Bovine lens aldehyde dehydrogenase

Purification and preliminary characterization

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Cytoplasmic aldehyde dehydrogenase from bovine lens was purified to apparent homogeneity by using ion-exchange and affinity chromatography. Sedimentation-equilibrium ultracentrifugation, gel-filtration chromatography and sodium dodecyl sulphate/polyacrylamide-gel electrophoresis show that the enzyme is a dimer of M_r 114 000, with subunits of M_r 57 000. The enzyme does not dissociate into monomers in the presence of Ca^{2+} or Mg^{2+} . The enzyme has a pI of 5.0, an activation energy of 35.1 kJ/mol and a pK value of 8.6 with acetaldehyde as substrate. The enzyme is a prolate ellipsoid with a Stokes radius of 4 nm. Progesterone, deoxycorticosterone and chlorpropamide inhibited enzyme activity, and this inhibition may play a role in cataract formation in patients maintained on systemic corticosteroids and in tablet-dependent diabetics.

Aldehyde dehydrogenase (EC 1.2.1.3) was first demonstrated in bovine liver by Racker (1949): it catalyses irreversible dehydrogenation of a large variety of aldehydes to their corresponding carboxylic acids with NAD^+ as coenzyme in the following reaction:

Its activity has been found in almost every mammalian organ, but its concentration is highest in the liver (Simpson & Lindahl, 1979; Inoue *et al.*, 1979, 1981; Goedde *et al.*, 1980; Lamboeuf *et al.*, 1981; Messiha, 1981).

The liver enzymes from horse, man, cattle and sheep have been purified and characterized. They are all tetramers with M_r values in the range 200 000–245 000 and share similar physical properties.

The exact role of aldehyde dehydrogenase has not been defined, except under special circumstances, i.e. ethanol metabolism in the liver (Peters, 1982). There is evidence that alcoholics have higher blood acetaldehyde concentrations than do non-alcoholic subjects when blood ethanol concentrations are maintained at the same value (Korsten *et al.*, 1975), and chronic ethanol consumption also decreased total and low- K_m mitochondrial aldehyde dehydrogenase activity in the baboon and rat liver (Lebsack *et al.*, 1981).

Specific members of this heterogeneous family of enzymes have been assumed to be important in oxidizing aldehyde metabolites of dopamine and noradrenaline (Berger & Weiner, 1977). It also appears that steroid acids are normal metabolites of corticosteroids via aldehyde dehydrogenase (Martin & Monder, 1978). Involvement of aldehydes in cataractogenesis, in which insoluble protein aggregates are formed, is of considerable interest (Crabbe *et al.*, 1982; Ting & Crabbe, 1982).

In the present paper we report the purification to apparent homogeneity of an aldehyde dehydrogenase from bovine lens and a partial characterization of the enzyme. The following paper (Ting & Crabbe, 1983) contains studies pertinent to the kinetic mechanism of the reaction.

Materials and methods

Materials

Fresh bovine lenses were obtained from the Alf Meade Slaughterhouse, Reading, Berks., U.K., and stored at -20°C before use.

NAD^+ (grades I and II) was obtained from Boehringer, Lewes, East Sussex, U.K. Grade-II NAD^+ was used for analyses of enzyme activities during purifications, and the special grade-I NAD^+ was used for characterization of the purified enzyme.

Acetaldehyde and propionaldehyde were redistilled from an AnalaR-grade solution (BDH Chemicals, Poole, Dorset, U.K.). Propionaldehyde

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was redistilled under O₂-free N₂. Chlorpropamide [1-(*p*-chlorobenzenesulphonyl)-3-propylurea] was from Pfizer, Sandwich, Kent, U.K.

All other chemicals were of the highest purity available. Glass-distilled water was used throughout.

Enzyme assay

Aldehyde dehydrogenase activity was determined with a Perkin-Elmer 555 double-beam spectrophotometer by monitoring the initial rate of NADH production at 340 nm with 0.2 mM-NAD⁺ and 1 mM-acetaldehyde in 0.05 M-sodium pyrophosphate buffer, pH 8.4, at 37°C. The reaction was initiated by the addition of enzyme. A molar absorption coefficient of $6.22 \times 10^3 \text{ M}^{-1} \cdot \text{cm}^{-1}$ was used for NADH. One unit of enzyme activity was defined as that amount of enzyme producing 1 μmol of NADH/min in the above assay system. The progress curve was linear for at least 3 min. A plot of initial velocity versus [enzyme] was linear over the range used for these studies.

Protein determination

For routine column monitoring protein concentration was estimated by measurement of A_{280} . Other samples were assayed by the Bradford (1976) method. Bovine serum albumin was used as standard.

Purification of aldehyde dehydrogenase

All buffers used in the purification were of pH 7.4 and contained NaN₃ (0.02%), EDTA (2 mM) and 2-mercaptoethanol (4 mM). All purification steps were performed at 4°C.

Bovine lenses (50) were homogenized in 5 vol. (v/w) of 0.1 M-potassium phosphate buffer. The homogenate was filtered through cheese-cloth and centrifuged at 30 000 *g* for 30 min. After (NH₄)₂SO₄ fractionation (45–60%-saturation fraction retained), the solution was centrifuged at 15 000 *g* for 30 min and the enzyme pellet resuspended in 0.02 M-potassium phosphate buffer. It was then dialysed overnight against 0.02 M-potassium phosphate buffer (4.5 litres). The dialysed preparation was loaded on a CM-Sephadex column (32 cm $\times$ 2 cm) pre-equilibrated with the 0.02 M-phosphate buffer. Enzyme was eluted in the first protein peak. Active fractions were pooled and loaded on a pre-equilibrated DEAE-Sephadex column (62 cm $\times$ 2.2 cm). Enzyme was eluted with a linear gradient of potassium phosphate buffer (0.02–0.2 M over 200 ml). Aldehyde dehydrogenase was eluted at about 0.1 M, in the first protein peak after gradient application, and was stable at 4°C for over 1 month.

An affinity column of NAD⁺-Sephacrose prepared as described by Mosbach *et al.* (1972) was used as the final step. Enzyme from the DEAE-Sephadex column (100 mg) was applied on a

NAD⁺-Sephacrose 4B column (9 cm $\times$ 1 cm). Non-enzyme protein was eluted with 0.2 M-KCl in 0.1 M-phosphate buffer. The enzyme was very tightly bound, as 1.2 M-KCl in 0.1 M-phosphate buffer did not release the enzyme from the column. The enzyme was successfully eluted with NAD⁺ (1 mM) in 0.1 M-phosphate buffer at a flow rate of 40 ml/h.

Enzyme solutions for experiment were prepared from the final NAD⁺ eluate by dialysis against phosphate buffer or by chromatography on Sephadex G-10.

Polyacrylamide-gel electrophoresis

For sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, Laemmli's (1970) method was adopted, with 6% acrylamide in the stacking gel and 12% acrylamide in the separating gel.

BDH marker proteins in the range M_r 14 300–71 500 were used for calibration. Gels were stained for protein with 0.25% Coomassie Brilliant Blue R-250 in a solution containing 50% (v/v) methanol and 10% (v/v) acetic acid for 1 h and destained overnight in a large volume of solution containing 5% (v/v) methanol and 7.5% (v/v) acetic acid.

Non-sodium dodecyl sulphate-containing polyacrylamide gels were prepared by modifying the procedure of Pietruszko & Vallari (1978). The 7.5%-acrylamide gel was prepared in 10 mM-sodium citrate buffer, pH 5.5, containing 1 mM-EDTA and 0.1% (v/v) 2-mercaptoethanol. Electrophoresis was performed at 4°C in 50 mM-sodium citrate buffer, pH 5.5, at constant voltage (100 V/eight tubes) for 4–5 h. The gel was stained for activity by incubation in the dark at 37°C for 30 min in 0.1 M-sodium pyrophosphate buffer, pH 8.5 (10 ml), containing NAD⁺ (6 mg), Nitro Blue Tetrazolium salt (4 mg), phenazine methosulphate (0.4 mg) and acetaldehyde (10 mM). Control incubations followed the same procedure except that substrate was not present in the incubation mixture. After being stained for activity, the gels were bisected longitudinally and one half was stained for protein as mentioned above. Electrophoresis at pH 7.5 gave similar results to that at pH 5.5 for activity staining.

M_r determinations

Sedimentation-equilibrium analysis was performed in a Beckman model E ultracentrifuge equipped with a photoelectric scanner. Runs were done at 10 000 rev./min for 2 days at 18°C with purified aldehyde dehydrogenase (0.5 mg/ml). All experiments were done in 0.02 M-phosphate buffer, pH 7.4. A partial specific volume of 0.75 cm³/g was used to calculate the M_r value of the enzyme. The partial specific volume was estimated from the amino acid composition.

The M_r and Stokes radius were also determined by gel filtration on a column (1.3 cm $\times$ 142 cm) of

Sephadex G-200 equilibrated at 0–4°C with 50 mM-Tris/HCl buffer, pH 7.5, containing KCl (0.1 M), EDTA (5 mM), 2-mercaptoethanol (10 mM) and NaN_3 (0.02%). Elution was performed at 6 ml/h and 1.2 ml fractions were collected. The standards used, with their M_r values, were: alcohol dehydrogenase, 120 000; bovine serum albumin, 68 000; ovalbumin, 45 000; cytochrome c, 12 270. The void volume (V_0) of the column was obtained by using Blue Dextran 2000 (Pharmacia, Uppsala, Sweden). The elution volume (V_e) was determined as the peak volume from the elution profile, and the M_r of aldehyde dehydrogenase was assessed by interpolation by using a plot of $\log M_r$ against V_e .

Acid hydrolysis

Desalted and freeze-dried protein specimens (1 mg) were hydrolysed in 1 ml of 6 M-HCl at 108°C for 18 h *in vacuo*. The hydrolysates were rotary-evaporated to dryness at 70°C and then redissolved in 0.5 ml of sodium citrate buffer, pH 2.2, containing 10.6 mM-phenol. This was then passed through a micropore filter to remove debris.

Samples (50–80 μ l) were applied to a Locarte amino acid analyser or an LKB model 4400 automated amino acid analyser. The amino acid composition was calculated by comparison with a chromatogram prepared at the same time from amino acid standards of known concentration.

Isoelectric focusing

Gels (5% acrylamide) containing a 16-fold dilution of Ampholines, pH range 3–10 (Pharmacia Fine Chemicals, Uppsala, Sweden), were freshly prepared before each experiment. Protein samples (3 ml) were desalted by chromatography on a Sephadex G-25 column (8 cm $\times$ 1.2 cm) and applied to the gel bed by soaking 50 μ l portions on to filter paper (0.6 cm $\times$ 0.4 cm) (Whatman 3MM Paratex) resting on the gel surface. Focusing was performed at 16°C with a Pharmacia FBE 3000 flat-bed apparatus and ECPS 3000/150 constant power supply at 50 W. Focusing time was 3700 V $\cdot$ h, with a maximum current of 150 mA and maximum voltage of 3000 V.

Results and discussion

Purification and criteria of purity

A typical purification based on 50 bovine lenses performed in accordance with the methods described is shown in Table 1.

The specific activity and yield differed among batches (0.9–1.4 units; 20–27%), higher yields and specific activities being obtained when all buffers used in the purification were saturated with N_2 before addition of 2-mercaptoethanol. Fluctuation of the specific activity of different preparations has been reported previously (Feldman & Weiner, 1972; Vallari & Pietruszko, 1981), this possibly being due to the intrinsic instability of the enzyme because of the high sensitivity of its thiol group(s) to oxidation. The importance of using nitrogenated buffer has been stressed in the purification of a human liver enzyme (Pietruszko & Yonetani, 1981).

Our preparation was judged to be homogeneous by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis and by sedimentation-equilibrium ultracentrifugation (Figs. 1 and 2). When the enzyme was subjected to electrophoresis with 7.5% polyacrylamide gel in the absence of sodium dodecyl sulphate, the enzyme activity staining corresponded exactly to the position of the only protein band.

The enzyme was of M_r 114 000 from the sedimentation-equilibrium analysis and of M_r 115 000 $\pm$ 2000 from the Sephadex G-200 gel filtration. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis showed only a single band with M_r 57 000 (Fig. 1); the natural enzyme is therefore a dimer. The high specific activity of our purified enzyme compared with the lower values obtained for the sheep enzymes under similar conditions may reflect the dimeric nature of our enzyme; the dimer prepared from the horse liver has a higher specific activity than the tetrameric form (Takahashi & Weiner, 1980).

The possibility that this dimeric enzyme is a product of dissociation of a polymeric enzyme of higher order was tested by Sephadex G-200 gel filtration of a crude lens homogenate. A 70 mg portion of soluble protein was applied to a column (1.2 cm $\times$ 150 cm), with 0.1 M-potassium phosphate

Table 1. *Purification of bovine lens aldehyde dehydrogenase*
For full experimental details see the text.

Step	Total protein (mg)	Total activity (units)	Specific activity (munits/mg of protein)	Purification (fold)	Yield (%)
Crude extract	36 773	51	1.39	1	100
45–60%-satn. $(\text{NH}_4)_2\text{SO}_4$	2388	36.3	15.2	10.9	71.2
CM-Sephadex	1410	33.4	23.7	17	65.5
DEAE-Sephadex	305	21.5	70.5	51	42.2
NAD^+ -Sephacrose	9.3	12.5	1339	963	24.5

Fig. 1. Sodium dodecyl sulphate/polyacrylamide- (12.5%) slab-gel electrophoresis of bovine lens aldehyde dehydrogenase at different stages of purification

For full experimental details see the text. Stage of purification: (a) after CM-Sephadex chromatography; (b) after DEAE-Sephadex chromatography; (c) buffer eluate from NAD^+ -Sephacrose column; (d) enzyme from NAD^+ -Sephacrose column eluted with 1 mM- NAD^+ .

buffer, pH 7.5, containing 2-mercaptoethanol (10 mM) and EDTA (2 mM) as elution buffer. The enzyme activity was found in the β_{H} -crystallin fractions, which were eluted with M_r values over the range 70 000–170 000, and so it seems unlikely that the enzyme was in a higher polymeric form *in vivo* and dissociated into dimers during purification.

This dimer was not due to the presence of bivalent ions such as Ca^{2+} or Mg^{2+} , which cause the dissociation of the mitochondrial horse liver tetrameric enzyme into the more active dimeric form (Takahashi & Weiner, 1980). As phosphate buffer and EDTA were used in the purification, they would either precipitate or chelate most of the bivalent ions present. Moreover, Ca^{2+} and Mg^{2+} inhibit both crude and purified bovine lens aldehyde dehydrogenase activity and crude human lens aldehyde

Fig. 2. Sedimentation-equilibrium ultracentrifugation of purified bovine lens aldehyde dehydrogenase

For full experimental details see the text. The M_r value calculated from the least-squares best-fit slope (0.278) was 114 000. The limiting slope gave minimum and maximum M_r values of 102 000 and 140 000.

dehydrogenase activity (Fig. 3). The presence of 0.1 mM- Ca^{2+} or 0.2 mM- Mg^{2+} decreased the enzyme activities by about 50%. This inhibition was not due to depolymerization. Gel filtration on Sephadex G-100 of the purified enzyme in 0.1 M-Tris/HCl buffer, pH 7.4, and in the presence of 0.2 mM- Mg^{2+} in the same buffer, did not dissociate the dimer into its subunits. Moreover, over 95% of the enzyme activity applied to the column was recovered in the void volume.

Similar inhibitory effects have been reported with human erythrocyte aldehyde dehydrogenase (Inoue *et al.*, 1979) and sheep liver cytoplasmic aldehyde dehydrogenase (Dickinson & Hart, 1982).

The purified enzyme therefore has M_r 114 000 with two subunits of M_r 57 000. This is different from all the aldehyde dehydrogenases isolated from other mammalian sources reported so far, with the exception of rat liver enzymes. The latter have isoenzymes with an M_r range of 67 000–320 000 (Siew *et al.*, 1976). A dimeric enzyme has been found in rat hepatoma with M_r 105 000 (Lindahl & Feinstein, 1976). All other mammalian aldehyde dehydrogenases reported are tetramers with M_r 200 000, but the isoelectric points are similar to that of the bovine lens enzyme.

Fig. 3. Effects of (a) Ca^{2+} and (b) Mg^{2+} on bovine lens aldehyde dehydrogenase activity

For full experimental details see the text. O, Crude human lens soluble extract; $\square$, purified bovine lens aldehyde dehydrogenase; $\blacksquare$, crude bovine lens soluble extract. Vertical bars represent standard deviations.

U.v. spectrum

The u.v. spectrum of the purified enzyme solutions after being dialysed exhaustively against 0.1% $(\text{NH}_4)_2\text{CO}_3$ at 4°C showed a maximum absorbance at 275 nm and no absorbance in the visible region. The absorption coefficient ($A_{1\text{cm}}^{1\%}$) at 280 nm was 0.98.

Stokes radius

This was calculated from gel filtration of the enzyme and marker proteins on Sephadex G-200. The Stokes radii of marker proteins were obtained from Siegel & Monty (1966) and Mantle (1978).

The Stokes radius obtained for aldehyde dehydrogenase was 4 nm. The unhydrated 'ideal' Stokes radius (assuming that the protein is spherical) was calculated from its M_r , determined by sedimentation-equilibrium centrifugation, and found to be 3.2 nm.

This difference of 20% between the values calculated is probably due to both a hydration effect

and the non-spherical nature of the protein. A dimeric protein with two globular subunits would probably have an overall prolate ellipsoidal shape (Cantor & Schimmel, 1980).

The maximum frictional coefficient ratio, or Perrin factor (F), was estimated from the ratio of the two Stokes radii obtained above as 1.25. This corresponds to a prolate ellipsoid with an axial ratio of 5 (Cantor & Schimmel, 1980). This approximation is likely to be slightly higher than the real value, as r_{sph} , used here was calculated for an unhydrated protein.

Isoelectric point

When staining for activity was performed with acetaldehyde and propionaldehyde as substrates, with both purified aldehyde dehydrogenase and crude bovine lens homogenate after high-voltage polyacrylamide-gel isoelectric focusing, only a band at pI 5 showed activity. However, the purified enzyme also showed a shadow band at pI approx. 5.2 after staining for protein with Coomassie Blue (Fig. 4). The nature of this band is still unknown; however, the absorbance ratio at 555 nm of this shadow band to the main band (pI 5) varied between runs.

Characterization of purified bovine lens aldehyde dehydrogenase

pH-activity profile. The enzyme activities were identical in phosphate and pyrophosphate buffers at pH 7.8. No difference in the rate was observed when the enzyme was assayed in glycine/HCl buffer, sodium barbiturate buffer, sodium pyrophosphate buffer and sodium carbonate buffer at pH 8.4 at I 0.1 at 37°C , but Tris/HCl buffer was inhibitory under the same conditions.

Within the pH range tested, the enzyme had a similar pH-activity profile (Fig. 5) to those of the horse liver cytoplasmic enzyme (Eckfeldt & Yonetani, 1976) and the sheep liver mitochondrial enzyme (Kitson, 1975). The profile suggested that the reaction involved a protein functional group with a pK value about 8.6, which resembled the cysteinyl thiol group, as in most other aldehyde dehydrogenases (Jakoby, 1963). The pH-activity profile only plateaued off at around 50% activity at the lower pH, and this would indicate that more than one group is involved in the reaction. Other functional groups might be involved in the direct binding of substrate or transfer reactions, or in a charge-relay system.

Effect of temperature. The reaction rate increased linearly with temperature from 27°C to 47°C at pH 8.4. The activation energy calculated under these conditions was 35.1 kJ/mol.

Amino acid composition. The amino acid composition of the purified bovine lens enzyme is shown

Fig. 4. Analytical isoelectric focusing showing stages of purification of bovine lens aldehyde dehydrogenase. For full experimental details see the text. (a) Purified aldehyde dehydrogenase (100 μ g) after NAD⁺-Sephadex chromatography; (b) as (a), but with 50 μ g; (c) inactive proteins eluted from NAD⁺-Sephadex column; (d) pooled active fractions after DEAE-Sephadex chromatography; (e) pooled active fractions after CM-Sephadex chromatography; (f) 45–60%-satn.-(NH₄)₂SO₄ fraction; (g) soluble lens extract. M, Isoelectric-point markers.

Fig. 5. pH-activity profile of purified bovine lens aldehyde dehydrogenase.

For full experimental details see the text. Ionic strength was 0.1. Δ and $\square$, Phosphate buffer; $\blacktriangle$ and $\blacksquare$, pyrophosphate buffer. Δ and $\blacktriangle$, Purified enzyme; $\square$ and $\blacksquare$, aldehyde dehydrogenase activity in crude bovine lens soluble extract. Vertical bars represent standard deviations.

in Table 2. The result was calculated from three sets of data obtained from different batches of the final preparation from NAD⁺-Sephadex purification. The average standard deviation for each amino acid was $\pm 5.64\%$.

The amino acid chromatogram showed no trace of citrulline or ornithine, typical contaminating amino acids from fingerprints (Hamilton, 1965).

The amino acid compositions of liver cytoplasmic aldehyde dehydrogenases of human, sheep, horse and ox are listed together with that from bovine lens in Table 2. All of them have high contents of aspartic acid and glutamic acid residues, which reflect the enzyme's generally acidic nature (i.e. pI 5). All of them also have a large amount of hydrophobic amino acids. The average 'hydrophobicity' of the enzyme proteins, calculated from their amino acid compositions in accordance with Bigelow & Channon (1976), was 4.00 ± 0.10 kJ/mmol. The 'Difference Index', 8, calculated in accordance with Metzger *et al.* (1968), indicated that the liver and lens proteins are similar.

Attempted N-terminal determination showed that the N-terminal amino acid residue was blocked.

Table 2. Amino acid composition of aldehyde dehydrogenases

Data for liver enzymes are taken from MacGibbon *et al.* (1979), the values given there being multiplied by 11.4 to give mol/114 000 g of protein. The data for the bovine lens enzyme are means $\pm$ S.D. of results from three determinations.

Amino acid	Amino acid composition (mol/114 000 g of protein)				
	Sheep liver	Horse liver	Human liver	Bovine liver	Bovine lens
Asp	82.1	74.1	84.4	84.4	90.3 $\pm$ 1.5
Thr	47.9	46.7	43.3	38.8	37.3 $\pm$ 4.1
Ser	57.0	46.7	49.0	53.6	65.5 $\pm$ 0.8
Glu	91.2	88.9	66.1	90.1	97.2 $\pm$ 1.6
Pro	50.2	55.9	69.5	47.9	87.2 $\pm$ 1.7
Gly	79.8	76.4	78.7	85.5	139.0 $\pm$ 10
Ala	68.4	63.8	67.3	75.2	67.5 $\pm$ 3.2
Val	62.7	65.0	63.8	66.1	38.8 $\pm$ 0.9
Met	17.1	17.1	10.3	18.2	10.7 $\pm$ 1.7
Ile	47.9	41.0	47.9	43.3	40.4 $\pm$ 4.2
Leu	63.8	65.0	55.9	66.1	62.7 $\pm$ 4.8
Tyr	25.1	23.9	21.4	27.4	17.9 $\pm$ 1.2
Phe	44.5	41.0	34.2	41.0	35.3 $\pm$ 1.3
His	12.5	11.4	13.7	12.5	15.2 $\pm$ 0.4
Lys	63.8	60.4	58.1	71.8	60.8 $\pm$ 1.8
Arg	31.0	28.5	29.6	37.6	25.6 $\pm$ 2.2

Table 3. Effect of steroids on purified bovine lens aldehyde dehydrogenase

All steroids were at a final concentration of 10 μ M. Data, expressed as percentage activity relative to a control without added steroid, are the means of results from three experiments. Standard deviations were 10% or less in all cases. All incubation mixtures contained 5% ethanol. The ethanol did not affect enzyme activity. For full experimental details see the text.

Steroid	Relative activity (%)
Control	100
Progesterone	25
Diethylstilboestrol	120
Oestrone	95
Corticosterone	88
Cortisol	96
Pregnenolone	94
Deoxycorticosterone	38
Dehydroisoandrosterone	70
Testosterone	65
Androsterone	85
Androsterone-3,17-dione	78

Effect of steroids and chlorpropamide on enzyme activity

Most steroids tested were inhibitory at 10 μ M, apart from a slight activation by diethylstilboestrol (Table 3). The effects of steroids were first reported in rabbit liver aldehyde dehydrogenase (Maxwell & Topper, 1961), but similar effects have also been reported in human and sheep liver enzymes (Kitson & Crow, 1982).

The most potent steroid inhibitors, progesterone

and deoxycorticosterone, share some common features, i.e. the 3-oxo group, 20-oxo group and 4,5-ene bond, which might be involved in the binding to the enzyme protein. This inhibition, however, is diminished by the presence of hydroxy groups. Corticosteroids also showed an inverse relationship between inhibition and number of hydroxy groups, implying a surface charge effect, also noticed with some thiol-blocking reagents (Ting & Crabbe, 1982).

Patients maintained on systemic corticosteroids for many years in treatment of asthma, in treatment of rheumatoid arthritis or as prophylaxis of a renal graft rejection may develop cataract. The cataract is typically posterior subcapsular and starts in the axial part of the lens. Inhibition of aldehyde dehydrogenase, resulting in increased concentrations of reactive aldehydes, may play a role in cataractogenesis, together with direct protein modification by steroids.

Chlorpropamide exhibited both slope and intercept inhibitory effects with respect to acetaldehyde and NAD⁺ on purified bovine lens aldehyde dehydrogenase activity (Fig. 6), with $K_{i(\text{slope})}$ = 0.243 mM and $K_{i(\text{intercept})}$ = 0.661 mM with respect to acetylaldehyde when NAD⁺ was saturating (0.5 mM), and $K_{i(\text{slope})}$ = 0.152 mM and $K_{i(\text{intercept})}$ = 0.69 mM with respect to NAD⁺ when acetaldehyde was saturating (10 mM).

This inhibition was similar to that found with human lens aldehyde dehydrogenase (Ting & Crabbe, 1982).

Sulphonylureas are administered daily to tablet-dependent diabetics to maintain a steady blood

Fig. 6. Inhibition of purified bovine lens aldehyde dehydrogenase by chlorpropamide

For full experimental details see the text. For primary plots: ●, no inhibitor; □, 0.05 mM-chlorpropamide; ▲, 0.1 mM-chlorpropamide; △, 0.2 mM-chlorpropamide. For replots: ●, intercept; △, slope. (a) $1/v$ versus $1/[\text{acetaldehyde}]$; (b) slope and intercept replot; (c) $1/v$ versus $1/[\text{NAD}^+]$; (d) slope and intercept replots. Vertical bars represent standard deviations. Where there are no vertical bars, standard deviations were within the plotted points.

glucose concentration. Sulphonylureas and their aldehyde metabolites are specific inhibitors of liver aldehyde dehydrogenase (Podgainy & Bressler, 1968; Petersen & Hjelle, 1982). The long-term continuous drug treatment is likely to cause accumulative aldehyde effects in tissues as a consequence of inefficient removal of some toxic aldehydes. Blood acetaldehyde concentrations were increased in patients treated with chlorpropamide after ethanol intake, and acetaldehyde binding to lens proteins was increased in the presence of chlorpropamide (Ting & Crabbe, 1982). This side effect of sulphonylurea suggests an alternative pathway for the formation of cataracts in tablet-dependent diabetics, apart from the osmotic and protein glycosylation hypotheses (Crabbe *et al.*, 1982).

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