Identification of Lactaldehyde Reductase and Aldehyde Reductase as Functions of the Same Enzyme Protein in Pig Kidney

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Abstract : Lactaldehyde reductase activity of pig kidney extract was potently inhibited by aldehyde reductase inhibitors such as barbital and diphenic acid. The lactaldehyde reductase and aldehyde reductase activities from this tissue were co-purified to apparent homogeneity, and co-migrated on isoelectric focusing and Sephadex G-100 filtration. These two enzymatic activities in the crude extract were almost completely immunoprecipitated by an antibody against the purified reductase. The results indicate that lactaldehyde reductase in pig kidney is identical to aldehyde reductase.

Keyphrases : lactaldehyde reductase, aldehyde reductase, aldose reductase, pig kidney

Mammalian tissues contain a NADPH-dependent enzyme which catalyzes the reduction of lactaldehyde to 1,2-propanediol.[1] The enzyme partially purified from pig kidney shows specificity for D-lactaldehyde,[2] and is classified as lactaldehyde reductase (EC 1.1.1.55). However, the enzymes from bovine placenta and seminal vesicles catalyze the reduction of several aldoses and have been suggested to function as aldose reductase (EC 1.1.1.21).[3] In addition, aldehyde reductases (EC 1.1.1.2) from rat liver,[4] kidney[5] and brain[6] of pigs have been reported to reduce D- or DL-lactaldehyde.

It may be questioned whether lactaldehyde reductase and aldehyde reductase or aldose reductase activities in animal tissues correspond to different enzymes or are functions of the same enzyme. In this communication, we reexamined lactaldehyde reductase activity in pig kidney, in which the enzyme was first identified.[1] The results indicate that the major active species of lactaldehyde reductase activity in this tissue is associated with aldehyde reductase.

Experimental

Materials Pig kidneys and skeletal muscle were obtained from a slaughterhouse. NADPH and pI markers were obtained from Oriental Yeast Co. (Tokyo, Japan); molecular weight markers, Sephadex

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G-100 and Q-Sepharose from Pharmacia Fine Chemicals; Matrex Red-A and ultrafiltration membranes from Amicon Co.; and HA-Ultrogel and Ampholine from LKB Products. D- and L-Lactaldehydes were synthesized by reacting L- and D-threonines, respectively, with ninhydrin according to the method of Zagalak *et al.*[7] The concentrations of lactaldehydes were determined by their bisulfite binding capacity.[8]

Enzyme Assay The activities of lactaldehyde reductase and aldehyde reductase were determined spectrophotometrically by measuring the oxidation rate of NADPH at 340 nm. The reaction mixture for lactaldehyde reductase assay consisted of 100 mM potassium phosphate, pH 7.4, 5 mM D-lactaldehyde, 0.1 mM NADPH and enzyme, in a total volume of 2.0 ml. For assay of aldehyde reductase, 100 mM potassium phosphate, pH 6.0, and 13 mM D-glucuronate were used as the buffer and substrate, respectively. One unit of enzyme activity was defined as the amount of enzyme catalyzing the oxidation of 1 μmol NADPH per min at 25°C.

Protein concentration was determined by the method of Lowry *et al.*[9] with bovine serum albumin as the standard.

Enzyme Purification Lactaldehyde reductase and aldehyde reductase were purified in five steps consisting of 1) ammonium sulfate precipitation followed by 2) gel filtration through Sephadex G-100, 3) chromatography on Q-Sepharose, 4) affinity chromatography on Matrex Red-A, and 5) chromatography on HA-Ultrogel. The steps reported below were carried out at 2 to 4°C.

Step 1——Pig kidney (120 g) was minced and homgenized with 200 ml of 50 mM Tris-HCl, pH 8.0, containing 5 mM EDTA, 5 mM 2-mercaptoethanol and 0.14 M KCl in a Waring blender. After centrifugation at 105,000×g for 1 h, the supernatant was fractionated by the addition of solid ammonium sulfate. The 35 to 70%-saturated ammonium sulfate precipitate was collected by centrifugation at 12,000×g for 15 min and dissolved in 30 ml of 10 mM Tris-HCl, pH 7.5, containing 0.5 mM EDTA and 5 mM 2-mercaptoethanol (buffer A).

Step 2——The solution of step 1 was dialyzed for 5 h against buffer A and passed through a 5×120 cm Sephadex G-100 column in buffer A plus 0.15 M KCl. The enzyme active fractions were concentrated in an Amicon ultrafiltration cell using a YM-10 membrane.

Step 3——The enzyme solution obtained in step 2 was dialyzed overnight against buffer A and applied to a 2.5×30 cm Q-Sepharose column which had equilibrated with buffer A. After washing with 3 bed volumes of buffer A, the enzymes were eluted with a 500-ml linear gradient of 0.0 to 0.1 M NaCl in the same buffer. The fractions with high enzyme activity were pooled.

Step 4——The enzyme solution obtained in step 3 was applied to a 1.2×10 cm Matrex Red-A column equilibrated with buffer A. The column was washed with 150 ml of this buffer containing 0.1 M NaCl, and the enzyme was eluted with the buffer containing 0.5 M NaCl. The fractions containing activity were concentrated as indicated in step 2, and dialyzed against buffer A without EDTA.

Step 5——The enzyme solution obtained in step 4 was added to the top of a 1.2×10 cm HA-Ultrogel column equilibrated with buffer A without EDTA. The enzyme was eluted out during washing

of the column with this buffer. The fractions containing high activity were combined. Pig muscle aldose reductase was purified to homogeneity by the method of Cromlish and Flynn.[10]

Gel Electrophoresis Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis[11] on a 12.5% slab gel or isoelectric focusing on a 7.5% polyacrylamide disc gel[12] was performed as described in the references cited. Lactaldehyde reductase and aldehyde reductase activities in the gels were stained with 5 mM D-lactaldehyde and 10 mM D-glucuronate as the respective substrates as described previously,[13] and protein in the gel was stained with Commassie Brilliant Blue R-250.

Molecular Weight Determination The relative molecular mass (M_r) of the native enzyme was estimated by gel filtration on a Sephadex G-100 column in buffer A plus 0.15 M KCl, and that of the denatured enzyme by SDS-polyacrylamide gel electrophoresis standarized by the use of M_r markers.

Immunochemical Experiments Antibody against the purified enzyme was raised in female rabbits as described.[14] The immunoglobulins from the antiserum were prepared by ammonium sulfate fractionation. Control IgG was prepared from a nonimmune rabbit serum. The enzyme solution obtained in step 2 was diluted to 0.3 unit/ml as aldehyde reductase activity with 20 mM potassium phosphate, pH 7.2, containing 0.15 M NaCl, and then subjected to immunoprecipitation using the immunoglobulin as described by Sawada *et al.*[14]

Results and Discussion

Using D-lactaldehyde and D-glucuronate as substrates, we have examined the reductase activities in the 105,000×g supernatant. It was found that both the D-lactaldehyde and D-glucuronate reductase activities were inhibited more than 90% by 5 mM barbital and 1 mM diphenic acid which are described as potent aldehyde reductase inhibitors,[4-6,15] and were not affected by 5 mM pyrazole. The results indicate that the predominant lactaldehyde reductase activity in pig kidney may be identical or related to aldehyde reductase. To explore this possibility, purification of lactaldehyde reductase by a scheme based on the method described for the purification of aldehyde reductase from pig kidney[16] was carried out. The D-lactaldehyde and D-glucuronate reductase activities were found to co-migrate on the four column chromatographies employed. Although two very minor peaks of the two reductase activities were separated from a predominant activity peak at the step of Q-Sepharose chromatography (Fig. 1), only the predominant enzyme species was purified because the minor peaks had less than 4% of the total D-lactaldehyde reductase activity recovered.

The purification procedures are summarized in Table 1. The data indicated that the activity ratios between D-lactaldehyde and D-glucuronate reductases were essentially constant at all purification steps and the purification folds and recoveries of these two reductase activities were almost the same. The specific activity of the final enzyme preparation is much higher than the value of the lactaldehyde reductase partially purified from pig kidney,[1,2] and is comparable to those of homogeneous aldehyde reductases from pig tissues.[5,15-17]

Table 1. Co-Purification of Lactaldehyde and Aldehyde Reductase Activities from Pig Kidney

Step	Total Protein (mg)	Lactaldehyde reductase			Aldehyde reductase			L/A[a)]
		Activity (units/mg)	Recovery (%)	Purification	Activity (units/mg)	Recovery (%)	Purification	
Crude extract	5170	0.085	100	1	0.068	100	1	0.80
Ammonium sulfate fraction	4680	0.085	91	1	0.068	91	1	0.80
Sephadex G-100	232	1.39	74	16	1.19	78	17	0.85
Q-Sepharose	16.7	16.5	63	194	13.5	64	199	0.84
Matrex Red-A	12.1	20.7	57	244	17.0	59	250	0.82
HA-Ultrogel	11.3	21.9	55	258	17.1	55	251	0.78

a) Activity ratio between lactaldehyde (L) and aldehyde reductases (A).

The enzyme preparation showed single coincident bands of protein stain and enzyme activities with D-lactaldehyde and D-glucuronate as substrates at pH 5.8 on polyacrylamide gel focusing. SDS-polyacrylamide gel electrophoresis of the enzyme preparation also resulted in a single protein band corresponding to M_r=38,000. Gel filtration of the enzyme preparation on Sephadex G-100 yielded a single protein peak corresponding to M_r=35,000, which contained both D-lactaldehyde and D-glucuronate reductase activities. It has been reported that pig kidney aldehyde reductase is a monomeric protein with a M_r of 38,000 to 40,200 and a pI value of 5.7.[5,16,17] Thus, the present results indicate that lactaldehyde reductase purified from pig kidney is indeed identical to aldehyde reductase.

Both D-lactaldehyde and D-glucuronate reductase activities of the present enzyme preparation showed almost identical pH dependencies which were optimum at pH 6.3 to 7.0. The pH optimum of the present enzyme is slightly more acidic than that reported for pig kidney lactaldehyde reductase,[1,2] but is similar to those of aldehyde reductases from pig tissues.[5,6,15-17] The present enzyme reduced aromatic aldehydes such as pyridine-4-aldehyde and 4-nitrobenzaldehyde, aliphatic aldehydes such as DL-glyceraldehyde and butyraldehyde, and α-diketones such as 2,3-butanedione and camphoquinone at rates similar to those for D-lactaldehyde and D-glucuronate, but was inactive towards D-glucose and D-galactose. The substrate specificity of the enzyme is similar to those of aldehyde reductases from animal tissues[4-6,15-17] but not to that of aldose reductase.[10,18] The present enzyme also reduced L-lactaldehyde and exhibited an apparent K_m value of 20 mM and V_{max} value of 30 units/mg, whereas the K_m and V_{max} values for D-lactaldehyde were 4.5 mM and 35 units/mg, respectively. The V_{max}/K_m value for D-lactaldehyde was about 5-fold higher than that for L-lactaldehyde, which indicates the enzyme's preference of D-form to L-form as the substrate. The stereochemical specificity of this enzyme is in agreement with that previously observed with pig kidney lactaldehyde reductase.[2] Since the physiological role of lactaldehyde reductase has not been identified, the reduction of lactaldehyde may be incidental to the broad substrate specificity of aldehyde reductase.

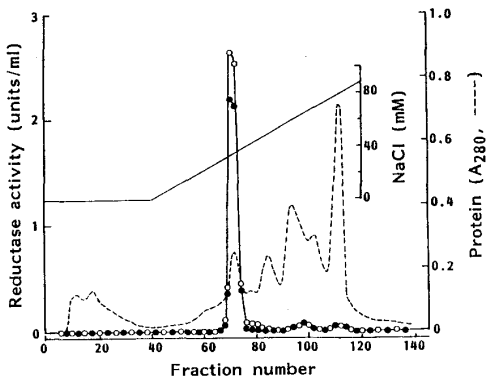

Fig. 1. Chromatography of Q-Sepharose of Kidney Lactaldehyde Reductase and Aldehyde Reductase

The reductase activities with D-lactaldehyde (●) and D-glucuronate(○) as substrates were co-eluted, but were resolved into three peaks during eluting with a linear 0 to 0.1 M NaCl gradient (——). The main peak (fractions 79-84) of activity was further purified.

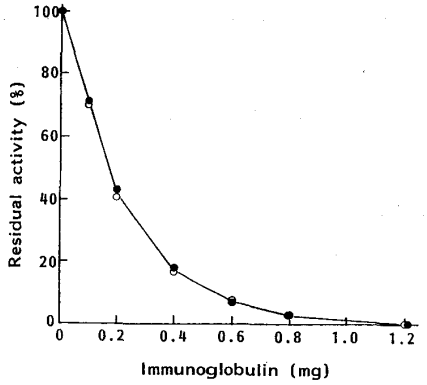

Fig. 2. Immunoprecipitation of Lactaldehyde and Aldehyde Reductase Activities by Anti-(Aldehyde Reductase) IgG

The ammonium sulfate fraction of pig kidney was incubated with the IgG at 4°C overnight. The D-lactaldehyde (●) and D-glucuronate (○) reductase activities in the supernatant after centrifugation of the mixture was determined.

Lactaldehyde reductases purified from bovine placenta and seminal vesicles have been suggested to be identical to aldose reductase.[3] We confirmed that the homogeneous aldose reductase from pig muscle reacted D-lactaldehyde at about twice the reduction rate for L-form. Aldose reductase, although its amount is much smaller than that of aldehyde reductase, has been detected in pig kidney.[10] The minor active species of D-lactaldehyde reductase detected on the Q-Sepharose chromatography might be aldose reductase or a minor form of aldehyde reductase which is formed by proteolytic cleavage of the native enzyme during the purification of pig kidney aldehyde reductase.[17] When an immunoprecipitation by the immunoglobulin against the purified aldehyde reductase was carried out against the ammonium sulfate fraction of pig kidney extract, both lactaldehyde reductase and aldehyde reductase activities were almost completely immunoprecipitated by the IgG (Fig. 2). This further supports the conclusion that aldehyde reductase is the major active species being capable of reducing lactaldehyde in this tissue.

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