Horse Liver Aldehyde Dehydrogenase

PURIFICATION AND CHARACTERIZATION OF TWO ISOZYMES*

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Two isozymes of horse liver aldehyde dehydrogenase (alddehyde, NAD oxidoreductase (EC 1.2.1.3)), F1 and F2, have been purified to homogeneity using salt fractionation followed by ion exchange and gel filtration chromatography. The specific activities of the two isozymes in a pH 9.0 system with propionaldehyde as substrate were approximately 0.35 and 1.0 pmol of NADH/min/mg of protein for the F1 and F2 isozymes, respectively.

The multiporosity polyacrylamide gel electrophoresis molecular weights of the F1 and F2 isozymes were approximately 230,000 and 240,000, respectively. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis gave subunit molecular weight estimates of 52,000 and 53,000 for the F1 and F2 isozymes, respectively. The amino acid compositions of the two isozymes were found to be similar; the ionizable amino acid contents being consistent with the electrophoretic and chromatographic behavior of the two isozymes.

Both isozymes exhibited a broad aldehyde specificity, oxidizing a wide variety of aliphatic and aromatic aldehydes and utilized NAD as coenzyme, but at approximately 300-fold higher coenzyme concentrations could use NADP. The F1 isozyme exhibited a very low Kₐ for NAD (3 pM) and a higher Kₐ for acetaldehyde (70 μM), while the F2 isozyme was found to have a higher Kₐ for NAD (50 μM) and a lower Kₐ for acetaldehyde (0.2 μM). The two isozymes showed similar chloral hydrate and p-chloromercuribenzoate inhibition characteristics, but the F1 isozyme was found to be several orders of magnitude more sensitive to disulfiram, a physiological inhibitor of acetaldehyde oxidation. Based on its disulfiram inhibition characteristics, it has been suggested that the F1 isozyme may be the primary enzyme for oxidizing the acetaldehyde produced during ethanol oxidation in vivo.

Biological oxidation of ethanol to acetate requires two NAD-linked dehydrogenases: alcohol dehydrogenase and aldehyde dehydrogenase. As a result of extensive studies over the past 25 years, it is now possible to discuss the structure-function correlation of alcohol dehydrogenase in detail. Studies on aldehyde dehydrogenase, however, are far less advanced. Since 1949 when Racker (1) showed that the dismutation of acetaldehyde into ethanol and acetate catalyzed by animal tissues was due to two separable enzymes, many NAD-linked aldehyde dehydrogenase enzymes from a number of sources have been studied (2, 3). Jakoby (4) first purified several isozymes of yeast to homogeneity but later showed the multiple isozymes to be due to in vitro proteolytic degradation of the native enzyme (5). Because liver is the primary site of mammalian ethanol oxidation in vivo, liver aldehyde dehydrogenase is considered to be of major importance in the regulation and control of ethanol metabolism. The presence of kinetically and physically distinct isozymes of aldehyde dehydrogenase in various subcellular fractions of rat liver is now well documented (3, 6-8). In order to understand the effects of ethanol on cellular metabolism, localization and characterization of each individual isozyme are important. We have developed a large scale method for purifying both major isozymes of horse liver to homogeneity and have investigated their physical and catalytic properties. This paper describes the purification and characterization of two isozymes, F1 and F2, of aldehyde dehydrogenase from horse liver, the latter of which appears to be identical with that studied by Okuda et al. (9) and purified to homogeneity by Feldman and Weiner (10, 11).

EXPERIMENTAL PROCEDURE

Materials—Reagent grade or purified chemicals were used throughout. Coenzymes were purchased from Sigma. Purified grade aldehydes were used as received; others were distilled under nitrogen. Ion exchange cellulose, Whatman CM52 and DE52, were obtained from Reef Angel and gel filtration resin, Bio-Gel A-1.5m, was obtained from Bio-Rad.

Activity Assay—To facilitate comparison to previous work, the activity assay of Feldman and Weiner (10) was used during the isolation procedure. One unit of aldehyde dehydrogenase activity has been defined as that amount of enzyme producing 1 pmol of NADH/min under these conditions (10).

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‡ Trainee in the United States Public Health Service Medical Scientist Training Program (GM 02046).
Purification Procedure

Fresh horse livers were obtained periodically from a local slaughter house. Within a few hours after removal, the liver was perfused with approximately 8 liters of 0.15 m NaCl to remove excess blood. The liver was then divided into approximately 800-g lots and frozen for later use.

Extraction—When beginning a preparation, an 800-g lot of liver was allowed to thaw partially and was then minced in a meat grinder. All subsequent procedures were performed below 5°, using nitrogen-saturated buffers and a nitrogen atmosphere where possible. To the minced liver, 1 liter of 0.25% mercaptoethanol containing 2 mM EDTA was added, and the mixture was homogenized for 3 min using a Torax Tissumizer. The insoluble debris was removed by centrifugation at 13,000 × g for 60 min.

Ammonium Sulfate Fractionation—To this extract, 2 ml of mercaptoethanol and 209 g/liter of solid ammonium sulfate were added. After dissolution of the ammonium sulfate, the slurry was allowed to stand 60 min before centrifugation at 24,000 × g for 60 min. The precipitate was discarded. To the supernatant, 129 g/liter of solid ammonium sulfate and 1 ml/liter of mercaptoethanol were added. After dissolution of the ammonium sulfate, the slurry was allowed to stand 90 min followed by centrifugation at 94,000 × g for 30 min. The supernatant was discarded, and the pellet suspended in approximately 400 ml of 10 mM sodium phosphate buffer, pH 6.3, containing 0.25% mercaptoethanol and 1 mM EDTA. This material was then dialyzed overnight against several 10-liter changes of the suspending buffer.

CM-cellulose Chromatography—The dialyzed material was reconcentrated at 24,000 × g for 30 min to remove insoluble material. The supernatant was then applied to a CM-cellulose column (4 × 15 cm) equilibrated with 10 mM sodium phosphate, pH 6.3, containing 0.25% mercaptoethanol and 1 mM EDTA. The column was equilibrated with the same buffer.

DEAE-column on which the Fl and F2 isozymes are separated is shown in Fig. 1. The Fl and F2 isozymes were combined as shown and purified further as described above. The final specific activities of the homogeneous F1 and F2 isozymes were generally 0.35 to 0.40 and 0.8 to 1.0 units/mg, respectively. Both purified isozymes appeared on acrylamide gel electrophoresis as single bands with less than 5% extraneous proteins. The Fl isozyme is particularly unstable when exposed to air even when EDTA and excess reducing agent (mercaptoethanol or sodium dodecyl sulfate) are present.

Table I

<table>
<thead>
<tr>
<th>Purification of two aldehyde dehydrogenase isozymes from horse liver</th>
<th>F1/F2 mixture</th>
<th>Total protein*</th>
<th>Total activity*</th>
<th>Specific activity</th>
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<tr>
<td></td>
<td>g</td>
<td>μmol NADH/min</td>
<td>μmol NADH/min</td>
<td>min/mg</td>
</tr>
<tr>
<td>Crude extract</td>
<td>61</td>
<td>1400</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>35% Ammonium sulfate</td>
<td>38</td>
<td>1100</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>50% Ammonium sulfate</td>
<td>24</td>
<td>800</td>
<td>33</td>
<td></td>
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<tr>
<td>CM-cellulose chromato-</td>
<td>12</td>
<td>500</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>graphy</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>F1 Isozyme</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>DEAE-cellulose chromato-</td>
<td>0.61*</td>
<td>100</td>
<td>160</td>
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<td>graphy imidazole buffer</td>
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<tr>
<td>Gel filtration chromato-</td>
<td>0.20*</td>
<td>74</td>
<td>370</td>
<td></td>
</tr>
<tr>
<td>graphy F2 Isozyme</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DEAE-cellulose chromato-</td>
<td>1.33*</td>
<td>200</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>graphy imidazole buffer</td>
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<td></td>
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<tr>
<td>Gel filtration chromato-</td>
<td>0.23*</td>
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<td>570</td>
<td></td>
</tr>
<tr>
<td>graphy F2 Isozyme</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DEAE-cellulose chromato-</td>
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<td>80</td>
<td>800</td>
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<td>graphy phosphate buffer</td>
<td></td>
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</tbody>
</table>

* By Lowry method (12) except as noted below.

* In the pH 9 sodium pyrophosphate buffer system with propionaldehyde substrate.

** Using E100 = 0.95 mg/ml cm-1 for the F1 isoenzyme.

** Using E100 = 1.08 mg/ml cm-1 for the F2 isoenzyme.
Lipid may be present in the purified samples, although possible
pared to the unextracted isozymes. These results suggest some
approximately 10% higher 280-nm extinction in 6 M guanidine
hydrochloride was observed after chloroform-methanol extrac-
tion. The lack of visible spectra and the high A280 coefficients at 280 nm in sodium phosphate, pH 7.0, were
stable, losing less than 5% activity per month.

Native Protein and Subunit Molecular Weights—As shown
in Fig. 2, multiporosity polyacrylamide gel electrophoresis gave molecular weight estimates for the F1 and F2 isozymes of
230,000 and 240,000. The results of sodium dodecyl sulfate-
polyacrylamide gel electrophoresis gave subunit molecular
weight estimates of 52,000 and 53,000.

Absorption Spectra—The absorption spectra of both iso-
zymes from 250 to 600 nm showed only a single peak at 280 nm
with no absorption in the visible region. The extinction
coefficients at 280 nm in sodium phosphate, pH 7.0, were
calculated to be 0.95 and 1.05 cm−1 mg/ml−1 for the Fl and F2
isozymes, respectively. The A280/A260 was 1.90 to 1.95 for both
the isozymes. The lack of visible spectra and the high A280/A260
indicate the isozymes are not flavoproteins nor do they
contain bound nucleotide as does glyceraldehyde-3-phosphate
dehydrogenase.

Amino Acid Composition—As seen in Table II, the two
isozymes have somewhat similar amino acid compositions.
Differences in ionizable amino acids agree with the electropho-
retic and chromatographic behavior. The cysteine contents as
measured by the mercury orange method of Sakai (18) were
calculated to be 1.1 and 0.8 free sulfhydryl groups/104 g of
protein for the Fl and F2 isozymes, respectively.

Because recoveries on amino acid analysis were slightly
lower than expected (81% for Fl and 82% for F2), tests for the
presence of lipids and carbohydrate were performed. An
approximately 10% higher 280-nm extinction in 6 M guanidine
hydrochloride was observed after chloroform-methanol extract-
tion of the distilled water-dialyzed, lyophilized proteins com-
pared to the unextracted isozymes. These results suggest some
lipid may be present in the purified samples, although possible
changes in water content caused by the extraction process
must be considered. No carbohydrate was detected in the
purified samples by the phenolsulfuric acid method of Hirs
(19).

Aldehyde Dehydrogenase Activity—As shown in Table III,
both isozymes show rather broad aldehyde specificity. The Km
values reported in Table III were derived from Lineweaver-
Burk plots with the aldehyde concentration in the solutions
being based either on the volume of pure aldehyde added or for
the low Km aldehydes, on NADH production after allowing the

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Fl isozyme</th>
<th>F2 isozyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>6.0</td>
<td>7.4</td>
</tr>
<tr>
<td>Threonine</td>
<td>4.3*</td>
<td>4.1*</td>
</tr>
<tr>
<td>Serine</td>
<td>4.1*</td>
<td>3.6*</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>7.8</td>
<td>8.1</td>
</tr>
<tr>
<td>Proline</td>
<td>4.9</td>
<td>4.4</td>
</tr>
<tr>
<td>Glycine</td>
<td>6.7</td>
<td>7.0</td>
</tr>
<tr>
<td>Alanine</td>
<td>5.6</td>
<td>7.2</td>
</tr>
<tr>
<td>Valine</td>
<td>5.3</td>
<td>6.6</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.5</td>
<td>1.7</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>3.6</td>
<td>3.0</td>
</tr>
<tr>
<td>Leucine</td>
<td>5.7</td>
<td>5.1</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>2.1</td>
<td>2.3</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>3.6</td>
<td>3.4</td>
</tr>
<tr>
<td>Lysine</td>
<td>5.5</td>
<td>4.2</td>
</tr>
<tr>
<td>Histidine</td>
<td>1.9</td>
<td>1.9</td>
</tr>
<tr>
<td>NH4</td>
<td>8.4</td>
<td>8.2</td>
</tr>
<tr>
<td>Arginine</td>
<td>2.5</td>
<td>2.9</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Cys SO3H</td>
<td>1.4</td>
<td>1.2</td>
</tr>
</tbody>
</table>

* These values represent extrapolation to zero hydrolysis time. All
others are the average of the three times.

* These values were obtained using performic acid-oxidized samples.

Fig. 1. Elution profile of aldehyde dehydrogenase isozymes from a
DEAE-cellulose column with a salt gradient in imidazole-hydrochlo-
ride. Following application of the dialyzed sample from the CM-cell-
ulose column, approximately 100 ml of 5 mM imidazole-hydrochloride
buffer, pH 7.2, containing 0.25% mercaptoethanol were passed
through. Then the aldehyde dehydrogenase isozymes were eluted using
a linear salt gradient prepared from 1 liter of 5 mM imidazole-hydro-
chloride, pH 7.2, and 1 liter of 5 mM imidazole-hydrochloride, pH 7.2,
with 200 mM NaCl, both solutions containing 0.25% mercaptoethanol.

Fig. 2. Molecular weight estimation of the native Fl and F2
isozymes. The molecular weights of the Fl and F2 isozymes were
estimated after electrophoresis on a thin slab polyacrylamide gel
containing a linear acrylamide concentration gradient perpendicular to
the direction of protein migration. Plots of log Rf versus position in the
concentration gradient were linear. The slopes of these semilog plots
versus the molecular weights of the protein standards are plotted
according to Hedrick and Smith (16). Protein standards are urease
(Sigma), rabbit muscle pyruvate kinase (gift of Drs. G. Reed and F.
Kayne, University of Pennsylvania School of Medicine), beef liver
catalase (Sigma), horse liver catalase (gift of Dr. R. Hershberg,
University of Pennsylvania School of Medicine), alcohol dehydrogenase
(Boehringer Mannheim), and bovine serum albumin (Sigma).

Table II

Aldehyde dehydrogenase isozymes
At least three separate samples of each isozyme were hydrolyzed for
24, 48, and 72 hours.

<table>
<thead>
<tr>
<th>Amino acid component</th>
<th>Fl isozyme</th>
<th>F2 isozyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>mol/100 g of protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>6.0</td>
<td>7.4</td>
</tr>
<tr>
<td>Threonine</td>
<td>4.3*</td>
<td>4.1*</td>
</tr>
<tr>
<td>Serine</td>
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<tr>
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<td>Proline</td>
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<tr>
<td>Glycine</td>
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<td>7.0</td>
</tr>
<tr>
<td>Alanine</td>
<td>5.6</td>
<td>7.2</td>
</tr>
<tr>
<td>Valine</td>
<td>5.3</td>
<td>6.6</td>
</tr>
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</tr>
<tr>
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<tr>
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<td>5.5</td>
<td>4.2</td>
</tr>
<tr>
<td>Histidine</td>
<td>1.9</td>
<td>1.9</td>
</tr>
<tr>
<td>NH4</td>
<td>8.4</td>
<td>8.2</td>
</tr>
<tr>
<td>Arginine</td>
<td>2.5</td>
<td>2.9</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Cys SO3H</td>
<td>1.4</td>
<td>1.2</td>
</tr>
</tbody>
</table>

* These values represent extrapolation to zero hydrolysis time. All
others are the average of the three times.

* These values were obtained using performic acid-oxidized samples.
zyme.

reaction to progress to completion. It is well known, however, that in aqueous solutions aldehydes exist as a mixture of the free aldehyde, RCHO, and the hydrate, RCH(OH)₂, the exact ratio being determined by the gem-diol equilibrium constant (20). It is likely that only one form is the true substrate for the dehydrogenase (21-23), and the large range in Kₘ values for different aldehydes may in part be due to the widely varying fractions existing as free aldehyde.

Both isozymes show a relative, but not absolute specificity for NAD over NADP as coenzyme. In sodium phosphate, pH 7.0, with 1 mM acetaldehyde as substrate, the Kₘ values for NAD and NADP were 3 μM and 700 μM, respectively, for the F₁ isozyme and 30 μM and approximately 10 mM, respectively, for the F₂ isozyme. For each isozyme, the Vₘₐₓ values with the two coenzymes were about the same.

**Inhibition Studies**—Chloral hydrate, p-chloromercuribenzoate, and disulfiram (tetraethylthiuram disulfide, Antabuse) were all found to be inhibitors of both isozymes. Using approximately 10 μg/ml of protein in the pH 7 system with 1 mM acetaldehyde and 1 mM NAD as substrates, the concentration of chloral hydrate resulting in 50% inhibition was approximately 0.25 mM and 0.4 mM for the F₁ and F₂ isozymes, respectively. Preincubation of dilute solutions of either isozyme with 1 μM p-chloromercuribenzoate without protective reducing agent gave complete inhibition. With disulfiram, a physiologically important inhibitor, the behavior of the F₁ and F₂ isozymes is markedly different. As shown in Fig. 3 approximately 30 μM disulfiram was required to give 50% inhibition of the F₂ isozyme in the above reaction mixture, while micromolar concentrations of disulfiram completely inhibited the F₁ isozyme. Further studies on the F₁ isozyme showed disulfiram to be an essentially stoichiometric inhibitor, requiring a single disulfiram per monomer for inhibition as shown in Fig. 4. Experiments showed the inhibition by disulfiram under these conditions to be complete within a minute; and that addition of a large excess of mercaptoethanol (0.4 M) leads to 90 to 100% reactivation of the inhibited enzyme.

**Discussion**

We have described a practical method for purification of large quantities of the two major isozymes of aldehyde dehydrogenase from horse liver. The principle molecular and catalytic properties have been ascertained. Both isozymes appear to be tetrameric with molecular weights about 200,000 to 250,000. There was no evidence on sodium dodecyl sulfate electrophoresis for nonidentical subunits in either isozyme. Both isozymes exhibit broad aldehyde specificity. One isozyme (F₁) had a very low Kₘ for NAD, and a higher Kₘ for acetaldehyde and a higher Kₘ for p-chloromercuribenzoate (PCMB). Approximately 30 μg of F₂ which had been extensively dialyzed to remove reducing agents were combined with p-chloromercuribenzoate (PCMB) or disulfiram (Di) in 1.5 ml of the standard pH 7.0 assay buffer. After 5 min incubation, 0.2 ml of 10 mM acetaldehyde/10 mM NAD was added to start the reaction. Inhibitor concentrations on the figure represent the final concentration in the 5.0-ml volume.

**Fig. 4 (right).** Disulfiram inhibition of the F₁ isozyme. Small samples (0.2 ml) of reducing agent free F₁ isozyme (0.5 mg/ml) were prepared using sodium phosphate buffer, pH 7.0, so as to contain increasing amounts of disulfiram (0 to 10 μM). A 5-min incubation, 20-μl aliquots were removed for assay in 1 ml of 5 mM acetaldehyde/0.5 mM NAD in the same pH 7.0 buffer.

**Fig. 3 (left).** Inhibition of the F₁ and F₂ isozymes by disulfiram and p-chloromercuribenzoate (PCMB). Approximately 30 μg of F₁ or 15 μg of F₂ which had been extensively dialyzed to remove reducing agents were combined with p-chloromercuribenzoate (●) or disulfiram (●) in 1.5 ml of the standard pH 7.0 assay buffer. After 5 min incubation, 0.2 ml of 10 mM acetaldehyde/10 mM NAD was added to start the reaction. Inhibitor concentrations on the figure represent the final concentration in the 5.0-ml volume.

**Table III.** Relative maximal velocities and Kₘ values of two isozymes for various aldehydes

<table>
<thead>
<tr>
<th>Aldehyde</th>
<th>R group</th>
<th>F₁ isozyme</th>
<th>F₂ isozyme</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>Relative Vₘₐₓ</td>
<td>Kₘ</td>
</tr>
<tr>
<td>Aliphatic aldehydes</td>
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<td>μM</td>
<td>μM</td>
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<tr>
<td>Isovaleraldehyde</td>
<td>(CH₃)₂CH CH₄—</td>
<td>1.1 0.5</td>
<td>0.6 &lt;0.1</td>
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<tr>
<td>Valeraldehyde</td>
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<td>0.7 &lt;0.1</td>
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<td>Propionaldehyde</td>
<td>CH₂CH₂CH₂—</td>
<td>1.2 0.5</td>
<td>1.4 0.3</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>CH₂—</td>
<td>1.0 70</td>
<td>1.0 0.2</td>
</tr>
<tr>
<td>Phenylacetaldehyde</td>
<td>(C₆H₅)CH₂—</td>
<td>1.9 4</td>
<td>1.4 0.3</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>H—</td>
<td>0.8 940</td>
<td>1.6 270</td>
</tr>
<tr>
<td>Glycolaldehyde</td>
<td>HOCH₂—</td>
<td>0.9 130</td>
<td>3.2 50</td>
</tr>
<tr>
<td>Chloroacetaldehyde</td>
<td>CHCl₂—</td>
<td>0.7 30</td>
<td>3.5 10</td>
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<tr>
<td>Conjugated aldehydes</td>
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<tr>
<td>Benzaldehyde</td>
<td>C₆H₅—</td>
<td>0.6 &lt;0.1</td>
<td>0.11 &lt;0.1</td>
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<tr>
<td>Cinnamaldehyde</td>
<td>(C₆H₅)CH—CH—</td>
<td>0.5 &lt;0.1</td>
<td>0.11 &lt;0.1</td>
</tr>
</tbody>
</table>

*The Vₘₐₓ values are relative to acetaldehyde as substrate for each isozyme. With 1 mM NAD and 1 mM acetaldehyde in this pH 7.0 buffer, the specific activities of the purified F₁ and F₂ isozymes are approximately 0.13 and 0.35 μmoles of NADH/min/mg, respectively.*
noted a minor activity peak eluting from a DEAE-column
before their major activity. We identify this minor peak as the
F1 isozyme. These previous investigators did not attempt to
purify or characterize it and because of its instability in
the presence of oxygen, probably underestimated its importance.
Because several reports of multiple isozymes, especially in
yeast, have subsequently been shown to be due to in vitro
modification of a native species with partial retention of
activity (5, 24, 25), this question was carefully considered. We
feel, however, that the evidence is overwhelmingly against one
of the two horse liver aldehyde dehydrogenase isozymes (F1 or
F2) being a degradation artifact. First, the F1 and F2 isozymes
are markedly different in their relative maximal velocities and $K_\text{m}$ values for the various substrates examined, and in their
sensitivities to disulfiram and phenylmethylsulfonylfluoride.3
In most cases of known proteolysis, the kinetic data from the
"isozymes" are very similar (5, 24). Second, no interconversion
of the purified F1 and F2 isozymes was ever detected, in
contrast to the case of proteolytic modification where inter-
conversion has been observed (5, 24). Third, subcellular localiza-
tion studies provided kinetic evidence that both isozymes are
present in the crude homogenate and that the F1 isozyme is
cytosolic and the F2 isozyme mitochondrial in origin (96, 97).
Finally, a fresh horse liver homogenate can be resolved into the
two isozymes within 30 min of cellular disruption using
DEAE-cellulose chromatography.4 In these latter experiments,
the F1 isozyme accounts for approximately 60% of the total
activity and hence is not a minor component in vivo.

From the physiological standpoint, the disulfiram inhibition
characteristics may be important in understanding ethanol
metabolism. Disulfiram (Antabuse) administration prior to
alcohol ingestion is known to produce a complex of very
unpleasant symptoms including nausea, vomiting, respiratory
distress, headaches, and so on (28). Classically, this drug is
thought to inhibit acetaldehyde dehydrogenase in vivo thereby
causing the greatly elevated blood acetaldehyde levels when
alcohol is consumed, and most, but not all, of the symptoms of
disulfiram administration followed by alcohol ingestion can be
obtained directly by acetaldehyde administration (28). Since
liver is the major site of alcohol and acetaldehyde metabolism,
it was surprising when Kraemer and Deitrich (30) first found
human liver aldehyde dehydrogenase to be relatively insensi-
tive to disulfiram. For such dramatic pharmacological effects,
one would suspect that if inhibition of acetaldehyde metabo-
lism were involved, the responsible enzyme(s) would be ex-
tremely sensitive to disulfiram. With the purification of the F1
isozyme, we now have a homogeneous enzyme that shows the
expected drug sensitivity. Similar disulfiram sensitive and
insensitive aldehyde dehydrogenase activities have been found
in rat liver homogenates (29).

While horse liver aldehyde dehydrogenase enzymes are
interesting in their own right from the standpoint of elucidating
enzyme mechanisms, they are primarily of interest as models for the physiology of alcohol metabolism with the
human enzyme system. While Kraemer and Deitrich (30) in a
partial purification of human liver aldehyde dehydrogenase
found evidence for only a single isozyme, Blair and Bodley (31)
more recently observed two isozymes which were separable on
DEAE-cellulose chromatography. Their study of the human
isozyme which was more firmly bound to the DEAE-cellulose
shows surprisingly many similarities to the F2 isozyme of horse
liver. Both show broad aldehyde specificity, sensitivity to
sulphydryl reagents, molecular weight near 200,000, high pH
optimum, micromolar range $K_\text{m}$ for aliphatic aldehydes, and
only moderate disulfiram inhibition (55% at 40 $\mu$M). While
more work on the human system is obviously needed, we are
encouraged that the readily available horse isozymes may
represent a good model system.

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