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TWO ALDEHYDE DEHYDROGENASES FROM HUMAN LIVER

ISOLATION VIA AFFINITY CHROMATOGRAPHY AND CHARACTERIZATION OF THE ISOZYMES

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Summary

Human liver extracts show two major bands with aldehyde dehydrogenase (Aldehyde:NAD^+ oxidoreductase, EC 1.2.1.3) activity via starch gel electrophoresis at pH 7.0. Both bands have been purified to apparent homogeneity via classical chromatography combined with affinity chromatography on 5'-AMP-Sepharose 4B. The slower migrating band, enzyme 1, when assayed at pH 9.5 has a low K_m for NAD (8 μM) and a high K_m for acetaldehyde (approx. 0.1 mM). It is very strongly inhibited by disulfiram at pH 7.0 with a K_i of 0.2 μM. The faster migrating band, enzyme 2, has a low K_m for acetaldehyde, (2—3 μM at pH 9.5), a higher K_m for NAD (70 μM at pH 9.5), and is not inhibited by disulfiram at pH 7.0. The two enzymes are very similar to the F1 and F2 isozymes of horse liver purified by Eckfeldt et al. (Eckfeldt, J., Mope, L., Takio, K. and Yonetani, T. (1976) J. Biol. Chem. 251, 236—240) in molecular weight, subunit composition, amino acid composition and extinction coefficient. Preliminary kinetic characterizations of the enzyme are presented.

Introduction

Acetaldehyde, the first product of ethanol metabolism, is more toxic than alcohol itself. The toxicity may be due to both direct effects of the metabolite on cellular organelles and to inhibition of the enzymes that act on other aldehydes with metabolic significance [1]. While there have been numerous studies on the alcohol dehydrogenases which produce acetaldehyde from ethanol, there have been relatively few studies on the aldehyde dehydrogenases (aldehyde:NAD^+ oxidoreductase, EC 1.6.1.3), the enzymes which convert acetaldehyde to acetate. Racker, in 1949, first demonstrated the presence of aldehyde dehydrogenases in bovine liver [2] and there subsequently have been numerous

studies of aldehyde dehydrogenases in various mammalian tissues [3,4]. There have been very few isolations of the enzyme, however, due to its instability. The yeast enzyme was the first to be purified to homogeneity [5] followed by one aldehyde dehydrogenase isozyme of horse liver [6]. Recently a second isozyme of horse liver has been purified by Eckfeldt et al. [7]. To date there have been no total purifications of human aldehyde dehydrogenases. Kraemer and Dietrich [8] isolated and partially purified one enzyme from human liver. They found no evidence for additional enzymes. Blair and Bodley [9] also partially purified aldehyde dehydrogenases from human liver and separated two enzyme activity peaks, one of which they partially characterized. In a subsequent publication, Sidhu and Blair [10] have further purified one of the activity peaks and characterized it kinetically. They estimate that their preparation is 30% pure. We present here the purification of two isozymes of aldehyde dehydrogenase from human liver to apparent homogeneity. The key step in the purification involves affinity chromatography on AMP-Sepharose. Partial characterizations and disulfiram susceptibility of the isozymes are discussed.

Materials and Methods

Materials. Nicotinimide adenine diphosphopyridine nucleotide (NAD) was obtained from Sigma Chemical Co. Acetaldehyde and propionaldehyde were obtained from Eastman Organic Chemicals and resdistilled before use. DE-32 cellulose was obtained from Reeve Angel and Co. Sephadex G-100, 5′-AMP-Sepharose 4B, CM-Sephadex C-50, and DEAE-Sephadex A-50 were obtained from Pharmacia Fine Chemicals. All other chemicals were reagent grade.

Activity assay. During enzyme isolation the activity assay of Feldman and Weiner [6] was used. This assay mixture contains 0.1 M sodium pyrophosphate buffer, pH 9.0, 68 μM propionaldehyde and 0.3 mg/ml NAD. One unit of enzyme activity is defined as the amount of enzyme producing 1 μmol of NADH per min.

Kinetics. Enzyme kinetics were done under two conditions. For comparison with the work on the human enzyme the assay system of Sidhu and Blair [10] was employed. The assay mixture contains 33 mM pyrophosphate buffer, pH 9.5, 2 mM NAD and varying concentrations of aldehydes for the measurement of the aldehyde K_m values, and 13.5 mM propionaldehyde with varying concentrations of NAD for the measurement of the K_m of NAD. The assay system of Eckfeldt et al. [7] was used for comparisons with the horse liver enzymes. This assay mixture contains 100 mM sodium phosphate buffer, pH 7.0, with 1 mM NAD and varying concentrations of aldehyde for the aldehyde Michaelis constants and 13.6 mM propionaldehyde with varying concentrations of NAD for the measurement of the K_m of NAD. Disulfiram inhibition experiments were performed at pH 7.0 under the same conditions as the determination of the K_m of NAD. However, the solutions contained various amounts of disulfiram dissolved in ethanol. Assays were initiated with enzyme. The final concentration of ethanol was 0.17%. Ethanol at this concentration has no effect on the rate of aldehyde dehydrogenase from human liver.

Before the kinetic experiments were performed the enzymes were dialyzed against glass-distilled, deionized water that was saturated with nitrogen to

remove mercaptoethanol, glycerol, EDTA and any traces of endogenous aldehydes. The K_m values were determined from Lineweaver-Burk plots and Hill plots [11].

Protein assay. Protein was assayed via the method of Lowry et al. [12] with bovine serum albumin as standard. Protein concentrations of the purified enzyme were determined using the ultraviolet extinction coefficient at 280 nm.

Protein absorption spectra. The purified proteins were dialyzed against deionized water that was flushed with nitrogen and the solutions were filtered though 0.22 Å filters supplied by Millipore Corp. to remove dust. The spectra were read at the resulting isoionic values. The protein concentrations of the solutions were determined by the microbiuret procedure of Goa [13] using crystalline bovine serum albumin (Sigma) as standard.

Amino acid analysis. Samples of the enzymes were dialyzed against ion-exchanged water and hydrolysed with 6 M HCl. Analyses were performed by R. Poretz Laboratories, Rutgers University, New Brunswick, N.J.

Starch gel electrophoresis. Starch gel electrophoresis was performed in gel trays prepared with 12% Otto-Hiller Electrostarch. The gels contained 5 mM sodium phosphate buffer, pH 7.0, the buffer reservoirs contained 50 mM sodium phosphate buffer. The samples were electrophoresed for 18 h at a constant voltage, approx. 100 V, that gave an initial current of 15 mA. The enzyme activities were visualized in 0.1 M sodium pyrophosphate buffer at pH 9.0 containing 0.3 mg/ml NAD, 13.6 mM propionaldehyde, and Nitroblue Tetrazolium and phenazine methosulfate as described by Crow et al. [16].

Polyacrylamide gel electrophoresis. Polyacrylamide disk gel electrophoresis was performed in an apparatus described by Davis [14]. Continuous systems employing the following buffers and gel concentrations were used: 0.263 M Tris/acetate, pH 9.07, with 5% acrylamide-bisacrylamide (30 : 0.8, v/v); 0.0338 M Tris/acetate, pH 7.3, with 5 and 7% acrylamide-bisacrylamide (30 : 0.8, v/v); and 0.025 M sodium phosphate buffer, pH 6.0, with 5% acrylamide-bisacrylamide (30 : 0.8, v/v). The gels were polymerized as described by Davis [14]. Materials for the electrophoresis were obtained from BioRad Laboratories. Before application of the enzyme samples the gels were prerun for 1 h with the above buffers containing 10 mM 2-mercaptoacetic acid. The buffers containing mercaptoacetic acid were also used during the protein electrophoresis. The gels were stained for protein with Coomassie Brilliant Blue. They were stained for activity as described above for the starch gels. Before activity staining the gels were washed with 0.1 M sodium pyrophosphate buffer for 0.5 h.

Molecular weight determination. The molecular weights of the intact enzymes were determined on multiporosity thin slabs of polyacrylamide obtained from Pharmacia Fine Chemicals using conditions suggested by the manufacturer of the gels. Lactate dehydrogenase, catalase and bovine serum albumin were used as standards. Their mobility agreed with the calibration chart supplied by Pharmacia Fine Chemicals. The sodium dodecyl sulfate method of Laemmli [15] was employed to determine the subunit molecular weight. However, thin slabs of polyacrylamide rather than tubes were utilized.

Purification procedure

Human liver tissue was obtained at autopsy 6—8 h following death. The tissues were cut into 1-inch cubes and frozen until use.

Extraction. 600 g of liver were partially defrosted and homogenized with 600 ml of buffer containing 30 mM sodium phosphate, 1 mM EDTA, 0.1% 2-mercaptoethanol, pH 6.0 (Buffer 1). The resulting slurry was centrifuged at 13 000 × *g* to remove insoluble debris and the supernatant was dialyzed for 24 h against Buffer 1 and centrifuged again to remove denatured proteins.

CM-Sephadex chromatography. The spun, dialyzed supernatant was applied to a 50 × 4 cm column of CM-Sephadex C-50 equilibrated with Buffer 1. The enzyme activity all appeared in the eluate following the void volume. Fractions containing enzyme activity were pooled.

DEAE-Sephadex chromatography. The pH of the active CM eluate was raised to 6.8 with the addition of small aliquots of saturated Tris base and was applied to a 30 × 4 cm column of DEAE-Sephadex A-50 equilibrated with 30 mM phosphate, 1 mM EDTA, 0.1% 2-mercaptoethanol, pH 6.8 (Buffer 2). The column was washed with Buffer 2 until all non-bound protein was eluted as monitored by the ultraviolet absorption at 280 nm. A non-linear salt gradient was applied to the column with 1000 ml of Buffer 2 in the mixing chamber and 1000 ml of Buffer 2 containing 1 M NaCl in the buffer reservoir. (The column flowed by gravity pressure. As one drop of buffer was drawn from the mixing chamber it was replaced by one drop from the buffer reservoir. This arrangement results in a non-linear concave gradient.) 20-ml fractions were collected. The fractions with enzyme activity were pooled, glycerol was added to 20% concentration and the sample was divided into five batches which were frozen until the next step.

AMP-Sepharose chromatography. A frozen aliquot was thawed and the pH was adjusted back to 6.0 with 1 M sodium monobasic phosphate. The sample was applied to a 1.5 × 10 cm column prepared from 6 g of 5′-AMP-Sepharose 4B dry weight swelled and equilibrated with Buffer 1. The column was washed with Buffer 1 until the extraneous proteins were eluted. The column was then extracted with buffer containing 100 mM phosphate, 1 mM EDTA, 0.1% 2-mercaptoethanol, pH 8.0. 7-ml fractions were collected. The active fractions were pooled and brought to a concentration of 20% glycerol for storage. The column was regenerated by washing with 6 M guanidine · HCl and reequilibrating with Buffer 1.

Sephadex chromatography. The five aliquots from the 5′-AMP-Sepharose 4B step were pooled and divided into two batches. Each batch was concentrated to 1 ml using a 200 ml Amicon ultrafiltration apparatus equipped with an Amicon PM-10 membrane and applied to a 90 × 2 cm column of Sephadex G-100 equilibrated with Buffer 1. 7-ml fractions were collected. The active fractions were pooled and glycerol was added to 20% for storage in the freezer.

Separation of isozymes. The Sephadex eluates were concentrated to 1 ml as above and applied to a 30 × 2 cm column of DE-32 cellulose equilibrated with 25 mM sodium phosphate, 1 mM EDTA, 0.1% 2-mercaptoethanol and the column was washed with the same buffer. The elution was monitored by an ISCO ultraviolet absorbance monitor. One protein peak, containing enzyme 1, eluted directly after the vold volume. The column was washed with one column volume of buffer and a second protein peak, enzyme 2 was eluted by changing the buffer to 80 mM sodium phosphate, 1 mM EDTA, 0.1% 2-mercaptoethanol, pH 6.0.

Storage. For long term storage glycerol was added to 20% concentration to both isozymes and the fractions were frozen.

Results

Starch gel electrophoresis of tissue homogenates. The soluble fraction of whole liver homogenates (1 : 1 with water) electrophoresed at pH 7.0 show two major bands with aldehyde dehydrogenase activity which migrate towards the anode. The slower migrating band is enzyme 1. The faster migrating band is enzyme 2 *.

Purification. The results of a typical purification experiment are shown in Table I. The key step in the purification is chromatography on 5'-AMP-Sepharose 4B which provides a greater than 10-fold purification with a 90% yield. Purification is effected by binding the protein to the resin at pH 6.0 and eluting at high pH. NAD at a concentration up to 5 mM only partially elutes the activity at pH 6.0. NADH at a concentration of 0.5 mM totally elutes the activity at pH 6.0, however, purification is not as great due to the coelution of extraneous protein.

The complete purification procedure yields approx. 20 mg of enzyme 1 and 50 mg of enzyme 2 from 600 g of human liver in a pure state representing approximately a 20% total yield. The two enzymes copurify throughout all steps until the last DE-32 cellulose column. The elution profile of this column is shown in Fig. 1.

Using the assay system of Feldman and Weiner [6] the specific activity of enzyme 1 ranges from 0.4 to 0.6 μmol NAD reduced/min per mg, while the specific activity of enzyme 2 is 0.8—1.05 μmol/min per mg. Both of the puri-

TABLE I

PURIFICATION OF ALDEHYDE DEHYDROGENASES FROM HUMAN LIVER

Step	Total (ml)	Rate (μmol/ min/ml)	Total activity	Specific activity (units/mg)	mg/ml	Yield	Purification
Dialysed-spun homogenate	600	0.64	376	0.019	32.9	100	1.0
CM-Sephadex	650	0.34	220	0.031	10.9	59	1.6
DEAE-Sephadex	300	0.56	167	0.057	9.8	45	3.0
AMP-Sepharose	175	0.77	135	0.64	1.2	36	33.6
Sephadex G-100	98	0.90	88	0.70	1.3	23	36.8
DE-32 Cellulose							
Enzyme 1	90	0.14	12	0.58	0.2	3	30.5
Enzyme 2	76	0.67	51	1.01	0.66	13.5	53

* Preliminary subcellular fractionation of human tissue samples obtained at autopsy showed traces of enzyme 2 in the mitochondrial fraction, while enzyme 1 seemed to derive from the soluble fraction; however, these results are complicated due to the lability of the mitochondrial membranes following death.

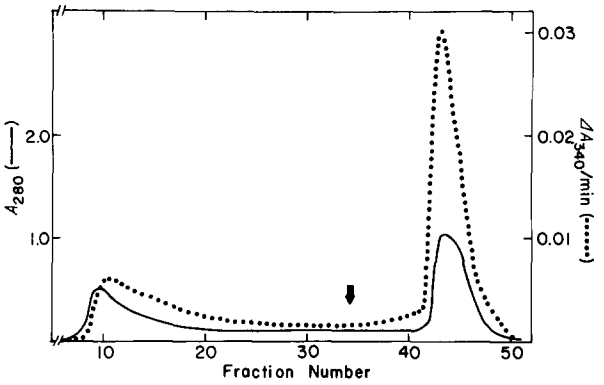

Fig. 1. The separation of human aldehyde dehydrogenases 1 and 2 on DE-32 cellulose. 7-ml fractions were collected. The arrow marks the beginning of the 80 mM phosphate buffer wash. 20 µl of enzyme were assayed in a final buffer volume of 3.02 ml.

fied isozymes appear homogeneous with less than 5% impurities on polyacrylamide gels under four different sets of pH and gel concentration. The enzymes separate from each other and each stain for activity at pH 6.0. At pH 7.0 both enzymes stain for activity but are superimposible on polyacrylamide gel with each other. At pH 9.0 the enzymes can once again be separated but neither stains for activity, possibly due to the instability of the enzymes at high pH. In the absence of stabilizing agents both isozymes are extremely labile and lose 50—90% activity overnight. The enzyme activity is also lost upon freezing and thawing. In the presence of 2-mercaptoethanol, 1 mM EDTA and 20% glycerol, however, the enzymes lose less than 10% activity per month.

Molecular weight. Multiporosity polyacrylamide electrophoreses give molecular weight estimates of 245 000 for enzyme 1 and 225 000 for enzyme 2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis gives subunit molecular weights of 54 800 and 54 200, respectively.

Amino acid composition. The amino acid frequency of acid hydrolysates of the two isozymes and of the horse liver isozymes [7] for comparison are shown in Table II. In amino acid composition, the isozymes are very similar both to each other and the horse enzymes.

Absorption spectra. The absorption spectra of both isozymes show one peak at 279 nm with a shoulder at 290 nm. Microbiuret determination of the protein concentrations gives an extinction coefficient at 280 nm of 0.96 and 1.00 for enzyme 1 and 2, respectively, at the respective isoionic pH values of 4.6 and 4.8. The 280 nm/260 nm ratios of the two isozymes at pH 6.0 in phosphate buffer are 1.73 and 1.67, respectively, suggesting an absence of bound cofactors. The similarity of the extinction coefficients for the human enzymes and the horse enzymes (0.95 and 1.05 respectively [7]) suggest a similar tryptophan content.

Kinetics. Table III shows the K_m values of the two enzymes for acetaldehyde, propionaldehyde and NAD. At pH 9.5 Lineweaver-Burk plots of the activity versus concentration are linear. Enzyme 2 has a high affinity for both acetaldehyde and propionaldehyde while enzyme 1 has a lower affinity for propionaldehyde and a very low affinity for acetaldehyde (approx. 0.1 mM). At pH 7.0

TABLE II

RELATIVE AMINO ACID FREQUENCY

Samples of each enzyme were hydrolyzed for 24, 48 and 72 h with 6 M HCl. The average of the three times is given with the exception of tyrosine and histidine which are the 24-h values. For purposes of comparison with the horse enzymes the data were normalized to the amino acid frequencies of Eckfeldt et al. [7] whose data represent the amino acid composition per 10 000 g.

Amino acid	Human		Horse	
	Enzyme 1	Enzyme 2	F1 Isozyme	F2 Isozyme
Aspartate	7.4	6.5	6.5	7.4
Threonine	3.8	3.9	4.3	4.1
Serine	4.3	5.5	4.1	3.6
Glutamate	5.8	3.5	7.8	8.1
Proline	6.1	5.3	4.9	4.4
Glycine	6.9	7.1	6.7	7.0
Alanine	5.9	6.7	5.6	7.2
Valine	5.6	7.1	5.3	6.6
Methionine	0.9	0.8	1.5	1.7
Isoleucine	4.2	3.1	3.6	3.0
Leucine	4.9	4.3	5.7	5.1
Tyrosine	1.9	1.4	2.1	2.3
Phenylalanine	3.0	3.0	3.6	3.4
Lysine	5.1	4.3	5.3	4.2
Histidine	1.2	1.8	1.0	1.0
Arginine	2.0	1.7	2.5	2.9

the K_m values for enzyme 2 are almost unchanged and Lineweaver-Burk plots are again linear. Enzyme 1, however, shows non-linear Lineweaver-Burk plots at pH 7.0 with apparent substrate activation or negative cooperativity for both acetaldehyde and propionaldehyde. The reciprocal plots for the activity as a function of NAD, however, are linear at both pH 7.0 and 9.5. The reciprocal plot of the variation in rate with acetaldehyde and propionaldehyde concentration of enzyme 1 is shown in Fig. 2. Over the concentration range of 0—50 μM the apparent K_m towards acetaldehyde and propionaldehyde are 30 and 5 μM, respectively. Over the range of 1—20 mM the apparent Michaelis constants rise to 2.5 and 1.0 mM, respectively.

TABLE III

KINETIC CONSTANTS OF HUMAN ALDEHYDE DEHYDROGENASES

Substrate	K_m(μM)				V	
	pH 7.0		pH 9.5		pH 9.5	
	Enz 1 *	Enz 2	Enz 1	Enz 2	Enz 1	Enz 2
Acetaldehyde **	2500 (30)	3.0	120	2.4	0.8	1.7
Propionaldehyde **	1000 (5)	0.7	11	1.2	0.9	1.2
NAD	40	70	8	70	—	—

* The Lineweaver-Burk plots are non-linear. The values listed represent the linear portion at high substrate concentration (1—20 mM). The values in parenthesis are the Michaelis constants over the concentration range 0—50 μM.

** The aldehyde concentrations are not corrected for hydration.

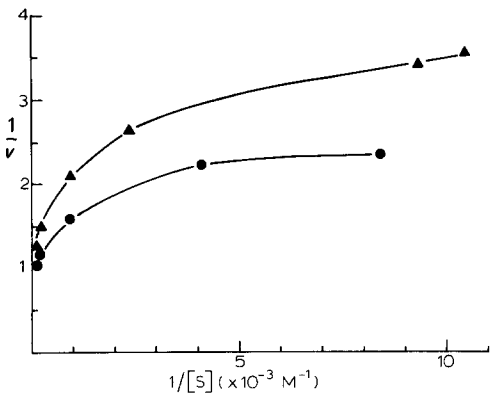

Fig. 2. The reciprocal plot of the activity of human aldehyde dehydrogenase 1 as a function of acetaldehyde and propionaldehyde concentration. ▲, acetaldehyde; ●, propionaldehyde.

A pH profile of the maximal rate with propionaldehyde as substrate for both enzyme 1 and enzyme 2 is shown in Fig. 3. Enzyme 1 has an activity maximum at pH 8.6 with a shoulder at pH 7.0. The percentage maximal activity at pH 7.0 is 66%. Enzyme 2, on the other hand, shows a single pH optimum above 9 and the activity drops to 15% maximal at pH 7.0.

Disulfiram inhibition. The inhibition of enzyme 1 and enzyme 2 by disulfiram, a drug used to promote alcohol aversion, was tested at pH 7.0 in 0.1 M sodium phosphate buffer with 13.6 mM propionaldehyde as substrate. Assays were initiated with enzyme. Under these conditions disulfiram does not inhibit enzyme 2 at a near saturating concentration of 40 μM. (The NAD concentration ranged from 0.04 to 4.0 mM.) In contrast, enzyme 1 is strongly inhibited by disulfiram which is a competitive inhibitor towards NAD with a K_i of 0.02 μM.

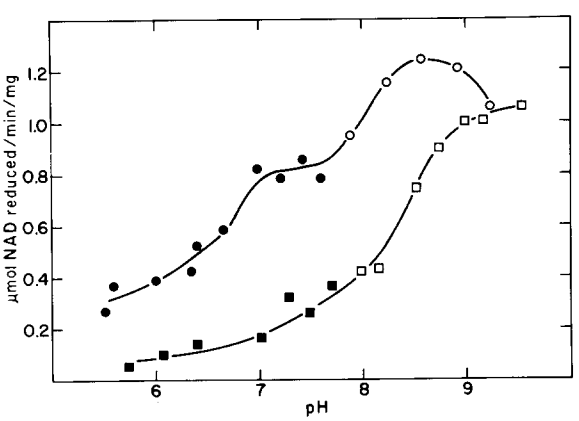

Fig. 3. The activity of human aldehyde dehydrogenases 1 and 2 as a function of pH. The assay mixture contained 0.02 M buffer, 0.9 M NaCl, 13.6 mM propionaldehyde and 0.3 mg/ml NAD. The open squares and circles represent data in sodium pyrophosphate buffer. The closed circles and squares represent data in sodium phosphate buffer. The upper curve (circles) is enzyme 1, the lower curve (squares) is enzyme 2.

Discussion

We have described a method for the purification of two aldehyde dehydrogenases from human liver. The key step involves chromatography on AMP-Sepharose. Since the two isozymes, while differing in charge and properties, copurified via this affinity method the procedure should be applicable to purify aldehyde dehydrogenases from different sources. The purification involves binding to the resin at low pH and eluting at pH 8.0 with a pH gradient. The two procedures give similar degrees of purification. Efforts to elute with NAD give low yields and poor purification. NADH at low concentrations leads to a high recovery of activity but poor purification, due to the coelution of nonspecific proteins. Kraemer and Deitrich [8] in a partial purification of human liver aldehyde dehydrogenase found evidence for only a single isozyme. Blair and Bodley [9], however, observed two activity peaks which were separable on DEAE-cellulose and probably correspond to enzyme 1 and enzyme 2. They only studied peak 2 due to the low yield and a purity of peak 1.

Enzyme 2 is very similar to the enzyme purified by Kraemer and Deitrich [8] and Blair and Bodley [9] in its high affinity for propionaldehyde and acetaldehyde and its pH maxima above 9.0. The enzyme 2 isolated herein appears to have a lower K_m for NAD (0.07 mM), however. Blair and Bodley [9] report a K_m of 0.2 mM and Kraemer and Deitrich [8] report a K_m of 0.6 mM. The differences in K_m values could represent interferences from contaminating proteins.

Blair and Bodley [9] suggested that their minor peak, peak 1, could be an isozyme of aldehyde dehydrogenase or may arise from partial proteolytic degradation of enzyme 2 or from binding of other molecules to yield complexes of different charge properties. Their first hypothesis, that the minor peak is an isozyme (enzyme 1), is probably correct as the enzymes isolated resemble each other in amino acid composition but differ greatly in kinetic properties and in sensitivity to disulfiram. Enzyme 1 is present in large quantities in the crude homogenate and indeed is the major component seen on starch gels and therefore probably does not represent a breakdown product of enzyme 2. Moreover, the purification procedure would tend to remove any bound impurities. The similarity of the ultraviolet spectra also suggest no differences in bound cofactors on the two enzymes.

The difficulty of previous investigators in purifying enzyme 1 may have several causes. First, the enzyme is unstable in the absence of glycerol and reducing agents. Second, the enzyme has a very high K_m for acetaldehyde at pH 9.5 so routine assays using buffers prepared with low acetaldehyde concentrations might not detect the activity. Finally, purified enzyme 1 is strongly inhibited by impurities present in commercial acetaldehyde and assays must be done with freshly distilled substrate. These impurities do not affect the activity of enzyme 2.

Recently, Eckfeldt et al. [7] have isolated two enzymes from horse liver that are similar to enzyme 1 and 2. One enzyme, F1, arises from the soluble fraction of the liver, has a high affinity for NAD and is strongly inhibited by disulfiram. It is similar to the human enzyme 1 and indeed their amino acid composition, spectra and molecular weight are very close. The second horse liver enzyme, F2,

arises from the mitochondrial fraction, has a high affinity for acetaldehyde and propionaldehyde and is only weakly inhibited by disulfiram. It is similar to enzyme 2. Two aldehyde dehydrogenases have similarly been isolated from sheep liver cytosol and mitochondria by Crow et al. [16] and a detailed paper on the inhibition of the sheep liver isozymes by disulfiram has been presented by Kitson [17].

An outstanding problem in alcohol metabolism is which enzyme is responsible for acetaldehyde oxidation in vivo. Eckfeldt et al. [7] suggest that in the horse the cytoplasmic enzyme (F1) is responsible due to its sensitivity to disulfiram, low K_m for NAD at pH 7.0 (3 μM) and reasonable K_m for acetaldehyde (70 μM). In the rat, however, it has been established that the mitochondria appear to be the major site of acetaldehyde metabolism [18—20]. At pH 7.0, human enzyme 1 has a low K_m for NAD (40 μM) and is strongly inhibited by disulfiram, however, the biphasic nature of the kinetics with acetaldehyde as substrate preclude a definitive answer as to its oxidative role in vivo. Biphasic kinetics have been observed for the kinetics of bovine brain [21] and pig brain [22] aldehyde dehydrogenases. Biphasic kinetics may have several causes. The first is that the enzymes exhibits cooperative behavior [11]. The second is that the enzyme preparation has several different isozymic components. The possibility of inhomogeneity of enzyme 1 cannot be positively ruled out; however, the enzyme is homogeneous in four different polyacrylamide gel systems and in two buffer systems on starch gel, and the kinetics with NAD and disulfiram inhibition do not show evidence of inhomogeneity. A third possibility is that the enzyme consists of non-equivalent subunits which have different affinities for acetaldehyde. Hopefully, future work on the kinetics and mechanism of the two enzymes will resolve the problem of their physiological roles.

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