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# Partial Purification and Some Properties of Mitochondrial Aldehyde Reductases from Chicken Liver

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The subcellular distribution of aldehyde reductase activity has been studied in chicken liver. Most of the activity with D-erythrose as a substrate appeared in cytosol, but 11% of the total activity appeared to be present in mitochondria. Two reduced nicotinamide adenine dinucleotide phosphate (NADPH)-dependent aldehyde reductases from chicken liver mitochondria have been partially purified and characterized. One enzyme (mitochondrial aldehyde reductase I) has a molecular weight of 29000 and has an isoelectric point of 7.0, whereas a second enzyme (mitochondrial aldehyde reductase II) has a molecular weight of 31000 and has an isoelectric point of 7.7. Substrate specificity studies showed that mitochondrial aldehyde reductase I and II are capable of reducing various aldehydes such as D-glyceraldehyde, D-erythrose, D-erythrose 4-phosphate and aromatic aldehydes. Unlike mitochondrial aldehyde reductase II, mitochondrial aldehyde reductase I very efficiently reduces D-glucuronic acid and succinic semialdehyde, and has higher $K_m$ values for aldehydes. Mitochondrial aldehyde reductase I activity is much more susceptible to inhibition by sodium valproate than mitochondrial aldehyde reductase II activity. With respect to substrate specificity and inhibitor sensitivity, mitochondrial aldehyde reductase I and II could be classified as high-$K_m$ aldehyde reductase and low-$K_m$ aldehyde reductase, respectively.

**Keywords**——aldehyde reductase; chicken liver mitochondria; subcellular distribution; purification; substrate specificity; inhibitor sensitivity; D-erythrose; D-erythrose 4-phosphate

It is known that tetritols such as erythritol are excreted in relatively large amounts in human and chicken urine, but the metabolic pathway for the formation of erythritol is unclear. In the course of our studies on the metabolism of tetroses we have found that D-erythrose and D-erythrose 4-phosphate are converted to erythritol and D-erythritol 4-phosphate, respectively, by enzymes in human, chicken[1] and beef[2] liver, and we showed that the enzymes responsible for this conversion are aldehyde reductases.

The aldehyde reductases (alcohol: nicotinamide adenine dinucleotide phosphate ($NADP^+$) oxidoreductase, EC 1.1.1.2) comprise a family of monomeric reduced $NADP^+$ (NADPH)-dependent oxidoreductases with wide specificity for the reduction of aldehyde and ketone groups to corresponding alcohols, and they have been found in the cytosol of a wide range of animal tissues. The physiological role of the aldehyde reductases remains to be defined, but it has been suggested that the aldehyde reductases function in the metabolism of biogenic amines (catecholamines and $\gamma$-aminobutyric acid)[3] and isocorticosteroids,[4] the conversion of prostaglandin $E_2$ into $F_2\alpha$,[5] the polyol pathway,[6] the uronic acid pathway,[7] drug detoxication[8] and the biosynthesis of plasmalogens.[9] As a continuation of our studies on the physiological significance of aldehyde reductases of chicken liver, we have found aldehyde reductases not only in the cytosol but also in the mitochondria of chicken liver.

In this paper, we report the finding of two aldehyde reductases in chicken liver mitochondria using D-erythrose as a substrate, and describe the partial purification and some of the properties of the enzymes.

### Experimental procedures

**Materials**——D-Erythrose was prepared from D-glucose by a slight modification of the method of Perlin and Brice.[10] Nicotinamide adenine dinucleotide ($NAD^+$), NADH and NADPH were obtained from Oriental Yeast Co. (Tokyo), and diphenylamine, Triton X-100, sodium pyruvate, D-xylose, *p*-nitrobenzaldehyde, pyridine 3-aldehyde, sodium barbital and hydroxylapatite were purchased from Wako Pure Chemical Industries, Ltd. (Osaka). Potassium glutamate, α-glycerophosphate, D-glucuronic acid, 4-benzoylpyridine, α-naphthoquinone, menadione, pyrazole, quercitrin and Coomassie brilliant blue G-250 were obtained from Nakarai Chemicals, Ltd. (Kyoto). Sodium valproate was purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo). Calf thymus deoxyribonucleic acid (DNA), D-glyceraldehyde, D-erythrose 4-phosphate, succinic semialdehyde, naloxone, bovine serum albumin (type IV), bovine pancreas α-chymotrypsinogen A (type II), whale skeletal muscle myoglobin (type II) and ovalbumin (type VII) were purchased from Sigma Chemical Co. Sephadex G-100, DEAE-Sephadex A-25, 2′5′ADP-Sepharose and Pharmalyte (pH 3—10) were bought from Pharmacia Fine Chemicals Co. All other chemicals were of the highest grade commercially available and were used without further purification.

**Subcellular Fractionation**——Nuclear fraction was isolated by the method of Chauveau *et al.*[11] Subcellular fractionation was performed by a modification of the method of Hogeboom.[12] 2 g sample of a single liver was used in each study. The sample was homogenized in 9 volumes of 0.25 M sucrose solution containing 3 mM Tris-HCl buffer, pH 7.4 (sucrose solution) with a Potter-Elvehjem glass homogenizer with Teflon pestle, and the homogenate was centrifuged at $700 \times g$ for 10 min. The pellet was discarded and the supernatant was centrifuged at $8000 \times g$ for 15 min. This pellet was suspended in 7.5 volumes of the sucrose solution and centrifuged as before. The pellet was retained as the "crude mitochondrial fraction." The combined supernatants were centrifuged at $110800 \times g$ for 60 min and the pellet was suspended in 10 volumes of the sucrose solution and centrifuged as above. The pellet was retained as the "microsomal fraction" and the combined supernatants were used as the "cytosolic fraction." The two particulate fractions were resuspended in 5 volumes of the sucrose solution.

**Extraction of Enzymatic Activity from the Crude Mitochondrial Fraction in the Presence of Cations**——The crude mitochondrial fraction was washed with cation-free or cation-containing isotonic sucrose solution by the method of Rosenthal *et al.*[13] and the materials were centrifuged at $12000 \times g$ for 12 min.

**Further Fractionation of the Crude Mitochondrial Fraction**——The crude mitochondrial fraction was further separated into lysosomal, mitochondrial and peroxisomal fractions by ultracentrifugation through a discontinuous sucrose density gradient, as described by de Duve.[14]

**Assay Methods**——Aldehyde reductase activity was assayed spectrophotometrically in a reaction mixture consisting of 67 μM NADPH, 10 mM D-erythrose and 100 mM sodium phosphate buffer, pH 7.0, in a total volume of 1.0 ml. The reaction was initiated by the addition of enzyme preparation, and the rate of NADPH oxidation was observed by following the decrease in absorbance at 340 nm at 25 °C with a Shimadzu UV-200S spectrophotometer. One unit of enzyme activity is defined as the amount that causes the oxidation of 1 μmol NADPH per minute under the assay conditions described above.

For the assay of DNA, the extraction procedure of Schneider[15] was used. DNA was determined by using the diphenylamine reaction of Dische,[16] and calf thymus DNA was employed as the standard. Protein concentration was determined by the dye-binding method of Bradfold using bovine serum albumin as the standard.[17] Glutamate dehydrogenase and lactate dehydrogenase were assayed by the methods of Strecker[18] and Kornberg,[19] respectively. Acid phosphatase was assayed according to the method of Wattiaux *et al.*[20]

In all assays for enzymatic activity, the reaction mixtures contained 0.5% (w/v) Triton X-100. Aldehyde substrates and flavonoid inhibitor (which were not sufficiently soluble in water) were dissolved in methanol, giving a final methanol concentration of 1% in the enzyme assay. This had no effect on the activity of the aldehyde reductases.

**Molecular Weight Determination**——Molecular weight values were determined by gel filtration on a Sephadex G-100 column (1.2 × 70 cm) in 100 mM sodium phosphate buffer, pH 7.0. Bovine serum albumin, ovalbumin, myoglobin and α-chymotrypsinogen A were used as standards.

**Isoelectric Point Determination**——Isoelectric focusing in 5% polyacrylamide gel containing 2% Pharmalyte (pH 3—10) was performed at 0 °C as described by Miyazaki *et al.*[21] Enzyme fractions (0.1 ml, containing 50 munits of activity) were applied at the cathode. To measure the pH value and enzyme activity, the gels were cut into 3 mm slices, and Pharmalyte and enzyme activity were eluted for 2 h at 0 °C into 0.5 ml of degassed water.

## Results

### The Subcellular Distribution of Aldehyde Reductase Activity in Chicken Liver

The subcellular distribution of aldehyde reducing activity in chicken liver was examined with D-erythrose as a substrate. The reasons for the use of D-erythrose as a substrate were that D-erythrose is a good substrate for aldehyde reductases in chicken and beef liver cytosol, and it

TABLE I. Subcellular Distribution of Aldehyde Reductase Activity in Chicken Liver

| Fraction | Aldehyde reductase | | | | Lactate dehydrogenase | |
|---|---|---|---|---|---|---|
| | Triton X-100 | | No addition | | | |
| | Activity (units) | Recovery (%) | Activity (units) | Recovery (%) | Activity (units) | Recovery (%) |
| Homogenate | 2.44 | 100 | 1.92 | 100 | 216 | 100 |
| Mitochondria | 0.255 | 11.4 | 0.112 | 5.83 | 10.8 | 5.00 |
| Microsomes | 0.181 | 8.08 | 0.023 | 1.18 | 11.0 | 5.09 |
| Cytosol | 1.81 | 80.8 | 1.78 | 92.7 | 194 | 89.8 |

The fractions, crude mitochondria, microsomes and cytosol, were prepared and assayed as described in the experimental procedures section. Recovery is expressed as a percentage of the sum of the recovered activities in all fractions. Values given are the means of determinations in eight separate experiments.

is stable. In addition, it is not a substrate (unlike *p*-nitrobenzaldehyde) for nitro reductase or (unlike pyridine 3-aldehyde) for carbonyl reductase.

The nuclear fraction obtained by the centrifugation at $700 \times g$ for 10 min was too turbid for measurement of aldehyde reductase activity to be possible. Thus, the nuclei were isolated by the method of Chauveau *et al.*[11] in order to examine whether or not the activity was present in the nuclei. About 40% of DNA was recovered from the crude extract, but no aldehyde reductase activity was detected (data not shown). Therefore, the pellet (containing the nuclei and cell debris) obtained by the centrifugation at $700 \times g$ for 10 min was discarded, and its supernatant was regarded as homogenate.

The distribution of the reducing activities towards D-erythrose in subcellular fractions is shown in Table I. Most of the activity was confined to cytosolic fraction, but significant activities were also detected in crude mitochondrial and microsomal fractions. The activities in the crude mitochondrial and microsomal fractions and the homogenate were increased when they were determined in the presence of 0.5% (w/v) Triton X-100. The results suggest that particulate fractions have appreciable enzymatic activities.

**Extraction of Enzymatic Activity from the Crude Mitochondrial Fraction in the Presence of Cations**

About 5% of total lactate dehydrogenase activity appeared in the crude mitochondrial fraction, as shown in Table I. This result raised the possibility that cytosolic aldehyde reductases were adsorbed on components of this fraction. However, this possibility is excluded by the following experiments. Rosenthal *et al.*[13] indicated that the absence of cations in the homogenization medium might result in adsorption of cytosolic enzymes on intracellular particulates, even though electrolyte-free media were essential for the effective separation of organella by fractional centrifugation. Thus, exposure of the separated particulate organellas to isotonic salt solution seemed to offer a simple check on the degree of association between cytosolic enzymes and particles. In the experiments listed in Table II, the crude mitochondrial fraction was washed with either cation-free or cation-containing sucrose solution. Treatment with monovalent (124 mM) or divalent (80 mM) cations extracted about 70% of lactate dehydrogenase from the crude mitochondrial fraction, while aldehyde reductase activity was 18 or 0% extracted and glutamate dehydrogenase activity was 0 or 4% extracted, respectively. These results obviously indicate that an appreciable amount of aldehyde reductase activity is present in the crude mitochondrial fraction.

**Further Fractionation of the Crude Mitochondrial Fraction**

The crude mitochondrial fraction can be separated into three fractions (lysosomal,

TABLE II. Release of Enzyme Activities from the Mitochondrial Fraction Following Treatment with Cations

| Cation | Concn. (mM) | Recovery (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Protein | | GDH | | LDH | | AL.R | |
| | | Sup | Ppt | Sup | Ppt | Sup | Ppt | Sup | Ppt |
| Control | | 1.88 | 98.1 | 0 | 100 | 0 | 100 | 0 | 100 |
| KCl | 7.8 | 0.81 | 99.1 | 0 | 100 | 9.26 | 90.8 | 11.2 | 88.8 |
| | 124 | 6.08 | 94.0 | 0 | 100 | 63.1 | 36.9 | 17.5 | 82.5 |
| $CaCl_2$ | 5.0 | 3.07 | 97.1 | 0 | 100 | 26.7 | 73.3 | 7.61 | 92.4 |
| | 80 | 15.7 | 84.3 | 4.20 | 95.8 | 75.2 | 24.8 | n.d. | n.d. |
| $MgCl_2$ | 5.0 | 2.02 | 97.8 | 0 | 100 | 26.3 | 73.7 | 7.69 | 92.3 |
| | 80 | 9.29 | 90.5 | 2.93 | 97.0 | 71.7 | 28.3 | 0 | 100 |

n.d., not detectable

The mitochondrial fraction was treated with two concentrations of various cations, and then centrifuged at 12000 × *g* for 12 min. The activities of glutamate dehydrogenase (GDH), lactate dehydrogenase (LDH) and aldehyde reductase (AL.R) and the concentrations of protein were measured in the pellet and supernatant. Details of the methods were as described in the experimental procedures section. Recovery is expressed as a percentage of the sum of the recovered activities or proteins.

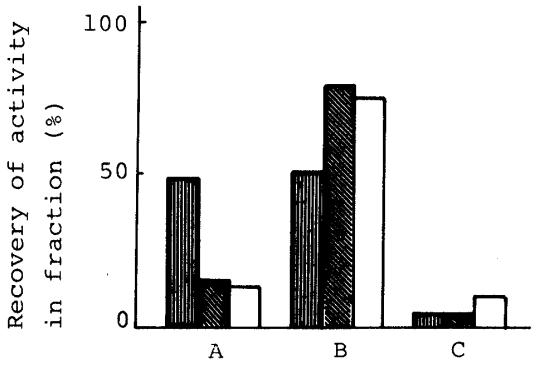


Fig. 1. Distribution of Enzyme Activities in Fractions Derived from the Crude Mitochondrial Preparation of Chicken Liver

The crude mitochondrial preparation was fractionated into lysosomal (A), mitochondrial (B) and peroxisomal (C) fractions by centrifugation on a discontinuous density gradient. The activities of aldehyde reductase (□), glutamate dehydrogenase (▨) and acid phosphatase (▥) were measured in each fraction. Details of the methods were as described in the experimental procedures section. Recovery of the activity in each fraction is expressed as a percentage of the activity of unfractionated crude mitochondrial preparation, and as the mean of determinations in two separate experiments.

mitochondrial and peroxisomal) by ultracentrifugation through a discontinuous density gradient consisting of equal layers of sucrose solutions (1.12—1.81 M). Acid phosphatase and glutamate dehydrogenase were selected as marker enzymes for the lysosomal fraction and the mitochondrial fraction, respectively. Acid phosphatase activity was distributed in both the A (1.27—1.45 M sucrose) and B (1.45—1.55 M sucrose) layers, but glutamate dehydrogenase activity was hardly present in the A layer, as shown in Fig. 1. Judging from these results, the A layer was regarded as the lysosomal fraction and the B layer as the mitochondrial fraction (containing some lysosomes). The C (1.55—1.81 M sucrose) layer might be peroxisomal fraction. About 80% of the aldehyde reductase activity was confined to the B layer and its distribution pattern was similar to that of glutamate dehydrogenase. These results show that the aldehyde reductase detected in the crude mitochondrial fraction is located in mitochondria, not in lysosomes and peroxisomes.

**Purification of Mitochondrial Aldehyde Reductases from Chicken Liver**

In order to characterize mitochondrial aldehyde reductase, a purification scheme was developed. Chickens were killed by decapitation and the livers were collected within 4 h of death and transported to the laboratory on ice. All subsequent procedures were carried out at 0—6 °C.

**Step 1: Preparation of Mitochondria**——The livers were minced and washed with

0.25 M sucrose solution containing 0.1 mM ethylenediaminetetraacetic acid (EDTA) and 3 mM Tris-HCl buffer, pH 7.4 (sucrose solution). The mince (200 g) was homogenized in 9 volumes of the sucrose solution in a Potter–Elvehjem glass homogenizer with Teflon pestle. The homogenate was centrifuged at $700 \times g$ for 10 min, and the pellet was discarded. The supernatant was centrifuged at $8000 \times g$ for 15 min. The resulting pellet was washed in the following order (with intervening centrifugations at $8000 \times g$ for 15 min): with one-half of the original volume of the sucrose solution, with one-fourth volume of 155 mM KCl solution containing 0.1 mM EDTA and 3 mM Tris-HCl buffer, pH 7.4, and with one-fourth volume of the sucrose solution. The final mitochondrial pellet was resuspended in 30 ml of 10 mM sodium phosphate buffer, pH 7.0, containing 1 mM EDTA (buffer A).

**Step 2: Extraction of Enzyme from Mitochondria**——To the mitochondrial suspension, 15% (w/v) Triton X-100 solution containing 1 mM EDTA was added to give the desired final concentration of 1 mg Triton X-100 per mg of mitochondrial protein. The solubilized mitochondria were treated three times with 90% (v/v) acetone to remove Triton X-100 and phospholipids as described below. To the solubilized mitochondria, cold acetone (−15 °C) was added to give 90% (v/v) and the mixture was stirred at 0 °C for 10 min. The resulting precipitate was collected by centrifugation at $10000 \times g$ for 10 min. The precipitate was suspended in 30 ml of buffer A in a Potter–Elvehjem glass homogenizer with a Teflon pestle. After that, the enzyme was extracted twice from the final pellet with 30 ml of buffer A, followed by centrifugation at $30000 \times g$ for 15 min. The clear supernatant was dialyzed against two changes of 1 l of ice-cold buffer A for 24 h, and then concentrated with an Amicon ultrafiltration apparatus using a PM-10 membrane to about 10 ml.

**Step 3: DEAE-Sephadex A-25 Column Chromatography**——After removal of the precipitate by centrifugation at $30000 \times g$ for 20 min, the dialyzed enzyme solution was applied to a column of DEAE-Sephadex A-25 (1.6 × 15 cm) equilibrated with buffer A. The enzyme activity was eluted with the same buffer at a flow rate of 30 ml/h, and 5 ml fractions were collected, and concentrated by ultrafiltration to about 10 ml.

**Step 4: Sephadex G-100 Gel Filtration**——The concentrated enzyme solution was applied to a column of Sephadex G-100 (2.0 × 110 cm) equilibrated with buffer A. Elution was performed with the same buffer at a flow rate of 15 ml/h, and 3.0 ml fractions were collected.

**Step 5: Hydroxylapatite Column Chromatography**——The pooled solution with en-

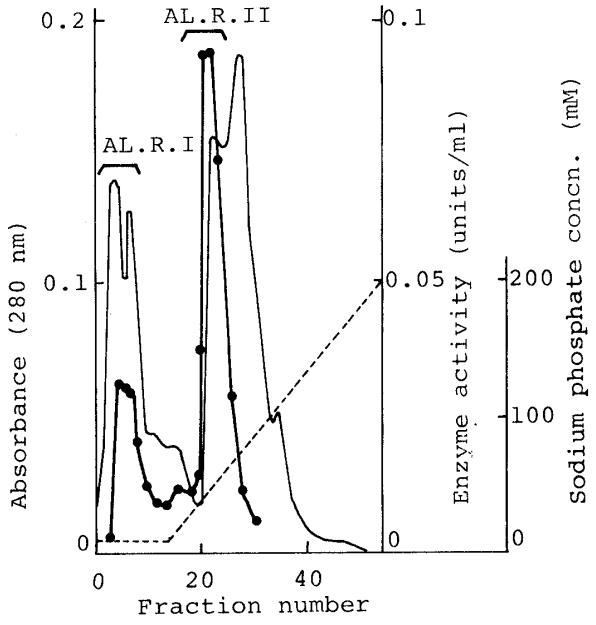


Fig. 2. Hydroxylapatite Column Chromatography of Aldehyde Reductases from Chicken Liver Mitochondria

Aldehyde reductases from the Sephadex G-100 gel filtration step were chromatographed on a hydroxylapatite column as described in the text. Absorbance at 280 nm (——) and enzyme activity (●—●) of each fraction were examined. The concentration gradient is indicated by the broken line.

Table III. Purification of Aldehyde Reductase I and II from Chicken Liver Mitochondria

| | Total protein (mg) | Total enzyme (units) | Specific activity (units/mg) | Recovery (%) | Purification (-fold) |
|---|---|---|---|---|---|
| Mitochondria | 3630 | 4.90 | 0.00135 | 100 | 1.00 |
| Acetone fraction | 461 | 3.50 | 0.00759 | 71.4 | 5.62 |
| DEAE-Sephadex | 177 | 1.83 | 0.0103 | 37.4 | 7.63 |
| Sephadex G-100 | 11.8 | 2.22 | 0.188 | 45.3 | 139 |
| Hydroxylapatite | | | | | |
| M.AL.R.I. | 2.30 | 0.418 | 0.182 | 8.53 | 137 |
| M.AL.R.II | 4.26 | 1.03 | 0.242 | 21.0 | 179 |
| 2′5′ADP-Sepharose | | | | | |
| M.AL.R.I. | 0.051 | 0.113 | 2.22 | 2.31 | 1644 |
| M.AL.R.II | 0.243 | 0.372 | 1.53 | 7.59 | 1133 |

zyme activity was applied to a column of hydroxylapatite (1.2 × 5.0 cm) equilibrated with buffer A. After elution with 25 ml of the same buffer, linear sodium phosphate gradient elution was carried out (10—200 mM sodium phosphate, 60 ml of each) at a flow rate of 5 ml/h, and 3.0 ml fractions were collected. Two aldehyde reductases, referred to here as aldehyde reductase I and II, were separated by this step (Fig. 2). Mitochondrial aldehyde reductase I (M.AL.R.I) appeared in the unadsorbed fraction, whereas mitochondrial aldehyde reductase II (M.AL.R.II) was retained on the column and eluted at about 50 mM sodium phosphate. The two enzyme fractions were pooled separately, and then dialyzed against 1 l of buffer A for 12 h. The dialyzed samples were concentrated by ultrafiltration to about 2 ml.

**Step 6: 2′5′-Adenosine Diphosphate (ADP)-Sepharose Column Chromatography**——The two enzyme fractions were adsorbed separately on 2′5′ADP-Sepharose columns (1.0 × 5.0 cm) equilibrated with buffer A. After washing of the columns with 40 ml of the same buffer, elution was carried out with a linear NADPH gradient (0—50 μM NADPH, 60 ml of each) at a flow rate of 10 ml/h, and 5 ml fractions were collected. The enzyme activity eluted from each column was separately pooled, and dialyzed against two changes of 1 l of buffer A for 48 h.

Table III summarizes a typical purification of mitochondrial aldehyde reductases. The specific activity of M.AL.R.I was 2.2 units/mg with D-erythrose as a substrate and represented about 1600-fold purification over the original mitochondrial preparation. In the case of M.AL.R.II, 1100-fold purification was achieved with a specific activity of 1.53 units/mg.

After the 2′5′ADP-Sepharose step, both enzyme preparations contained two very minor protein contaminants, as judged by polyacrylamide gel electrophoresis in the presence of 20 μM $NADP^+$ at pH 9.4.

**Substrate Specificity of Aldehyde Reductases from Chicken Liver Mitochondria**

Table IV shows the substrate specificity of the two aldehyde reductases from mitochondria. The two reductases utilized NADPH as a cofactor and rapidly reduced D-glyceraldehyde, D-erythrose, D-erythrose 4-phosphate and aromatic aldehydes, while no activity was detected with substrates such as ketone and quinone compounds, which were reduced by carbonyl reductase. M.AL.R.I was able to reduce D-glucuronic acid and succinic semialdehyde, but M.AL.R.II was not.

Michaelis constants for some typical substrates are listed in Table V. The $K_m$ values for these substrates of M.AL.R.I were greater than those of M.AL.R.II. There are two major aldehyde reductases in tissues and they are commonly referred to as the high-$K_m$ and low-$K_m$ aldehyde reductases, respectively.[22] With respect to substrate specificity and $K_m$ values, the

TABLE IV. Substrate Specificity of Aldehyde Reductases from Chicken Liver Mitochondria

| Substrate | Relative $V$ (%) | | |
|---|---|---|---|
| | Concn. (mM) | M.AL.R.I. | M.AL.R.II |
| D-Glyceraldehyde | 3.3 | 76 | 83 |
| D-Erythrose | 3.3 | 100 | 100 |
| D-Erythrose 4-phosphate | 3.3 | 77 | 44 |
| D-Xylose | 100 | 23 | 9 |
| D-Glucuronic acid | 3.3 | 170 | 6 |
| *p*-Nitrobenzaldehyde | 0.5 | 105 | 79 |
| Pyridine 3-aldehyde | 3.3 | 209 | 156 |
| 4-Benzoylpyridine | 3.3 | 0 | 0 |
| Naloxone | 3.3 | 0 | 0 |
| α-Naphthoquinone | 0.5 | 0 | 0 |
| Menadione | 0.5 | 0 | 0 |
| Succinic semialdehyde | 3.3 | 106 | 15 |
| NADH[a)] | 0.067 | 0 | 0 |

*a*) The activity was measured with D-erythrose as a substrate and the velocity with 67 μM NADH is given relative to that with 67 μM NADPH.

Activity was measured as described in the experimental procedures section. Relative $V$ value is expressed as a percentage of that obtained with NADPH and D-erythrose.

TABLE V. Michaelis Constants for Various Substrates of Aldehyde Reductases from Chicken Liver Mitochondria

| Substrate | $K_m$ (mM) | |
|---|---|---|
| | M.AL.R.I | M.AL.R.II |
| D-Erythrose | 3.37 | 1.31 |
| *p*-Nitrobenzaldehyde | 0.835 | 0.128 |
| Pyridine 3-aldehyde | 4.76 | 0.045 |
| Succinic semialdehyde | 0.036 | n.d. |

n.d., not determined.

Activity was measured as described in the experimental procedures section. Michaelis constants were determined from Lineweaver-Burk double-reciprocal plots.

TABLE VI. Effects of Inhibitors on Aldehyde Reductases from Chicken Liver Mitochondria

| Inhibitor | Inhibition of reductases (%) | | |
|---|---|---|---|
| | Concn. (mM) | M.AL.R.I | M.AL.R.II |
| Pyrazole | 10 | 16.5 | 8.3 |
| Sodium barbital | 1.0 | 90.0 | 65.5 |
| Sodium valproate | 1.0 | 91.6 | 13.2 |
| Quercitrin | 0.1 | 93.9 | 92.7 |

The inhibitor at the final concentration indicated was added to the reaction mixture containing buffer and enzyme and the whole was incubated for 2 min at 25 °C. Remaining activity was then determined by the addition of NADPH and D-erythrose as described in the experimental procedures section.

two aldehyde reductases (M.AL.R.I and M.AL.R.II) found here could be classified as high-$K_m$ aldehyde reductase and low-$K_m$ alehyde reductase, respectively.

**Inhibitor Sensitivity**

Table VI shows the inhibitor sensitivity of the aldehyde reductases. Both reductases were only slightly inhibited by pyrazole (10 mM). This indicates that they are not alcohol dehydrogenase. Quercitrin (0.1 mM) inhibited both reductases, while sodium barbital (1 mM) was a much better inhibitor of M.AL.R.I than of M.AL.R.II. Sodium valproate, an inhibitor of the high-$K_m$ aldehyde reductase, was a potent inhibitor of M.AL.R.I at the concentration of 1 mM, whereas it had only a slight effect on M.AL.R.II.

The above results of substrate specificity and inhibitor sensitivity studies show that M.AL.R.I exhibits the same properties as the high-$K_m$ aldehyde reductase. On the other hand, the properties of M.AL.R.II are similar to those found for the low-$K_m$ aldehyde reductase.

**Molecular Weights and Isoelectric pH's of the Enzymes**

The molecular weights of the two enzymes were estimated by Sephadex G-100 gel filtration, and the values obtained were 29000 for M.AL.R.I. and 31000 for M.AL.R.II.

Isoelectrofocusing experiments on polyacrylamide tube gels were carried out with pH 3—10 Pharmalyte, and the pI values of 7.0 and 7.7 were obtained for M.AL.R.I and M.AL.R.II, respectively.

## Discussion

This is the first report on the purification of mitochondrial aldehyde reductase. von Wartburg *et al.*[23] reported previously that the low-$K_m$ form of aldehyde reductase was mitochondrial in rat brain, using *p*-nitrobenzaldehyde as a substrate. However, Ryle and Tipton[24] have suggested that this substrate may be inappropriate since an active nitro reductase activity is apparently associated with the mitochondrial inner membrane in ox brain. Thus, it seemed extremely important to select an appropriate substrate for the detection of aldehyde reductase activity. We therefore chose D-erythrose as a substrate, and found two aldehyde reductases in chicken liver mitochondria. These aldehyde reductases did not have any nitro reductase activity.

The two aldehyde reductases (M.AL.R.I and M.AL.R.II) were purified from chicken liver mitochondria approximately 1600-fold with a recovery of 2.3%, and approximately 1100-fold with a recovery of 7.6%, respectively. Mitochondrial aldehyde reductase I can be differentiated from mitochondrial aldehyde reductase II by its isoelectric pH of 7.0 as opposed to 7.7. In addition, the substrate specificities, kinetic properties and elution pattern from a hydroxylapatite column of M.AL.R.I are quite different from those of M.AL.R.II. The characteristics of these two enzymes correspond well to those of a high-$K_m$ aldehyde reductase (M.AL.R.I) and of a low-$K_m$ aldehyde reductase (M.AL.R.II.).

The physiological role of the mitochondrial aldehyde reductases in aldehyde metabolism remains to be defined. However, Anderson *et al.*[3d] observed that normetanephrine conversion to its glycol metabolite occurred in a mitochondrial preparation and suggested the possible presence of a mitochondrial aldehyde reductase responsible for this process. On the other hand, a number of reports have indicated that the binding of aldehyde compounds as well as drugs, xenobiotics, and their metabolites to thiol and amino groups of cellular macromolecules (nucleic acids and proteins) may be a mechanism of toxicity, perhaps due to impairment of macromolecular function.[25] Therefore, it is biologically desirable that aldehyde compounds are instantly metabolized to nontoxic substances. In fact, the concentrations of D-erythrose 4-phosphate and D-glyceraldehyde 3-phosphate (which are sugars containing free aldehyde groups) are very low as compared with those of other intermediates

of the pentose phosphate pathway and the Embden Meyerhoff pathway.[26] From this point of view, it is possible that the aldehyde reductases may play a general role in the removal of potentially toxic and reactive aldehydes, and may be distributed in virtually all organella. It seems significant in relation to aldehyde metabolism that the enzymes are actually present not only in the cytosol but also in mitochondria and microsomes.

In order to determine whether these mitochondrial aldehyde reductases are indeed distinct from cytosolic aldehyde reductases, we compared their response to various substrates and inhibitors. The two mitochondrial aldehyde reductases have similarities to some of the six cytosolic aldehyde reductases (unpublished observations). In an accompanying paper, further comparisons of the enzymatic and immunological properties of mitochondrial and cytosolic aldehyde reductases will be reported.

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