Catalytic Amine Oxidation under Ambient Aerobic Conditions: Mimicry of Monoamine Oxidase B**


Abstract: The flavoenzyme monoamine oxidase (MAO) regulates mammalian behavioral patterns by modulating neurotransmitters such as adrenaline and serotonin. The mechanistic basis which underpins this enzyme is far from agreed upon. Reported herein is that the combination of a synthetic flavin and alloxan generates a catalyst system which facilitates biomimetic amine oxidation. Mechanistic and electron paramagnetic (EPR) spectroscopic data supports the conclusion that the reaction proceeds through a radical manifold. This data provides the first example of a biorelevant synthetic model for monoamine oxidase B activity.

Monoamine oxidase (MAO) is a mitochondrial flavin-dependent oxidoreductase enzyme which oxidizes a range of important amines to imines, for example, the neurotransmitters serotonin, histamine, and noradrenaline.[1] With such an integral role in the neurochemical network, MAO function has been pinpointed as an underlying rationale for a range of behavioral, evolutionary, and physiological observations. For example, variations in the MAO A gene can lead to increased aggression, known as the “warrior gene”, ultimately impacting human evolution.[2] Inhibition of MAO has been an important area for medicinal chemistry with MAO inhibitors (MAOIs) acting as potent antidepressants and having potential applications as neuroprotective agents.[3] Mechanistic studies have also helped in understanding the role of lysine-specific demethylase 1 (LSD1), a key epigenetic modulator, with MAOIs impacting a number of key biological processes.[4]

It is remarkable that no consensus has been reached with respect to a mechanism of action, despite over 45 years of investigation.[5] There are two isozymes of MAO: MAO-A and MAO-B. While the flavin active sites are identical, each form displays a different substrate and inhibitor profile, and the mechanistic basis of this selectivity unknown.[6]

2H primary kinetic isotope (KIE) effects have been observed for the C–H bond cleavage step(s) with both MAO A and B. In principle, rate-contributing cleavage may be envisaged as proceeding by either H²⁻, H⁻, or H⁻ transfer mechanisms (Scheme 1). These options have been widely discussed,[3] with rate-contributing C–H cleavage by H⁻ transfer being the most prevalent mechanistic description. Two mechanistic postulates have been developed to account for the requisite increase in acidity of the relevant α-amino C–H bond: the formation of a covalent flavin–amine conjugate,[7] and the formation of an aminium radical cation[8] after single-electron transfer from amine to flavin. As both mechanisms require discrete steps prior to the rate-contributing C–H cleavage, it is notable that no intermediates accumulate to observable populations. C–H cleavage in the context of a direct hydride transfer has also been suggested.[9] However, such a synchronous event would not be consistent with the 13N KIE measured for amine oxidation by MAO B, thus pointing to an absence of synchronicity between C–H cleavage and sp²–sp³ nitrogen atom re-hybridization.[10]

Finally, H⁻ transfer from the substrate to the flavin has been
suggested.\[11\] This possibility was discarded on the grounds that no hydrogen-atom abstracting moiety, which was reactive enough to overcome relevant α-amino C–H bond dissociation energies, could be identified in the enzyme active site.\[12\]

Studies using synthetic flavins have played a crucial role in elucidating flavoenzyme mechanisms.\[13\] Accordingly, insight gained from studying model cofactors is a valid strategy to unlocking mechanistic problems in flavoenzymology. Pioneering work on primary amines by various groups supported the polar, proton-transfer mechanism, but the low turnover, tendency of catalysts to decompose, and requirement of heating in an enriched O₂ atmosphere for several days meant that they are perhaps of limited relevance to biological processes.\[14\] Wc,\[15\] and others,\[16\] have previously applied cationic flavin catalysts in biomimetic monoxygenase contexts, as well as donor–acceptor chemistry,\[17\] and now report the oxidation of biologically pertinent amines as a vehicle to understanding MAO-B mechanism.

Initial exploratory studies demonstrated catalytic aerobic oxidation of benzylamine, with formation of the imine 4a being consistent with oxidase rather than monoxygenase-like reactivity (Table 1).\[18\] Excellent yields of 4a were obtained if a thioether additive (Me₂S) and a cocatalyst, alloxan (3a), were used (Table 1). Initially 3a was present as an undetected by-product from the synthesis of 2a, however, was found to be crucial for this transformation. N,N-dimethylalloxan (3b) was found to be inactive (entry 3) despite possessing structural similarity to 3a. Additionally, cobalamin synthase, BluB, has been implicated in the cannibalization of flavin mononucleotide to form alloxan, which acts as a crucial multifunctional redox catalyst in the biosynthesis of vitamin B12.\[19\] A series of substituted benzylamines, typical substrates for MAO-B, have been examined.

Generally, high yields of imine products are attainable, although substrates with a strongly electron-withdrawing para-substituent group (entry 9) are less reactive, thus mirroring MAO-B reactivity trends.

Upon attempted in situ ¹H NMR analysis, the inability to locate the lock signal suggested paramagnetic behavior. Accordingly, EPR studies at the X-band were initiated. Mixing 2a and Me₂S generated the flavin radical cation 2a’ (Figure 1). The structure was further confirmed by pulsed EPR studies. In particular, the protonation state of 2a’ was assessed by electron spin echo envelope modulation (ESEEM), and is a rare example of an aerobically generated flavin semiquinone, having demonstrable relevance to catalysis, observed by EPR spectroscopy.\[20\] The use of a strong hydrogen-bonding solvent, trifluoroethanol, may aid the stabilization of the semiquinone formation, as discussed by Massey and co-workers, for flavins with amino acids.\[21\] Upon sequential addition of alloxan and amine, a new EPR spectrum was observed and characterized as the radical 4a’, and is consistent with charge-transfer-initiated hydrogen-atom abstraction from 4a. Hybrid-DFT and post-Hartree Fock calculations were performed on 2a’ and 4a’ and the spin density isosurfaces are shown in Figure 1.\[22\] Importantly, the theoretical calculations quantify the local spin density distribution, thus further corroborating the simulations of the continuous-wave EPR spectra.\[23\]

Kinetic studies provided additional important mechanistic information with the transformation being first order in benzylamine\[24\] and showing a KIE of k_H/k_D = 1.9 when using PhCD₂NH₂ (7), thus supporting rate-contributing C–H bond cleavage (Figure 2). A range of studied para-substituted benzylamines provided a negative Hammett correlation (ρ = −2).

The observed rates of reaction were found to be independent of the Me₂S concentration. Kinetic analysis for

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**Table 1: Flavin-organocatalyzed amine oxidation.**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate</th>
<th>R¹</th>
<th>Product</th>
<th>Yield [%]</th>
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<tr>
<td>1</td>
<td>1a</td>
<td>Ph</td>
<td>4a</td>
<td>94</td>
</tr>
<tr>
<td>2</td>
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<td>4c</td>
<td>95</td>
</tr>
<tr>
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<td>1d</td>
<td>4-BrC₆H₄</td>
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<td>92</td>
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<td>4-FC₆H₄</td>
<td>4e</td>
<td>98</td>
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<tr>
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<tr>
<td>17</td>
<td>1o</td>
<td>1-naphthyl</td>
<td>4o</td>
<td>72</td>
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</tbody>
</table>

[a] Reaction conditions: 2a (2 mol %), 3a (2 mol %), Me₂S (10 equiv), 5 h. [b] 2b used. [c] 3b used. [d] 18 h. [e] 2a (4 mol %), 3a (4 mol %) used.
3a did not demonstrate a simple reaction order, with saturation behavior observed over the concentrations examined (see the Supporting Information). The kinetic order in 2a was probed by means of a \( \ln(k_{\text{obs}}) \) versus \( \ln([\text{flavin}]) \) plot, which was linear with a slope of 0.25 and consistent with de-aggregation of a higher order resting state, but with a monoergic semiquinone being catalytically active. Significantly, the less oxidizing flavin 2b also mediates this reaction (\( k_{\text{obs2b}} = 2.96 \)) with an electrochemical reduction potential of +66 mV vs. SHE, which parallels MAO-B at +40 mV (Table 1, entry 2).[29] Therefore, the flavin catalysts 2a,b offer themselves as realistic mimics of MAO through the neutral N(5)-H semiquinone.[26]

A mechanism that accounts for EPR and kinetic data is underpinned by the realization that rate-determining C–H cleavage is mediated by 2a' (Scheme 2). The radical cation 2a' is formed by a proton-coupled electron transfer from MeS, as observed by EPR. BnNH2 promotes the formation of 2a' by mediating the de-aggregation and deprotonation of 2a', thus generating the neutral semiquinone 2a'', with subsequent 1a' transfer, initiated by a charge-transfer event, from 1a to 2a''. An \( \alpha \)-amino radical is formed (1a''), and it acts as a potent reductant,[23] thus reducing alloxan and forming 1a'. Electron transfer from \( \alpha \)-amino radicals to vicinal dicarbonyl compounds is regarded as one of the fastest reactions between a radical and a neutral closed-shell organic molecule.[24] Alloxan (3a) reacts as an amide tautomer, thus allowing stabilization of a developing oxyanion character, a feature which is impossible for the inactive 3b (Table 1, entry 3). This captodative-stabilized radical[29] subsequently reacts with O\(_2\), thus generating 5. The peroxy radical 5 oxidizes 2a'' to 2a', thus forming the hydroperoxide 5' and completing the catalytic cycle. Formation of stoichiometric DMSO is observed. Therefore MeS mediates the reduction of 5' to alloxan. Additionally, a purple by-product, consistent with the dye murexide (6; UV/vis \( \lambda_{\text{max}} = 521 \) nm; lit = 520 nm),[30] is observed to accumulate from 3a'', 3a, and ammonia. This observation is consistent with a two-electron over-reduction of 3a, thus leading to catalyst deactivation and suggesting that 3a'' is not a catalytically active species.

This model study supports a homolytic C–H bond cleavage mediated by a flavin semiquinone, and with a substrate preference for benzylamines, it has prompted us to ask whether any reasonable insight into the enzymatic mechanism of MAO B can be achieved through consideration of this currently presented model system. A linear correlation exists between the substrate pK\(_a\) value and steady-state \( k_{\text{cat}} \) for MAO B (Figure 3).[31] and is consistent with a neutral amine substrate. It is significant that Hammett electronic correlations for MAO B are only apparent at pH 9.0. The similarity of the model’s KIE and Hammett profiles to the equivalent B isozyme data, when the enzyme kinetics are measured at pH 9.0, which is similar to this unbuffered system, is notable, (MAO B: \( k_{\text{obs}}/k_{\text{cat}} = 2.25, \rho = -0.9 \) at pH 9.0).[32]

Our proposal for the MAO B mechanism is informed by the presented data, the substrate reactivity trends, and the pH sensitivity of MAO B.[34] This mechanistic suggestion centers upon a charge-transfer event promoted by the free-base substrate interacting with an electron-rich phenol of Y398 near the flavin acceptor, as demonstrated by Scrutton and coworkers.[33] This acceptor is itself activated by the H\(_2\)O–K296 hydrogen-bonding motif. The neutral semiquinone thus formed can mediate hydrogen-atom transfer from the substrate, with the tyrosinyl radical cation now able to accept the second substrate electron, in direct analogy to the role played
by alloxan in the currently discussed model. Indeed, both components can be viewed as redox-active hydroxylated units.

In summary, an aerobic, catalytic oxidation of benzylamines which mimics MAO B activity proceeding through charge-transfer-initiated substrate H abstraction has been developed. EPR spectroscopy has revealed the operation of an aerobically generated flavin semiquinone. KIE and Hammett studies have demonstrated a pH-dependent kinetic parallel to MAO B activity. This model system has opened up an additional mechanistic model of MAO B activity, that is, a charge-transfer event is harnessed to access a reactive neutral flavin semiquinone as the C–H abstracting species in MAO B.

Keywords: amines · enzymes · EPR spectroscopy · oxidation · reaction mechanisms

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[23] For full discussion of EPR spectroscopy and calculations, see the Supporting Information.
[24] The reaction surprisingly becomes apparently zero order in amine after 1h of reaction time, perhaps because oxygen becomes rate contributing.
[26] ES-MS monitoring did not reveal any catalyst–amine adducts that would be expected from polar mechanisms.

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