Immunochemical characterization of aldo-keto reductases from human tissues

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Aldose reductase, aldehyde reductase and carbonyl reductase constitute a family of monomeric NADPH-dependent oxidoreductases with similar physical and chemical properties. Characterization of the enzymes from human tissues by immunotitration and an enzyme immunoassay indicated that, despite their apparent likeness, the three reductases do not cross-react immunochemically.

Aldose reductase *Aldehyde reductase* *Carbonyl reductase* *Immunochemical properties* *(Human)*

1. INTRODUCTION

Aldose reductase together with aldehyde reductase and carbonyl reductase constitute the enzyme family of the aldo-keto reductases in human tissues [1]. Interest in this enzyme has been generated because in such hyperglycemic conditions as diabetes mellitus and galactosemia excess amounts of glucose and galactose are reduced to the corresponding polyols which, in turn, produce a hyperosmotic effect and subsequent swelling of the cell (review [2,3]). This process has been shown to initiate cataract formation in diabetic animals, and evidence is mounting that it may also be involved in the etiology of other diabetic and galactosemic complications [3].

Investigations of the distribution of aldose reductase in human tissues have been impeded by the overlapping substrate specificity and similar physicochemical properties of the other aldo-keto reductases, notably aldehyde reductase [4]. A solution to the problem was offered when we observed that antibodies to aldose reductase from human brain do not precipitate aldehyde reductase and carbonyl reductase [5]. Here we describe the further characterization of the antibodies by immunotitration and an enzyme immunoassay and show that by these criteria they do not cross-react with the other aldo-keto reductases.

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2. EXPERIMENTAL

2.1. *Materials*

Aldose reductase [5], aldehyde reductase [6] and carbonyl reductase [7] from human tissues were purified following previously described procedures. Protein A-peroxidase conjugate was bought from Dr Bommeli Laboratories (Bern, Switzerland). NADPH was obtained from Sigma (St. Louis, MO). Other chemicals were purchased from Fluka AG (Buchs, Switzerland) or from Merck (Darmstadt, FRG). Polystyrene Cooke microtiter plates were obtained from Dynatech (Kloten, Switzerland).

2.2. *Enzyme assay*

Aldo-keto reductase activity was measured spectrophotometrically by following the decrease in absorbance of NADPH at 340 nm. The reaction mixture consisted of 0.1 M sodium phosphate buffer (pH 7.0), 0.1 mM NADPH and the appropriate substrate, i.e. 100 mM D-xylose for aldose reductase, 10 mM sodium D-glucuronate for aldehyde reductase and 0.2 mM menadione for carbonyl

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reductase. The assays were routinely started by the addition of enzyme.

2.3. *Preparation of antibodies*

Rabbits were injected with an initial dose of 0.5 mg aldose reductase, aldehyde reductase and carbonyl reductase, respectively, emulsified in Freund's complete adjuvant, and 4 weeks thereafter with 2–3 weekly doses of 0.2 mg enzyme in adjuvant. 10 days after the last immunization the rabbits were killed by bleeding, and antibodies (immunoglobulins G) were isolated by ammonium sulfate precipitation and DEAE-Sephadex chromatography [8]. The antibodies to aldose reductase were further purified by immunoaffinity chromatography. Homogeneous aldose reductase (5 mg) was coupled to CNBr-activated Sepharose 4B (10 ml) following the procedure recommended by Pharmacia [9]. Binding of the antibodies to the affinity matrix was allowed to proceed over night at 4°C. The next day the Sepharose was thoroughly washed with 2 M NaCl before the antibodies were eluted with 0.1 M glycine/HCl (pH 2.8). The eluate was neutralized with solid Tris base and dialyzed against 0.1 M NaCl containing 15 mM sodium azide. The concentration of antibodies was estimated from the absorbance at 280 nm, using an extinction coefficient of 1.35 $l^{-1} \cdot g^{-1} \cdot cm$.

2.4. *Enzyme-linked immunosorbent assay*

The general procedure outlined by Engvall [10] for the detection of antibodies using enzyme-labelled protein A was applied. Experimental details are given in the legend to fig.2.

3. RESULTS AND DISCUSSION

The absence of precipitate formation, e.g. on Ouchterlony plates, between antibodies raised against a specific protein and 'foreign' proteins is often taken as a measure for the specificity of the antibodies. To our knowledge, this has so far been the only criterion to assess the specificity of antibodies to aldose reductase from human [5,11,12] and animal [13–15] tissues. It must be remembered, however, that the formation of precipitates is only a reflection of the primary antigen-antibody interaction and does not directly correspond to it, especially with antigens having few antigenic determinants. Katiyar and Porter [16], for instance, recently showed that polyclonal antibodies to glucose-6-phosphate dehydrogenase inhibit dihydrofolate reductase and fatty acid synthase without precipitating these enzymes.

To investigate whether aldose reductase and the other aldo-keto reductases exhibit immunochemical cross-reactions which do not give rise to precipitates, the antibodies to aldose, aldehyde and carbonyl reductase, respectively, were tested by immunotitration and an enzyme immunoassay. Fig.1, depicting the results from the immunotitration experiments, shows that all 3 antibody preparations selectively reacted with the reductase against which they had been raised and did not affect the other enzymes. Parallel to the decrease in enzymatic activity, the formation of a fine precipitate was detectable. Although markedly de-

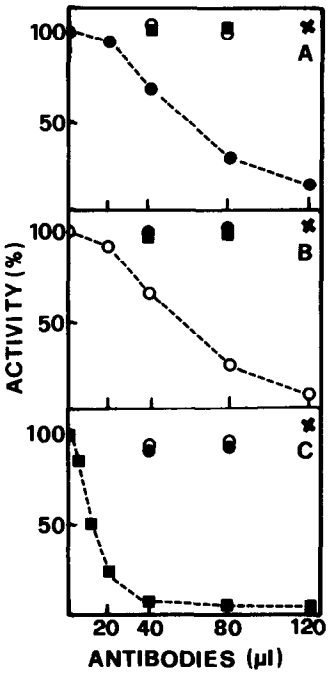

Fig.1. Immunotitration of aldo-keto reductases. Aliquots (20 µl) of purified (A) aldose reductase, (B) carbonyl reductase and (C) aldehyde reductase were incubated with the indicated amounts of antibodies to aldose reductase (●), carbonyl reductase (○), aldehyde reductase (■) and immunoglobulins from non-immunized rabbits (×) at 4°C for 24 h. Enzyme activity was assayed after centrifugation and is expressed as a percentage of the activity determined with a control in the absence of immunoglobulins.

decreased, the enzymatic activity of the precipitated enzymes was not abolished.

The same specificity of the 3 antibody preparations as in the immunotitration experiments was observed when the binding to immobilized antigen was tested by the enzyme immunoassay. Fig.2 shows that the antibodies against aldose reductase did not bind to immobilized carbonyl and aldehyde reductase and, vice versa, the antibodies of aldehyde and carbonyl reductase did not recognize aldose reductase.

The present results indicate that, despite their apparent enzymatic and physicochemical likeness, aldose, aldehyde and carbonyl reductase have no antigenic determinants in common. Further structural information, however, is needed to interpret this finding on a protein chemical and phylogenetic basis. Nevertheless, the absence of immunochemical cross-reactions between the 3 aldo-keto reductases allows one to determine aldose reductase by immunochemical methods, such as an enzyme immunoassay, without interference of the 2 other reductases.

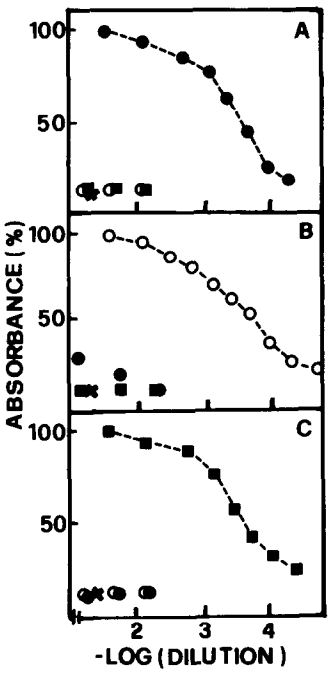

Fig.2. Demonstration of the specificity of antibodies to human aldo-keto reductases by an enzyme-linked immunosorbent assay. The wells of a microtiter plate were coated with (A) aldose reductase, (B) carbonyl reductase and (C) aldehyde reductase (all 2 μg/ml) and subsequently with bovine serum albumin. Thereafter progressively diluted antibodies to aldose reductase (●), carbonyl reductase (○), aldehyde reductase (■) and immunoglobulins from non-immunized rabbits (×) were added. The amount of antibodies bound by the immobilized antigens was determined using horseradish peroxidase linked to protein A and is expressed as a percentage of the maximum absorbance at 486 nm.

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