Purification and Properties of NADPH-dependent Aldehyde Reductase from Human Liver*

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An aldehyde reductase (EC 1.1.1.2) from human liver has been purified to homogeneity. The enzyme is NADPH-dependent, prefers aromatic to aliphatic aldehydes as substrates, and is inhibited by barbiturates and hydantoins. The following physicochemical parameters were determined: molecular weight, 36,200; sedimentation coefficient, 2.9 S; Stokes radius, 2.65 nm; isoelectric point, pH 5.3; extinction coefficient at 280 nm, 54,300 M⁻¹ cm⁻¹. Results from polyacrylamide gel electrophoresis with and without sodium dodecyl sulfate, gel filtration, and ultracentrifugation suggest a monomeric structure. One molecule of NADPH binds to the enzyme causing a red shift of the coenzyme absorption maximum from 340 to 352 nm. The amino acid composition has been determined and a partial specific volume of 0.74 was computed from these data. An α-helicity of 7 and 18% was estimated from the ellipticities at 208 and 222 nm, respectively. Combination of the most reactive thiol group with P-mercuribenzoate does not cause loss of catalytic activity. Inactivation occurs when more than one thiol group is modified. The presence of NADPH or NADP⁺ prevents loss of activity by thiol modification. The comparison of structural features of aldehyde reductase with other monomeric and oligomeric dehydrogenases suggests similarities of aldehyde reductase with octopine dehydrogenase.

Aldehyde reductases are pyridine nucleotide-dependent oxidoreductases, catalyzing the reduction of a variety of aromatic and medium chain aliphatic aldehydes to their corresponding alcohols. The occurrence in brain (1-3), kidney (4-6), heart (7, 8), lens (9), skeletal muscle (10), and liver (11) of several mammalian species is well documented. Special attention has been paid to the enzymes from brain because of their preferential role for the aldehydes derived from the neurotransmitters octopamine and norepinephrine and their possible physiological role in the metabolism of these two neurotransmitters. The scarcity of this enzyme in other tissues has, however, prevented the complete purification and detailed physicochemical studies. Liver and kidney, on the other hand, contain more than 10 times higher aldehyde reductase concentrations, and the enzyme has therefore first been purified to homogeneity from pig kidney cortex (5). Contrary to the functionally similar alcohol dehydrogenase and to most other pyridine nucleotide-dependent dehydrogenases, aldehyde reductases are single chain proteins with molecular weights between 30,000 (2, 7) and 44,000 (3). With the exception of octopine dehydrogenase (12), an NADH-dependent enzyme occurring in mollusc muscle, no other monomeric dehydrogenase has, to our knowledge, been purified and subjected to physicochemical studies.

Considering a recently proposed model of an evolutionary tree of pyridine nucleotide-dependent dehydrogenases (13) it seems possible that monomeric dehydrogenases represent a link between a hypothetical ancestral dehydrogenase and today's diversified oligomeric enzymes. Structural and functional comparisons between the monomeric aldehyde reductase and oligomeric dehydrogenases might give evidence for such relations. In this paper we report on the purification and some properties of human liver aldehyde reductase, and comparisons with other dehydrogenases are made. A preliminary account of this work has been presented.¹

EXPERIMENTAL PROCEDURES

Materials

Human livers from both sexes were obtained from legal autopsies; death causes being traffic accidents, homicides, or cardiovascular diseases. The organs were frozen 6 to 20 h after death and stored at -20°C. All chemicals were of the highest grade commercially available and used without further purification. Proteins used for Sephadex column and polyacrylamide gel calibration were purchased from Sigma (St. Louis, Mo.) and from Boehringer (Mannheim, Germany). Boehringer also furnished the coenzymes, 5-phenyl-5-ethylbarbituric acid was bought from Siegfried AG (Zofingen, Switzerland) and 5-phenyl 5-ethylhydantoin was a gift from Dr. Wander AG (Bern, Switzerland). All other chemicals were supplied by Fluka (Buchs, Switzerland) or by Merck (Darmstadt, Germany).

DEAE-cellulose (DE52) was bought from Whatman (Maidstone, Great Britain), hydroxyapatite (Bio-Gel HTP) and Bio-Gel P-100 from Bio-Rad (Richmond, Calif.), and Sephadex resins from Pharmacia (Uppsala, Sweden). Ampholyte (Ampholine) for isoelectric focusing were purchased from LKB Producer AB (Bromma, Sweden). Deionized water was used for all buffers. Aldehyde and alcohol substrates which were not sufficiently soluble in water were dissolved in a methanol/water solution to give a final methanol concentration of 1.2%. This concentration had no effect on the catalytic activity of aldehyde reductase.

Methods

Enzyme Assay

Aldehyde reductase activity was assayed by recording the change of NADPH absorption as a function of time at 334 nm in an Eppen-
Amino Acid Analyses

Human Liver NADPH-dependent Aldehyde Reductase

do not hallucinate.
Polyacrylamide gel electrophoresis. Two protein and corresponding activity bands were obtained from freshly purified enzyme. A single band was observed when the enzyme sample had been treated with thiol compounds such as 2-mercaptoethanol, dithioerythritol, or glutathione prior to the electrophoresis (Fig. 1). The presence of oxidizing substances such as oxygen or o-iodosobenzene, on the other hand, favored the formation of new bands. No changes in specific activity were observed upon interconversion of the oxidized and reduced enzyme forms. Oxidized and reduced aldehyde reductase was also separated on polyacrylamide gels in the presence of sodium dodecyl sulfate. As in the gels without the detergent the band corresponding to the oxidized enzyme showed a higher migration rate. Since polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate separates proteins according to their molecular weights it may be assumed that the observed faster migrating band is due to the formation of one or more intramolecular disulfide bridges which retain the enzyme in a partially folded conformation giving rise to a lower apparent molecular weight.

Liver cells contain approximately 5 mM glutathione which is present almost exclusively in its reduced form (23). This concentration has been shown to completely reduce aldehyde reductase, suggesting that in vivo the enzyme is present in its reduced form. Consequently, all physicochemical studies were carried out with the reduced enzyme.

**Substrate Specificity and Inhibitor Sensitivity**—Aldehyde reductase catalyzed the reduction of a number of aromatic and medium chain length aliphatic aldehydes and ketones in the presence of NADPH (Table II). The values for the Michaelis constants were calculated by the method of Lineweaver and Burk (24). Straight lines were obtained for all substrates in the double reciprocal plots. Substituted benzaldehydes carrying a partial positive charge on the carbonyl carbon were the most active substrates and displayed the lowest $K_m$ values. Aliphatic aldehydes were generally poor substrates but displayed considerably higher maximal velocities in cases where the substrate contained a hydroxyl or oxo group adjacent to the aldehyde function. The reactions proceeded at least 5% of the rates observed with NADPH when the same concentration of NADH was used as coenzyme. No enzymatic activity could be measured in the presence of NADPH with acetaldehyde, pyridoxal, pyridoxal phosphate, glyceraldehyde phosphate, glucose, or fructose. The reverse reaction, i.e. the dehydrogenation of alcohols to their aldehydes, proceeded at a rate less than 2% of the rate measured with p-NO$_2$-benzaldehyde in the forward reaction (turnover number $= 570$ min$^{-1}$). The turnover numbers for the oxidation of alcohols (100 mM) by aldehyde reductase in the presence of 1 mM NADP$^+$ were: 4-hydroxy-3-methoxybenzyl alcohol, 8.8; phenylethanol, 4.8; benzyl alcohol, 4.4; propene-1,2-diol, 2.6; phenylethanol, 1.7; and glyceral, 1.7. No activity was detected with ethanol, cyclohexanol, phenylpropyl alcohol, and glyceral phosphate.

The pH optima for the forward and reverse reaction differed by approximately 3 pH units. The rate of reduction of p-nitrobenzaldehyde in the presence of NADPH was maximal at pH 6.6 in sodium phosphate or imidazole/Cl buffer. A 1.6-fold increase of the maximum rate was observed when the phosphate buffer concentration was raised from 10 to 500 mM. Oxidation of benzyl alcohol in the presence of NADP$^+$ was fastest at pH 9 to 9.5 in 0.1 mM sodium pyrophosphate buffer.

Inhibition of aldehyde reductase was measured in presence of pyrazole, a known inhibitor of alcohol dehydrogenase, and two anticonvulsive drugs which are good inhibitors for the aldehyde reductases from brain (25) and heart (7). Pyrazole inhibited aldehyde reductase only 12% at a concentration of 10 mM. Ethylphenylhydantoin and phenobarbital on the other hand gave 50% inhibition at concentrations of 30 and 50 mM, respectively.

**Physicochemical Properties**—Table III summarizes several physicochemical properties of human liver aldehyde reduc-

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**Figure 1.** Polyacrylamide gel electrophoresis of human liver aldehyde reductase. Ten micrograms of enzyme were applied per gel and electrophoresis was carried out as described under "Experimental Procedures." A to C, enzyme after standing in presence of oxygen for 2 days; D to F, enzyme treated with 1% mercaptoethanol. A and D were stained for protein, B and E for activity. C and F are protein stains of gels run in presence of sodium dodecyl sulfate.
The molecular weight, given in the table as an average of 36,200, was determined by three independent methods. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis on 10% and 12.5% gels gave identical values of 39,400 (Fig. 2). Sugar residues, which can give aberrant results with this method could not be detected when the gels were stained for carbohydrates.

From the two gel filtration resins Sephadex G-100 and Bio-Gel P-100, the reductase was eluted in both cases shortly after the marker enzymes ovalbumin and β-lactoglobulin. From the calibration curve depicted in Fig. 3A a molecular weight of 36,600 was estimated. By the same experiments the Stokes radius of the enzyme was obtained, when in Fig. 3A the values of the Stokes radii instead of the molecular weights were plotted against the elution volume (Fig. 3B).

Siegel and Monty have pointed out that upon gel filtration the Stokes radii correlate better with the elution volume than the molecular weights and that in fact these former values together with the sedimentation coefficient should be used to compute the molecular weight of a protein (26). By this approach we obtained a molecular weight of 33,600 for the aldehyde reductase. Finally, ultracentrifugal studies gave a weight average molecular weight of 36,300 as calculated from two meniscus depletion sedimentation equilibrium runs. The sedimentation coefficient was determined in 50 mM potassium phosphate, pH 7.6, in the presence of 5 mM glutathione at protein concentrations of 0.4 and 0.6 mg/ml.

<table>
<thead>
<tr>
<th>Molecular weight</th>
<th>36,200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stokes radius</td>
<td>2.65 nm</td>
</tr>
<tr>
<td>Sedimentation coefficient</td>
<td>2.9 S</td>
</tr>
<tr>
<td>Diffusion constant</td>
<td>7.5·10⁻⁷ cm² s⁻¹</td>
</tr>
<tr>
<td>Frictional ratio</td>
<td>1.18</td>
</tr>
<tr>
<td>Isoelectric point</td>
<td>pH 5.3</td>
</tr>
<tr>
<td>Extinction coefficient</td>
<td>54,300 M⁻¹ cm⁻¹</td>
</tr>
<tr>
<td>α helicity</td>
<td>12%</td>
</tr>
<tr>
<td>Partial specific volume</td>
<td>0.74 cm³/g</td>
</tr>
</tbody>
</table>

* Average from determinations by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, gel filtration, and ultracentrifugation.

Table III

Physicochemical properties of human liver aldehyde reductase

Fig. 2. Molecular weight determination of human liver aldehyde reductase by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The following marker proteins were used to calibrate the gels: 1, ovalbumin; 2, horse liver alcohol dehydrogenase; 3, aldolase; 4, mitochondrial malate dehydrogenase; 5, glyceraldehyde 3-phosphate dehydrogenase; 6, chymotrypsinogen. The mobility of aldehyde reductase relative to the standard proteins is marked by the vertical bar. The line was calculated by the method of linear least squares.

Fig. 3. Determination of molecular weight (A) and Stokes radius (B) of human liver aldehyde reductase by gel filtration on Sephadex G-100. The column (50 × 0.9 cm) was equilibrated with 50 mM sodium phosphate buffer, pH 7.0. The following marker proteins (0.5 mg/0.5 ml) were used to calibrate the column: 1, dimer of ovalbumin; 2, bovine serum albumin; 3, hemoglobin; 4, ovalbumin; 5, β-lactoglobulin; 6, chymotrypsinogen. The elution position of aldehyde reductase is marked by the vertical bars. The lines were calculated by the method of linear least squares.

Fig. 4. Isoelectric focusing of human liver aldehyde reductase. Of aldehyde reductase, 0.1 mg was focused as described under "Experimental Procedures." A mixture of ampholine pH 4 to 6 and ampholine pH 5 to 7 was used to form the pH gradient. The cathode was at the top and the anode at the bottom of the column. Enzyme activity (●) and pH value (■) were determined in each fraction.
assuming a molecular weight of 36,200. A value of 54,300 
$\text{m}^{-2}\text{cm}^{-1}$ was obtained.

Circular Dichroism – In order to get some information on the secondary structure of aldehyde reductase, circular dichroism spectra were recorded. The shape of the spectrum shown in Fig. 5 is similar to that given by pure a helical structures but the negative peaks are much smaller. The percentage of helical structures was calculated from the values of the mean residue ellipticity at the two negative peaks. From the value at 208 nm using the formula of Greenfield and Fasman the a helix content was 7% (27) and from the value at 222 nm according to Chen et al. it was 18% (28).

Amino Acid Composition – The amino acid composition of aldehyde reductase is shown in Table IV. Of the cysteine residues, 3.4 could be carboxymethylated by iodoacetamide in the reduced native enzyme. However, 6.4 cysteine residues were obtained when the reductase had been denatured in 8 M urea prior to carboxymethylation. This latter value agreed well with results from analyses of performic acid-oxidized enzyme. A partial specific volume of 0.14 cm$^3$/g was calculated from the partial specific volumes of the single amino acids by the method of Cohn and Edsall (29).

Titrination of Enzyme with NADPH – Formation of a binary complex between NADPH and aldehyde reductase caused a red shift of the absorption maximum of the coenzyme from 340 to 352 nm (Fig. 6A). The difference spectrum of free and enzyme-bound NADPH showed a minimum at 323 nm, a maximum at 376 nm, and an isosbestic point at 347 nm (Fig. 6B). The spectral titration of aldehyde reductase with NADPH is depicted in Fig. 7. A stoichiometry of 0.85 mol of NADPH/mol of enzyme was estimated by graphical extrapolation.

Reaction of Thiol Modifying Reagents – As shown by Flynn et al. (6), a 50-fold excess of p-mercuribenzoate over the concentration of enzyme thiol groups was needed to inactivate the aldehyde reductase from pig kidney. Fig. 8 shows the influence of this thiol modifying reagent on human liver aldehyde reductase activity in dependence of the time and the reagent concentration. Addition of 1 eq of p-mercuribenzoate/m of

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**TABLE IV**

Amino acid composition of human liver aldehyde reductase

Mean values determined from eight analyses and two enzyme preparations assuming a molecular weight of 36,200. Standard deviations are expressed in parentheses.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Value (mol %)</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asparagine or aspartic acid</td>
<td>27.9 (0.68)</td>
<td>Isoleucine 13.9 (0.19)</td>
</tr>
<tr>
<td>Threonine$^a$</td>
<td>12.0 (0.37)</td>
<td>Leucine 34.9 (0.63)</td>
</tr>
<tr>
<td>Serine$^a$</td>
<td>16.1 (0.73)</td>
<td>Tyrosine 13.0 (0.52)</td>
</tr>
<tr>
<td>Glutamine or glutamic acid</td>
<td>34.9 (0.62)</td>
<td>Phenylalanine 8.3 (0.27)</td>
</tr>
<tr>
<td>Proline</td>
<td>26.7 (1.62)</td>
<td>Histidine 8.7 (0.49)</td>
</tr>
<tr>
<td>Glycine</td>
<td>21.1 (0.81)</td>
<td>Lysine 19.6 (0.64)</td>
</tr>
<tr>
<td>Alanine</td>
<td>28.8 (0.38)</td>
<td>Arginine 15.4 (0.44)</td>
</tr>
<tr>
<td>Valine</td>
<td>21.1 (0.42)</td>
<td>Cysteine$^c$ 6.3 (0.31)</td>
</tr>
<tr>
<td>Methionine$^b$</td>
<td>3.7 (0.27)</td>
<td>Tryptophan$^a$ 7</td>
</tr>
</tbody>
</table>

$^a$ Extrapolated to zero hour.

$^b$ Determined as methionine and as sulfone derivative.

$^c$ Determined as carboxymethylcysteine and as cysteic acid.

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**Fig. 5.** Circular dichroism spectrum of aldehyde reductase from human liver. The spectrum was recorded on a Dichrograph II under conditions described under "Experimental Procedures." A mean residue weight of 112, calculated from the amino acid composition, was used to convert each value of observed ellipticity into mean residue ellipticity ($\theta$).

**Fig. 6.** Absorption spectrum of free NADPH and NADPH bound to aldehyde reductase. The spectra were recorded on a Unicam SP 1800 spectrophotometer. The concentration of NADPH, dissolved in 30 mM sodium phosphate buffer, pH 7.0, was 14 $\mu$M. A, curve a represents the spectrum of NADPH alone and curve b the spectrum of enzyme-bound NADPH in the presence of 21 $\mu$M aldehyde reductase. B, difference spectrum of enzyme-bound and free NADPH.

**Fig. 7.** Spectral titration of human liver aldehyde reductase by NADPH. The titration was carried out in 30 mM sodium phosphate buffer, pH 7.0. The aldehyde reductase concentration was 27 $\mu$M and the concentration of the NADPH stock solution was 1.4 mM. Difference spectra were recorded and $\Delta A$ was calculated as difference between the minimal (323 nm) and the maximal (376 nm) absorbance (see Fig. 6).
enzyme had only a minimal effect on the activity. Simultaneous spectral titration carried out according to Boyer (30), however, showed that a quantitative reaction had occurred instantaneously after the addition of the reagent. Addition of further equivalents resulted in a gradual and time-dependent inactivation of the enzyme and a concomitant appearance of turbidity which hampered the following of the reaction spectrophotometrically. Loss of activity was prevented by preincubation of the enzyme with 0.1 mM NADPH. More than 10 times higher NADP+ concentrations were needed to obtain the same protective effect and NADH, NADH, or p-N02-benzaldehyde at concentrations of 1 mM did not prevent loss of catalytic activity. Dialysis of inactivated enzyme against β-mercaptoethanol did not, in contrast to the pig kidney enzyme, reactivate human liver aldehyde reductase. A qualitatively similar inactivation pattern was observed with N-ethylmaleimide. This reagent was, however, ineffective in stoichiometric amounts and concentrations above 1 mM were needed to obtain comparable inactivation of aldehyde reductase. Iodoacetamide, finally, did not affect the catalytic activity of aldehyde reductase even at concentrations of 50 mM and over a period of several hours.

**DISCUSSION**

The presence of two NADPH-dependent aldehyde reducing enzymes in human liver has been described by Bosron and Prairie (31). They differed with respect to their molecular weight, their substrate specificity, and inhibitor sensitivity. Judged by these criteria identity between the smaller enzyme and the enzyme described in this paper can be assumed. No attempts have been made to purify and characterize the second enzyme. By preparing aldehyde reductase from human brain Rls and von Warburg have separated three isoenzymes by DEAE-cellulose chromatography (3). No evidence for such a heterogeneity was, however, found for the liver enzyme which was eluted from DEAE-cellulose as a single peak. According to its substrate and coenzyme specificity, as well as its sensitivity toward inhibition by anticonvulsive drugs, the liver enzyme corresponds to the isoenzyme 4.3 from brain (3). Both enzymes have the following characteristics: p-nitrobenzaldehyde is one of the best and aliphatic aldehydes are poor substrates, a strict requirement for NADPH as coenzyme and barbiturates and hydantoins are potent inhibitors. In this respect, both enzymes resemble the aldehyde reductases isolated from bovine (1), pig (2), and rat brain (2, 3), pig kidney (5), and sheep heart (7).

The value obtained for the average molecular weight of human liver aldehyde reductase agreed within 10% with the values obtained for the enzymes from pig kidney (6), beef heart (8), bovine lens (9), rabbit muscle (10), and rat liver (11). Somewhat lower values were reported for the enzyme from pig brain (2) and sheep heart (7), while for the human brain isoenzymes approximately 20% higher values were found (3).

X-ray diffraction studies of several pyridine nucleotide-dependent dehydrogenases have revealed the presence of a domain of great structural similarity around the coenzyme binding site of these enzymes (13, 32). Where no structural data are available, evidence for homologies among different proteins can be gained by comparing their amino acid compositions. Difference indices according to Metzger et al. (33), calculated from the amino acid compositions of human liver aldehyde reductase and those of eight other pyridine nucleotide-dependent dehydrogenases are compiled in Table V. An index of less than 10 suggests possible homology between two proteins (46), values above 10, however, do not exclude homology.

The value of 6.4 obtained from the comparison of the amino acid compositions of human liver and pig kidney aldehyde reductases indicates homology between these two enzymes. An even lower value of 4.3 was obtained by comparing the amino acid compositions of human liver aldehyde reductase and rat

![Figure 8](http://www.jbc.org/)

**FIG. 8.** Inactivation of human liver aldehyde reductase by p-mercaptoethanol. Aldehyde reductase (9.6 μM) in 10 mM sodium phosphate buffer, pH 7.0, was incubated with the following concentrations of p-mercuribenzoate: 0, 9.6 μM; A, 19.2 μM; B, 38.4 μM; C, 38.4 μM in the presence of 100 μM NADPH. The temperature was kept constant at 25°C and aliquots were taken for activity determinations after specified time intervals.

**Table V**

Comparison of human liver aldehyde reductase with other pyridine nucleotide-dependent dehydrogenases

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Source</th>
<th>Subunits</th>
<th>Difference indices according to Metzger et al. (33)</th>
<th>Proline content</th>
<th>Shift of absorption maximum at 340 nm of coenzyme after binding to enzyme</th>
<th>Most reactive binding site for catalytic activity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldehyde reductase</td>
<td>Human liver</td>
<td>1</td>
<td>0</td>
<td>Red</td>
<td>No</td>
<td>This paper</td>
<td>31</td>
</tr>
<tr>
<td>Daunorubicin reductase</td>
<td>Rat liver</td>
<td>1</td>
<td>3.4</td>
<td>7.93</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aldehyde reductase</td>
<td>Pig kidney</td>
<td>1</td>
<td>6.4</td>
<td>6.69</td>
<td></td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Octopine dehydrogenase</td>
<td>Pecten maximus</td>
<td>1</td>
<td>11.1</td>
<td>6.02</td>
<td>Red</td>
<td>No</td>
<td>12, 34</td>
</tr>
<tr>
<td>Alcoholic dehydrogenase</td>
<td>Human liver</td>
<td>2</td>
<td>17.4</td>
<td>5.23</td>
<td>Blue</td>
<td></td>
<td>35, 36</td>
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<tr>
<td>Alcohol dehydrogenase</td>
<td>Horse liver</td>
<td>2</td>
<td>21.2</td>
<td>5.35</td>
<td>Blue</td>
<td>Yes</td>
<td>37-39</td>
</tr>
<tr>
<td>Supernatant malate dehydrogenase</td>
<td>Mammalian heart</td>
<td>2</td>
<td>13.4</td>
<td>4.39</td>
<td></td>
<td></td>
<td>40, 42</td>
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<tr>
<td>Mitochondrial malate dehydrogenase</td>
<td>Mammalian heart</td>
<td>2</td>
<td>14.8</td>
<td>6.24</td>
<td>Red</td>
<td>No</td>
<td>40, 42</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>Bovine muscle</td>
<td>4</td>
<td>18.2</td>
<td>4.03</td>
<td>Blue</td>
<td>Yes</td>
<td>43-45</td>
</tr>
</tbody>
</table>
liver daunorubicin reductase (11). Although some differences in the physicochemical properties and the substrate specificity of these two enzymes are observed, a close structural resemblance may nevertheless be assumed from the similarities of the amino acid compositions. The value of 11.1 found by comparing human liver aldehyde reductase and octopine dehydrogenase, another monomeric dehydrogenase, is only slightly above 10, suggesting that a sequence comparison between aldehyde reductase and octopine dehydrogenase would be worthwhile. The difference indices obtained with the oligomeric dehydrogenases are all well above 10 and homology of the primary sequences therefore is less probable. It must, however, be kept in mind that during the evolution of oligomeric enzymes greater changes in the amino acid composition occurred in the areas of newly formed subunit interfaces (13). Other domains as for instance the coenzyme binding site may still show close homology in monomeric and oligomeric enzymes.

The proportions of proline residues in human liver and pig kidney aldehyde reductase and rat liver daunorubicin reductase are higher than those of the oligomeric dehydrogenases listed in Table V. This may account for the low estimated degree of α-helicity of aldehyde reductases (6, 9).

Binding of NADPH to human liver aldehyde reductase induces a red shift of the absorption maximum of the coenzyme. Similar red shifts were reported to occur upon binding of reduced coenzyme to octopine dehydrogenase (33), mitochondrial malate dehydrogenase (41), and glutamate dehydrogenase (47). Contrary, the absorption maximum of the reduced coenzyme is shifted toward shorter wavelengths by binding to human (36) and horse (38) liver alcohol dehydrogenase and to lactate dehydrogenase (44). According to Velick the absorption maximum of the reduced coenzyme is determined by the relative structural contributions of the quinoid and of the polar part of the coenzyme binding site (49). Similar red shifts were reported to occur upon binding of reduced coenzyme to octopine dehydrogenase (34), mitochondrial malate dehydrogenase (41), and glutamate dehydrogenase (47). Contrary, the absorption maximum of the reduced coenzyme is shifted toward shorter wavelengths by binding to human (36) and horse (38) liver alcohol dehydrogenase and to lactate dehydrogenase (44). According to Velick the absorption maximum of the reduced coenzyme is determined by the relative structural contributions of the quinoid and of the polar part of the coenzyme binding site (49).

Aldehyde reductase from human liver resembles the reductase from pig kidney (6), octopine dehydrogenase (12), supernatant and mitochondrial malate dehydrogenase (42), and isocitrate dehydrogenase (50) in that combination of the most reactive thiol groups with p-mercuribenzoate is not accompanied by loss of catalytic activity. These enzymes differ in this respect from alcohol dehydrogenase (39) and lactate dehydrogenase (45) which are inactivated by the reaction of 1 molecule of p-mercuribenzoate/subunit of enzyme. Like octopine dehydrogenase, mitochondrial malate dehydrogenase and isocitrate dehydrogenase inactivation of human liver aldehyde reductase occurs when further —SH groups react with p-mercuribenzoate.

Differences in the reactivity of thiol groups essential for the catalytic activity were also noted among aldehyde reductases from different sources. Three of four isoenzymes of human brain were not inhibited by 1 mM p-mercuribenzoate (3). The same behavior was observed by Boisson and Prairie for the enzyme from pig kidney (5) and Flynn et al. needed a 50-fold excess of the reagent for complete inactivation of the same enzyme (6). Inactivation with a slow concomitant denaturation of human liver aldehyde reductase on the other hand occurred after the stoichiometric addition of more than 1 molecule of p-mercuribenzoate/molecule of enzyme (Fig. 8).

An objective of this work has been to compare human liver aldehyde reductase with other dehydrogenases. To summarize the results presented and discussed above we find that human liver aldehyde reductase displays many similarities with the monomeric octopine dehydrogenase and the dimeric malate dehydrogenase, while greater deviations from alcohol dehydrogenase and lactate dehydrogenase are observed.

Acknowledgments—We are indebted to Professor J. Engel and Mr. A. Lustig of the Biocentral, Basel, for the use of the ultracentrifuge and to Dr. P. Lavanich and Mr. Ch. Bührmann of the Eidgenössische Versuchsanstalt für Milchwirtschaft, Bern, for the use of the amino acid analyzer. We thank Miss A. Süsser and Miss A. Lüthi for their excellent technical assistance.

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