Evidence against a Nucleophilic Mechanism for Monoamine Oxidase-Catalyzed Amine Oxidation

Richard B. Silverman* and Xingliang Lu

Department of Chemistry and Department of Biochemistry, Molecular Biology, and Cell Biology Northwestern University, Evanston, Illinois 60208-3113

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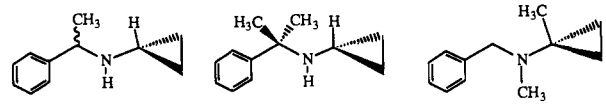
Monoamine oxidase (MAO, EC 1.4.3.4) catalyzes the oxidative deamination of a variety of amine neurotransmitters; benzylamine also is an excellent substrate for the enzyme. Results over the last 13 years with the purified MAO B isozyme and a variety of amines have provided experimental evidence consistent with an electron-transfer mechanism of amine oxidation.[1] A recent paper by Kim et al.[2] describing model chemistry to support a polar nucleophilic mechanism for the oxidation of benzylamine by 3-methyllumiflavin prompts this communication, which provides evidence to indicate that this model chemistry is probably unrelated to the mechanism of MAO-catalyzed amine oxidation.

Evidence provided by Kim et al.[2] appears to substantiate a nucleophilic mechanism in their model reaction. However, the drastic conditions of this reaction (wet acetonitrile containing 10 mM HCl heated to 80 °C for 7 days to give a 30% conversion of benzylamine to *N*-benzylbenzaldimine) are far from the conditions of the enzyme-catalyzed reaction, which has a rather high pH optimum (pH 9.0) for an enzyme. The model reaction does not proceed either in the absence of HCl or $MgCl_2$ or at 25 °C. Furthermore, when the secondary amine, *N*-methylbenzylamine, was used in the model study, no reaction occurred. *N*-Methylbenzylamine, however, is as good a substrate for MAO B as is benzylamine.[3] The tertiary amine used in the model study (*N*-methyl-*N*-((trimethylsilyl)methyl)benzylamine) was completely unreactive. However, some tertiary amines are good substrates for MAO B.[4] An internal deuterium isotope effect (k_H/k_D) for benzylamine-d_1 ($PhCHDNH_2$) oxidation in the model reaction (dark, 80 °C, 6 days) was 4.3 and for a known photochemical SET reaction[5] ($\lambda > 320$ nm, 20 °C, 1 h) was 1.6. A reference was cited to the MAO B-catalyzed oxidation of benzylamine and [α,α-$^2H_2$]benzylamine[6] which showed an isotope effect of 8.7 at 25 °C for the reductive half reaction, and it was pointed out by Kim et al.[2] that the nucleophilic reaction isotope effect (4.3) was closer to the enzymatic isotope effect (8.7) than was the SET isotope effect (1.6). However, enzyme-catalyzed reactions can be very different from solution reactions, particularly when carried out under such different conditions. The observed chemical and enzymatic isotope effects are determined from the relative rates of the *two* steps in the reaction (either SET followed by deprotonation, if it is a SET mechanism, or nucleophilic addition and deprotonation, if it is a nucleophilic mechanism) *and* the pK_a of the proton acceptor.[7] The rates of each of the two steps in the enzymatic reductive half reaction can be different from those in solution, and the pK_a of the acceptor will, most likely, be different. When the pK_a of the proton acceptor is matched with the acidity of the proton being removed, maximal isotope effects are observed.[7] It is not surprising, then, that there is a large difference between the isotope effects obtained in the two model reactions as well as with the enzyme-catalyzed reaction. In the study where the enzymatic isotope effect was measured[6] it also was shown that the isotope effect for the first step when β-phenylethylamine is the substrate was only 3. If C–H bond cleavage were the only consideration in an enzyme-catalyzed reaction, then the isotope effect on β-phenylethylamine would have been at least as large as that for benzylamine. Therefore, a direct comparison of isotope effects in solution and on an enzyme often is misleading. Finally, no C-4a flavin adduct has been reported by those who have attempted to detect this by stopped-flow techniques; if it exists, it must be very short-lived.

The observation by Kim et al.[2] that the order of the rate of amine oxidation via a polar nucleophilic mechanism (primary > secondary ≫ tertiary; actually, there was *no* reaction with the tertiary amine) is consistent with steric hindrance to C-4a flavin addition by the amine. Steric hindrance, however, apparently is not very important in, at least, certain MAO-catalyzed amine oxidations because *N*-cyclopropyl-α-methylbenzylamine (**1**),[1f] *N*-cyclopropyl-α,α-dimethylbenzylamine (**2**),[1f] and *N*-methyl-*N*-(1-methylcyclopropyl)benzylamine (**3**)[1e] are all oxidized by MAO; compound **1** is converted by MAO to the expected oxidation



1 **2** **3**

products, acetophenone and cyclopropylamine, in addition to causing enzyme inactivation.[1f] Two other, even more sterically hindered amines, *N*-(1-methylcyclopropyl)-α-methylbenzylamine (**4**; R = H) and N,α-dimethyl-*N*-(1-methylcyclopropyl)benzylamine (**4**; R = CH_3) now have been synthesized.[8] Both are

* Address correspondence to this author at the Department of Chemistry.

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(8) *N*-(1-Methylcyclopropyl)-α-methylbenzylamine hydrochloride (**4**; R = H) was synthesized by the same method used to synthesize *N*-(1-methylcyclopropyl)benzylamine hydrochloride[1e] and *N*-cyclopropyl-α-methylbenzylamine hydrochloride[1f] except using 1-methylcyclopropylamine and acetophenone: mp 202–204 °C; NMR (D_2O) δ 7.54 (5 H, s), 4.76 (1 H, q), 1.73 (3 H, d), 1.49 (3 H, s), 1.05 (1 H, m), 0.805 (1 H, m), 0.609 (2 H, m). Anal. Calcd for $C_{12}H_{18}ClN$: C, 68.09; H, 8.51; N, 6.62. Found: C, 67.96; H, 8.44: N, 6.61. N,α-Dimethyl-*N*-(1-methylcyclopropyl)benzylamine (**4**; R = CH_3) was prepared from *N*-(1-methylcyclopropyl)-α-methylbenzylamine by reductive amination with aqueous formaldehyde in methanol and sodium cyanoborohydride: colorless liquid (82–85 °C/8 mm Torr); NMR ($CDCl_3$) δ 7.30–7.35 (5 H, m), 7.23 (1 H, m), 3.66 (1 H, m), 2.19 (3 H, s), 1.45 (3 H, m), 1.17 (3 H, s), 0.913 (1 H, m), 0.571–0.604 (1 H, m), 0.529 (1 H, m), 0.295 (1 H, m). Anal. Calcd for $C_{13}H_{19}N$: C, 82.54; H, 10.05; N, 7.41. Found: C, 82.97; H, 10.15; N, 7.52.

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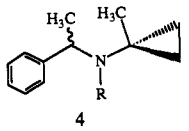

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excellent time-dependent inactivators of MAO;[9] the K_I and k_{inact} values are 39 μM and 0.091 min^{-1} for **4** (R = H) and 16 μM and 0.71 min^{-1} for **4** (R = CH_3). Note that at saturation the maximal inactivation rate constant (k_{inact}) is 8 times *greater* for the tertiary amine than for the secondary amine. Furthermore, after enzyme inactivation the flavin absorption spectrum shows reduced flavin, indicating that a two-electron oxidation of the amines has occurred, even though these are highly sterically hindered substrates. These results are consistent with an electron transfer mechanism, but not with a nucleophilic mechanism.

Other evidence consistent with an electron transfer mechanism is the reaction of MAO B[1i] and cytochrome P-450 with 1-phenylcyclobutylamine. MAO B catalyzes the conversion of 1-phenylcyclobutylamine to 2-phenyl-1-pyrroline, a reaction precedented by known nonenzymatic one-electron chemistry.[10] When this reaction is carried out in the presence of a radical spin trap, the expected triplet of doublets at g = 2.00 in the EPR spectrum was observed,[1k,11] again suggesting a radical intermediate in the reaction. Furthermore, cytochrome P-450, an enzyme for which there is considerable support in favor of a radical mechanism, catalyzes the identical reaction with 1-phenylcyclobutylamine as does MAO B.[13]

Recently, we reported the mechanism of MAO-catalyzed oxidation of (*R*)- and (*S*)-1-phenyl[2,2-$^2H_2$]cyclopropylamine.[1s] A normal secondary deuterium isotope effect on ring opening was observed, which is consistent with a mechanism involving ring cleavage followed by radical combination. A nucleophilic mechanism would involve attack of the active-site cysteine on the cyclopropyl ring concomitant with elimination of the reduced flavin; this mechanism would give an inverse secondary isotope effect (more crowded transition state), which is not observed.

In summary, the study by Kim et al.[2] provides the first evidence that, if conditions are set up appropriately, a nucleophilic reaction of primary amines with a flavin model is possible. However, it is our opinion that the large body of evidence to date suggests that this mechanism is not relevant to the reaction catalyzed by monoamine oxidase.

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(9) The kinetic constants were determined as previously described.[1e,1f]

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(11) Reference 1k describes the reaction of an analogue of 1-phenylcyclobutylamine, namely, 1-benzoylcyclobutylamine; however, the same EPR observation was made for 1-phenylcyclobutylamine.[12]

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