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THE PRESENCE OF TWO NADPH-LINKED AROMATIC ALDEHYDE–KETONE REDUCTASES DIFFERENT FROM ALDEHYDE REDUCTASE IN RABBIT LIVER

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Abstract—Three NADPH-linked aldehyde reductases (F_1, F_2 and F_3) could be separated by DEAE–cellulose chromatography from rabbit liver cytosol. These enzymes could be distinguished in regard to molecular weight, mobility in polyacrylamide gel electrophoresis, pH optima, substrate specificity and inhibitor sensitivity. Molecular weights of 33,000, 29,000 and 32,000 were estimated for F_1, F_2 and F_3, respectively, by gel filtration on Sephadex G-100. The F_2 was identical to aldehyde reductase (EC 1.1.1.2) from the substrate specificity for aromatic aldehydes and D-glucuronate and inhibition by barbiturates. The F_1 and F_3 were reductases for aromatic aldehydes and ketones, and had a higher affinity for aromatic ketones than for aromatic aldehydes, and were inhibited by sufhydryl reagents but not by barbiturates.

Several NADPH-linked enzymes capable of reducing xenobiotic and aromatic ketones have been characterized regarding substrate specificity in mammalian tissues [1–4]. For example, there is aromatic α-keto acid reductase in beef liver [1], aromatic aldehyde–ketone reductase in rabbit kidney [2], α, β-unsaturated ketone reductase in dog erythrocyte and human liver [3], and *p*-nitroacetophenone reductase in human erythrocyte [4]. In addition to these enzymes, mammalian tissues contain a group of cytoplasmic enzymes which catalyze reduction of xenobiotic and endogenous aldehydes, certain ketones and aldoses, called aldehyde reductases (EC 1.1.1.2), but the ketones are poor substrates for these enzymes [5–7]. The presence of NADPH-dependent reductases for ketones in which the carbonyl group is adjacent to an aromatic ring was found in rabbit liver cytosol [8, 9]. In the previous communication we reported the existence of enzyme activity to reduce 2-(2-amino-5-bromobenzoyl)pyridine(ABBP), a metabolite of bromazepam, to 2-amino-5-bromo-2′-azabenzhydrol in rabbit and guinea pig liver cytosol [10]. This study reports the isolation and some properties of two aromatic aldehyde–ketone reductases and an aldehyde reductase from the cytosol of rabbit liver.

MATERIALS AND METHODS

All chemicals were analytical reagent grade and were obtained from Wako Pure Chemical Industries (Tokyo) unless otherwise stated. Coenzymes, glucose-6-phosphate and glucose 6-phosphate dehydrogenase were obtained from Oriental Yeast Co. Ltd (Tokyo), and pyridine-3-aldehyde, pyridine-4-aldehyde and pyrazole were from Nakarai Chemicals (Tokyo). ABBP was supplied through the courtesy of Nippon Roche K.K. (Tokyo), oxisuran through the courtesy of Warner–Lambert Research Institute (Morris. NJ) and metyrapone through the courtesy of Ciba Pharmaceutical Co. (Summit, NJ). Ovalbumin and equine skeletal muscle myoglobin were purchased from Sigma Chemical Co. (London). Purified acid phosphatase was isolated from human prostate by the method of Ostrowski [11]. Hydroxylapatite was prepared by the method of Bernardi [12].

Polyacrylamide gel electrophoresis. The purified enzymes were subjected to electrophoresis in 7 per cent (w/v) acrylamide gels at 4° according to the method of Hedrick and Smith [13] except that the concentrate gels were omitted. Proteins in 5 per cent sucrose were layered on the gels, the gels were stained with Coomassie Brilliant Blue and destaining was performed as described by Weber and Osborn [14]. Bromphenol blue was used as a tracking dye and the gels were cut off at its position before staining. Enzyme activities of the fresh run gels were measured. The gels were removed from the glass tubes, chilled in a freezer for 10 min, and cut into 1-mm slices. Each slice was suspended in 0.3 ml of 0.1 M phosphate buffer, pH 6.0, at 37° for about 30 min. Each 0.1 ml of 1 mM NADPH and 5 mM substrate(pyridine-4-aldehyde, 4-benzoylpyridine and D-glucuronate, respectively) was added to, and the incubation was continued for 10 min at 37°. The remaining NADPH was assayed by transferring its electrons to *p*-iodonitrotetrazolium via Meldola Blue by following the procedures, the reaction was stopped by the addition of 0.5 ml of the mixture of 0.1 mM Meldola Blue and 2% (w/v) Triton X-100 and 1 mM *p*-iodonitrotetrazolium, and the incubation was continued for 5 min at 37°, and then 1 ml of 1% (w/v) Triton X-100 containing 0.05 M HCl was added. The enzyme activity was calculated from the decrease of the extinction of the reduced formazan at 500 nm(ε = 19.4 cm^2/μmol) against the reaction mixture that control gel slices was incubated without substrates but with NADPH.

Gel filtration. The molecular weight of the enzyme was estimated by gel filtration on a column (1.9 × 47 cm) of Sephadex G-100 according to the method of Andrews [15]. Calibration proteins used and

their molecular weights were: myoglobin (17,800), ovalbumin (45,000), bovine serum albumin (67,000) and acid phosphatase (102,000). Blue Dextran was used to determine the column void volume.

Enzyme assays. Two different techniques were employed to measure reductase activity. Method A: 2-amino-5-bromo-2′-azabenzhydrol formed by the enzymatic reduction of ABBP was quantitated colorimetrically by the Bratton–Marshall method after separating ABBP as described previously [10].

Method B (assay of aldehyde reductase activity): this method measured the rate of NADPH oxidation by continuously recording the decrease in absorbance at 340 nm in Hitachi model-100 spectrophotometer fitted with a Hitachi model-056 recoder. The assays were performed at 30° in a 1 cm path-length cell: 80 mM phosphate buffer, pH 6.0, or 100 mM citrate buffer, pH 5.2, 0.16 mM NADPH and 1 mM *p*-nitrobenzaldehyde, to 2.5 ml. The reaction was routinely initiated by the addition of enzyme. A similar assay system was used to measure the reduction of other aldehydes, and ketones replaced *p*-nitrobenzaldehyde in the reaction mixture.

Protein was determined by the method of Lowry *et al.* [16] with bovine serum albumin as the standard. The eluates of the chromatographic column were determined spectrophotometrically at 280 nm.

Enzyme purification. Livers were obtained from Japan white rabbit after killing by decapitation and were subject to portal perfusion with 0.15 M KCl. All procedures were carried out at 0°–4° and all buffers contained 1 mM 2-mercaptoethanol to stabilize enzyme. In a typical experiment, 108 g of livers was homogenized with 3 vol. of 0.15 M KCl containing 10 mM Tris buffer, pH 7.5, in a Waring Blender for 2 min. The homogenate was centrifuged at 105,000 *g* for 60 min. To the supernatant fluid, ammonium sulfate was added to 35 per cent saturation. After stirring for 2 hr, the mixture was centrifuged at 12,000 *g* for 20 min. To precipitate reductase enzyme, ammonium sulfate was added to the supernatant solution to 75 per cent saturation. The mixture was stirred and centrifuged as described above. The precipitate was dissolved in a small volume of 20 mM phosphate buffer, pH 6.5, and dialyzed overnight against the same buffer. The dialyzate was applied to a CM-Sephadex C-50 column (3 × 70 cm) equilibrated with 20 mM phosphate buffer, pH 6.5, and washed with the above buffer. The reductases for ABBP and *p*-nitrobenzaldehyde were not adsorbed. The washed fractions with enzyme activity were pooled, and the enzymes were precipitated by addition of ammonium sulfate to 90 per cent saturation and the precipitate was collected by centrifugation at 12,000*g* for 20 min and was dissolved in a minimum volume of 10 mM Tris buffer, pH 7.5. This solution was applied to a column (4.8 × 90 cm) of Sephadex G-100. The column was washed with 10 mM Tris buffer, pH 7.5. The enzyme activities with both ABBP and *p*-nitrobenzaldehyde as substrates emerged as a single broad peak at the same elution position. The enzyme-rich fractions eluted from Sephadex G-100 were applied to a DEAE–cellulose column (1.8 × 20 cm) equilibrated with 10 mM Tris buffer, pH 7.5. The column was washed with 10 mM Tris buffer containing 0.01 M NaCl, pH 7.5, until A_{280} of the effluent approached zero; about 300 ml of this buffer was required. No reductase activity for ABBP and *p*-nitrobenzaldehyde was found in the effluent. The adsorbed proteins were eluted with 300 ml of a linear gradient from 0.01 to 0.1 M NaCl at a flow rate of 30 ml/hr. As shown in Fig. 1, three distinct peaks of reductase activity for *p*-nitrobenzaldehyde emerged, and were denoted F_1, F_2 and F_3 in their order of elution, respectively. The F_1 and F_3 contained activity with ABBP, but the F_2 had very little activity with ABBP. Each enzyme fraction was applied to a hydroxylapatite column (1 × 7 cm) equilibrated with 10 mM Tris buffer containing 1 mM phosphate buffer, pH 7.5. The F_1 was adsorbed and eluted with 100 ml of a linear salt gradient from 1 to 80 mM phosphate buffer, pH 7.5, after washing with three column volumes of the Tris buffer. On the other hand, the F_2 and F_3 were not adsorbed and appeared in the

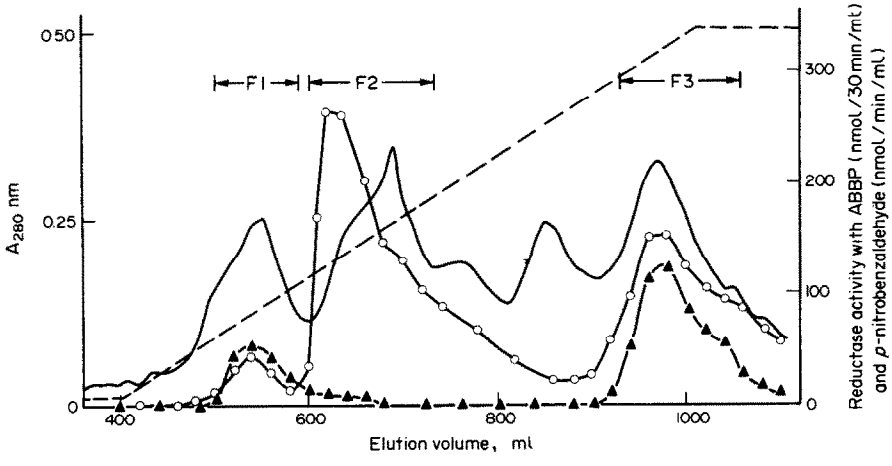

Fig. 1. Separation of reductases for ABBP and *p*-nitrobenzaldehyde on DEAE–cellulose. The column (1.8 × 20 cm) was eluted with a linear salt gradient from 0.01 M NaCl (volume 400 ml) to 0.1 M NaCl (volume 1000 ml), (— — —). ABBP reductase activity (▲) was measured by the method A and represents as the reduced product (nmol)/30 min/ml, and *p*-nitrobenzaldehyde reductase activity (○) represents as NADPH (nmol) oxidized/min/ml.

effluent and washings. No ABBP reductase activity of the purified F_2 was found, but the F_1 and F_3 had the activity to reduce both ABBP and *p*-nitrobenzaldehyde and both activities eluted on the column showed a good correspondence with each other. Each enzyme-rich fraction from the hydroxylapatite chromatography was concentrated to about 1 mg protein/ml, and stored frozen in small portions at −20°, and was stable under these conditions for at least 2 weeks.

RESULTS

Purification of enzymes. In preliminary experiments we found that most of the ABBP reductase activity was contained in the soluble fraction of rabbit liver [10], in which high aldehyde reductase activity with *p*-nitrobenzaldehyde as a substrate was also found.

The partial purification of two ABBP reductases and an aldehyde reductase from the soluble fraction of rabbit liver is summarized in Table 1. Almost all of both reductase activities were recovered in the protein fraction precipitated with ammonium sulfate between 35 and 75 per cent. The aldehyde reductase activity decreased by subsequent fractionation on CM-Sephadex, whereas considerable reductase activity with ABBP remained. Gel-filtration on Sephadex G-100 could not separate both activities. However, the activity to reduce *p*-nitrobenzaldehyde was separated into three fractions on DEAE–cellulose chromatography (Fig. 1). The main fraction (F_2) of the enzyme activity with *p*-nitrobenzaldehyde was eluted at about 0.05 M NaCl concentration. The minor fraction (F_1), eluted ahead of the F_2, and the last eluted fraction (F_3) showed a high affinity for ABBP and *p*-nitrobenzaldehyde. Each enzyme fraction was purified further by hydroxylapatite chromatography, but no evidence for a further heterogeneity was found.

Molecular weight determination. The molecular weight of three enzymes was determined by gel filtration on a previously calibrated Sephadex G-100 column with calibration proteins. The F_1 and F_3 had the similar molecular weight of about 33,000 and 32,000, respectively, but the F_2 had a slightly lower molecular weight of about 29,000 (Fig. 2).

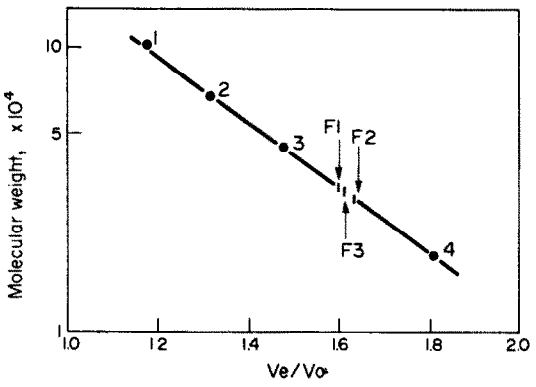

Fig. 2. Molecular weight determination of rabbit liver reductases by gel filtration on a Sephadex G-100 column. Plot of elution volume per void volume, V_e/V_o, against log (molecular weight) for proteins. The reference proteins used are as follows: (1) acid phosphatase, (2) bovine serum albumin, (3) ovalbumin, (4) myoglobin. The arrow indicates the position of three reductases.

Properties of enzymes. Only NADPH, and not NADH, was utilized as a cofactor for the reduction of *p*-nitrobenzaldehyde and ABBP by three enzymes.

Similar pH rate profiles with ABBP as substrate were observed for F_1 and F_3 with broad optimum pH between 7.2 and 8.0, but all other tested substrates possessed the acid optimum pH between 5.0 and 5.4. Substrate-dependent optimum pH might be the result of the difference in the structure of substrate and in the enzyme assay method. On the other hand, the optimal pH for the F_2 was found to range from 5.8 to 6.2 when *p*-nitrobenzaldehyde and D-glucuronate were used as substrate.

It was confirmed that 1 mol of pyridine-3-aldehyde was reduced for every mol of NADPH oxidized by all

Table 1. Purification of ABBP and aldehyde reductases from rabbit liver

Purification step		ABBP reductase* Specific activity (nmol/min/mg)	ABBP reductase* Yield (%)	*p*-Nitrobenzaldehyde reductase† Specific activity (nmol/min/mg)	*p*-Nitrobenzaldehyde reductase† Yield (%)	Total protein (mg)
105.000 *g* supernatant		0.097	100	48.4	100	9720
Ammonium sulfate fractionation (35–75% saturation)		0.180	104	74.7	87	5500
CM-Sephadex C-50 chromatography		0.400	89.6	59.0	36.5	2910
Sephadex G-100 chromatography		2.85	64.9	327	22.2	317
DEAE–cellulose chromatography	F_1	3.40	9.8	28.9	1.7	27.7
	F_2	0.43	2.7	621	8.0	60.9
	F_3	12.9	37.6	668	4.0	27.7
Hydroxylapatite chromatography	F_1	11.0	1.7	170	0.04	1.12
	F_2	0.00	0.0	2780	5.6	7.36
	F_3	57.4	22.6	4140	3.2	3.75

* Activity was measured by method A and represents nmol of 2-amino-5-bromo-2′-azabenzhydrol formed/min/mg of protein.

† Activity was measured by method B and represents nmol of NADPH oxidized/min/mg of protein.

Table 2. Substrate specificity of rabbit liver aldehyde reductases

Substrate	Relative activity* F_1†	F_2‡	F_3†
Acetaldehyde	3	1	0
n-Butyraldehyde	38	15	10
DL-Glyceraldehyde	30	33	0
D-Glucuronate	0	70	0
γ-D-Glucuronolactone	0	34	0
Benzaldehyde	51	4	0
p-Nitrobenzaldehyde	37	116	150
Pyridine-3-aldehyde	70	119	67
Pyridine-4-aldehyde	100	100	100
p-Nitroacetophenone	105	0	75
p-Chloroacetophenone	32	0	4
2-Benzoylpyridine	73	0	140
3-Benzoylpyridine	25	0	120
4-Benzoylpyridine	124	0	227
Oxisuran	51	0	110
Metyrapone	24	0	6
Cyclohexanone	110	0	67
ABBP§	2	0	2

* Initial velocity of substrates at 1 mM concentration expresses relative to the initial velocity of pyridine-4-aldehyde.
† Assayed with 80 mM citrate buffer (pH 5.2).
‡ Assayed with 80 mM phosphate buffer (pH 6.0).
§ Activity was measured by method A described in Methods.

Table 3. Apparent Michaelis constants with rabbit liver aldehyde reductases

Substrate	K_m (mM) F_1*	F_2†	F_3*
DL-Glyceraldehyde	2.63	1.70	n.d.‡
Benzaldehyde	0.12	6.8	n.d.
p-Nitrobenzaldehyde	0.013	0.18	4.3
Pyridine-3-aldehyde	0.14	0.093	4.5
Pyridine-4-aldehyde	0.016	0.14	2.1
p-Nitroacetophenone	0.093	n.d.	3.6
2-Benzoylpyridine	0.018	n.d.	0.84
4-Benzoylpyridine	0.016	n.d.	0.33
Oxisuran	0.64	n.d.	4.9
Cyclohexanone	0.034	n.d.	1.6
D-Glucuronate	n.d.	2.6	n.d.
ABBP§	0.24	n.d.	0.31

* Assayed with 80 mM citrate buffer (pH 5.2).
† Assayed with 80 mM phosphate buffer (pH 6.0).
‡ n.d., not determined.
§ Assayed by method A.

enzymes according to the method of Bosron and Prairie [5]. However, utilization of A_{340} of NADPH (Method B) as a measure of enzyme activity with ABBP was not possible. No accurate stoichiometric relation could be found between the amount of product formed and NADPH oxidized, because ABBP shows an absorption maximum at 398 nm ($\log \varepsilon = 3.75$) and the reduced product shows a weak absorption maximum at 304 nm.

Less than one per cent of the reducing activity found with *p*-nitrobenzaldehyde and NADPH could be observed for the reverse reaction with *p*-nitrobenzylalcohol (5 mM) and NADP (0.4 mM) by any enzymes. Furthermore, no detectable activity was observed in the presence of either NADP or NAD (0.4 mM) with ethanol (10 mM) as substrate at pH 8.5. This indicates that alcohol dehydrogenase activity is absent in these enzyme preparations.

Substrate specificity. As shown in Table 2, there exist remarkable differences between these enzymes with regard to substrate specificity. The F_1 and F_3 reduced aromatic aldehydes, aromatic ketones and cyclohexanone, whereas the F_2 reduced aromatic and aliphatic aldehydes, D-glucuronate and γ-D-glucuronolactone but not aromatic ketones and cyclohexanone. None of these enzymes reduced aldoses (D-ribose, D-galactose and D-arabinose) or aliphatic ketones (acetone and butanone).

The apparent K_m values reported in Table 3 were derived from Lineweaver–Burk plots with concentrations of the aldehydes and ketones at saturating coenzyme concentration (0.16 mM). The K_m values for aromatic ketones with F_3 were lower than that for aromatic aldehydes, and F_1 revealed the lower K_m values for all substrates than F_3. The F_2 had a low K_m value for aromatic aldehydes, but a high K_m value for D-glucuronate. The K_m values for NADPH with F_1, F_2 and F_3, and with pyridine-4-aldehyde (5 mM) as a substrate, were below 8 μM.

Polyacrylamide disc gel electrophoresis. Purity of the three enzymes was evaluated by polyacrylamide disc gel electrophoresis. All enzymes showed one main

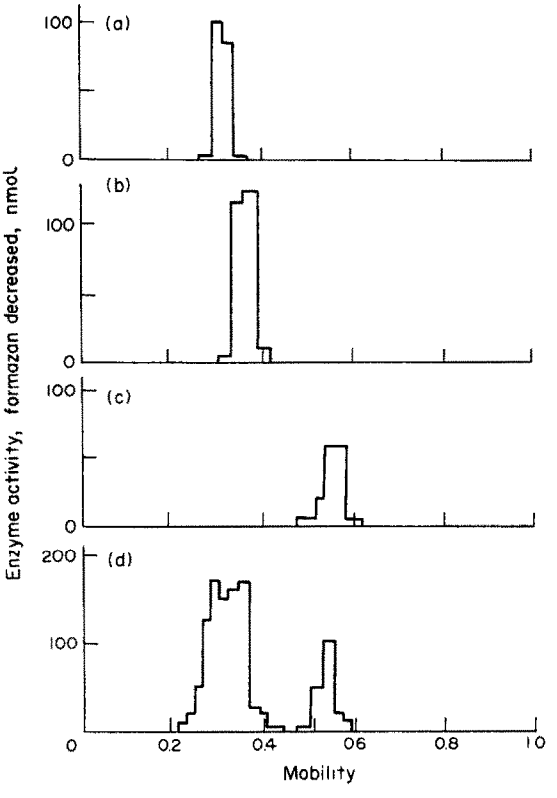

Fig. 3. Detection of reductase activity for three different substrates after polyacrylamide gel electrophoresis. After F_1(a), F_2(b), F_3(c) and a mixture of three enzymes (d) were run on identical gels in parallel, the gel was sliced and the activity assayed as described in the text. Substrates used were 4-benzoylpyridine (a and c), D-glucuronate (b) and pyridine-4-aldehyde (d).

Table 4. Effect of various inhibitors on rabbit liver aldehyde reductases

Inhibitor	Concentration (mM)	Inhibition(%)*		
		F_1†	F_2‡	F_3†
Sodium barbital	1.0	0	41	0
Sodium phenobarbital	1.0	0	42	5
p-Chloromercuribenzoate	0.1	19	79	99
p-Chloromercuribenzoate	0.001	0	10	97
$CuSO_4$	0.1	57	75	98
2.2′-Dipyridyl	1.0	0	3	0
Disulfiram	0.04	0	5	29
Chloral hydrate	1.0	0	0	0
Pyrazole	1.0	1	6	0

* Activity was measured with pyridine-4-aldehyde as substrate as described in Methods and assayed by the addition of NADPH after the preincubation for 1 min with inhibitor.
† Assayed with 80 mM citrate buffer (pH 5.2).
‡ Assayed with 80 mM phosphate buffer (pH 6.0).

and several minor protein bands when stained for protein. After electrophoresis the activity of unstained gel slices was assayed. As shown in Fig. 3, the F_1, F_2 and F_3 showed one activity peak with 4-benzoylpyridine or D-glucuronate, that was coincident with each main protein band, respectively. Even when a mixture of three enzymes was run, three activity peaks with pyridine-4-aldehyde as substrate emerged, and the activity peaks with pyridine-4-aldehyde and 4-benzoylpyridine were coincided for F_1 and F_3. Hence it would seem for F_1 and F_3 that the same protein is responsible for reduction of both aromatic aldehydes and ketones respectively. The mobility of activity with D-glucuronate was identical to that with pyridine-4-aldehyde on electrophoresis of F_2. Thus, F_2 was responsible for reduction of both aromatic aldehydes and D-glucuronate.

Inhibitor studies. The different effect of inhibitors on rabbit liver reductases is shown in Table 4. The F_1 was not affected by various inhibitors with the exception of moderately inhibitory effects of *p*-chloromercuribenzoate and cupric sulfate. The inhibition caused by phenobarbital and barbital was moderate for F_2. The F_3 was very sensitive to *p*-chloromercuribenzoate and inhibited 63 per cent at 10^{-7} M and this inhibition by *p*-chloromercuribenzoate (10^{-7} M) was found to be almost completely reversed by the addition of 2-mercaptoethanol and glutathione (10^{-6} M). Disulfiram slightly inhibited the F_3, but chloralhydrate, an inhibitor of aldehyde dehydrogenase as well as disulfiram [17], had no effect. This indicates that the inhibition of disulfiram may not be dependent on the presence of aldehyde dehydrogenase. Pyrazole, an inhibitor of alcohol dehydrogenase [18], did not inhibit these rabbit liver reductases.

DISCUSSION

The results in the present paper demonstrate that the reduction of aromatic aldehydes in rabbit liver is due to at least three proteins (F_1, F_2 and F_3). They can be distinguished by several criteria, including pH optima, substrate specificity, inhibitor sensitivity, molecular weight and mobility in gel electrophoresis.

NADPH-dependent aldehyde reductases in various mammalian tissues have been known to catalyze reduction of physiological substrates including D-glyceraldehyde, D-glucuronate, glucuronolactone and long chain aliphatic aldehydes, in addition to a number of xenobiotic aromatic and aliphatic aldehydes, but ketones are poor substrates for the enzymes (5–7, 19, 20). They are inhibited by barbiturates. From the results of substrate and cofactor specificity and inhibitor sensitivity, one of the purified enzymes (F_2) may be identical to aldehyde reductase. The F_2 has a low molecular weight of 29,000, and is compatible with the molecular weight of aldehyde reductases in mouse liver [21], pig kidney [5] and brain [6]. Physiological roles of the enzyme may be reduction of D-glucuronate to L-gulonate in the production of L-ascorbic acid [22], and glycerol metabolism [20], and detoxication of biogenic aldehydes as brain and heart aldehyde reductases [6, 19].

Borson and Prairie [23] reported the existence of high and low molecular weight aldehyde reductases in rabbit liver. The multiple molecular forms of the enzyme are separated by ion-exchange chromatography from human and rat brain [24]. Recently, two isozymes of the aldehyde reductase have been isolated from mouse liver [21]. During purification on CM-Sephadex step, the reductase activity with *p*-nitrobenzaldehyde decreased, suggesting that aldehyde reductases with charge difference are present in rabbit liver.

The other two enzymes (F_1 and F_3) that were purified as ABBP reductases give similar substrate specificity for aromatic ketones and aldehydes, and it is confirmed for F_1 and F_3 that a protein is responsible for reduction of aromatic ketones and aromatic aldehydes from coincidence of activities with both substrates on polyacrylamide gel electrophoresis. Thus, two enzymes are aromatic aldehyde–ketone reductase isozymes that have a higher affinity for aromatic ketones than for aromatic aldehydes; these isozymes are not due to differences in molecular weight but to charge differences, as previously noted for human brain enzymes [24]. The F_1 has very low K_m values for all tested substrates, and the F_3, a major isozyme in rabbit liver, may be a sulfhydryl enzyme. The existence of NADPH-dependent reductases for acetophenone and metyrapone [8], and oxisuran [9] in rabbit liver has been reported, and the reduction is probably mediated by these isozymes.

Classical liver alcohol dehydrogenase (EC 1.1.1.1) exhibits a broad substrate specificity; many aldehydes and cyclic ketones can be reduced [25, 26]. The F_1 and F_3 which actively reduce cyclohexanone, however, are differentiated from alcohol dehydrogenase, since NADH was not utilized as cofactor, and pyrazole did not inhibit these enzymes. Furthermore, ethanol was not oxidized by these enzymes under conditions favorable for liver alcohol dehydrogenase. With respect to substrate specificity, these enzymes are differentiated from α, β-unsaturated ketone reductase [3] and aromatic α-keto acid reductase [1]. Human erythrocyte *p*-nitroacetophenone reductase has reduced some aromatic aldehyde [4], but the F_1 and F_3 are probably different from the erythrocyte enzyme, because these enzymes were purified from perfused livers and ABBP reductase activity could not detected in rabbit hemolyzate. Ris and Von Wartburg [24] have separated four aldehyde reductase isozymes by DEAE–cellulose chromatography; the F_1 and F_3 resemble the human brain isozyme 4.1 and 4.4 with respect to cofactor and substrate specificity and inhibitor sensitivity, respectively. Based on the catalytic properties, they may be identical to aromatic aldehyde–ketone reductase which Culp and McMahon [2] purified from rabbit kidney. The only major difference between the kidney enzyme and the liver enzymes is that the liver enzymes were sensitive to *p*-chloromercuribenzoate but not to metal binding reagents. No claim for such a heterogeneity was made for the kidney enzyme. However, it was found in our studies that rabbit kidney enzymes showed a heterogeneity like the liver enzymes.*

The function of these reductases for aromatic aldehydes and ketones may be detoxication of aldehydes and ketones in drug metabolism, because they could reduce some drugs, such as oxisuran, metyrapone and ABBP. In spite of many reports on the enzyme activities that reduce aromatic ketones as reviewed by Bachur [27], the responsible reductases have not yet been characterized in other animals except for rabbit. Further studies on the aromatic aldehyde–ketone reductase with respect to biogenic substrate and comparative biochemical studies are needed in order to fully understand the function of these enzymes.

* Unpublished work.

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