Comparative Computational Approach To Study Enzyme Reactions Using QM and QM-MM Methods

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Supporting Information

ABSTRACT: Choline oxidase catalyzes oxidation of choline into glycine betaine through a two-step reaction pathway employing flavin as the cofactor. On the light of kinetic studies, it is proposed that a hydride ion is transferred from α-carbon of choline/hydrated-betaine aldehyde to the N5 position of flavin in the rate-determining step, which is preceded by deprotonation of hydroxyl group of choline/hydrated-betaine aldehyde to one of the possible basic side chains. Using the crystal structure of glycine betaine–choline oxidase complex, we formulated two computational systems to study the hydride-transfer mechanism including main active-site amino acid side chains, flavin cofactor, and choline as a model system. The first system used pure density functional theory calculations, whereas the second approach used a hybrid ONIOM approach consisting of density functional and molecular mechanics calculations. We were able to formulate in silico model active sites to study the hydride-transfer steps by utilizing noncovalent chemical interactions between choline/betaine aldehyde and active-site amino acid chains using an atomistic approach. We evaluated and compared the geometries and energetics of hydride-transfer process using two different systems. We highlighted chemical interactions and studied the effect of protonation state of an active-site histidine base on the energetics of transfer. Furthermore, we evaluated energetics of the second hydride-transfer process as well as hydration of betaine aldehyde.

1. INTRODUCTION

Glucose–methanol–choline oxidoreductase (GMC oxidoreductase) superfamily are flavin cofactor-containing enzymes that catalyze the oxidation of primary and secondary alcohols into corresponding aldehydes and ketones.1–5 GMC oxidoreductase enzymes are of value in biotechnology as they were utilized in various bio-related applications such as in biosensing,6 organic synthesis,7 and biocatalysis.8 In this family, choline oxidase (EC 1.1.3.17) is an exception that it catalyzes biosensing,6 organic synthesis,7 and biocatalysis.3 In this family, utilized in various bio-related applications such as ineductase enzymes are of value in biotechnology as they were

The mechanism of choline oxidation has been studied extensively by Gadda et al., and a great deal of mechanistic information have been obtained from numerous studies.8,11–15 Based on these studies, oxidation of choline to glycine betaine was proposed to occur via hydride-transfer mechanism as opposed to radical and carbanion mechanisms (Figure 2). Accordingly, oxidation of choline is initiated with the removal of the alcohol proton (O–H) from choline (step 1 in Figure 2) by an active-site base to generate an activated alkoxide species. In the next step (step 2 in Figure 2), a hydride ion transfers from α-carbon of choline (C–H) to flavin adenine dinucleotide (FAD), leading to betaine aldehyde and reduced flavin. In the following step, the hydrated gem-diol form of betaine aldehyde is further oxidized to glycine betaine through a second hydride-transfer step to FAD yielding reduced FAD (step 3 in Figure 2). Since betaine aldehyde can act as both an intermediate and a substrate toward choline oxidase, steady-state kinetic studies involving kinetic isotope effects of substrate and product indicated that the kcat values for choline are about 2 times lower than those for betaine aldehyde, indicating that a kinetic step involving the oxidation of choline into aldehyde should be partially the rate-limiting step.11 The rate-limiting step was elaborated by means of studies involving kinetic isotope effects of substrate and product.

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It was observed that the rate of FAD reduction decreased 9 times upon switching to the deuterated choline substrate, and the rate did not change appreciably upon switching to the deuterated solvent. These two observations led to the conclusions that the hydride-transfer and proton-transfer steps are not occurring simultaneously, and because of the considerable primary isotope effect, hydride transfer should be indeed the limiting factor in the enzyme turnover. In another study, the pH dependence of initial reaction rates for the oxidation of choline to betaine aldehyde suggested that a base with a $pK_a$ value of 7.5 should be responsible for the deprotonation of the $O^-$ group of choline for the catalytic activity. To identify this catalytic base, the histidine site chain, His466, was replaced by alanine on the ground that His466 is conserved in the GMC superfamily, and its equivalent side chain, His548, in aryl alcohol oxidase was shown to act as the catalytic base. Even though a 60-fold decrease in $k_{cat}$ was observed, the pH dependence of $k_{cat}/K_m$ indicated that another base is still acting for the catalytic activity. It was also argued that His466 should stay protonated during the hydride-transfer process on the basis of the observation that partial recovery of $k_{cat}$ occurred for the alanine variant in the presence of imidazolium cation rather than neutral imidazole. In another study, His351 side chain was mutated to alanine and it gave rise to similar observations to the His466 variant. Based on these two mutagenesis studies, it was suggested that both His351 and His466 contribute to the catalysis, but another active-site base should act as the main source of proton acceptor. However, the resolution of the crystal structure of choline oxidase with the glycine betaine as the enzyme–product complex (PC) led to the revisiting of the concept of His351 and His466 as the possible active-site bases for the deprotonation of $O^-$ as a result of the close interaction of these residues with the glycine betaine. Genetic modification of His466 with glutamine led to the conclusion that His466 is the probable catalytic base. A plausible scenario was offered to explain why alanine variant could not verify the role of His466 in the catalysis process. In the alanine variant, it is suggested that there is enough space for an external hydroxide base to diffuse due to switching from histidine to alanine, which is smaller than histidine. However, in the glutamine variant, due to its larger size compared to alanine, it is likely that there is not enough room for an external molecule, which can act as a base. This explanation agrees with the observed lack of any enzymatic activity in the alanine variant.

Mechanistic insights into the oxidation of choline entail invaluable importance in the elucidation of mechanism of alcohol oxidation with the similar enzymes. Computational methods, in principle, could furnish supplementary mechanistic interpretations, which might be difficult to obtain in vitro. There are a number of computational studies related to the oxidation of alcohols by different enzymes such as liver alcohol dehydrogenase, aryl alcohol oxidase, and galactose oxidase. Among computational methods, density functional...
theory (DFT)-based quantum mechanical (QM) calculations can provide a variety of qualitative and quantitative information pertaining to the enzymatic reactions, such as binding energies, noncovalent interactions, probing different proposed mechanisms, calculations of activation barriers, and rate constants. Conclusions and reasoning deduced from experimental studies could be probed with the calculations involving DFT methods, and novel insights could be gained into interpreting the enzyme mechanism. One prominent drawback of DFT calculations in the mechanistic enzyme studies is related to its expensive calculations, and as a result, smaller and simplified active-site models should be used. Therefore, this might bring about an inevitable question related to how much realistic can be the structure of an active site based on DFT calculations without the three-dimensional presence and constraints of all surrounding residues. To circumvent this issue, hybrid approaches utilizing quantum mechanics and molecular mechanics (MM) such as ONIOM in the calculations might be employed. The species directly taking part in the enzymatic reactions could be portrayed with DFT methods, and the surrounding residues could be treated with molecular mechanics methods to include noncovalent interactions as well as provide a realistic framework around the active sites.

Choline oxidase is an ideal model system to study the reaction mechanism due to a number of reasons. First of all, the crystal structure of reaction product, glycine betaine, in complex with the enzyme is available, and it reveals a number of noncovalent interactions between active-site residues and glycine betaine. These interactions between the same residues and the substrate, choline, might be employed to study the hydride-transfer processes computationally. Besides, it is expected that DFT methods might portray a reasonable picture of active site with the substrate, choline, by means of its ability to recognize multiple noncovalent interactions of active-site residues with the substrate. Second, the proposed reaction mechanism involves proton and hydride-transfer steps from the substrate, and experimental studies suggests that proton transfer is not synchronous with the hydride transfer in a similar enzyme. It is possible to study the energetics of these processes computationally in different order to gain a better understanding of the oxidation mechanism. Finally, since it is proposed that His466 is the catalytic base deprotonating choline, and it is also proposed that His466 stays protonated during the hydride-transfer step, DFT calculations might probe the protonation status of His466 energetically.

2. COMPUTATIONAL DETAILS AND METHODOLOGY

In this study, the proposed hydride-transfer step (step 2 in Figure 2) from choline/betaine aldehyde to FAD was evaluated with two different approaches. In the first case, model systems were developed based on pure DFT functionals, B3LYP and M06-2X, using Gaussian 09 package. B3LYP functional is one of the mostly utilized DFT functionals providing complementary results to the experimental studies in the mechanistic studies of enzymes. To include dispersion correction to B3LYP functional, D3 version of Grimme’s dispersion term was utilized. The reasoning to utilize M06-2X functional is that this functional gives better results compared to B3LYP in the main-group chemistry. The geometries of reactants, products, and transition states (TSs) were optimized in the gas phase using 6-31g basis set, and then optimizations with a larger basis set, 6-31+g(d,p), were performed for some selected models. Transition states (TSs) are validated with the frequency calculations requiring one negative eigenvalue, and reactants and products were validated without any negative eigenvalues. Thermodynamic analyses were performed with the frequency calculations at 25 °C and 1 atm. Optimized TS structures were subjected to intrinsic reaction coordinate (IRC) calculations to confirm that reactants and products could be connected through the TS while moving in forward and backward pathways. Potential energy surface (PES) scans were run to locate the TS structures using optimized reactant complex (RC) by scanning bond coordinates, and then the structures corresponding to the maximum energy points in the scans were optimized using the Berny algorithm. In the second approach, a two-layer ONIOM method—comprising DFT functionals, B3LYP or M06-2X, for the substrate and active-site residues, and AMBER force field for the other residues located in a radius of 10 Å around the substrate—was adopted. N-terminal and C-terminal residues were capped with acetyl and N-methyl groups to preserve the electrostatic environment of the model active site. Capping the terminals prevents inclusion of any unnatural charged residues or H-bonding interactions around the model active site, which might radically alter the chemical interactions. To locate and characterize reactants, products, and TS structures, similar PES to those in the first approach were employed. Since D3 dispersion correction is not available for ONIOM method for B3LYP functional, all of the ONIOM results with this functional do not include any correction term.

For the first approach, the crystal structure of choline oxidase complexed with glycine betaine was used to generate the model systems for the pure DFT calculations. An initial model structure was extracted from crystal structure including main active-site amino acids having electrostatic, H-bonding, hydrophobic, and cation-π interactions with glycine betaine and FAD (Figure 3). In this structure, all amino acids and FAD were simplified to have a reasonable number of atoms that could be handled in the calculations. The main active-site amino acids in our calculations comprised His466, His351, Glu312, and Trp331. Since the optimized model systems did not reveal any considerable H-bonding interactions for Ser101 and Asn510 with choline, these two residues were not included in the calculations.
the model systems. Furthermore, Trp61 and Tyr465 were also excluded from the model systems based on their lack of any appreciable interaction with the choline in the optimized model geometries.

To model the initial hydride-transfer step (step 2 in Figure 2) from choline to FAD in the first approach, the carbonyl group of glycine betaine in Figure 3 was modified to the alkoxy functionality to generate an initial reactant complex (RC) between FAD and the deprotonated choline while excluding all amino acid residues. This structure was subjected to the geometry optimization producing RC between FAD and choline. So as to locate the TS structure corresponding to the hydride-transfer process, a PES scan using the distance between H atom connected to α-C in choline and N atom in FAD was performed. This calculation was run on the basis of the fact that hydride-transfer process entails the breaking of α-C–H bond in choline and requires the formation of N–H bond in FAD. The geometry corresponding to the highest energy point in the PES scan was selected to optimize the TS structure. Product complex (PC) of the hydride-transfer process corresponding to the reduced FAD and betaine aldehyde was optimized using an appropriate downhill geometry in the PES scan. RC and PC obtained with the PES scans were validated with the IRC calculation on the optimized TS structure. More PES scans were performed to locate the TS structures in the presence of main amino acid residues forming the active site His466, His351, Trp331, and Glu312 to the RC between choline and FAD. Amino acid residues were placed around the RC based on their locations in the crystal structure of choline oxidase with glycine betaine. Calculations involving the second approach utilizing the ONIOM method adopted a similar strategy to the first approach. However, the model system included all of the residues around the substrate in a radius of 10 Å. FAD and choline were kept all of the time in the quantum mechanics (QM) region, and main active-site residues—His466, His351, Trp331, and Glu312—were incorporated one by one to the QM region while placing all other residues into molecular mechanics (MM) region. Our second approach to model the hydride-transfer process did not utilize simplified versions of residues. Simply, FAD, choline, and essential residues, His466, His351, Glu312, and Trp331, around active site were included in the QM region, while the rest of the residues in a radius of 10 Å around choline were placed in the MM region in the ONIOM calculations. A total of 93 atoms were placed in the DFT region with a net negative charge and 83 residues totaling 1073 atoms were placed in the MM region with an overall negative charge.

3. RESULTS AND DISCUSSION

In this study, we aimed at evaluating the proposed hydride-transfer mechanism for choline oxidase first with pure DFT calculations, and then with a hybrid method involving two later ONIOM (QM-MM) calculations. In this sense, we tried to address several key features involving the details of the reaction mechanism, noncovalent interactions, and the energetics of the hydride-transfer processes. The main objective was to formulate a model active site including main amino acids that has considerable chemical interactions with the substrate, choline, and then to study the hydride-transfer process from choline to FAD. After analyzing the crystal structure of choline oxidase in complex with glycine betaine, there are a number of prominent interactions between active-site residues and glycine betaine (Figure 3). One of the carboxylate O atom in glycine betaine is in close proximity to the two histidine residues, His466 and His351, Ser101, and Asn510, suggesting that choline might display H-bonding interactions with these residues during the hydride-transfer process. Calculations involving Ser101 and Asn510 did not reveal any H-bonding interaction with choline’s O atom with either QM or QM-MM approach. It is visible that the negatively charged Glu312 is in close proximity to glycine betaine’s positively charged tetrasubstituted N atom, suggesting that an electrostatic interaction between choline and this residue might exist during the hydride-transfer process. Moreover, the tetramethyl ammonium portion of the glycine betaine faces Trp331 closely, and this interaction is very similar to cation–π interaction occurring between tetramethylated lysines and aromatic amino acids. Another observation is that Tyr465 is in close proximity to methyl groups on FAD and choline, and this suggests possible van der Waals interactions.

3.1. Formulation of Enzyme–Substrate Complex. The initial geometry of model enzyme–substrate complex, termed as the reactant complex (RC), between choline and choline oxidase for the calculations was derived from the crystal structure of enzyme complexed with the glycine betaine (Figure 3). Glycine betaine was converted to choline by transforming the carboxylate group into alkoxy functionality considering that the hydride-transfer step occurs after the deprotonation of the alcohol group in the choline. All amino acid residues and FAD were simplified in the DFT calculations for the first approach, termed as QM approach (Figure 3). Histidines were kept as neutral, and glutamate was kept as negatively charged. Optimization of the model geometry including choline, FAD, and all amino acid residues, including His466, His351, Glu312, and Trp331, failed to converge in a reasonable time using 6-31g basis set. It is more likely that the initial geometry extracted from crystal structure is far from an ideal starting point for the optimization process. We did not utilize any semiempirical or molecular mechanical methods to obtain a better initial geometry since the accuracy of DFT methods is expected to provide more reliable initial structures. To circumvent initial convergence problems, we adopted an atomistic approach to reach the optimized geometries in a reasonable time for the model active site together with choline and FAD. In the first step, the hydride-transfer process from choline to FAD was studied in the absence of all amino acid residues. After excluding all residues, we were able to obtain an optimized structure of FAD–choline complex (Figure 4).

To generate model enzyme–substrate complexes, amino acid residues—His466, His351, Trp331, and Glu312—were incorporated into the optimized complex between FAD and choline (Figure 4) in four steps in a successive manner one by one at the positions roughly similar to their positions in the crystal structure (Figure 3), and in each step, geometry optimization process was repeated without any constraints. Thus, we were able to reach five optimized geometries for the model enzyme–substrate complexes in a systematic way. Five model systems will be discussed in the rest of text as follows: QM-1 including only FAD and choline; QM-2 including FAD, choline, and His461; QM-3 including FAD, choline, His461, and His351; QM-4 including FAD, choline, His461, His351, and Trp331; and QM-5 including FAD, choline, His461, His351, Trp331, and Glu312.

Figure 5, the optimized geometry of RC for QM-5, reveals two close H-bonding interactions between deprotonated choline and two histidine residues. H atoms connected to N3 atoms at His351 and His466 residues interacts with O atom in choline. The H-bonding distance for His466, 1.50 Å, is shorter
The positioning and binding of choline in the active site.

relative positions of residues with respect to FAD and each other in our optimized model complex (Figure 5) are very similar to their positions—with the exception of their orientations—in the crystal structure (Figure 3), in which glycine betaine is in complex with the active-site residues. It has to be noted that our model active site could only be optimized in the presence of substrate, choline. It should be also noted that the model QM systems for the enzymatic reactions entail simplified, flexible, and isolated active-site residues in the calculations. However, in real cases, the flexibility of residues is limited, and the contribution of the other residues is grossly ignored. It is the recognition of the noncovalent interactions between each residue and choline by the QM methods that yields an optimized model active site, which is similar to the crystal structure. Without choline, optimization might not converge or might converge to a totally unrelated structure. Another consideration might involve a possible noncovalent interaction between FAD and choline. The distance between the O atom in choline and C4a atom in FAD is 2.56 Å in the RC in Figure 5 and 2.15 Å in the RC in Figure 4. This observation suggests that there might be a dipole interaction between negatively charged O atom in choline and the electropositive C4a atom in FAD. This observation is supported by the formation of C4a−OH and C4a−OOH adducts during the oxidation of reduced FAD in the presence of H2O and O2. The relative positions of residues with respect to FAD and each other in our optimized model complex (Figure 5) are very similar to their positions—with the exception of their orientations—in the crystal structure (Figure 3), in which glycine betaine is in complex with the active-site residues. It has to be noted that our model active site could only be optimized in the presence of substrate, choline. It should be also noted that the model QM systems for the enzymatic reactions entail simplified, flexible, and isolated active-site residues in the calculations. However, in real cases, the flexibility of residues is limited, and the contribution of the other residues is grossly ignored. It is the recognition of the noncovalent interactions between each residue and choline by the QM methods that yields an optimized model active site, which is similar to the crystal structure. Without choline, optimization might not converge or might converge to a totally unrelated structure. Another consideration might involve a possible noncovalent interaction between FAD and choline. The distance between the O atom in choline and C4a atom in FAD is 2.56 Å in the RC in Figure 5 and 2.15 Å in the RC in Figure 4. This observation suggests that there might be a dipole interaction between negatively charged O atom in choline and the electropositive C4a atom in FAD. This observation is supported by the formation of C4a−OH and C4a−OOH adducts during the oxidation of reduced FAD in the presence of H2O and O2. To address the limitations of model active sites obtained through QM calculations, we adopted a hybrid approach, ONIOM, incorporating not only more residues around active site but also rigidity and three dimensionality. In addition, this may eliminate the need to incorporate a protein solvation model into QM calculations. In the same fashion, this approach might, in principle, provide a better snapshot of the active site. To generate model systems, FAD and choline were kept always in QM region, while four amino acid residues—His466, His351, Trp331, and Glu312—were incorporated into QM region one by one in four steps in a successive manner, and in each step, geometry optimization process was repeated. In total, 83 residues were always kept in the MM region. Thus, we were able to reach optimized geometries for the model enzyme—substrate complexes, RC, in a systematic way leading to five model systems. Similar to QM models, five model systems will be discussed in the rest of the text as follows: QM-MM-1 including only FAD and choline in QM region while confining all residues to MM region; QM-MM-2 including FAD, choline, and His461 while confining all residues to MM region; QM-3 including FAD, choline, His461, and His351 while confining all residues to MM region; QM-4 including FAD, choline, His461, His351, and Trp331 while confining all residues to MM region; and QM-5 including FAD, choline, His461, His351, Trp331, and Glu312 while confining all residues to MM region. The geometries of each model enzyme—substrate complexes were included in the supporting document.

The optimized geometry of RC for QM-MM-5 (Figures 6 and 7) displays similar interactions as in the case of QM-5 model system. Relative positions of residues with respect to FAD and choline are similar to QM-5 system. As expected, the orientation of residues is different from QM-5, which is imposed by the MM restriction; the orientations and locations of residues bear more similarity with the crystal structure. A notable difference exists for the FAD cofactor between QM and QM-MM models. In the QM-5 model, the isoalloxazine ring is nearly planar. However, in the QM-MM-5 model, an obvious bent is apparent between benzene and pyrimidine moieties.
previous study, this case was observed for a flavo-enzyme, cholesterol oxidase, in the presence of its native substrate, cholesterol. The crystal structures of bacterial cholesterol oxidase in the presence and absence of cholesterol indicated that binding of substrate to the active site causes bending of isalloxazine ring through N5−N10 axis as a result of interaction between three aromatic residues, flavin, and cholesterol. Our second computational approach, QM-MM model, corroborates this finding, and this particular observation is actually a computational verification of an experimental result. In future studies, we are planning to reserve more focus on this subject.

It is also noteworthy to point out that QM-MM-1 model system, which places all residues into MM region except FAD and choline, did not furnish appreciably H-bonding distances between choline and His466−351 (4.32 and 3.67 Å, respectively, between O atom in choline and N3 in His residues), whereas placement of His466 and His351 into QM region resulted in a H-bonding distance in the range of 1.70−2.62 Å. For Trp331 and Glu312 residues, both QM and MM methods predicted similar interactions with choline based on their distances.

3.2. Formulation of the Transition State for the Hydride-Transfer Process. According to the proposed oxidation mechanism, a hydride ion is transferred from the deprotonated choline to FAD yielding betaine aldehyde and reduced FAD (step 2 in Figure 2). To study the energetics of hydride transfer, a PES scan was employed using the reactant complex, RC, for QM-1 model in Figure 4. Considering that the α−C−H bond in choline will break and a new N−H bond will form in FAD, a PES scan was run by decreasing the distance between H connected to α-C of choline and N atom in FAD over a number of steps to a reasonable N−H bond length distance. The geometry of the highest energy point in the PES scan was used to locate the TS structure (Figure 8) corresponding to the hydride transfer.

The PES scan process was repeated four more times to include all of the main amino acid residues, His466, His351, Trp331, and Glu312, in the active site to locate the TS structure corresponding to the hydride-transfer step. The structure of TS in the presence of all amino acid residues is given in Figure 9. The structures of RC, TS, and PC with all model systems are provided in the Supporting Information.

Both IRC calculation and PES scan resulted in similar PC and RC structures for the hydride-transfer process. A closer analysis of TS structure (Figure 9) reveals similar noncovalent interactions as in the case of RC in Figures 6 and 7. The two histidine residues are in H-bonding interactions with the...
choline’s O atom in the TS structure with the distances of 1.89 and 1.75 Å between O atom in choline and H atoms attached to N3 positions of histidines. It was also observed that the H-bonding distances between His466 and His351 with O atom in choline increased from RC (Figure 6) to TS (Figure 9). This illustrates that H-bonding interactions in the RC is stronger than in the TS, which is a quite expected phenomenon for a negatively charged O atom in RC compared to a neutral aldehyde-like TS. Both O atoms in Glu312 are around 2.00 Å away from the H atoms on the methyl groups of the choline, suggesting that an electrostatic interaction exists between choline’s positively charged N atom and Glu312’s O atoms in the TS. It is also visible that Trp331 has cation−π interaction with the quaternary N atom in choline.

The optimized geometry of the TS for QM-MM-5 (Figure 10) reveals similar interactions as in the case of QM-5 model. As clearly visible, the isoalloxazine ring is also distorted, and the increase in the H-bonding distances from RC to TS between His residues and O atom in choline is more prominent. In addition, the H-bonding distances in TS structures for QM-5 and QM-MM-5 reveal that the distances are longer for QM-MM-5 models (2.27 vs 1.89 Å for His351, and 1.94 vs 1.75 Å for His466) pointing out that molecular mechanics restricted the mobility of residues.

3.3. Energy Profile of the Hydride-Transfer Process. The energetics of the initial hydride-transfer process are summarized in Table 1 in terms of Gibbs free-energy change (ΔG) between TS and RC/PC for forward and reverse processes for all model systems. For QM calculations, adding amino acid residues of His466 and His351 to the model systems increased the activation barriers for forward process, whereas Glu312 caused a considerable decrease. A reasonable interpretation might be related to the positive charge density on the α-C atom of choline in the TS structure. Hydride transfer from choline to FAD involves transfer of the negatively charged H ion, and it is expected that the positive charge density on the α-C atom of the choline during the flight of hydride ion will increase. The deprotonated negatively charged O atom in choline is expected to stabilize this positive charge density. Inclusion of His466, which forms QM-2 (Table 1), increased the activation barrier by 5.40 kcal/mol for B3LYP functional and by 7.93 kcal/mol for M06-2X functional. Since His466 forms H-bonding interaction with the negatively charged O atom in choline, the negative charge density on the O atom is expected to decrease. This, in turn, leads to decrease in the stabilization of the developing positive charge density on the α-C atom of choline in the TS structure. The effect, despite being moderate, could be stated for the QM-MM-2 model having 2.60 kcal/mol more energy than QM-MM-1. In the same fashion, addition of Glu312 to QM-5 model, which forms QM-5 (Table 1), exert a similar effect on the activation barrier. Since Glu312 carries a net negative charge and has an electrostatic interaction with choline, it is expected that it might decrease the positive charge density on the α-C atom of the choline, thereby leading to a decrease in the activation barrier. Indeed, 6−7 kcal/mol decreases were observed upon the introduction of Glu312 for QM-5 models, and 3.07 kcal/mol for QM-MM-5. This observation aligns with the study reporting lack of choline oxidase activity in the mutant enzyme in which Glu312 was replaced with alanine.4 Inclusion of Tyr465 and Asn510 did not affect the activation barrier to a significant extent. Furthermore, the corresponding RC structure with these two residues did not reveal any prominent chemical interaction with either FAD or choline. For that reason, we did not include these two residues in the QM region for the ONIOM calculations.

There was not any notable trend in the activation barrier of the reverse hydride-transfer process for QM models. However, inclusion of first residue causes a significant decrease in the activation energy, and further addition of residues causes erratic increases or decreases for both QM models, B3LYP and M06-2X. This might be due to the absence of any constraints to preserve the geometry of model active site. A careful analysis of PC structures through QM-1 to QM-5 models with both functionals (Figures S1−S8) supports this reasoning. Even though mobility of residues in the RC and TS models are limited, PC structures through QM-1−5 show considerable mobility and variability in terms of residues and substrate. In contrast, ONIOM calculations (QM-MM models)
calculations for QM-MM models with M06-2X functional. All results, except $\Delta G$:

The structure of PC reveals analyzed the optimized structure of PC obtained through PC between betaine aldehyde and other components, we the interaction with choline. To understand the extra stability of aldehyde and active-site residues including FAD is tighter than This observation suggests that the interaction between betaine

residues to QM region steadily increased the activation barrier. Inclusion of residues to QM region steadily increased the activation barrier. This observation indicates that calculations with 6-31g basis set can provide proper

gave a clear trend in the reverse activation barrier. Inclusion of residues to QM region steadily increased the activation barrier. This observation suggests that the interaction between betaine aldehyde and active-site residues including FAD is tighter than the interaction with choline. To understand the extra stability of PC between betaine aldehyde and other components, we analyzed the optimized structure of PC obtained through ONIOM PES scan (Figure 11). The structure of PC reveals that the H-bonding distances for His351 and His466 in PC are longer than (Figure 11) those in RC (Figure 6). However, Trp331 and Glu312 are closer to betaine aldehyde than choline. This might explain part of the stability conferred to PC. Moreover, a considerable interaction exists between reduced FAD and betaine aldehyde to such an extent that a distance of 2.36 Å between O4 in the isoalloxazine and the carbonyl C of aldehyde suggests a strong dipole–dipole interaction. In addition, it is clear that negative charge on FAD can be stabilized and delocalized through ring system (Figure 11). One more consideration has to be kept in mind that the transition from RC to TS does not entail any major shift in the relative position of choline with respect to FAD. However, the transition from TS to PC brings about considerable shifts of betaine aldehyde and the surrounding residues relative to FAD in QM-MM models. As we switch more residues from MM region to QM region, a significant decrease in the energies of PC models is visible through QM-1 to QM-5 models in Table 1. The optimized geometries were very close to each other obtained with these two basis sets, and the difference in the forward and backward activation barriers was in the range of 0.70−2.93 kcal/mol for M06-2X and B3LYP functionals. This observation indicates that calculations with 6-31g basis set can provide proper quantitative and qualitative mechanistic information for the hydride-transfer process.

Table 1. Energy Profile for the Hydride-Transfer Process for the Different Model Systems with B3LYP, M06-2X, and Hybrid ONIOM Calculations along with the Imaginary Frequencies for the Transition States

| functional system | model system | $\Delta G^f$ | $\Delta G^r$ | $\Delta(\Delta G^f)$ | imaginary frequency (i) |
|-------------------|--------------|-------------|-------------|----------------------|-------------------------|
| B3LYP (QM)        | 1            | 11.18       | 27.83       | −1184.38             |
|                   | 1*           | 13.10       | 26.70       | −1272.35             |
|                   | 2            | 16.58       | 16.99       | 5.40                 |
|                   | 2*           | 27.88       | 11.80       | −718.48              |
|                   | 3            | 22.32       | 21.87       | 5.64                 |
|                   | 3*           | 23.01       | 14.92       | 0.79                 |
|                   | 5            | 16.62       | 17.11       | −6.39                |
|                   | 5*           | 12.93       | 30.95       | −999.40              |
|                   | 2*           | 20.86       | 17.87       | 7.93                 |
|                   | 3*           | 33.94       | 24.83       | −958.73              |
|                   | 4*           | 21.64       | 26.92       | 0.78                 |
|                   | 4*           | 24.67       | 20.56       | 3.03                 |
|                   | 5*           | 18.10       | 22.35       | −6.57                |
|                   | 5*           | 13.55       | 6.78        | −849.79              |
|                   | 1*           | 10.95       | 6.08        | −993.16              |
|                   | 1**          | 13.39       | 6.78        | −910.11              |
|                   | 2            | 16.15       | 10.88       | 2.60                 |
|                   | 3            | 15.02       | 20.53       | −1.13                |
|                   | 4            | 15.76       | 31.99       | 0.74                 |
|                   | 5            | 12.69       | 27.36       | −3.07                |
|                   | 5**          | 19.46       | 28.68       | −1127.52             |

$\Delta G^f$: Gibbs free energy of activation for the forward reaction in kcal/mol; $\Delta G^r$: Gibbs free energy of activation for the reverse reaction in kcal/mol; $\Delta(\Delta G^f)$: difference of the Gibbs free energy of activation for the forward reaction in kcal/mol between each consecutive model active site in the first column. * Denotes calculations with 6-31+g(d,p) basis set, ** denotes calculations with protonated His466, and # denotes ONIOM calculations for QM-MM models with M06-2X functional. All results, except * models, were obtained with 6-31g basis set.

**Figure 11.** Structure of optimized PC in the presence of choline, four amino acid residues, and FAD while excluding all residues to MM region (model QM-MM-5) obtained with M06-2X functional with 6-31g basis set using tube models excluding all H atoms except the ones shown in green.
3.4. Protonation State of His466 during Hydride-Transfer Process. His466 was suggested to be the most plausible catalytic base, which is responsible for the deprotonation of choline’s hydroxyl group before the hydride-transfer process (step 1 in Figure 1). Furthermore, during the hydride-transfer step, it was also suggested that His466 stays protonated based on a study in which His466 was replaced with alanine. The mutant form did not show any appreciable enzymatic activity, and a partial recovery of activity was observed in the presence of imidazolium cation. However, addition of neutral imidazole did not contribute any recovery to the enzymatic activity.

In the previous model systems, which were formulated for the hydride-transfer step, His466 was kept neutral and all of the calculated activation energies were based on the assumption that His466 is neutral during the hydride-transfer process. To study the effect of protonation state of His466 on the energetics of hydride-transfer process, His466 N1 position was protonated. Simply, a H atom was added to N1 position of His466 to produce an RC for a model system (QM-2) described in Table 1 and Figure 12. Then, the resulting systems were subjected to geometry optimizations, followed by PES scans to locate TS structures representing hydride-transfer process in the presence of protonated His466.

The optimized RC (Figure 12) revealed a striking observation with respect to the location of proton originally placed at N3 position of His466. Instead of N3 position of His466, the proton is attached to O atom in choline. This observation is not surprising considering the pKa values of choline, 13.9, and the conjugate acid of histidine’s imidazole ring, 6.6. Considering that the microenvironment of the active site does not alter the pKa values of acidic groups significantly, if

Figure 12. Optimized structures of RC and TS for the hydride-transfer process for the model active site QM-2 in the presence of protonated His466 obtained with the M06-2X functional with 6-31g basis set using tube models excluding all H atoms except the ones shown in green.

Figure 13. Optimized structures of RC, TS-1H2O, TS-2H2O (for two water molecules), and PC for the hydration of betaine aldehyde in the presence of one water molecule obtained with the M06-2X functional with 6-31g basis set using tube models excluding all H atoms except the ones shown in green.
there is a proton placed between choline’s O and histidine’s N3 position, it will prefer to be attached to O atom due to choline’s being less acidic and more basic than imidazole. The same phenomenon was observed for the model active sites QM-2 with B3LYP functional. These results highlight that His466 group, which is in close proximity of a deprotonated choline molecule, cannot stay protonated at the N3 position, and that proton prefers to be attached to choline’s O atom.

Another interesting observation was revealed for the TS structure for the hydride-transfer process from protonated choline to FAD for the model represented in Figure 12. The optimized TS structure (Figure 12) corresponds to the flight of hydride ion from the deprotonated choline to FAD, indicating that the proton should have been already transferred from O atom in choline to the N3 position of His466 somewhere before the TS geometry. Indeed, the analysis of PES scan, which was obtained by scanning the bond coordinate between α-C and H as the C–H distance was increased, showed the transfer of the proton connected to choline’s O atom to N3 position of His466 before the highest energy point in the PES scan. According to this model, it could be concluded that the deprotonation of choline and the hydride transfer are coupled and the deprotonation happens on the way while the hydride ion moves from choline. Furthermore, as the alcohol transforms to an aldehyde group as the hydride ion transfers, it is normal that the proton connected to O atom in a semi-aldehyde (pKₐ around −6 for a protonated aldehyde) would transfer to much more basic His466 (pKₐ around 6 for the conjugate acid of imidazole ring of the histidine). However, a collection of experimental studies indicated that the deprotonation of choline and the transfer of hydride steps are decoupled and deprotonation should occur in a separate step before the hydride transfer. 

The activation barrier for the hydride-transfer step from protonated choline to FAD was found to be always 10–15 kcal more than the deprotonated models (QM-2 models vs QM-2 in Table 1). These results clearly point out that energetically the hydride transfer is more favorable when choline is deprotonated and His466 is neutral. It is quite likely that His466 is indeed responsible for the deprotonation of choline along with a couple of residues who might accept proton from His466 in a cascade of proton-transfer steps.

3.5. Hydration of Betaine Aldehyde. According to the proposed mechanism, the second hydride-transfer process is preceded by the hydration of betaine aldehyde into gem-diol (step 2 in Figure 1). It has been established that a water molecule should exist in the active site toward this end, and hydration of betaine aldehyde should occur in a kinetically fast step. Hydration of aldehydes occurs via three general routes such as uncatalyzed, general acid-catalyzed, and general base-catalyzed. We formulated a model system consisting of water, betaine aldehyde, and FAD to test uncatalyzed and base-catalyzed hydration of betaine aldehyde.

By means of exploiting PES scan calculations, we located a TS structure corresponding to addition of one water molecule to betaine aldehyde using M06-2X functional with 6-31g basis set (TS-1H₂O in Figure 13). Following PES scan, we obtained optimized structures of RC and PC, neutral gem-diol, for the hydration process. The activation energy turned out to be a very high value, 40.39 kcal/mol. A closer analysis of TS-1H₂O structure reveals the apparent reason for this high value. The TS structure looks like a highly strained four-membered ring, which is expected to have a high ring strain. We envisaged that a second water molecule might decrease the activation barrier through forming less strained six-membered ringlike TS structure (TS-2H₂O in Figure 13). Indeed, the calculated activation energy turned out to be 12.74 kcal/mol. The energetics of these two models can be compared to QM-1 models (QM-1 for M06-2X in Table 1) for the initial hydride-transfer step since they do not contain any residue. The hydride-transfer process requires 12.93 kcal/mol activation energy, and a value of 12.74 kcal/mol activation energy for the hydration step seems not very probable given that the overall turnover number did not change in deuterated solvent. It is also possible that there might not be enough room for a second water molecule. We also tested these two model systems with QM-MM calculations and we observed similar high activation energies for a kinetically fast step.

From this observation, it seems more logical that a general base-catalyzed water addition should be operative in the hydration of betaine aldehyde considering similar enzymatic processes involving aldehyde/ketone hydration as an intermediate step. To this end, instead of a water molecule, we placed a hydroxide molecule for the PES scan to study the energetics of hydration process. Without any activation barrier as in a downhill process, hydration occurs through bond formation between O atom at hydroxide ion and α-C of betaine aldehyde. It is likely that His466 or His351 residues might act as base catalyst deprotonating water molecule and forming hydroxide ion. It should not be also ruled out that a hydroxide anion might diffuse to active site. We tried to locate a TS structure through PES scans for a model system including a hydroxide ion, a protonated His residue, FAD, and betaine aldehyde to simulate a model system as if His residue was the basic residue that deprotonated the water molecule. However, the protonated His residue could not hold its proton, and it was transferred to hydroxide ion forming water molecule. Through the PES scan, we could not locate a TS structure forming gem-diol form of aldehyde. Interestingly, if we employ the same model system with a neutral His residue, again a downhill energy profile leading to gem-diol formation was obtained. This observation suggests that one of the His residues might still act as the basic residue that deprotonates the water molecule provided that the proton on the His residue subsequently needs to be transferred to another residue or group.

3.6. Hydride Transfer from Hydrated-Betaine Aldehyde. Following the hydration of betaine aldehyde, it was proposed that a second hydride transfers to FAD, leading to the formation of glycine betaine and reduced FAD (step 3 in Figure 2). Since betaine aldehyde acts also as a good substrate for choline oxidase, much of the mechanistic information was obtained through kinetic and spectroscopic studies. It has been shown that a residue with a pKₐ value of 6.7 should be involved in the oxidation of betaine aldehyde as unprotonated based on pH dependence of kₐp/kₐm value. It was argued that this residue, more likely His466, acts as a catalytic base deprotonating one of the hydroxyl group of hydrated-betaine aldehyde. Furthermore, this activated deprotonated hydrated-betaine aldehyde loses its hydride at the α-C position forming final products. Overall, it is expected that the second hydride-transfer process should be very similar to the first hydride-transfer process on the grounds that both processes happen at the same active site, and the substrates are chemically similar, one is alcohol and the other is gem-diol.

To study the energetics of second hydride transfer, we formulated a model system including hydrated-betaine
aldehyde, and FAD using M06-2X functional with 6-31g basis set. We run PES scans to locate TS structure corresponding to the transfer of hydride at α-C from hydrated-betaine aldehyde to N5 position at FAD. We tested three different formulations. In the first instance, we kept OH groups of hydrated-betaine aldehyde with their protons and run PES scan varying the distance between hydride ion and N5 atom was accompanied with a continuous uphill energy profile without a TS structure candidate. This

Figure 14. Optimized geometries of RC, TS, and PC for the second hydride transfer from hydrated-betaine aldehyde to FAD obtained with the M06-2X functional with 6-31g basis set using tube models excluding all H atoms except the ones shown in green.

|          | RC  | TS  | PC  |
|----------|-----|-----|-----|
| αC-H1    | 1.10| 1.32| 3.19|
| O1-H2    | 1.54| 1.71| 1.85|
| O1-H2    | 1.54| 1.83| 2.00|
| N5-H1    | 2.28| 2.19| 1.01|
| C2-C3    | 3.41| 3.41| 3.44|
| O2-C1    | 3.11| 3.14| 3.74|

Figure 15. Optimized geometries of RC, TS, and PC for the second hydride transfer from hydrated-betaine aldehyde to FAD in the presence of four amino acid residues obtained with the M06-2X functional with 6-31g basis set using tube models excluding all H atoms except the ones shown in green. The distances are in the table in angstrom unit.
finding suggests that hydride transfer is not energetically favorable when the OH groups in the gem-diol are neutral. In the second case, we used a gem-diol with one of the OH deprotonated. The ensuing PES scan produced RC, TS, and PC for the hydride-transfer process (Figure 14).

Based on the Gibbs free-energy calculations, the activation energy is 13.66 kcal/mol for the forward step and 42.18 kcal/mol for the reverse step. Clearly, incorporating deprotonated gem-diol (O1 deprotonated in Figure 14) into the model system had such a dramatic effect that an energetically unfavorable uphill process became an energetically feasible process with a considerable exergonic character. In parallel, QM-MM version of this model predicted activation energies of 14.01 kcal/mol for forward steps and 29.11 kcal/mol for reverse steps. The PC reveals an interesting observation such that N5 atom in FAD accepts carboxylic proton of glycine. The difference of activation energy between the first hydride-transfer step and the second hydride-transfer step is very small (12.93 kcal/mol for the first hydride transfer, QM-1 for M06-2X in Table 1, versus 13.66 kcal/mol for the second hydride transfer, Figure 14). To gain more insight into geometries and energetics of the second hydride-transfer process, we formulated QM-5 version of this process including His351, His466, and Glu312 (Figure 15) using M06-2X functional with 6-31g basis set.

Figure 15 reveals that the second hydride transfer has indeed very similar RC, TS, and PC structures to the first hydride-transfer process (Figures 6, 9, and S8). It is obvious that similar noncovalent interactions can be seen in all three structures. Carboxylic proton did not transfer to N5 as in the case of QM-1 version of second hydride transfer without any residue (Figure 14). This might be presumably as a result of tight interaction of residues with betaine aldehyde preventing proton transfer electronically and sterically. Furthermore, this process is accompanied with a 21.75 kcal/mol forward and 33.08 kcal/mol reverse activation barriers. The initial hydride transfer had 18.10 kcal/mol forward and 22.35 kcal/mol reverse activation barriers for the similar QM-5 model (QM-5 for M06-2X in Table 1). There is 3.65 kcal/mol for the forward and ~10 kcal/mol for the reverse process. However, as discussed previously, QM-5 model might not also portray the geometry and energetics of second hydride transfer in a suitable way given that residues are free to move to find more stable geometries without any physical constraint. For this reason, we formulated an ONIOM model corresponding to QM-MM-5 version for the second hydride-transfer process including four amino acid residues that have considerable chemical interactions with substrate (Figure 16).

Similar interactions for RC and TS structures were observed in Figure 16 as in the case of QM-5 version (Figure 15) of the second hydride-transfer process. His351 and His466 have H-bonding interactions with the O atom in hydrated-betaine aldehyde (H2–O1 and H3–O1 in Figure 16). Glu312 has electrostatic interaction, and Trp312 has cation–π interaction with tetramethyl ammonium cation portion of the hydrated-betaine aldehyde. However, PC converged to a somewhat different geometry than its counterpart in the first hydride-transfer process.
transfer process (Figure 11 as QM-MM-5 formulation), and its counterpart in the second hydride transfer process in QM-5 formulation (PC in Figure 15). The first striking difference is the transfer of carboxylic proton from glycine betaine to reduce FAD. This case had already been witnessed in the QM-1 version of second hydride-transfer process (Figure 14). Furthermore, the H-bonding interaction of His351 with the carboxylate O atom in glycine betaine is disrupted (O1–H2 distance in Figure 16), whereas His466 still continues to have this interaction. Finally, Trp331 in PC does not have any more cation−π interaction as it moved away from positively charged N atom. Proton transfer from carboxylic group to FAD, presumably, changed the electronic interactions of product, glycine betaine, with the FAD and with surrounding residues to a great extent, and then the active-site residues were reorganized. The resulting negatively charged carboxylate anion and positively charged N atom in FAD interact to a significant extent that the PC in this model was stabilized more than its PC counterpart in the QM-5 version of second hydride-transfer process (PC in Figure 15). This could be deduced from the comparison of activation energy of the reverse steps (41.79 vs 33.08 kcal/mol). The role of this strong electrostatic interaction can also be verified from the QM-1 version of the second hydride-transfer process (Figure 14). Also in this model, carboxylic proton was transferred to N5 position of FAD, leading to a high value of reverse activation barrier, 42.18 kcal/mol. Since this model does not contain any residue, the apparently high value of activation barrier confirms the role of PC stabilization through this particular electrostatic interaction.

Comparison of energetics of first hydride transfer from choline (QM-MM-5@ with M06-2X functional having 19.46 kcal/mol activation energy, Table 1) with similar model of the second hydride transfer from hydrated-betaine aldehyde (16.59 kcal/mol in Figure 16) reveals that the contribution of the first and second hydride-transfer steps to the overall turnover of the choline oxidase are considerable. A ≃3 kcal/mol difference of the activation barrier based on QM-MM-5 models between the first and second hydride-transfer processes agrees with the measured $k_{\text{cat}}$ values of 93 s$^{-1}$ for choline and 135 s$^{-1}$ for betaine aldehyde.$^{13}$ It has to be regarded that inclusion of MM region into calculations portrays and give more quantitative description of the activation energy difference between the first and second hydride transfers.

As the last model system, we tested the protonation state of a His residue in a formulation in which we included a deprotonated gem-diol and positively charged protonated His466 at N3 positions. In this formulation, we probed the protonation status of His466 during the flight of the hydride as well as the possible base catalyst role of His466 to deprotonate the gem-diol. As we run the PES scan, it was found that the proton transfers from His466 to gem-diol, and the model system turns into the first model system we tested earlier. This trial suggests that as the hydride ion moves from α-C of gem-diol to FAD, His466 cannot stay protonated, which is a similar case to the first hydride ion transfer from choline to FAD. We tested this approach with the QM-MM formulation and obtained similar results.

**CONCLUSIONS**

In this study, we employed some computational approaches analyzing hydride-transfer processes from choline and betaine aldehyde to FAD as well as the hydration process of betaine aldehyde prior to second hydride transfer. Two major model sets, QM and QM-MM, were utilized to analyze geometries and energetics of the reactions. QM models offered guidelines to determine the residues interacting with the substrates and cofactor, and as a result, more plausible active-site geometries were estimated through QM-MM models. Through QM-MM models, we were able to include a considerable number of residues around active site to impose a real rigid protein environment. It was found that four amino acid residues, choline/betaine aldehyde, and FAD form a very stable preorganized reactant complex that have strong chemical interactions. In these complexes, a hydride ion transfers from choline/betaine aldehyde to FAD. According to our calculations, hydride-transfer process is more energetically feasible from deprotonated choline and gem-diol. Furthermore, it is also found that His466 cannot stay protonated during the hydride-transfer processes. Through QM-MM calculations, it was suggested that the isoalloxazine ring of FAD is distorted.

Our models might be adapted to study other alcohol and aldehyde oxidases in specific and in general to all enzyme reactions, and invaluable mechanistic information and interpretations might be obtained. In future studies, we are planning to extend our calculations toward the deprotonation mechanism of choline and FAD planarity.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b02638.

Model systems (Figures S1–S13); Gibbs free energy, enthalpy, and zero-point corrected electronic energies of each species (Tables S1–S29); and Cartesian coordinates of all species (PDF)

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**Notes**

The authors declare no competing financial interest.

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**REFERENCES**

1. Dijkman, W. P.; de Gonzalez, G.; Mattevi, A.; Fraaije, M. W. Flavoprotein Oxidases: Classification and Applications. *Appl. Microbiol. Biotechnol.* 2013, 97, 5177–5188.
2. Wongnate, T.; Chaiyen, P. The Substrate Oxidation Mechanism of Pyranose 2-Oxidase and Other Related Enzymes in the Glucose–Methanol−Choline Superfamily. *FEBS J.* 2013, 280, 3009–3027.
3. Bankar, S. B.; Bule, M. V.; Singhal, R. S.; Anantharanayan, L. Glucose Oxidase — an Overview. *Biotechnol. Adv.* 2009, 27, 489–501.
4. Goswami, P.; Chinnadaddy, S. S. R.; Chakrabortty, M.; Kumar, A. K.; Kakoti, A. An Overview on Alcohol Oxidases and Their Potential Applications. *Appl. Microbiol. Biotechnol.* 2013, 97, 4259–4275.
5. Romero, E.; Gadda, G. Alcohol Oxidation by Flavoenzymes. *Biomol. Concepts* 2014, 5, 299.
Choline Oxidase; Accumulation of Glycinebetaine and Enhanced Bio-
Choline Oxidase with Betaine Aldehyde and Its Isosteric Analogue 3,3-
by
T. Osmoprotective Properties and Accumulation of Betaine Analogues
Montgomery, J. A.; Morokuma, K.; Frisch, M. J. Combining Quantum
Oxidase.
the Oxidation of Choline to Betaine-Aldehyde Catalyzed by Choline
Gadda, G. Identification of the Catalytic Base for Alcohol Activation in
Proton Transfers in the Reaction of Flavin Oxidation Catalyzed by
Cholinesterase from Arthrobacter globiformis. Biochim. Biophys. Acta,
Proteins Proteotemesis 2003, 1646, 112–118.
Gadda, G. Kinetic Mechanism of Choline Oxidase from
Choline Oxidase. Biochemistry 2013, 52, 1221–1226.
(15) Smitherman, C.; Rungserunsirayachai, K.; Germann, M. W.;
Gadda, G. Identification of the Catalytic Base for Alcohol Activation in
Choline Oxidase. Biochemistry 2015, 54, 413–421.
(16) Gadda, G. Ph and Deuteron Kinetic Isotope Effects Studies on the
Oxidation of Choline to Betaine-Aldehyde Catalyzed by Choline Oxidase.
Biochim. Biophys. Acta, Proteins Proteotemesis 2003, 1650, 4–9.
(17) Ghaneh, M.; Gadda, G. On the Catalytic Role of the Conserved
Active Site Residue His466 of Choline Oxidase. Biochemistry 2005, 44,
893–904.
(18) Hernández-Ortega, A.; Lucas, F.; Ferreira, P.; Medina, M.;
Guallar, V.; Martinez, A. T. Role of Active Site Histidines in the Two
Half-Reactons of the Aryl-Alcohol Oxidase Catalytic Cycle. Biochemistry
2012, 51, 6595–6608.
(19) Rungserunsirayachai, K.; Gadda, G. On the Role of Histidine 351
in the Reaction of Alcohol Oxidation Catalyzed by Choline Oxidase.
Biochemistry 2008, 47, 6762–6769.
(20) Salvi, F.; Wang, Y. F.; Weber, I. T.; Gadda, G. Structure of
Choline Oxidase in Complex with the Reaction Product Glycine
Betaine. Acta Crystallogr., Sect. D: Biol. Crystallogr. 2014, 70, 405–13.
(21) Agarwal, P. K.; Webb, S. P.; Hammes-Schiffer, S. Computational
Studies of the Mechanism for Proton and Hydroxide Transfer in Liver
Alcohol Dehydrogenase. J. Am. Chem. Soc. 2000, 122, 4803–4812.
(22) Hernández-Ortega, A.; Borrelli, K.; Ferreira, P.; Medina, M.;
Martínez, A. T.; Guallar, V.; Substrate Diffuse and Oxidation in GMC
Oxidoreductases: An Experimental and Computational Study on
Fungal Aryl-Alcohol Oxidase. Biochim. Biophys. Acta 2011, 13, 427–435.
(23) Hernández-Ortega, A.; Ferreira, P.; Merino, P.; Medina, M.;
Guallar, V.; Martinez, A. T. Stereoselective Hydroxide Transfer by
Aryl-Alcohol Oxidase, A Member of the Gmc Superfamily. ChemBioChem
2012, 13, 427–435.
(24) Verma, P.; Pratt, R. C.; Storr, T.; Wasinger, E. C.; Stack, T. D. P.
Sulfanyl Stabilization of Copper-Bonded Phenoxyls in Model
Complexes and Galactose Oxidase. Proc. Natl. Acad. Sci. U.S.A.
2011, 108, 18600–18605.
(25) Volz, G. Computational Approaches for Studying Enzyme
Mechanisms; Academic Press: 2016; Vol. 577.
(26) Lonsdale, R.; Harvey, J. N.; Mulholland, A. J. A Practical Guide to
Modelling Enzyme-Catalysed Reactions. Chem. Soc. Rev. 2012, 41,
3025–3038.
(27) Vreven, T.; Byun, K. S.; Komáromi, I.; Dapprich, S.;
Montgomery, J. A.; Morokuma, K.; Frisch, M. J. Combining Quantum
Mechanics with Molecular Mechanics Methods in ONIOM. J. Chem.
Theor. Comput. 2006, 2, 815–826.
(28) Chen, J.; Wang, J.; Zhang, Q.; Chen, K.; Zhu, W. Prospecting Origin
of Binding Difference of Inhibitors to Mdm2 and Mdmx by Polarizable
Molecular Dynamics Simulation and QM/MM-GBSA Calculation. Sci.
Rep. 2015, 5, No. 17421.
(29) Chen, J.; Wang, J.; Zhang, Q.; Chen, K.; Zhu, W. A Comparative
Study of Trypsin Specificity Based on QM/MM Molecular Dynamics
Simulation and QM/MM GBSA Calculation. J. Biomol. Struct. Dyn.
2015, 33, 2606–2618.
(30) Chung, L. W.; et al. The Oniom Method and Its Applications.
Chem. Rev. 2015, 115, 5678–5796.
(31) Lundberg, M.; Morokuma, K. The Oniom Method and Its
Applications to Enzymatic Reactions. In Multi-Scale Quantum Models
for Biocatalysis: Modern Techniques and Applications; Yor, D. M., Lee,
T.-S., Eds.; Springer: Netherlands, 2009; pp 21–55.
(32) Menon, V.; Hsieh, C. T.; Fitzpatrick, P. F. Substituted Alcohols as
Mechanistic Probes of Alcohol Oxidase. Bioorg. Chem. 1995, 23,
42–53.
(33) Becke, A. D. Density-Functional Thermochemistry. III. The
Role of Exact Exchange. J. Chem. Phys. 1993, 98, 5648–5652.
(34) Zhao, Y.; Trulhar, D. The M06 Suite of Density Functionals for
Main Group Thermochemistry, Thermochemical Kinetics, Non-
covalent Interactions, Excited States, and Transition Elements: Two
New Functionals and Systematic Testing of Four M06-Class
Functionals and 12 Other Functionals. Theor. Chem. Acc. 2008, 120,
215–241.
(35) Frisch, M. J. et al. Gaussian 09; Gaussian, Inc.: Wallingford, CT,
2009.
(36) Meunier, B.; de Visser, S. P.; Shaik, S. Mechanism of Oxidation
Reactions Catalyzed by Cytochrome P450 Enzymes. Chem. Rev. 2004,
104, 3947–3980.
(37) Grimme, S.; Antony, J.; Ehrlich, S.; Krieg, H. A Consistent and
Accurate Ab Initio Parameterization of Density Functional Dispersion
Correction (DFT-D) for the 94 Elements H-Pu. J. Chem. Phys. 2010,
132, No. 154104.
(38) Zhao, Y.; Trulhar, D. G. Applications and Validations of the
Minnesota Density Functionals. Chem. Phys. Lett. 2011, 502, 1–13.
(39) Hratchian, H. P.; Schlegel, H. B. Finding Minima, Transition
States, and Following Reaction Pathways on Ab Initio Potential Energy
Surfaces A2 - Dykstra, Clifford E. In Theory and Applications of
Computational Chemistry; Frenking, G., Kim, K. S., Scuseria, G. E.,
Eds.; Elsevier: Amsterdam, 2005; Chapter 10, pp 195–249.
(40) Peng, C.; Ayala, P. Y.; Schlegel, H. B.; Frisch, M. J. Using
Redundant Internal Coordinates to Optimize Equilibrium Geometries
and Transition States. J. Comput. Chem. 1996, 17, 49–56.
(41) Hornak, V.; Abel, R.; Okur, A.; Strockbine, B.; Roitberg, A.;
Simmerling, C. Comparison of Multiple Amber Force Fields and
Development of Improved Protein Backbone Parameters. Proteins
2006, 65, 712–25.
(42) Yildiz, I. A Computational Insight into the Interaction of
Methylated Lysines with Aromatic Amino Acid Cages. J. Phys. Org.
Chem. 2016, No. e3660.
(43) Quaye, O.; Lountos, G. T.; Fan, F.; Orvile, A. M.; Gadda, G.
Role of Glu312 in Binding and Positioning of the Substrate for the
Hydroxide Transfer Reaction in Choline Oxidase. Biochemistry 2008,
47, 243–256.
(44) Orville, A. M.; Lountos, G. T.; Finneghan, S.; Gadda, G.;
Prabhakar, R. Crystallographic, Spectroscopic, and Computational
Analysis of a Flavin C4a-Oxygen Adduct in Choline Oxidase.
Biochemistry 2009, 48, 729–728.
(45) Lyubimov, A. Y.; Heard, K.; Tang, H.; Sampson, N. S.; Viteklin,
A. Distortion of Flavin Geometry Is Linked to Ligand Binding in
Cholesterol Oxidase. Protein Sci. 2007, 16, 2647–2656.
(46) Wood, E. J. Data for Biochemical Research (Third Edition) by
R M C Dawson, D CElliott, W H Elliott and K M Jones, Pp 580. Oxford
Science Publications, OUP, Oxford, 1986. £35/$59. Isbn 0-19-
853588-7. Biochem. Educ. 1987, 15, 97.
(47) Sander, E. G.; Jencks, W. P. Equilibria for Additions to the Carbonyl Group. J. Am. Chem. Soc. 1968, 90, 6154–6162.
(48) Fleming, S. M.; Robertson, T. A.; Langley, G. J.; Bugg, T. D. H. Catalytic Mechanism of a C–C Hydrolase Enzyme: Evidence for a Gem-Diol Intermediate, Not an Acyl Enzyme. Biochemistry 2000, 39, 1522–1531.
(49) Li, J.-J.; Li, C.; Blindauer, C.; Bugg, T. D. H. Evidence for a gem-Dioi Reaction Intermediate in Bacterial C–C Hydrolase Enzymes BphD and MhpC from 13C NMR Spectroscopy. Biochemistry 2006, 45, 12461–12469.