
Et-octanoate + NAD^+	1.50	0
Et-octanoate + LADH	3.06	0
Et-octanoate + NAD^+ + LADH	3.35	4.18
NAD^+ + LADH	0	0.563

Rates of hydrolysis of ethyl octanoate (200 μM) in 0.10 M KCl were measured at pH 8.2 in the presence or absence of LADH (5.0 μM) and/or NAD^+ (1.0 mM). Octanoic acid liberation was measured titrimetrically by the base (1.0 mM NaOH) consumption and ethanol formation was followed spectrophotometrically at 340 nm according to the reaction sequences:

Ethyl octanoate → Ethanol + Octanoic acid

NAD^+ → $NADH + H^+$

Acetaldehyde

RESULTS AND DISCUSSION

An aqueous solution of ethyl octanoate underwent spontaneous hydrolysis and the rate of hydrolysis was facilitated by LADH (Table 1). The hydrolysis products of ethyl octanoate were monitored by following octanoic acid liberation and ethanol production indirectly *via* NADH formation. The stoichiometry of the LADH catalyzed hydrolysis of ethyl octanoate shows that the liberation of 1 mole of octanoic acid is accompanied by the reduction of 1 mole of NAD^+ by ethanol produced.

p-Nitrophenyl esters provide a convenient and direct spectrophotometric assay for esterases. A number of dehydrogenases have been shown to hydrolyze NPA (5,11,12). However this common substrate was not found to be hydrolyzed by LADH at pH 7.4 (7). At pH 8.2, NPA was hydrolyzed at 1/3-1/4 of the rate of NPO. This is hardly surprising since the reactivity of aldehydes in the LADH catalyzed dismutation increases with an increase in carbon chain to reach the optimum at octanal (3). Fig. 1 shows that NPO was readily hydrolyzed by LADH and the rate of hydrolysis was proportional to LADH concentrations. The specific activity of LADH to hydrolyze NPO at pH 8.2 is 1/500-1/1000

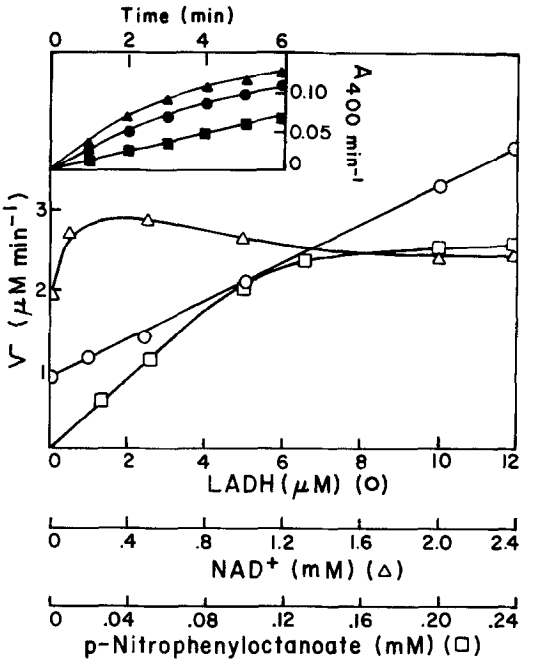

Fig. 1. Hydrolysis of NPO by LADH

Effect of LADH (O), NAD^+ (Δ) and NPO (□) on the rates of p-nitrophenol release in 0.10M borate buffer, pH 8.2 were measured spectrophotometrically at 400 nm. Experimental conditions were:
(O), 100 μM NPO with varied LADH concentrations
(Δ), 5.0 μM LADH + 100 μM NPO with varied NAD^+ concentrations
(□), 5.0 μM LADH with varied NPO concentrations
All rates were corrected for spontaneous hydrolysis. Inset shows time courses for p-nitrophenol release at pH 8.2 for spontaneous hydrolysis (■, NPO = 100 μM), LADH (2.5 μM) catalyzed hydrolyses of NPO (100 μM) in the absence (●) and presence (▲) of NAD^+ (0.10mM).

that of the dehydrogenase activity. Rates of spontaneous and LADH catalyzed hydrolyses of octanoate esters were slightly enhanced by NAD^+, however the co-enzyme was not essential for the esterolytic activity of LADH.

To examine the molecular identity between the esterolytic activity and LADH, the dehydrogenase was subjected to a CM-cellulose column chromatography which separates four ethanol-active isozymes. Fig. 2 shows the intimate correspondence between the esterase activity and dehydrogenase activity for all isoyzmes suggesting that the two activities presumably derive from the same protein molecule.

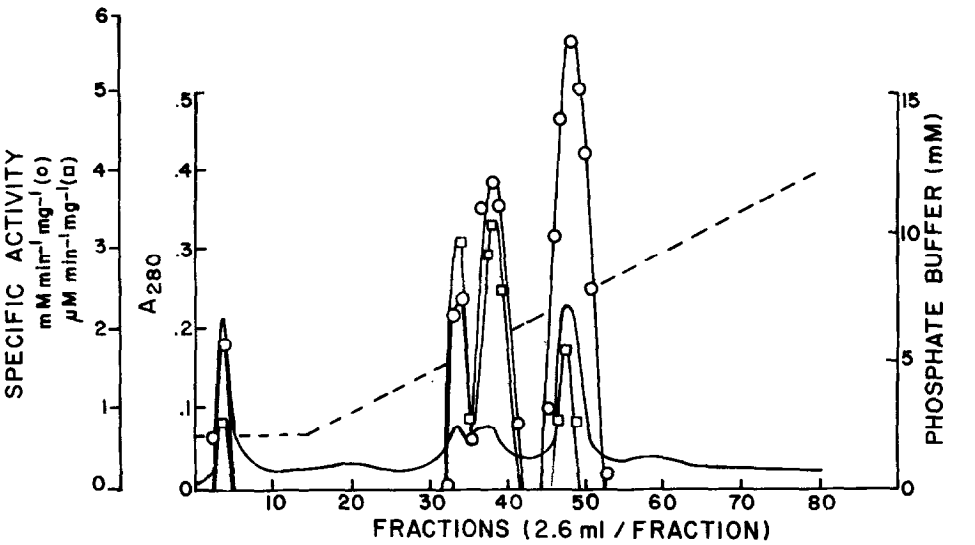

Fig. 2. CM-Cellulose Column Chromatography of LADH

Ten mg of LADH was dialyzed against 2.0 mM phosphate buffer, pH 7.0 overnight and delivered onto a CM-52 column (Sephadex K9/30). The enzyme was eluted with a linear gradient of an increased phosphate buffer (pH 7.0) concentrations from 2.0 mM to 20 mM. Buffer concentrations (---) of eluates was estimated from the conductivity and protein (——) was determined at 280 nm. The dehydrogenase (O) and esterase (□) activities were assayed spectrophotometrically at 340 nm and 400 nm (NPO) respectively as described in the text.

To further substantiate that LADH is the enzyme responsible for the esterase activity, effects of a number of inhibitors of LADH were tested (Table 2). o-Phenanthroline and thiocyanide which chelate to the active-site Zn^{++} (13) inhibited the esterase activity of LADH. The requirement of Zn^{++} for the esterase activity was also demonstrated by a decreased esterolytic activity of Cd^{++} - replaced LADH (14). Pyrazole and i-butyramide which competed with ethanol and acetaldehyde for LADH respectively (15,16), likewise, inhibited the LADH catalyzed hydrolysis of NPO. Treatment of LADH with iodoacetate (17) or pCMB (14) results in an inactivation of the esterase activity implicating the requirement of sulfhydryl group(s) for both activities.

It is noted that the esterase activity of aldehyde dehydrogenase and alcohol dehydrogenase differs in several aspects. NAD^{+} which greatly

Table II. Effect of LADH Inhibitors on Esterase Activity

Inhibitor	specific activity ($\mu M\ min^{-1}\ mg^{-1}$)	% Inhibition
None (control)	2.51	
1.0μM Pyrazole	0.752	69.9
500μM i-Butyramide	1.75	30.3
50μM o-Phenanthroline	1.25	50.2
50μM thiocyanide	1.50	40.2
0.05mM Cadmium acetate (2m.)	1.00	60.2
2.5mM Iodoacetate (10 min.)	0.752	69.9
0.50mM pCMB (15 min)	1.25	50.2

Alcohol dehydrogenase (5.0 μM) catalyzed hydrolysis of NPO (100 μM) in 0.10 M borate buffer, pH 8.2 were studied in the presence of inhibitors at the concentrations given. To study effects of cadmium ion (14), iodoacetate (15) and pCMB (14), LADH was incubated with these reagents at the concentration indicated for the specific time periods prior to assay. All rates were corrected for spontaneous hydrolysis.

promotes the hydrolytic reaction of aldehyde dehydrogenase, produces only a slight enhancement on the esterolytic reaction of alcohol dehydrogenase. i-Butyramide which inhibits both dehydrogenation and hydrolysis reactions of LADH was ineffective toward aldehyde dehydrogenase (18). No metal ion has been reported to be essential for aldehyde dehydrogenase, whereas Zn^{++} is essential for the dehydrogenase as well as esterase activities of LADH. However, both aldehyde and alcohol dehydrogenases require the sulfhydryl group(s) for the dual activities.

Thus, LADH was shown to possess esterolytic activity toward octanoate esters at pH 8.2, in addition to its oxidation-reduction activity. All isozymes exhibited both activities. Inhibition studies furnished evidence that the octanoate ester was hydrolyzed presumably at the same domain for the dehydrogenase activity of LADH.

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