Computational Study of the pK_a Values of Potential Catalytic Residues in the Active Site of Monoamine Oxidase B†

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† This manuscript is dedicated to Professor Wilfred F. van Gunsteren on the occasion of his 65th birthday.

ABSTRACT

Monoamine oxidase (MAO), which exists in two isozymic forms, MAO A and MAO B, is an important flavoenzyme responsible for the metabolism of amine neurotransmitters such as dopamine, serotonin and norepinephrine. Despite extensive research effort, neither the catalytic nor the inhibition mechanisms of MAO have been completely understood. There has also been dispute with regard to the protonation state of the substrate upon entering the active site, as well as the identity of residues that are important for the initial deprotonation of irreversible acetylenic inhibitors, in accordance with the recently proposed mechanism. Therefore, in order to investigate features essential for the modes of action of MAO, we have calculated pK_a values of three relevant tyrosine residues in the MAO B active site, with and without dopamine bound as the substrate (as well as the pK_a of the dopamine itself in the active site). The calculated pK_a values for Tyr188, Tyr398 and Tyr435 in the complex are found to be shifted upwards to 13.0, 13.7 and 14.7, respectively, relative to 10.1 in aqueous solution, ruling out the likelihood that they are viable proton acceptors. The altered tyrosine pK_a values could be rationalized as an interplay of two opposing effects: insertion of positively charged bulky dopamine that lowers tyrosine pK_a values, and subsequent removal of water molecules from the active site that elevates tyrosine pK_a values, in which the latter prevails. Additionally, the pK_a value of the bound dopamine (8.8) is practically unchanged compared to the corresponding value in aqueous solution (8.9), as would be expected from a charged amine placed in a hydrophobic active site consisting of aromatic moieties. We also observed potentially favorable cation–π interactions between $–NH_3^+$ group on dopamine and aromatic moieties, which provide stabilizing effect to the charged fragment. Thus, we offer here theoretical evidence that the amine is most likely to be present in the active site in its protonated form, which is similar to the conclusion from experimental studies of MAO A (Jones et al. *J. Neural Trans.* **2007**, *114*, 707–712). However, the free energy cost of

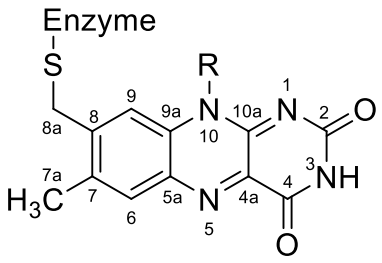
transferring the proton from the substrate to the bulk solvent is only 1.9 kcal mol^{-1}, leaving open the possibility that the amine enters the chemical step in its neutral form. In conjunction with additional experimental and computational work, the data presented here should lead towards a deeper understanding of mechanisms of the catalytic activity and irreversible inhibition of MAO B, which can allow for the design of novel and improved MAO B inhibitors.

KEYWORDS

MAO B, flavoenzymes, enzyme catalysis, free energy calculations, dopamine degradation

INTRODUCTION

Flavoenzymes are enzymes that operate with either flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD) cofactors. Prominent members of this family include the monoamine oxidases (MAOs), which metabolize biogenic amines towards the corresponding imines. They are located in the outer mitochondrial membranes of the brain, liver, intestinal, placental cells and platelets.[1–3] In MAOs, the FAD coenzyme is covalently bound to a cysteine through an 8α-thioether linkage.[4–6] The enzyme exists in two isozymic forms, MAO A and MAO B,[7–9] which differ in substrate and inhibitor specificities, as well as in their tissue distribution.[1–3] MAOs have the role of regulating the concentrations of neurotransmitters in living cells, and are a very promiscuous family of enzymes, since they act on a number of diverse primary, secondary and tertiary alkyl and arylamines, although their preference is for primary amines. MAO A is the more abundant isoform in humans, and is mainly responsible for the oxidation of noradrenaline and serotonin. The imbalance in noradrenaline/serotonin levels is known to cause depression-like symptoms and other mood disorders.[2] Hence, the selective inhibition of this isoform results in elevated noradrenaline and serotonin concentrations, thus gradually improving the symptoms of depression. In contrast, MAO B is responsible for the metabolism of histamine's metabolite *N*–methylhistamine and dopamine.[1] The latter is an important neurotransmitter involved in the control of voluntary movement. It has been established that insufficient dopaminergic stimulation of the basal ganglia is characteristic for Parkinson's disease.[4] Hence, inhibition of MAO B is one of the strategies for the treatment of the latter illness.[10] Most MAO B inhibitors that are in clinical use nowadays are irreversible.[10,11]



Scheme 1. Atom numbering of the flavin moiety, without which MAO enzymes are catalytically inactive. "R" denotes the ribityl adenosine diphosphate group, which is not shown here for clarity.

In our previous work, we studied the mechanism of the irreversible inhibition of MAO B by the acetylenic inhibitors rasagiline and selegiline.[12] In terms of the calculated barrier heights and the overall exergonicity of the reaction, our study elucidated that the polar anionic mechanism is the most probable, where the rate limiting step involves nucleophilic attack of the deprotonated inhibitor onto the flavin. The chemical reaction takes place on the N5 atom of the flavin (Scheme 1), in accordance with the available X-ray structures.[9,13,14] It followed that the latter reaction is preceded by a facile enzymatic proton abstraction from the inhibitor's terminal acetylene site. However, it has not been possible to experimentally determine the identity of the relevant proton acceptor, which we also did not determine in our computational study as it was performed on a model system involving the flavin and inhibitors. Therefore, as a preliminary step towards a deeper understanding of the chemical and the inhibition mechanisms, insight into the pK_a values of potentially catalytically relevant residues would be beneficial.

Three different potential catalytic mechanisms have been proposed to date: (1) a hydride mechanism, (2) a radical mechanism and (3) a polar nucleophilic mechanism. In other words, it is assumed that the

catalytic rate-limiting step involves either the heterolytic H^- abstraction in (1), or the homolytic $H^\bullet$ extraction in (2), or deprotonation of H^+ in (3), all from the α–carbon atom of the substrate in the vicinity of the amino group. A common feature of all three mechanisms is that the mentioned activating stage is performed by N5 atom on the flavin and that dopamine enters the reaction in the neutral form. Erdem et al.[15] assumed that the hydride mechanism is unlikely to take place, because hydride transfer is kinetically unfavorable.[16] Using kinetic and structural analysis, and employing Taft correlation to a series of benzylamine analogs, Miller and Edmondson[17] provided strong experimental evidence that proton transfer is an integral part of the rate limiting step, contrary to hydride anion abstraction. This has led Edmondson and co-workers to propose the polar nucleophilic mechanism for MAO enzymes, [17–24] although the latter has been disputed in the literature, mostly by Silverman,[25–29] Ramsay,[30–34] Scrutton[35] and their co-workers, in favor of the radical mechanism. Finally, in a very recent study Erdem and Büyükmenekşe[36] investigated a biradical mechanism for MAO catalysis, but in the same paper the authors declared it as improbable concluding that their results "present negative evidence for the modelled biradical mechanism". Nevertheless, it still remains a fact that, despite a huge amount of research devoted to MAOs in the last couple of decades, there is still no consensus in the literature about the exact mechanisms of the catalytic activity of MAO and its irreversible inhibition.

Several important structural features of MAO B have been thoroughly emphasized when assessing mechanisms of the catalysis/inhibition, but one is particularly relevant for the present work: the hydrophobic nature of the MAO active site composed of aromatic moieties, that include tyrosines (called the aromatic cage) and the FAD co-factor.[37,38] It should be stressed that hydrophobicity of an active site is not a black and white concept, it is difficult to define it, but on the other hand one can

relatively safely assume that it depends on the nature of the moieties comprising the active site. The active site hydrophobicity was proposed to determine the protonation state of the substrate in MAO active site, since MAO substrates are protonated in the cytoplasm, and are present as monocations under physiological conditions. Edmondson and coworkers argued[39] that because the free energy cost associated with the transfer of a charged moiety into the hydrophobic active site is expected to be too high, the substrate must enter the enzyme in its neutral form. However, experimental pH profiles for kynuramine oxidation by MAO A and phenylethylamine degradation by MAO B would suggest that the amine is most likely present in the active site in its protonated form,[40] though contradicting arguments have been presented by Scrutton and co-workers,[41] who, based on their pH dependent measurements of kinetic isotope effects in MAO A, suggested that the active site is believed to be organized for the activation of the neutral rather than charged form of the substrate. However, both groups agree that the neutral form must enter the chemical step. The aromatic cage surrounding the flavin co-factor also plays an important role in MAO enzymes. X-ray analysis revealed two tyrosyl residues (Tyr398 and Tyr435 in human MAO B), constituting the aromatic cage, which both lie almost perpendicular to flavin,[7,39] suggesting a functional role in catalysis. It was proposed that they are responsible for the orientation of a substrate towards the flavin, [37,38] but could also have direct involvement in the proton transfer reactions.

Therefore, for all reasons stated, it is critical to know the pK_a values of relevant residues and the substrate within the MAO active site in order to progress in understanding catalytic and inhibition mechanisms. However, these values are difficult to determine experimentally,[42] and, similarly, while experimental pH profiles can provide tremendous insight, it can be hard to conclusively determine the identity of residues whose protonation state is being affected. Although there are many experimental

methods that enable determination of the overall titration curve of a protein, only a few spectroscopic techniques posses sufficient resolution to allow for the determination of pK_a values of individual residues in a protein.[43] For MAO enzymes, a lot of research efforts has been devoted by Scrutton,[41] Edmondson,[44] Ramsay[45] and their co-workers to experimentally measure pK_a values, but only data for several residues that are close to the surface of MAOs, and which are believed to form the so-called "*entrance*" and "*substrate*" cavities[7,39,46–48] were obtained. In addition, pK_a calculations continue to provide a significant challenge to computations.[49–52] In the present work, we have investigated pK_a values of three tyrosine residues (Tyr188, Tyr 398 and Tyr 435) and the dopamine molecule within MAO B active site. Both the free enzyme and the enzyme complexed with dopamine were considered. We hope that the obtained acidity/basicity parameters will offer new insight into features of MAO enzymes and help elucidating exact mechanisms of their activity and irreversible inhibition.

COMPUTATIONAL METHODS

The starting point for our calculations was the high-resolution (1.6 Å) X-ray structure of MAO B in complex with 2-(2-benzofuranyl)-2-imidazoline),[13] which was obtained from the Protein Data Bank[53] (accession code 2XFN). All ligands present in the crystal structure were removed and we manually placed physiologically relevant dopamine monocation (Figure 1) in the active site, as it is a characteristic substrate metabolized by MAO B.

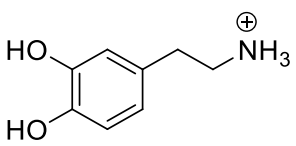


Figure 1. Chemical structure of the dopamine molecule in its physiological monocationic form.

pK_a calculations were performed using the semi-macroscopic protein dipole / Langevin dipole approach of Warshel and coworkers, in its linear response approximation version (PDLD/S-LRA),[49,54–56] To parameterize the charge distribution of oxidized FAD and dopamine, electrostatic potential derived atomic charges were obtained on the optimized structures at the (PCM)/B3LYP/6–31G(d) level of theory in conjunction with the UFF radii as implemented in Gaussian09 program.[57] The essence of the PDLD/LRA pK_a calculation is to convert the problem of evaluating a pK_a in a protein to evaluation of the change in "solvation" energy associated with moving the charge from water to the protein. One must consider the thermodynamic cycle described by the following equation: $\Delta G^{p}\left(AH_p \rightarrow A_p^- + H_w^+\right) = \Delta G^{w}(AH_w \rightarrow A_w^- + H_w^+) + \Delta G_{sol}^{w\rightarrow p}(A^-) - \Delta G_{sol}^{w\rightarrow p}(AH)$ where *p* and *w* denote protein and water, respectively. This equation can be rewritten for each ionizable residue *i*, as: $pK_{a,i}^{p} = pK_{a,i}^{w} - \frac{\bar{q}_i}{2.3RT}\Delta\Delta G_{sol}^{w\rightarrow p}(AH_i \rightarrow A_i^-)$ where the ΔΔG term consist of the last two terms of the previous equation, qi is the charge of the ionized form of the given residue, for acids $\bar{q}_i = -1(q(AH) = 0, q(A^-) = -1)$ and for base $\bar{q}_i = +1(q(AH) = +1, q(A^-) = 0)$. The p$K_a$ calculations are reduced to two free energy calculations in addition to the experimental value in aqueous solution. The first simulation is mutation of a neutral residue to its ionized analog in aqueous solution and the other is in the protein environment. The philosophy underlying the applied approach is the same as in calculation of activation free energies, where catalytic effect always refers to the reference reaction in aqueous solution. This approach calculates pK_a shifts relative to aqueous solution by taking into account the protein environment dependent stabilization effects for the Brønsted acid and its conjugate base.**Fehler! Textmarke nicht definiert.**[54] This method has previously been successfully applied to a wide range of systems of biological relevance, such as the aquaporin channel, carbonic anhydrase and

the bovine pancreatic trypsin inhibitor, to name a few examples.[52,58–61]

The protein studied here was first explicitly solvated using the surface constrained all atom solvent (SCAAS) model,[54] employing a water grid with a radius of 20 Å around the investigated residue. Long range interactions were treated using the local reaction field (LRF) approach.[62] The resulting system was equilibrated by running a 50 ps molecular dynamics simulation using a 0.5 fs time step at 300 K. After that, we evaluated pK_a values using the PDLD/S-LRA approach, employing full atomic charges, by averaging the corresponding values over the results obtained for 20 protein configuration windows, connecting charged and uncharged states, each averaged over 25 ps of simulation with a 1 fs time step, giving rise to a total simulation time of 500 ps for the entire thermodynamic perturbation.

Calculated pK_a values are sensitive to the applied external dielectric constant during the simulations. The choice of the correct dielectric constant to describe the protein interior is a very complicated issue, which has been the subject of heated debates over the years. A variety of values were suggested, ranging from ε = 2–80. For example, van Gunsteren and co-workers performed molecular dynamics simulation using the GROMOS force field, and obtained a value of ε = 30 for the interior of lysozyme.[63] In our work we employed ε = 8–12 based on the discussion in reference 55. All PDLD/S-LRA calculations were performed using the ENZYMIX force field and the MOLARIS simulation package.[54]

RESULTS AND DISCUSSION

The results of pK_a calculations of relevant residues in the MAO active site are shown in Table 1, and

the orientation of the relevant residues is illustrated in Fig. 2, as well as the corresponding pK_as of the tyrosine sidechain and dopamine in aqueous solution. Before we start analyzing the calculated results, it is useful to bring about the fact that experimental aqueous solution pK_a values of tyrosine (side chain –OH deprotonation) and dopamine (aminoethyl $-NH_3^+$ deprotonation) assume 10.1[64] and 8.9,[65] respectively. As a consequence, it follows that under physiological conditions tyrosine is a rather weak acid and is mostly present in the neutral Tyr–OH form, and that dopamine assumes monocationic form, being protonated at the free aminoethyl group.

Table 1. Calculated pK_a values at different dielectric constants ε.[a] All values are averaged over 20 starting conformations, with the corresponding standard deviations shown in parentheses.

	MAO B free enzyme					**MAO B in complex with protonated dopamine**					**pK_w**
	ε = 8	ε = 9	ε = 10	ε = 11	ε = 12	ε = 8	ε = 9	ε = 10	ε = 11	ε = 12	
Tyr188	11.2 (0.019)	11.1 (0.018)	11.0 (0.020)	10.4 (0.022)	10.4 (0.020)	13.6 (0.030)	13.3 (0.022)	13.1 (0.018)	12.5 (0.024)	12.3 (0.019)	
Tyr398	10.7 (0.020)	10.5 (0.023)	10.3 (0.020)	10.2 (0.020)	10.1 (0.018)	14.8 (0.019)	14.3 (0.019)	13.8 (0.016)	13.0 (0.020)	12.8 (0.016)	
Tyr435	10.2 (0.018)	10.2 (0.016)	10.2 (0.019)	9.7 (0.040)	9.8 (0.017)	15.6 (0.022)	15.2 (0.019)	14.8 (0.019)	14.0 (0.020)	13.8 (0.017)	
tyrosine											10.1
dopamine						8.7 (0.037)	8.7 (0.036)	8.7 (0.039)	8.9 (0.026)	8.9 (0.024)	8.9

[a] pK_w denotes the corresponding experimental value in aqueous solution.

The apparent pK_a value of each amino acid is influenced by the micro-environment provided by the protein's structure. The latter reflects inter-residue, residue-solvent and long-range electrostatic interactions with other charged residues in the protein or salt ions in solution. In the free enzyme, the pK_as of the tyrosine sidechains considered in this work are within 0.8 pK_a unit of the corresponding value in aqueous solution (Table 1). The former assume 10.8, 10.4 and 10.0 for Tyr188, Tyr398 and

Tyr435, respectively, obtained as the average of the five calculated dielectric constant dependent values (ε = 8–12). However, placing a protonated dopamine into the active site (Figure 2) causes an upward pK_a shift of up to 4.7 pK_a units, as would be expected from placing a positively charged species next to them into a hydrophobic active site, and in line with the suggestion of Edmondson and coworkers.[37,38] It turns out that in the complex, the hydroxy groups are made less acidic, which further favors the neutral form relative to the aqueous solution. We can conclude that it is very unlikely that these tyrosines could serve as proton acceptors either from the protonated substrate or particularly during the initial deprotonation of irreversible acetylenic inhibitors. It turns out that, before the protonated substrate enters the enzyme, its active site is not markedly hydrophobic resulting in unchanged tyrosine pK_a values. However, once dopamine monocation is positioned in the active site, its steric requirements demand removal of nearby water molecules, which enhances the hydrophobicity of the environment. As a consequence, the resulting tyrosine pK_a values are controlled by two opposing effects. Firstly, binding of the cation, which favors deprotonated form of tyrosine sidechain, thus lowering its pK_a values, and secondly the increased hydrophobic nature of the active site, which works towards an increase in tyrosine pK_a values. Our results demonstrate that the latter effect prevails resulting in an overall upwards shift of tyrosine acidity constants.

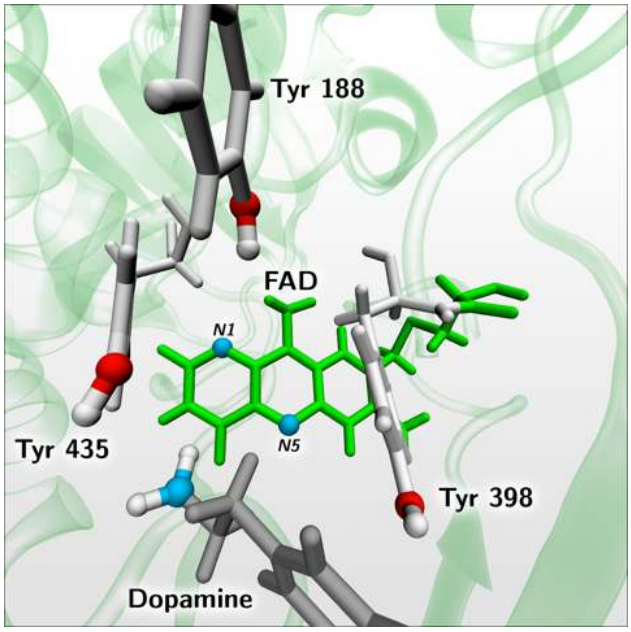


Figure 2. Structure of the MAO B active site in complex with dopamine.

Visualization of the relevant crystal structures as well as the simulation trajectories reveals that all three investigated tyrosine residues are found in front of the *re* side of flavin. Two of those (Tyr398 and Tyr435) form the so-called "aromatic cage" and the third one (Tyr188) is found between them, just a bit further away from the flavin (Figure 2). Additionally, despite the hydrophobic nature of the active site consisting of aromatic moieties, there appears to nevertheless still be a few water molecules present, which are hydrogen bonded to the aforementioned tyrosines, and connecting the tyrosine sidechains and the substrate with the N1 atom (Scheme 1) of the flavin. This is important, because this location could serve as the potential proton accepting site, which together with N5 position forms two reactive centers on flavin. These are, for example, found both hydrogenated in the reduced form of the flavin $FADH_2$. This is in agreement with the recent study by North and co-workers,[66] who used Mulliken population analysis on several substituted flavins and showed that the N1 atom bears more negative atomic charge and is more nucleophilic compared with the N5 atom,

which, on the other hand, shows electrophilic nature. Moreover, a large number of flavoenzymes have the N1 atom of FAD interacting with positively charged residues.2 As a result, there is a possibility of proton transfer from the dopamine to the bulk water, allowing for any of the suggested mechanisms of catalysis and inhibition.

From Table 1, it can be seen that when dopamine is bound to MAO B it assumes a pK_a value of 8.8 (Table 1), which would be expected from an amine bound in a hydrophobic active site consisting of aromatic moieties. This is in accordance with experimental studies on MAO A by Ramsay and coworkers.[40] Scrutton and co-workers,[41] however, considered several MAO A substrates and showed that, upon binding to the active site, the corresponding amine pK_a values were downshifted by as much as two pK_a units. Therefore, in order to verify our calculated pK_a shift using the PDLD/S-LRA approach, we also calculated the pK_a shift of the dopamine using the free energy perturbation adiabatic charging (FEP/AC) approach[67,68] and the thermodynamic cycle outlined in Figure 3 of reference 49 (see also references 69 and 70). That is, the charges of the dopamine were perturbed from its charged to neutral form (with the proton being replaced by a dummy atom with no charge in the neutral form) in 51 mapping frames of 50 ps length each (total simulation time 2.55 ns) in both aqueous solution and the MAO B active site. This gave solvation free energy differences of –53.7 and –54.1 kcal mol^{-1} in aqueous solution and in MAO B, respectively, which corresponds to a negligible downward pK_a shift of 0.3 kcal mol^{-1}, using the relationship $pK_a^p = pK_a^w + \Delta\Delta G/2.3RT$ (see for example reference 70), where pK_a^p and pK_a^w denote pK_a values of dopamine in protein and in water, in the same order. This is in good agreement with our PDLD/S-LRA calculations (Table 1), again suggesting that the dopamine is present in the active site in its protonated form. From this value, it is also possible to obtain the free energy cost for transferring a proton from the dopamine monocation to the

bulk using the relationship:[71] $\Delta G_{PT}^{p} = 2.303RT(pK_a^p(donor) - pH)$, which implies that, even if the dopamine is present in its protonated form, it would be fairly easy to deprotonate it by proton transfer to the bulk solvent, with a free energy cost of about 1.9 kcal mol^{-1} at physiological pH (7.4). It is also worth noting that analysis of the simulation trajectory reveals specific interactions of the protonated amino group with the aromatic cage (Figure 3), where, in a typical snapshot, the observed distance between the dopamine nitrogen atom and the centre of the phenyl ring on tyrosine residues assumes values between 4–5 Å. This, in conjunction with the elevated tyrosine pK_a values, suggests that these residues might play an important role in stabilizing the protonated amine, either directly through hydrogen bonding interactions with the relevant sidechains, or through cation–π interactions with the aromatic cage. The former can be as strong as hydrogen bonding interactions,[72] and are a well-established pattern of molecular recognition in the systems of biological interest.[73 – 76] As an illustration, the relevant experimentally determined gas-phase binding energy between protonated methylamine $MeNH_3^+$ and benzene is as high as –18.8 kcal mol^{-1}.[72] Also, it was demonstrated that cation–π interactions are crucial, for instance, in promoting binding of cationic agonists and antagonists to the nicotinic acetylcholine receptor.[73]

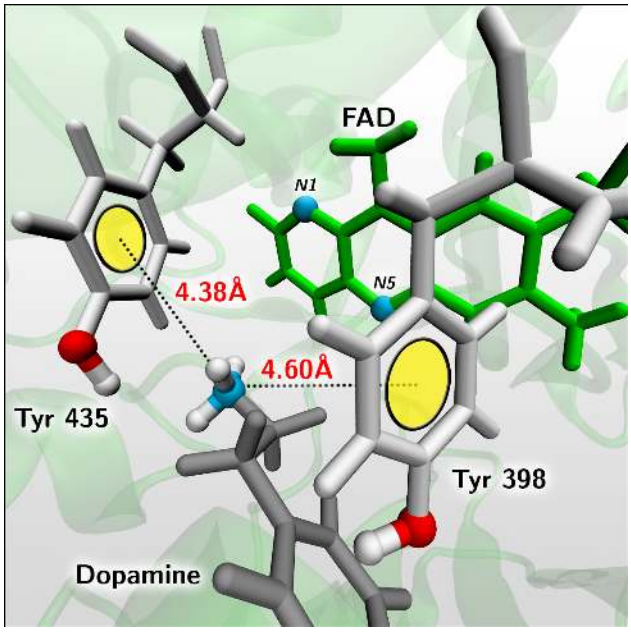


Figure 3. Typical snapshot of the simulation trajectory, giving some evidence of stabilizing cation–π interactions between the tyrosine aromatic cage and the protonated dopamine amino group.

Together with practically unchanged dopamine pK_a values, our study would strongly suggest that dopamine is predominantly present in the active site as monocation. This idea is in full agreement with the observed tendency that analogous benzyl alcohols are poor MAO substrates,[77] some of them even being MAO inhibitors.[78,79] A possible explanation is that alcohols cannot easily be protonated under physiological conditions (most protonated alkyl alcohols have pK_a values below –2),[80] which is in accordance with the work by Ramsay and co-workers[40] who demonstrated that even though the substrate is most likely protonated, neutral inhibitors form tighter binders. In addition, it should be stressed that the precise nature of binding of those benzyl alcohols to MAO enzymes is not yet known. However, the low free energy cost associated with dopamine deprotonation to the bulk implies that dopamine can easily be deprotonated prior to chemical step. This is in harmony with all three proposed catalytic mechanisms that all require neutral dopamine as a starting point.

CONCLUDING REMARKS

In this article we provide what is, to the best of our knowledge, the first systematic study of the pK_a values of titratable groups present in the active site of Monoamine oxidase B (MAO B). We have considered here both the holoenzyme and the enzyme supplemented with the dopamine molecule to form enzyme–substrate Michaelis complex, giving rise to the prechemical step of dopamine degradation. Specifically, we have examined the pK_as of the three tyrosine residues that constitute the so-called aromatic cage,[7,39] the pK_a value of the dopamine itself, as well as the free energy cost of potential deprotonation of the dopamine by bulk solvent. The calculations were performed using the full dimensionality of the protein and extensive sampling.

It was demonstrated that, for the investigated tyrosine residues, their pK_a values span the range between 13.0–14.7 pK_a units, which are increased from the corresponding water solution value of 10.1 providing strong support to the idea of the hydrophobic nature of the active site put forward by Edmondson and co-workers,[17] which could help in understanding the precise mechanism of the catalytic step and the inhibition reaction of MAO enzymes. The altered tyrosine pK_a values could be rationalized as an interplay of two opposing effects: insertion of positively charged bulky dopamine that lowers the tyrosine pK_a values, and therewith associated removal of water molecules from the active site that promotes hydrophobicity and elevates the tyrosine pK_a values, in which the latter prevails. Similarly, calculated pK_a values for the dopamine suggest that the pK_a of this species is relatively unaffected by the change of environment, which would again be consistent with an amine placed in a hydrophobic active site consisting of aromatic residues, and implying that the

corresponding deprotonation of the dopamine monocation by the bulk solvent would be fairly facile, with a free energy cost of 1.9 kcal mol^{-1} at room temperature and physiological pH. It is worth noting that, during the entire simulation time, few water molecules were present at the active site, and were constantly exchanging with the bulk water molecules. This gives some additional evidence about the nature of the active site, which is not conventionally water-free hydrophobic, but rather involves the aromatic cage that is capable to form favorable quadrupolar interactions with dipolar (water) and cationic (protonated substrate) species. Additional proof was provided by visualization of the simulation trajectories showing a favorable orientation of the charged dopamine towards tyrosine residues. We would like to emphasize at this stage that Edmondson and co-workers[39] did raise a valid point about the high free energy cost associated with transporting a protonated dopamine through a membrane, so at this stage it is unclear precisely how the protonated dopamine could enter the MAO B active site. However, this is an issue that is out of the scope of the present work, and our calculated results tie in with experimental studies on MAO A,[40] suggesting that the amine is present in the active site in its protonated form, but that the relatively low cost of proton loss to the bulk is consistent with all three proposed catalytic mechanisms that all require neutral dopamine to enter the chemical step.

Future studies will be directed towards elucidation of the exact catalytic and the inhibition mechanisms of MAO B on the QM/MM level with proper thermal averaging and appropriate free energy calculations.[67] Quantization of the nuclear motion should yield values of the H/D kinetic isotope effects that will discriminate between several possible mechanisms. In order to facilitate this, the present results shed new light on features of MAO B active site and provide relevant constrains for upcoming calculations. It remains a future challenge to apply the molecular dynamics methodology for simulations at the constant pH[81] and quantum dynamical treatment of proton dynamics[82–84] to

MAO B active site, which we plan to address. Our ultimate goal will be to rationalize substrate and inhibitor specificity and design novel reversible and irreversible inhibitors that are all potential drugs, used in the clinical treatment of depression and certain neurological disorders like Parkinson disease.[85]

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REFERENCES

(1) Joosten, V.; van Berkel, W. J. *Curr. Opin. Chem. Biol.* **2007**, *11*, 195–202.

(2) Fraaije, M. W.; Mattevi, A. *Trends Biochem. Sci.* **2000**, *25*, 126–132.

(3) Costa, E.; Green, A. R.; Koslow, S. H.; LeFevre, H. F.; Revuelta, A. V.; Wang, C. *Pharmacol. Rev.* **1972**, *24*, 167–190.

(4) Johnston, J. P. *Biochem. Pharmacol.* **1968**, *17*, 1285–1297.

(5) Wu, H. F.; Chen, K.; Shih, J. C. *Mol. Pharmacol.* **1993**, *43*, 888–893.

(6) Nandigama, R. K.; Edmondson, D. E. *J. Biol. Chem.* **2000**, *275*, 20527–20532.

(7) Binda, C.; Hubalek, F.; Li, M.; Edmondson, D. E.; Mattevi, A. *FEBS Lett.* **2004**, *564*, 225–228.

(8) Edmondson, D. E.; Mattevi, A.; Binda, C.; Li, M.; Hubalek, F. *Curr. Med. Chem.* **2004**, *11*, 1983–1993.

(9) De Colibus, L.; Li, M.; Binda, C.; Lustig, A.; Edmondson, D. E.; Mattevi, A. *Proc. Nat. Acad. Sci. USA* **2005**, *102*, 12684–12689.

(10) Hefti, M. H.; Vervoort, J.; van Berkel, W. J. *Eur. J. Biochem.* **2003**, *270*, 4227–4242.

(11) Goodman, L. S.; Brunton, L. L.; Chabner, B.; Knollmann, B. C. *Goodman & Gilman's Pharmacological Basis of Therapeutics*, McGraw-Hill, New York, 12th edn., 2011.

(12) Borštnar, R.; Repič, M.; Kržan, M.; Mavri, J.; Vianello, R. *Eur. J. Org. Chem.* **2011**, 6419–6433.

(13) Bonivento, D.; Milczek, E. M.; McDonald, G. R.; Binda, C.; Holt, A.; Edmondson, D. E.; Mattevi, A. *J. Biol. Chem.* **2010**, *285*, 36849–36856.

(14) Binda, C.; Hubálek, F.; Li, M.; Herzig, Y.; Sterling, J.; Edmondson, D. E.; Matevi, A. *J. Med. Chem.* **2005**, *48*, 8148–8154.

(15) Erdem, S. S.; Karahan, O.; Yildiz, I.; Yelekci, K. *Org. Biomol. Chem.* **2006**, *4*, 646–658.

(16) Binda, C.; Wang, J.; Li, M.; Hubálek, F.; Mattevi, A.; Edmondson, D. E. *Biochemistry* **2008**, *47*, 5616–5625.

(17) Miller, J. R.; Edmondson, D. E. *Biochemistry* **1999**, *38*, 13670–13683.

(18) Edmondson, D. E. *Xenobiotica* **1995**, *25*, 735–753.

(19) Edmondson, D. E.; Bhattacharrya, A. K.; Xu, J. *Biochim. Biophys. Acta* **2000**, *1479*, 52–58.

(20) Edmondson, D. E.; Bhattacharyya, A. K.; Walker, M. C. *Biochemistry* **1993**, *32*, 5196–5202.

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

(22) Nandigama, R. K.; Edmondson, D. E. *Biochemistry* **2000**, *39*, 15258–15265.

(23) Walker, M. C.; Edmondson, D. E. *Biochemistry* **1994**, *33*, 7088–7098.

(24) Upadhyay, A. K.; Wang, J.; Edmondson, D. E. *Biochemistry* **2008**, *47*, 526–536.

(25) Silverman, R. B.; Lu, X.; Zhou, J. J. P.; Swihart, A. *J. Am. Chem. Soc.* **1994**, *116*, 11590–11591.

(26) Silverman, R. B.; Lu, X. *J. Am. Chem. Soc.* **1994**, *116*, 4129–4130.

(27) Silverman, R. B. *Acc. Chem. Res.* **1995**, *28*, 335–342 and references cited therein.

(28) DeRose, V. J.; Woo, J. C.; Hawe, W. P.; Hoffman, B. M.; Silverman, R. B.; Yelekci, K. *Biochemistry* **1996**, *35*, 11085–11091.

(29) Silverman, R. B. *Biochem. Soc. Trans.* **1991**, *19*, 201–206.

(30) Ramsay, R. R.; Sablin, S. O.; Bachurin, S. O.; Singer, T. P. *Biochemistry* **1993**, *32*, 9025–9030.

(31) Rigby, S. E.; Hynson, R. M.; Ramsay, R. R.; Munro, A. W.; Scrutton, N. S. *J. Biol. Chem.* **2005**, *280*, 4627–4631.

(32) Sablin, S. O.; Ramsay, R. R. *J. Biol. Chem.* **1998**, *273*, 14074–14076.

(33) Ramsay, R. R. *Biochemistry* **1991**, *30*, 4624–4629.

(34) Kay, C. W.; El Mkami, H.; Molla, G.; Pollegioni, L.; Ramsay, R. R. *J. Am. Chem. Soc.* **2007**, *129*, 16091–16097.

(35) Scrutton, N. S. *Nat. Prod. Rep.* **2004**, *21*, 722–730.

(36) Erdem, S. S.; Buyukmenekse, B. *J. Neural Transm.* **2011***, 118*, 1021–1029.

(37) Li, M.; Binda, C.; Mattevi, A.; Edmondson, D. E. *Biochemistry* **2006**, *45*, 4775–4784.

(38) Akyuz, M. A.; Erdem, S. S.; Edmondson, D. E. *J. Neural Transm.* **2007**, *114*, 693–698.

(39) Binda, C.; Wang, J.; Pisani, L.; Caccia, C.; Carotti, A.; Salvati, P.; Edmondson, D. E.; Mattevi, A. *J. Med. Chem.* **2007**, *50*, 5848–5852.

(40) Jones, T. Z. E.; Balsa, D.; Unzeta, M.; Ramsay, R. R. *J. Neural Transm.* **2007**, *114*, 707–712.

(41) Dunn, R. V.; Marshall, K. R.; Munro, A. W.; Scrutton, N. S. *FEBS J.* **2008**, *275*, 3850–3858.

(42) Grimsley, G. R.; Scholtz, J. M.; Pace, C. N. *Protein Sci.* **2009**, *18*, 247–251 and references cited therein.

(43) Juffer, A. H. *Biochem. Cell Biol.* **1998**, *76*, 198–209.

(44) Milczek, E. M.; Binda, C.; Rovida, S.; Mattevi, A.; Edmondson, D. E. *FEBS J.* **2011**, *278*, 4860–4869.

(45) Ramsay, R. R. *Vopr. Med. Khim.* **1997**, *43*, 457–470.

(46) Ramsay, R. R.; Dunford, C.; Gillman, P. K. *Br. J. Pharmacol.* **2007**, *152*, 946–951.

(47) Ramsay, R. R.; Koerber, S. C.; Singer, T. P. *Biochemistry* **1987**, *26*, 3045–3050.

(48) Ramsay, R. R.; Sablin, S. O. *Neurobiol.* **1999**, *7*, 205–212.

(49) Kamerlin, S. C.; Haranczyk, M.; Warshel, A. *J. Phys. Chem. B* **2009**, *113*, 1253–1272 and references cited therein.

(50) Vogel, H. J.; Juffer, A. H. *Theor. Chem. Acc.* **1999**, *101*, 159–162.

(51) Harris, T. K.; Turner, G. J. *Life* **2002**, *53*, 85–98.

(52) Kato, M.; Warshel, A. *J. Phys. Chem. B* **2006**, *110*, 11566–11570.

(53) Bernstein, F. C.; Koetzle, T. F.; Williams, G. J.; Meyer Jr., E. E.; Brice, M. D.; Rodgers, J. R.; Kennard, O.; Shimanouchi, T.; Tasumi, M. *J. Mol. Biol.* **1977**, *112*, 535–542.

(54) Lee, F. S.; Chu, Z. T.; Warshel, A. *J. Comp. Chem.* **1993**, *14*, 161–185.

(55) Sham, Y. Y.; Chu, Z. T.; Tao, H.; Warshel, A. *Proteins* **2000**, *39*, 393–407.

(56) Kato, M.; Pisliakov, A. V.; Warshel, A. *Proteins* **2006**, *64*, 829–844.

(57) Gaussian 09, Revision A.1, Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, Jr., J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, N. J.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. Gaussian, Inc., Wallingford CT, 2009.

(58) Schutz, C. N.; Warshel, A. *Proteins* **2001**, *44*, 400–417.

(59) Braun–Sand, S.; Burykin, A.; Chu, Z. T.; Warshel, A. *J. Phys. Chem. B* **2005**, *109*, 583–592.

(60) Burykin, A.; Warshel, A. *Biophys. J.* **2003**, *85*, 3696–3706.

(61) Burykin, A.; Kato, M.; Warshel, A. *Proteins* **2003**, *52*, 412–426.

(62) Lee, F. S.; Warshel, A. *J. Chem. Phys.* **1992**, *97*, 3100–3107.

(63) Smith, P. E.; Brunne, R. M.; Mark, A. E.; van Gunsteren, W. F. *J. Phys. Chem.* **1993**, *97*, 2009–2014.

(64) Nespoulous, C.; Pernollet, J. C. *Int. J. Pept. Protein. Res.* **1994**, *43*, 154–159.

(65) Armstrong, J.; Barlow, R. B. *Br. J. Pharmac.* **1976**, *57*, 501–516.

(66) North, M. A.; Bhattacharyya, S.; Truhlar, D. G. *J. Phys. Chem. B* **2010**, *114*, 14907–14915.

(67) Warshel, A. *Computer Modeling of Chemical Reactions in Enzymes and Solutions*, Wiley–Interscience, New York, 1997.

(68) Warshel, A.; Sussman, F.; King, G. *Biochemistry* **1986**, *25*, 8368–8372.

(69) Glennon, T. M.; Villa, J.; Warshel, A. *Biochemistry* **2000**, *39*, 9641–9651.

(70) Fothergill, M.; Goodman, M. F.; Petruska, J.; Warshel, A. *J. Am. Chem. Soc.* **1995**, *117*, 11619–11627.

(71) Warshel, A. *Biochemistry* **1981**, *20*, 3167.

(72) Deakyne, C. A.; Meot-Ner (Mautner), M. *J. Am. Chem. Soc.* **1985**, *107*, 474–479.

(73) Ma, J. C.; Dougherty, D. A. *Chem. Rev.* **1997**, *97*, 1303–1324, and references cited therein.

(74) Dougherty, D. A. *Science* **1996**, *271*, 163–168.

(75) Gaberšček, Mavri, J. *Chem. Phys. Lett.* **1999**, *308*, 421–427.

(76) Mavri, J.; Koller, J.; Hadži, D. *J. Mol. Struct. (Theochem)* **1993**, *283*, 305–312.

(77) McEwen Jr., C. M.; Sasaki, G.; Lenz Jr., W. R. *J. Biol. Chem.* **1968**, *243*, 5217–5225.

(78) McEwen Jr., C. M. *J. Biol. Chem.* **1965**, *240*, 2011–2018.

(79) Severina, I. S. *Eur. J. Biochem.* **1973**, *38*, 239–246.

(80) Smith, M. B.; March, J. *March's Advanced Organic Chemistry: Reactions, Mechanisms and Structure*, 5th ed.; John Wiley & Sons: New York, 2001.

(81) Bürgi, R.; Kollman, P. A.; van Gunsteren, W. F. *Proteins* **2002**, *47*, 469–480.

(82) Mavri, J.; Berendsen, H. J. C.; van Gunsteren, W. F. *J. Phys. Chem.* **1993**, *97*, 13469–13476.

(83) Billeter, S. R.; van Gunsteren, W. F. *Comp. Phys. Comm.* **1997**, *107*, 61–91.

(84) Billeter, S. R.; van Gunsteren, W. F. *J. Phys. Chem. A* **1998**, *102*, 4669–4678.

(85) Westlund, K. N. in *Monoamine oxidase inhibitors in neurological diseases*, ed. A. N. Lieberman, CRC Press, **1994**, pp. 1–20.