RESOLUTION OF SPERMIDINE DEHYDROGENASE FROM SERRATIA MARCESCENS: REQUIREMENTS FOR FLAVIN ADENINE DINUCLEOTIDE AND AN ADDITIONAL ELECTRON CARRIER

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Recent work from this laboratory described a partially purified enzyme from Serratia marcescens that carries out the oxidation of spermidine according to the following formulation (Bachrach, 1962):

Spermidine + O_2 $\longrightarrow$ 1,3-diaminopropane + Δ^1-pyrroline + H_2O_2.

We have now found that this reaction requires flavin adenine dinucleotide (FAD) and an additional electron carrier. This enzyme differs strikingly from the known monoamine and diamine oxidase preparations (Zeller, 1963; Blaschko, 1963), since these do not require any added electron carriers to react with molecular oxygen.

Figure 1 demonstrates the requirement of a 10-fold purified enzyme preparation[1] for an electron carrier. The effect of phenazine methosulfate concentration on the enzyme activity is given in Figure 1A. The activity of the enzyme was followed spectrophotometrically at 435 mμ by measuring the yellow color produced by the reaction of Δ^1-pyrroline with o-aminobenzaldehyde (Holmstedt, et al, 1961; Bachrach, 1962). The

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[1]The conditions of growth have been modified so that the specific activity of the sonicate is 20-fold higher than that of the sonicate used in the previous work (Bachrach, 1962). S. marcescens, obtained from Dr. Bachrach, was grown with aeration for 20 hours at 30° in the basic medium described by Vogel and Bonner (1956), plus (per liter) 1.0 g of disodium succinate as a carbon source and 0.1 g of bis-(3-aminopropyl)-amine. A 10-fold purification over the crude sonicate was obtained by collecting the ribosomal fraction by differential centrifugation, solubilization of the pellet by treatment with EDTA at pH 9.5 (Adams and Newberry, 1961), ammonium sulfate fractionation, and chromatography on Sephadex G-200.

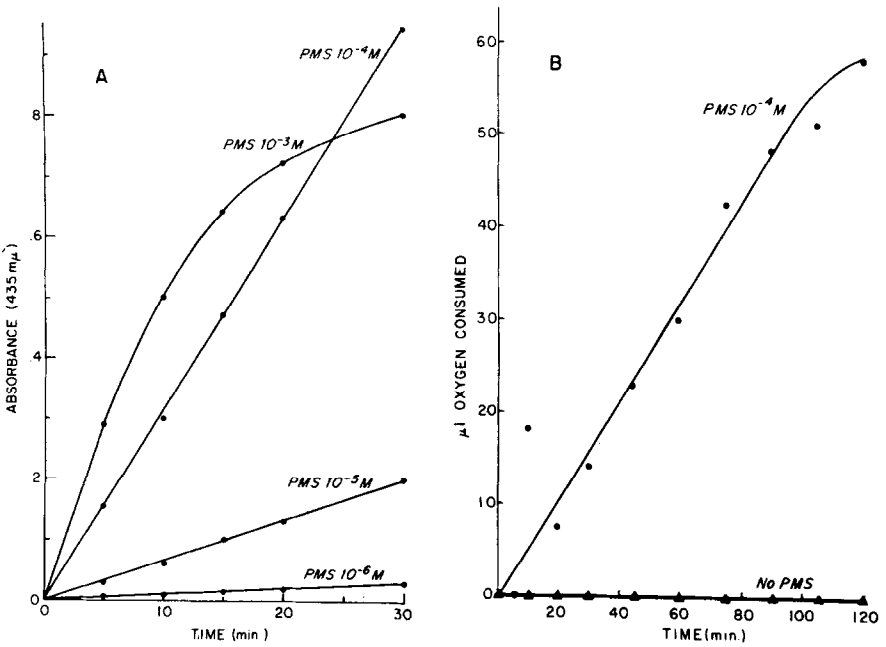

FIGURE I. Electron carrier (phenazine methosulfate) requirement for spermidine oxidation by S. marcescens preparations.

Figure 1A. The effect of phenazine methosulfate (PMS) concentration on Δ^1-pyrroline formation. The incubation mixture contained 1 μmole of spermidine trihydrochloride, 0.3 mg of a 10-fold purified enzyme, 0.8 μmole of o-aminobenzaldehyde, phenazine methosulfate as indicated, and 160 μmoles of sodium phosphate buffer (pH 6.5) in a final volume of 1 ml. The incubations were carried out at 25° for 30 minutes. The incubation mixtures were aerated at 10-minute intervals by gentle inversion.

Figure 1B. The effect of phenazine methosulfate on oxygen consumption. The incubation mixture contained (in 3 ml) 6 μmoles of spermidine trihydrochloride, 260 μmoles of sodium phosphate buffer, pH 6.5, 1.1 mg of a 10-fold purified enzyme, and 10^{-4} M phenazine methosulfate (temperature, 30°). Oxygen consumption was not altered by addition of catalase.

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purified enzyme had no activity unless phenazine methosulfate was added (Figure 1A). In contrast, the crude sonicate showed very little stimulation by added phenazine methosulfate. The requirement for an electron carrier was also shown by measuring oxygen consumption manometrically (Figure 1B). No oxygen consumption occurred without added phenazine methosulfate.

The enzymatic oxidation of spermidine can also be followed by the reduction of 2,6-dichlorophenolindophenol. The reduced dye was measured by its absorption at 600 mμ (Armstrong, 1964). The experimental cuvette contained 0.03 mg of a 10-fold purified enzyme, 160 μmoles of sodium phosphate buffer (pH 6.5), 60 mμmoles of dichlorophenolindophenol, and 20 mμmoles of spermidine trihydrochloride in a 1 ml volume. The reaction was complete in 2 minutes, with the formation of 19.4 mμmoles of reduced dichlorophenolindophenol. No dye reduction occurred in the absence of spermidine or of enzyme. Ferricyanide could also serve as an electron acceptor.

TABLE I

Flavin adenine dinucleotide requirement for oxidation of spermidine by a partially purified enzyme preparation

Δ^1-Pyrroline formation was measured as in Fig. 1A, using 0.04 mg of enzyme, 10^{-4} M phenazine methosulfate, and a 30 min reaction time.

		Δ Absorbance at 435 mμ
Unresolved enzyme		0.64
Resolved enzyme[1, 2]		0
Resolved enzyme + FAD[3]	(0 min preincubation)	0.10
Resolved enzyme + FAD	(20 hrs' preincubation)	0.30
Resolved enzyme + FAD	(40 hrs' preincubation)	0.29
Resolved enzyme + FMN[4]	(20 hrs' preincubation)	0

[1]The purified enzyme described above was chromatographed on a DEAE-Sephadex column. This material was then precipitated twice with 55% ammonium sulfate at pH 4.

[2]This enzyme remained inactive after 60 hours at 0°.

[3]One ml of the resolved enzyme (2 mg of protein dissolved in 0.1 M sodium phosphate buffer, pH 7.1) was preincubated with 100 mμmoles of FAD at 0° for the indicated times.

[4]One ml of the resolved enzyme (2 mg of protein dissolved in 0.1 M sodium phosphate buffer, pH 7.1) was preincubated at 0° with 1 μmole of FMN for the indicated time. Lower concentrations of FMN were also without any effect.

The requirement of the enzyme for FAD was demonstrated by subjecting a purified preparation to a series of two ammonium sulfate precipitations at pH 4. Table I shows the complete loss of activity of the enzyme. Preincubation of this inactive enzyme with FAD resulted in the recovery of 46 per cent of the initial activity. FMN was inactive.

Thus, in contrast to previously described systems for the oxidation of amines, spermidine oxidation by the partially purified enzyme from S. marcescens is linked to an electron transport system, and the enzyme should be considered as a spermidine dehydrogenase. FAD serves as a cofactor in this reaction.

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