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Structural Properties of Monoamine Oxidase B Complexed with Adrenaline in the Deamination Reaction: A Theoretical Study

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Abstract

Monoamine Oxidase B (MAO B) is a flavoenzyme that regulates and oxidizes monoamine neurotransmitters such as adrenaline, noradrenaline and dopamine. The oxidation reaction also produces hydrogen peroxide that can be harmful to dopaminergic neurons leading to neurological disorders such as Alzheimer's disease and Parkinson's disease. MAO B inhibitor is consequently used for preventing those neurological disorders. The structural details insight into the oxidation reaction mechanism of MAO B are vital to design specific inhibitors. A theoretical study is used to explain structural details of theoretical states in molecular level. In this work, the deamination reaction of adrenaline by MAO B was carried out to gain molecular-level details via ONIOM technique at M062X/6–31+G(d,p):PM6 level of theory. According to ONIOM calculation results, there are 4 key MAO B active site residues which are Leu171, Phe343, Tyr398 and Tyr435. Residues that bind adrenaline via hydrophobic interactions are Leu171, Phe343 and Tyr398 while Tyr435 binds via hydrogen bond. Additionally, NBO analysis suggests that the mechanism of this reaction is a hybrid between hydride transfer and polar nucleophilic characters.

Keywords: Monoamine Oxidase B, Adrenaline, ONIOM technique, Deamination reaction, Hydride transfer, Nucleophilic mechanism, Inhibitor

Introduction

Monoamine Oxidase B (MAO B) is a flavoenzyme that degrades several catecholamine molecules such as adrenaline, dopamine, phenethylamine and benzylamine [1]. Structures of those catecholamine molecules can be seen in **Figure 1**. The catalyst also produces the hydrogen peroxide (H_2O_2) [2] which can lead to the cellular damage. In advanced age, the MAO B level is increased significantly compared to adolescent [3] causing the rising of dopamine metabolism which is mainly served by glial MAO B [4]. Moreover, the production of hydrogen peroxide is risen leading to the death of dopaminergic neuronal cell where dopamine is produced. This depletion of dopaminergic neuronal cell results in the dopamine reduction that can further cause Alzheimer's disease and Parkinson's disease especially in aging people. So, the inhibition of MAO B has been considered to protect the decreasing of the dopamine level.

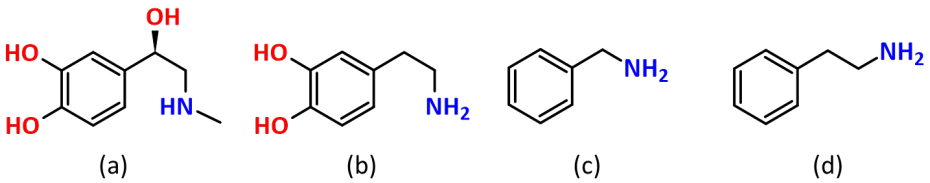

Figure 1 Examples of MAO B substrates as (a) Adrenaline, (b) Dopamine, (c) Benzylamine and (d) Phenethylamine.

The understanding of MAO B-ligand binding interactions and the reaction mechanism of the deamination are a guide that can be further investigated to develop specific MAO B inhibitors. However, the structural properties of the MAO B-ligand complex in molecular level are still required for developing

more specific inhibitors. Therefore, a theoretical study is called for the molecular-level details to explain these issues. One of techniques that can be used effectively in the theoretical calculation is the our own n-layered integrated molecular orbital and molecular mechanics or ONIOM technique. It can save time in the calculation but still gives an acceptable result [5]. This technique has been successfully applied to investigate the reaction mechanism of a serotonin oxidation by MAO B. It resulted that the mechanism of the serotonin oxidation by MAO B is a hybrid mechanism which is a mix between polar nucleophilic and hydride transfer, but it is mainly hydride transfer [4].

Adrenaline (ADR), a neurotransmitter, is one of the substrates of MAO B [6]. ADR has a hydroxyl functional group in the chain whereas other substrates have no this functional group as shown in **Figure 1**. This structural property of ADR might play an important role in the binding between MAO B and ADR leading to the new inhibitor design. Anyway, there have still no reports about the structural properties of the MAO B-ADR complex.

In this study, structural details of the ADR in the degradation by MAO B are carried out by using the ONIOM technique to go deeper into the binding details of the MAO B-ADR complex for further study of the inhibitor design.

Research methods and computational details

In order to generate the MAO B-ADR complex, the 3-dimension structure of MAO B was obtained from the Protein Data Bank, PDB code as 2BYB, while the structure of ADR was created and optimized by mean of M062X/6–311++G(d,p) level of theory. The MAO B-ADR complex was produced by removing the complexed inhibitor of 2BYB. Then, ADR was located instead the inhibitor. This initial MAO B-ADR complex was subjected to optimization using the ONIOM technique.

In this study, the 2-layer ONIOM optimization was employed. The inner layer of the ONIOM consists of ADR and the Flavin Adenine Dinucleotide (FAD) coenzyme. This FAD is covalently bound to Cys397. Remarkably, the FAD carbon atom that bound to Cys397 was set as a link atom. Additionally, the FAD was modified by representing its ribityl side chain with the methyl group. The active site residues of 2BYB as Tyr60, Leu171, Cys172, Tyr188, Ile198, Gln206, Tyr326, Cys397, Tyr398 and Tyr435 were selected and set as the outer layer of the ONIOM. The illustration of inner and outer layers of the ONIOM calculation system can be seen in **Figure 2**. The inner layer ONIOM was treated by DFT M062X method with 6–31+G(d,p) basis set while the outer layer ONIOM was calculated by mean of semi-empirical PM6.

In order to maintain the MAO B binding site cavity, the carbon atom of substituted methyl group on FAD and backbone atoms of active site amino acids as alpha-carbon, carbonyl carbon, carbonyl oxygen and amine nitrogen atoms were kept freezing during the optimization.

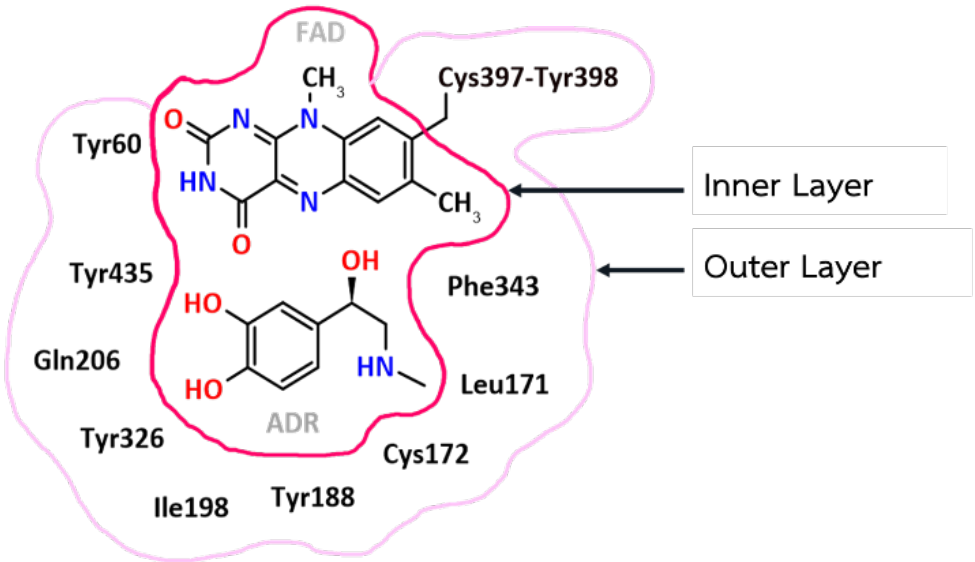

Figure 2 The ONIOM layer for optimization calculations of the MAO B-ADR complex.

To investigate structural details and the change in the complex energy along the deamination reaction take place, the reaction coordinate (R_c) was defined as presented in **Figure 3**.

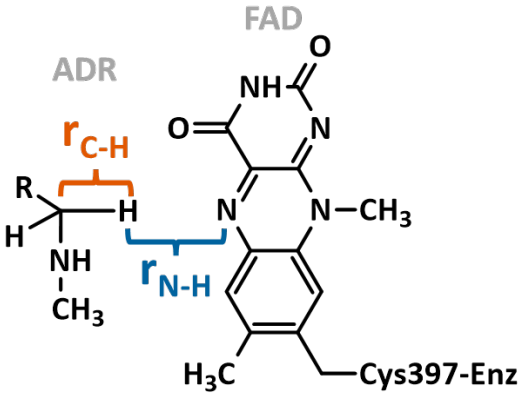

Figure 3 The reaction coordinate for studying the hydrogen transfer from ADR to MAO B.

The reaction coordinate of ADR deamination reaction by MAO B was defined by the difference of 2 distances as the length between the transferred hydrogen atom to the donor carbon atom of ADR or R(C–H) and the distance of the transferred hydrogen atom to the acceptor nitrogen atom of FAD or R(H– N). This can be written in the equation as

$$R_c = R(C–H)–R(H–N). \quad (1)$$

Notably, the ONIOM technique were applied to perform the optimization of all MAO B-ADR complexes obtained from the reaction coordinate.

The vibrational frequency calculations were calculated for all optimized MAO B-ADR complexes along the reaction coordinate at M062X/6–31+G(d,p) level of theory. The reactant, intermediate and product complexes were characterized by all real frequencies while the transition structure was identified by only 1 imaginary frequency. The transition complex was subjected to the 2nd order perturbation theory analysis of Fock matrix in the Natural Bond Orbital (NBO) basis at M062X/6–31+G(d,p) level of theory [7]. The stabilization energy $E^{(2)}$ values associated with the donor NBO (i) and the receptor NBO (j) interactions were calculated according to the following equation:

$$E^{(2)} = \Delta E_{i,j} = q_i \frac{(F_{i,j})^2}{E_j - E_i} \quad (2)$$

where q_i is occupancy, E_i and E_j are diagonal elements and $F(i,j)$ is the off diagonal NBO Fock matrix element.

All optimization and stabilization energy calculations were performed by Gaussian 09 program [8]. The intermolecular interactions of MAO B-ADR complexes along the oxidation reaction were investigated by using Protein-Ligand Interaction Profiler [9] and ProteinPlus web servers [10]. For preparing the input files of the web servers, the coordinates of all optimized complexes were converted to the PDB format. Then, the PDB files were submitted to Protein-Ligand Interaction Profiler web tool and ProteinPlus web tool. After calculations, intermolecular interaction results can be obtained from the web tools.

Results and discussion

Along the oxidation reaction of ADR by MAO B, reaction states were investigated through the relative reaction energy profile obtained from the reaction coordinate as presented in **Figure 4**.

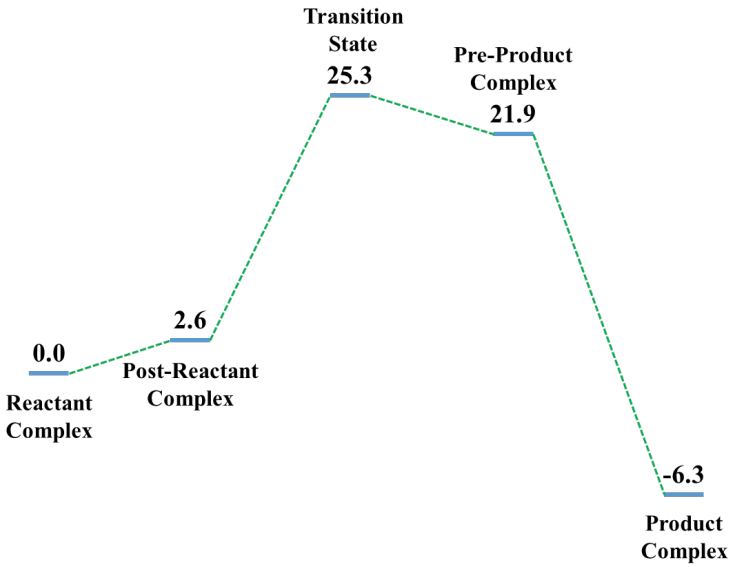

Figure 4 The relative energy (kcal mol^{-1}) profile of ADR deamination reaction by MAO B revealed by M062X/6−31+G(d,p):PM6 level of accuracy.

With regard to the relative reaction energy profile, 5 reaction states of the oxidation reaction of ADR by MAO B as reactant complex, post-reactant complex, transition state, pre-product complex and product complex, have been observed. Considering the relative reaction energy values, this reaction is the exothermal reaction since the product complex energy value related with the reactant complex energy is −6.3 kcal mol^{-1}. Additionally, the activated energy of this reaction is 25.3 kcal mol^{-1} observed from the relative reaction energy value of the transition state.

The optimized structures of oxidation reaction states of ADR by MAO B have been shown in **Figure 5**. It is found that ADR located in front of the face of FAD by turning the αC(ADR) atom to N5(FAD) atom throughout the reaction. For the reactant complex presented in **Figure 5**(**a**), the αC(ADR)−N5(FAD) distance is 3.48 Å and the αC(ADR)−αH(ADR)−N5(FAD) angle is 148.07º. The orientation of ADR in the post-reactant complex, shown in **Figure 5**(**b**), slightly differs from the reactant complex. However, the αC(ADR)–αH(ADR)–N5(FAD) angle of the post-reactant complex orientated to 180.0º preparing the transfer of the αH(ADR) atom to the N5(FAD) atom. The αC(ADR)–N5(FAD) distance of the post-reactant complex is 3.48 Å.

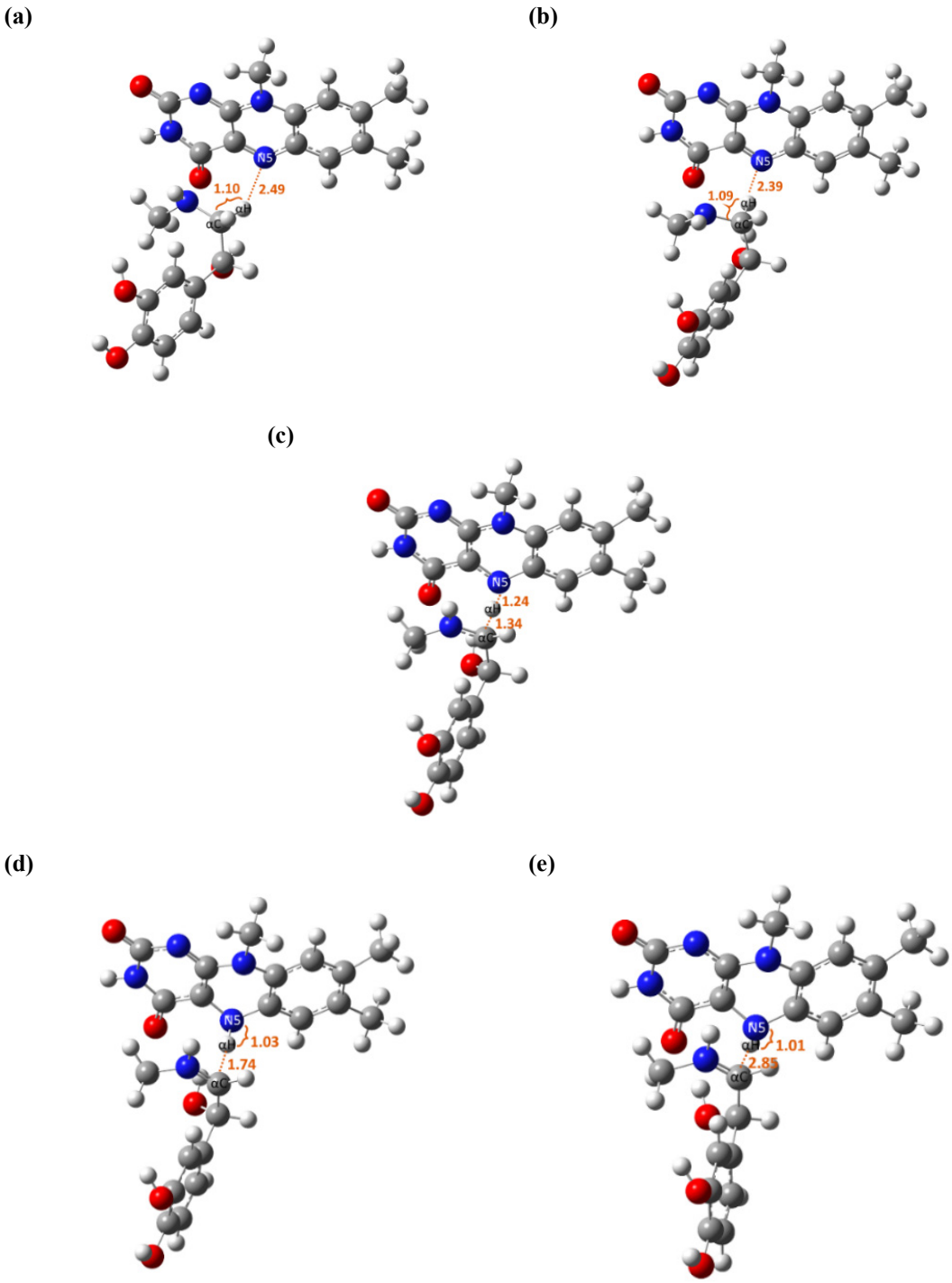

Figure 5 The optimized structures of oxidation reaction states of ADR by MAO B as (a) Reactant complex, (b) Post-reactant complex, (c) Transition state, (d) Pre-product complex and (e) Product complex.

The optimization structure of the transition state of ADR deamination reaction by MAO B has been displayed in **Figure 5**(**c**), the αC(ADR)–αH(ADR) and αH(ADR)–N5(FAD) distances were measured as 1.34 Å and 1.24 Å, respectively as well as the αC(ADR)–αH(ADR)–N5(FAD) angle is 180.0º. In **Figure 5**(**d**), the structural details of the pre-product complex have been presented. The αH(ADR) atom had completely transferred to the N5(FAD) atom with the αH(ADR)–N5(FAD) distance as 1.03 Å. After the hydrogen transfer, ADR transformed itself to the imine structure, the αC(ADR) atom has been bound to the N(ADR) atom with a double bond. The αC(ADR)–N5(FAD) distance of this state is 2.77 Å. According to the structural details of the product complex shown in **Figure 5**(**e**), the transferred αH(ADR) atom orientated from the face of FAD to behind. In addition, the αC(ADR)−N5(FAD) distance of the product complex as 2.41 Å, is shorter when compared of that with the pre-product complex.

The intermolecular interaction details of each reaction state were revealed by Protein-Ligand Interaction Profiler and ProteinPlus web servers as presented in **Figure 6**. On the reactant complex, ADR is held on the MAO B active site by the hydrophobic interaction of Leu171, a hydrogen bond interaction of Ile198 and 2 hydrogen bond interactions of Tyr435 as shown in **Figure 6**(**a**). The intermolecular interactions of the post-reactant complex have been discussed through **Figure 6**(**b**). The side chain of ADR has been found standing in front of FAD and locating between 2 tyrosyl residues as Tyr398 and Tyr435. ADR has been held by a hydrophobic interaction of Tyr398 and a hydrogen bond interaction of Tyr435. The existence of FAD, Tyr398 and Tyr435 is known as “aromatic cage” playing an important role on the catalysis of MAO B [11]. For the catechol part of ADR in the post-reactant complex, it has been embedded with a hydrophobic interaction of Leu171 and 3 hydrogen bond interactions of MAO B active site residues as Cys172, Gln206 and Tyr435. In **Figure 6**(**c**), the intermolecular interactions of the transition complex have been revealed. ADR is enhanced to perform the hydrogen transfer process. It is remained in the process by hydrophobic interactions of Leu171, Phe343 and Tyr398 and a hydrogen bond interaction of Tyr435 residue. This can be indicated that not only aromatic cage has been contributed the hydrogen transfer but also Leu171 and Phe343 have been attended in this process. Interestingly, those 4 residues interact at the catechol part of ADR only.

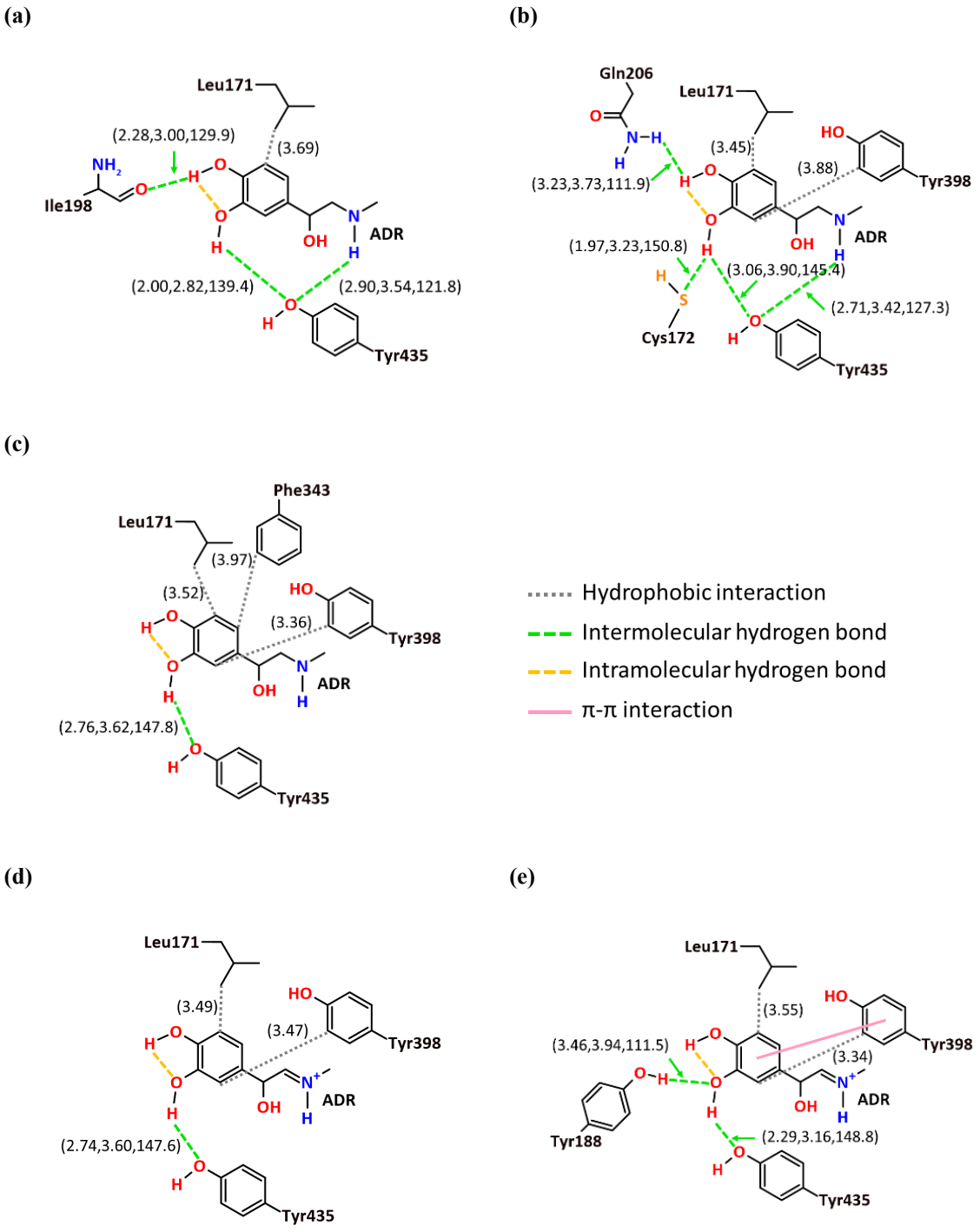

Figure 6 The intermolecular interaction details of ADR deamination reaction by MAO B as (a) Reactant complex, (b) Post-reactant complex, (c) Transition state, (d) Pre-product complex and (e) Product complex.

After the hydrogen transfer process, the pre-product complex has been formulated as **Figure 6**(**d**). there are 2 intermolecular interaction types between the imine and MAO B active site residues. Firstly, hydrophobic interactions of Leu171 and Tyr398. Secondly, a hydrogen bond interaction of Tyr435. For the product complex, the imine has been carried on the MAO B active site by hydrophobic interactions of Leu171 and Tyr635 and hydrogen bond interactions of Tyr188 and Tyr435 as well as a π–π interaction between the catechol part of ADR and Tyr398. Those interactions have been displayed in **Figure 6**(**e**).

With regard to the obtained intermolecular interaction details of all reaction states, it can indicate that Leu171, Phe343, Tyr398 and Tyr435 are key residues of ADR deamination reaction by MAO B. They enhance the ADR to perform the hydrogen transfer and handle the imine in MAO B active site after the process. The intermolecular interaction details of these key residues are crucial information for designing more specific MAO B inhibitors.

The stabilization energy of the MAO B-ADR transition state has been calculated using M062X method with 6–31+G(d,p) basis set and presented in **Table 1**. The calculated stabilization energy values were used to identify the reaction mechanism of ADR deamination by MAO B.

Table 1 The stabilization energy values of the MAO B-ADR transition state calculated at DFT M062X method with 6–31+G(d,p) basis set.

Donor NBO (*i*)	Acceptor NBO (*j*)	$E^{(2)}$ (kcal/mol)	$E(j) - E(i)$ (a.u.)	$F(i,j)$ (a.u.)
N5-C4a	H15	127.08	0.47	0.242
N5	H15	51.38	0.55	0.176
C13	H15	464.27	0.27	0.335
N11	C13	213.45	0.07	0.142

The strong stabilization energies can be observed at the electron transitions from N11 atom of ADR to C13 atom of ADR and C13 atom of ADR to H15 atom of ADR. This electron transfer characters are agreeable to the hydride mechanism [12,13]. As a result, ADR deamination by MAO B can be proposed as the hydride mechanism. The involved atoms in the mechanism have been labeled and presented in **Figure 7**.

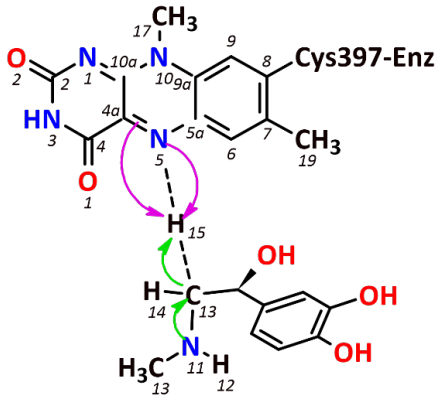

Figure 7 The 2 directions of electron flow between MAO B and ADR.

Surprisingly, not only the hydride mechanism character has been found, but also the character of the nucleophilic mechanism has been discovered. According to stabilization energy data in **Table 1**, there are the dominant stabilization energies of the electron transitions from the C4a–N5 bond to H15 atom of ADR and N5 atom of FAD to H15 atom of ADR. These characters are agreeable to the nucleophilic mechanism [14,15].

With regard to the presented stabilization energy data, there are 2 directions of electron flowing between MAO B and ADR illustrated in **Figure 7**. This existence can indicate that the reaction mechanism of ADR oxidation by MAO B is mixed between the hydride transfer and nucleophilic mechanisms so called the hybrid mechanism. Additionally, this hybrid mechanism has also been observed in the serotonin degradation mechanism MAO B [4]. However, the stabilization energy values of the

hydride character are larger than that of the nucleophilic character. Therefore, the major mechanism in the mixture reaction mechanism is the hydride mechanism.

Conclusions

The ONIOM technique has been successfully applied to investigate structural properties along the ADR deamination reaction by MAO B. Five reaction states as reactant, post-reactant, transition, pre-product and product states, have been revealed by mean of M062X/6–31+G(d,p):PM6 level of theory. During the reaction pathway, hydrophobic and hydrogen bond interactions between ADR and MAO B active site residues have been encouraged the ADR deamination reaction. ADR has been enhanced to perform a hydrogen transfer process by the intermolecular interactions of Leu171, Phe343, Tyr398 and Tyr435. Additionally, ADR has been handled in the MAO B active site by making the hydrophobic interaction with Leu171 and the hydrogen bond interactions with Tyr435 through the reaction pathway. These results can be indicated that Leu171, Phe343, Tyr398 and Tyr435 are the key residues in ADR deamination reaction by MAO B. The revealed structural properties in this study can be used for further designing novel specific MAO B inhibitors. In addition, the NBO analysis can be used to support that the ADR degradation by MAO B is performed in the mixed mechanism as hydride transfer and polar nucleophilic mechanisms.

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