Purification of the high- K_m aldehyde reductase from rat brain and liver and from ox brain

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A procedure is described that yields an apparently homogeneous preparation of the high- K_m aldehyde reductase from rat brain. This procedure is also applicable to the purification of this enzyme from rat liver and ox brain. In the latter case, however, the purified preparation could be resolved into two protein bands, both of which had enzyme activity, by polyacrylamide-gel electrophoresis. Since a sample of the ox brain enzyme from an earlier step in the purification procedure only showed the presence of a single band of activity after electrophoresis, this apparent multiplicity probably results from modification of the enzyme, possibly by oxidation, during the final step of the purification. A number of properties of the rat brain enzyme were determined and these were compared with those of the enzyme from rat liver. The two preparations were similar in their stabilities, behaviour during purification, kinetic properties, electrophoretic mobilities and amino acid compositions. Antibodies to the rat liver enzyme cross-reacted with that from brain and the inhibition of both these preparations by the antiserum was similar, further supporting the view that the enzymes from these two sources were closely similar if not identical.

Two major forms of the enzyme aldehyde reductase (alcohol: NADP⁺ oxidoreductase, EC 1.1.1.2) have been found to be present in tissues from a number of species including pig, rat and ox brain (Turner & Tipton, 1972; Ris & von Wartburg, 1973; Erwin *et al.*, 1972) and mouse and rat liver (Tulsiani & Touster, 1977; Rivett *et al.*, 1981). The functions of the minor form (which has been termed AR2 or the low- K_m form) may include the reduction of the aldehydes derived from the oxidation of the biogenic amines (Turner *et al.*, 1974; Anderson *et al.*, 1976). The role of the major form (AR1 or the high- K_m form) is, however, not clear although it has been suggested that it may be involved in the reduction of succinic semialdehyde (Kaufman *et al.*, 1979; Branlant & Biellmann, 1980; but cf. Rivett *et al.*, 1981), or general aldehyde detoxification and drug metabolism (Turner & Hick, 1976; Felsted *et al.*, 1977; Branlant & Biellmann, 1980).

Abbreviation used: SDS, sodium dodecyl sulphate.

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Several of the earlier studies on the properties of the high- K_m aldehyde reductase are difficult to evaluate because of failure to take account of the presence of the two forms of the enzyme (see Turner & Tipton, 1972) and it has been difficult to compare the published properties of more highly purified preparations because of differences in the substrates used and assay conditions used. As part of a study on the possible functions of the high- K_m aldehyde reductase we have purified the enzyme from several sources and the present paper describes a procedure that appears to be applicable to the enzyme from liver and brain and also presents evidence for the close similarity of these enzymes from these organs of the rat.

Materials and methods

Materials

Succinic semialdehyde, *p*-nitrobenzaldehyde, 5,5-diphenylhydantoin sodium salt, chlorpromazine hydrochloride, bovine serum albumin and ovalbumin were obtained from Sigma; *o*-carboxybenzaldehyde and γ -hydroxybutyrate were from Aldrich. NNN'-Tetramethylethylenediamine, acrylamide, D-glucuronate, DL-glyceraldehyde, acetaldehyde, γ -aminobutyric acid, propane-1,2-diol, *p*-carboxybenzaldehyde, 5-hydroxytryptamine,

creatinine sulphate and 5-hydroxyindoleacetic acid were obtained from BDH. L-Noradrenaline bitartrate and 4-hydroxy-3-methoxymandelic acid were from Koch-Light. Pyrazole and *NN'*-methylenebisacrylamide were from Eastman Chemicals and sodium barbitone was from May & Baker. Co-enzymes and commercial enzyme preparations were obtained from Boehringer and agarose was from MicroBio Laboratories, London, U.K. DEAE-cellulose (DE-52) was from Whatman and NADP-agarose (AG NADP type 4) was from P-L Biochemicals. Hydroxyapatite was prepared by the method of Levin (1962). Other laboratory chemicals were of AR grade whenever this was obtainable.

p-Nitrobenzaldehyde (m.p. 105–107°C) and *o*-carboxybenzaldehyde (m.p. 98°C) were recrystallized from water before use. γ -Hydroxybutyrate (m.p. 145–146°C) was recrystallized from ethanol. Solutions of *p*-nitrobenzaldehyde were made up in methanol/water (1:1, v/v) and diphenylhydantoin was dissolved in ethanol/water (1:1, v/v). The concentration of succinic semialdehyde in solution was estimated enzymically by using succinic semialdehyde dehydrogenase (Rivett *et al.*, 1981) and aldehyde dehydrogenase was used to determine the concentration of *p*-nitrobenzaldehyde (Racker, 1957). NADPH and NADH concentrations were estimated from the A_{340} by using a molar absorption coefficient of $6.22 \times 10^3 \text{ M}^{-1} \cdot \text{cm}^{-1}$. The concentration of NADP⁺ was estimated in a similar manner after reduction by glucose 6-phosphate in the presence of glucose-6-phosphate dehydrogenase.

Brains and livers from albino Wistar rats of either sex were used for enzyme preparations and antibodies were raised in New Zealand White rabbits.

Assay methods

Aldehyde reductase activity was assayed spectrophotometrically at 30°C by following the decrease in A_{340} accompanying the oxidation of NADPH. Unless otherwise stated the reaction mixture contained 0.1M-sodium phosphate buffer, pH 7.0, 100 μM -NADPH, aldehyde and the enzyme preparation. For routine assays during purification of the enzyme D-glucuronate was used as the substrate at a final concentration of 10mM. For assays in the reverse direction the reaction mixture contained 0.1M-glycine/NaOH buffer, pH 9.5, 1mM-NADP⁺, the enzyme preparation and either 100mM-propane-1,2-diol or 100mM- γ -hydroxybutyrate.

Protein concentration was determined by the Lowry method with bovine serum albumin as the standard. Amino acid analysis was carried out on freeze-dried samples of the enzyme that had been hydrolysed in 3M-*p*-toluenesulphonic acid in 2% (w/v) 3-(2-aminoethyl)indole at 110°C for 25h

under vacuum using a Jeol JLC-SAH amino acid analyser.

Electrophoresis

Polyacrylamide-gel electrophoresis was carried out in 7% (w/v) polyacrylamide gels at pH 8.5 by the method of Davis (1964). Gels were stained for aldehyde reductase activity by incubation at room temperature in the dark for 30min in a mixture containing, in a total volume of 15ml: 6mg of NADP⁺, 1ml of propane-1,2-diol, 8mg of Nitro Blue Tetrazolium, 0.5mg of phenazine methosulphate and 0.1M-sodium phosphate buffer, pH 7.4. Gels were stained for protein by incubation for about 30 minutes in a mixture of 2% (w/v) Coomassie Brilliant Blue in acetic acid/methanol/water (1:5:4, by vol.). The excess stain was removed by soaking the gels in acetic acid/methanol/water (7:10:83, by vol.).

Polyacrylamide slab-gel electrophoresis in the presence of SDS was carried out in a slab-gel apparatus (Studier, 1973) with gels prepared with 0.1% SDS and with acrylamide and *NN'*-methylenebisacrylamide concentrations of 15% and 0.087% respectively. The stacking gel contained 0.1% SDS, 5.75% acrylamide and 0.15% *NN'*-methylenebisacrylamide. The resolving gel contained 750mM-Tris/HCl buffer, pH 8.7, the stacking gel contained 140mM-Tris/HCl buffer, pH 6.8, and the running buffer was 400mM-glycine/50mM-Tris buffer, pH 8.3, containing 0.1% SDS.

Protein samples (1mg/ml) were prepared for electrophoresis by dilution 5-fold with 25mM-Tris/HCl, pH 6.8, containing 2% (w/v) SDS, 5% (v/v) glycerol, 0.01% 2-mercaptoethanol and 0.0012% Bromophenol Blue. This mixture was heated at 100°C for 2min and allowed to cool before 50 μl samples were applied to the gel. Electrophoresis was carried out at 50V overnight after which time the Bromophenol Blue band had almost reached the bottom of the gel. After electrophoresis the gels were fixed by soaking for 30min in acetic acid/ethanol/water (1:5:4, by vol.). They were then stained for 30min with 1.25% (w/v) Coomassie Blue in the same solvent and the excess stain was removed by soaking in methanol/acetic acid/water (10:7:83, by vol.). The following marker proteins were used for the determination of the subunit molecular weight: chymotrypsin (25 000), lactate dehydrogenase (35 000), aldolase (40 000), ovalbumin (45 000), pyruvate kinase (57 200) and bovine serum albumin (69 000). The values in parentheses are the subunit molecular weights, taken from Darnall & Klotz (1972).

Preparation and use of antiserum

A solution (1mg/ml) of purified rat liver aldehyde reductase (from step 4 of the purification procedure

described below) was emulsified with an equal volume of Freund's complete adjuvant by vortex mixing. Doses of 1mg of the enzyme were given to young adult rabbits at intervals of 2 weeks. The first dose was given intramuscularly, 1ml into each thigh, and subsequent injections were given subcutaneously at four sites on the back. Antibodies were detectable 4 weeks after the first injection.

Blood samples of up to 30ml were taken from the ear marginal veins and were allowed to clot at 30°C. The serum was decanted, centrifuged in a Minifuge for 10min and stored frozen. A sample of the control serum (pre-serum) was obtained before the course of injections was started. Immunoglobulin preparations were obtained by a modification of the method of Mayer & Walker (1978). $(\text{NH}_4)_2\text{SO}_4$ was added to the antiserum at 0°C to give 50% saturation. After stirring for about 30min the immunoglobulin precipitate was isolated by centrifugation in a Minifuge for 10min and it was then washed repeatedly with 1.75M- $(\text{NH}_4)_2\text{SO}_4$ at 0°C until all coloured contaminants had been removed. It was then dissolved in 0.1M-sodium phosphate buffer, pH 7.0, and dialysed against water at 4°C overnight. Precipitated protein was removed by centrifugation in a Minifuge for 10min and the supernatant was dialysed against 20mM-sodium phosphate buffer, pH 7.0.

Immunodiffusion (Ouchterlony, 1968) was carried out in 15% (w/v) agarose in phosphate-buffered saline [8% (w/v) NaCl, 0.2% KCl, 1.15% (w/v) disodium hydrogen phosphate and 0.2% potassium dihydrogen phosphate] which has been allowed to set on glass microscope slides.

Preparation of the high- K_m aldehyde reductase

The enzyme was purified from rat brain by a modification of the procedure of Felsted *et al.* (1974) with the addition of an affinity chromatography step on NADP-agarose. The use of brains that had been stored frozen was generally more convenient for the purification and gave similar results to those obtained with fresh brains. All procedures were carried out at 0–4°C unless otherwise stated.

Step 1. Homogenization and centrifugation. Rats were killed by decapitation and the brains were removed rapidly, rinsed in 50mM-Tris/HCl buffer, pH 8.6, blotted dry and weighed. Frozen brains were thawed overnight at 4°C and then used in the same way. Then, 50–150 brains were homogenized in 4 vol. of the same buffer for 2×30 s at full speed in a Kenwood Chef blender. For smaller preparations involving 20–25 brains a Dounce homogenizer was used. The homogenate was centrifuged at 48000g for 1h and the pellet was discarded.

Step 2. $(\text{NH}_4)_2\text{SO}_4$ fractionation. To the supernatant, which had a protein concentration of 6–12mg/ml, solid $(\text{NH}_4)_2\text{SO}_4$ was added with continuous

stirring to give 35% saturation (20.9g/100ml). Stirring was continued for a further 30min and the precipitate was removed by centrifugation at 48000g for 15min, and discarded. The supernatant was made to 65% saturation by addition to solid $(\text{NH}_4)_2\text{SO}_4$ (19.9g/100ml) in the same way. The precipitate was recovered by centrifugation and dissolved in 10mM-Tris/HCl buffer, pH 8.6, by gentle use of a Dounce homogenizer. This solution was then dialysed overnight against 100–200 vol. of the same buffer.

Step 3. DEAE-cellulose chromatography. The dialysed $(\text{NH}_4)_2\text{SO}_4$ fraction (10–20mg/ml) was applied to a column of DEAE-cellulose which had been equilibrated in 10mM-Tris/HCl buffer, pH 8.6. The column was then washed several times with the sample volume of this buffer before the enzyme was eluted by using a linear gradient, from 0–0.1M, of NaCl in the same buffer. For a preparation from 100 brains a column with a bed volume of about 100ml was used, the total gradient volume was 3.0 litres and 20ml fractions were collected. An example of the elution profile from such a column is shown in Fig. 1.

Fractions containing more than 15% of the peak activity of aldehyde reductase were pooled and solid $(\text{NH}_4)_2\text{SO}_4$ was added slowly with stirring to give 90% saturation (65.7g/100ml). After stirring on ice for 1h the precipitate was removed by centrifugation at 48000g for 15min and dissolved in a small volume of 10mM-sodium phosphate buffer, pH 7.4. This solution was then dialysed overnight against 100–200 vol. of this buffer with one change.

Step 4. Hydroxyapatite treatment. Hydroxyapatite equilibrated in 10mM-sodium phosphate buffer, pH 7.4, was subjected to a short low-speed centrifugation to remove the excess buffer. It was then added batchwise to the enzyme solution (1–2mg of protein/ml), stirring gently on ice, to give a final ratio of the volume of enzyme solution to that of the packed hydroxyapatite of about 2:1. Aldehyde reductase activity was not absorbed under these conditions and after stirring for 30min the hydroxyapatite was removed by centrifugation and was washed with a small volume of 10mM-sodium phosphate buffer, pH 7.4. The two supernatants were combined and centrifuged at 48000g for 10min to remove any remaining hydroxyapatite.

Step 5. NADP-agarose chromatography. The enzyme solution from the previous step, which had a protein concentration of 0.1–0.2mg/ml, was applied to a column of NADP-agarose (bed volume about 1ml) which had been equilibrated in 10mM-sodium phosphate buffer, pH 7.4. The column was washed with this buffer and a linear gradient of 0–0.1M-KCl in a total volume of 30ml of the same buffer was used to elute the enzyme. Fractions with aldehyde reductase activity were pooled, dialysed overnight

Fig. 1. *Chromatography of rat brain aldehyde reductase on DEAE-cellulose*

The procedure followed was that described in step 3 of the purification procedure. Elution was effected by a linear gradient from 0–0.1 M-NaCl in 10 mM-Tris/HCl buffer, pH 8.6; ○ shows the conductivity measurements (in arbitrary units). Protein concentration was estimated from the A_{280} (□) and enzyme activity (given in arbitrary units) was determined with 10 mM-D-glucuronate as the aldehyde substrate (▲). The horizontal bar indicates the fractions that were pooled for further purification.

Table 1. *Purification of the high- K_m aldehyde reductase from rat brain*

The table shows the purification of aldehyde reductase from 100 rat brains (approx. 170 g). Enzyme activity was assayed with 10 mM-D-glucuronate and 100 μ M-NADPH in 0.1 M-sodium phosphate, pH 7.0, to a total volume of 3 ml. One unit of activity is defined as the amount of enzyme required to catalyse the oxidation of 1 μ mol of NADPH/min.

Step	Volume (ml)	Activity (units/ml)	Total activity (units)	Protein (mg/ml)	Specific activity (units/mg)	Purification (-fold)	Recovery (%)
1. Supernatant	700	0.056	39.2	11.3	0.005	1.0	(100)
2. Desalted 35–65% (NH ₄) ₂ SO ₄ fraction	120	0.291	34.9	18.0	0.0162	3.24	89
3. DEAE-cellulose eluate	495	0.053	26.2	0.21	0.252	50.4	67
Desalted 90% (NH ₄) ₂ SO ₄ fraction	40	0.520	20.8	1.64	0.317	63.4	53
4. Enzyme after hydroxyapatite treatment	61	0.310	18.9	0.11	2.82	564	48
5. NADP-agarose column	19.3	0.959	18.5	0.10	9.59	1920	47

against 10 mM-sodium phosphate buffer, pH 7.4, and then concentrated to about 1 mg of protein/ml using either a Millipore immersible molecular separator or by ultrafiltration using an Amicon UM2 membrane.

Results

Purification and properties of rat brain aldehyde reductase

The results of a typical purification procedure are shown in Table 1. During the final dialysis and concentration the enzyme lost about 20% of its

activity. It was found to be unstable in dilute solution but at concentrations of about 1 mg/ml it lost activity only slowly over several weeks when stored at 4°C. When stored frozen there was negligible loss of activity after 6 months, but repeated freezing and thawing resulted in loss of activity. The enzyme was thus routinely stored frozen in small aliquots.

When samples (10–40 μ g) of the purified enzyme were subjected to polyacrylamide-gel electrophoresis only one protein band was observed after staining with Coomassie Brilliant Blue. This band coincided with the enzyme activity, which was detected in a

duplicate gel. When a sample of the rat brain supernatant was subjected to electrophoresis in the same way only a single band of activity stained with propane-1,2-diol and this had the same relative

Fig. 2. Determination of the subunit molecular weight of the purified high- K_m aldehyde reductase from rat brain by polyacrylamide-gel electrophoresis in the presence of SDS

The standard proteins used were chymotrypsinogen (O), lactate dehydrogenase (□), ovalbumin (●), pyruvate kinase (■) and bovine serum albumin (▲). The arrow shows the mobility of the aldehyde reductase.

mobility as that of the purified enzyme. No activity band was detected in the absence of propane-1,2-diol.

Polyacrylamide-gel electrophoresis in the presence of SDS gave only one protein-staining band. A comparison of its mobility with those of a number of marker proteins is shown in Fig. 2, and a value for the subunit molecular weight of 36 300 was calculated from these data. Enzyme samples purified from fresh and frozen brain preparations and a sample of the purified enzyme that had been stored for 6 months behaved identically on electrophoresis in the presence of SDS.

The amino acid composition of the purified rat brain aldehyde reductase is compared with the published compositions of the enzyme from other sources in Table 2.

Catalytic properties

Kinetic parameters determined for several substrates in the presence of 100 μ M-NADPH are shown in Table 3. At concentrations of succinic semialdehyde and *p*-nitrobenzaldehyde above about 10 times the K_m value, high-substrate inhibition was observed. No activity could be detected when either *o*-carboxybenzaldehyde or acetaldehyde was used as the substrate. No significant rate was observed when 100 μ M-NADH replaced the NADPH with *p*-nitrobenzaldehyde, succinic semialdehyde or D-

Table 2. Comparison of the amino acid composition of rat brain high- K_m aldehyde reductase with those reported for preparations from other sources

Results are expressed as the number of residues per 36 300g and are given to the nearest whole number.

Amino acid	Composition of aldehyde reductase from:				
	Rat brain	Rat liver*	Mouse liver†	Human liver‡	Pig kidney§
Lysine	20	21	21	20	20
Histidine	10	11	9	9	7
Arginine	21	15	17	15	19
Aspartic acid	27	27	31	28	31
Threonine	15	14	12	12	15
Serine	27	20	18	16	14
Glutamic acid	37	33	36	35	41
Proline	22	26	24	27	22
Glycine	22	19	22	21	22
Alanine	29	29	30	29	31
Cysteine	3	4	3	6	5
Valine	21	27	19	21	26
Methionine	3	3	4	4	3
Isoleucine	11	12	11	14	15
Leucine	32	36	34	35	35
Tyrosine	13	13	11	13	9
Phenylalanine	8	8	8	8	9
Tryptophan	6	9	5	7	4

* Felsted *et al.* (1974).
† Tulsiani & Touster (1977).
‡ Wermuth *et al.* (1977).
§ Flynn *et al.* (1975).

Table 3. *Kinetic constants of rat brain aldehyde reductase*

The maximum velocity values (V) are expressed as percentages of that obtained with *p*-nitrobenzaldehyde.

Varied substrate	K_m (μM)	V (relative)
<i>p</i> -Nitrobenzaldehyde	80	100
D-Glucuronate	4500	87
DL-Glyceraldehyde	1500	41
Succinic semialdehyde	130	83
NADPH	3–5	—

glucuronate as the aldehyde substrate. The K_m value for NADPH was found to be about 3–5 μM using any of these aldehyde substrates.

A rate of γ -hydroxybutyrate oxidation of about 5–10% of the maximum rate of succinic semialdehyde reduction was observed in 0.1M-glycine/NaOH buffer, pH 9.5, containing 1mM-NADP⁺ and 100mM- γ -hydroxybutyrate. A similar rate was observed when γ -hydroxybutyrate was replaced by propane-1,2-diol, but no activity was detectable when ethanol was used or when the NADP⁺ was replaced by NAD⁺.

The activity of the enzyme was found to be increased by increasing concentrations of sodium phosphate buffer, pH 7.0, as shown in Fig. 3. The effects of pH on the initial velocities of the reduction of *p*-nitrobenzaldehyde, succinic semialdehyde and D-glucuronate are shown in Fig. 4. The enzyme was shown to be stable at the pH values used for times corresponding to those used in the assays.

When rat brain supernatant was heated at 40°C for 10 min prior to assay it retained about 90% of its activity towards D-glucuronate but after 10 min at 50°C the activity had been decreased to less than 5%.

The activity of the enzyme in the standard assay was not significantly affected by 0.5mM concentrations of γ -aminobutyrate, serotonin or noradrenaline; 0.1mM-acetaldehyde, 200mM-ethanol, 200mM-methanol, 0.5% Triton X-100, 0.25mM-chlorpromazine and 2mM-pyrazole was also without effect but the activity was inhibited by 75% and 80% respectively by sodium barbitone and diphenylhydantoin, each at a concentration of 0.1mM. 5-Hydroxyindoleacetic acid and 3-methoxy-4-hydroxymandelic acid were also inhibitory, giving 49% and 20% inhibition respectively at concentrations of 0.5mM.

Purification of rat liver and ox brain aldehyde reductase

The procedure described for the purification of the rat brain enzyme could be applied to the preparation of the high- K_m aldehyde reductase from these two sources. The only modification made for

Fig. 3. *Effects of phosphate buffer concentration on the activity of rat brain high- K_m aldehyde reductase*

Assays were carried out as described in the text with 100 μM -NADPH and 450 μM -succinic semialdehyde and the initial velocities are expressed relative to the value obtained at a total sodium phosphate concentration of 100mM.

Fig. 4. *Effects of pH on the activity of rat brain high- K_m aldehyde reductase*

Activity was determined as described in the text with 100 μM -NADPH and 330 μM *p*-nitrobenzaldehyde (●), 450 μM -succinic semialdehyde (▲) or 10mM-D-glucuronate (■).

the purification of the ox brain enzyme was that the enzyme was eluted from the NADP-agarose column with NADP⁺ (0.05 mg/ml) instead of with a salt gradient. Polyacrylamide-gel electrophoresis of the

purified ox brain enzyme revealed the presence of two bands of protein which were of approximately equal density and were very close together. Both these bands stained for enzyme activity. When a sample of ox brain aldehyde reductase from step 4 of the purification procedure was subjected to electrophoresis in the same way only a single band of enzyme activity could be detected.

With rat liver aldehyde reductase it was found that a procedure involving steps 1–4 of the purification scheme, which is quite similar to that used by Felsted *et al.* (1974), was adequate in some cases to give a preparation that appeared to be homogeneous on electrophoresis in the presence and absence of SDS. On other occasions a few very minor bands that stained for protein but not for enzyme activity could be detected after electrophoresis in the absence of SDS, suggesting that the use of the final NADP–agarose affinity chromatography step would be a wise procedure in order to ensure that homogeneous enzyme preparations were obtained routinely.

Comparison of rat liver and brain aldehyde reductases

The high- K_m aldehyde reductases from rat liver and brain were found to be very similar. They behaved in a similar way during purification and showed similar stabilities. When samples of the purified liver and brain enzymes were mixed and subjected to electrophoresis on polyacrylamide gels only a single activity-staining band could be detected. The pH–activity profile of the rat liver enzyme with succinic semialdehyde as the substrate was found to be very similar to that shown in Fig. 4 and the K_m values of the rat liver enzyme for *p*-nitrobenzaldehyde, succinic semialdehyde and D-glucuronate were not significantly different from those shown in Table 3. The comparison of amino acid compositions in Table 2 also suggests close similarities between the enzyme preparations from these two sources.

Immunological studies

When antiserum to the purified rat liver enzyme was tested against the purified rat brain enzyme by Ouchterlony (1968) double-diffusion in agar gel a single precipitin band was observed. No precipitin reaction was observed when pre-serum was used, but the results obtained with an immunoglobulin fraction were identical to those obtained with the antiserum itself. Assays of enzyme activity in the presence of increasing amounts of antiserum were carried out after preincubation of the enzyme with antiserum for 15 min at 30°C. Controls were carried out by incubating the enzyme with pre-serum and corrections were made for any loss of activity. Neither preincubation for 30 min nor centrifugation for 5 min

Fig. 5. Inhibition of purified aldehyde reductase preparations by antiserum prepared against the purified high- K_m aldehyde reductase from rat liver

The enzyme preparations were incubated with the antiserum for 15 min at 30°C. Other details were as described in the text. (a) Inhibition of rat liver aldehyde reductase, (b) inhibition of rat brain aldehyde reductase.

in a Minifuge after a 15 min preincubation caused any significant further decrease in activity. The results shown in Fig. 5 show that both the rat brain and liver enzymes behaved similarly towards the antiserum.

Discussion

The modifications of the procedure of Felsted *et al.* (1974) with the addition of an affinity chromatography step using NADP–agarose results in a

convenient purification method that can be applied to the production of apparently homogeneous preparations of the high- K_m aldehyde reductase from rat liver and brain. In a comparison of the use of different ligands for the affinity chromatography of aldehyde reductase Turner & Hryszko (1980) found that Cibacron Blue F3GA, Procion Red HE3B, 2',5'-ADP and NADP⁺ were all effective when immobilized on Sepharose. Rather similar procedures to that reported here were used to purify the enzyme from rat liver (Turner & Hick, 1976) and pig liver (Branlant & Biellmann, 1980). Both of these methods, however, incorporated an additional gel filtration step.

The procedure used here appears to be applicable to the purification of the enzyme from ox brain, but in this case two components with enzyme activity could be resolved when the purified material was subjected to polyacrylamide-gel electrophoresis. Multiple bands of aldehyde reductase on polyacrylamide gels have been observed with preparations from other sources (Bosron & Prairie, 1972; Flynn *et al.*, 1975; Wermuth *et al.*, 1977). Since a sample of the enzyme after step 4 of the purification procedure revealed only a single band staining for activity after polyacrylamide-gel electrophoresis, the apparent multiplicity of the more highly purified preparation is probably an artefact. This may arise from partial oxidation of the enzyme during the final purification step, since von Wartburg *et al.* (1977) have shown that multiple bands of human liver aldehyde reductase can result from oxidation during electrophoresis.

Many of the properties of the high- K_m aldehyde reductase from rat brain are similar to those of the enzyme from other sources. The enzyme has been reported to be monomeric and the molecular weight determined here lies within the 30 000–40 000 range of values that have been reported for preparations from other sources (Bosron & Prairie, 1972; Felsted *et al.*, 1974; Wermuth *et al.*, 1977; Tulsiani & Touster, 1977; Davidson *et al.*, 1978; Branlant & Biellmann, 1980). Activation by increasing buffer concentration has also been reported for the enzyme from other sources (Bosron & Prairie, 1972; Felsted *et al.*, 1977; Wermuth *et al.*, 1977). The dependence of activity on pH is quite similar to that determined with preparations from other sources, although rat liver aldehyde reductase has an alkaline pH optimum with daunorubicin as the substrate (Felsted *et al.*, 1977) and the pH profiles are apparently different for the enzymes from widely different species (Davidson *et al.*, 1978). The effects of inhibitors reported here are also similar to the results obtained with other preparations. Chlorpromazine has been reported to be an inhibitor of the enzyme (Bronaugh & Erwin, 1972; Ris & von Wartburg, 1973) but the present and several other studies

suggest that this is not the case (Turner & Tipton, 1972; Felsted *et al.*, 1977; Kaufman *et al.*, 1979).

There has been considerable variation in the K_m values that have been reported for the enzyme from different sources. In some studies this may have been due to a failure to separate the low- and high- K_m forms of the enzyme (see Turner & Tipton, 1972) and differences in the assay conditions employed may also have affected the values. With succinic semialdehyde as the substrate, Felsted *et al.* (1977) reported a K_m value of 10.9 mM for the rat liver enzyme, which is very different from that of 130 μ M determined in this work. Enzymes capable of catalysing the interconversion of succinic semialdehyde and γ -hydroxybutyrate have been purified from hamster liver and brain by Kaufman *et al.* (1979). The inhibitor sensitivities and molecular weights reported for these preparations were similar to those reported for the high- K_m aldehyde reductase, as were the kinetic parameters for several substrates, but the K_m value for succinic semialdehyde (37 μ M) was considerably lower than that determined in the present work. It is possible that these variations in the K_m values for succinic semialdehyde may be due in part to impurities that have been found to be present in samples of succinic semialdehyde prepared by some commonly used procedures (Rivett *et al.*, 1981).

The high- K_m aldehyde reductases from rat liver and brain appear to be closely similar in their behaviour during purification, stabilities and kinetic properties. The amino acid compositions of preparations from these two sources were also quite similar (Table 2) and their mobilities on polyacrylamide-gel electrophoresis were identical. The two preparations also appear to have similar immunological properties, as indicated by the cross-reaction of antibodies produced against the liver enzyme with the enzyme from brain and their similar inhibition curves when treated with this antiserum. Although these data are insufficient to establish identity they do suggest that results obtained with the rat liver enzyme will probably be applicable to that from rat brain.

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