The Action of Plasma Amine Oxidase on β-Haloamines

EVIDENCE FOR PROTON ABSTRACTION IN THE OXIDATIVE REACTION*

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The action of plasma amine oxidase upon β-Br-ethylamine β-Cl-ethylamine, β-OH-phenylethylamine, and β-Cl-phenylethylamine was examined. β-Br-ethylamine is a substrate and irreversible inactivator of the enzyme. The enzyme becomes covalently labeled by the inactivator. Approximately 2 mol of inactivator are incorporated per mol of enzyme (MW 170,000). The reduced enzyme is not inactivated. The enzyme catalyzes the elimination of HCl from β-Cl-phenylethylamine to produce phenylacetaldehyde. The rate of the elimination reaction is comparable to the normal oxidative reaction. We conclude that the occurrence of this elimination reaction establishes the ability of the enzyme to catalyze proton abstraction from C-1 of the substrate and that proton abstraction occurs during the catalytic oxidation normally catalyzed by plasma amine oxidase. β-Cl-ethylamine is only oxidized to corresponding aldehyde. β-OH-phenylethylamine is neither oxidized, nor does elimination occur. It is a competitive inhibitor in the oxidation of benzylamine and in the elimination of HCl from β-Cl-phenylethylamine.

Many biological oxidations involve the general structural transformation shown by Equation 1:

\[
\text{H} \quad \text{-C-} + \text{A} \rightarrow \text{-C-} + \text{HA} + \text{H}^+ \\
\text{X} \quad \text{XH} \quad \text{X} \quad \text{X} \quad \text{X} \quad \text{X}
\]

\[\text{A}_{\text{oxid}}\text{ can be a cofactor as, for instance NAD}^+, \text{FMN, or a prosthetic group on the enzyme. To describe the mechanism of such reactions, it is important to decide whether the hydrogen bonded to carbon is abstracted as a proton, a hydride ion, or as a hydrogen radical. We have recently proposed an experimental test which, we believe, can determine whether proton abstraction takes place (1–3). It involves the use of substrates in which a good leaving group is bonded to the carbon at which oxidation occurs. Abstraction of a proton could lead to an elimination as shown in Equation 2. We consider occurrence of the elimination reaction evidence that the enzyme has the capability of abstracting a proton and that this is involved in the oxidative process. Several flavoproteins, D- and L-amino acid oxidase, and lactic oxidase can catalyze elimination reactions (1–3). We have now examined plasma amine oxidase.}

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EXPERIMENTAL PROCEDURES

Chemical Syntheses

β-Chloro-β-phenylethylamine-HCl—the synthesis of β-chloro-β-phenylethylamine-HCl from β-hydroxy-β-phenylethylamine-HCl was adapted from that of Barnett et al. (5). A solution of 1.5 ml of freshly distilled thionyl chloride in 1.5 ml of benzene was added slowly with stirring to a solution of 2 g of β-hydroxy-β-phenylethylamine-HCl in 1.5 ml of benzene at about 0°C in an ice bath. After continued stirring for 2 hours, the reaction mixture was evaporated under reduced pressure.
The product was recrystallized from acetone-ethanol and melted at 162-163° (uncorrected; literature value, 157-158° (5) and 163-164° (6)). NMR spectroscopy performed in D_2O gave the following signals: singlet at 7.45 ppm (5H, phenyl protons); triplet at 5.3 ppm (1H, J=7 Hz, /3 proton); doublet at 3.55 ppm (2H, J=7 Hz, methylene).

The stability of /3-chloro-/3-phenylethylamine-HCl in different phosphate buffers was tested by NMR analysis. A solution of 30 mg of this compound in 0.5 ml of 10 mM potassium phosphate buffer, pH 6.0 (made by mixing the buffer solutions with H_3PO_4) showed no change in the NMR spectrum for 24 hours; at pH 7.4, however, there was an instant appearance of crystals which dissolved slowly within a few hours and gave rise to the NMR spectrum of /3-hydroxy-/3-phenylethylamine with the following signals: singlet at 7.42 ppm (5H, phenyl protons); doublet at 5.0 ppm (1H, J=5.7 and 7.5 Hz, /3 proton); multiplet at 3.22 ppm (2H, methylene).

/3-Chlorophenylacetaldehyde—The compound was synthesized by a modification of the method of Stevens et al. (7). In a flask equipped with a reflux condenser and dropping funnel were placed 0.113 mol of phenylacetaldehyde (b.p. 75-76°) in 6.0 ml of methylene dichloride. The solution was chilled to 0° in an ice and a solution of sulphuryl chloride (0.12 mol) in 2.0 ml of methylene dichloride added drop-wise while maintaining the temperature at 15-20°. After completion of the addition, the reaction mixture was stirred for 30 min at room temperature and then heated to reflux for 30 min. Methylene dichloride and sulphuryl chloride were removed in a rotary evaporation under vacuum. The residue was distilled at 13 mm pressure and the 2-chlorophenylacetaldehyde collected at 98-102° (literature value: 98-100° (8)). The 2,4-dinitrophenylhydrazone melted at 145-147° (literature value: 147° (8)).

/3-Br-[U-14C]ethylamine-HBr—/3-Br-[U-14C]ethylamine-HBr was prepared by a modification of the procedure of Couteau (9). To 50 μCi of /3-hydroxy-[U-14C]ethylamine-HCl (78 mCi/mmol, 0.64 μmol) in 0.1 ml of ethanol/H_2O (1/1) was added 5.7 mg (58.4 μmol) of /3-hydroxy-ethylamine-HCl carrier. The ethanol was removed by evaporation under vacuum and the residue dissolved in 10 ml of HBr (specific gravity, 1.58). The reaction mixture was refluxed overnight and the HBr then evaporated under vacuum. Descending paper chromatography of the product on Whatman No. 3 paper with 1-butanol-acetic acid/water (196/30/50) showed only one amine peak with ninhydrin that was coincident with authentic /3-Br-ethylamine-HBr and with the major radioactive peak. The final specific activity, determined by ninhydrin assay (10), was 714 x 10^3 cpm/pmol of /3-Br-ethylamine.

Other Reagents and Enzymes—Plasma amine oxidase was purified from beef blood according to a modification of the procedure of Yamada and Yasunobu (4). This enzyme was reported to be at least 714 x 10^3 cpm/pmol of /3-Br-ethylamine.

RESULTS

Reactions with /3-Br-ethylamine—Fig. 1 shows that when /3-Br-ethylamine is added to amine oxidase, the initial rate of conversion of 2-chloro-2-phenylethylamine to the hydrazine-HCl. After 20 hours the enzyme was precipitated with 1 ml of concentrated HCl and removed by centrifugation. A solution of 50 mg of 2-4-dinitrophenylhydrazine in 0.5 ml of concentrated HCl was diluted with 2 ml of water, cleared by centrifugation, and added to the reaction mixture. After 20 min at room temperature, the hydrazone was extracted with benzene, the solvent was evaporated, and the sample was applied to a small (0.5 x 4.0 cm) column of neutral aluminum oxide. The elution with benzene gave compounds that were separated by thin layer chromatography on silica gel plates using a 1/1 mixture of benzene and petroleum ether (60-110°). Each spot was eluted from the plates and analyzed with Fourier Transform NMR. The spectrum of authentic 2,4-dinitrophenylhydrazone of phenylacetaldehyde using chloroform as a solvent has the following signals: singlet at 7.3 ppm (5H, phenyl protons); doublet at 3.75 ppm (1H, J = 5.5 Hz, methylene protons); triplet at 7.6 ppm (1H, J = 5.5 Hz, aldehyde proton), singlet at 11.05 ppm (1H, NH proton), doublet at 9.1 ppm (1H, J = 2.5 Hz, C5 proton), doublet at 8.92 ppm (1H, J = 9 and 2.5 Hz, C5 proton), and doublet at 7.92 ppm (1H, J = 9 Hz, C6 proton).

Fig. 1. Effect of /3-Br-ethylamine on the oxidation of benzylamine. The reaction mixtures contained 10 mM benzylamine (O) or 4 mM /3-Br-ethylamine (x). 0.1 mM phosphate buffer (pH 7.0), and 92.5 millimoles of amine oxidase in 0.5 ml. In the reaction with /3-Br-ethylamine, an additional 92.5 millimoles of amine oxidase was added at the point indicated by the first arrow and 10 mM benzylamine at the second arrow.

Fig. 2. Anaerobic inactivation of amine oxidase by Br-ethylamine. Enzyme, 58 milliunits, in 0.25 ml of 0.05 M potassium phosphate buffer, pH 7.0, was incubated at 0 °C under a stream of Argon for 2 hours. At t = 0, 5 μl of 0.2 M (anaerobic) benzylamine HCl solution were added, and the temperature was raised to 25°. At t = 15 min and 30 min, 10-μl aliquots of (anaerobic) 0.2 M bromoethylamine HBr were added. Aliquots, 20 μl, were removed at various times and assayed in an oxygen electrode containing 50 mM KPO_4 (pH 7) and 1.7 mM glycine ethyl ester. Air was admitted to the enzyme inactivator mixture at t = 53 min. The control does not contain Br-ethylamine.

Assays

Enzymatic activity was normally assayed with benzylamine (10 mM) in 0.1 M phosphate buffer, pH 7.2, by spectrophotometric assay of increase of absorbance at 250 nm due to benzaldehyde formation. The difference of the molar extinction coefficients was taken as 12,800. The NMR spectrum of /3-chloro-2-phenylethylamine-HCl, /3-Br-ethylamine-HCl, and /3-Br-ethylamine-HBr was purchased from Calbiochem.

Identification of Aldehyde Produced during Conversion of 2-Chloro-2-phenylethylamine

Plasma amine oxidase (0.60 units) was incubated in 5 ml of 35 mM piperazine/HCl buffer, pH 6.0, with 50 mg of 2-chloro-2-phenylethylamine-HCl. After 20 hours the enzyme was precipitated with 1 ml of concentrated HCl and removed by centrifugation. A solution of 50 mg of 2,4-dinitrophenylhydrazine in 0.5 ml of concentrated HCl was diluted with 2 ml of water, cleared by centrifugation, and added to the reaction mixture. After 20 min at room temperature, the hydrazone was extracted with benzene, the solvent was evaporated, and the sample was applied to a small (0.5 x 4.0 cm) column of neutral aluminum oxide. The elution with benzene gave compounds that were separated by thin layer chromatography on silica gel plates using a 1/1 mixture of benzene and petroleum ether (60-110°). Each spot was eluted from the plates and analyzed with Fourier Transform NMR. The spectrum of authentic 2,4-dinitrophenylhydrazone of phenylacetaldehyde using chloroform as a solvent has the following signals: singlet at 7.3 ppm (5H, phenyl protons); doublet at 3.75 ppm (1H, J = 5.5 Hz, methylene protons); triplet at 7.6 ppm (1H, J = 5.5 Hz, aldehyde proton), singlet at 11.05 ppm (1H, NH proton), doublet at 9.1 ppm (1H, J = 2.5 Hz, C5 proton), doublet at 8.92 ppm (1H, J = 9 and 2.5 Hz, C5 proton), and doublet at 7.92 ppm (1H, J = 9 Hz, C6 proton).

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Enzyme, 58 milliunits, in 0.25 ml of 0.05 M potassium phosphate buffer, pH 7.0, was incubated at 0 °C under a stream of Argon for 2 hours. At t = 0, 5 μl of 0.2 M (anaerobic) benzylamine HCl solution were added, and the temperature was raised to 25°. At t = 15 min and 30 min, 10-μl aliquots of (anaerobic) 0.2 M bromoethylamine HBr were added. Aliquots, 20 μl, were removed at various times and assayed in an oxygen electrode containing 50 mM KPO_4 (pH 7) and 1.7 mM glycine ethyl ester. Air was admitted to the enzyme inactivator mixture at t = 53 min. The control does not contain Br-ethylamine.
### Table I

**Kinetic constants for reaction of substrates with plasma amine oxidase**

Each measurement was done at 25° in a volume of 1.0 ml (0.5 ml where the O₂-electrode was used) of air-saturated buffer containing the appropriate substrate. The reaction was started with amine oxidase. All buffers were potassium phosphate.

| Substrate                             | Relative \( V_{\text{max}} \) | \( K_{\text{m}} \) | Buffer                  |
|---------------------------------------|-------------------------------|--------------------|-------------------------|
| Benzylamine¹                          | 1.00                          | 1.0 mM             | 0.2 M phosphate, pH 7.4 |
| Benzylamine¹                          | 0.61                          | 6.7 mM             | 0.1 M phosphate, pH 6.0 |
| 2-Chloro-2-phenyl-ethylamine²         | 0.25                          | 0.8 mM             | 0.1 M phosphate, pH 6.0 |
| 2-Hydroxy-2-phenyl-ethylamine²,³      | <0.01                         |                    | 0.2 M phosphate, pH 7.4 |
| 2-Phenyl-ethylamine²                  | 0.66                          | 0.5 mM             | 0.2 M phosphate, pH 7.4 |
| 2-Bromoethylamine³                    | 0.61                          | 1.0 mM             | 0.1 M phosphate, pH 7.0 |
| 2-Chloroethylamine³ [3.8 mM]          | 3.12                          |                    | 0.07 M phosphate, pH 7.2 |
| 2-Hydroxyethylamine³ [4.2 mM]         | 0.42                          |                    | 0.07 M phosphate, pH 7.2 |

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Each measurement was done at 25° in a volume of 1.0 ml (0.5 ml where the O₂-electrode was used), of air-saturated buffer containing the appropriate substrate. The reaction was started with amine oxidase. All buffers potassium phosphate.

**Assay - Methods**

1) Change of absorbance at 250 nm
2) Coupled assay with liver alcohol dehydrogenase + NADH
3) Oxygen electrode

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**Fig. 3.** (left). Double reciprocal plot of the inhibition of the conversion of \( \beta \)-Cl-\( \beta \)-phenylethylamine to phenylacetaldehyde by \( \beta \)-OH-\( \beta \)- phenylethylamine. Reaction was carried out in 0.1 M potassium phosphate buffer, pH 6.0, in a total volume of 1 ml containing 46.2 milliunits of amine oxidase. The reaction was followed in a coupled assay with liver alcohol dehydrogenase and NADH as described under "Experimental Procedures."

**Fig. 4.** (right). Double reciprocal plot of the inhibition of the oxidation of benzylamine by \( \beta \)-HO-\( \beta \)-phenylethylamine. Reaction conditions were the same as in Fig. 2, except that 48.5 milliunits of amine oxidase were used.

\( O_2 \) consumption is about 60% that of benzylamine. \( O_2 \) uptake gradually decreases to a background level, suggesting enzyme inhibition. Addition of the same amount of enzyme, at this point, almost reproduces the first reaction curve. Addition of benzylamine, however, does not lead to resumption of \( O_2 \) uptake. The amount of \( O_2 \) consumed is independent of the \( \beta \)-Br-ethylamine concentration and proportional to the amount of the enzyme. On the average each enzyme molecule turns over about nine times before it becomes inactive, assuming a molecular weight of 170,000 (12) and a specific activity of 0.140 units/mg.¹ These results show that \( \beta \)-Br-ethylamine is a substrate and probably also an irreversible inhibitor of the enzyme. The fact that the total \( O_2 \) consumption which takes place up to the point of enzyme inactivation is independent of substrate concentration indicates \( K_{\text{m, inactivation}} = K_{\text{m, oxidation}} \) and suggests that the same substrate enzyme complex leads to inactivation and oxidation.

¹ Previously a specific activity of up to 0.12 unit/mg had been reported (4). We have obtained preparations with specific activities as high as 0.14 unit/mg.
The irreversible inhibition was confirmed by incubating 1.02 units of enzyme in 0.5 ml 0.1 M potassium phosphate buffer, pH 7.0, with 3 mM \( \beta \)-Br-[\(^{14}C \)]ethylamine. After 30 min an aliquot was removed and assayed for amine oxidase activity. It was found that 5% of the initial activity remained. The enzyme was then applied to a Sephadex G-15 column (1.7 \( \times \) 45 cm, 10 mM potassium phosphate buffer, pH 7.0). Part of the radioactivity eluted with the protein and no catalytic activity was recovered. The amount of radioactivity associated with the protein corresponded to 1.95 mol of inhibitor/170,000 g of enzyme. When the protein peak was pooled and dialyzed 18 hours against 1 liter of 20 mM potassium phosphate buffer, pH 7.0, 10% of the radioactivity was lost. In another experiment where the incubation lasted for 3 hours, 2.5 mol of inhibitor/mol of enzyme were incorporated and 12% was lost upon dialysis. The enzyme inactivated with \( \beta \)-Br-[\(^{14}C \)]ethylamine was reduced with NaBH\(_4\) and then hydrolyzed with pronase 18 hours, 37\(^{\circ}\) in 0.5% NH\(_2\)HCO\(_3\) and subsequently 6N HCl. A radioactive compound was isolated which was not \( \beta \)-Br-ethylamine but appeared to be a modified amino acid. The structure of this compound is now under investigation. We conclude that Br-ethylamine irreversibly inactivates amine oxidase and in the process the enzyme becomes covalently labeled. Two moles of inactivator are incorporated per mole of enzyme. It has been reported that the enzyme is a dimer of identical subunits (12).

If enzyme-catalyzed chemical modification of the inactivator is required for the inactivation process, the reduced enzyme should not be subject to inactivation. This point was tested and the results of such an experiment\(^2\) are shown in Fig. 2. Br-ethylamine was added to the reduced enzyme under anaerobic conditions. The amount of inhibitor added was sufficient to inactivate the enzyme under aerobic conditions within 1 min. No significant inactivation is observed. The reduced enzyme slowly loses activity under the experimental conditions, but the rate of activity loss is comparable to that of the control (anaerobic, no inhibitor). Admission of air leads to rapid inactivation. It is, therefore, concluded that the reduced enzyme is not inactivated by \( \beta \)-Br-ethylamine.

**Reaction with \( \beta \)-Cl-\( \beta \)-phenylethylamine**—When \( \beta \)-Cl-\( \beta \)-phenylethylamine is added to the enzyme, no \( O_2 \) uptake is observed. However, when this compound is added in the presence of alcohol dehydrogenase and DPNH, DPNH is consumed. These results suggested that \( \beta \)-Cl-\( \beta \)-phenylethylamine is nonoxidatively converted to a carbonyl compound by plasma amine oxidase. This compound was identified (see below) as \( \beta \)-phenylacetaldehyde. The \( K_m \) for \( \beta \)-Cl-\( \beta \)-phenylethylamine and \( V_{max} \) for its conversion to phenylacetaldehyde are shown in Table 1 together with corresponding constants for other substrates. The kinetic constants for \( \beta \)-Cl-\( \beta \)-phenylethylamine are similar to those obtained for "normal" substrates, i.e. those which are oxidized. In contrast, \( \beta \)-OH-\( \beta \)-phenylethylamine is neither oxidized, nor does it undergo elimination reaction. It is, however, a competitive inhibitor in the oxidation of benzylamine (\( K_i = 0.67 \) mM; 0.2 M potassium phosphate buffer, pH 7.4). \( \beta \)-Cl-\( \beta \)-phenylethylamine is an uncompetitive inhibitor. Clearly, the nature of the substituent in the \( \beta \) position has a profound effect on the course of the reaction catalyzed by amine oxidase.

The effect of \( \beta \)-OH-\( \beta \)-phenylethylamine upon the conversion of \( \beta \)-Cl-\( \beta \)-phenylethylamine to phenylacetaldehyde was examined. Due to the instability of the \( \beta \)-chloro compound, these experiments were done at pH 6.0. It was found (Figs. 3 and 4) that the \( \beta \)-OH compound is a competitive inhibitor with \( K_i = 2.9 \) mM (pH 6.0) which is about the same as that obtained when benzylamine was the substrate (\( K_i = 2.2 \) mM). To test further the effect of inhibitors upon the reaction of \( \beta \)-Cl-\( \beta \)-phenylethylamine, a sample of amine oxidase was incubated with 4 and 12 mM aminoacetoniitride for 10 min.\(^4\) It was inactivated 48 and 96% as determined by the oxidation of benzylamine. The conversion of \( \beta \)-Cl-\( \beta \)-phenylethylamine to phenylacetaldehyde was inhibited 47 and 97%. Thus, both reactions were inhibited to the same extent.

Experiments were carried out to establish the effect of the oxidation state of the enzyme upon the reaction of \( \beta \)-Cl-\( \beta \)-phenylethylamine. The results are summarized in Table II. The reaction proceeds essentially as well under aerobic conditions as under anaerobic conditions. This is expected since the reaction is not an oxidative process. However, when the enzyme was reduced by the addition of 1 mM benzylamine (\( K_m = 6.7 \) mM) under anaerobic conditions, no conversion \( \beta \)-Cl-\( \beta \)-phenylethylamine to \( \beta \)-phenylacetaldehyde took place. The amount of benzylamine added was not enough to abolish the reaction of \( \beta \)-Cl-\( \beta \)-phenylethylamine through competitive inhibition. We, therefore, conclude that the enzyme must be in an oxidized state in order to catalyze the formation of phenylacetaldehyde. Furthermore, since the reaction of \( \beta \)-Cl-\( \beta \)-phenylethylamine proceeds under anaerobic conditions, it can be concluded that this substrate does not reduce the enzyme.

To confirm that \( \beta \)-Cl-\( \beta \)-phenylethylamine is converted to phenylacetaldehyde, the enzyme was incubated with the substrate for 60 min under experimental conditions of Table I. The reaction mixture then was treated with 2,4-dinitrophenyldrazine, as described under "Experimental Procedure," and the resulting 2,4-dinitrophenyldrazine was subjected to thin layer chromatography in two solvent systems. Table III indicates that from 2 to 3 spots were detected, one of which (\( R_f: 0.37 \), Solvent A; 0.74, Solvent B) corresponds to the authentic hydrazone of phenylacetaldehyde. The other spots are from impurities of the substrate. The derivative from the enzymically produced product separated distinctly from the 2,4-dinitrophenyldrazone of \( \beta \)-Cl-phenylacetaldehyde. A similar experiment was repeated on a larger scale and the compound with \( R_f 0.37 \) (Solvent A) was eluted and examined by Fourier Transform NMR. The spectrum showed the same signals as the authentic derivative of phenylacetaldehyde and a few signals between 1.0 and 2.8 ppm which are attributed to

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\(^2\)We wish to thank R. Suva for carrying out this experiment.

\(^4\)Aminoacetoniitride irreversibly inactivates plasma amine oxidase.

A. Maycock, R. Suva, and R. H. Abeles, unpublished observation.
impurities of the preparation. The spectrum, therefore, confirms the identity of the enzymically produced material. 

Since plasma amine oxidase catalyzes the elimination of HCl from \( \beta \)-Cl-\( \beta \)-phenylethylamine, it might also catalyze a similar elimination reaction from \( \beta \)-Cl-ethylamine. The oxidation of this compound is catalyzed by plasma amine oxidase. Table IV shows the amount of \( O_2 \) consumed when plasma amine oxidase acts on \( \beta \)-Cl-ethylamine as well as the amount of NADH consumed when the enzyme acts on \( \beta \)-Cl-ethylamine in the presence of liver alcohol dehydrogenase. A similar experiment was done, as control, with benzylamine, a compound which cannot undergo an elimination reaction. The amount of aldehyde produced from \( \beta \)-Cl-ethylamine, as measured with alcohol dehydrogenase, is identical to the amount expected from oxygen consumption. Therefore, \( \beta \)-Cl-ethylamine does not undergo a nonoxidative elimination reaction to any significant extent.

**DISCUSSION**

The results reported here establish that plasma amine oxidase catalyzes the elimination of HCl from \( \beta \)-Cl-\( \beta \)-phenylethylamine. \( V_{\text{max}} \) and \( K_m \) for the process is comparable to \( V_{\text{max}} \) and \( K_m \) for substrates which undergo oxidation, such as benzylamine or phenylethylamine. \( \beta \)-OH-\( \beta \)-phenylethylamine is a competitive inhibitor for the oxidation of benzylamine and for the elimination of HCl from \( \beta \)-Cl-\( \beta \)-phenylethylamine with a very similar \( K_i \) for the inhibition of both types of reaction.

Finally, the reduced enzyme does not catalyze the elimination reaction. These results indicate that \( \beta \)-Cl-\( \beta \)-phenylethylamine binds to the oxidized enzyme at the same site as substrates subject to oxidation, and therefore, probably both substrates interact with similar functional groups at the active site. The elimination reaction most probably involves abstraction of hydrogen as a proton from the carbon bearing the amino group.

We propose that similar proton abstraction occurs at some stage during the catalysis of the oxidative reaction.

A fundamental question concerning the mechanism of action of plasma amine oxidase is whether the reduced form of the enzyme is an intermediate or whether the enzyme catalyzes a direct oxidation of the substrate by oxygen without the intermediate formation of the reduced enzyme. Available evidence favors intermediate formation of a reduced enzyme. Addition of substrate under anaerobic conditions or reducing agents produces a spectral change in the enzyme (13). Under anaerobic conditions, 1 mol of oxidation product is formed per mole of enzyme (15). We also have shown that the addition of substrates under anaerobic conditions produces a change in the enzyme which prevents inactivation by propargylamine (14), 3 \( \alpha \)-aminoacetonitrile, and \( \beta \)-bromoethylamine. The elimination of HCl from \( \beta \)-chlorophenylethylamine described in this paper also does not occur when the enzyme is reduced. The most reasonable interpretation of these observations is that addition of substrate produces a reduced enzyme which is not subjected to inactivation and cannot catalyze elimination reaction. Kinetic evidence also has been obtained by others which points to an ordered process in which product is released prior to reaction of the reduced enzyme with \( O_2 \) (15). The results reported here indicate that at some point in the reaction sequence leading to the formation of reduced enzyme, a proton is abstracted from the carbon to which the amino group is bonded. Little further can be said about the mechanism, primarily because the nature of the functional group on the enzyme which is reduced is unknown. A number of proposals have been made regarding the nature of this functional group. Initially, it was suggested that the enzyme contains pyridoxal phosphate, or a closely related molecule (13). Oxidation of the substrate then could proceed via an initial transamination between the enzyme bound pyridoxal and the substrate and subsequent reoxidation of the enzyme bound pyridoxamine. We have been unable to confirm the presence of pyridoxal phosphate or a related compound in this enzyme. Others also have expressed doubts regarding the existence of such a group (16). It also has been proposed that a sulfenic acid residue at the active site functions as an oxidized agent (17). We have considered a modification of that mechanism and have postulated the existence of a \(-S\tilde{S}\tilde{S}\) group at the active site which functions as an electron acceptor as illustrated in Scheme 1.

**Scheme 1**

All of these mechanisms involve intermediate proton abstractions from the carbon bearing the amino group. However, it does not appear profitable to speculate further upon the mechanism of the oxidation until the nature of the group of the enzyme is identified.

For the inactivation of plasma amine oxidase by \( \beta \)-Br-ethylamine, we propose the mechanism shown in Scheme 2.
According to this mechanism, the enzyme oxidizes bromo-ethylamine to bromoacetaldehyde, resulting in the formation of a reduced enzyme-BrCH₂CHO complex. This complex can undergo one of two processes. The complex is oxidized (a, in Scheme 2) and releases BrCH₂CHO. This is a normal oxidative pathway. In approximately one out of nine turnovers, release of products does not occur and the enzyme becomes alkylated and inactivated. The inactivation can occur through either of two processes (b, b', Scheme 2). Both mechanisms of inactivation require the formation of the oxidation product of β-Br-ethylamine (Br-acetaldehyde) at the active site. This is consistent with the observation that the reduced enzyme is not subject to inactivation by β-Br-ethylamine, although alternate interpretations, such as failure of the inactivator to bind, could be involved. It seems reasonable on chemical grounds that oxidation precedes inactivation since the oxidation products of β-Br-ethylamine, i.e. β-Br-ethylamine or β-Br-acetaldehyde, are more effective alkylating agents. If this interpretation of the inactivation is correct, bromoethylamine can be considered a “suicide” inhibitor, i.e. the inhibitory molecule is activated at the active site through the catalytic action of the enzyme. For such an inhibitor to be effective, it is necessary that the alkylation of the enzyme proceeds at a rate comparable to product release. A similar type of inactivation by allyl alcohol of alcohol dehydrogenase has recently been described (18).

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