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His190 might directly bridge Yz and Mn-cluster: Mechanistic consequences for water oxidation deduced from DFT calculation

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Introduction

Utilizing light energy to oxidize water into oxygen is the unique ability of photosystem II (PSII). This reaction is accomplished through two distinct processes. One is the primary reaction occurring at the reaction center of PSII. This reaction converts the light energy into electrochemical potential energy. The obtained high redox potential $P_{680}^{+\bullet}$ is the thermodynamic driving force for water oxidation. The second process is the catalytic water oxidation on the Mn-cluster located at the lumen side of PSII. How to couple these two

processes is crucial for the performance of the unique function of PSII. Tyr161 (Yz) and His190 on the D1 protein are key components for this coupling. Experiments have demonstrated that His190 interacts

Scheme 1



with Yz through a H-bond (Mamedov et al., 1998; Debus, 2001). The turnover of Yz-His190 during water oxidation is shown in scheme 1.

Extensive studies have been done on Yz-His190, but the coupling mechanism is still unclear. One major problem is the uncertainty concerning the structural interaction between Yz-His190 and the Mn-cluster, as well as with the substrates. In this paper, we investigate this issue by using quantum chemistry DFT calculations. Combining the new PSII structure data and experimental findings on Yz with our calculations, we suggest that Yz can indeed interact with that His190 in active PSII, and His190 bridging Yz and the Mn-cluster is a promising model for the interaction of Yz and the Mn-cluster. Based on this, a new mechanism for Yz-His190 controlling the water oxidation is proposed.

Calculation details

The calculations were performed in two steps. First, a full optimization of geometry was performed using the B3LYP method, which is a density-function theory (DFT) type of calculation based on hybrid functionals. Double- ζ basis sets were used in this step. In the second step, energy evaluation on some models was performed at the B3LYP level.



Fig. 1 Structure for the reduction and oxidation states of Tyr-His systems

The calculation models Tyr-His and Tyr-His-Mn(X) (X is the valence of Mn ion, including II, III, IV) are shown in Fig.1 and Fig. 2. Tyr and His were simplified to p-methyl-phenol and imidozle, respectively. We selected the Mn ion close to Yz to represent the Mn-cluster. Except for one coordinating His, H₂O and OH⁻ were used as ligands to saturate the coordination space of the Mn ion.

Results

I) Tyr-His system

Considering the possible interactions between Yz with one H₂O or other residues, three models of Tyr-His, H₂O-Tyr-His and CH₃OH-Tyr-His were studied (structures shown in Fig. 1). The energy levels of the highest occupied molecule orbital (HOMO) of these models are listed in Table 1. From the orbital analysis, we found that the HOMO mainly came from the phenol group in all these models. When H_2O or CH_3OH were added to interact with Tyr, the HOMO energy levels of the model systems decreased (table 1). Since a higher HOMO energy means that the system is more ready to give out an electron, the electron donating ability of Tyr decreased when it interacted directly with either H₂O or CH₃OH.

After one electron is lost from these systems, the phenolic proton moved from phenol to His in all these systems (Fig. 1). This resulted in the formation of a neutral radical Tyr[•]. The spin density on the Tyr radical is listed in Table 1.

II) Tyr-His-Mn system

In the Tyr-His-Mn system we studied, we chose to let the His form a bridge between Tyr and the Mn ion (Fig. 2). Thus, the His is a Mn ligand in our models. The distance between the phenolic O atom to Mn is



Tyr•-[His-Mn(III)]⁺ Tyr•-[His-Mn(III)] Tyr-His-Mn(III) Tyr-His-Mn(IV)

Fig. 2 structural models for Tyr-His-Mn(III) in different states

6.5-6.7Å. The 2.6-2.7Å H-bond length (Table 1) indicates a strong H-bond between Tyr and His. Similarly to the Tyr-His system, the HOMO of Tyr-His-Mn was also dominated by Tyr. However, the HOMO energy level of Tyr-His-Mn was increased (Table 1). The amplitude increase was dependent on the valence of the Mn ion. The lower oxidation state Mn(III) increased the energy level more than Mn(IV). Therefore, the binding of a Mn ion to His

His, and spin density on Tyr in different models						
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Model	HOMO reduced	H–Bond (Ty	H–Bond (Tyr–His) (Å)	
-)	state	reduced	oxidized	oxidized state
	(Hartree)	state	state	
Tyr	-0.2261	-	-	1.000
[Tyr-His]	-0.1966	2.687	2.593	1.006
[H ₂ O-	-0.2069	2.641	2.734	1.008
Tyr-His]		a (00)		1.007
[CH ₃ OH- Tvr-His]	-0.2078	2.600	2.716	1.007
[Tyr-	-0.1833	2.647	2.682	1.005
His+Mn(III)]				
[Tyr-	-0.1925	2.677	2.650	1.005
His+Mn(IV)]				

increased the electron donating ability of the Tyr.

After Tyr-His-Mn(X) has given out one electron, the phenolic proton of Tyr moves to His, which resultes in the formation of one neutral radical Tyr[•], while one positive charge is delocalized on the Mn ion and its ligands, i.e. Tyr^{\bullet} -[His-Mn(X)]⁺ (Fig. 2). All the configurations of Tyr[•]-[His-Mn(X)]⁺ are stable in our calculations.

Since the positive charge can promote the proton release from H_2O bound to Mn ion, we also investigated the proton release from Tyr^{\bullet} -[His-Mn(III)]⁺. We found that the total energy of Tyr^{\bullet} -[His-Mn(III)] and Tyr^{\bullet} -[His-Mn(IV)] (Fig. 2) were very close (only 16 kcal/mol difference).

Discussion

I) Possibility for His190 binding directly to the Mn-cluster

It is generally accepted that the His190 only interacts with Yz and acts as a base to receive the proton released upon Yz oxidation. Mutagenesis studies (Debus, 2001; Diner, 2001) showed that the replacement of His190 with stronger basic residues, e.g. Arg or Lys, decreased the water oxidation reaction greatly. This indicates that His190 might not only take the role of proton acceptor. In fact, all other mutants at His190 position failed to assemble the Mn-cluster, which indicate that His190 could bind to the Mn-cluster, and there are very few data that can rule this hypothesis out.

Therefore, we compared our earlier structure model (Svensson et al., 1996) with the new Xray data (Zouni et al., 2001). Our comparison indicates two important differences: i) in the theoretical model, the C-helix on the D1 protein with Yz should be moved ~1.0Å towards the D-helix on the D1 protein to match the crystal structure; ii) the real CD-helix is ~3-4 residues longer than the modeled CD-helix. However, it is not clear where it starts and ends. This opens the possibility that His190 is wrongly positioned in the structural model. With this in mind, we analyzed the structural freedom of His190 and found it is structurally attractive, that His190 directly bridges Yz and the closest Mn ion (in the X-ray structure).

Thus, we have studied the alternative that Yz interacted with the Mn-cluster through His190. In our calculation, we therefore introduced the His bridging the Tyr and the Mn-cluster. The structural opimization for this model gave the distance of 6.4-6.8Å between the phenolic O and the Mn ion, which is very close to the distance observed in X-ray data (7.0Å, Zouni et al., 2001). In addition, we found that the His accepted the phenolic proton of Tyr upon its oxidation, which means that the His remained acting as a base in this model, as well as being a ligand of the Mn ion. Meanwhile, the calculation indicated that the electron donating ability of Tyr was increased upon Mn binding to the His. According to this, it can be well explained that Yz is a better electron donor to P680⁺ than Y_D.

II) Possible function of the positive charge

In previous models, the positive charge was a by-product of Yz oxidation, without further function for water oxidation. It either released to bulk on a ns time-scale (Tommos and Babcock, 1998), or stayed nearby (Haumann et al., 1999).

According to our calculations, binding of His to the Mn-cluster resulted in the delocalization of positive charge on the Mn-cluster instead of only on the His. Comparing with the His group, the Mn-cluster is more effective at stabilizing the positive charge. This led to the higher free energy change (ΔG) for the Tyr oxidation reaction. Moreover, Mn ions at different oxidation levels have various stabilizing effects at the positive charge, which means different driving forces for the oxidation of Tyr. This prediction is consistent with the experimental observation that the oxidation of Yz is dependent on the existence and oxidation states of the Mn-cluster. Meanwhile, it is worth mentioning that the positive charge delocalized on the Mn ion could be directly used to promote the proton release from water bound to the Mn ion. Therefore, we suggest that the positive charge might have important functions for both Yz oxidation and water oxidation in PSII.

III) Mechnism for water oxidation controlled by Yz-His190

Calculations on the His bridging Tyr and Mn-cluster model show that one neutral radical Tyr[•] was formed upon the oxidation of Tyr-His-Mn, which is consistent with EPR studies on PSII. Considering the short H-bond length (2.6-2.7Å) and the small pKa variance ($\Delta pKa = 1 \sim 2$) between the Tyr and the His bound to the Mn ion, the H-bond of Tyr-His in our model is similar to the Low-Barrier Hydrogen-Bond (Cleland and Kreevoy, 1994). It is believed that the active energy of the H atom movement along this kind of H-bond is rather low. The very close energy for Tyr[•]-[His-Mn(III)] and Tyr-[His-Mn(IV)] models obtained also indicated that a H-atom could move readily along the H-bond in our model. It should be pointed out that this possible H-atom movement only occurs after the proton release from H₂O bound to the Mn ion. This is in agreement with kinetic studies of PSII (Haumann and Junge, 1996).

On the basis of our DFT calculations and structural comparisons to locate His190, we are currently working with a new mechanism for the function of Yz-His190 in controlling the

water oxidation processes in active PSII, and Fig. 3 shows a hypothetical scheme along these lines.

If the His190 really is a bridging between Yz and the Mn-cluster, this might implicate a following mechanism: upon the oxidation of Yz, the phenolic proton of Yz shuttles to His190, which results in the formation of Yz[•] and one positive charge delocalized on Mn-cluster. The



Fig.3 Hypothetical H-nucleus shuttle mechanism for water oxidation in PSII: dashed arrows indicate the moving direction of the H-nucleus; • signifies the neutral radical; for clarity, one Mn ion binding one H_2O is shown for the Mn-cluster; only $S_0 \rightarrow S_1$ is illustrated as an example for the proposed mechanism.

positive charge drives the proton release from H_2O bound to Mn-cluster. After the release of the proton, the H-atom shuttles back to Yz^{\bullet} , which results in the reduction of Yz^{\bullet} and oxidation of the Mn-cluster. Thus, Yz would interact with His190 but not with H_2O , which is essential, and the reaction would be similar in all the S-states (Tommos and Babcock, 1998).

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