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Distribution and characterization of dihydrodiol dehydrogenases in mammalian ocular tissues

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The immunological relationship of two forms of dihydrodiol dehydrogenase (DD) in pig lens to pig muscle aldose reductase and kidney aldehyde reductase has been studied. Although the minor enzyme form, a monomer of M_r 35000, was identical with aldose reductase, the major enzyme form, a dimer of M_r 65000, was distinct from the two reductases. The two enzyme species, although their amounts were low, were distributed in the cornea, iris–ciliary body, retina and choroid of the pig eye. In other mammals, rabbit lens exhibited much higher DD activity than did lens of mice, rats, cats, hamsters, guinea pigs and monkeys, and contained large amounts of the M_r-65000 enzyme form as well as the minor enzyme form of M_r 35000. In contrast, only the M_r-35000 form of the enzyme was found in the lens of other species, except that a small amount of the high-M_r enzyme was detected in mouse lens. The high-M_r enzyme, purified from rabbit lens, was functionally and immunologically similar to dimeric DD of pig lens. The low-M_r enzyme forms, isolated or partially purified from these animal lenses, showed several features in common with aldose reductases from mammalian tissues. The dimeric enzymes of pig and rabbit lenses were $NADP^+$-specific, whereas the low-M_r enzymes exhibited dual cofactor specificity and their activities with NAD^+ were more than 3-fold higher than those with $NADP^+$.

INTRODUCTION

Dihydrodiol dehydrogenase (DD; EC 1.3.1.20) catalyses the oxidation of dihydrodiols of aromatic hydrocarbons to the corresponding catechols. The enzymes in mammalian liver and some extrahepatic tissues [1–9] have been extensively purified and characterized from the viewpoint of metabolic detoxification of carcinogenic polycyclic aromatic hydrocarbons [10]. These studies have shown that the enzyme exists in multiple forms in most tissues, and that there are marked species or tissue differences in the multiplicity and the nature of the enzyme.

In 1967, van Heyningen & Pirie [11] and Rees & Pirie [12] suggested that DD in tissues of the rabbit eye plays an important role in the pathogenesis of naphthalene-induced cataract and damage to the retina by producing 1,2-dihydroxynaphthalene, which is readily autoxidized to cytotoxic 1,2-naphthoquinone and H_2O_2. Naphthalene is also cataractogenic in the rat [13], but monophenol oxidase (EC 1.10.3.1) in the iris has been thought to be essential for the formation of the toxic metabolite, 1,2-dihydroxynaphthalene, because of the high susceptibility of pigmented rats to the drug-induced cataract, compared with the albino animals [14], and of low activity of DD in the lens of these animals [15]. Although substrate specificity and pH optimum of DD have been reported with the crude extracts of the rabbit lens and iris [11,15], no information is available on the isolation and further characterization of the enzyme in the rabbit and rat lens.

In order to understand the species difference in the enzymes implicated in the bioactivation of naphthalene, it is necessary to elucidate the distribution and multiplicity of DD in mammalian ocular tissues, and to compare the properties of the purified enzymes from the tissues of the rabbit and rat. We have recently isolated two DDs associated with aldehyde reductase activity from pig lens [16]. The predominant enzyme form in the tissue is a dimer of M_r 65000, whereas another form is a monomer of M_r 34000 which exhibits properties similar to those of aldose reductase. Since a dimeric aldehyde reductase that immunologically cross-reacts with aldose reductase has been isolated from some human tissues [17], we first examined the immunological relationship between the two pig lens DDs, pig muscle aldose reductase and pig kidney aldose reductase, by using the antibodies against the respective enzymes. We further investigated the distribution of the two forms of DD in pig ocular tissues and other mammalian lenses by gel filtration and by immunological means with the specific antibodies. Here we report the comparative analyses of the multiplicity of DD in mammalian ocular tissues and describe the purification and properties of DDs from the rabbit and rat lens.

EXPERIMENTAL

Chemicals

trans-1,2-Dihydrobenzene-1,2-diol (benzenedihydrodiol) and 1,2-dihydro-1,2-dihydroxynaphthalene (naphthalenedihydrodiol) were synthesized as described by Platt & Oesch [18,19]. Blue Sepharose was prepared by the method of Heyns & De Moor [20]. Other chemicals were as specified elsewhere [7,16].

Animals and preparation of tissue extracts

Pig eyes, kidneys and muscle were obtained from a slaughterhouse. Eyes were also obtained from adult albino rabbits of both sexes, male ddY mice, male Sprague–Dawley rats, male golden hamsters, male Hartley guinea pigs, male cross-bred cats and Japanese monkeys of both sexes. Aqueous and vitreous humours, cornea, iris–ciliary body, lens, retina and choroid were collected from the eyes of the pig, and only lens was removed from the eye of other species. Liver, kidney, lung, spleen, brain and heart were removed from the rabbits. The tissues (1–15 g) were minced and homogenized with 4 vol. of 20 mM-potassium phosphate, pH 7.5, containing 5 mM-EDTA, 5 mM-2-mercaptoethanol and 0.15 M-KCl (buffer A), and the homogenate

Abbreviation used: DD, dihydrodiol dehydrogenase.

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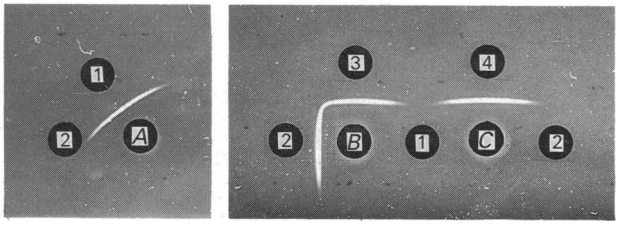

Fig. 1. Immunodiffusion of pig DDs, aldose reductase and aldehyde reductase to immunoglobulins against the respective enzymes

Wells: *A*, anti-(dimeric DD) IgG; *B*, anti-(aldose reductase) IgG; *C*, anti-(aldehyde reductase) IgG; 1, lens dimeric DD; 2, lens monomeric DD; 3, muscle aldose reductase; and 4, kidney aldehyde reductase.

Table 1. Distribution of DD activity in pig ocular tissues

The value represents the mean ± S.D. for determinations with 12000 *g* supernatants of the three tissue homogenates.

Tissue	DD activity (m-units/mg of protein)	DD activity (m-units/g wet wt. of tissue)
Lens	2.3±0.3	436±150
Iris–ciliary body	4.5±2.1	66±22
Retina	2.2±1.0	46±2
Choroid	1.8±0.9	28±1
Cornea	1.6±0.7	12±2

was centrifuged at 12000 *g* for 15 min. The supernatants were analysed for protein [21] and DD activity. All steps involving homogenization and centrifugation were carried out at 0–4 °C.

Enzyme assay

Dehydrogenase and reductase activities were assayed spectrophotometrically or fluorimetrically by measuring the production and oxidation of NADPH [7,16]. The standard assay mixture for DD contained 0.1 M-glycine/NaOH, pH 10.0, 0.25 mM-$NADP^+$, 1.8 mM-naphthalenedihydrodiol and enzyme in a total volume of 2.0 ml. In the experiments with inhibitors, the DD activity was determined with 0.1 M-potassium phosphate buffer, pH 7.5, instead of the glycine/NaOH buffer, and the inhibitors were added to the assay mixture before the reaction was initiated by addition of the enzyme solution. For the assay of aldose reductase activity, 10 mM-DL-glyceraldehyde was used as a substrate. The pH-dependence of the enzyme activity was determined in 0.1 M buffers (pH 5–11) which were prepared by mixing solutions of H_3PO_4 and K_3PO_4. One unit of activity was defined as the oxidation or production of 1 μmol of NADPH/min at 25 °C.

Immunochemical experiments

Pig lens DDs were purified to homogeneity as described previously [16], and pig muscle aldose reductase and pig kidney aldehyde reductase were isolated by the methods of Cromlish & Flynn [22] and Flynn *et al.* [23] respectively. Antibodies against the purified enzymes were raised in female rabbits as previously described [24]. The immunoglobulin fractions from the antisera were prepared by $(NH_4)_2SO_4$ fractionation. Immunodiffusion and immunoprecipitation were performed as described previously

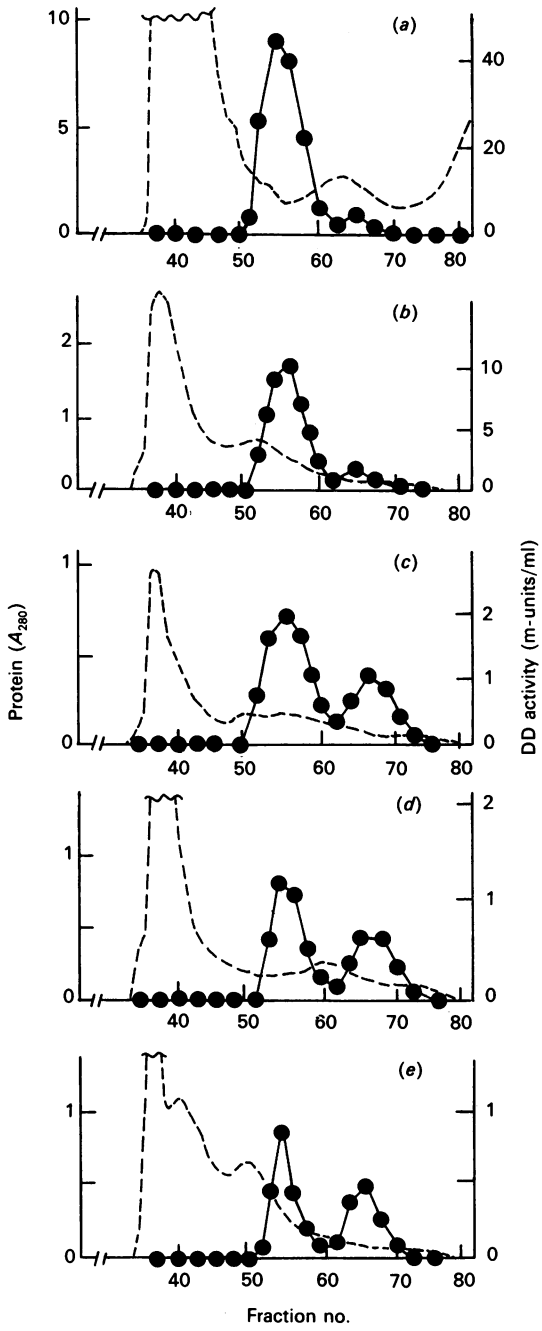

Fig. 2. Sephadex G-100 filtration of DD activity of pig ocular tissues

The $(NH_4)_2SO_4$ fractions of the extracts from lens (*a*), retina (*b*), cornea (*c*), iris–ciliary body (*d*) and choroid (*e*) were applied to the column, equilibrated and eluted with buffer A. The fractions were analysed for protein (---) and activity (●——●).

[24]. For Western immunoanalysis, samples were subjected to SDS/PAGE [25] in a 12/5% (w/v)-acrylamide slab gel. Transfer of the proteins to nitrocellulose and Western analysis using the antibodies were carried out as described previously [26].

Gel filtration

The 12000 *g* supernatants of the homogenates of lens and other tissues (1–25 g) were prepared as described above, and then the proteins in the supernatant were fractionated by addition of $(NH_4)_2SO_4$. The 35–70%-satd. $(NH_4)_2SO_4$ precipitate was collected by centrifugation at 12000 *g* for 15 min, dissolved in a

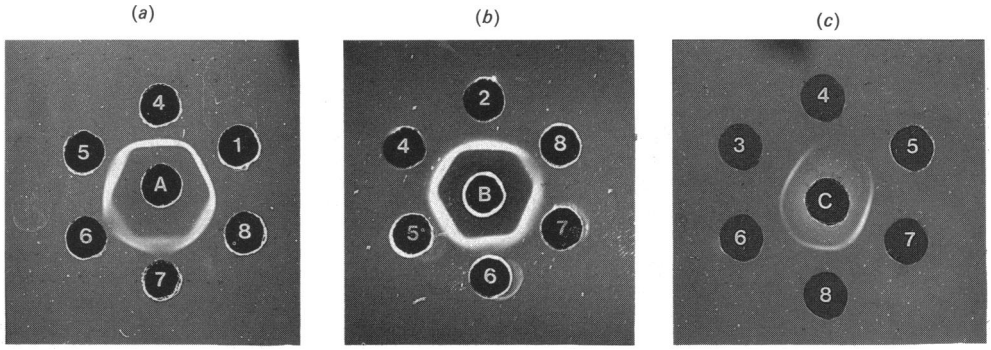

Fig. 3. Ouchterlony plates demonstrating the presence of dimeric DD, aldose reductase and aldehyde reductase in pig ocular tissues

(*a*) Immunodiffusion of the high-M_r DD fractions of the tissues against anti-(dimeric DD) IgG (A). (B) and (C), Immunodiffusion of the low-M_r DD fractions of the tissues against anti-(aldose reductase) IgG (B) and anti-(aldehyde reductase) IgG (C) respectively. Peripheral wells contained purified preparations of lens dimeric DD (1), muscle aldose reductase (2) and kidney aldehyde reductase (3), and other wells contained the high-M_r DD or low-M_r DD fractions from lens (4), retina (5), iris–ciliary body (6), choroid (7) and cornea (8).

small amount of buffer A, and dialysed overnight against buffer A at 4 °C. The dialysed solution was applied to a Sephadex G-100 column (2.0 × 90 cm) in buffer A at a flow rate of 14 ml/h and at 4 °C. The column was standardized by use of M_r markers. The high-M_r DD and low-M_r DD fractions were separately concentrated by ultrafiltration using an Amicon YM-10 membrane.

Enzyme purification

DD was purified from 20 g of rabbit lens by using, with minor modification, a procedure for the purification of the enzymes from pig lens [16]. This method consisted of homogenization of the tissue, followed by $(NH_4)_2SO_4$ fractionation of the 12000 *g* supernatant as described above, gel filtration on Sephadex G-100, affinity chromatography on Matrex Red A, and chromatography on DEAE-Sephacel. The enzyme activity was resolved into two peaks by Sephadex G-100 gel filtration, and the high-M_r and low-M_r DDs were purified separately. For rat lens DD, the enzyme was purified as follows at 0–4 °C. Rat lenses (10 g) were homogenized with an equal volume of 20 mM-Tris/HCl, pH 7.5, containing 10 mM-2-mercaptoethanol and 0.5 mM-EDTA (buffer B). The homogenate was centrifuged at 12000 *g* for 15 min. The supernatant was passed through a Sephadex G-100 column (2.5 cm × 90 cm) in buffer B. The enzyme fractions were directly applied to a Blue-Sepharose column (1.2 cm × 20 cm) equilibrated with buffer B. After the column was washed with the buffer containing 0.1 M-NaCl, the enzyme was eluted with buffer B containing 0.5 M-NaCl. The enzyme fractions were concentrated by ultrafiltration as described above, dialysed against buffer B without EDTA, and then applied to a Q-Sepharose column (1.2 cm × 10 cm) equilibrated with the same buffer. The enzyme was eluted with a linear 0–0.1 M-NaCl gradient in the buffer. The enzyme fractions were concentrated by ultrafiltration and stored at 4 °C.

RESULTS

Immunological properties of pig lens DDs

The homogeneous preparations of pig muscle aldose reductase and kidney aldehyde reductase exhibited low DD activities of 0.3 and 1.2 units/mg respectively, and the respective K_m values for naphthalenedihydrodiol were 1.2 and 23 mM. The immunodiffusion test was used to examine the immunological relationship among the dimeric and monomeric DDs of pig lens, muscle aldose reductase and kidney aldehyde reductase. The anti-(dimeric DD) IgG reacted only with the antigen (Fig. 1). The anti-(aldose reductase) IgG formed fused precipitin lines against both the monomeric DD and aldose reductase, but the anti-(aldehyde reductase) IgG did not react with the two pig lens enzymes. The DD activities of the dimeric and monomeric DDs were completely immunoprecipitated by addition of the immunoglobulins against dimeric DD and aldose reductase respectively.

Distribution of DD in pig ocular tissues

The DD activities in the tissue extracts of the pig eye are summarized in Table 1. The specific activity per mg of protein decreased in the order iris–ciliary body > lens > retina > choroid > cornea. No activity was detected in the aqueous and vitreous humours. When the activity was compared in terms of its tissue content, the value for the lens was more than 6-fold higher than those of the other tissues.

DD activity in the $(NH_4)_2SO_4$ fractions of the ocular tissues was resolved into two peaks of M_r 65000 and M_r 35000 on Sephadex G-100 filtration (Fig. 2). The retina and lens showed similar elution profiles, in which the high-M_r DD was predominant, whereas the activity ratios of the high-M_r DD to the low-M_r DD were about 1:1 in the cornea, iris–ciliary body and choroid. When the enzyme fractions of the ocular tissues were subjected to immunodiffusion test with the antibodies against the dimeric DD, aldose reductase and aldehyde reductase, the high-M_r DD fractions of these tissues formed precipitin lines which fused with that for pig lens dimeric DD (Fig. 3*a*), whereas the low-M_r DD fractions all cross-reacted with both aldose reductase (Fig. 3*b*) and aldehyde reductase (Fig. 3*c*). About 60% of the DD activity in the low-M_r DD fractions from the tissues was inhibited by 1 mM-diphenic acid, which is a specific inhibitor for aldehyde reductase [27], although the activities of lens low-M_r DD and the high-M_r DDs of all the tissues were not affected by the inhibitor. The results indicate that dimeric DD is distributed in the ocular tissues, and suggest that the activity in the low-M_r DD fractions of the tissues, except lens, are due to both aldose reductase and aldehyde reductase.

Distribution of DD in mammalian lenses

The contents of DD activity in lenses of the rabbit, cat, rat, mouse, hamster, monkey and guinea pig were 1720 ± 320, 97 ± 15, 68 ± 15, 50 ± 13, 48 ± 9, 30 ± 12 and 27 ± 10 m-units/g wet wt. (n = 3–6) respectively. Sephadex G-100 filtration revealed the existence of the two DDs of M_r 65000 and M_r 35000 in rabbit and mouse lenses, whereas the other animal lenses contained

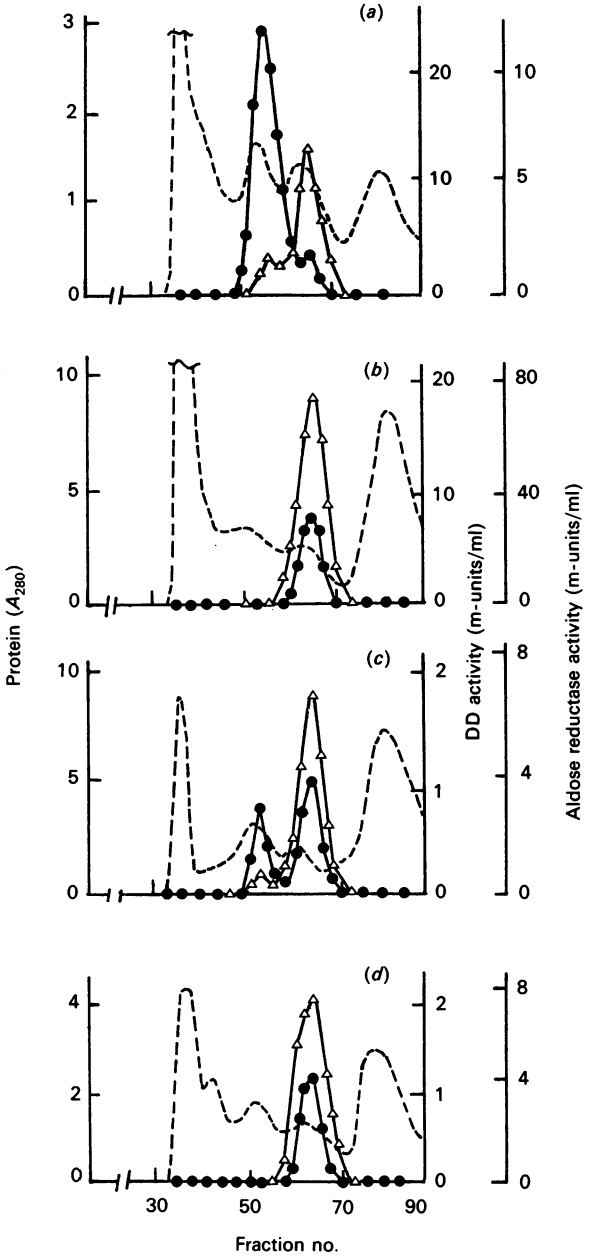

Fig. 4. Gel-filtration patterns of DD and aldose reductase activities of rabbit, rat, mouse and cat lenses on a Sephadex G-100 column

The $(NH_4)_2SO_4$ fractions of the extracts from lenses of the rabbit (*a*), rat (*b*), mouse (*c*) and cat (*d*) were passed through the column. The fractions (2.8 ml) were analysed for protein (---) and for activities of DD (●) and aldose reductase (△).

only the low-M_r enzyme species, which was co-eluted with aldose reductase activity. Representative results for the rabbit, mouse, rat and cat lenses are shown in Fig. 4.

Since the rabbit lens contained high amounts of DD activity, we examined the distribution of the enzyme in other tissues of the rabbit. The contents of the enzyme activity in liver, lung, kidney, spleen, heart, muscle and brain were 2800 ± 700, 140 ± 32, 120 ± 25, 94 ± 14, 34 ± 3, 30 ± 2 and 17 ± 2 m-units/g wet wt. ($n = 3$) respectively. However, no high-M_r DD was detected on Sephadex G-100 filtration of the $(NH_4)_2SO_4$ fractions of these rabbit tissue extracts, which all showed a single activity peak around M_r 35000 (results not shown). The results suggest that the high-M_r DD occurs specifically in lens, of all rabbit tissues.

Table 2. Purification of high-M_r and low-M_r DDs from rabbit lens

At the DEAE-Sephacel step for the high-M_r DD, half of the enzyme activity obtained from the Matrex Red A column was chromatographed.

Step	Protein (mg)	Activity: Total (units)	Activity: Specific (units/mg)	Yield (%)
Extract	5860	33.0	0.0056	100
$(NH_4)_2SO_4$ fraction	4090	26.0	0.0064	79
Sephadex G-100				
High-M_r DD	596	15.5	0.026	47
Low-M_r DD	250	1.4	0.0056	4.2
Matrex Red A				
High-M_r DD	3.0	15.0	5.0	45
Low-M_r DD	4.2	1.1	0.26	3.3
DEAE-Sephacel				
High-M_r DD	0.66	1.6	2.4	4.8
Low-M_r DD	2.1	0.61	0.29	1.8

Table 3. Co-purification of DD and aldose reductase activities from rat lens

At each stage of purification, the activities of DD and aldose reductase (AR) were assayed.

Step	Protein (mg)	Specific activity (units/mg): DD	Specific activity (units/mg): AR	DD/AR activity ratio
Extract	1640	0.0003	0.0025	0.12
Sephadex G-100	112	0.0046	0.039	0.12
Blue Sepharose	6.6	0.064	0.51	0.12
Q-Sepharose	0.9	0.14	1.78	0.08

Purification and properties of rabbit and rat lens DDs

The results of purification of DDs from rabbit lens and rat lens are shown in Tables 2 and 3 respectively. In the purification of rabbit lens DD, the high-M_r enzyme was unstable and lost most of the activity when half of the enzyme solution was chromatographed on the DEAE-Sephacel column. Therefore the preparation of the high-M_r DD obtained in the Matrex Red A chromatography was used for subsequent experiments. In the purification of rat lens DD, DD and aldose reductase activities were co-purified, because the two enzymes had been expected to be identical from the Sephadex G-100 filtration analyses. The ratios of DD activity to aldose reductase activity were essentially constant at all the purification steps.

On SDS/PAGE the purified preparations of rabbit lens high-M_r DD, rabbit lens low-M_r DD and rat lens DD gave almost single protein bands of M_r 39000, M_r 34000 and M_r 36000 respectively (Fig. 5*a*). In the immunodiffusion, the low-M_r enzymes of rabbit and rat lenses did not react with the antibody against pig muscle aldose reductase, but the high-M_r DD of rabbit lens formed a faint precipitin line against anti-(pig lens dimeric DD) IgG, although its photographic demonstration was not possible. In addition, as evidenced by the Western immunoanalysis of rabbit lens high-M_r DD, the anti-(pig lens dimeric DD) IgG detected one positive band at the same M_r as that of pig lens dimeric DD (Fig. 5*b*), although the reactivity of the rat lens enzyme with the IgG was much lower than that of the pig enzyme (Fig. 5*c*). The results indicate that the low-M_r DDs

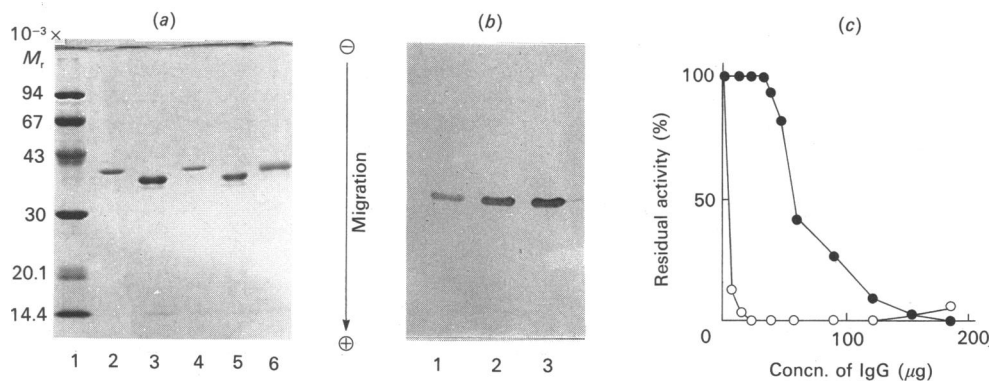

Fig. 5. Electrophoresis of the purified lens DDs of the pig, rabbit and rat, and immunological relationship between pig and rabbit dimeric DDs

(*a*) SDS/PAGE. Proteins were stained with Coomassie Brilliant Blue R-250. Lanes: 1, M_r standards; 2, pig dimeric DD; 3, pig monomeric DD; 4, rabbit high-M_r DD; 5, rabbit low-M_r DD; 6, rat DD. (*b*) Western immunoanalysis using anti-(rabbit dimeric DD) IgG. Lanes 1, rabbit dimeric DD; 2, mixture of pig dimeric DD and pig high-M_r DD; 3, pig high-M_r DD. (*c*) Immunoprecipitation of rabbit lens high-M_r DD (●) and pig lens dimeric DD (○) by the anti-(pig dimeric DD) IgG. The enzymes (0.2 unit) were incubated overnight with the IgG at 4 °C. After centrifugation of the mixture at 3000 *g* for 10 min, the activity in the supernatant was determined.

Table 4. Substrate specificities of DDs of rabbit and rat lenses

The NADP$^+$-linked dehydrogenase and NADPH-linked reductase activities were assayed in 0.1 M-potassium phosphate, pH 7.5 and pH 6.0 respectively. The relative activities and apparent K_m values for NAD(P)$^+$ were determined with 1.8 mM-naphthalene dihydrodiol, and those for NAD(P)H with 0.5 mM-camphorquinone. The values are means for two determinations. n.d., no activity was detected.

		Rabbit lens high-M_r DD		Rabbit lens low-M_r DD		Rat lens DD	
Substrate	Concn. (mM)	Relative activity (%)	K_m (mM)	Relative activity (%)	K_m (mM)	Relative activity (%)	K_m (mM)
Naphthalenedihydrodiol	1.8	100	0.29	100	1.6	100	1.2
Naphthalenedihydrodiol*	1.8	n.d.		278	0.65	347	1.4
Benzenedihydrodiol	1.8	41	0.69	9	0.58	6	
Benzyl alcohol	1.0	n.d.		33	3.4	9	
Butan-1-ol	5.0	n.d.		11		3	
Glycerol	5.0	n.d.		5		2	
NADP$^+$	0.25	100	0.002	100	0.001	100	0.002
NAD$^+$	2.5	n.d.		278	0.25	347	0.28
Camphorquinone	0.5	92	0.03	261	0.005	411	0.005
3-Nitrobenzaldehyde	1.0	44	1.49	226	0.004	421	0.005
Butane-2,3-dione	1.0	39	0.67	254	0.33	562	0.56
Pyridine-3-aldehyde	1.0	13	1.3	377	0.002	874	0.004
DL-Glyceraldehyde	10	24		500		996	
D-Xylose	10	n.d.		226	11	162	13
D-Galactose	10	n.d.		68		28	
D-Glucose	10	n.d.		47	91	22	
NADPH	0.08	92	0.003	261	0.001	411	0.001
NADH	0.12	n.d.		50		72	

* The activity was determined with 2.5 mM-NAD$^+$ as a cofactor.

of rabbit and rat lenses are monomeric and the high-M_r enzyme of rabbit lens is structurally and immunologically similar to pig lens dimeric DD.

Table 4 compares the substrate specificity of rabbit and rat lens DDs in the forward and reverse reactions. The rabbit lens high-M_r DD specifically oxidized dihydrodiols of naphthalene and benzene with NADP$^+$ as a strict cofactor, whereas the low-M_r DDs of rabbit and rat lenses were active towards several alcohols, including the dihydrodiols, in the presence of NADP$^+$ or NAD$^+$. The three enzymes did not oxidize hydroxysteroids such as 17β-hydroxy-5β-androstan-3-one, 3α-hydroxy-5β-androstan-17-one and 5β-androstane-3α,17β-diol, which have been reported as substrates for DDs from rabbit [6] and rat liver [1,2]. Rabbit lens high-M_r DD showed a lower K_m value for naphthalenedihydrodiol than did the low-M_r enzymes. In the reverse reaction, rabbit lens high-M_r DD reduced α-dicarbonyl compounds, some aromatic aldehydes and DL-glyceraldehyde in the presence of NADPH as a cofactor, whereas the low-M_r enzymes of rabbit and rat lenses exhibited high NADPH-linked reductase activities towards various aldehydes and aldoses.

The maximal NADP$^+$-linked naphthalenedihydrodiol oxidation rates by the high-M_r and low-M_r DDs of rabbit and rat lenses were broadly observed at pH 7.5–10.0, whereas the NAD$^+$-linked activities of the low-M_r DDs were higher than their NADP$^+$-linked activities and were optimal around pH 10.0 (Fig. 6). Aldose reductases from pig lens and muscle also showed

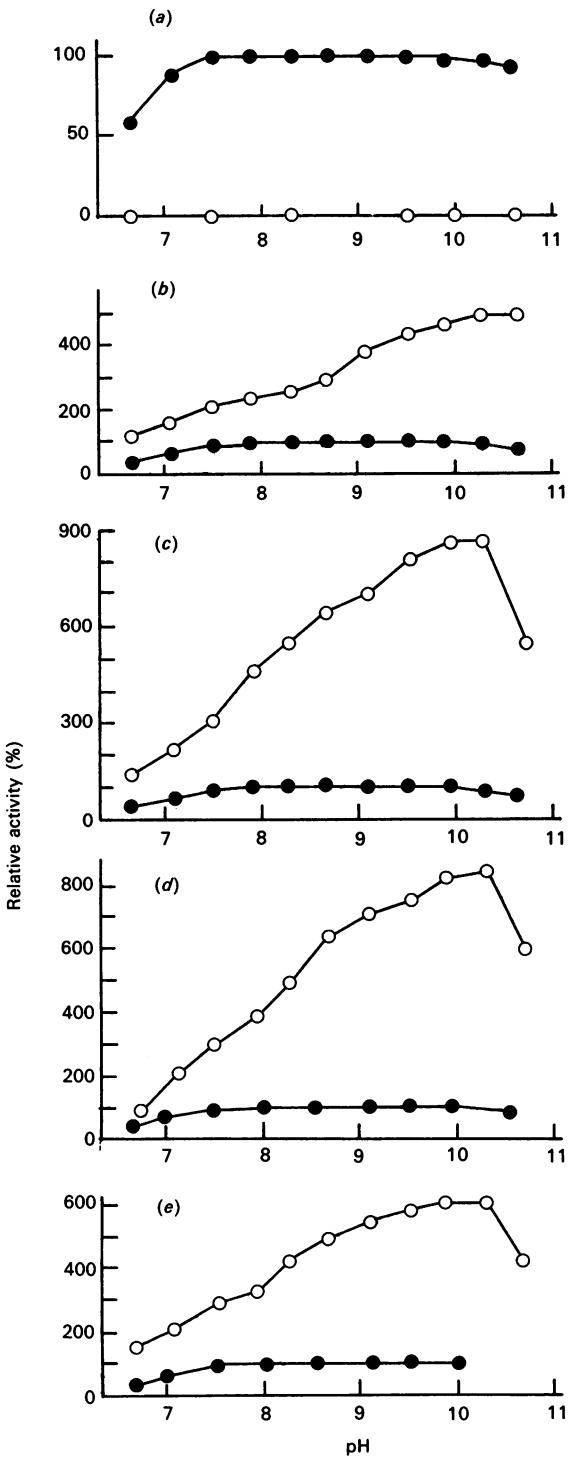

Fig. 6. pH-dependencies of NADP$^+$- and NAD$^+$-linked naphthalenedihydrodiol dehydrogenase activities of the purified DDs from rabbit, rat, cat and mouse lenses

The activity was assayed with 0.25 mM-NADP$^+$ (●) or 2.5 mM-NAD$^+$ (○) as a cofactor and is expressed as the relative activity to the maximal NADP$^+$-linked activities of rabbit high-M_r DD (*a*), rabbit low-M_r DD (*b*), rat DD (*c*), cat DD (*d*) and mouse low-M_r DD (*e*).

similar high NAD$^+$-dependent DD activity, but the NAD$^+$-dependent DD activity of pig kidney aldehyde reductase was only 3% of the NADP$^+$-dependent activity. In the NADPH-linked camphorquinone reduction, the rabbit lens high-M_r DD showed a broad pH optimum from 6.0 to 8.5, and the low-M_r DDs exhibited narrow pH optima, around 6.0.

Table 5. Effects of various compounds on DDs of rabbit and rat lenses

The dehydrogenase activity was assayed in 0.1 M-potassium phosphate, pH 7.5, with or without the compounds. The values are means ± S.D. for three determinations.

Compound	Concn. (mM)	Relative activity (%) Rabbit High-M_r DD	Rabbit Low-M_r DD	Rat DD
p-Chloromercuribenzenesulphonate	0.001	48±12	98±2	78±2
Quercitrin	0.01	66±9	5±1	2±1
Sorbinil	0.01	99±2	24±4	1±1
Diphenic acid	1.0	99±1	94±2	96±3
Barbital	1.0	98±2	73±5	43±2
Valproic acid	1.0	99±2	92±4	68±1
$(NH_4)_2SO_4$	300	30±6	133±5	132±8

The high-M_r DD of rabbit lens was inhibited by *p*-chloromercuribenzenesulphonate, quercitrin and $(NH_4)_2SO_4$, whereas the low-M_r DDs of rabbit and rat lenses were highly sensitive to quercitrin and Sorbinil and activated by $(NH_4)_2SO_4$ (Table 5).

Properties of DDs in other mammalian lenses

Since DDs of livers in the guinea pig [4], mouse [3] and hamster [5] have been reported to be identical with hydroxysteroid dehydrogenases and aldehyde reductase, we examined the substrate specificity and inhibitor sensitivity of the DD fractions obtained from mouse, cat, hamster, monkey and guinea-pig lenses by gel filtration. The high-M_r DD fraction of mouse lens oxidized the dihydrodiols of naphthalene and benzene at an optimal pH of 10.0, but not hydroxysteroids such as 17β-hydroxy-5β-androstan-3-one, 3α-hydroxy-5α-androstan-17-one and 5β-androstane-3α,17β-diol. The enzyme activity was not affected by the addition of 1 mM-barbital, diphenic acid and 0.1 mM-Sorbinil, but was slightly inhibited by 0.3 M-$(NH_4)_2SO_4$. The low-M_r fractions of the five species were also inactive towards the hydroxysteroids, but their naphthalenedihydrodiol dehydrogenase activities with 2.5 mM-NAD$^+$ were more than 3-fold higher than those with 0.25 mM-NADP$^+$. The representative pH-dependencies of the NADP$^+$- and NAD$^+$-linked activities of the mouse and cat enzymes are shown in Fig. 6. Sorbinil (0.1 mM) inhibited 76–85% of the activity of the lens low-M_r enzymes of the five species. Barbital and diphenic acid at concentrations of 1 mM caused 52% and 31% inhibitions respectively of the activity of the mouse lens enzyme, but the respective inhibitions for the enzymes of the other species were < 37 and 10% respectively. The activities of all the low-M_r DDs were activated about 1.3-fold by the addition of 0.3 M-$(NH_4)_2SO_4$.

DISCUSSION

We have presented immunological evidence here which confirms our previous suggestion that the monomeric DD in pig lens is aldose reductase [16]. On the other hand, the dimeric DD was immunologically distinct from aldose and aldehyde reductases. This is in contrast to human dimeric aldehyde reductase, which has been shown to comprise aldose reductase and another non-enzymic protein [17]. Recently, Nakayama *et al.* [28] have purified dimeric DDs, which are immunologically

and catalytically identical with the lens DD, from various extraocular tissues of the pig. This study also revealed that the dimeric enzyme is distributed in several pig ocular tissues. The ubiquitous distribution of the dimeric enzyme in the pig tissues suggests the enzyme's normal physiological function is other than drug metabolism, although the endogenous substrates of the enzyme remain unknown.

DD activity in the rabbit lens was much greater than that in the lens of other species, as previously reported [15]. The enzyme in rabbit and mouse lenses existed in high-M_r and low-M_r forms, in contrast with the occurrence of only the low-M_r enzyme form in the lens of the other species. The high-M_r enzyme form was predominant in rabbit lens, but the amount of this enzyme form was low in mouse lens. The high-M_r DD of rabbit lens was a dimer which is functionally and immunologically similar to pig lens dimeric DD. It is clear that the high DD activity of the rabbit lens is due to the presence of large amounts of the dimeric enzyme.

The substrate specificity, activation by sulphate ion and inhibitor sensitivity of the purified low-M_r DD from rabbit lens are similar to those reported for aldose reductases of rabbit lens [29] and muscle [30], which indicates that the purified low-M_r DD is very probably identical with aldose reductase. The low-M_r DD of rat lens was also judged to be aldose reductase by the co-purification of the two enzyme activities and by its functional properties. Although the rat enzyme was inhibited more effectively than rabbit low-M_r DD by Sorbinil, a similar difference in susceptibility to inhibition by this drug has been observed with rabbit [30] and rat [31] aldose reductases. The activity in the low-M_r DD fractions of cat, mouse and hamster lenses may be mainly due to aldose reductase, because of its dual cofactor specificity, activation by sulphate ion and sensitivity to Sorbinil. Aldose reductases and low-M_r DDs from these animal lenses exhibited greater DD activity with NAD^+ as a cofactor than with $NADP^+$. Since the concentrations of NAD^+ plus NADH in mammalian lenses are much higher than those of $NADP^+$ plus NADPH [32], aldose reductase is the predominant DD in animal lenses which do not contain large amounts of dimeric DD.

Dimeric DD was detected only in the lens of rabbit tissues and exhibited a lower K_m value for naphthalenedihydrodiol than did aldose reductase. This strongly supports the previous suggestion that DD, especially the dimeric enzyme, plays an important role in the pathogenesis of naphthalene-induced cataract in the rabbit [11,33]. On the other hand, a great variation of the rabbit lens's susceptibility to naphthalene has been noted [34]. Since the individual difference in DD activity of rabbit lenses was relatively low, the variable response to naphthalene may not depend on differences in the enzyme content in the lens, but may be caused by differences in other intra-lenticular factors such as glutathione, which plays a central role against oxidative damage [35], and ascorbic acid, which is accumulated in the lens of naphthalene-fed rabbits [36] and influences the activity of dimeric DD [37].

Monophenol oxidase has been suggested to be the important enzyme responsible for the formation of naphthalene-induced cataract in the pigmented rat [14]. However, albino rats develop naphthalene-induced cataract which is less consistent and severe than that of the pigmented strains, and it has been thought that there may be other pathways responsible for less severe changes in the lens of naphthalene-fed albino rats [14,33]. Hockwin *et al.* [38] have reported that concomitant administration of an aldose reductase inhibitor, AL-1576, prevents naphthalene-induced cataract formation in the pigmented rat. In addition, although the $NADP^+$-dependent DD activity in the albino rat lens was low, we identified the primary DD as aldose reductase, which exerted high DD activity with NAD^+ as the cofactor. Therefore the oxidation of naphthalenedihydrodiol by aldose reductase may be a major route to naphthalene-induced cataract formation, at least in the albino rat.

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