CRYSTALLINE TYRAMINE OXIDASE FROM SARCINA LUTEA

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The oxidation of tyramine by cells of various bacteria has already been reported (Gale, 1942; Pershin, et al., 1963; Maki, et al., 1964). It has been assumed that tyramine is oxidized to p-hydroxyphenylacetaldehyde by a monoamine oxidase. However, the properties of bacterial monoamine oxidase remain unknown. In the course of investigations on the amine oxidases of microorganisms we obtained from cells of Sarcina lutea, a crystalline protein which catalyzed the oxidation of tyramine and dopamine in a manner reminicent of the monoamine oxidase of mitochondria in animal organs. We wish to report purification and crystallization of the tyramine oxidase from Sarcina lutea and some of its properties.

Purification and Crystallization.

Sarcina lutea (IAM 1099) was grown in 200 liters of a medium containing 1.0% of peptone, 1.0% of meat extract, 0.5% of NaCl and 0.5% of yeast extract, with shaking, for 24 hours at 30°. The cells were harvested, washed and suspended in 0.01 M phosphate buffer, pH 7.0. All the subsequent procedures were performed at 0-5°. Step I. The cell suspension was subjected to ultrasonic oscillation (20 kc, 1 hour) and centrifuged. Step II. The supernatant solution was fractionated with ammonium sulfate

(0-80% saturation), followed by dialysis against 0.01 M phosphate buffer, pH 7.0. Step III. The dialyzate was fractionated with ammonium sulfate (40-60% saturation), followed by dialysis against 0.01 M phosphate buffer, pH 7.0. Step IV. One tenth volume of 6% protamine sulfate solution at pH 8.0 was added to the dialyzate and the precipitate formed was centrifuged off. Step V. The supernatant solution was applied on a DEAE-cellulose column (6 x 53 cm) equilibrated with 0.01 M phosphate buffer, pH 7.0. After the column was washed with 2 liters of 0.1 M phosphate buffer, pH 7.0, the enzyme was eluted stepwise with 0.1 M phosphate buffer, pH 7.0, containing 0.05 and 0.15 M NaCl. The active fractions were combined and concentrated by the addition of ammonium sulfate (70% saturation). The precipitate was collected and dialyzed against 0.01 M phosphate buffer, pH 7.0. Step VI. The dialyzate was fractionated with ammonium sulfate (40-55% saturation), followed by dialysis against 0.003 M phosphate buffer, pH 7.0. Step VII. The dialyzate was applied to a hydroxylapatite column (5 x 15 cm) equilibrated with 0.003 M phosphate buffer, pH 7.0. The enzyme was eluted stepwise with 0.003 M and 0.03 M phosphate buffers, pH 7.0. The active fractions containing enzyme of specific activity greater than 200 were combined and concentrated by the addition of ammonium sulfate (70% saturation). Step VIII. The precipitate was dissolved in 0.01 M phosphate buffer, pH 7.0 and passed through a sephadex G-150 column (2 x 100 cm) equilibrated with 0.01 M phosphate buffer, pH 7.0. The active fractions containing enzyme of specific activity greater than 300 were combined and concentrated by the addition of ammonium sulfate (70% saturation). The precipitate was collected and dialyzed against 0.01 M phosphate buffer, pH 7.0, containing 0.001 M dithiothreitol. Step IX. The dialyzate was fractionated with ammonium sulfate (35-40% saturation) and the precipitate was dissolved in 0.01 M phosphate buffer, pH 7.0, containing 0.001 M dithiothreitol. Step X. Finely powdered ammonium sulfate was cautiously added to the enzyme so-

Table I. Purification of Tyramine Oxidase from Sarcina lutea

The enzyme assays were based on the initial rates of oxygen uptake by reaction mixtures containing enzyme, 15 µg of catalase, 5 µmoles of tyramine and 30 µmoles of phosphate buffer, pH 7.0, in a total volume of 3 ml. The oxygen uptake was measured in air, by a conventional Warburg apparatus at 30°. A unit of enzyme activity was defined as the amount of enzyme which gave an oxygen uptake of 1 µl per 30 minutes (caluculated from the initial rate). Specific activity was expressed in units per mg of protein. The protein concentrations were determined by the spectrophotometric method of Kalckar (1947).

Step	Fraction	Total protein (mg)	Total units	Specific activity
I	Cell extract	330,000	160,000	0.48
II	Ammonium sulfate	248,000	151,000	0.61
III	Ammonium sulfate	48,000	124,000	2.60
IV	Protamine sulfate	16,700	114,000	6.80
V	DEAE-cellulose	2,030	81,200	40.0
VI	Ammonium sulfate	830	71,100	84.0
VII	Hydroxylapatite	160	51,100	320
VIII	Sephadex G-150	67	40,300	600
IX	Ammonium sulfate	10	27,400	2,740
X	Crystallization	5	19,650	3,930
	Recrystallization	3	12,450	4,150

lution until it became slightly turbid, and the mixture was placed in an ice bath. Crystallization began after about 6 hours and was virtually completed within a week. Crystals appeared as minute, highly refractive needles with a yellow color. Recrystallization was carried out by repeating the last step. A summary of typical purification procedures is presented in Table I.

Properties.

The crystalline enzyme preparation was found to be homogeneous upon ultracentrifugation and disc electrophoresis. The sedimentation constant ($S_{20,w}$) of the enzyme was caluculated to be 6.18×10^{-13} (cm/sec) assuming a partial specific volume of 0.75.

Spectrophotometric investigation on the crystalline enzyme preparation revealed that the visible spectrum of the enzyme was very similar to that of free FAD except that the absorption peaks were shifted slightly to 368 and 466 mμ ($E_{280\ m\mu}/E_{466\ m\mu}$ = 16.6). Addition of tyramine to the enzyme under anaerobic conditions produced a bleaching of the visible color (Fig. 1). Treatment of the crystalline enzyme preparation with heat resulted in liberation of a yellow compound which displayed the same R_F values as those of FAD by paper chromatography (Sutton, 1955). Assuming the extinction coefficient of the enzyme-bound FAD at 466 mμ as for free FAD at 450 mμ, the FAD content of enzyme was caluculated to be 7.9 mμmoles per mg of enzyme. Analysis for metals in the crystalline enzyme preparation by atomic absorption spectrophotometry revealed that copper was the only such component and that its content was approximately 22.0 mμatoms per mg of enzyme.

The oxidation of tyramine by tyramine oxidase proceeded in accordance with the following equation: Tyramine + O_2 + $H_2O \rightarrow$ p-hydroxyphenylacetaldehyde + NH_3 + H_2O_2. Ammonia and hydrogen peroxide were formed in stoichiometric amounts. Dopamine was also oxidized at almost the same

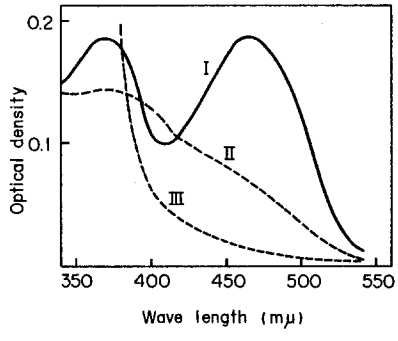

Fig. 1. Absorption spectra of tyramine oxidase of Sarcina lutea in 0.01 M phosphate buffer, pH 7.0. In each experiment, 1 ml of the enzyme solution containing 2 mg of protein was used. I represents the native enzyme, II the reduced enzyme after the addition of 1 μmoles of tyramine and III the reduced enzyme after the addition of 10 μmoles of sodium dithionite.

rate with tyramine. However, other amines and amino acids were not oxidized at all. Optimal enzyme activity was observed at pH 7.0 with tyramine and dopamine as substrates.

The enzyme was inhibited by phenylhydrazine but not by other carbonyl reagents such as hydroxylamine, hydrazine, semicarbazide and isoniazid. The enzyme was inhibited by pCMB and iproniazid. The inhibition by iproniazid was not immediate but required preincubation of the enzyme with the inhibitor. The enzyme was inhibited by metal chelating reagents such as 8-hydroxyquinoline, 2,2'-bipyridyl, cuprizone and o-phenanthroline. A similar specificity towards inhibitors has been reported with the monoamine oxidase of mitochondria in animal organs (Gorkin and Romanova, 1959; Barbato and Abood, 1963; Nara, et al., 1966a).

The results presented in this paper suggest that copper and FAD are prosthetic groups of tyramine oxidase from Sarcina lutea and that these are involved in the catalytic activity. Some amine oxidases of bacterial origin have been purified and it was demonstrated that these require FAD (Campello, et al., 1965; Yamada, et al., 1965; Adachi, et al., 1966). Moreover, the monoamine oxidase of mitochondria of beef liver has been reported to be a copper-flavoprotein (Nara, et al., 1966b). FAD has been suggested as a direct hydrogen acceptor in such amine oxidases. However, detailed mechanisms of the enzymatic oxidation of amines remain to be elucidated.

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