How are Biogenic Amines Metabolized by Monoamine Oxidases?

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Keywords: Enzyme catalysis / Computer chemistry / Metabolism / Flavoenzymes / Oxidoreductases

Monoamine oxidases (MAOs) are flavoenzymes important in regulating amine neurotransmitter levels and are the central pharmacological targets in treating depression and Parkinson's disease. On the basis of quantum chemical calculations, we have proposed a new two-step hydride mechanism for the MAO-catalysed oxidative deamination of amines. In the rate-limiting first step, through its N5 atom, the flavin abstracts a hydride anion from the substrate α-carbon atom and forms a strong covalent adduct with the thus created cation. This is followed by flavin N1 deprotonation of the substrate amino group, facilitated with two active-site water molecules, to produce fully reduced flavin, FADH2, and neutral imine. We have demonstrated that our mechanism is in agreement with available experimental data and provided evidence against both traditional polar nucleophilic and single-electron radical pathways. These results provide valuable information for mechanistic studies on other flavoenzymes and for the design of new antidepressants and anti-parkinsonian drugs.

Introduction

Monoamine oxidase (MAO) is a mitochondrial outer-membrane-bound flavoenzyme that catalyses the oxidative deamination of a large variety of biogenic amine neurotransmitters, such as adrenaline, noradrenaline, dopamine and serotonin, into their corresponding imines, which are then non-enzymatically hydrolysed to the final carbonyl compounds and ammonia. The enzyme itself is regenerated to its active form by molecular oxygen (O2), which is in turn reduced to hydrogen peroxide (H2O2). MAOs operate by using the flavin adenine dinucleotide (FAD) cofactor, which is, in contrast to the majority of other flavoenzymes, covalently bound to a cysteine through an 8α-thioether linkage (Figure 1).[1] Despite having around 70% sequence identities and identical cofactor structure, the A and B isoforms of the enzyme exhibit differing substrate and inhibitor specificities,[2] but it is assumed that they act by the same mechanism. Despite tremendous research efforts devoted to MAOs over several decades, neither the catalytic nor the inhibition mechanisms of MAO have been unambiguously established.

Once available, the three-dimensional structures of MAO–A,[3] and MAO–B,[4,5] together with the wide-ranging kinetic and spectroscopic studies on mutant enzymes,[6,7] have led researchers to propose three possible catalytic scenarios: (a) the radical mechanism, (b) the polar nucleophilic mechanism and (c) the direct hydride mechanism (Scheme 1). As the key step, these include the abstraction of either a hydride anion (H–), a hydrogen atom (H·) or a proton (H+) from the α-carbon atom of the substrate,[2,8–10] commonly by the flavin N5 atom, the latter also being the target for irreversible acetylenic MAO inhibitors such as rasagiline[11,12] and selegiline.[13] The possibility of the hydride mechanism operating for MAOs (Scheme 1, c) was suggested on the basis of their structural similarities to flavoprotein amino acid oxidases, which, through a series of kinetic measurements[13,14] and related calculations, were determined to operate by either H· or a single electron transfer. However, Erdem et al.[15] assumed that the hydride mechanism is unlikely, because in MAO it would be associated with a barrier too high to be
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(a) RADICAL MECHANISM

(b) POLAR NUCLEOPHILIC MECHANISM

(c) HYDRIDE MECHANISM

Scheme 1. Three mechanisms for MAO catalytic activity proposed in the literature, initiated by either (a) abstraction of a hydrogen atom, (b) deprotonation (H⁺ abstraction) or (c) removal of a hydride anion, all from the substrate α-carbon atom. The final products from all three mechanisms are the protonated iminium cation and semi-reduced flavin.

readily crossed. This mechanism was further ruled out by Matsson and co-workers who measured 15N kinetic isotope effects (KIEs) for the MAO-B oxidation of benzylamine and its 1,1-dideutero analogue to show that C–H bond-breaking is not concerted with nitrogen rehybridization, as required for traditional H⁺ transfer (Scheme 1, c).

According to Silverman and co-workers, the radical mechanism is initiated by a single-electron ionization of the substrate by the flavin and the subsequent or concerted deprotonation of the resulting aminium radical cation intermediate (Scheme 1, a). The main supporting evidence for the radical mechanism is the observation that both MAOs are inactivated by cyclopropylamine analogues with subsequent ring-opening, a process characteristic of radical reactions. However, experiments by Edmondson and co-workers as well as related EPR studies and stopped-flow kinetic determinations failed to provide evidence for radical intermediates, and no influence of the magnetic field on the kinetics of enzyme reduction was observed. On the other hand, the study of Miller and Edmondson on benzylamines showed that attaching electron-withdrawing groups to the substrate para position increases the rate of the reaction in MAO-A, with the Taft correlation factor assuming values of 1.5–2.0, which implies negative charge build-up on the substrate α-carbon atom and suggests that proton transfer is an integral part of the rate-limiting step. This led authors to propose the polar nucleophilic mechanism for MAO-A (Scheme 1, b), which first involves the creation of a highly energetic substrate–flavin adduct formed by amine nucleophilic attack on the flavin C4a atom. The resulting adduct then decomposes to the protonated imine with proton abstraction concerted with either adduct or product formation.

Apart from knowing how MAO enzymes work, a detailed knowledge of their catalytic activity is also important for a successful design of novel MAO inhibitors as transition-state analogues. Because MAOs regulate the concentration of amine neurotransmitters, the selective inhibition of both MAO-A and MAO-B isoforms is used in clinical practice as the primary strategy for relieving the symptoms of depression and Parkinson’s disease respectively. Therefore, for all the reasons stated, a thorough insight into the chemical steps of amine degradation would be of paramount importance for various future studies on flavoenzymes, which represents the focus of this manuscript.

Results and Discussion

The starting point for our calculations was the high-resolution (1.6 Å) X-ray structure of MAO-B complexed with 2-(2-benzofuranyl)-2-imidazoline obtained from the Protein Data Bank (accession code 2XFN). We truncated the enzyme to the flavin moiety of the FAD cofactor (isoalloxazine group) and three tyrosine side-chains (p-hydroxytoluenes of Tyr188, Tyr398 and Tyr435), which all form the hydrophobic “aromatic cage”, an important
structural feature of MAO enzymes. Previously, we calculated the p$	ext{K}_a$ values of the Tyr residues with bound dopamine, employing the full dimensionality of the protein,[34] and obtained an upward shift to 13.0–14.7 (10.1 in aqueous solution). This clearly confirmed the hydrophobic nature of the active site, which indicates that gas-phase calculations on truncated MAOs are quite reliable. Also, we showed that the p$	ext{K}_a$ value of bound dopamine changes to only 8.8 (8.9 in aqueous solution), a result of stabilizing cation–π interactions with the tyrosine side-chains.[34] This implies that dopamine binds to the MAO active site as a protonated monocation, but the free-energy cost to deprotonate it to the bulk, being as low as 1.9 kcal mol$^{-1}$, allows it to enter the chemical step either as an ionized or neutral molecule, which led us to consider both alternatives here. However, a few water molecules are still present within the active site, as revealed from both the available crystal structures[11] and our simulations,[34] which is why we included four water molecules in our model (HOH2157, HOH2181, HOH2329 and HOH2372). It turns out that two of those solvent molecules are chemically involved during catalysis. For the substrates, we first considered the truncated ethylamines 2 and 3, and refined the approach later with the full-size dopamine 1 for the most feasible mechanism. All three compounds 1–3 were separately placed within the active site resulting in the corresponding initial stationary-point (SP) complexes (Figure 2).

For $\text{I}_{\text{SP1}}$ we first considered the single-electron radical mechanism (Scheme 1, a), which would initially create a biradical system either in the singlet or triplet electronic state. (CPCM)/UM06-2X/6-31+G(d) calculations for the triplet state produced the system 54.2 kcal mol$^{-1}$ higher in energy than $\text{I}_{\text{SP1}}$. For the singlet state, we employed “symmetry-broken” open-shell calculations, defining dopamine and the rest of the system as two different fragments each having an unpaired electron of opposite spin. This resulted in a stable wavefunction with both the energy and the electron distribution identical to that in $\text{I}_{\text{SP1}}$. Overall, this suggests that the singlet-state biradical is non-existent, whereas the free-energy cost to generate the triplet is too high for an efficient catalysis. In addition, the M2 model (see Exp. Section) yielded an ionization energy (IE) for isolated dopamine (1) of 148.2 kcal mol$^{-1}$ and an electron affinity (EA) for the flavin 4a of 78.5 kcal mol$^{-1}$. The latter, however, increases to 87.3 kcal mol$^{-1}$ when the structure of the whole MAO cluster is considered, still leaving a gap of around 60 kcal mol$^{-1}$ between these two quantities and revealing a thermodynamic imbalance for a feasible electron-transfer process. This agrees well with the observed mismatch between the oxidation/reduction potentials of the FAD cofactor, which is too low (–0.2 V)[35] for it to be an effective oxidant of the neutral amine (around +1.0–1.5 V)[36] although it was argued[39] that the former value could change somewhat in the presence of the substrate and for each enzyme. Still, on top of the fact that all X-ray analyses have established that in MAOs there are no classical radical initiators to start the reaction and that there is no experimental evidence for a radical intermediate, our results suggest that it is very unlikely that a radical pathway is feasible and we did not consider it further.

The polar nucleophilic mechanism is another alternative for the amine oxidation and involves proton abstraction from the benzyl α-carbon atom as the key step (Scheme 1, b).[27] The crucial issue relating to this mechanism is what moiety on the enzyme would be a strong enough base to perform this task, because the p$	ext{K}_a$ of a benzyl proton is expected to be around 25.[37] Structural analysis of both MAO isoforms shows there are no active-site basic residues that could act as proton acceptors[3,38] Edmondson and co-workers upheld their arguments by saying that in MAOs the flavin is bent by around 30° from planarity about the N5–N10 axis, which enhances the basicity of the N5 atom and depletes the electron density on the C4a atom, thus facilitating substrate–flavin complex formation with the latter flavin site making subsequent proton abstraction by N5 possible. Still, on top of the fact that all X-ray analyses have established that in MAOs there is no classical radical initiators to start the reaction and that there is no experimental evidence for a radical intermediate, our results suggest that it is very unlikely that a radical pathway is feasible and we did not consider it further.

Figure 2. Structures of relevant stationary points for the newly proposed two-step MAO catalytic hydride mechanism for the degradation of dopamine 1. Geometries of the corresponding systems with the truncated substrate 2 are analogous.

For $\text{I}_{\text{SP2}}$ we first considered the single-electron radical mechanism (Scheme 1, a), which would initially create a biradical system either in the singlet or triplet electronic state. (CPCM)/UM06-2X/6-31+G(d) calculations for the triplet state produced the system 54.2 kcal mol$^{-1}$ higher in energy than $\text{I}_{\text{SP1}}$. For the singlet state, we employed “symmetry-broken” open-shell calculations, defining dopamine and the rest of the system as two different fragments each having an unpaired electron of opposite spin. This resulted in a stable wavefunction with both the energy and the electron distribution identical to that in $\text{I}_{\text{SP1}}$. Overall, this suggests that the singlet-state biradical is non-existent, whereas the free-energy cost to generate the triplet is too high for an efficient catalysis. In addition, the M2 model (see Exp. Section) yielded an ionization energy (IE) for isolated dopamine (1) of 148.2 kcal mol$^{-1}$ and an electron affinity (EA) for the flavin 4a of 78.5 kcal mol$^{-1}$. The latter, however, increases to 87.3 kcal mol$^{-1}$ when the structure of the whole MAO cluster is considered, still leaving a gap of around 60 kcal mol$^{-1}$ between these two quantities and revealing a thermodynamic imbalance for a feasible electron-transfer process. This agrees well with the observed mismatch between the oxidation/reduction potentials of the FAD cofactor, which is too low (–0.2 V)[35] for it to be an effective oxidant of the neutral amine (around +1.0–1.5 V)[36] although it was argued[39] that the former value could change somewhat in the presence of the substrate and for each enzyme. Still, on top of the fact that all X-ray analyses have established that in MAOs there are no classical radical initiators to start the reaction and that there is no experimental evidence for a radical intermediate, our results suggest that it is very unlikely that a radical pathway is feasible and we did not consider it further.

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Leu and Trp led to enzymes that maintained a substantial level of catalytic functionality.\textsuperscript{[60]} We feel that, even if all of these effects are operational, it would still be insufficient to downshift the substrate pK\textsubscript{a} value by around 10–15 units in order to make the α–C–H bond acidic enough for an efficient catalysis. Moreover, inspection of 1\textsubscript{SP1} shows that the flavin ring is not bent, but is almost perfectly planar with the C10α–N10–N5–C5α dihedral angle of 174.3\textdegree{} and the N(dopamine)···C4α(flavin) bond length as long as 2.827 Å. Furthermore, with the M2 model, a relaxed-geometry scan of the latter bond, by compressing it with 0.1 Å increments, showed no indication of the formation of a stable complex, accompanied only by an increase in the total energy of the system to values above 45 kcal mol\textsuperscript{−1} for bond lengths shorter than 1.327 Å. In addition, NBO charges on the flavin C4α and N5 sites, and the substrate N atom in 1\textsubscript{SP1} are 0.13, −0.35 and −0.97\textit{e}, respectively, being not much changed from the values of 0.10, −0.34 and −0.93\textit{e} found in isolated flavin 4a and dopamine 1. This all suggests that neutral amines do not exhibit the necessary nucleophilicity to readily add to the flavin C4α position, in agreement with the fact that no direct evidence for a stable amine–flavin adduct has been found experimentally. Lastly, should a stable adduct be formed, the subsequent C–H deprotonation (Scheme 1, b) would additionally increase its stability due to the enhanced electrostatic attraction between the two such created charged species, which would very likely prevent further catalysis. Taken all together, these results also led us to rule out this mechanism as feasible.

The geometry of stationary structure 1\textsubscript{SP1} (Figure 2) suggests that two pathways are possible. The substrate amino group is connected through two active-site water molecules to the flavin N1 atom. According to our calculations, the latter represents the most basic position within the isoalloxazine moiety as the corresponding N1, O(C2), O(C4), N5 and N3 protonation free energies in 4a are −165.6, −164.6, −156.3, −154.9 and −133.3 kcal mol\textsuperscript{−1}, respectively (M2 model). This implies that the substrate could be first activated by amino deprotonation to the flavin N1 atom. Also, the α-C(substrate)···N5(flavin) bond length in 1\textsubscript{SP1} is 3.198 Å, being sufficiently short to suggest that the substrate is properly oriented for a direct α–CH abstraction as the initial step of our novel hydride transfer mechanism.

Deprotonation of the substrate amino group is feasible, but the process is associated with a large barrier of 37.3 kcal mol\textsuperscript{−1} (Figure 3, a). The transition-state structure 2\textsubscript{TS1}\textsubscript{N} has one imaginary frequency of 270i cm\textsuperscript{−1} representing proton transfer to the flavin N1 atom assisted by two water molecules by the de Grotthuss mechanism.\textsuperscript{[39]} Such a high energy requirement is rationalized by the large difference in the pK\textsubscript{a} values between donor proton and acceptor sites. For the flavin N1–H deprotonation this was measured to be around 7.0,\textsuperscript{[40]} whereas amine deprotonation typically has a pK\textsubscript{a} of around 35\textsuperscript{[37]} Upon proton removal, the anionic substrate turns nucleophilic and covalently binds to the flavin C4α atom, accompanied by a reduction in the corresponding N(substrate)···C4α(flavin) distance from 4.063 (2\textsubscript{SP1}) and 1.550 Å (2\textsubscript{TS1}\textsubscript{−N}) to 1.508 Å in 2\textsubscript{SP2}\textsubscript{−N}. The complex formed facilitates subsequent H\textsuperscript{−} abstraction from the α–CH group, requiring only 13.2 kcal mol\textsuperscript{−1} to arrive at the transition-state structure 2\textsubscript{TS2}\textsubscript{−N}, characterized with one imaginary frequency of 871i cm\textsuperscript{−1}. Abstraction of the H\textsuperscript{−} is concerted with the loosening of the N(substrate)···C4α(flavin) bond from 2.439 Å in 2\textsubscript{TS2}\textsubscript{−N} to 3.505 Å in the final products 2\textsubscript{SP3}, being reduced flavin FADH\textsubscript{2} and neutral imine CH\textsubscript{2}CH=NH. This pathway is associated with a collective activation energy of 44.6 kcal mol\textsuperscript{−1}, which is too high to be feasible for the mechanism of MAO activity.

Direct substrate α-hydride abstraction by the flavin N5 atom turned out to be much more feasible (Figure 3, b). In the transition-state structure 1\textsubscript{ST1}, the transferring hydrogen atom is placed between the leaving α-carbon atom and the accepting flavin N5 atom in an almost symmetrical fashion, the corresponding bond lengths assuming 1.349 and 1.269 Å, respectively, with one imaginary frequency of 1291i cm\textsuperscript{−1}. The free energy required for this process is...
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24.4 kcal mol\(^{-1}\), after which the system relaxes to an intermediate \(1_{\text{SP2}}\). NBO atomic charges reveal that this process is indeed associated with the transfer of H\(^+\). Initially, the total charges on the dopamine and the flavin in \(1_{\text{SPI}}\) are 0.03 and 0.01[e], respectively, changing to 0.31 and –0.29[e] in \(1_{\text{TSl}}\), respectively. In addition, the charge on the flavin N5 atom changes from –0.35[e] in \(1_{\text{SPI}}\) to –0.50[e] in \(1_{\text{TSl}}\), which, taken together, support H\(^+\) transfer. This agrees well with the electrophilic nature of the flavin N5 atom, which was revealed after we demonstrated that irreversible MAO–B inhibition by acetylenic inhibitors rasagiline and seleagine proceeds by nucleophilic attack of the terminally deprotonated anionic inhibitor onto the flavin N5 atom,\(^{[41]}\) the resulting complex matching the available X-ray structures\(^{[3,11,12]}\).

According to Scheme 1, c, the traditional notion of hydride abstraction should (a) generate positive charge on the substrate \(\alpha\)-carbon atom and (b) be concerted with the substrate amino nitrogen atom rehybridization from sp\(^3\) to sp\(^2\). The first process would contrast the aforementioned positive Taft correlation by Miller and Edmondson\(^{[27]}\) that suggested negative charge development on the stated carbon \(\alpha\)-carbon atom, is also negative as \(\alpha\)-carbon atom changes from –0.25[e] in \(1_{\text{SPI}}\) to –0.11[e] in \(1_{\text{TSl}}\), surprisingly preserving enough negative charge for this reaction to be facilitated by the electron-withdrawing \(\alpha\)-substituents on the aromatic ring. Moreover, the charge on the dopamine \(\beta\)-carbon atom, which is in terms of \(\alpha\)-substituent effects analogous to the benzylamine \(\alpha\)-carbon atom, is also negative as it only moderately changes from –0.49[e] \((1_{\text{SPI}})\) to –0.50[e] \((1_{\text{TSl}})\), putting our results in firm agreement with the work of Miller and Edmondson.\(^{[27]}\) We should also mention that Banerji and co-workers\(^{[42]}\) studied the non-enzymatic oxidation of benzylamine derivatives by hexamethylenetetramine/bromine and obtained a negative Taft correlation with

\[
\text{DP}_N [\%] = [360° - \sum_i a_i] / 0.9
\]

The maximal pyramidalization (100%) occurs if all three bond angles are 90°, whereas it is 0% for a planar trigonal atom. The M2 geometries reveal that DP\(_N\) for the dopamine nitrogen atom is 37.4% in isolated \(1\) and 36.5% in \(1_{\text{SPI}}\), values typical of primary amines. These change to 4.2 and 13.9% in \(1_{\text{TSl}}\) and \(1_{\text{SP2}}\), respectively. It follows that in the transition-state structure \(1_{\text{TSl}}\), the substrate still retains 4.2% of its nitrogen pyramidalization, which is then, surprisingly, even increased to 13.9% following H\(^+\) removal. The latter occurs, because – after the flavin accommodates H\(^+\) – its N5 atom becomes sp\(^3\)-hybridized with excess negative charge, having enough nucleophilicity to form a covalent bond with the thus formed cationic substrate (Figure 2 and Scheme 2). Macheroux et al.\(^{[40]}\) measured the pK\(_{a}\) value of the fully hydrogenated flavin N(5)–H2 moiety to be pK\(_{a}\) ≈ 4, which indicates that the N(5)–H\(^+\) group possesses sufficient basicity to act as a base. The calculated substrate–flavin interaction free energy in \(1_{\text{SP2}}\), obtained as the difference in the total free energy of the complex and that of both isolated components, is as high as 27.7 kcal mol\(^{-1}\) (M2 results), which, together with

Scheme 2. Complete two-step mechanism for MAO catalysed amine degradation. The first step involves H\(^+\) abstraction from the substrate to form the flavin–substrate adduct, which then decomposes to the final products, namely neutral imine and fully reduced flavin, FADH\(_2\), a reaction promoted by amine deprotonation facilitated by two water molecules.
the corresponding N5–C(α) bond length of only 1.703 Å, suggests that the complex formed is rather strong. In 1SP2, the dopamine nitrogen atom maintains its amine –NH2 features, which restores its pyramidalization back to 13.9% leaving its planarization only for the upcoming step, thus strongly tying in with the experimental results of Matsson and co-workers. This all gives a convincing indication that α-hydride abstraction is not the only and last step during MAO catalysis. For product release and subsequent enzyme regeneration, another chemical reaction must take place. This idea represents an important advantage over other mechanistic proposals that all advise protonated imine as the final product of a one-step catalysis (Scheme 1). We disagree with this latter suggestion for the following reasons. First, it would be difficult for a protonated imine to leave the active site, because on its way out it would strongly bind to the “aromatic cage” through favourable cation–π interactions. Secondly, it is well established that the final imine hydrolysis to aldehydes occurs non-enzymatically outside the MAO. However, the protonated product would immediately be hydrolysed by the nearest water molecule within the enzyme, because in organic chemistry this reaction readily proceeds with the protonated imine under acidic conditions.

The next step involves deprotonation of the substrate amino group by the flavin N1 atom with an activation free energy of 9.4 kcal mol⁻¹ (Figure 3, b). In 1SP2, the flavin N1 atom is well prepared for this proton abstraction assuming an atomic charge of -0.70e. The loss of the amine proton is concerted with the weakening of the N5(flavin)···Cα-(dopamine) bond, which elongates from 1.703 Å in 1SP2 to 2.707 Å in 1TS2, and further to 3.121 Å in 1SP3. The transition-state structure 1TS2 has one imaginary frequency of 933i cm⁻¹, again describing a de Grotthuss-type proton transfer proceeding with the two active-site water molecules, but has both dopamine imine protons still close to the nitrogen atom with N–H bond lengths of 1.032 and 1.063 Å. This yields a nitrogen DPN value of 0.1% and represents the stage at which full nitrogen sp³-sp² rehybridization occurs. The aforementioned proton transfer would also be possible even if, instead of only a cluster model, the full structure of the enzyme was used, because the relative positions of these two water molecules both before this catalytic step are identical to those found in the MAO–B crystal structure. Upon deprotonation the system is stabilized by 10.1 kcal mol⁻¹ to 1SP3, making the whole reaction energetically feasible and yielding the neutral trans-imine and the reduced flavin (FADH2) as the final products (Figure 2 and Scheme 2). It has to be strongly emphasized that the presence of the aforementioned acidic amine N–H bond enables the completion of MAO turnover and explains why many alkyl- and arylamines change from being MAO substrates to MAO inhibitors upon N,N-dimethylation. The fact that dopamine is converted into a neutral imine is significant, because this suggests that it will predominantly remain unprotonated in the hydrophobic active site, based on consideration of the pKa values of similar unconjugated imines, which are, as a rule, found to be below the physio-

![Figure 4](image-url)
ionic charge over the whole N1-protonated FADH⁺. Still, the collective activation barrier for this process is notably higher than in the previous case, making this mechanism of amine metabolism less plausible.

Conclusions

Our suggested hydride mechanism for MAO-catalysed amine degradation is a two-step process in which in the rate-limiting first step the flavin N5 atom abstracts a hydride anion from the substrate α-carbon atom with an activation free energy of 24.4 kcal mol⁻¹. This result is in agreement with those of a few other flavoenzymes, which were determined to operate by the hydride abstraction mechanism.¹⁷,¹⁸ In the following step, the flavin N1 atom deprotonates the amino group of the cationic substrate, which is facilitated by the two active-site water molecules. The overall process produces fully reduced flavin (FADH₂) and neutral trans-imine, which is then easily released from the enzyme. To the best of our knowledge, this is the first mechanistic study that has revealed the complete pathway of MAO catalysis involving two steps. Strictly speaking, the results presented herein hold for the catalytic activity of MAO–B, because its crystal structure was used to build up the model. However, knowing that both isoforms are structurally very similar, particularly in the active-site region, having identical cofactors and metabolizing a large variety of substrates equally well,¹⁹ we feel that the possibility that the two enzymes function through different mechanisms is very unlikely.

The results of this work were obtained by applying a quantum mechanical cluster approach to a relatively small but well-chosen part of the enzyme. It is clear that this computational methodology could be further improved by either including a larger portion of the enzyme within this approach or by considering the full dimensionality of MAOs employing any of the established QM/MM techniques. However, we have shown that our mechanism is the most feasible out of five possible scenarios, the other four being associated with free-energy costs of at least 43 kcal mol⁻¹, which is almost twice as large as found for the hydride mechanism. Therefore, it is very doubtful to expect that improvements in the methodology could reverse this picture in favour of any other pathway. Although being a reasonable compromise, the cluster method used has certain limitations, for example, it lacks thermal averaging. The proper study should involve ab initio free-energy calculations using metadynamics,¹⁹ or the recently improved par-dynamics approach, which are, however, associated with high computational costs. Still, we plan to proceed with the QM/MM treatment of the MAO mechanism by using the empirical valence bond (EVB) scheme,¹⁵ which proved to be successful in a large number of cases.²⁰ To further refine the activation free energy for the proposed mechanism, one could proceed with the quantization of the hydrogen nuclear motion, giving rise to tunnelling which could additionally lower the barrier. The experimental value of the H/D kinetic isotope effect (KIE) for MAO–B is between 6 and 13,²⁷ which suggests significant tunnelling and provides additional evidence in support of the polar hydride mechanism. Higher KIEs would substantiate non-polar radical mechanisms, such as in the enzyme soybean lipooxygenase, for which the KIE is around 81 for the rate-limiting hydrogen atom transfer.⁵⁰ It also indicates that during the reactive step, the charge distribution does not change significantly, which implies that the reaction is controlled by intrinsic hydride dynamics rather than by fluctuations in the environment, thus justifying the use of a truncated cluster model. The total dipole moments for the reactant (15.0 D) and the transition state (11.4 D), obtained with the M2 model, confirm this hypothesis. It remains a challenge to study nuclear quantum effects in MAO by a combination of QM/MM and path integral approaches.⁵⁰–⁶⁰

The results provided herein shed an important new light on the activity of this class of biological systems and pave the way for future studies on MAOs and other members of the large family of flavoenzymes. The data obtained could be of significant importance for the pharmaceutical industry in terms of the design and synthesis of novel effective MAO inhibitors as transition-state analogues, which are all potential drugs employed clinically as antidepressants and in the treatment of Parkinson’s disease.
Acknowledgments

R. V. would like to thank the Croatian Ministry of Science, Education and Sports (Grant No. 098-0982933-2932). M. R. and J. M. would like to thank the Slovenian Research Agency (Contract No. J1-2014; program group P1-0012). Part of this work was supported by COST Action CM1103.

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Received: August 16, 2012
Published Online: October 25, 2012