CRYSTALLIZATION AND PROPERTIES OF DIAMINE OXIDASE FROM PIG KIDNEY

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The purification of diamine oxidase from pig kidney has been reported by several workers (Stephenson, 1943; Laskowsky, 1945; Tabor, 1951; Swedin, 1958; Uspenskaia et al., 1958; Mondovi et al., 1964). Apparently homogeneous preparations have been obtained by Mondovi et al. (1967) and by Goryachenkova et al. (1967). The present communication describes a method for obtaining a crystalline preparation of the diamine oxidase from pig kidney, and some properties of the crystalline enzyme.

Purification and Crystallization

All operations were carried out at 5°. Step I. Fresh pig kidney cortex (1.1 kg) was homogenized in 1.100 ml of 0.03 M phosphate buffer, pH 7.0, for 2 minutes in a Waring blendor and centrifuged. Step II. The supernatant solution was fractionated with ammonium sulfate (30-60% saturation), followed by dialysis against 0.03 M phosphate buffer, pH 7.0. Step III. The dialyzate was applied to a DEAE-sephadex A-50 column (6 x 60 cm) equilibrated with 0.03 M phosphate buffer, pH 7.0. After the column was washed with 0.03 M phosphate buffer, pH 7.0, the enzyme was eluted stepwise with 0.07 M and 0.1 M phosphate buffers, pH 7.0. The active fractions were combined and concentrated by the addition of ammonium sulfate (60% saturation).

The precipitate was collected and dialyzed against 0.1 M phosphate buffer, pH 7.0. Step IV. The dialyzate was fractionated with ammonium sulfate (35-55% saturation), followed by dialysis against 0.1 M phosphate buffer, pH 7.0. Step V. The dialyzate was applied to a hydroxylapatite column (5 x 15 cm) equilibrated with 0.1 M phosphate buffer, pH 7.0. After the column was washed with 0.1 M phosphate buffer, pH 7.0, containing 0.2 M ammonium sulfate, the enzyme was eluted with 0.1 M phosphate buffer, pH 7.0, containing 0.5 M ammonium sulfate. The active fractions containing enzyme of specific activity greater than 0.200 were combined and concentrated by the addition of ammonium sulfate (60% saturation). Step VI. The precipitate was dissolved in 0.1 M phosphate buffer, pH 7.0 and passed

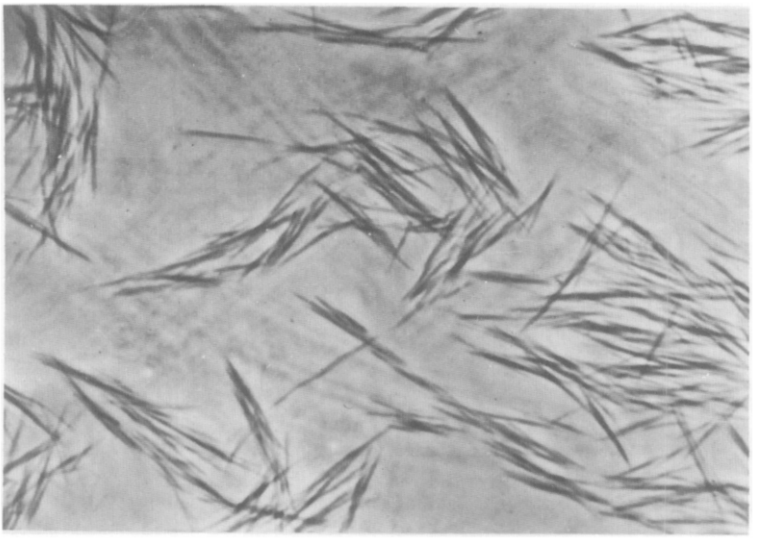

Fig. 1. Crystalline diamine oxidase from pig kidney (x 600).

through a sephadex G-150 column (2 x 100 cm) equilibrated with 0.1 M phosphate buffer, pH 7.0. The active fractions containing enzyme of specific

Table I. Purification of Diamine Oxidase from Pig Kidney.

The enzyme activity was determined by measuring the formation of pyrroline, an oxidation product of putrescine. The assay was carried out colorimetrically as described by Yamada et al. (1965), except that it was carried out at pH 7.4 rather than pH 8.0. A unit was defined as the amount of enzyme which catalyzed the formation of 1 µmole of pyrroline per minute under the standard assay conditions. The protein concentration was determined spectrophotometrically by measuring the absorbancy at 280 mµ. An E value of 1.63 for 1 mg per ml and for 1 cm light path was used throughout which was obtained by absorbancy and dry weight determinations.

Step	Fraction	Total protein (mg)	Total units	Specific activity
I	Homogenate	113,000	89.2	0.0008
II	Ammonium sulfate (I)	34,900	73.3	0.0021
III	DEAE-sephadex	5,700	59.5	0.0104
IV	Ammonium sulfate (II)	3,350	49.0	0.0146
V	Hydroxylapatite	138	42.3	0.372
VI	Sephadex G-150	46.1	31.1	0.673
VII	Crystals (I)	22.4	23.3	1.042
	Crystals (II)	17.7	19.1	1.079

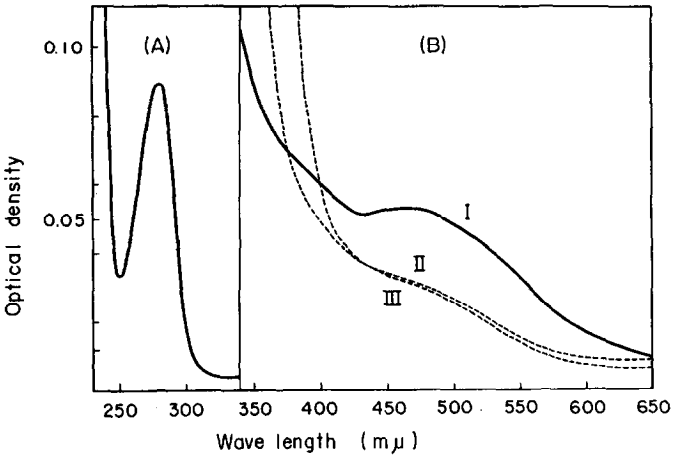

Fig. 2. Absorption spectra of diamine oxidase from pig kidney. The crystalline enzyme with a specific activity of 1.042 was used at concentrations of 0.34 mg/ml (A) and 2.76 mg/ml (B) in 0.1 M potassium phosphate buffer, pH 7.4. Curve I represents the native enzyme; curves II and III represent the reduced enzyme after the addition of 10 µmoles of putrescine and sodium dithionite, respectively, to 3.0 ml of the enzyme solution.

activity greater than 0.500 were combined and concentrated by the addition of ammonium sulfate (60% saturation). The precipitate was collected and dissolved in 0.1 M phosphate buffer, pH 7.0. Step VII. Finely powdered ammonium sulfate was cautiously added to the enzyme solution until it became slightly turbid, and the mixture was placed in an ice bath. Crystallization began after about 3 hours and was virtually completed within a week. Crystals appeared as fine, highly refractive needles with a faint pink color (Fig. 1). Recrystallization was carried out by repeating the last step. A summary of typical purification procedures is shown in Table I.

Properties

The crystalline enzyme preparation migrated as a single band upon Tiselius electrophoresis carried out at 4° in sodium phosphate-sodium chloride buffer (Miller et al., 1950) of 0.1 ionic strength and pH of 7.4. The enzyme preparation sedimented as a single symmetric peak in the ultracentrifuge in 0.1 M potassium phosphate buffer, pH 7.4, and at 16°. Extrapolation of the data obtained from four ultracentrifuge runs to zero protein concentration gave an $S^0_{20,w}$ of 9.90×10^{-13} (cm/sec); the sedimentation constant decreased by 0.03×10^{-13} (cm/sec) per mg with increasing protein concentration. A diffusion constant, $D_{20,w}$ of 5.14×10^{-7} (cm^2/sec) was determined for a 6.18 mg/ml solution of protein. A value of 185,000 was calculated for the molecular weight of the enzyme, assuming a partial specific volume of 0.75.

Spectrophotometric investigation of the crystalline enzyme preparation revealed that the enzyme was pink in color and that the pink color was associated with the absorption maximum at 470 mμ. The pink color was discharged by putrescine as well as by sodium dithionite (Fig. 2), and restored by bubbling oxygen through the solution. Analysis for metals in the crystalline enzyme by both atomic absorption spectrophotometry and chemical analysis revealed that copper was the only such component and that its content was

11.7 mμatoms per mg of the enzyme. This value corresponds to 2.17 g-atoms of copper per mole of the enzyme.

The crystalline enzyme preparation oxidized cadaverine, putrescine, 1,6-diaminohexane, histamine, agamatine and 1,3-diaminopropane, at relative rates of 100, 97, 61, 59, 40 and 17, respectively. The enzyme activity was determined manometrically by measuring the uptake of oxygen at 38°, in a reaction mixture containing 100 μg of the enzyme, 50 μg of catalase, 10 μ-moles of substrate and 100 μmoles of potassium phosphate buffer, pH 7.4, in a total volume of 3 ml. Under these conditions, the crystalline enzyme with specific activity of 1.079 oxidized 2.04 μmoles of cadaverine per minute per mg of the enzyme.

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