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ISOLATION OF CRYSTALLINE PIG KIDNEY ALDOLASE B

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SUMMARY

A procedure is described for the isolation of crystalline aldolase (fructose-1,6-diphosphate D-glyceraldehyde-3-phosphate-lyase, EC 4.1.2.13) from pig kidney cortex. A study of some of those properties which permit distinction between aldolases A, B and C, shows that the purified enzyme can be considered as a type B aldolase.

The existence of three distinct parental aldolases (fructose-1,6-diphosphate D-glyceraldehyde-3-phosphate-lyase, EC 4.1.2.13; ketose-1-phosphate aldehyde-lyase, EC 4.1.2.7) in vertebrate tissues has been clearly established[1]. Suitable methods for the isolation of these enzymes from skeletal muscle (aldolase A), liver (aldolase B) and brain (aldolase C) have been published (for references see ref. 2). Since aldolase B is also present in kidney tissue[1], we decided to isolate the pig kidney cortex enzyme, which has been crystallized by the following method. All operations were performed at 4° unless otherwise stated.

Two frozen pig kidneys were allowed to thaw at room temperature and were freed from gross fat, connective tissue, and medulla. The kidney cortex was then minced, homogenized for 2 min in a Waring Blendor with 2 vol. of cold 1.0 mM EDTA (pH 8.0)–10 mM β-mercaptoethanol, and centrifuged for 30 min at 37 000 $\times$ g. The supernatant was filtered through cotton wool and then it was adjusted to pH 4.0 with 1.7 M acetic acid. The solution was stirred for 5 min and then centrifuged for 20 min at 5000 $\times$ g. The supernatant was then adjusted to pH 7.5 with 2 M Tris, and the precipitate formed was discarded by centrifuging for 20 min at 5000 $\times$ g. The supernatant was fractionated with solid ammonium sulfate (required amounts of salt were calculated by the method of NOLTMANN *et al.*[3]), and the precipitate obtained between 0.52 and 0.65 saturation was collected, redissolved with 4–6 ml of 50 mM Tris–HCl (pH 8.0) containing 10 mM β-mercaptoethanol and 1 mM EDTA, and dialyzed against two changes of 1000 ml of the same buffer. The dialyzed solution was applied at room temperature to a P-cellulose column (1.8 cm $\times$ 25 cm) equilibrated with 50 mM Tris–HCl (pH 8.0) containing 1 mM EDTA, and the column

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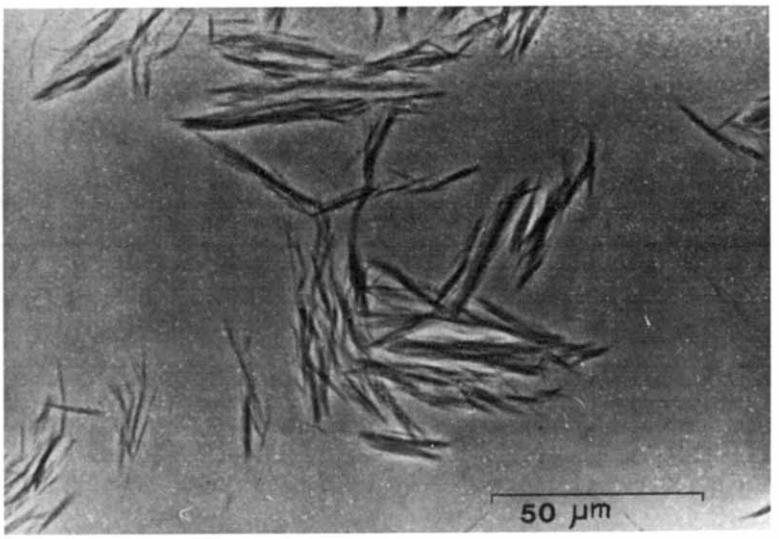

Fig. 1. Crystals of pig kidney cortex aldolase B. The magnification is indicated by the 50-μm line drawn on the photograph.

was washed with the same buffer until the effluent was free of protein (measured by the absorbance at 280 nm). Aldolase was then eluted with the same buffer, containing 0.1 M NaCl, and precipitated with ammonium sulfate to 0.90 saturation. After 1 h equilibration, the precipitate was collected, redissolved with 2 ml of 50 mM Tris–HCl (pH 8.0) containing 10 mM β-mercaptoethanol and 1 mM EDTA, and dialyzed against three changes of 1000 ml of the same buffer. The dialyzed solution was precipitated at 0° by the addition of solid ammonium sulfate to 0.65 saturation. The precipitate was then sequentially extracted with 2-ml aliquots of solution which were 0.57, 0.51, 0.47, 0.42, 0.37 saturation in ammonium sulfate (prepared in 50 mM Tris–

TABLE I

SUMMARY OF THE PURIFICATION PROCEDURE*

Aldolase activity was assayed by the method of RAJKUMAR *et al.*[5] except that 33 mM Tris–HCl buffer (pH 8.0) was used instead of glycylglycine buffer, and 3.3 mM β-mercaptoethanol was added to the reaction mixture. A unit of aldolase activity is defined as the cleavage of 1 μmole of Fru-1,6-P_2 per min. Protein concentrations were determined spectrophotometrically at 280 nm using an extinction coefficient of 0.89 (ref. 2) except for the crude fractions (Fractions 1–3), whose protein content was estimated by the biuret method[6]. Specific activity is expressed in terms of units/mg of protein.

Fraction	*Vol. (ml)*	*Total units*	*Protein (mg/ml)*	*Specific activity*	*Yield (%)*
1. Extract	590	342	26.3	0.02	100
2. Acid supernatant	560	325	11.5	0.05	94
3. Ammonium sulfate fraction	23	177	42.5	0.18	51
4. P-cellulose eluate	5.6	81	14.5	1.00	23
5. Crystals	2.6	37	9.7	1.46	11

* Initially, 268 g of pig kidney cortex.

HCl, pH 8, 10 mM β-mercaptoethanol and 1 mM EDTA)[4]. Following suspension, the mixtures were centrifuged at 4°. The supernatants were poured into tubes which were placed in a 250-ml bath at 0° and then were allowed to warm slowly to room temperature over a period of 6 h. Crystals appeared in the tube containing the supernatant of 0.42 saturation in ammonium sulfate after 1–2 days at room temperature. To complete crystallization the suspension was kept at room temperature for 6 days. The needle type crystals (Fig. 1) were then collected by centrifugation, washed twice with a solution of 0.45 saturation ammonium sulfate in 50 mM Tris–HCl (pH 8) containing 10 mM β-mercaptoethanol and 1 mM EDTA, and finally suspended in the same solution.

A summary of the data obtained for a typical preparation is given in Table I. Kidney aldolase isolated as described above showed a single cathodic band of protein, as well as of aldolase activity, when subjected to cellulose acetate electrophoresis on the Millipore PhoroSlide system, under conditions similar to those previously described[7]. By using the gel filtration technique on Sephadex G-200 (see ref. 8), the molecular weight of pig kidney aldolase was found to be similar to that of crystalline rabbit skeletal muscle aldolase (Sigma), which was used as a standard (molecular weight of 158 000, see ref. 9).

A study of some of those properties which permit distinction between aldolases A, B and C (Table II), shows that the enzyme isolated by the procedure described herein can be clasified as a type B aldolase.

TABLE II

COMPARATIVE PROPERTIES OF PURIFIED MAMMALIAN ALDOLASES

Unless otherwise indicated, the data for muscle, liver and brain aldolases are those reported by PENHOET *et al.*[10]. The same experimental conditions were used for studying the properties of crystalline pig kidney aldolase.

	Muscle	*Liver*	*Brain*	*Kidney*
v_{max} (Fru-1,6-P_2 cleavage)*	2900	250	1000	240
K_m (M) Fru-1,6-P_2	$3 \cdot 10^{-6}$	$1 \cdot 10^{-6}$	$3 \cdot 10^{-6}$	$1 \cdot 10^{-5}$
K_m (M) Fru-1-P	$1 \cdot 10^{-2}$	$3 \cdot 10^{-4}$**	$4 \cdot 10^{-3}$	$1.1 \cdot 10^{-3}$
Fru-1,6-P_2/Fru-1-P activity ratio	50	1	10	1
% Residual Fru-1,6-P_2 activity after carboxypeptidase treatment	3	46	7	100
% Residual activity after glyceraldehyde-3-P treatment	15***	100†		100

* Moles of Fru-1,6-P_2 cleaved per mole of enzyme per min at 25°.

** Values ranging from this value to $1.5 \cdot 10^{-3}$ M have been reported[11,12].

*** Data taken from BROX *et al.*[13].

† Data taken from LAI *et al.*[14] (Table I, Expt. 2). The same experimental conditions were used for the kidney enzyme.

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