THE OXIDATION OF POLYAMINES
BY NEISSERIA PERFLAVA*

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Neisseria perflava and Hemophilus parainfluenzae degrade spermine and spermidine to 1,3-propanediamine during growth (1). One or both of these polyamines are essential growth factors or are growth-stimulatory to a number of microorganisms (2–8), and these compounds occur in many microorganisms (1) and in most mammalian tissues (9).

There have been few studies on the enzymatic degradation of polyamines. Hirsch (10) reported the oxidation of spermine and spermidine by beef or sheep serum, and Tabor et al. (11) purified an enzyme from beef plasma which oxidizes these compounds as well as a number of monoamines. Silverman and Evans (12) showed that Pseudomonas pyocyanea could oxidize spermidine, and some diamine oxidase preparations are slightly active on spermine (13).

This paper is concerned with the oxidation of polyamines by an enzyme from N. perflava. The enzyme is adaptive in nature and oxidatively degrades a number of polyamines with the stoichiometric formation of a diamine, an aldehyde, and hydrogen peroxide. This enzyme differs from plasma amine oxidase in its substrate specificity and in the products of the reaction.

EXPERIMENTAL

Materials—Spermine was obtained from Hoffmann-La Roche, Inc. Spermidine, N-(3-aminopropyl)-1,3-propanediamine, and N-(3-aminopropyl)-1,5-pentanediamine were synthesized by Dr. Aldo M. Pulito, The Ames Laboratories, Inc., South Norwalk, Connecticut. N-(4-Aminobutyl)-1,4-butanediamine was kindly supplied by Dr. E. A. Zeller, Northwestern University Medical School, Chicago, and n-butylamino-1,4-butanediamine was synthesized by Dr. L. H. Amundsen, University of

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Connecticut, Storrs. The ethylenediamine derivatives were obtained from the Carbide and Carbon Chemicals Corporation. The amines were used as neutral solutions of the hydrochlorides.

Purified horse radish peroxidase was obtained from the Worthington Biochemical Corporation.

Preparation of Cells and Cell Extracts—N. perflava, obtained from Dr. M. J. Pelczar, Department of Bacteriology, University of Maryland, College Park, was used as the enzyme source. A synthetic growth medium (3) was used, since cells grown in crude medium (peptone-yeast) yielded a much lower enzyme activity. The enzyme is adaptive in this organism and was induced by adding spermine to the growth medium. An inoculum was prepared by growing cells in a 30 ml. volume for 24 hours at 34°. This volume was then transferred to 3 liters of medium in a 6 liter Florence flask containing 0.5 μmole of spermine per ml. After incubation for 18 hours at 34° with shaking, the cells were harvested in a refrigerated Sharples centrifuge and washed once with 0.033 M phosphate buffer (pH 7.3). Cold water was then added to form a thick suspension which was immediately and rapidly frozen and lyophilized to dryness. The cells were stored in a desiccator at −10°, and the enzyme is stable for months under these conditions. Lyophilized cell suspensions can be used without further treatment in enzyme experiments, but a cell-free extract prepared by sonic oscillation was used in most experiments. Suspensions containing 20 mg. of lyophilized cells per ml. in either distilled water or 0.02 M potassium phosphate buffer (pH 7.0) were treated for 10 minutes in a 10 kc. Raytheon oscillator which was cooled with ice water. The extracts were dialyzed for 18 hours at 5° against 100 volumes of either water or phosphate buffer and then centrifuged at 15,000 × g for 30 minutes at 0°. The supernatant fluids containing the enzyme were usually used immediately, but they can be frozen and stored at −10° with little loss of activity, at least in the 1st few days.

Enzyme Assay—Enzyme activity was measured manometrically with a conventional Warburg apparatus at 37.1°; the gas phase was air. The incubation mixtures usually contained 1 ml. of dialyzed N. perflava sonic extract (equivalent to 20 mg. of cells), 60 μmoles of potassium phosphate buffer (pH 7.0), and 4 μmoles of spermine hydrochloride (added from the side arm after equilibration) in a total volume of 3 ml. The rate of oxygen uptake was measured, and the activity is usually expressed as microliters of oxygen consumption per 20 mg. of cells in the initial 30 minutes.

General—Ammonia was determined by Conway's method (14). Paper electrophoresis was carried out as described previously (15) with 0.03 M citrate buffer. 1,3-Propanediamine on paper electrophoresis strips was determined by a ninhydrin method used previously (1).
Results

Spermine Oxidation—Fig. 1 shows the oxidation of spermine by a sonic extract of spermine-adapted *N. perflava* cells. Spermine oxidation under these conditions is linear almost to the end of the reaction. The enzyme preparations used in most other experiments had a higher activity than that used in Fig. 1. Although the enzyme would appear to be saturated initially in this experiment, higher spermine concentrations (6 to 8 μmoles per flask) gave a slight increase in the oxidation rate. However, incon-

Fig. 1. The oxidation of spermine by a cell-free extract of spermine-adapted *N. perflava*. The system contained 1 ml. of dialyzed *N. perflava* sonic extract (equivalent to 20 mg. of cells), 60 μmoles of potassium phosphate buffer (pH 7.0), and 4 μmoles of spermine hydrochloride (added from the side arm after equilibration) in a total volume of 3 ml.; KOH was in the center well.

Fig. 2. The effect of pH on spermine oxidation by a cell-free extract of spermine-adapted *N. perflava*. The conditions were the same as in Fig. 1.

sistencies result with higher spermine concentrations; this may be due to the low solubility of spermine phosphate.

*pH Optimum*—The enzyme in a cell-free extract has an optimal pH of 6.8 to 7.0 as shown in Fig. 2.

*Inhibitors*—The effect of a number of carbonyl reagents which are common inhibitors of amine oxidases (13) was tested on this enzyme. The results are shown in Table I. The enzyme is apparently a typical amine oxidase since it is sensitive to these reagents at appropriate concentrations.

*Substrate Specificity*—A number of monoamines, diamines, and polyamines were tested as substrates for the enzyme. None of a series of aliphatic monoamines from methylamine to amylamine was oxidized. Benzylamine, a good substrate for plasma amine oxidase (11), was not oxidized by the *N. perflava* enzyme. A series of aliphatic diamines from
ethylenediamine to hexamethylenediamine was also tested. None of these compounds was oxidized to a measurable extent. Table II shows the relative rate of oxidation of a number of polyamines. Only three of the polyamines tested were oxidized to an appreciable extent, but the results give some insight into the essential structure characteristics of the substrate. Since spermine (Compound 9), spermidine (Compound 6), and $N$-(4-
aminobutyl)-1,4-butanediamine (Compound 8) are oxidized, while there is no activity on N-(3-aminopropyl)-1,3-propanediamine (Compound 5) or N-(3-aminopropyl)-1,5-pentanediameine (Compound 7), the substrate must contain a 4-carbon diamine unit. Furthermore, since putrescine (1,4-butanediamine) is not oxidized, the 4-carbon diamine unit must be N-substituted. Since N-butylputrescine (Compound 4) is not oxidized and the N-aminoalkylputrescines (Compounds 6, 8, and 9) are oxidized, the side chain must have an amino group. Since the enzyme is most active on spermine (Compound 9), the putrescine unit obviously can be N,N'-disubstituted.

Stoichiometry—the data in Table III indicate that 1 μatom of oxygen is consumed per micromole of spermine or spermidine oxidized. Most amine oxidases studied previously were shown to form hydrogen peroxide as a product (11, 13, 16–19). If this enzyme were a typical amine oxidase, the theoretical oxygen consumption should be 1 μmole per μmole of substrate oxidized. It was suspected that hydrogen peroxide was being formed but was being destroyed by endogenous catalase, thus releasing 1 μatom of oxygen per micromole of peroxide; this would account for the observed oxygen uptake. By using the assay method of Herbert and

### Table III

**Stoichiometry of Oxygen Consumption, Ammonia Production, and Propanediamine Production**

Conditions for the measurement of oxygen consumption were essentially the same as in Fig. 1. After the reaction was complete, the protein was precipitated with trichloroacetic acid and removed by centrifugation. Other determinations were carried out on the protein-free filtrate after extraction of the excess acid with ether. In the Conway method, KOH was used for alkalination, and the ammonia was titrated with HCl after absorption into borate buffer. 1,3-Propanediamine was determined by electrophoresis of 0.05 ml. aliquots of the samples followed by quantitative estimation by the ninhydrin method.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>O2 consumption μmole</th>
<th>NH3 production μmole</th>
<th>PD* production μmole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spermine</td>
<td>4</td>
<td>3.7†</td>
<td>0</td>
</tr>
<tr>
<td>Spermidine</td>
<td>4</td>
<td>3.2†</td>
<td>3.6‡</td>
</tr>
</tbody>
</table>

* PD = 1,3-propanediamine.
† The oxygen consumption in the basic reaction is probably twice this figure. See the text for an explanation.
‡ It is doubtful that this was actually ammonia. Under the conditions used in the Conway determination, low molecular weight amines or amine derivatives react like ammonia. In addition, since the reaction apparently involves only one oxidative step and since 1,3-propanediamine is a product of the reaction, it seems unlikely that any ammonia could be formed except by a non-oxidative reaction.
Pinsett (20), sonic extracts of *N. perflava* cells were shown to have a very active catalase. The formation of hydrogen peroxide in the reaction mixture was shown indirectly by a coupled reaction involving peroxidase and a suitable acceptor as described by Kohn (19). As seen in Fig. 3, the addition of the peroxidase system to the reaction mixture resulted in almost a 2-fold increase in oxygen consumption. A primary product of the reaction, therefore, is probably hydrogen peroxide, and the oxygen consumption in the basic reaction is 1 μmole per μmole of substrate.

1 μmole of 1,3-propanediamine is produced per micromole of either spermine or spermidine oxidized (Table III). Spermine could theoretically yield 2 μmoles of the diamine, but the detection of only 1 is in agreement with the data on oxygen consumption, which indicates that only one oxidation is involved.

From the data on oxygen consumption and 1,3-propanediamine production, the oxidation of spermine and spermidine might proceed according to the following equation:

\[
RCH_2NH(CH_2)_2NH_2 + O_2 + H_2O \rightarrow RCHO + H_2N(CH_2)_2NH_2 + H_2O_2
\]

According to the above equation, γ-aminobutyraldehyde would be formed in the oxidation of spermidine. This compound is a product of the action of diamine oxidase on putrescine and undergoes a spontaneous cyclization under physiological conditions to form Δ⁴-pyrroline (21–23). Since no aldehyde could be detected by the 2,4-dinitrophenylhydrazine method of Friedemann and Haugen (24) on the reaction mixture after spermidine oxidation, a similar cyclization might take place in this case. Similarly,
if the equation postulated above is correct, the other product of spermine oxidation would be \(N\)-(3-aminopropyl)-\(\gamma\)-aminobutyraldehyde. This compound might undergo a cyclization similar to that of \(\gamma\)-aminobutyraldehyde to form \(N\)-(3-aminopropyl)-\(\Delta^2\)-pyrroline. A chemical oxidation of spermine analogous to that postulated for this enzyme reaction has been reported. Wrede et al. (25) identified 1,3-propanediamine and \(N\)-(3-aminopropyl)-\(\Delta^2\)-pyrroline (the position of the double bond was not established) as products of the oxidation of spermine by air in the presence of copper powder. This oxidation was carried out with minor changes, and the products of the reaction were compared with those of the enzymatic oxidation of spermine. Electrophoresis of the reaction mixture after the enzymatic oxidation of spermine reveals a ninhydrin-positive compound on the paper strips with electrophoretic properties identical with those of the cyclic compound resulting from the oxidation of spermine with air.

**DISCUSSION**

The polyamine oxidase of *N. perflava* differs from amine oxidases described previously. While it apparently catalyzes a typical amine oxidase reaction, it is distinguished by its substrate specificity. The enzyme preparation was inactive on all the monoamines and diamines tested and therefore has no monoamine oxidase or diamine oxidase activity. It also differs from the plasma amine oxidase studied by Tabor et al. (11) which oxidizes spermine and spermidine. The latter enzyme is also active on a number of monoamines, and the evidence indicates that only one enzyme is involved. In addition, the stoichiometry of the degradation of spermine and spermidine by the *N. perflava* enzyme differs markedly from that of plasma amine oxidase. On spermine oxidation by the latter enzyme, 2 moles of oxygen are consumed with the formation of an aminooaldehyde, hydrogen peroxide, and putrescine (11, 26). In addition, spermidine is an intermediate in the oxidation of spermine (11). This degradation, therefore, obviously involves a different attack on the spermine molecule from that of the *N. perflava* system.

The results of the studies on the substrate specificity of the *N. perflava* enzyme are in agreement with those from studies on the degradation of polyamines by growing cells of *N. perflava* and *H. parainfluenzae* (1). Spermine, spermidine, and \(N\)-(4-aminobutyl)-1,4-butanediamine, all substrates for the enzyme system, are degraded to diamine during growth, while \(N\)-(3-aminopropyl)-1,3-propanediamine, a compound which is not oxidized by the enzyme system, is not appreciably degraded by growing cells. The metabolism of the products of polyamine degradation by these organisms remains to be investigated. The product, in addition to 1,3-propanediamine, of spermine degradation by *H. parainfluenzae* and...
N. perflava is metabolized by growing cells, since it does not accumulate in
growth experiments (1) as it does in the enzyme reaction mixture. The
role of this enzyme in microbial metabolism is not clear, but it does serve to
detoxify polyamines (1).

The inhibition of this enzyme by carbonyl reagents indicates that an
active carbonyl group is present on the enzyme or coenzyme. Tabor et al.
(11) suggested that the coenzyme of plasma amine oxidase might be
pyridoxal phosphate, and Davison (27) has obtained some evidence which
indicates that pyridoxal phosphate may be involved in diamine oxidase
action.

A thorough study of the distribution of this enzyme has not been made.
Evidence has been obtained which indicates that the enzyme can be in-
duced in Pasteurella tularensis as well as in N. perflava and H. parainfluenzae
(1). The observation that yeast, Azotobacter vinelandi, Neurospora
crassa, and some mammalian tissues contain 1,3-propanediamine (1)
suggests that the enzyme may be more widely distributed.

SUMMARY

1. An adaptive amine oxidase from Neisseria perflava which oxidatively
degrades polyamines to diamines was studied manometrically in a cell-free
extract of spermine-adapted cells.

2. The enzyme is apparently specific for polyamines since no monoamines
or diamines were oxidized. Polyamines such as spermine, spermidine,
and N-(4-aminobutyl)-1,4-butanediamine which contain a 4-carbon
diamine unit are attacked by the enzyme.

3. Spermine and spermidine are oxidized with the consumption of 1
mole of oxygen and the production of 1 mole of 1,3-propanediamine, 1
mole of hydrogen peroxide, and 1 mole of an aldehyde.

4. The enzyme has an optimal pH of 6.8 to 7.0 and is inhibited by car-
bonyl reagents.

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