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Purification and characterization of the multiple forms of aldehyde reductase in ox kidney

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Aldehyde reductase from ox kidney cytosol has been fractionated into four forms, two of which have been purified to apparent homogeneity. One of the minor forms is shown to be heterogenous on polyacrylamide-gel electrophoresis. The substrate specificities of the four forms using a variety of aldehydes and ketones are presented. The sensitivity of the various forms to inhibition by sodium valproate, sodium barbitone and various benzodiazepines has been determined. The relationships of these forms to the previously described hexonate dehydrogenase, aldose reductase and prostaglandin dehydrogenase is discussed.

Aldehyde reductases are NADPH-dependent oxidoreductases which reduce aldehydes to their corresponding alcohols. They possess a wide species and tissue distribution (Davidson *et al.*, 1978). Multiple forms of aldehyde reductase have been described in brain (Tabakoff & Erwin, 1970; Turner & Tipton, 1972; Ris & von Wartburg, 1973) and in liver (Tulsiani & Touster, 1977; Sawada & Hara, 1979; Sawada *et al.*, 1979, 1980; Branlant & Biellmann, 1980), while Sawada *et al.* (1979) have reported multiple forms of aldehyde/ketone reductase in guinea-pig kidney.

The physiological role(s) of these multiple forms remain obscure and have been variously suggested as functioning in the catabolism of biogenic amines (Turner & Tipton, 1972; Tabakoff & von Wartburg, 1975), the polyol pathway (Hers, 1960), drug detoxification (Bachur, 1976) and the inactivation of prostaglandins E and F (Wermuth, 1981; Chang & Tai, 1981). The major barbiturate-sensitive form of aldehyde reductase appears to be involved in ascorbate biosynthesis, reducing D-glucuronate to L-gulonate (Flynn *et al.*, 1975). Since the ascorbate biosynthetic pathway is localized in liver or kidney, depending on the species (Chatterjee, 1973), the high levels of the major form of aldehyde reductase reported in these tissues (Davidson *et al.*, 1978) can be interpreted as further evidence of its role in ascorbate biosynthesis.

Most aldehyde reductases, including the major form, have M_r values of approx. 30000; however, higher molecular weight forms (M_r 50000–110000) have also been described (Bosron & Prairie, 1973; Cash *et al.*, 1979; Sawada *et al.*, 1980).

Although the major form of aldehyde reductase has been purified and studied in detail from pig kidney (Flynn *et al.*, 1975; Morpeth & Dickinson, 1980), an analysis of the kinetic mechanism has been restricted to the substrates glyceraldehyde and pyridine-3-aldehyde (Davidson & Flynn, 1979; Morpeth & Dickinson, 1981). We have purified the major form of ox kidney aldehyde reductase (since this species, unlike certain species of bats and primates, is capable of the biosynthesis of ascorbate) to enable a detailed examination of the kinetic mechanism with D-glucuronate as substrate. Details of the purification procedure are described in this paper. While this study was in progress we noted the presence of three minor forms of aldehyde reductase and this paper also presents details of their substrate specificities and inhibitor sensitivities.

Abbreviation used: SDS, sodium dodecyl sulphate.

Experimental

Materials

NADPH was purchased from Boehringer, 4-nitrobenzaldehyde was obtained from Merck and 2′,5′-ADP–Sepharose 4B was supplied by Pharmacia. All other chemicals were of the highest purity available. Glass distilled deionized water was used throughout. Procion Orange MX-G was generously given by Dr. C. V. Stead, ICI Organics Division, Blackley, Manchester, U.K. Benzodiazepines were a gift from Roche, Welwyn Garden City, Herts., U.K. and the prostaglandins were supplied by Dr. J. Pike, Upjohn, Kalamazoo, MI 49001, U.S.A.

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Enzyme assay

Aldehyde reductase activity was routinely monitored by measuring the decrease in A_{340} with 75 μM-NADPH and either 20 mM-glucuronic acid or 0.33 mM-4-nitrobenzaldehyde in 0.1 M-sodium phosphate buffer, pH 7.0 at 30°C. Stock solutions of 4-nitrobenzaldehyde, 4-carboxybenzaldehyde, menadione and cyclohexanone were made up in methanol. The benzodiazepines were also made up in methanol, with the exception of flurazepam which was made up in buffer. The prostaglandins were added from stock solutions in ethanol. A control experiment showed that the amounts of methanol and ethanol added did not affect activity towards D-glucuronic acid.

Protein measurement

For routine column monitoring protein concentration was estimated by measurement of the A_{280}. Other samples were assayed by either the biuret method (Gornall *et al.*, 1949) or the microbiuret method of Goa (1953). Bovine serum albumin was used as standard in both assays.

Homogenization and separation of aldehyde reductase forms

Ox kidneys, obtained from a local slaughterhouse, were processed within 2 h of killing. On occasions, after cutting the tissue into small pieces (approx. 25 cm^3), it was stored at −20°C. All operations were carried out at 4°C. Quantities of 1 kg of tissue were homogenized in 3 vol. of 0.25 M-sucrose/2.0 mM-EDTA/2.5 mM-2-mercaptoethanol/10 mM-sodium phosphate, pH 7.2, in a Kenwood domestic blender. The homogenate was centrifuged at 16000 *g* for 10 min. The supernatant was centrifuged at 45000 *g* for 30 min. The supernatant was decanted and 0.25 vol. of 0.5 M-sodium phosphate buffer, pH 7.2, was added. $(NH_4)_2SO_4$ (280 g/l) was added slowly and the suspension was stirred for 20 min. After centrifugation at 16000 *g* for 20 min, the pellet was discarded and $(NH_4)_2SO_4$ (199 g/l) was slowly added to the supernatant. After centrifugation the pellet was resuspended in a small volume of 10 mM-sodium phosphate/2 mM-EDTA/2.5 mM-2-mercaptoethanol, pH 7.2. This solution was dialysed overnight against 20 litres of the same buffer. The non-dialysable material was applied to a DEAE-cellulose column (14 cm × 7 cm) equilibrated with 10 mM-sodium phosphate buffer, pH 7.2, containing 2.5 mM-2-mercaptoethanol. The majority of the enzyme activity did not bind to the column. Two further peaks of activity were eluted with a linear 0–0.2 M-KCl gradient (2 × 1 litre). The three peaks of activity were denoted F1, F2 and F3 in order of their elution.

Further purification of fraction F1

F1 was applied to a DEAE-cellulose column (14 cm × 7 cm) equilibrated with 10 mM-Tris/HCl, pH 7.5, containing 2.5 mM-2-mercaptoethanol. Activity was eluted with a linear 0–0.1 M-KCl gradient (2 × 1 litre). Active fractions were applied to a Procion Orange MX-G–Sepharose 4B column [10 cm × 7 cm; synthesized by the method of Baird *et al.* (1976)], equilibrated with 10 mM-Tris/HCl, pH 7.5, containing 2.5 mM-2-mercaptoethanol. Activity was eluted with a linear 0–1 M-KCl gradient (2 × 1.0 litre). Active fractions were concentrated by ultrafiltration under nitrogen in an Amicon unit equipped with a UM-10 membrane. The enzyme was then gel-filtered on a Sephadex G-100 column (2.5 cm × 90 cm), equilibrated with 10 mM-Tris/HCl/0.1 M-KCl/2 mM-EDTA/2.5 mM-2-mercaptoethanol, pH 7.5. Active fractions were pooled, concentrated to a protein concentration of 1 mg/ml and stored at −20°C. The purified enzyme is described as AR1.

Further purification of fraction F3

F3 from the DEAE-cellulose column was precipitated by addition of $(NH_4)_2SO_4$ to give 85% saturation. After centrifugation at 16000 *g*, the pellet was resuspended in a small volume of 10 mM-sodium phosphate buffer, pH 7.2, containing 2.5 mM-2-mercaptoethanol and dialysed overnight against 5 litres of the same buffer. The nondialysable material was concentrated by ultrafiltration to a volume of approx. 15 ml, and applied to a Sephadex G-100 column (2.5 cm × 98 cm) equilibrated with 10 mM-sodium phosphate/0.1 M-KCl/2.5 mM-2-mercaptoethanol, pH 7.2. Two peaks of activity are separated by this procedure. The lower-M_r peak, AR 3, was applied to a Procion Orange MX-G–Sepharose 4B column (10 cm × 2 cm) equilibrated with 10 mM-sodium phosphate buffer, pH 7.2, containing 2.5 mM-2-mercaptoethanol and eluted with a linear 0–1.5 M-KCl gradient (2 × 75 ml). The enzyme was diluted with 10 mM-sodium phosphate buffer, pH 7.2, to give a final salt concentration of 0.1 M and applied to a 2′,5′-ADP–Sepharose column (7 cm × 1 cm) equilibrated in 10 mM-sodium phosphate buffer, pH 7.2. The enzyme did not bind to this column. Active fractions were pooled and concentrated to give a final protein concentration of approx. 1 mg/ml. The enzyme was stored at 4°C in the presence of 0.01% NaN_3.

AR4, the higher-M_r peak, was not purified further. It was stable at 4°C for at least 1 month.

Further purification of fraction F2

F2 from the DEAE cellulose step was gel-filtered on a Sephadex G-100 column (2.5 cm × 98 cm) equilibrated in 10 mM-sodium phosphate buffer, pH 7.2, containing 0.1 M-KCl and 2.5 mM-2-mercapto-

ethanol. The gel-filtered enzyme (G2) was stable at 4°C for approx. 2 weeks.

SDS/polyacrylamide-gel electrophoresis

SDS/polyacrylamide-gel electrophoresis was performed on 15% slab gels with the buffer systems of Laemmli (1970). Bovine serum albumin, ovalbumin, chymotrypsinogen A and cytochrome *c* were used as M_r standards. Gels were stained with 1.25% Coomassie Blue R250 in methanol/acetic acid/water (5:1:4, by vol.) and destained in methanol/acetic acid/water (10:4:83, by vol.). Non-denaturing polyacrylamide-gel electrophoresis was performed at pH 8.9 on 7.5% rod gels by the method of Maizel (1971). Gels were stained for protein as described above and for aldehyde reductase activity by the method of Turner & Tipton (1972). Gels were stained for ketone reductase activity by substitution of 1ml of cyclohexanol per 10ml of assay mix for the propane-1,2 diol. Gels were stained for prostaglandin dehydrogenase activity by the method of Braithwaite & Jarabak (1975) with prostaglandin B_1 substituted for prostaglandin E_1 and $NADP^+$ for NAD.

M_r determination

M_r values were determined by gel filtration on a Sephadex G-100 column (2.5cm × 98cm) in 10mM-sodium phosphate buffer (pH 7.2) containing 0.1M-KCl and 2.5mM-2-mercaptoethanol. Bovine serum albumin, ovalbumin, chymotrypsinogen A and cytochrome *c* were used as standards.

Immunological studies

Purified AR1 (1ml of 1mg/ml) was thoroughly mixed with 1ml of Freund's complete adjuvant by vortex-mixing for 1h. The mixture was injected into multiple sites on the back of a New Zealand White rabbit. Further injections of 1mg of AR1 in Freund's incomplete adjuvant and prepared as described above were carried out after 2 weeks and again after 4 weeks. Blood samples were collected by cardiac puncture from 1 week after the second injection. The blood was allowed to clot overnight at room temperature and then was centrifuged at 1000*g* for 10min. The serum was stored at −20°C. Cross-reaction between enzyme and antibody was examined by immunodiffusion on plates coated with 1% (w/v) agarose in 0.85% (w/v) NaCl.

Results

Separation and purification of the aldehyde reductase forms

Aldehyde reductase activity from ox kidney cytosol was resolved into three peaks of activity by chromatography on DEAE-cellulose (Fig. 1). The first peak, F1, comprised approx. 93% of the total activity, with F2 and F3 contributing approx. 3% and 4% respectively. When the homogenization and dialysis steps were carried out in the presence of the proteinase inhibitor phenylmethanesulphonyl fluoride (0.1mM), no change in the elution profile was observed. F1 was further purified by chromatography on DEAE-cellulose, Procion Orange MX-G–Sepharose 4B and finally by gel filtration on Sephadex G-100. The results of a typical purification are summarized in Table 1. The purified enzyme, AR1, was stable for at least 6 months when stored at −20°C but lost all activity after storage at 4°C for 1 month.

The purity of the enzyme was examined by SDS/polyacrylamide-gel electrophoresis and by polyacrylamide-gel electrophoresis at pH 8.9. At a loading of 25μg only one band was visible following SDS/polyacrylamide-gel electrophoresis. A similar result was obtained after non-denaturing polyacrylamide gel electrophoresis at pH 8.9, when the gels were stained for either protein or enzyme activity.

F3 was further purified by gel filtration on Sephadex G-100. The low-M_r fraction (AR3) was purified to homogeneity by chromatography on Procion Orange MX-G–Sepharose 4B and 2′,5′-ADP–Sepharose. A typical purification is summarized in Table 2. The recovery of AR3 from the DEAE-cellulose step was poor (35%, using xylose as substrate) but this was due, at least partly, to selective pooling of active fractions to avoid F2 contamination. After the Procion Orange MX-G–Sepharose 4B step only two proteins were present, as judged by polyacrylamide-gel electrophoresis at pH 8.9. These were identified as AR3 and biliverdin reductase (Phillips & Mantle, 1981) by staining for aldehyde reductase activity with propane-1,2-diol and by assaying for biliverdin reductase activity in homogenized gel slices. The enzymes were separated by 2′,5′-ADP–Sepharose chromatography, since biliverdin reductase binds to this gel while AR3 is not retarded. AR3 lost most of its activity when stored at −20°C but was stable for at least 1 month at 4°C in the presence of NaN_3.

Polyacrylamide-gel electrophoresis of purified AR3 at pH 8.9 gave rise to a single band on staining either for protein or for enzyme activity. A single protein band was also obtained following SDS/polyacrylamide-gel electrophoresis.

The higher -M_r fraction AR4 obtained by gel filtration of F3 was not purified further. Following electrophoresis at pH 8.9 a single band was obtained on staining AR4 for aldehyde reductase activity with propane-1,2-diol.

The F2 fraction from the DEAE-cellulose step was subjected to polyacrylamide-gel electrophoresis at pH 8.9. The gels were stained for aldehyde reductase, ketone reductase and 15-oxoprostaglandin reductase activity and the results are shown

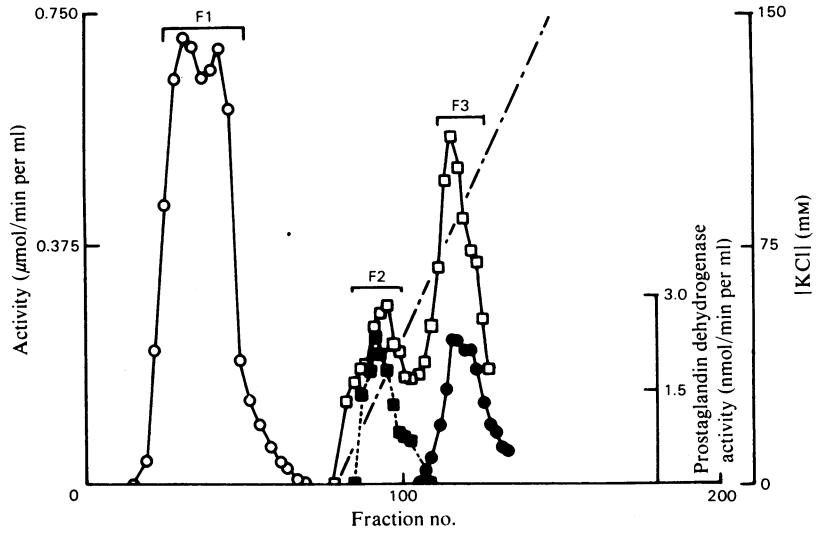

Fig. 1. *DEAE-cellulose chromatography of aldehyde reductase with 10 mM-sodium phosphate, pH 7.2*
Aldehyde reductase assays were performed with 4-nitrobenzaldehyde (○) and D-xylose. Note that the activity of F2 and F3 with 4-nitrobenzaldehyde (□) and D-xylose (●), which is described in units of μmol/min per ml, has been multiplied 10-fold. Prostaglandin dehydrogenase activity (■) was assayed with prostaglandin B_1, as described in the text. —·—, KCl gradient.

Table 1. *Purification of AR1*
All assays were performed with 20 mM-D-glucuronic acid and 75 μM-NADPH in 0.1 M-sodium phosphate buffer, pH 7.0, as described in the text. Units are μmol of NADPH/min.

Procedure	Volume (ml)	Activity (units/ml)	Total activity (units)	Protein (mg/ml)	Specific activity (units/mg)	Yield (%)	Purification (-fold)
High speed supernatant	3680	0.339	1245.7	16	0.021	(100)	(1)
35–65% satd. $(NH_4)_2SO_4$ fraction, dialysed	415	2.336	969.5	34.5	0.068	78	3.2
DEAE-cellulose in phosphate, pH 7.2	520	1.768	919.6	16.25	0.109	74	5.1
DEAE-cellulose in Tris/HCl, pH 7.5	430	1.157	497.7	2.0	0.579	40	27.4
Procion Orange MX-G–Sepharose 4B	145	2.315	335.7	0.8	2.894	27	136.8
Sephadex G-100	35	7.331	256.6	0.82	8.940	21	424.0

schematically in Fig. 3(*a*). Four bands were obtained on staining with propane-1,2-diol (the 'aldehyde' substrate), but on staining with either prostaglandin B_1 or cyclohexanol (the 'ketone' substrate), five bands were obtained in similar positions. Addition of 2-mercaptoethanol had no effect on the pattern obtained, and neither did the addition of phenylmethanesulphonyl fluoride (0.1 mM) during the preparation of F2. Following gel filtration of F2 on Sephadex G-100, the majority of the aldehyde reductase activity eluted at a position corresponding to an M_r of 26 300. This fraction was denoted G2. A higher-M_r peak was also present but this was found to have a similar substrate specificity to AR4. The G2 fraction was subjected to polyacrylamide-gel electrophoresis at pH 8.9. As shown schematically in Fig. 3(*b*) the gels were stained for aldehyde reductase, ketone reductase and 15-oxoprostaglandin reductase activity. In each case, three bands in similar positions appeared on the gels.

Substrate specificities of the forms

The substrate specificities of the various aldehyde reductase forms are summarized in Table.3. AR1 was active with a variety of aldehydes, including 4-nitrobenzaldehyde, 4-carboxybenzaldehyde, D-glucuronic acid and succinic semialdehyde. The

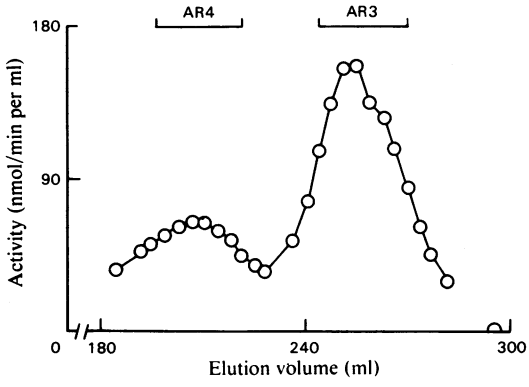

Fig. 2. *Gel filtration of fraction F3 on Sephadex G-100*
Aldehyde reductase assays were performed with 4-nitrobenzaldehyde.

enzyme was not active with neutral sugars or with ketones. AR3 was also active with a variety of aldehydes; however, with pyridine-3-aldehyde as substrate the ratio $k_{cat.}/K_m$ is larger for AR3 than for AR1, the reverse being the case with substrates possessing carboxylic groups. AR3 is capable of reducing neutral sugars, although with xylose, ribose and arabinose nonlinear, downwardly curving double-reciprocal plots were obtained (Fig. 4). It should be noted that double reciprocal plots obtained using AR3 with other substrates were linear. Although the G2 fraction appeared to consist of several forms, linear double reciprocal plots were obtained with a variety of substrates. This fraction appeared to be active with ketones, such as cyclohexanone and menadione, in addition to aldehydes. The G2 fraction also reduced 15-oxoprosta-

Table 2. *Purification of AR3*
All assays were performed with 50mM-D-xylose + 75μM-NADPH in 0.1M-sodium phosphate buffer, pH 7.0, with 0.5mM-sodium barbitone. These results were obtained with 1kg of ox kidney. Units are μmol of NADPH/min.

Step	Volume (ml)	Activity (units/ml)	Total activity (units)	Protein (mg/ml)	Specific activity (units/mg)	Yield (%)	Purification (-fold)
35–65% satd. $(NH_4)_2SO_4$ fraction, dialysed	178	0.076	13.53	35.625	0.0021	—	—
DEAE-Cellulose	275	0.0176	4.84	2.95	0.0060	35	2.8
Sephadex G-100	28	0.072	2.02	1.32	0.055	15	26
Procion Orange MX-G–Sepharose 4B	77	0.0224	1.72	0.075	0.30	13	143
2′,5′-ADP–Sepharose	12.5	0.083	1.04	0.15	0.55	8	262

Table 3. *Substrate specificities of the multiple forms of aldehyde reductase*
Relative $V_{max.}$ values are percentages of those obtained with 4-nitrobenzaldehyde. Actual $V_{max.}$ values were: 8.9, 0.081, 1.45 and 0.026 μmol/min per mg for AR1, G2, AR3 and AR4 respectively. n.d., no activity detected.

	AR1		G2		AR3		AR4	
Substrate	K_m (mM)	Relative $V_{max.}$	K_m (mM)	Relative $V_{max.}$	K_m (mM)	Relative $V_{max.}$	K_m (mM)	Relative $V_{max.}$
4-Nitrobenzaldehyde	0.071	100	0.129	100	0.0084	100	0.262	100
4-Carboxybenzaldehyde	0.018	83	—	—	0.258	28	—	—
Succinic semialdehyde	0.029	62	0.036	37	0.064	15	0.028	60
Pyridine-3-aldehyde	0.934	111	0.751	28	0.008	159	5.0	159
D-Glucuronic acid	1.29	104	2.48	26	2.99	34	—	—
D-Xylose*	n.d.	—	n.d.	—	(1) 41.0	75	n.d.	—
					(2) 1.84	4		
Cyclohexanone	n.d.	—	1.18	34	n.d.	—	36.77	168
Menadione	n.d.	—	0.030	77	n.d.	—	0.009	56
NADPH	0.002	—	0.003	—	0.008	—	0.007	—

* With this substrate, AR3 gave biphasic double reciprocal plots. Two K_m and $V_{max.}$ values were determined by fitting the data to the equation:

$$V = \frac{V_{max.1}[S]}{K_{m_1} + [S]} + \frac{V_{max.1}[S]}{K_{m_2} + [S]}$$

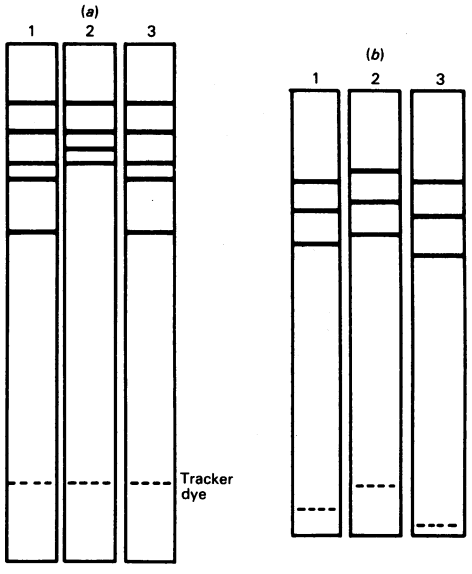

Fig. 3. *Non-denaturing polyacrylamide-gel electrophoresis of F2 and G2*

(*a*) Approx. 200 μg of protein from the F2 fraction was applied to each gel. Gel 1 was stained with prostaglandin B_1, gel 2 with propane-1,2-diol and gel 3 with cyclohexanol. (*b*) Approx. 60 μg of protein from the G2 fraction was applied to each gel. Gel 1 was stained with propane-1,2-diol, gel 2 with cyclohexanol and gel 3 with prostaglandin B_1.

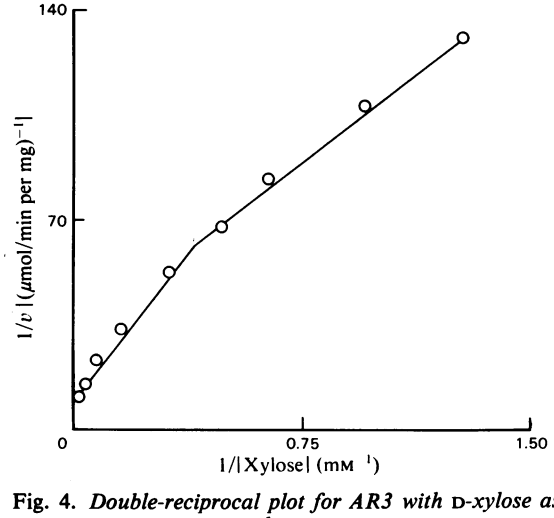

Fig. 4. *Double-reciprocal plot for AR3 with* D-*xylose as substrate*

All assays were performed with purified AR3 using 100 μl of a 0.21 mg/ml solution in a final volume of 2 ml.

Table 4. *Effect of inhibitors on the aldehyde reductase forms*

Assays were conducted with 0.33 mM-4-nitrobenzaldehyde and 75 μM-NADPH in 0.1 M-sodium phosphate buffer, pH 7 at 30°C

Inhibitor	Concn. (mM)	Inhibition (%) of: AR1	G2	AR3	AR4
Sodium barbitone	0.5	90	52	0	19
Sodium valproate	1.0	97	52	0	39
Clonazepam	0.25	0	0	0	—
Flurazepam	7.5	0	0	0	—
Nitrazepam	0.25	0	0	0	—

Table 5. *M_r values for AR1, G2, AR3 and AR4*

Enzyme form	M_r by: Gel filtration	SDS/polyacrylamide-gel electrophoresis
AR1	30000 ± 800	33500 ± 400
G2	25500 ± 800	—
AR3	31000 ± 500	34700 ± 200
AR4	56000 ± 3000	—

glandin B_1, which at a concentration of 45 μM was 30% as active as 4-nitrobenzaldehyde under saturating conditions. AR4 was similar to G2 in reducing cyclohexanone and menadione; however, it was inactive with 15-oxoprostaglandin B_1. The effect of sodium barbitone and sodium valproate on the activity of the various forms is summarized in Table 4. AR1 was potently inhibited by both compounds. G2 was also inhibited but to a lesser extent. AR3 was not inhibited by either compound while AR4 was slightly inhibited by sodium valproate but not by sodium barbitone. None of the forms were inhibited by the benzodiazepines, flurazepam, clonazepam or nitrazepam at any of the concentrations tested.

M_r determinations

The native M_r values of AR1, AR3, AR4 and G2 and the subunit M_r of AR1 and AR3 are summarized in Table 5.

Immunological studies

Antibody to AR1 was shown to cross-react with AR1 by immunodiffusion. No cross-reaction was obtained with G2, AR3 or AR4.

Discussion

There are at least six NADPH-dependent enzymes (AR1, AR3, AR4 and the three electrophoretically separable forms in G2) which reduce aldehydes to alcohols in ox kidney. The nature of the three additional bands of activity in F2 (see Fig. 3*a*) is unclear (although one is probably contamination by AR4) and will not be discussed further. These results contrast with those of previous workers who have found only one form of aldehyde reductase in pig kidney (Branlant & Biellmann, 1980; Morpeth & Dickinson, 1980). This apparent discrepancy may be due to the relatively low activity of the minor forms or they may indeed be absent from the pig. Sawada *et al.* (1979) have separated three aldehyde/ketone reductases from guinea-pig liver and state that a similar pattern is obtained for guinea-pig kidney.

AR1 appears to be similar to the aldehyde reductase previously described in pig kidney (Bosron & Prairie, 1972; Flynn *et al.*, 1975; Morpeth & Dickinson, 1980), to the high-K_m aldehyde reductase in pig, rat and ox brain (Turner & Tipton, 1972; Rivett *et al.*, 1981) and to hexonate dehydrogenase from rat liver (Mano *et al.*, 1961; Mano & Shimazono, 1970). All of these preparations have a native M_r of approx. 30000, similar substrate specificities (including high activity with glucuronic acid) and sensitivity to sodium barbitone. The function of this form may be the reduction of D-glucuronate in the biosynthesis of ascorbic acid.

AR3 appears to be similar to the enzyme previously described as aldose reductase (Hers, 1957). Like aldose reductase, AR3 is active with sugars and both have M_r values of approx. 30000. This enzyme has been previously found to occur in kidney papilli by Gabbay & O'Sullivan (1968). AR3 is also similar to the low-K_m aldehyde reductase described by Turner & Tipton (1972) having a K_m value for 4-nitrobenzaldehyde an order of magnitude lower than that obtained with AR1. In this respect pyridine 3-aldehyde is even more discriminatory between ox kidney AR1 and AR3. 4-Carboxybenzaldehyde behaves in the reverse manner, having a lower K_m for AR1. Aldose reductase has been previously reported to give non-linear Lineweaver–Burk plots with a variety of substrates (Dons & Doughty, 1976; Schaeff & Doughty, 1976; Crabbe & Halder, 1980; Hoffmann *et al.*, 1980). Ox kidney AR3 gives similar plots when xylose, ribose, arabinose or glucose are used as substrates; however, linear plots are obtained for other aldehydes such as 4-nitrobenzaldehyde and pyridine-3-aldehyde. These results suggest that different kinetic mechanisms may operate depending on the substrate used.

G2 consists of three closely related enzyme forms. Based on the results of gel electrophoresis experiments, all these forms appear to be active with aldehydes, ketones and prostaglandins. The exact nature of these forms remains unclear. However, they all appear to have similar M_r values and may be similar kinetically, since linear double-reciprocal plots were obtained for several substrates. G2 appears similar to the carbonyl reductase from human brain described by Wermuth (1981) which reduces aldehydes, ketones and prostaglandins. The carbonyl reductase was found to consist of three electrophoretically different forms which on separation were found to have similar amino acid compositions and kinetic properties. It is possible that the G2 forms are similarly related. Chang & Tai (1981) have recently suggested that prostaglandin 9-oxo reductase and 15-hydroxyprostaglandin dehydrogenase are minor forms of aldehyde reductase.

AR4 has a higher M_r value than the other forms and is active with aldehydes and with ketones such as cyclohexanone, but not with sugars or with 15-oxoprostaglandin B_1. In this respect it is similar to the high-M_r aldehyde reductase described by Bosron & Prairie (1973) and to the F_4 form described by Sawada *et al.* (1980).

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