REFERENCES
Chan, W. W.-C., Mort, J. S., Chong, D. K. K., & Macdonald, P. D. M. (1973) *J. Biol. Chem. 248*, 2778–2784.
Edelhoch, H., Rodwell, V. W., & Grisolia, S. (1957) *J. Biol. Chem. 228*, 891–903.
Girg, R., Jaenicke, R., & Rudolph, R. (1983a) *Biochem. Int. 7*, 433–441.
Girg, R., Rudolph, R., & Jaenicke, R. (1983b) *FEBS Lett. 163*, 132–135.
Grisolia, S. (1962) *Methods Enzymol. 5*, 236–242.
Grossman, S. H., Pyle, J., & Steiner, R. J. (1981) *Biochemistry 20*, 6122–6128.
Hermann, R., Jaenicke, R., & Rudolph, R. (1981) *Biochemistry 20*, 5195–5201.
Hermann, R., Rudolph, R., Jaenicke, R., Price, N. C., & Scobbie, A. (1983) *J. Biol. Chem. 258*, 11014–11019.
Holbrook, J. J., Liljas, A., Steindel, S. J., & Rossmann, M. G. (1975) *Enzymes, 3rd Ed. 11*, 191–292.
Jaenicke, R. (1982) *Biophys. Struct. Mech. 8*, 231–256.
Jaenicke, R. (1984) *Angew. Chem., Int. Ed. Engl. 23*, 395–413.
Jaenicke, R., & Rudolph, R. (1977) in *Pyridine Nucleotide-Dependent Dehydrogenases* (Sund, H., Ed.) pp 351–367, de Gruyter, Berlin and New York.
Jaenicke, R., & Rudolph, R. (1983) in *Biological Oxidations* (Sund, H., & Ullrich, V., Eds.) pp 62–90, Springer-Verlag, Berlin and Heidelberg.
Jaenicke, R., Krebs, H., Rudolph, R., & Woenckhaus, C. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 1966–1969.
Krebs, H., Rudolph, R., & Jaenicke, R. (1979) *Eur. J. Biochem. 100*, 359–364.
Price, N. C., & Jaenicke, R. (1982) *FEBS Lett. 143*, 283–286.
Ray, W. J., Jr., & Peck, E. T., Jr. (1972) *Enzymes, 3rd Ed. 6*, 407–477.
Rudolph, R., Engelhard, M., & Jaenicke, R. (1976) *Eur. J. Biochem. 67*, 455–462.
Rudolph, R., Westhof, E., & Jaenicke, R. (1977) *FEBS Lett. 73*, 204–206.
Schultze, I. T., & Colowick, S. P. (1969) *J. Biol. Chem. 244*, 2306–2316.
Seifert, T., Bartholmes, P., & Jaenicke, R. (1985) *Biochemistry 24*, 339–345.
Winn, S. I., Watson, H. C., Harkins, R. N., & Fothergill, L. A. (1981) *Philos. Trans. R. Soc. London, Ser. B 293*, 121–130.
Yamoto, S., & Murachi, T. (1979) *Eur. J. Biochem. 93*, 189–195.

Human Brain Monoamine Oxidase Type B: Mechanism of Deamination As Probed by Steady-State Methods†

L. Bruce Pearce and Jerome A. Roth*
Department of Pharmacology and Therapeutics, State University of New York at Buffalo, School of Medicine, Buffalo, New York 14214
Received August 1, 1984

ABSTRACT: Recently, evidence has been published which suggests that [Husain, M., Edmondson, D. E., & Singer, T. P. (1982) *Biochemistry 21*, 595–600] monoamine oxidase [amine:oxygen oxidoreductase (MAO), EC 1.4.3.4] deaminates phenylethylamine and benzylamine via two distinct kinetic pathways which involve either binary or ternary complex formation, respectively. These conclusions were drawn largely from stopped-flow kinetic analysis performed on purified enzyme removed from its native membrane and in the presence of the inhibitory detergent Triton X-100. In this study, *d*-amphetamine and alternative substrates were used as steady-state probes of the kinetics of deamination by the B form of human brain MAO using native membrane-bound enzyme. Initial velocity studies showed mixed-type patterns for amphetamine inhibition of phenylethylamine, tryptamine, and tyramine when either amine or oxygen was the varied substrate. Slope and intercept vs. amphetamine concentration replots were linear in all cases except for phenylethylamine (hyperbolic); K_i values obtained from linear replots of slope or intercept values were comparable. In contrast, amphetamine was a competitive inhibitor of benzylamine deamination when amine concentration was varied and uncompetitive when oxygen concentration was varied; slope and intercept replots were linear for both. When benzylamine was the alternative substrate inhibitor and tyramine and tryptamine deamination was measured, mixed-type inhibition patterns were obtained when either amine or oxygen concentration was varied; replots of slope and intercept were linear in all cases. These results strongly support the proposal that phenylethylamine and benzylamine are deaminated via two distinct mechanisms and further suggest that tyramine and tryptamine, like phenylethylamine, are deaminated via exclusively binary complex formation whereas benzylamine deamination uniquely involves the formation of a ternary complex with the reduced form of the oxidase.

Numerous studies have suggested that deamination of amines by the membrane-bound outer mitochondrial enzyme monoamine oxidase [amine:oxygen oxidoreductase (MAO), EC 1.4.3.4] occurs via a ping-pong mechanism (Tipton, 1968; Oi et al., 1970, 1971; Houslay & Tipton, 1973; Fowler & Oreland, 1979; Roth, 1979). However, Husain et al. (1982) have recently proposed that the mechanism of deamination of monoamines by bovine liver type B MAO may be substrate dependent, and they suggest that deamination of benzylamine and phenylethylamine proceeds via two distinct pathways. According to their model, deamination may involve either

† This study was supported by a grant from the National Institutes of Health (GM 7145-10).

0006-2960/85/0424-1821$01.50/0

ternary or binary complex formation with the reduced form of MAO ($E_{red.}$). These authors concluded that benzylamine deamination proceeds via a ternary complex intermediate, E_{red}–aldehyde–oxygen, whereas phenylethylamine deamination involves formation of binary complexes indicative of a ping-pong mechanism. These findings were obtained by using steady-state and stopped-flow techniques, the latter requiring the presence of the detergent Triton X-100 to suspend the enzyme in solution for measurement of the half-reactions.

A significant body of literature exists which suggests that many membrane-bound proteins are sensitive to perturbations of their membrane environment (Sandermann, 1978; Coleman, 1973), and in this regard, several studies have revealed that alterations of the lipid content of preparations of MAO may modify the enzymatic properties of the oxidases (Nandaswami & D'Iorio, 1978; Fowler & Oreland, 1980; Yu, 1979; Haung & Faulkner, 1981; Sawyer & Greenawalt, 1979; Houslay, 1980). For example, Housley & Tipton (1975) have demonstrated that solubilization with the nonionic detergent Triton X-100 resulted in a change in the kinetic mechanism of rat liver MAO. Triton X-100 has also been shown by a number of authors to inhibit oxidase activity at concentrations well below those employed by Husain et al. (1982) noted above (Yu, 1981; Achee & Gabay, 1981). In addition, Roth & Eddy (1980) have demonstrated that following Triton X-100 solubilization of both forms of human brain MAO, the activation energies and true K_m values for a number of substrates were changed. In support of the argument that the activity of MAO may be dependent on its lipid environment are results of studies in our laboratory which demonstrate that low concentrations of the relatively mild nonionic detergent octyl glucoside (below that which solubilizes the outer mitochondrial enzyme) will inhibit deamination by the B form of human brain MAO (Pearce & Roth, 1983). In light of the above evidence, the possibility exists that the findings of Husain et al. (1982) which demonstrate that benzylamine and phenylethylamine deamination proceeds via different reaction pathways may have been a consequence of alterations in the properties of the enzyme during purification and/or the presence of the inhibitory detergent Triton X-100 during kinetic analysis. Accordingly, it is important to establish whether the mechanism proposed by Husain et al. (1982) is, in fact, indicative of the reaction with native membrane-bound enzyme in the absence of any inhibitory detergent or modifications of the lipid microenvironment. In this paper, *d*-amphetamine and alternative substrate inhibitors have been employed as probes of the mechanism of deamination by native mitochondrial preparations of MAO. The results of these experiments provide steady-state kinetic evidence which supports the mechanism proposed by Husain et al. (1982) and also provide a simple steady-state technique for determining the pathway of deamination for any MAO substrate.

Materials and Methods

Human brain frontal lobes were obtained at autopsy within 12 h after death, and crude mitochondria were isolated as described previously (Roth, 1976). To prepare solutions containing exclusively the B-type MAO activity, mitochondrial enzyme was incubated for 30 min at 37 °C in the presence of the A-selective irreversible inhibitor clorgyline (5 μM). To remove excess inhibitor, reaction mixtures were immediately centrifuged at 10000*g* for 30 min, and the washed precipitate was resuspended in buffer and stored at −20 °C for later use. Pretreatment of mitochondria in this manner resulted in complete inhibition of the A form of human brain MAO as reported previously (Pearce & Roth, 1983).

MAO was assayed by the procedure described previously (Roth & Feor, 1978). Briefly, reaction mixtures were incubated in the presence or absence of amphetamine and contained one of the ^{14}C-labeled substrates (tryptamine, tyramine, or benzylamine) and various concentrations of enzyme in a total volume of 0.4 mL of 0.05 M potassium phosphate buffer, pH 7.4. Reactions were initiated by the addition of the appropriate enzyme preparation and terminated by the addition of 50 μL of 0.4 M HCl. Blank values were determined in reactions performed in the presence of 1 mM pargyline. To determine the effect of oxygen on the reaction kinetics, experiments were performed in a manner similar to that described above, except prior to incubation flasks were sealed with rubber septums and flushed for 3 min with various concentrations of oxygen as indicated in the text.

Protein concentrations were determined by the method of Lowry et al. (1951), and kinetic constants were determined by computer analysis using nonweighted linear regression analysis of Lineweaver–Burk plots. Reaction velocities were measured on the linear portion of the velocity curve with respect to time and protein concentration.

Radioactively labeled [^{14}C]tryptamine bisuccinate (50.3 mCi/mmol), [^{14}C]phenylethylamine hydrochloride (48.3 mCi/mmol), and [^{14}C]tyramine (7.7 Ci/mmol) were purchased from New England Nuclear, Boston, MA, and [^{14}C]benzylamine hydrochloride (56 mCi/mmol) was obtained from Amersham Corp., Arlington Heights, IL. Unlabeled tyramine hydrochloride, phenylethylamine hydrochloride, tryptamine hydrochloride, benzylamine hydrochloride, and pargyline hydrochloride were obtained from Sigma Chemical Co., St. Louis, MO. Bio-Rex 70 was obtained from Bio-Rad Laboratories, Richmond, CA. Clorgyline was a gift from May and Baker, England.

Results

Inhibition of Deamination by d-Amphetamine. Previous results from our laboratory (Roth, 1979) indicated that amphetamine produces a mixed pattern of inhibition on double-reciprocal plots when amine is the varied substrate. As shown in Figure 1, amphetamine inhibition of phenylethylamine deamination when the second substrate, oxygen, was varied resulted in a mixed-type pattern with a linear intercept replot and a hyperbolic slope replot consistent with previous observations when amine was the varied substrate. In contrast, double-reciprocal plots for amphetamine inhibition of benzylamine deamination when amine was the varied substrate are indicative of a competitive inhibition pattern with a linear slope vs. amphetamine concentration replot. As illustrated in Figure 2, when oxygen was the varied substrate and the concentration of benzylamine was held constant, a family of parallel plots was obtained at varying amphetamine concentrations.

The observed kinetic patterns of amphetamine inhibition of phenylethylamine described suggest that two dead-end complexes are formed, one with the oxidized and the other with the reduced form of MAO. This observation is consistent with a ping-pong mechanism for phenylethylamine deamination. However, amphetamine apparently does not inhibit benzylamine deamination by the same mechanism since the kinetic data suggest that a nonproductive complex forms with the oxidized form (E_{ox}) only. The lack of dead-end complex formation between amphetamine and the reduced form ($E_{red.}$), when benzylamine is substrate, can readily be rationalized on the basis of the proposal of Husain et al. (1982). As these authors suggest, free $E_{red.}$ does not exist during benzylamine deamination. In this case, the ternary complex involving $E_{red.}$–aldehyde–oxygen prevents the substrate analogue

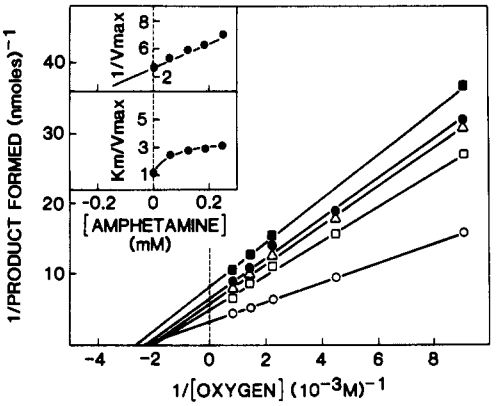


FIGURE 1: Lineweaver–Burk plot of the inhibition of phenylethylamine deamination by *d*-amphetamine with oxygen as the varied substrate. Varying concentrations of oxygen were incubated along with 5 μM phenylethylamine in the absence (○) or the presence of 62.5 (□), 125 (△), 187.5 (●), and 250 μM (■) amphetamine at 37 °C for 10 min. Enzyme (20–60 μg of protein, 200 μL) in 0.1 M potassium phosphate buffer, pH 7.4, was injected into the sealed tubes to initiate the incubations which were terminated by the addition of 50 μL of 0.4 M HCl. Assay for deaminated products by cation-exchange chromatography is presented in the text. Kinetic constants and plots were determined by computer analysis using nonweighted linear regression analysis of double-reciprocal plot data.

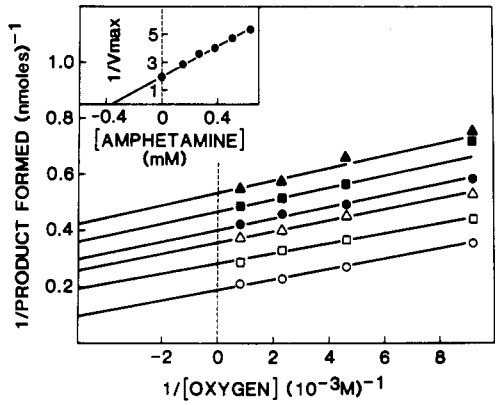


FIGURE 2: Lineweaver–Burk plot of the inhibition of benzylamine deamination by *d*-amphetamine with oxygen as the varied substrate. Varying concentrations of oxygen were incubated along with 100 μM benzylamine in the absence (○) or presence of 125 (□), 250 (△), 375 (●), 500 (■), and 625 μM (▲) amphetamine at 37 °C for 60 min. Enzyme (40–120 μg of protein, 200 μL) in 0.1 M potassium phosphate buffer, pH 7.4, was injected into the sealed tubes to initiate the incubations which were terminated by the addition of 50 μL of 0.4 M HCl. Deaminated products formed were assayed as described in the text.

amphetamine from binding to $E_{red.}$ at the site probably blocked by aldehyde.

The data presented above reveal that amphetamine might be useful as a probe of the mechanism by which any amine is deaminated by the B form of MAO. This approach assumes that amphetamine inhibition of deamination which gives rise to a competitive pattern on double-reciprocal plots is indicative of a reaction proceeding via a ternary complex intermediate whereas a mixed pattern of inhibition is diagnostic of a reaction involving exclusively binary complexes (i.e., ping-pong). Accordingly, the mechanism of deamination of two other MAO substrates, tyramine and tryptamine, by the B form of MAO was examined. Figures 3 and 4 illustrate the results of amphetamine inhibition of tyramine and tryptamine deamination, when amine was the varied substrate, respectively. A mixed-type inhibition pattern was observed in both cases, and replots of slope and intercept data were linear. As expected, when amphetamine inhibition of tyramine and

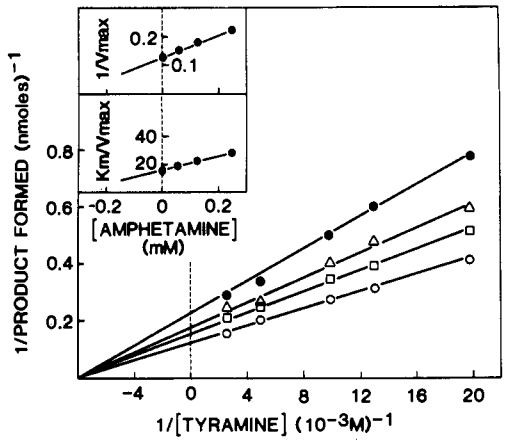


FIGURE 3: Lineweaver–Burk plot of the inhibition of tyramine deamination by *d*-amphetamine when the varied substrate was tyramine. Mitochondrial MAO was incubated in the presence of 5 μM clorgyline for 30 min at 37 °C, and the mitochondrial pellet was washed as described under Materials and Methods. Varying amounts of tyramine were incubated in the absence (○) or presence of 62.5 (□), 125 (△), and 250 μM (●) amphetamine at 37 °C for 60 min. Enzyme (150–250 μg of protein, 200 μL) in 0.1 M potassium phosphate buffer, pH 7.4, was added to initiate incubations which were terminated by the addition of 50 μL of 0.4 M HCl. Deaminated product formed was assayed as described in Figure 1.

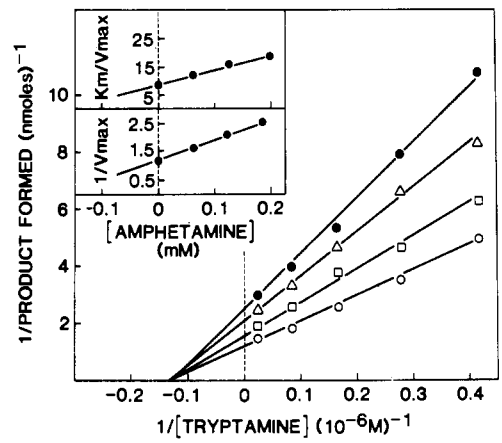


FIGURE 4: Lineweaver–Burk plot of the inhibition of tryptamine deamination by *d*-amphetamine when the varied substrate was tryptamine. Mitochondrial MAO was incubated in the presence of 5 μM clorgyline for 30 min at 37 °C, and the mitochondrial pellet was washed as described under Materials and Methods. Varying amounts of tryptamine were incubated in the absence (○) or presence of 62.5 (□), 125 (△), and 250 μM (●) amphetamine at 37 °C for 60 min. Clorgyline-pretreated enzyme (150–250 μg of protein, 200 μL) in 0.1 M potassium phosphate buffer, pH 7.4, was added to initiate incubations which were terminated by the addition of 50 μL of 0.4 M HCl. Deaminated product formed was assayed as described in Figure 1.

tryptamine deamination was examined with varied oxygen concentration, a mixed pattern was obtained for both substrates, and replots were again linear (data not shown).

Alternative Substrate Inhibition of Deamination. The results described above are consistent with the deamination of tyramine and tryptamine proceeding via binary complex formation involving E_{ox} and $E_{red.}$ identical with the model proposed for the reaction pathway for phenylethylamine deamination. While these findings are in complete agreement with the findings of Husain et al. (1982) and provide evidence in support of their proposal, experiments utilizing alternative substrate inhibition were undertaken to further test the hypothesis that deamination by MAO-B may involve two distinct pathways and that tyramine deamination and tryptamine deamination follow the same pathway as that of phenyl-

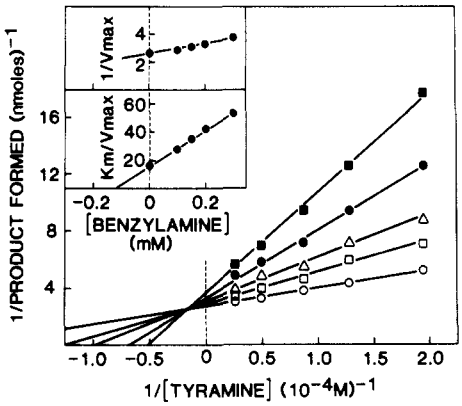


FIGURE 5: Lineweaver–Burk plot of the inhibition of tyramine deamination by benzylamine when tyramine was the varied substrate and when only the product of tyramine deamination was measured. Mitochondrial MAO was incubated in the presence of 5 μM clorgyline at 37 °C for 30 min, and the mitochondrial pellet washed as described under Materials and Methods. Varying amounts of tyramine were incubated in the absence (○) or presence of 100 (□), 150 (△), 200 (●), and 300 μM (■) benzylamine at 37 °C for 60 min. Clorgyline-pretreated enzyme (150–250 μg of protein, 200 μL) in 0.1 M potassium phosphate buffer, pH 7.4, was added to initiate incubations which were terminated by the addition of 50 μL of 0.4 M HCl and cooling in an ice–water bath. Deaminated product formed was assayed as described in Figure 1.

ethylamine. The equations for inhibition by the alternative substrate (A′) for a ping-pong mechanism in which either A or B is the varied substrate (Segel, 1975) are the following:

$$v/V_{max} = [A]/\{K_A(1 + K_{B'}[A']/K_{A'}[B] + [A']/K_{A'}) + [A](1 + K_B/[B])\} \quad (1)$$

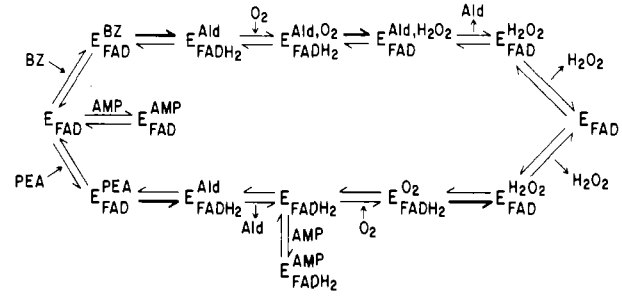
$$v/V_{max} = [B]/\{K_B(1 + K_{B'}K_A[A']/K_BK_{A'}[A]) + [B](1 + K_A/[A] + K_A[A']/K_{A'}[A])\} \quad (2)$$

where eq 1 is for varied [A] and eq 2 is for varied [B]. If the alternative substrate A′ only binds to a single form of the enzyme, as the above equations indicate, then A′ would be a competitive inhibitor of the deamination when [A] is varied and a noncompetitive inhibitor when [B] is varied. Illustrated in Figure 5 are the results of benzylamine (A′) inhibition of tyramine (A) deamination when tyramine was the varied substrate and only the product of tyramine deamination was measured. In contrast to the predicted inhibition pattern based on the equations, a mixed pattern of inhibition was obtained, and replots of slope and intercept data were linear. Similarly, inhibition of tyramine deamination by the alternative substrate benzylamine (A′) was also examined when oxygen (B) was the varied substrate, and these results reveal a mixed-type pattern of inhibition (data not shown). These data suggest that benzylamine most likely interacts with more than one form of the enzyme. Similarly, benzylamine inhibition of phenylethylamine deamination yielded a mixed-type inhibition pattern (Roth, 1976). Inhibition of benzylamine deamination by phenylethylamine could not be examined since phenylethylamine deamination, at the concentration needed to produce reasonable inhibition of benzylamine, was too extensive, resulting in significant changes in the concentrations of the inhibitor (phenylethylamine) during the incubation. However, when tyramine or tryptamine was used to inhibit benzylamine deamination, a simple competitive pattern of inhibition was observed (data not shown).

Discussion

The majority of the evidence from kinetic studies suggests that MAO proceeds via a binary complex mechanism consistent with a double-displacement reaction pathway. Recent stopped-flow kinetic studies by Husain et al. (1982), as discussed above, are compatible with this mechanism when phenylethylamine is substrate but suggest formation of the ternary complex when benzylamine is substrate for the B form of bovine liver MAO. While these authors have provided new insight into the mechanism of benzylamine and phenylethylamine deamination, their method unfortunately required the use of purified enzyme and the presence of detergent during stopped-flow analyses. As discussed in the introduction, a number of factors may have contributed to the proposed mechanistic differences that they observed for the deamination of phenylethylamine and benzylamine, and stopped-flow analysis may not be directly applicable to determination of the kinetic behavior of the native membrane-bound state of the B form of MAO.

Scheme I



Earlier reports from our laboratory and reports of others (Roth, 1979; Edwards & Burns, 1978) provided evidence that a number of drugs, including tricyclic antidepressants and amphetamine, inhibit the deamination of phenylethylamine and benzylamine by apparently different mechanisms. These drugs were consistently mixed inhibitors of phenylethylamine deamination and competitive inhibitors of benzylamine metabolism. These observations in light of the studies of Husain and co-workers (Husain et al., 1982) and those described in this paper are now reconcilable and ostensibly suggest that these drugs could be used as probes to differentiate the mechanism of deamination of any number of substrates. The reversible inhibitor of deamination, amphetamine, was chosen for this study because it is a substrate analogue of phenylethylamine which probably inhibits MAO via a selective interaction with the active site as suggested by previous findings in our laboratory (Roth, 1979). Initial experiments reported herein focused on the kinetics of inhibition of phenylethylamine and benzylamine deamination by the substrate analogue amphetamine. The kinetics of amphetamine inhibition of phenylethylamine deamination indicate that two inhibitory complexes are formed involving E_{ox} and $E_{red.}$ whereas with benzylamine apparently only a single inhibitor complex is formed involving E_{ox}. These findings are entirely consistent with the pathways for deamination proposed by Husain et al. (1982) as depicted in Scheme I. Accordingly, amphetamine can function as a useful probe of the mechanism of deamination by the B form of MAO. When amphetamine was utilized in this fashion, deamination of tyramine and tryptamine was shown to occur via a pathway indistinguishable from that followed by phenylethylamine.

To further test the hypothesis that there exist two distinct pathways for the deamination of phenylethylamine and benzylamine, the kinetics of alternative substrate inhibition of MAO were investigated. As discussed under Results, an alternative substrate, A′, in a ping-pong mechanism should act as a simple competitive inhibitor when deamination of substrate A is measured (eq 1). However, when phenylethylamine

was used as substrate and benzylamine was used as the alternative substrate inhibitor, a mixed-inhibition pattern was obtained. These results suggest that benzylamine not only is acting as a simple competitive inhibitor involving only E_{ox} but also is inhibiting by binding to $E_{red.}$ as proposed for the inhibition by amphetamine. Substrate binding to $E_{red.}$ has been proposed as the mechanism for substrate inhibition observed when phenylethylamine is substrate. Additionally, substrate inhibition involving E_{ox} or its equivalent is characteristic of ping-pong systems (Segel, 1975). Predictably, when benzylamine inhibited the deamination of either tyramine or tryptamine, again the kinetic patterns were the same as that seen for benzylamine inhibition of phenylethylamine deamination. In addition, the absence of an intercept effect on double-reciprocal plots (competitive inhibition) of either tyramine or tryptamine inhibition of benzylamine deamination is consistent with the existence of a ternary complex preventing the interaction of these substrates with the reduced form of MAO when benzylamine was the varied substrate.

The results of this study also clarify a heretofore inexplicable difference between benzylamine and phenylethylamine deamination in that only phenylethylamine, at high concentrations (approximately 5 times the K_m for human brain enzyme), will inhibit its own deamination by forming a nonproductive complex with the reduced form of the oxidase. Numerous attempts in our laboratory and others have failed to demonstrate substrate inhibition by benzylamine with the B form of human MAO. It was assumed that benzylamine would not bind to the reduced form of the oxidase; yet as noted above, benzylamine inhibits the deamination of phenylethylamine, tyramine, and tryptamine in a manner which can best be interpreted on the basis of its interaction with both E_{ox} and $E_{red.}$. These observations can now be readily rationalized in terms of the existence of a ternary complex with $E_{red.}$ when benzylamine is the sole substrate, which precludes binding of benzylamine to the free reduced form of the enzyme since it does not exist. This accounts for the absence of substrate inhibition when benzylamine is substrate for the B form of MAO.

Another important observation taken from this study is the fact that benzylamine is unique among the substrates examined in its ability to form a detectable ternary complex with $E_{red.}$. The reason for the singularity of benzylamine cannot be definitively addressed at present although several possible explanations exist. One possibility is that the structure of the intermediate or the benzylamine-derived aldehyde product may uniquely distort the reduced flavin ring system to bring about a change in the rate at which reoxidation occurs. Thus, when benzylamine is the substrate, reoxidation of the flavin occurs more rapidly than loss of product from the reduced enzyme complex. An alternative explanation precludes the existence of a unique imine intermediate generated during deamination of benzylamine. Of the four possible imine intermediates produced from the oxidation of benzylamine, tyramine, tryptamine, and phenylethylamine, only benzylamine is resonance stabilized due to conjugation with the aromatic ring system of the flavin. Accordingly, the predictably greater stability of the benzylimine–$FADH_2$ complex may account for the kinetically detectable ternary complex. Furthermore, conjugation with the flavin prosthetic group could be responsible for the accelerated reoxidation observed by Husain et al. (1982).

The findings presented herein demonstrate that kinetic analysis of the inhibition of deamination by amphetamine and alternative substrates provides a convenient method for examining the kinetics of deamination by the B form of MAO. Such analyses utilizing enzyme in its native membrane and in the absence of detergent provide strong evidence supported the conclusions of Husain et al. (1982) that deamination of benzylamine and phenylethylamine deamination occur via two distinct mechanisms involving ternary and binary complex formation, respectively. Our results have extended mechanistic studies to include two additional endogenous substrates for the B form of the oxidase and, for the first time, demonstrate that tyramine and tryptamine are deaminated by a ping-pong-like mechanism indistinguishable from that followed by phenylethylamine.

Registry No. MAO, 9001-66-5; phenylethylamine, 64-04-0; benzylamine, 100-46-9; tyramine, 51-67-2; tryptamine, 61-54-1; oxygen, 7782-44-7; *d*-amphetamine, 51-64-9.

References

Achee, F. M., & Gabay, S. (1981) *Biochem. Pharmacol. 30*, 3151–3157.

Bright, H. J., & Porter, D. J. T. (1975) *Enzymes, 3rd Ed. 12*, 421–503.

Coleman, R. (1973) *Biochim. Biophys. Acta 300*, 1–30.

Edwards, D. J., & Burns, M. O. (1974) *Life Sci. 15*, 2045–2058.

Fowler, C. J., & Oreland, L. (1979) in *Monoamine Oxidase: Structure, Function and Altered Functions* (Singer, T. P., Vonkorff, R. W., & Murphy, D. L., Eds.) pp 145–151, Academic Press, New York.

Fowler, C. J., & Oreland, L. (1980) *J. Pharm. Pharmacol. 32*, 681–688.

Haury, R. H., & Faulkner, R. (1981) *J. Biol. Chem. 256*, 9211–9215.

Hellerman, L., & Erwin, V. G. (1968) *J. Biol. Chem. 243*, 5234–5243.

Houslay, M. D. (1980) *Biochem. Pharmacol. 29*, 3211–3213.

Houslay, M. D., & Tipton, K. F. (1973) *Biochem. J. 135*, 735–750.

Houslay, M. D., & Tipton, K. F. (1975) *Biochem. J. 145*, 311–321.

Husain, M., Edmondson, D. E., & Singer, T. P. (1982) *Biochemistry 21*, 595–600.

Kandaswami, C., & D'Iorio, A. (1978) *Arch. Biochem. Biophys. 198*, 847–849.

Lowry, O. H., Rosenbrough, A., Farr, L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 267–275.

Oi, S., Shimada, K., Inamasu, M., & Yasunobu, K. T. (1970) *Arch. Biochem. Biophys. 139*, 28–37.

Oi, S., Yasunobu, K. T., & Westley, J. (1971) *Arch. Biochem. Biophys. 145*, 557–564.

Palmer, G., & Massey, V. (1968) in *Biological Oxidations* (Singer, T. P., Ed.) pp 264–267, Wiley, New York.

Pearce, L. B., & Roth, J. A. (1983) *Arch. Biochem. Biophys. 224*, 464–472.

Roth, J. A. (1976) *J. Neurochem. 27*, 1107–1112.

Roth, J. A. (1979) in *Monoamine Oxidase: Structure, Function and Altered Functions* (Singer, T. P., VonKorff, R. W., & Murphy, D. L., Eds.) pp 153–168, Academic Press, New York.

Roth, J. A., & Feor, J. (1978) *Biochem. Pharmacol. 27*, 1606–1608.

Roth, J. A., & Eddy, B. J. (1980) *Arch. Biochem. Biophys. 205*, 260–266.

Sanderman, H. J. (1978) *Biochim. Biophys. Acta 515*, 209–237.

Sawyer, S. T., & Greenwalt, J. W. (1979) *Biochem. Pharmacol. 28*, 1737–1744.

Segel, I. H. (1975) in *Enzyme Kinetics Behavior and Analysis*

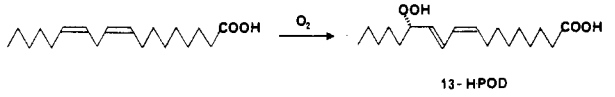
of Rapid Equilibrium and Steady State Enzyme Systems, pp 793–813, Wiley, New York.
Tipton, K. F. (1968) *Eur. J. Biochem. 5*, 316–320.
Yu, P. H. (1979) in *Monoamine Oxidase: Structure, Function and Altered Functions* (Singer, T. P., Vonkorff, R. W., & Murphy, D. L., Eds.) pp 233–244, Academic Press, New York.
Yu, P. H. (1981) *Biochem. Pharmacol. 30*, 1283–1297.

Inhibition of Soybean Lipoxygenase 1 by *N*-Alkylhydroxylamines†

Charles H. Clapp,* Amit Banerjee,‡ and Susan A. Rotenberg§
Department of Chemistry, Brown University, Providence, Rhode Island 02912
Received July 12, 1984

ABSTRACT: Micromolar concentrations of *N*-octylhydroxylamine dramatically increase the induction period in the conversion of linoleic acid to 13(*S*)-hydroperoxy-*cis*-9,*trans*-11-octadecadienoic acid (13-HPOD) catalyzed by soybean lipoxygenase 1. The induction period produced by *N*-octylhydroxylamine is abolished by 13-HPOD but not by the corresponding hydroxy acid. Addition of a catalytic amount of lipoxygenase to a mixture of 13-HPOD and *N*-octylhydroxylamine results in consumption of approximately 1 μmol of 13-HPOD/μmol of *N*-octylhydroxylamine present. These results can be explained by a model in which 13-HPOD oxidizes the enzyme from an inactive ferrous form to an active ferric form, as proposed by previous workers, and *N*-octylhydroxylamine reduces the enzyme back to the ferrous form. Consistent with this model, the ESR signal at $g = 6.1$ characteristic of ferric lipoxygenase is rapidly abolished by *N*-octylhydroxylamine and can be regenerated by 13-HPOD. These results provide additional support for earlier proposals that ferric lipoxygenase is the catalytically active form and also establish a novel method of inhibiting enzymes in this class. The octyl group of *N*-octylhydroxylamine appears to contribute to binding near the iron, since hydroxylamine and *N*-methylhydroxylamine do not extend the induction period. In the *n*-RNHOH series, activity passes through an optimum at R = decyl.

Soybean lipoxygenase 1 catalyzes the oxygenation of linoleic acid to 13(*S*)-hydroperoxy-*cis*-9,*trans*-11-octadecadienoic acid (13-HPOD)[1] [for reviews, see Axelrod (1974) and Veldink et al. (1977)].



13-HPOD

The enzyme will also oxygenate other fatty acids and fatty acid derivatives that have a *cis*,*cis*-1,4-diene unit that begins on the sixth carbon from the methyl terminus. Lipoxygenases are widespread in the plant kingdom, and lipoxygenase 1 is one of three isoenzymes that have been purified from soybeans. Interest in the mechanism and inhibition of lipoxygenases has been stimulated by recent discoveries of several physiologically and pharmaceutically interesting lipoxygenase reactions in mammalian arachidonic acid metabolism (Bailey & Chakrin, 1981).

Lipoxygenase 1 has a molecular weight of about 100 000 and contains one atom of non-heme iron per molecule (Chan, 1973; Roza & Francke, 1973; Pistorius & Axelrod, 1973). Removal of the iron results in loss of catalytic activity (Pistorius & Axelrod, 1974). The purified enzyme is ESR silent, and magnetic susceptibility measurements indicate that the iron is in the high-spin ferrous state (Slappendel et al., 1982b; Cheesbrough & Axelrod, 1983). Treatment of the enzyme with 1 equiv of its product, 13-HPOD, results in oxidation of the iron to a high-spin ferric form that exhibits an ESR signal near $g = 6$ and a weak ultraviolet absorption at 320 nm (De Groot et al., 1975a,b; Pistorius et al., 1976; Slappendel et al., 1981).

Under some assay conditions, lipoxygenase 1 exhibits a short induction period that appears to be due to activation of the enzyme by product, since no induction period is observed if low concentrations of 13-HPOD are present at the start of the reaction (Haining & Axelrod, 1958). The corresponding alcohol, 13-HOD, does not eliminate the induction period (Gibian & Galaway, 1976; Funk et al., 1981). The induction period can be extended by addition of glutathione peroxidase in the presence but not in the absence of glutathione, presumably due to consumption of 13-HPOD (Smith & Lands, 1972).

The apparent product activation can be reconciled with the physical studies by the hypothesis that the ferric enzyme is the catalytically active form (De Groot et al., 1975a; Pistorius et al., 1976). Further support for this notion comes from the observation that the spectroscopic features of the ferric enzyme can be eliminated by treatment with linoleic acid under anaerobic conditions (De Groot et al., 1975a; Egmond et al., 1977). Vliegenthart and co-workers have proposed a catalytic cycle consistent with these facts (De Groot et al., 1975a).

† Presented in preliminary form at the 186th National Meeting of the American Chemical Society, Washington DC, Aug 28–Sept 2, 1983 (Clapp et al., 1983). This work was supported by the National Institutes of Health through a research grant (GM 28347) to C.H.C. and a Biomedical Sciences Research Support Grant to Brown University. The ESR spectrometer was purchased with a grant from the National Science Foundation.
* Address correspondence to this author at the Department of Chemistry, Haverford College, Haverford, PA 19041-1392.
‡ Present address: Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104.
§ Present address: Division of Molecular Biology and Virology, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

[1] Abbreviations: 13-HPOD, 13(*S*)-hydroperoxy-*cis*-9,*trans*-11-octadecadienoic acid; 13-HOD, 13(*S*)-hydroxy-*cis*-9,*trans*-11-octadecadienoic acid; ESR, electron spin resonance; NMR, nuclear magnetic resonance; UV, ultraviolet; SDS, sodium dodecyl sulfate.

0006-2960/85/0424-1826$01.50/0