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Theoretical Study of Hydrogen–Bonded Network and Proton Transfer in the Active Site of Reduced Cytochrome *c* Oxidase

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Theoretical Study of Hydrogen–Bonded Network and Proton Transfer in the Active Site of Reduced Cytochrome *c* Oxidase[#]

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Abstract

Motivation. There are many proposals of reaction mechanisms of O₂–reduction catalyzed by cytochrome *c* oxidase (CcO) and the mechanism is not still conclusive. We have previously proposed new ‘water–proton transport’ (WPT) mechanism in which H₂O hydrogen–bonded to His290 and Tyr244 transports a proton to FeOO to yield FeOOH. The path through which a proton is transferred to this H₂O molecule in the active site of CcO is investigated from theoretical viewpoint in this work.

Method. The moderate size of model of the active site in CcO was constructed based on the geometry of fully reduced form 1OCR in PDB. All histidines were modeled by imidazoles and Tyr244 by phenol. The key fragments concerned with the hydrogen–bonded network were geometrically optimized. The hybrid exchange–correlation functional B3LYP method was employed with double–zeta basis set.

Results. The H₂O molecule exists between farnesylethyl and Thr316 by hydrogen bonds and the hydrogen–bonded network, which is connecting from Lys319 to the active site of O₂–reduction, is formed in the reduced CcO. The proton necessary for O₂–reduction is provided through this hydrogen–bonded network. The proton is trapped on farnesylethyl of heme *a*₃, subsequently the proton is transferred to Tyr244 to give a proton to the H₂O molecule between His290 and Tyr244. The energy barrier of the proton transfer is qualitatively estimated to be 12 kcal/mol.

Conclusions. The proton path, which is necessary to perform the O₂–reduction, was made clear from the theoretical viewpoint.

Keywords. Cytochrome *c* oxidase; hydrogen bond; reduction; proton transfer; B3LYP.

1 INTRODUCTION

Cytochrome *c* oxidase (CcO) is a terminal oxidase of cellular respiration system to catalyze the reduction of oxygen molecule to water molecules. At the same time, electrons are sequentially transferred from heme *a* to heme *a*₃, and protons are transferred from the matrix side of mitochondrial membrane toward the cytosolic side (so called proton pumping) [1–3]. There are fully oxidized and reduced forms which have common active site composed of heme *a*₃(Fe) and

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Cu_B binuclear system. The three-dimensional structures of both forms of bovine muscle have been determined by X-ray crystallographic studies [4–6]. The oxidized form [Fe³⁺, Cu_B²⁺] does not catalyze the O₂-reduction, while the reduced form [Fe²⁺, Cu_B⁺] does.

The O₂ molecule in the triplet state is initially bound to Fe atom of heme *a*₃ in the reduced form to yield the oxy intermediate [Fe–OO, Cu_B] [2,3,7,8]. The subsequent intermediate observed experimentally is the oxo intermediate [Fe=O, Cu_B] [3,7–13]. Two electrons and two protons are necessary to yield the oxo intermediate with generating H₂O molecule. There is now consensus that first two protons are provided from K-channel that begins from Lys319 and ends to Thr316 through hydrogen bonds of water molecule, peptide bonds, and amino residues [14,15].

In the previous paper [16], we have proposed “Water-Proton Transport” (WPT) mechanism of O₂-reduction in the active site of the reduced CcO from theoretical viewpoint. It has been shown that there exists a H₂O molecule between His290 and Tyr244 with the hydrogen bonds. The H₂O molecule plays a role as a proton carrier to yield the ferric-hydroperoxo intermediacy [Fe–OOH, Cu_B²⁺]²⁺ from the oxy intermediate. However, in our mechanism, H₂O molecule in the network of the hydrogen bonds was abruptly changed to hydronium ion with assumption that a proton is provided through K-channel. The barrier of the proton transfer was not also estimated.

In this paper, the barrier of the proton transfer from farnesyethyl to the H₂O molecule between His290 and Tyr244 will be qualitatively estimated. It is shown that the barrier is smaller than the binding energy of O₂ by Fe.

2 MODELS AND METHODS

2.1 Model of the Active Site

The model of the active site of CcO has been discussed in details in the previous paper [16]. Here, we like to show the construction procedure of the model briefly. The structure of the active site of the reduced form 1OCR [6] in PDB was employed to construct the model of the active site.

All histidine residues, which coordinate to Fe of heme *a*₃ in the axial direction and Cu atom, were modeled by imidazoles. Tyr244, which is bonded covalently to His290, is modeled by phenol. The formyl and vinyl groups are left in the porphyrin ring due to possibility of the π -resonance. The farnesyethyl group was modeled by –CH₂OH which has possibility to make hydrogen bond to phenol (Tyr244). The geometries of heavy atoms in the model are same as those given by 1OCR of X-ray crystallographic study. The hydrogen atoms, which are not observed in 1OCR, were added by the usual way. The constructed model is shown in Figure 1.

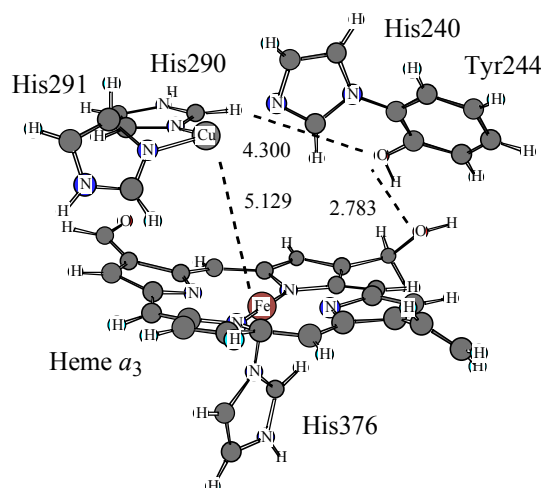


Figure 1. Model of active site in fully reduced CcO (1OCR in PDB).

2.2 Calculation Procedures

In the reduced form of CcO, Fe and Cu have formal charges of +2 and +1, respectively. Since porphyrin ring has formal charges of –2, the total charge of the model should be given by +1, such as $[\text{Fe}^{2+}, \text{Cu}_B^{+}]^+$. The full geometry optimization was not performed in order to hold the geometry of the reaction field in the active site of reduced CcO. The key fragments (H_2O and H atoms of OH in phenol and $-\text{CH}_2\text{OH}$), which are directly concerned with the network of the hydrogen bonds, were optimized. We employed the hybrid exchange–correlation functional B3LYP [17–20] method. Wachters double zeta basis set [21] was employed for Fe and Cu atoms. The 6–31G* basis set was used for key oxygen atoms of O_2 molecule, phenol and H_2O molecule, and 3–21G basis set for C, N, H, and other O atoms in the model of the active site of fully reduced CcO. All calculations were carried out using the program package Gaussian 94 [22].

3 RESULTS AND DISCUSSION

It has been shown in our previous work [6] that there exists a H_2O molecule between His 290 and Tyr244 through hydrogen bonds. This H_2O molecule receives a proton to give the hydronium ion that transports a proton to FeOO to produce FeOOH. The protonated geometry in which H_2O molecule receives a proton is a transient state not a stable state. However, a proton is abruptly added to H_2O molecule as a terminal point of the proton transfer through K–channel. The previous work was based on assumption that the H_2O molecule is connecting to the K–channel by hydrogen bonds through phenol (Tyr244), farnesylethyl (heme a_3), and Thr316. It is, therefore, interesting to investigate whether the proton transfer is available or not through the K–channel.

The total energies of the singlet state and triplet state shown in Figure 1 are estimated to be –5395.72502 a.u. and –5395.72187 a.u., respectively, under the optimization of H atom of OH in

phenol (Tyr244), indicating that the singlet state is lower in energy by only 2.0 kcal/mol. The electronic configurations of the singlet and triplet states are given by $(d_{xy})^2(d_{xz})^2(d_{yz})^2$ and $(d_{xy})^2(d_{xz})^2(d_{yz})^1(d_z)^1$ of Fe atom, respectively. Two unpaired electrons are localized on Fe atom of porphyrin ring in the triplet state. Heme a_3 is composed of the five-coordinate Fe^{2+} porphyrin complex in which Fe is ligated by axial histidine ligand. This energy difference is conflict with other DFT calculations that the triplet state of five-coordinate Fe^{2+} porphyrin complex without the Cu_B moiety is lower than the singlet state [23–26]. This discrepancy shows that the positive charge of Cu^+ stabilizes the singlet state rather than the triplet state. The electronic structures of the Cu_B moiety are, however, same between the singlet and triplet state, although the reaction with the triplet oxygen molecule proceeds on the potential energy surface of the singlet state. As the proton transfer is expected to occur on the Cu_B moiety, calculations of the proton transfer have been performed on the potential energy surface of the single state in order to avoid the time-consuming.

From the geometry of the fully reduced CcO by X-ray crystallographic study [6], the distance between O-atom of OH in farnesylethyl group and O-atom of OH in Thr316 is given by 4.760 Å, indicating the possibility that another H_2O molecule exists between farnesylethyl group and Thr316 through the hydrogen bonds, compared with H_2O molecule hydrogen-bonded to His290 and Tyr244. Thr316 is a terminal amino residue of K-channel beginning from Lys319. Therefore, two H_2O molecules are added into geometry shown in Figure 1 to investigate the hydrogen-bonded network connecting with phenol and $-CH_2OH$.

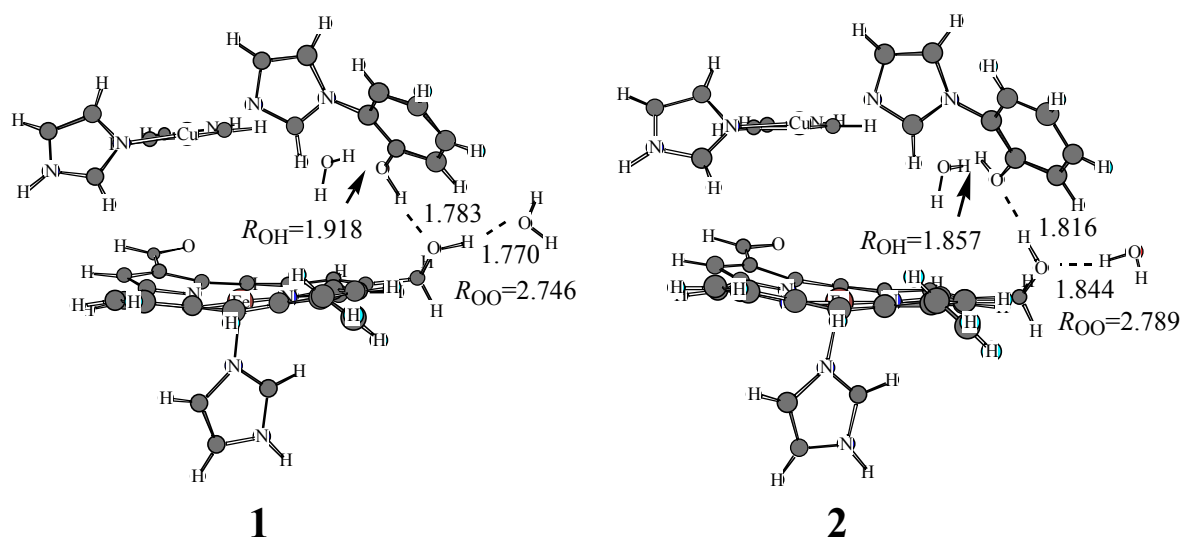


Figure 2. Hydrogen-bonded networks formed by two water molecules in the vicinity of active site of CcO.

Figure 2 shows the optimized locations of two H_2O molecules. It can be easily seen from Figure 2 that there are two ways in the networks of the hydrogen bonds. One (1 shown in Figure 2) is a hydrogen-bonded network that H-atom of OH in Tyr244 is hydrogen-bonded to O-atom of $-CH_2OH$ in farnesylethyl group, and another (2 in Figure 2) is that H-atom of $-CH_2OH$ in

farnesyethyl is hydrogen-bonded to O-atom of phenol in Tyr244. Geometry **1** is lower in energy by 9.2 kcal/mol than geometry **2**. The removals of two H₂O molecules from geometries **1** and **2** give two different geometries in the directions of H-atoms of phenol and -CH₂OH. The removal of them from geometry **1** leads to lower energy by 12.1 kcal/mol than that from geometry **2**. These results indicate that the hydrogen bond of OH in phenol and -CH₂OH is preferable rather than the hydrogen bond OH in -CH₂OH and O in phenol.

The H₂O molecules hydrogen-bonded to -CH₂OH in geometries **1** and **2** are directed to Thr316 which is a terminal residue of the K-channel, indicating that H₂O molecule exists between farnesyethyl group and Thr316 and connects with the K-channel. In the vicinity of Thr316, Ile312 exists with the distance of 2.898 Å between O-atom of OH in Thr316 and O-atom of C=O in Ile312. It is reasonable to consider that H-atom of OH in Thr316 is directed to O-atom of C=O in Ile312 to form the hydrogen bond. It is, therefore, expected that lone-pair orbital of O-atom in Thr316 is directed to O-atom of OH in farnesyethyl group. Two H atoms of H₂O are faced to O-atom of OH in Thr316 in geometry **1**, while O-atom of H₂O is faced to Thr316 in geometry **2**. H₂O in geometry **1** has a possibility to form the hydrogen bonds to the lone-pair orbital of O-atom in Thr316. It is, however, expected that H₂O in geometry **2** is not stable because of repulsive interactions between the lone-pair orbitals of O atoms of H₂O and Thr316. The network of the hydrogen bonds in **1** is, therefore, more preferable than that in **2**. This consideration shows that the stability of **1** could be enhanced by the hydrogen bond to Thr316 more than energy difference of 9.2 kcal/mol between **1** and **2**.

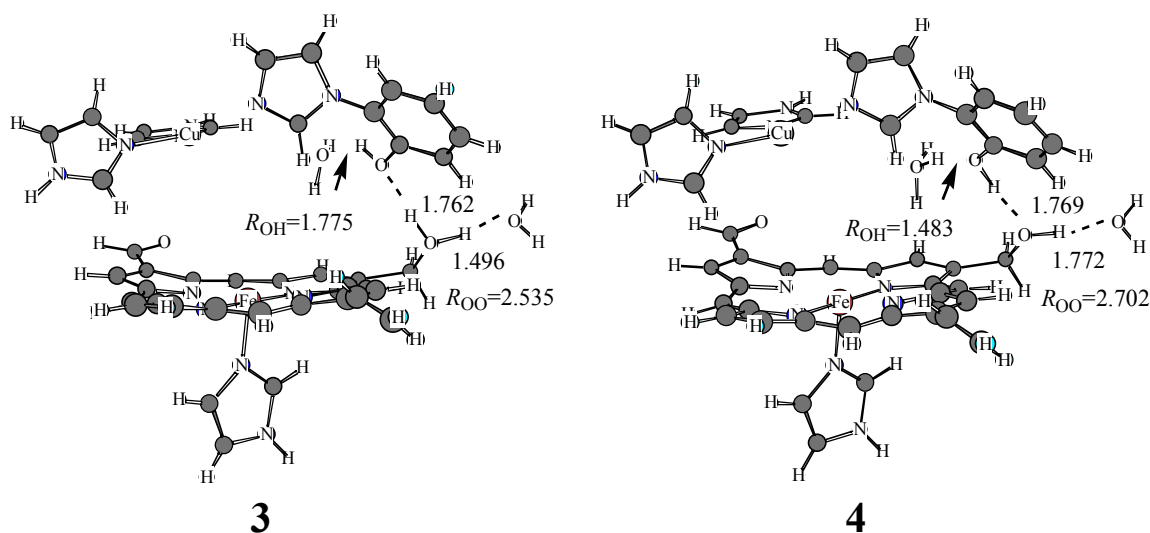


Figure 3. Geometries in which a proton is captured.

When a proton is transferred to the reaction site through K-channel, there are four possibilities to capture the proton. Those are H₂O between -CH₂OH and Thr316, -CH₂OH, phenol, and H₂O between His290 and Tyr244. Only two geometries in which a proton is captured on -CH₂OH and

H₂O between His290 and Tyr244 were found, as shown in Figure 3. Geometry **3** is lower in energy by 2.4 kcal/mol than **4**. When Fe binds oxygen molecule at early stage of O₂-reduction, the H₂O between His290 and Tyr244 transports a proton to FeOO at the same time that H₂O captures a proton [16]. However, H₂O in geometry **4** enable to capture a proton without binding O₂ by Fe.

The charge density of OH₂ of –CH₂OH₂ in **3** is estimated to be 0.308*e*, while the charge density of H₃O in **4** is 0.726*e*. In the case of **3**, the positive charges are increased by 0.588*e* and 0.579*e* from OHs of **1** and **2**, respectively. It is, therefore, found that a proton is localized on OH₂ and H₃O in **3** and **4**, respectively, consistent with decreases of distances of the hydrogen bonds from $R_{\text{OH}} = 1.770 \text{ \AA}$ to $R_{\text{OH}} = 1.496 \text{ \AA}$ in **3** and from $R_{\text{OH}} = 1.918 \text{ \AA}$ to $R_{\text{OH}} = 1.483 \text{ \AA}$ in **4**.

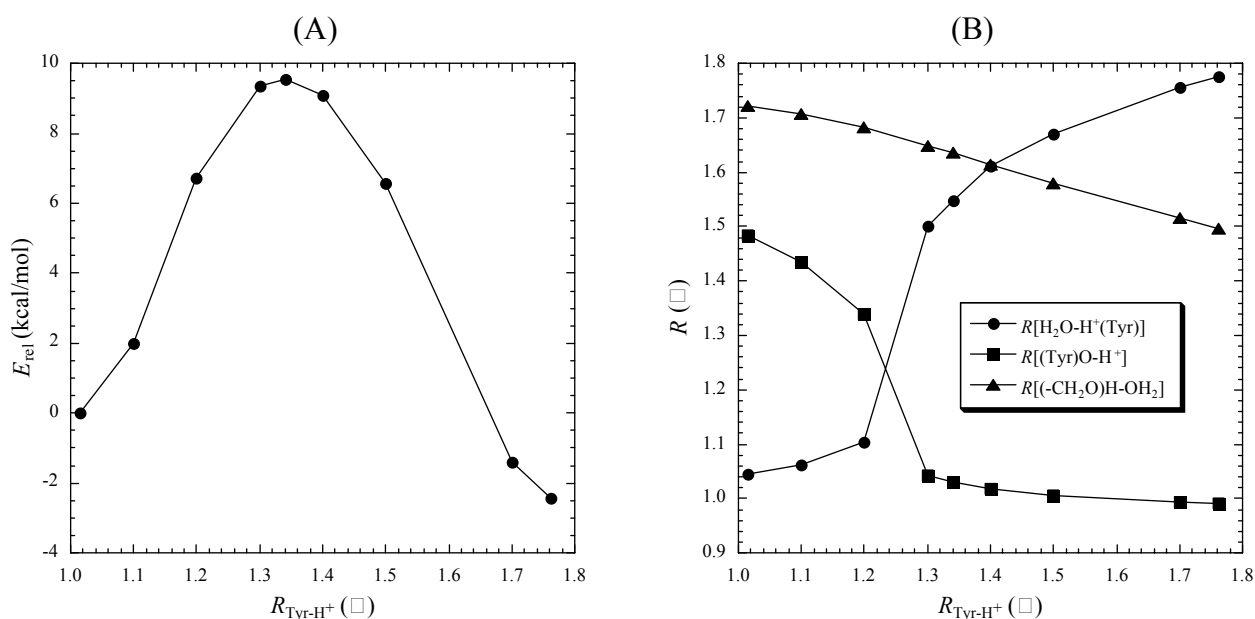


Figure 4. Variations of relative energy and selected distances to a transferring proton through the proton transfer between phenol and –CH₂OH.

It is expected that geometrical change from **3** to **4** is induced by the proton-transfer from –CH₂OH₂ to OH of phenol. We selected the distance H⁺ and O of phenol as a geometrical parameter of the proton transfer. Figure 4 shows the variations of the relative energy and selected distances via the stepwise change of distance of H⁺ and O of phenol. **3** corresponds to $R = 1.762 \text{ \AA}$ and **4** to $R = 1.041 \text{ \AA}$. The highest point in energy is estimated to be $R = 1.341 \text{ \AA}$ by three-point extrapolation from $R = 1.2 \text{ \AA}$, 1.3 \AA , and 1.4 \AA . It can be found from Figure 4 that barrier height of the proton transfer from **3** to **4** is 12.0 kcal/mol. It was reported in the case of $[\text{H}_2\text{O-H-OH}_2]^+$ that the optimized O–O distance is about 2.4 Å with a single minimum on the potential energy surface [27–29]. It was also reported that the elongation of O–O distance changes the potential energy surface a double well with a significant barrier, in agreement with our result shown in Figure 4. Our barrier height 12.0 kcal/mol at $R_{\text{OO}} = 2.783 \text{ \AA}$ (in Figure 1) of phenol and –CH₂OH is comparable with about 13 kcal/mol at $R_{\text{OO}} = 2.8 \text{ \AA}$ of $[\text{H}_2\text{O-H-OH}_2]^+$. It seems that 12.0 kcal/mol is high to transfer

a proton. However, this energy barrier is lower than binding energy of 30 kcal/mol of Fe to OO [16], indicating that the proton can be easily transferred after the early stage of reduction at which heme a_3 binds oxygen molecule.

It can be seen from Figure 4(B) that until the proton reaches the highest point ($R = 1.341 \text{ \AA}$) of potential energy curve starting from **3**, H-atom of OH in phenol is entirely immovable with maintaining the OH bond. The H_2O molecule between His290 and Tyr244 becomes close to this H atom with gradually decreasing the distance. It can be found that this H atom of OH in phenol moves rapidly to H_2O as a proton after the barrier is passed.

The oxygen molecule is bound to Fe atom in a real reaction system before the proton transfer occurs [16]. Since **4** is a transient state under existence of FeOO , it can be considered that the proton-transfer from phenol to the H_2O molecule between His290 and Tyr244 and the proton-transportation by H_2O to FeOO will occur simultaneously with transferring a proton from farnesyethyl to phenol. FeOOH is finally formed in heme a_3 by concerted movement of a proton and H_2O .

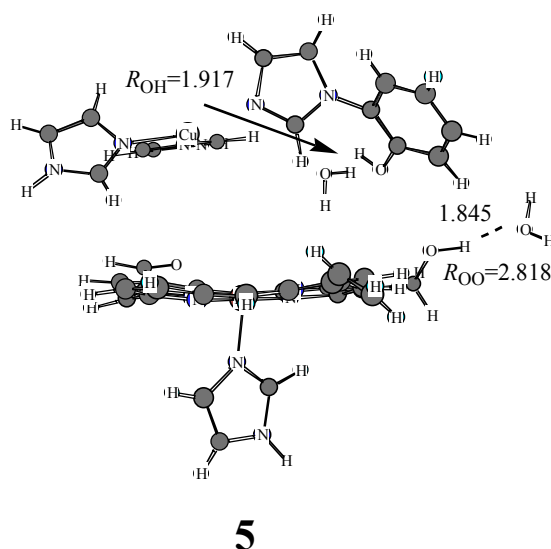


Figure 5. Geometry in which H-atom of phenol is rotated.

It is expected to be reasonable that the proton moves to H_2O hydrogen-bonded to $-\text{CH}_2\text{OH}$ in **2** to obtain **3** through the proton transfer from K-channel. Since the geometry **2** protonated to H_2O is, in fact, a transient state, **3** is easily obtained by the proton transfer to $-\text{CH}_2\text{OH}$. It is necessary to obtain from **1** to **2** that two $-\text{OH}$ groups of phenol and $-\text{CH}_2\text{OH}$ should be rotated and H_2O molecule between His290 and Tyr244 is also. The energy barriers of the rotations are large. Figure 5 shows the geometry that is corresponding to the rotation of OH of phenol from **1**. This geometry is higher in energy by only 8.7 kcal/mol than **2** with the rotation barrier of about 22 kcal/mol, indicating still lower energy than the FeOO binding energy. It can be seen from Figure 5 that this geometry has a pocket to receive a proton to give **3**. It may be expected in a real system that the

energy barrier from **1** to **3** becomes lower due to concerted manner of the rotation of OH in phenol and the proton-transfer to $-\text{CH}_2\text{OH}$.

After the FeOOH is formed, the network of the hydrogen bonds is returned to the type of geometry **1**. There is consensus that first two protons are migrated through K-channel to the active site to produce the first H_2O . The transfer of the second proton should be, therefore, started from the hydrogen-bonded network of **1**. Although the path of the proton movement to $-\text{CH}_2\text{OH}$ to provide **3** is not identified in this work, it should be thoroughly investigated by inclusion of Ile312 and Thr316. In general, the patterns of the hydrogen-bonded network are different before and after the proton transfer. It is an important problem in the biological system to investigate the driving force to reverse the pattern of the hydrogen-bonded network before the proton transfer.

4 CONCLUSIONS

In the previous work [16], it was shown that H_2O molecule exists between His290 and Tyr244 by hydrogen bonds. In this work, it has been shown that another H_2O molecule exists between farnesylethyl and Thr316 with hydrogen bonds. From the experimental observation that Thr316 is a terminal residue of K-channel beginning from Lys319, it has been also found that the hydrogen-bonded network, which is connecting from Lys319 to the active site of O_2 -reduction, is formed in the reduced CcO. The proton necessary for O_2 -reduction is provided through this hydrogen-bonded network. The proton is trapped on farnesylethyl of heme a_3 , subsequently the proton is transferred to Tyr244 to give a proton to H_2O molecule between His290 and Tyr244. The energy barrier of the proton transfer is qualitatively estimated to be 12 kcal/mol, showing that the proton is easily transferred to the active site due to the lower barrier than the binding energy of Fe and oxygen molecule.

In this work, the proton path, which is necessary to perform the O_2 reduction, was made clear from the theoretical viewpoint. This result is important to obtain the conclusive mechanism for yielding the first product, H_2O molecule.

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