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Purification and Characterization of Human-Brain Aldose Reductase

Bendicht WERMUTH, Hanspeter BÜRGISSER, Kurt BOHREN, and Jean-Pierre VON WARTBURG

Medizinisch-chemisches Institut der Universität, Bern

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Aldose reductase (EC 1.1.1.21) from human brain has been purified to apparent homogeneity. The enzyme catalyzes the NADPH-dependent reduction of several physiological and xenobiotic aldehydes. Isocorticosteroids, e.g. isocortisol and isocorticosterone, are the best substrates ($K_m < 1$ μm), followed by aromatic and arylalkylaldehydes, including biogenic aldehydes ($K_m = 3-15$ μM). The activity towards aldoses is highest with glyceraldehyde ($K_m = 25$ μM) and decreases with increasing number of carbon atoms of the sugar.

Flavonoids, e.g. quercetin and rutin, inhibit aldose reductase ($IC_{50} = 2-5$ μM). Sulfate ions, on the other hand, stimulate the enzyme activity. Thiol-modifying reagents, e.g. 4-hydroxymercuribenzoate and iodoacetate, cause a time-dependent inactivation.

Aldose reductase consists of a single polypeptide chain with a molecular weight of 38000 and an isoelectric point of 5.9. In the presence of thiol reagents the isoelectric point is shifted to 5.1.

Antibodies against aldose reductase do not cross-react with other carbonyl reductases, Nevertheless, the comparison of structural and enzymic properties of aldose reductase with those of other carbonyl reductases suggests a relationship between aldose reductase and aldehyde reductase (EC 1.1.1.2).

Aldose reductase (alditol:$NADP^+$ 1-oxidoreductase) catalyzes the conversion of aldoses and a number of other aldehydes to the corresponding alcohol metabolites. It is one of several cytosolic, monomeric, NADPH-dependent aldehyde and ketone reductases of wide substrate specificity that are generally referred to as carbonyl reductases [1].

Aldose reductase together with sorbitol dehydrogenase constitutes the polyol pathway, a minor route of carbohydrate metabolism converting glucose to fructose [2]. Under normal conditions there is little flux through this pathway. However, in diabetic hyperglycaemic conditions the flux increases dramatically, leading to elevated cellular concentrations of the intermediate sorbitol, which in turn cause a hyperosmotic effect and swelling of the cell. This process has been shown to initiate cataract formation in diabetic animals [3], and it may also play a role in the pathogenesis of other diabetic complications, such as neuropathy [4].

In addition to its function as one of the enzymes of the polyol pathway, aldose reductase appears to play a role in the metabolism of the biogenic amines. In the nervous system, biogenic amines, which bear a hydroxyl group adjacent to the amine function, e.g. noradrenaline, are catabolized to the glycol products, whereas the amines lacking the hydroxyl group, such as serotonin and dopamine, are mainly converted to the acid metabolites [5]. The bifurcation into an oxidative and reductive pathway results from the action of specific dehydrogenases and reductases on the aldehyde metabolites derived from the amines by monoamine–oxidase-catalyzed deamination. Evidence is accumulating that the reduction of the aldehydes derived from β-hydroxylated amines is catalyzed by aldose reductase [6, 7].

The potential involvement of aldose reductase in the pathogenesis of diabetic complications and its possible role in the biogenic amine metabolism have stimulated the investigation of this enzyme, leading to its isolation and characterization from a number of species and tissues. The occurrence of aldose reductase in brain was first reported by Moonsammy and Stewart who studied the enzyme from ox brain [8]. In 1973 Ris and von Wartburg demonstrated in human brain multiple molecular forms of an NADPH-dependent aldehyde reductase of wide substrate specificity [9]. Hoffman et al. [6] and O'Brien and Schofield [10] subsequently identified the enzyme form, which had been named aldehyde reductase 2 by Ris and von Wartburg, as aldose reductase.

Here we report on the purification of this reductase and some of its physicochemical and kinetic properties. We also give evidence that the enzyme may be involved in the metabolism of corticosteroids.

EXPERIMENTAL PROCEDURE

Materials

Human tissue from both sexes was obtained from legal medical autopsies. The tissue was frozen 6–20 h post mortem and stored at −20 °C.

Pyridine nucleotide cofactors were purchased from Sigma (St Louis, USA). Substrates, with the exception of the biogenic aldehydes and the isocorticosteroids, came from Fluka AG (Buchs, Switzerland) or from Merck AG (Darmstadt, FRG). Biogenic aldehydes were prepared as described by Tabakoff et al. [11] by incubating the appropriate amine with monoamine oxidase from calf liver. Isocorticosteroids were a gift from Dr C. Monder (The Population Council, New York, USA). Flavonoids were kindly supplied by Zyma SA (Nyon, Switzerland) and sodium valproate was a gift from

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Abbreviations. Isocortisol, 11β,17β,20α/β-trihydroxy-3-oxo-pregn-4-en-21-al; isocorticosterone, 11β,20α/β-dihydroxy-3-oxo-pregn-4-en-21-al; IC_{50}, concentration of inhibitor causing 50% inhibition under standard assay conditions.

Enzymes. Aldose reductase (polyol dehydrogenase) or alditol:$NADP^+$ 1-oxidoreductase (EC 1.1.1.21); aldehyde reductase or alcohol:$NADP^+$ oxidoreductase (EC 1.1.1.2); glucuronate reductase (L-hexonate dehydrogenase) or L-gulonate:$NADP^+$ 1-oxidoreductase (EC 1.1.1.19); carbonyl reductase (EC 1.1.1.–).

Labaz AG (Basel, Switzerland). Other inhibitors were from Fluka. Phenylglyoxal and dimethylsuberimidate dihydrochloride were bought from Aldrich (Beerse, Belgium).

DEAE-cellulose was purchased from Whatman (Maidstone, Great Britain). Sephadex G-100 and Sepharose 4B were obtained from Pharmacia (Uppsala, Sweden). Ampholine polyacrylamide gel plates for thin-layer gel isoelectrofocusing were a product of LKB Producter AB (Bromma, Sweden).

Cibacron-blue–Sepharose 4B was prepared by the following two-step procedure. (a) 6-Aminohexanoic acid (26 g) was coupled to 1 l Sepharose 4B by use of cyanogen bromide [12]. (b) The derived Sepharose was suspended in an equal volume of water and 4 g each of Cibacron blue F3 G-A (gift from Ciba-Geigy AG, Basel, Switzerland) and 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (Fluka AG) were added. The slurry, adjusted to pH 5, was agitated for 20 h; then the Sepharose was washed thoroughly on a sintered-glass funnel with 0.1 M NaOH, 0.1 M HCl, 2 M NaCl and H_2O until the dye became nondetectable in the effluent. The washed Cibacron-blue–Sepharose was of purple color.

Enzyme Assay

Reaction mixtures consisted of 0.1 M sodium phosphate, pH 7.0, 2–200 µl enzyme solution, 0.08 mM NADPH and substrates at various concentrations as indicated in the results section in a total volume of 1.0 ml. The reaction was followed spectrophotometrically by recording the decrease in absorbance of NADPH at 340 nm. Blanks without substrate and blanks without enzyme were routinely included.

In crude extracts other dehydrogenases, notably aldehyde reductase, may contribute to the rate of NADPH oxidation. Interference with aldehyde reductase can be diminished by inclusion of 0.5 mM sodium valproate or 0.1 mM diphenylhydantoin into the assay medium.

1 enzyme unit (U) is defined as the amount of protein catalyzing the oxidation of 1 µmol NADPH/min.

Protein Concentration

Aldose reductase, purified by the procedure outlined below, contains tightly bound adenine nucleotides, which interfere with spectrophotometric methods. The protein content of solutions containing pure enzyme was, therefore, estimated by quantifying the protein bands on polyacrylamide gels stained with Coomassie brilliant blue, using horse liver alcohol dehydrogenase as a standard. Gels were scanned on a Camag electrophoresis scanner (Camag AG, Muttenz, Switzerland). During enzyme purification the protein concentration was estimated from the absorbance at 280 nm and 260 nm [13].

Polyacrylamide Gel Electrophoresis

Sodium dodecyl sulfate/polyacrylamide gel electrophoresis was performed on 10% gels prepared in 0.375 M Tris/Cl, pH 8.9, containing 0.1% sodium dodecyl sulfate and 2-mercaptoethanol as indicated in Fig. 1 and 2. Protein bands were stained with Coomassie brilliant blue.

Polyacrylamide gel electrophoresis in the absence of sodium dodecyl sulfate was carried out on 7.5% acrylamide gels using the same buffer system. Enzyme activity was revealed by incubating the gels in 0.1 M Tris/Cl, pH 8.5, containing 10 mM benzyl alcohol, 0.1 mM $NADP^+$, 0.4 mM nitrotetrazolium blue chloride and 0.15 mM phenazine methosulfate, at 40 °C. Alternatively the gels were incubated at pH 7.5 in the presence of 0.1 mM NADPH, 10 µM 9,10-phenanthrenequinone and 0.4 mM nitrotetrazolium blue chloride. In this system the tetrazolium salt is reduced to formazan by the phenanthrenehydroquinone produced in the enzymic reaction.

Isoelectric Focusing

Isoelectric focusing of purified aldose reductase was performed on thin-layer polyacrylamide gels pH 3.5–10 and 4–6 as described by the manufacturer. Protein bands were visualized by staining with Coomassie brilliant blue.

Preparation of Aldose Reductase Antibodies

Antibodies to human brain aldose reductase were raised in rabbits. Animals were injected with an initial dose of 200 µg purified enzyme in complete Freund's adjuvant, and then after 4 weeks with 3 weekly doses of 100 µg enzyme. Immunoreactivity of aldose reductase was tested by double immunodiffusion.

Purification Aldose Reductase

The whole purification was carried out at 4–10 °C, and all buffers contained 0.1 mM EDTA and 1 mM 2-mercaptoethanol. Sodium azide (0.02%) was added to all enzyme solutions that were not immediately processed. D-Xylose was routinely used to screen the column effluent for aldose reductase activity.

Step 1: Extraction. Approximately 300 g brain tissue were homogenized with an equal volume (v/w) of 0.1 M sodium phosphate buffer, pH 7.4, in a Waring blendor for 2–3 min. The homogenate was centrifuged for 2 h at $20000 \times g$. The precipitate was re-extracted with the same volume of buffer. After centrifugation, the supernatant solutions were combined and dialyzed against three changes, 10 l each, of 5 mM sodium phosphate buffer pH 7.4. Usually some precipitate formed during dialysis and was removed by centrifugation (1 h, $20000 \times g$).

Step 2: DEAE-cellulose Chromatography. The dialyzed extract was applied to a column (2 × 80 cm) of DEAE-cellulose, equilibrated with dialysis buffer. The cellulose was washed with at least two column volumes of the same buffer before a linear gradient (2 × 1 l) of 5–100 mM sodium phosphate, pH 7.4, was applied. Fractions containing aldose reductase activity were pooled.

Step 3: Gel Filtration. The pooled fractions (maximum 150 ml/run) were passed over a column (4 × 180 ml) of Sephadex G-100, equilibrated with 10 mM sodium phosphate buffer, pH 6.2, at a flow rate of 50 ml/h. Fractions containing xylose reductase activity were pooled.

Step 4: Affinity Chromatography. The pooled fractions were applied to a column (2 × 20 cm) of Cibacron-blue–Sepharose, equilibrated with 10 mM sodium phosphate buffer, pH 6.2. The gel was washed with the two column volumes of the same buffer, followed by one volume of 100 mM sodium phosphate, pH 6.2, and again one volume of the original buffer. Aldose reductase was eluted with a gradient of 0–150 µM NADPH in the 10 mM phosphate buffer. Fractions containing enzyme activity were pooled and stored at 4 °C.

Table 1. *Purification of human brain aldose reductase*
Activity was measured using 0.1 M D-xylose as substrate

Purification step	Volume	Activity	Protein	Specific activity	Recovery
	ml	μmol min^{-1}	mg	nmol $min^{-1}mg^{-1}$	%
Extract	770	7.45	5930	1.3	100
DEAE-cellulose	130	4.48	420	10.7	60
Sephadex G-100	238	3.70	31	119	50
Blue-Sepharose	44	2.48	3.1	800	33

RESULTS

Purification

Table 1 summarizes the results of a typical purification of human brain aldose reductase. The initial steps, which are essentially the same as those reported by Ris and von Wartburg [9], resulted in the separation of aldose reductase from other aldehyde and ketone reductases, particularly aldehyde reductase (alcohol:NADP oxidoreductase, EC 1.1.1.2). The major part of aldose reductase activity eluted from the DEAE-cellulose column just ahead of aldehyde reductase (cf. [6]). With some preparations a second peak of aldose reductase activity emerged from the column immediately after aldehyde reductase. On the subsequent Sephadex G-100 column both aldose reductases yielded the same molecular weight, and displayed very similar catalytic properties (H. P. Bürgisser, M. S. Thesis, 1981); the second enzyme form, however, was not included in this study. Final purification of aldose reductase was achieved by dye-ligand affinity chromatography on Cibaron-blue–Sepharose. The enzyme eluted from the column in a single peak of activity at the beginning of the NADPH gradient.

The purified enzyme was stable for several weeks at 4 °C. Loss of activity and concomitant precipitation of protein occurred upon freezing and thawing and at temperatures above 50 °C. Loss of activity was also observed at pH values below 6. At pH 5.1 the activity decreased to one-half within 90 min.

Molecular Forms

Multiple molecular forms of aldose reductase have originally been found in bovine lens [14] and several rabbit tissues [15]. A more recent study on the bovine lens enzyme, on the other hand, has yielded only one enzyme form [16]. Similarly, only one form was observed with the pig brain enzyme [17].

In this study we obtained evidence of two molecular forms of homogeneous human brain aldose reductase. In the absence of reducing agents, polyacrylamide gel electrophoresis yielded a single band (Fig. 1 A), whereas in the presence of 2-mercaptoethanol or dithioerythritol a second, faster-migrating band became detectable (Fig. 1 B). Both bands showed enzyme activity, which corresponded approximately to the amount of protein (Fig. 1 C). This finding rules out the possibility that in the presence of the thiol some contaminating protein is released.

In agreement with the above findings, isoelectric focusing on polyacrylamide gels yielded one band at pH 5.9 in the absence and two bands at pH 5.9 and 5.1 in the presence of thiols (Fig. 2).

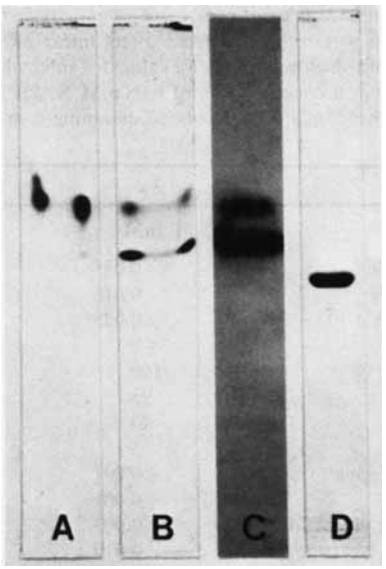

Fig. 1. *Polyacrylamide gel electrophoresis of human brain aldose reductase.* About 10 μg enzyme were applied per gel and electrophoresis was carried out as described under Experimental Procedure. (A) Native enzyme in the absence of thiol; stained for protein. (B, C) Native enzyme in the presence of 1% 2-mercaptoethanol; stained for protein and activity respectively. (D) Gel run in the presence of 1% sodium dodecyl sulfate

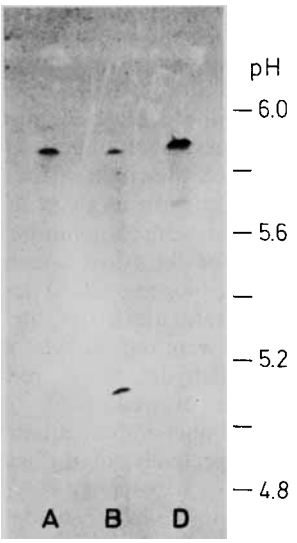

Fig. 2. *Polyacrylamide gel isoelectric focusing of human brain aldose reductase.* About 5 μg enzyme in 20 μl 5 mM ammonium bicarbonate were applied evenly between the electrode strips and electrofocusing was carried out for 90 min. (A) Enzyme in the absence of thiol. (B) Enzyme preincubated with 1% 2-mercaptoethanol. (D) Thiol-free enzyme exposed to oxygen for 24 h

Electrophoresis in the presence of sodium dodecyl sulfate gave only one band corresponding to a molecular weight of 38000, irrespective of the concentration of thiols (Fig. 1 D). This finding, in conjunction with previous reports of a molecular weight of 44000 for the native enzyme [6, 9], indicates that the two enzyme forms do not represent monomeric and dimeric forms of aldose reductase.

Substrate Specificity

Table 2 illustrates that aldose reductase was active with a wide range of aldehydes. Typically for aldose reductases, the

Table 2. *Substrate specificity of human brain aldose reductase*
Kinetic constants were estimated from direct linear plots according to Eisenthal and Cornish-Bowden [25]. K_m values for aldehydes and NADPH were determined at a concentration of 0.08 mM NADPH and 100 mM D-xylose respectively. k_{cat} was calculated assuming a molecular weight of 38000

Substrate	K_m	k_{cat}
	mM	s^{-1}
D-Glyceraldehyde	0.035	0.55
L-Glyceraldehyde	0.018	0.59
DL-Glyceraldehyde	0.025	0.64
D-Xylose	5.1	0.51
L-Xylose	100	0.28
D-Glucose	95	0.44
D-Galactose	40	0.40
D-Glucuronate	5	0.42
20α-Isosorticosterone[a]	<0.001	0.64
20α-Isocortisol[a]	<0.001	0.64
4-Hydroxyphenylglycolaldehyde	0.003	0.47
4-Hydroxyphenylacetaldehyde	0.010	0.59
Indole-3-acetaldehyde	0.016	0.48
4-Nitrobenzaldehyde	0.002	0.73
Phenylglyoxal	0.005	0.76
NADPH	0.002	–

[a] Similar kinetic constants were obtained for the 11-deoxy derivatives and the 20β-epimers

Michaelis constant for aldoses increased and the maximal velocity decreased with increasing number of carbon atoms of the sugar. Thus, of all carbohydrate substrates glyceraldehyde was most efficiently reduced. The best substrates, however, were isocorticosteroids. These compounds, which bear a 17-aldol instead of the 17-ketol side-chain of the corticosteroids [18], were reduced at the same rate as glyceraldehyde, their Michaelis constants, however, were two or more orders of magnitude lower than the K_m for the triose. Aromatic and arylalkylaldehydes, such as the biogenic aldehydes, were also excellent substrates. Pure aliphatic aldehydes, on the other hand, e.g. decanal and butanal, were only slowly metabolized.

In addition to aldehydes, aldose reductase also reduced quinones. The rate of reduction of 2,3-bornanedione (0.25 mM) and 9,10-phenanthrenequinone (0.05 mM) was 100% and 40%, respectively, of the velocity with 100 mM D-xylose.

The reverse reaction, i.e. the oxidation of alcohols by $NADP^+$, was negligible at 25 °C either at pH 7.0 or 9.0. At 37 °C enzyme activity could be measured with benzyl alcohol, glycerol and xylitol.

Aldose reductase was specific for NADPH as coenzyme. Less than 5% of the NADPH-linked activity was detectable with NADH (80 μM) as the coenzyme.

The substrate specificity of the homogeneous enzyme differs slightly from that of the partially purified enzyme described previously [6]. Decanal, for instance, was a better substrate of the partially purified than the homogeneous enzyme. Moreover, the non-homogeneous enzyme could use NADH as coenzyme, whereas the enzyme used in this study was specific for NADPH. These findings suggest that the partially purified enzyme contained traces of a hitherto unidentified $NADH^+$-linked dehydrogenase.

Influence of Effectors

Several compounds, known to affect the activity of carbonyl reductases, were tested as inhibitors of the homogeneous human brain aldose reductase. Flavonoids, which are potent inhibitors of aldose reductase from several sources [19,20] as well as of other aldehyde and ketone reductases [21,22], also effectively inhibited human brain aldose reductase. Quercetin and rutin, for instance, decreased the enzyme activity to one-half at a concentration of 2 μM and 5 μM respectively. Barbiturates and hydantoins, typical inhibitors of aldehyde reductase, also affected aldose reductase. With phenobarbitone 50% inhibition was obtained at a concentration of 120 μM. This value is only two to three times higher than the one determined with aldehyde reductase. Sodium valproate, on the other hand, which is an equally good inhibitor of aldehyde reductase as phenobarbitone [7], inhibited aldose reductase by only 20% at a concentration of 1 mM. Valproic acid may, therefore, be used to specifically inhibit aldehyde reductase in crude extracts.

Typically for aldose reductase, sulfate ions (200 mM) activated the enzyme by 40–60%.

Modification of cysteine residues by SH-specific reagents caused a time-dependent inactivation of aldose reductase. 0.5 μM 4-hydroxymercuribenzoate decreased the activity to 50% within 5 min. Iodoacetic acid caused the same inactivation at a concentration of 0.15 mM.

Incubation with the arginine-specific reagent phenylglyoxal (10 mM) or with dimethylsuberimidate (10 mM), which modifies amino groups, also decreased the activity of aldose reductase.

Kinetics

Conflicting results have been reported regarding the kinetic behavior of aldose reductase. Homogeneous enzyme from bovine lens [16] and calf brain [23] and the partially purified enzyme from human brain [6] yielded concave downward curved lines in a double-reciprocal plot of initial velocity versus the substrate concentration. Contrary to these findings, homogeneous aldose reductase from pig brain followed Michaelis-Menten kinetics [24].

In this study all substrates tested (cf. Table 2) yielded straight lines in a double-reciprocal plot. The reason for the different kinetic behavior of the partially purified and the homogeneous human brain enzyme is not clear at present, but might be explained by the removal of a contaminating reductase in the final affinity chromatography step (see above).

Controversial results have also been reported with respect to the product inhibition of aldose reductase. O'Brien and Schofield [10], working with partially purified aldose reductase from human brain, found competitive inhibition by glycerol when glyceraldehyde was varied. Boghosian and McGuinness, on the other hand, obtained non-competitive inhibition of pig brain aldose reductase by xylitol [24].

In order to verify this discrepancy we have carried out product-inhibition studies with xylose and glyceraldehyde, respectively, as substrate. Fig. 3 illustrates that only the two coenzymes were mutually exclusive, whereas xylitol clearly gave non-competitive inhibition with respect to xylose as well as NADPH. Similarly, glycerol was a non-competitive inhibitor relative to glyceraldehyde. Our results thus corroborate the findings of Boghosian and McGuinness, favoring an ordered bi-bi mechanism for aldose reductase.

Substrate inhibition of aldose reductase was observed with several aldehydes. It was most pronounced with glucuronate, which caused a deviation from linearity of the Lineweaver-Burk plot already at half-maximal velocity. With

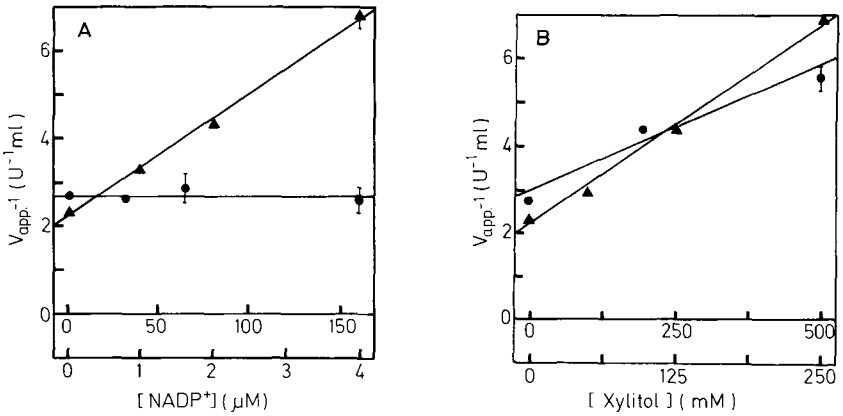

Fig. 3. *Product inhibition of human brain aldose reductase (secondary plots).* Apparent maximal velocities were estimated from direct linear plots according to Eisenthal and Cornish-Bowden [25]. Each point represents the median intersection from 6–7 determinations. Standard deviations were calculated as outlined by Wilkinson [26]. Lower scales (●) NADPH varied (0.5–10 K_m), D-xylose = 100 mM; upper scales (▲) D-xylose varied (0.2–2 K_m), NADPH = 80 μM. (A) Inhibition by $NADP^+$. (B) Inhibition by xylitol

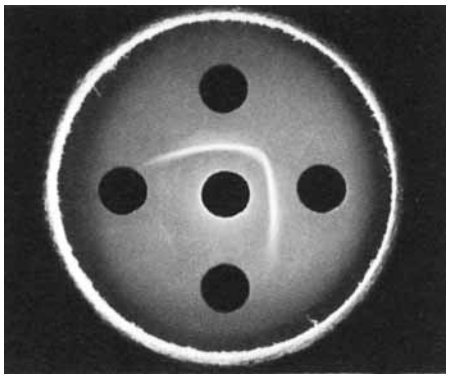

Fig. 4. *Immunogenicity of human brain aldose reductase.* Antibodies to aldose reductase were placed in the center well. The outer wells contained (clockwise from the top) purified aldose reductase, human brain extract, aldehyde reductase, and carbonyl reductase

glyceraldehyde and xylose inhibition became detectable only at concentrations 20 times above the K_m value.

Immunogenicity

Fig. 4 illustrates that antibodies against human brain aldose reductase gave fused precipitin lines between the homogeneous enzyme and brain extracts, but that they did not cross-react with either aldehyde or carbonyl reductase. Similarly, antibodies against aldehyde and carbonyl reductase did not cross-react with purified aldose reductase.

DISCUSSION

Aldose reductase was first demonstrated and its role in the polyol pathway established by Hers in the course of his studies on the metabolism of fructose in sheep seminal vesicles and placenta [2]. The presence of the enzyme in other species and tissues was established soon after and it has subsequently been purified to apparent homogeneity from several sources, including human placenta [27], calf [23] and pig [17] brain and bovine lens [16].

In his pioneering work Hers observed that several tissues, e.g. liver, contained another enzyme which also reduced glyceraldehyde and other aldoses, but was inactive towards glucose, and named it aldehyde reductase [2]. Mano et al. shortly after purified an enzyme from rat liver which reduced D-glucuronate to L-gulonate, the key metabolite for ascorbic acid synthesis and the glucuronate pathway, respectively, and demonstrated its identity with aldehyde reductase [28].

Detailed studies on aldose and aldehyde reductase have revealed a number of common properties. Both enzymes consist of a single polypeptide chain of molecular weight 30000–40000, use NADPH as the coenzyme and are localized in the cytosol. Most striking, however, is their almost identical substrate specificity. In addition to aldoses and glucuronate, they catalyze the reduction of biogenic aldehydes [11, 29] and isocorticosteroids [30] as well as a number of xenobiotic carbonyl compounds [1].

The similar substrate specificity and physicochemical properties of the two reductases may explain many of the discrepancies in the literature concerning relative velocities and K_m values of aldose reductase. Since aldehyde reductase constitutes the major activity in most tissues, including brain, even a small contamination of a preparation of aldose reductase can markedly affect the kinetic parameters. Many ambiguities, however, can be avoided if appropriate substrate concentrations are used. Aldose reductase exhibits Michaelis constants for most substrates, e.g. aldoses, biogenic aldehydes and isocorticosteroids, which are one to two orders of magnitude lower than those of aldehyde reductase. Exceptions are aldehydes bearing a negative charge, e.g. glucuronate and succinic semialdehyde, which are preferentially metabolized by aldehyde reductase.

The similarity between aldose and aldehyde reductase also raises the question of their physiological role(s). In seminal vesicles and the liver, from where the two reductases have originally been isolated, they participate in fructose and ascorbic acid synthesis respectively. These metabolic routes play, if at all, a marginal role in the central nervous system, and one may pose the question of the physiological substrate(s) in this highly specialized organ. Aldehyde reductase [11] and, more recently, aldose reductase [7] have been associated with the metabolism of biogenic amines in brain tissue. However, the contribution of each enzyme to the total reduction capacity *in vivo* has not yet been assessed. On kinetic grounds one would expect aldose reductase to be responsible for the reduction of the aldehydes under normal

Table 3. *Amino acid composition of human brain aldose reductase*
Aldose reductase was oxidized by performic acid [33] before hydrolysis in 6 M HCl, containing 0.1% phenol, for 24 h at 115 °C. Analyses of amino acids were performed on a Liquimat III amino acid analyzer (Kontron AG, Zürich, Switzerland), using a one-column system. Tryptophan was determined spectrophotometrically according to Edelhoch [34]. The amino acid composition was calculated from the analyses of two enzyme preparations assuming a molecular weight of 38000

Amino acid	No. residues	Amino acid	No. residues
Asparagine or aspartic acid	32	Isoleucine	17
Threonine	15	Leucine	34
Serine	16	Tyrosine	9
Glutamine or glutamic acid	36	Phenylalanine	12
Proline	24	Histidine	12
Glycine	18	Lysine	26
Alanine	21	Arginine	15
Valine	25	Cysteine	10
Methionine	6	Tryptophan	7

conditions. Aldehyde reductase, on the other hand, could act as a scavenger of these reactive metabolites at times of high production. Isocorticosteroids, which are the best substrates of aldose reductase known to date, may constitute another class of substrates in the central nervous system as well as in other tissues.

Aldose and aldehyde reductases have most extensively been studied in rat, bovine and porcine tissues, where they constitute the main cytosolic NADPH-linked carbonyl reductase activities. Human brain contains another enzyme, which shows the typical features of aldose and aldehyde reductase, i.e. a molecular weight of about 30000, dependence on NADPH, localization in the cytosol, and a wide specificity for aldehydes and ketones [9,21]. This enzyme is identical with human prostaglandin 9-ketoreductase and xenobiotic ketone reductase, and is referred to here as carbonyl reductase.

Aldose, aldehyde and carbonyl reductase are often taken together in the family of carbonyl reductases. The three enzymes, however, are not equal members of the family. Aldose and aldehyde reductase preferentially reduce 2-hydroxyaldehydes and show little activity towards ketones. Carbonyl reductase, on the other hand, reduces ketones, especially quinones, as well as aldehydes. Moreover, during catalysis aldose and aldehyde reductases catalyze the transfer of the *pro-4R* hydrogen atom of the nicotinamide ring of NADPH to the substrate [6], whereas carbonyl reductase is specific for the *pro-4S* hydrogen atom [21].

The differences between the three reductases are further substantiated by comparing their amino acid compositions (Table 3) [21,31]. The difference index according to Metzger et al. [32] is 8.3 for the aldose/aldehyde reductase pair, but about 12 when carbonyl reductase is compared with either aldose or aldehyde reductase. Metzger et al. have pointed out that a difference index below 10 suggests structural homology between two proteins. If the index is above 10, homology is less likely, but cannot be excluded.

Summarizing our present knowledge of the relationship between the three reductases we may conclude that at an earlier stage in the evolution carbonyl reductase has branched off from an ancestral aldose/aldehyde reductase, which in turn more recently has separated into aldose and aldehyde reductases. A more definite statement, however, has to await elucidation of the enzymes' primary and tertiary structures.

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B. Wermuth, H. Bürgisser, K. Bohren, and J.-P. von Wartburg, Medizinisch-Chemisches Institut der Universität Bern, Bühlstrasse 28, CH-3000 Bern 9, Switzerland