OXIDATION OF SPERMIDINE BY A PSEUDOMONAS

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There have been a number of reports on the oxidation of polyamines by microorganisms. The instability of the probable aldehydes formed from the oxidation of the polyamines presents a particular difficulty in identifying the oxidation products, and the identity of these oxidation products has not yet been well established. Only very recently Bachrach (1962) was able to show that Serratia marcescens, which had been adapted to grow on a medium containing spermidine, could oxidize this amine to 1,3-diaminopropane and γ-aminobutyraldehyde. It had been generally believed that all bacterial cleavage of spermidine yielded these two products.

A different pathway for the bacterial oxidation of spermidine is reported in this brief note. Putrescine and 3-aminopropionaldehyde have been identified as the cleavage products.

$$NH_2\text{-}(CH_2)_4\text{-}NH\text{-}(CH_2)_3\text{-}NH_2 \xrightarrow{O_2} NH_2\text{-}(CH_2)_4\text{-}NH_2 + OHC\text{-}(CH_2)_2\text{-}NH_2 + H_2O_2$$

Materials and Methods

A Pseudomonas sp. which had been isolated in this laboratory was adapted to grow on spermidine as the sole carbon and nitrogen source. The crude homogenate was prepared from freshly harvested or frozen cells by sonication in four parts of 0.1 M Tris-HCl buffer, pH 8.5. The cleaving enzyme activity was found mainly in the particulate fraction after centrifugation at 144,000 x g for 60 minutes,

and this particulate enzyme could be partially solubilized by further sonication.

The enzyme activity was measured either by the amount of putrescine formed using diamine-αKG transaminase coupled with o-aminobenzaldehyde (Kim, 1964), or by the amount of 3-aminopropionaldehyde thiosemicarbazone formed. This thiosemicarbazone derivative has an ultraviolet absorption maximum at 256 mμ.

Spermidine was purchased from K and K Laboratories. The T-spermidine was prepared by the Wilzbach method by the New England Nuclear Corp. and was purified by paper chromatography (Herbst, 1958). 3-Aminopropionaldehyde diethylacetal was synthesized by the method of Becke (1953).

Results and Discussion

When the crude homogenate or the partially purified enzyme was incubated with spermidine and thiosemicarbazide at pH 8.5, stoichiometric amounts of putrescine and 3-aminopropionaldehyde thiosemicarbazone were obtained (Table I). The putrescine formed was

TABLE I

Stoichiometry of Spermidine Oxidation

Spermine oxidized	0.27 μm
3-Aminopropionaldehyde formed	0.24 μm
Putrescine formed	0.25 μm

The reaction mixture contained 0.2 ml of partially purified enzyme, 20 μmoles Tris buffer, pH 8.5, 5 μmoles spermidine·3 HCl and 1 μmole of thiosemicarbazide. The reaction was stopped by the addition of 0.3 ml of 20% TCA after one hour incubation at room temperature.

isolated by paper chromatography and by paper electrophoresis (Herbst, 1958). Tritium labelled putrescine obtained from T-spermidine was used for the further identification of the putrescine. As shown in Table II, the benzamide derivative of T-putrescine

TABLE II

Identification of Putrescine

Number of Recrystallizations	Benzamide of Putrescine(cpm/mg)	Benzamide of 1,3-diaminopropane(cpm/mg)
3	2270	490
4	2280	280
5	2270	164

T-putrescine obtained from T-spermidine oxidation was used to make the benzamide derivative. Approximately 708,000 cpm was used with carrier putrescine (100 mg) and 1,3-diaminopropane (100 mg). Theoretical cpm were 2090 and 1836 respectively.

obtained from the enzymatic reaction could be recrystallized to constant specific activity. Diamine-αKG transaminase, which is relatively specific for putrescine, was also used to identify this product (Figure 1). It is clear from these experiments that one of the cleavage products is putrescine.

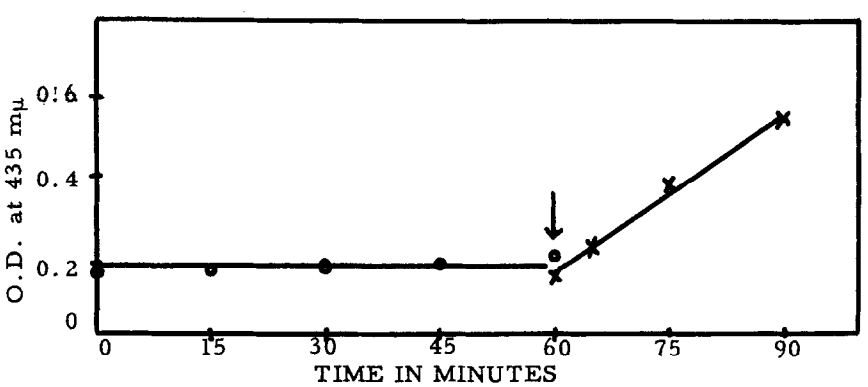

Figure 1. The reaction mixture contained 0.1 ml extract, 20 μmoles Tris buffer, pH 8.5, 5 μmoles spermidine·3 HCl, 0.1 μmoles pyridoxal phosphate and 2 μmoles o-aminobenzaldehyde in a total volume of 0.9 ml. Diamine-αKG transaminase and α-ketoglutaric acid were added where it is indicated.

3-Aminopropionaldehyde was identified by its thiosemicarbazone derivative. The absorption spectrum of this derivative formed from the enzymatic reaction and that of the authentic compound, which was chemically synthesized, are shown in Figure 2. Since the thiosemi-

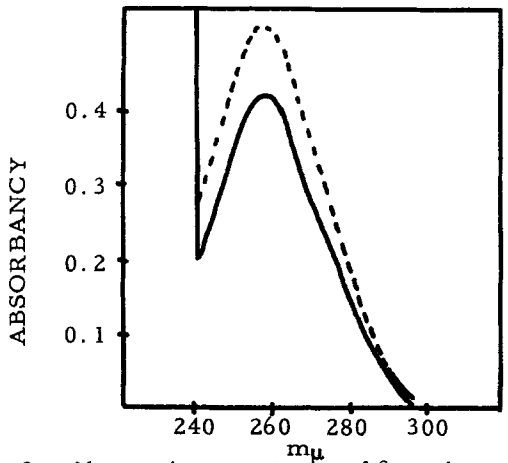

Figure 2. Absorption spectrum of 3-aminopropionaldehyde thiosemicarbazone obtained from the enzymatic reaction (----). Absorption spectrum of 3-aminopropionaldehyde thiosemicarbazone derivative obtained from 3-aminopropionaldehyde diethyl acetal (——).

carbazone of 3-aminopropionaldehyde and that of γ-aminobutyraldehyde have similar absorption spectra, it was essential to further identify this cleavage product. Unemoto (1964) suggested the formation of 3-aminopropionaldehyde in the serum amine oxidase reaction as one of the cleavage products of spermidine on the basis of absorption spectra only without identifying the other product. Contrary to this report, Tabor, et.al. (1964) reported that plasma amine oxidase oxidized only primary amino groups rather than the secondary amino group of spermidine. In order to further characterize the thiosemicarbazone obtained from the reaction mixture, T-labelled 3-aminopropionaldehyde thiosemicarbazone was hydrolized and oxidized to T-β-alanine by using dilute sulfuric acid and sodium dichromate. The T-β-alanine which was obtained from the oxidation has the theoretical specific activity of tritium. Extracts of this bacterium also oxidized 3-aminopropionaldehyde to β-alanine in the presence of DPN^+. Detailed studies of this enzyme will be presented elsewhere. The formation of hydrogen peroxide was detected by the use of catalase (Keilin and Hartree, 1945).

Summary

A Pseudomonas sp. which has been adapted to grow on spermidine as the sole carbon and nitrogen source oxidizes spermidine to 1,4-diaminobutane (putrescine) and 3-aminopropionaldehyde. These products were identified by chromatography and by the formation of derivatives.

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