RESEARCH ARTICLE

Why Does Monoamine Oxidase (MAO) Catalyze the Oxidation of Some Tetrahydropyridines?

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Abstract: Results pertaining to the mechanism of the oxidation of the tertiary amine 1-methyl-4-(1-methyl-1-H-pyrrol-2-yl)-1,2,3,6-tetrahydropyridine (MMTP, a close analog of the Parkinsonism inducing compound MPTP) by 3-methyllumiflavin (3MLF), a chemical model for the FAD cofactor of monoamine oxidase, are reported. MMTP and related compounds are among the few tertiary amines that are monoamine oxidase B (MAO-B) substrates. The MMTP/3MLF reaction is catalytic in the presence of O_2 and the results under anaerobic conditions strongly suggest the involvement of radical intermediates, consistent with a single electron transfer mechanism. These observations support a new hypothesis to explain the MAO-catalyzed oxidations of amines. In general, electron transfer is thermodynamically unfavorable, and as a result, most 1° and 2° amines react via one of the currently accepted polar pathways. Steric constraints prevent 3° amines from reacting via a polar pathway. Those select 3° amines that are MAO substrates possess certain structural features (e.g., a C-H bond that is α- both to nitrogen and a C=C) that dramatically lower the pK_a of the corresponding radical cation. Consequently, the thermodynamically unfavorable electron transfer equilibrium is driven towards products by an extremely favorable deprotonation step in the context of Le Chatelier's principle.

Introduction

Monoamine oxidase A (MAO-A) and -B (MAO-B) catalyze the oxidation of various neurotransmitters, including dopamine, norepinephrine, epinephrine and serotonin. The overall reaction is a two-electron α-carbon oxidation, $R'NH\text{-}CH_2R \rightarrow R'N{=}CHR$, that is coupled to the two-electron reduction of the flavin cofactor FAD to $FADH_2$. Several mechanisms have been proposed to account for the initial stages of the mechanism of MAO-catalyzed oxidations[1] including conventional "two-electron/polar" pathways such as nucleophilic addition[2] and hydride transfer,[3] as well as a single electron transfer (SET) pathway that involves radical intermediates.[3-4] 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a selective MAO-B substrate, and a precursor to the Parkinsonian syndrome-inducing neurotoxic pyridinium species MPP^+ (Figure 1).[5] Moreover, MPTP is one of the very few tertiary amines that are MAO substrates.

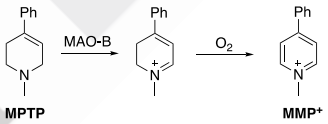

Figure 1. MAO-B catalyzes the oxidation of MPTP to MMP^+, which induces symptoms of Parkinson's disease in humans. MPTP (and some derivatives) are rare in that they are the only tertiary amines known to be oxidized by MAO.

In general, tertiary amines are not MAO substrates,[6] which makes sense in the context of the currently proposed and generally accepted mechanisms of catalysis. As noted, these mechanisms include a polar, nucleophilic addition pathway (which may be stepwise or concerted), or a hydride transfer pathway.[1a, 1b, 7] Current thinking is that both mechanisms proceed via the same transition state, with the difference being in the direction of electron flow (Figure 2).[8] Because of steric issues, tertiary amines cannot react by either of these mechanisms which require the nitrogen lone-pair of the amine, and C_{4a} of the flavin to be in close proximity in the transition state.

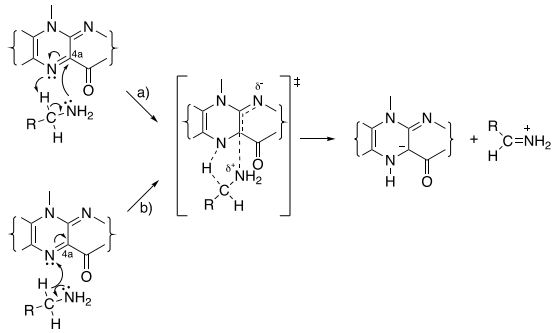

Figure 2. Proposed concerted mechanisms for MAO catalysis: a) a polar, nucleophilic pathway where the amine acts as a nucleophile, attacking C_{4a} of the flavin, and b) a hydride transfer pathway. Both mechanisms may proceed through the same five-membered transition state, with the difference being the direction of electron flow.

Single electron transfer mechanisms for MAO catalysis have been proposed by Silverman,[4a] Castagnoli,[9] and others.[10] The most compelling evidence for single electron transfer came from the behavior of MAO substates equipped with a single electron transfer "probe," essentially a compound with an *N*-cyclopropyl[4a, 4b, 9] or *N*-cyclobutyl group[11] which (because of ring strain) would undergo ring opening when a radical cation was produced. This is illustrated in Figure 3 with the *N*-cyclopropyl derivative of MPTP which is an irreversible MAO inhibitor. The rationale for inhibition is that ring opening produces a 1° radical which disrupts the active site of MAO through covalent bond formation.

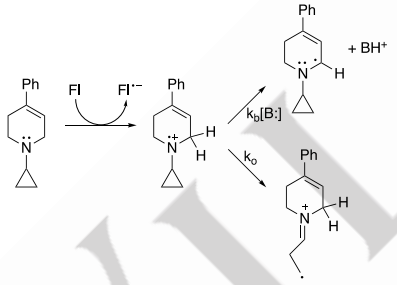

Figure 3. Use of a single electron transfer probe to examine the mechanism of MAO-catalyzed oxidations. The *N*-cyclopropyl group diverts the initially produced radical cation forming a 1° radical that disrupts the active site thereby deactivating the enzyme. Fl represents the flavin cofactor in MAO.

One of the classic arguments against the SET pathway is that the initial single electron transfer reaction is thermodynamically unfavorable,[12] and with a few exceptions,[1c, 10] the SET pathway seems to have fallen out of favor.[8a, 13] In 2020, we reported results for the oxidation of 1-methyl-4-(1-methyl-1*H*-pyrrol-2-yl)-1,2,3,6-tetrahydropyridine **(**MMTP, an MPTP derivative and MAO-B substrate with no known human toxicity) using a flavin biomimetic that provided compelling evidence that this class of compounds may react through an SET process. The reaction of 5-ethyl-3-methylumiflavinium perchlorate (Fl^+, Figure 4) with MMTP was monitored using NMR and EPR spectroscopy. The results suggested the intermediacy of radicals, an insight that came from studying this reaction under aerobic--but more importantly, under anaerobic conditions.[14]

In the absence of O_2, the reaction of MMTP with Fl^+ occurred nearly instantaneously and resulted in the disappearance of all ^{1}H NMR signals corresponding to Fl^+ and MMTP. (The sole species present in the ^{1}H NMR spectrum was the protonated starting material, $MMTPH^+$). However, no oxidation products were detected, and the reaction became dormant. During this dormant period however, a persistent flavin radical was detected by EPR. When O_2 was introduced (as much as three weeks later), the reaction resumed and resulted in the formation of the final oxidation product MMP^+. The ^{1}H NMR spectrum was remarkably similar to that observed when the reaction was conducted under aerobic conditions. To explain what was happening during this dormant period, an extension of the "persistent radical effect"[15] was proposed, wherein the "persistent" neutral flavin radical coupled reversibly to neutral reactive radical derived from MMTP (Figure 4).[14]

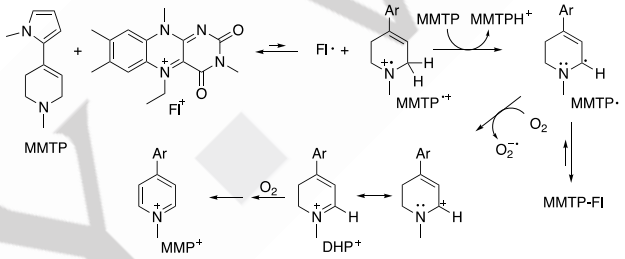

Figure 4. The reaction mechanism for the oxidation of MMTP by 5-ethyl-3-methyllumiflavinium perchlorate (Fl^+) proposed by Nakamura, et al.[14] A single electron transfer step is coupled to an extremely favorable proton transfer to a second molecule of MMTP, forming $MMTPH^+$ (detected by ^{1}H NMR) and MMTP• and Fl• (which are "invisible" in ^{1}H NMR). MMTP• and Fl• are believed to exist in equilibrium with a covalent adduct (MMTP-Fl), causing the reaction to enter a “dormant” phase. Upon O_2 addition, the equilibrium (MMTP• + Fl• = MMTP-Fl) is shifted towards the left and MMTP• is quickly consumed by O_2 to complete the oxidation of the substrate. Note: Ar refers to the N-methylpyrrole moiety in MMTP.

We believe that it is the unique structure of MPTP and its analogs that makes the electron transfer pathway feasible. Compared to typical tertiary amines (which are not MAO substrates), the additional resonance stability provided by the β-unsaturation makes the radical cation exceptionally acidic. For the oxidation of tertiary amines such as MPTP by monoamine oxidase, we propose that a reversible, thermodynamically unfavorable electron transfer followed by (and potentially coupled to) an extremely favorable deprotonation from the α-position of the aminyl radical cation (pK_a ca. -5) [14, 16] provides the driving force for the single electron transfer mechanism (Figure 5).

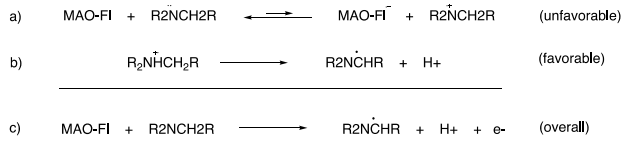

Figure 5. Modified single electron transfer (SET) hypothesis. a) Electron transfer between the flavin moiety and an amine is thermodynamically unfavorable. b) For some substrates, the radical resulting from deprotonation is very stable and the pK_a of the radical cation is exceptionally low. c) In the context of Le Chatelier's principle, the overall result of coupling an unfavorable electron transfer (a) and a very favorable proton transfer (b) drives the overall electron transfer/proton transfer process (c).

The biomimetic study discussed above utilized the highly reactive and electrophilic 5-ethyl-3-methyllumiflavinium (Fl^+) perchlorate, rather than a neutral flavin such as 3-methyllumiflavin (3MLF, Figure 6). Oxidation potentials reported by Sichula, et al.[17] suggests that an analogous N_{10}-ethyl flavinium species has an oxidation potential of +0.17 V, while the neutral flavin analog has an oxidation potential from -0.95 V (vs. $Ag/AgNO_3$). The increased oxidation potential of the flavinium (vs. neutral flavin moiety) introduces a bias favoring the SET process on the order of 25 kcal/mol!

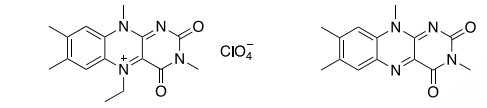

Figure 6. Structures of 5-ethy-3-methyllumiflavinium perchlorate and 3-methyllumiflavin.

In addition, the Fl^+/MMTP reaction was not catalytic. Upon reduction, Fl^+ decomposes and much of its fate is unknown. (The only detectable product was a benzimidazolium byproduct,[14] formed in a low yield). The objective of the present work was to examine the reactivity of a biomimetic that is more closely related to the neutral flavin moiety in MAO, to determine whether an electron transfer process remained feasible, and if so, whether the oxidation was catalytic. Towards this end, we report the results from a study of the reaction of 3-methyllumiflavin (3MLF) with MMTP. Our earlier work with Fl^+ and MMTP demonstrated that O_2 played a critical role in the reaction mechanism. Perhaps more importantly though, it was only possible to detect radicals when O_2 was excluded. Consequently, the 3MLF/MMTP chemistry was studied under both aerobic and anaerobic conditions.

Results

Reaction of MMTP and 3MLF in the Presence of O_2

The progress of both the aerobic and anaerobic reactions of MMTP and 3MLF was monitored by ^1H NMR. Authentic spectra for MMTP, 3MLF, and the reaction product MMP^+ and the observed chemical shifts and multiplicities for these compounds are provided in the supporting information (Figure S1). The peaks at 8.3, 6.0, and 4.0 ppm were particularly diagnostic, well-resolved and used to monitor relative concentrations of MMP^+, MMTP, and 3MLF, respectively, and for quantification purposes when an internal standard was used.

Aerobic reactions were prepared directly in an NMR tube using stock solutions of 3MLF and MMTP in CD_3CN and monitored by ^1H NMR. The solutions were continuously agitated on a mixing table to ensure thorough incorporation of O_2 into the solution and shielded from light to avoid any photo-induced reactions of 3MLF. The reaction of a 1:1 MMTP/3MLF mixture (2.50 mM each) was followed by ^1H NMR. In about four hours, all the MMTP was oxidized with ~50% conversion to MMP^+. During this time, there was no apparent change in the 3MLF concentration, demonstrating that 3MLF behaves as a catalyst in this reaction. Additionally, there was no ^1H NMR evidence for the presence of the reduced form of 3MLF at any time during the reaction.

To further probe catalysis, an 8:1 MMTP/3MLF reaction ([MMTP] = 4.80 mM, [3MLF] = 0.60 mM) was monitored by ^1H NMR for 240 hours. Again, the concentration of 3MLF remained constant for the duration of the reaction, and the MMTP was oxidized completely, indicating catalytic behavior over multiple turnovers (Figure 7). As a control, under identical conditions, it was shown that there was no significant reaction between MMTP and O_2 in the absence of 3MLF.

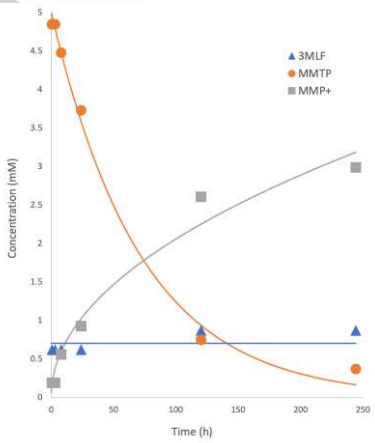

Figure 7. A plot of concentration vs. time for an aerobic 8:1 MMTP:3MLF reaction is shown here. The flavin demonstrates catalytic behavior while the substrate achieves ~50% conversion to the fully oxidized product. One turnover is estimated to take 4 hours. Concentrations were monitored by ^1H NMR with a benzene internal standard.

In summary, results from the reaction of 3MLF and MMTP under aerobic conditions demonstrated a) that 3MLF was an effective oxidant, b) that the reaction was catalytic, and most significantly c) that 3MLF was a good biomimetic for the flavin in the active site in MAO-B. However, as was found earlier with Fl^+/MMTP, important mechanistic features of the reaction became apparent when the reaction was conducted in the absence of O_2.

Reaction of MMTP and 3MLF in the absence of O_2

Solutions for the anaerobic reactions were also prepared directly in NMR tubes using stock solutions of 3MLF and MMTP in CD_3CN. The solutions were freeze-pump-thaw degassed immediately after mixing (to remove O_2), and the NMR tubes were flame-sealed. The reaction mixtures were kept in the dark unless being actively analyzed by NMR. (Analysis of the mixture shortly after mixing revealed the reaction did not proceed to a significant extent during the time period associated with sample preparation.)

The reaction of 1:1 MMTP and 3MLF (2.50 mM each) in CD_3CN in the absence of O_2 was monitored qualitatively by ^1H NMR for several hours. Within ca. four hours, all peaks corresponding to 3MLF disappeared from the ^1H NMR spectrum, leaving only peaks attributable to solvent, MMTP, and the reaction product MMP^+ (Figure 8). This behavior was reminiscent of the work of Nakamura, et al.,[14] but with some notable exceptions and nuances. The signals associated with the aromatic protons of 3MLF at δ = 7.7 and 7.9 ppm, and the aromatic methyl groups (2.4 and 2.5 ppm) disappeared "immediately." By immediate, we mean in the time it takes to prepare the sample and record the first spectrum, usually within fifteen minutes. However, as shown in Figure 9, rather than disappearing, the peaks associated with the N_3- and N_{10}-methyl groups of 3MLF exhibited line broadening at early reaction times, before disappearing into the baseline after about four hours (although the N_3 methyl was the last to completely vanish). After four hours, no peaks attributable to 3MLF (or any derived reduction product) were apparent in the reaction mixture. Nonetheless, the reaction progressed and MMTP was slowly oxidized to MMP^+.

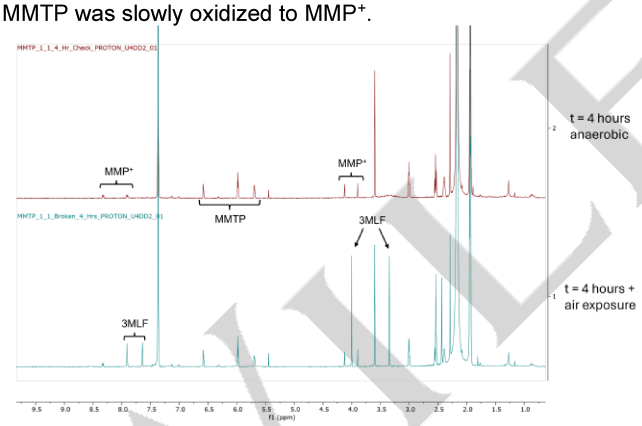

Figure 8. ^1H NMR spectrum of a reaction mixture of 3MLF and MMTP (1:1) under anaerobic conditions at t = 4 hours (top) demonstrates the disappearance of 3MLF from the ^1H NMR. Upon introduction of O_2, the flavin signals quantitatively re-emerge (bottom).

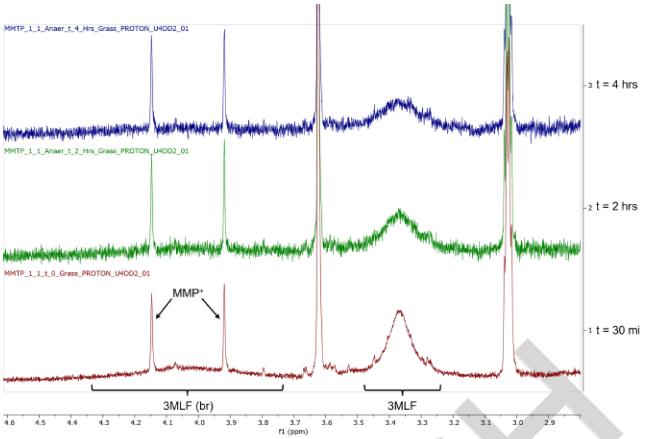

Figure 9. ^1H NMR spectra of 1:1 reaction of MMTP and 3MLF (2.50 mM each) in CD_3CN recorded as a function of time. The flavin N_3 and N_{10} methyl peaks disappear over the course of 4 hours while the MMTP decreases in intensity and the MMP^+ increases.

If O_2 was introduced at any time during the reaction, 3MLF reappeared in the ^1H NMR spectrum quantitatively, Figure 8. This phenomenon piqued a particular interest in uncovering the fate of the flavin over the course of the anaerobic reaction. We propose that during this early four hour period that the 3MLF ^1H NMR signals were broadening and decreasing in intensity, the concentration of flavin-derived radicals was steadily increasing.

The reaction continued until all the MMTP was consumed. After several days (3 - 5 depending on starting concentrations of MMTP and 3MLF), an orange precipitate formed. To isolate this precipitate, the NMR tube was opened in a glove box (to maintain anaerobic conditions). Direct characterization of this precipitate proved difficult, which was found to be insoluble in D_2O, $CDCl_3$, DMSO, and only sparingly soluble in CD_3CN. However, when suspended in CD_3CN and exposed to air, the precipitate readily dissolved, and the recorded ^1H NMR was a perfect match for 3MLF. This leads to the conclusion that the orange precipitate was a reduced form of the flavin, most likely dihydroflavin ($3MLFH_2$) or its deprotonated form ($3MLFH^-$) either of which upon exposure to O_2, would regenerate 3MLF.

Spectra collected in the absence of O_2 suggest that 3MLF is consumed but not degraded over the course of the reaction with MMTP because when O_2 is added, 3MLF is regenerated. This contrasts with the work done by Nakamura wherein the flavinium compound decomposed into a benzimidazolium ion as a function of time.[14] Unlike this earlier study, peaks attributable to 3MLF do not all disappear from the ^1H NMR spectrum simultaneously. Instead, the disappearance follows a consistent pattern of line broadening into the baseline as a function of time for each unique 3MLF signal.

We suggest that the observed line broadening and disappearance of signals associated with 3MLF provides indirect evidence for formation of a 3MLF derived radical or radical ion resulting from single electron transfer, whose concentration increases during the first few hours or so of reaction. Specifically, we suggest that during this period, approximately one equivalent of electrons have been transferred from MMTP to 3MLF producing 3MLFH• or $3MLF^{\bullet-}$. To confirm this hypothesis, EPR

spectroscopy was used to directly probe for the formation of flavin radicals in solution.

A 1:1 MMTP : 3MLF (2.50 mM each) anaerobic reaction solution was prepared and monitored by EPR spectroscopy. Initially, no EPR signals were observed. However, after about 40 minutes, the first sign of a signal appeared, and this signal continued to grow, reaching a maximum intensity after about four hours (Figure 10). After several days, the EPR signal vanished, coinciding with the formation of precipitate in the reaction vessel (i.e., a fully reduced form of 3MLF, vide supra). The shape and linewidth of the observed EPR signal is reminiscent of flavin derived radicals.[18]

It is particularly noteworthy that during the course of this reaction, the growth of the EPR signal associated with the flavin parallels the broadening and disappearance of the 3MLF signals in the ^{1}H NMR. During this same period, signals associated with the fully oxidized product (MMP^+) steadily grow in intensity in the ^{1}H NMR spectra. At longer times, the EPR signal diminishes and completely disappears when the reaction is complete (and the orange precipitate is formed).

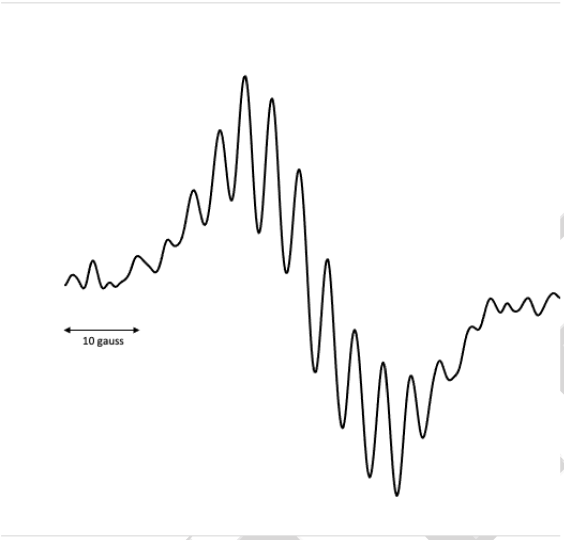

Figure 10. The EPR spectrum of a 1:1 MMTP:3MLF (2.50 mM each) reaction at t = 225 minutes. This spectrum matches the line shape and width of a flavin derived radical.

Oxidation of MMTP to MMP^+ requires a total of four electrons, and 3MLF (or species derived therefrom) are clearly effecting this oxidation. The EPR results and peculiar ^{1}H NMR observations point to the intermediacy of flavin derived radicals. To reconcile these observations, we propose that single electron transfer is occurring, and that the flavin radical observed by EPR is either $3MLF^{\bullet-}$, or if protonated, 3MLFH-1• or 3MLFH-2•. The difference between the latter two being the site of protonation. Figure 11 illustrates these structures, and also, shows the spin density surface and calculated spin density at hydrogen for each (M06-2X/6-311G*).[19] It is noteworthy that, regardless of which flavin structure is formed, the spin density at the N_3-methyl is nearly zero, and these are the last signals in the ^{1}H NMR to disappear. At longer times, the oxidation continues (MMTP → MMP^+), the EPR signals vanish, and a precipitate is formed (assigned to the fully reduced flavin $3MLFH_2$, vide supra).

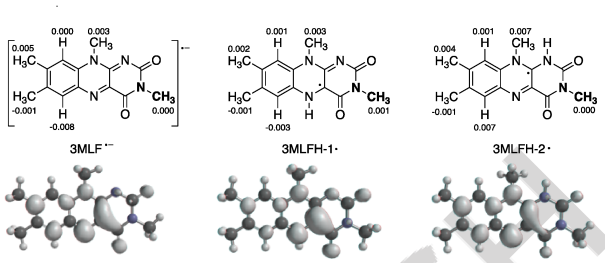

Figure 11. M06-2X/6-311G* calculated spin density surfaces of several flavin radicals; the numbers summarize the spin densities at each hydrogen. For all these species, the bulk of the spin density is associated with the left end of the fused-ring system with little to no spin density at the N_3-methyl group (boldfaced).

The line broadening observed in the ^{1}H NMR of the *N*-methyl groups of 3MLF during the early stages of the reaction (< 4 hours) is likely the result of a rapid exchange process between unreacted 3MLF and partially reduced 3MLF ($3MLF^{\bullet-}$ or 3MLFH•) as shown in Figure 12. Evidence for this rapid exchange was provided by variable temperature ^{1}H NMR and EPR.

$$3MLF + 3MLFH\cdot \rightleftharpoons 3MLFH\cdot + 3MLF$$
$$3MLF + 3MLF^{\bullet-} \rightleftharpoons 3MLF^{\bullet-} + 3MLF$$

Figure 12. Proposed self-exchange reactions for flavin radicals with neutral 3MLF starting material are shown.

The results of the variable temperature NMR experiments for a 1:2 MMTP : 3MLF reaction at three hours are shown in Figure 13. At room temperature, the signal for the N_{10} methyl group is not observed, and that for the N_3 is broadened and weak. As the temperature is reduced (10 degree increments to -30 °C, the N_3 and N_{10} methyl groups start to reappear in the opposite order in which they disappeared (N_{10}, followed by N_3). At -30 °C, even some peaks associated with protons on the aromatic ring appear to be emerging from the baseline. (The results obtained with variable temperature EPR were complementary. As the temperature was lowered, the EPR signal of the flavin radical disappeared, but reappeared when the temperature was brought back to ambient temperature.)

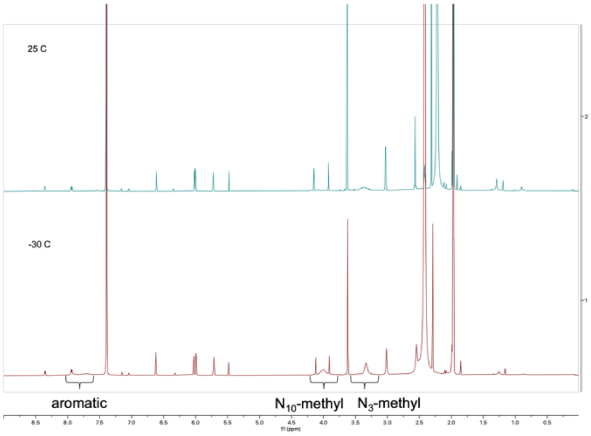

Figure 13. ^{1}H NMR spectrum of a 1:1 (MMTP:3MLF, 2.05 mM) reaction, taken after approximately four hours, is shown at two different temperatures, 25 ºC (top) and -30 ºC (bottom). Both the N_3- and N_{10}-methyl groups from 3MLF are much sharper and greater in intensity at low temperatures, indicating that the self-exchange reaction of 3MLF and its radical form has slowed down significantly.

Discussion

The oxidation of MMTP to MMP^+ is a net four electron process. Under aerobic conditions, the reaction is catalyzed by 3MLF which requires the reaction stoichiometry shown in Eq. 1. Under anaerobic conditions, the oxidation of MMTP by 3MLF is stoichiometric, requiring the stoichiometry shown in Eq. 2. In both cases, MMP^+ must be paired with a negatively charged counterion, HOO^- in the case of the catalyzed reaction and $3MLFH^-$ for the stoichiometric reaction (or hydroxide ion if $3MLFH^-$ is protonated by small amounts of water present in the CD_3CN NMR solvent.)

$$MMTP + 2\,O_2 \longrightarrow MMP^+ + H_2O_2 + HO_2^- \quad (1)$$

$$MMTP + 2\,3MLF \longrightarrow MMP^+ + 3MLFH_2 + 3MLFH^- \quad (2)$$

The results unambiguously point to the intermediacy of radical species formed via single electron transfer. We propose the mechanism depicted in Figure 14a for the anaerobic reaction. An unfavorable electron transfer between 3MLF and MMTP produces the radical anion/radical cation pair ($3MLF^{\bullet-}$ and $MMTP^{\bullet+}$). $MMTP^{\bullet+}$ is extremely acidic, and $3MLF^{\bullet-}$ is the strongest base in solution, so proton transfer leads to the neutral radicals MMTP• and 3MLFH•, the latter of which is persistent under anaerobic conditions and detected EPR. The 3MLFH• concentration increases steadily during the first few hours of reactions reflected by the intensifying EPR signal. Rapid exchange between 3MLFH• and 3MLF (or $3MLF^{\bullet-}$ and 3MLF) leads to line broadening and disappearance of the 3MLF signals in the ^{1}H NMR spectrum. These lines reappear and sharpen as the temperature, and thus rate of exchange, is lowered.

Meanwhile, MMTP• is sequentially oxidized in a series of electron transfer/proton transfer steps leading to MMP^+. Figure 14a does not explicitly identify the secondary or tertiary one-electron oxidant, but it is most certainly a flavin derived species in its oxidized (3MLF) or partially reduced form (3MLFH•), the latter of which is likely more easily reduced.[20] Similarly, the proton transfers likely involve the partially or fully reduced forms of the flavin, $3MLF^{\bullet-}$ and/or $3MLFH^-$. Over the course of longer time periods (days) the reaction continues until most of MMTP is converted to MMP^+ and a precipitate is formed ($3MLFH_2$ or $3MLFH^-$). The identity of this precipitate is confirmed by the fact that it reverts cleanly to the fully oxidized form (3MLF) when exposed to oxygen.

With oxygen present, the same mechanism operates up to and including the formation of MMTP• and 3MLFH•. Now however, as suggested in our earlier work,[14] MMTP• reacts with O_2 yielding DHP^+ (presumably at a diffusion-controlled rate),[21] and further oxidation leads to MMP^+ (Figure 14b). Because 3MLF remains at its initial concentration throughout the course of the aerobic reaction (i.e., it is a catalyst), we believe 3MLFH• reacts with O_2 to regenerate 3MLF. (The is no indication of any partially or fully reduced flavin in the ^{1}H NMR.)

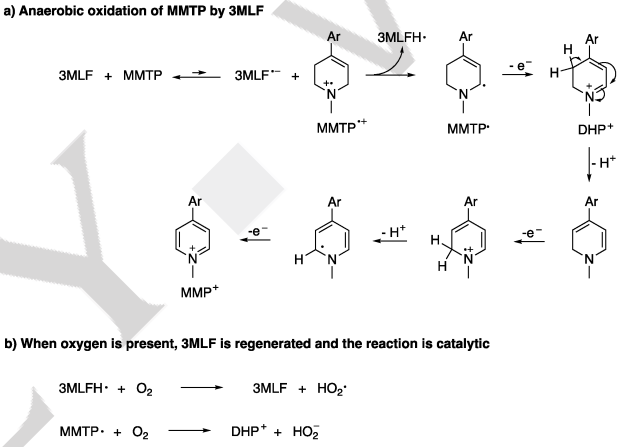

Figure 14. Proposed mechanism for the reaction of 3MLF with MMTP. Under anaerobic conditions (a), the reaction is stoichiometric and $3MLFH_2$ is a by-product. (b) In the presence of O_2, the reaction is catalytic; O_2 can also oxidize MMTP• to DHP^+.

It is not clear whether the initially formed $3MLF^{\bullet-}$ and $MMTP^{\bullet+}$ are freely diffusing and can separate, or are formed as a radical anion/radical cation caged pair. If the latter, then electron transfer/proton transfer sequence may not be discrete steps and the overall process may be tending toward a proton coupled electron transfer,[22] where electron transfer and proton transfer are concerted. Future work will address this issue in more detail.

Conclusion

These results are of direct relevance to oxidations of tertiary amines catalyzed by MAO. Unlike our earlier work with Fl^+ which is strongly oxidizing, 3MLF is a more realistic biomimetic for the neutral flavin in the active site of MAO. Moreover, 3MLF functions as a catalyst for oxidation. These results strengthen our hypothesis (Figure 5) that for MAO-catalyzed oxidations, the SET pathway is always available and present, but only becomes important when the resulting radical cation is very acidic. As noted, consistent with currently accepted mechanisms, tertiary amines are not generally MAO substrates. The exceptions are tertiary

amines based upon the tetrahydropyridine framework (i.e., MPTP, MMTP, etc.). Assuming that MAO inhibitors interact with the enzyme via a mechanism similar to that of substrates (i.e., mechanism-based inhibition), it may be particularly significant that there are a number of tertiary amines that a) are MAO inhibitors and b) have structural features related to the tetrahydropyridines (i.e., a CH_2 moiety α- both to nitrogen and either a C=C or C≡C, Figure 15.) These structural features dramatically lower the pK_a of the corresponding radical cations because they impart resonance stabilization to the resulting free radicals,[16] and we believe, activate the SET pathway.

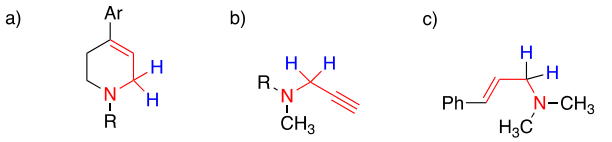

Figure 15. Tertiary amines that interact with MAO-B. The reactive C-H bonds are depicted in blue, and critical functionality in red: a) tetrahydropyridines and related compounds that are MAO substrates or inhibitors,[6a] b) MAO inhibitors based on 3° propargylic amines such as pargyline and deprenyl,[23] and c) analogs of *N*,*N*-dimethylcinnamylamine that are MAO inhibitors.[24]

Finally, it should be noted that both 5-ethyl-3-methyllumiflavinium perchlorate (Fl^+) and 3MLF have been used previously as biomimetics to better understand the mechanism of MAO-catalyzed oxidations by Mariano, et al.[25] All of their results were consistent with a polar (nucleophilic addition) mechanism. For 3MLF, the reactivity order parallels that of MAO in terms of amine structure, i.e., 1° > 2° > 3°, which makes sense because of steric effects. Moreover, using Fl^+ and benzylamine as substrate, a nucleophilic addition product was isolated which resulted from nucleophilic addition to the 4a position, as expected for the polar mechanism. However, unlike the present study, none of the amines examined in this earlier work possessed the structural features that we believe activates the electron transfer pathway, specifically a CH_2 moiety α- both to nitrogen and either a C=C or C≡C (Figure 15). Based upon our results and this earlier work, it appears that 3MLF faithfully mimics the reactivity patterns found with monoamine oxidase and is an outstanding biomimetic for understanding the mechanism of MAO-catalyzed oxidations.

In the recent MAO literature, there appears to be an (almost) general consensus that some sort of polar mechanism explains the catalytic pathway(s) by which MAOs convert 1° and some 2° amines to the corresponding imines ($R_2CHNH_2 \rightarrow R_2C{=}NH$), and we agree that this is most likely the case for "natural" or biogenic amines. However, there is also other compelling evidence for single electron transfer, based mainly on the behavior of certain compounds with *N*-cyclopropyl or *N*-cyclobutyl groups as electron transfer probes. These observations are not irreconcilable. Based upon these (and earlier results with flavin biomimetics), we suggest that the electron transfer pathway may always be available as an unfavorable equilibrium and is accessible to certain activated substrates which a) cannot react via the polar mechanism and, b) possess structural features that lower the pK_a of the corresponding radical cation. In the context of Le Chatelier's principle: an unfavorable equilibrium (electron transfer) is driven towards products by of an extremely favorable follow up step (deprotonation).

Supporting Information

The authors have cited additional references within the Supporting Information.[26]

Acknowledgements

This work was supported by the National Science Foundation (CHE-2106188).

Keywords: monoamine oxidase (MAO) • single electron transfer • MAO catalysis • radicals • biomimetic

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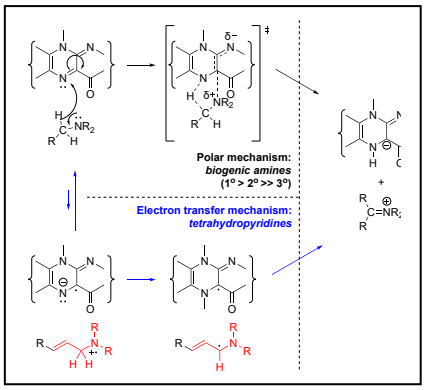

A unifying hypothesis for the mechanism of monoamine oxidize (MAO) catalyzed oxidations is presented. Most 1° and 2° amines are oxidized by MAO via a polar pathway; 3° amines are generally not substrates. However, for tetrahydropyridines (and similarly structured amines), an otherwise unfavorable electron transfer pathway is driven by a facile deprotonation (Le Chatelier's principle) because the resulting neutral radicals are exceptionally stable.

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