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Review

Low-barrier hydrogen bond plays key role in active photosystem II — A new model for photosynthetic water oxidation

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Abstract

The function and mechanism of Tyr_Z in active photosystem II (PSII) is one of the long-standing issues in the study of photosynthetic water oxidation. Based on recent investigations on active PSII and theoretical studies, a new model is proposed, in which D1-His190 acts as a bridge, to form a low-barrier hydrogen bond (LBHB) with Tyr_Z, and a coordination bond to Mn or Ca ion of the Mn-cluster. Accordingly, this new model differs from previous proposals concerning the mechanism of Tyr_Z function in two aspects. First, the LBHB plays a key role to decrease the activation energy for Tyr_Z oxidation and $Tyr_Z^{\cdot}$ reduction during photosynthetic water oxidation. Upon the oxidation of Tyr_Z, the hydrogen bond between Tyr_Z and His190 changes from a LBHB to a weak hydrogen bond, and *vice versa* upon $Tyr_Z^{\cdot}$ reduction. In both stages, the electron transfer and proton transfer are coupled. Second, the positive charge formed after Tyr_Z oxidation may play an important role for water oxidation. It can be delocalized on the Mn-cluster, thus helps to accelerate the proton release from substrate water on Mn-cluster. This model is well reconciled with observations of the S-state dependence of Tyr_Z oxidation and $Tyr_Z^{\cdot}$ reduction, proton release, isotopic effect and recent EPR experiments. Moreover, the difference between Tyr_Z and Tyr_D in active PSII can also be readily rationalized. The His190 binding to the Mn-cluster predicted in this model is contradictious to the recent structure data, however, it has been aware that the crystal structure of the Mn-cluster and its environment are significantly modified by X-ray due to radiation damage and are different from that in active PSII. It is suggested that the His190 may be protonated during the radiation damage, which leads to the loss of its binding to Mn-cluster and the strong hydrogen bond with Tyr_Z. This type of change arising from radiation damage has been confirmed in other enzyme systems.

Keywords: Photosystem II; $Tyrosine_Z$; His190; Mn-cluster; Low-barrier hydrogen bond; Proton and electron transfer

1. Introduction

Photosynthetic oxygen evolution is a unique function of the Photosystem II (PSII) in higher plants, algae and cyanobacteria, which sustains most life in this planet. PSII is a multi-subunit membrane protein complex. The arrangement of the main cofactors involved in photoinduced charge separation and charge stabilization in the reaction center is shown in Fig. 1 [1–5]. Upon excitation, the primary electron donor (P_{680}) donates one electron to the primary electron acceptor (Pheo) to produce the $P_{680}^{+\cdot}$ and $Pheo^{-\cdot}$ charge pair. $Pheo^{-\cdot}$ then transfers the electron to the primary and secondary quinones (Q_A and Q_B) in sequence at the acceptor side. The high redox potential of $P_{680}^{+\cdot}$ drives the water oxidation in the Mn-cluster containing four Mn ions, one Ca^{2+} and one or more Cl^- ions (See reviews [6–9]). The turnover of the Mn-cluster leading to water oxidation involves five different states (S_{0-4}), wherein S_0 is the lowest state and S_1 is the dark stable state. S_2 and S_3 states are more oxidizing states (see reviews [10–14]). S_4 is the transient state, which has been detected recently [15,16].

D_1-Tyr_{161} (Tyr_Z) is the secondary electron donor located between P_{680} and Mn-cluster, and plays the role of tuning the one-electron photochemical reaction and the four-electron catalytic water oxidation process [17]. However, the spectroscopic studies of the intermediate state of Tyr_Z oxidation and $Tyr_Z^{\cdot}$ radical formed after its oxidation by $P_{680}^{+\cdot}$ have always been suffered from its fast turnover and lack of spectroscopic

Abbreviations: EPR, electron paramagnetic resonance; ImH, imidazole; LBHB, low-barrier hydrogen bond; P_{680}, primary electron donor of PSII; Pheo, pheophytin; PSII, photosystem II; Q_A and Q_B, primary and secondary quinone electron acceptors, respectively; Tyr_Z, tyrosine 161 of the D_1 protein; Tyr_D, tyrosine 160 of the D_2 protein

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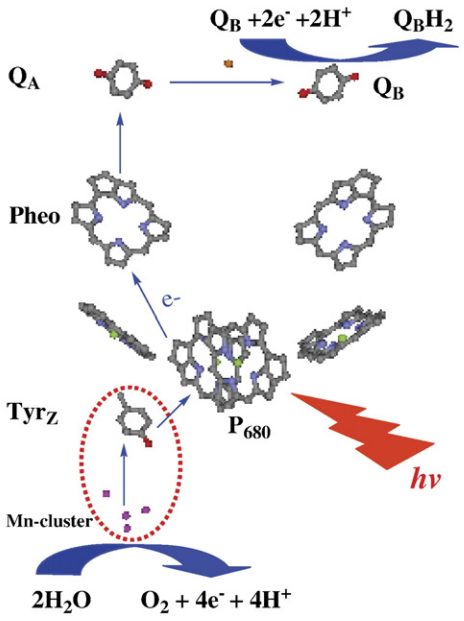

Fig. 1. Main cofactors and reactions in PS II. The pathway of electron transfer is shown in blue lines and arrows. The X-ray sensitive area is marked with red cycle.

characteristics [18–20]. Thus most of our present knowledge on Tyr_Z came from investigations on inhibited PSII (such as Mn-[21–23], Ca-depleted [24,25] PSII).

D1-His190 is known to interact with Tyr_Z through a hydrogen bond, and is crucial to the assembly of the Mn-cluster [18–20]. It is believed that His190 plays a role of base to accept the phenolic proton of Tyr_Z upon its oxidation. However, there is a debate whether His190 could act as a ligand of the Mn-cluster [2,26,27]. In addition, the strength of the hydrogen bond between Tyr_Z and His190 has not been well defined [18–20].

Substrate water molecule is another possible specie to interact with Tyr_Z in active PSII. Babcock and co-workers [28,29] suggested that Tyr_Z interacted directly with water, and proposed a hydrogen-atom abstraction mechanism wherein $Tyr_Z^{\cdot}$ radical abstracted the hydrogen atom from substrate H_2O bound to Mn ions. This model has been widely cited, and the idea of the direct interaction between Tyr_Z and substrate H_2O has been incorporated in many other models [30]. However, it should be pointed out that most evidences for this model were obtained from experiments on inhibited PSII samples (for example, Mn-[21–23], or Ca-[24,25] depleted PSII samples). Apparently, detailed information on the structural relationship between Tyr_Z and the Mn-cluster in active PSII, as well as, their local environments (such as water, and His190, etc.) is crucial to understand the function and mechanism of Tyr_Z.

D2-Tyr160 (Tyr_D), a symmetrical residue to Tyr_Z, is another redox active tyrosine residue in PSII. It has been well documented that Tyr_D is located in a hydrophobic environment, and interacts with D2-His189, analogous to D1-His190, through a hydrogen bond [18,31]. Similarly, Tyr_D can also be oxidized by $P_{680}^{+\cdot}$ and form the neutral $Tyr_D^{\cdot}$ radical. However, the oxidation and reduction of Tyr_D are much slower than that of Tyr_Z in active PSII at physiological pH value [18,31]. The reason for this difference is still unclear.

The crystal structural data of PSII reported by several groups in various resolutions have revealed most of the structures of proteins and cofactors in PSII [1–5] recently. However, the possible relationship between Tyr_Z and Mn-cluster is still far from an unambiguous demonstration due to two main reasons. Firstly, the resolution of structure is still not high enough to show the detailed structure of the Mn-cluster and its ligands (such as, Cl, substrate water, etc.). Secondly, the current structural data on Mn-cluster and its environment could be significantly different from that in active PSII. Yano et al. [32] and Grabolle, et al. [33] have reported recently that the oxidation state and the structure of the Mn-cluster as well as its environment (including ligands) were significantly modified due to serious radiation damage arising from the X-ray during the crystal structure determination.

Recently, a relatively high yield of Tyr_Z oxidation in S_0 and S_1 states induced by visible light has been observed at cryogenic temperature in active PSII [34–36]. The magnetic interaction between $Tyr_Z^{\cdot}$ and S_0 or S_1 state of the Mn-cluster gives rise to two distinctive EPR signals attributed to $S_0Tyr_Z^{\cdot}$ and $S_1Tyr_Z^{\cdot}$, respectively. The lifetime of $Tyr_Z^{\cdot}$ formed is a few minutes at cryogenic temperature, which is about 10^7 times longer than that at room temperature. Meanwhile, the oxidation of Tyr_Z in S_2 and S_3 states induced by NIR light has also been detected at cryogenic temperature [37–41]. Based on these new observations, a low barrier hydrogen bond (LBHB) between Tyr_Z and a base (His190 is the most likely candidate) has been proposed [34,35,42]. This proposal is in line with the similar suggestion from optical kinetic study of $P_{680}^{+\cdot}$ reduction [43]. More recently, the pH dependence study on Tyr_Z oxidation and $Tyr_Z^{\cdot}$ reduction in active PSII has revealed that there is probably no water molecule interacting directly with Tyr_Z in active PSII [44]. These data provide new insight into the structure and function of Tyr_Z in active PSII.

2. New mechanism for photosynthetic water oxidation in active PSII

On the basis of these recent observations, a new structure model for the relationship between Tyr_Z and Mn-cluster in active PSII [27] is proposed, in which His190 acts as a bridge to form a LBHB with Tyr_Z on one side, and to coordinate to the Mn-cluster on the other side. A new mechanism for the function of Tyr_Z in active PSII is also suggested (see Fig. 2). This model differs from previous proposals concerning the mechanism of Tyr_Z function in two aspects. First, LBHB plays a key role to decrease the activation energy for the Tyr_Z oxidation and $Tyr_Z^{\cdot}$ reduction during photosynthetic water oxidation. Upon the oxidation of Tyr_Z, the hydrogen bond between Tyr_Z and His190 changes from LBHB to weak hydrogen bond; and *vice versa* upon $Tyr_Z^{\cdot}$ reduction. In both stages, the electron transfer and proton transfer are coupled. Second, the oxidation of Tyr_Z by $P_{680}^{+\cdot}$ leads to H^+ transfer which carries the positive charge may play an important functional role for water oxidation. It can be

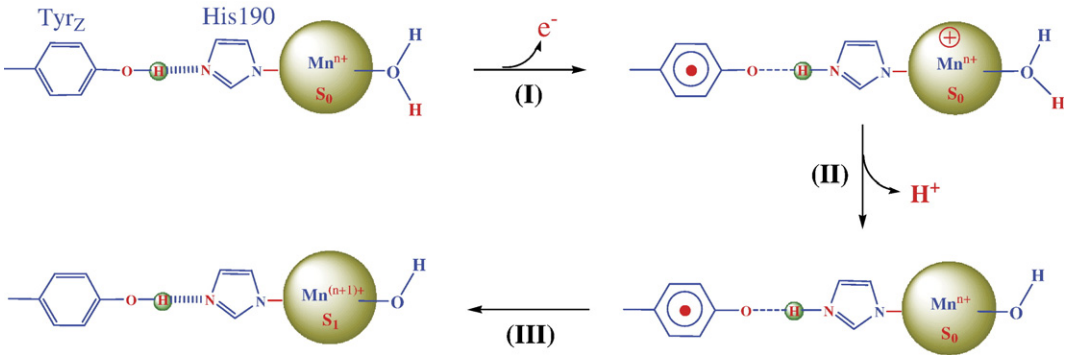

Fig. 2. New mechanism for photosynthetic water oxidation in PSII: (I) oxidation of Tyr_Z; (II) proton release from substrate water; (III) reduction of $Tyr_Z^{\cdot}$ and oxidation of the Mn-cluster. The thick dashed line indicates the low-barrier hydrogen bond (LBHB); the thin dashed line indicates weak or normal hydrogen bond. For clarity, only one Mn ion and one H_2O are shown to represent the Mn-cluster. Only $S_0 \rightarrow S_1$ is illustrated as an example for the proposed mechanism, other state transitions are following the same mechanism. The electron and proton transfer in step (I) and (III) are coupled.

delocalized on the Mn-cluster, thus, helps to accelerate the proton release from substrate water on Mn-cluster.

In the following, the main features of this model will be illustrated in detail.

2.1. Position of substrate water

It was thought that the substrate water could interact directly with Tyr_Z [28–30]. However, there has been no any evidence from active PSII yet. It is also impossible to get direct information for the binding position of the substrate water based on present PSII structure data, due to the low resolution and radiation damage of the crystal as mentioned above. To tackle the question of possible interaction between substrate water molecules and Tyr_Z, investigations on the pH dependence of Tyr_Z oxidation and $Tyr_Z^{\cdot}$ reduction in active PSII were performed by using the method developed recently [44]. Surprisingly, change of the pH in bulk had no effect on the Tyr_Z oxidation and $Tyr_Z^{\cdot}$ reduction in active PSII, which was dramatically different from that in Mn-depleted PSII [18–20,45–48]. Furthermore, DFT theoretical studies on model Tyr–His and Tyr–His–H_2O systems indicated that Tyr became difficult to be oxidized when the H_2O molecule interacted with it [27,44]. In view of the pH dependence study, theoretical study on model system, as well as, observation of the faster substrate water exchange rate by time-resolved mass spectroscopy [49], it was proposed that Tyr_Z might be located in a hydrophobic environment [44,50] without substrate water molecules accessible to it in the S_0 and S_1 states of the active PSII [44]. These results challenge the previous postulation of direct interaction between Tyr_Z and the substrate water during water oxidation. On the other hand, one would speculate that the separation of the Tyr_Z and substrate water (as shown in Fig. 2) might have advantage to protect Tyr_Z from some uncontrollable reactions with highly active intermediate species formed during water oxidation process.

2.2. Position of His190 in active PSII

For long, it was thought that the His190 only acted as a base to accept proton from Tyr_Z during water oxidation. However, mutagenesis studies [19,20] have shown that the replacement of His190 with stronger basic residues, e.g. Arg or Lys, decreased the water oxidation reaction greatly. In fact, all the mutations of His190 abolish photo-autotrophic growth, which implies that His190 might act as a ligand of the Mn-cluster. Therefore the dual role of His190 has been proposed [26,27] that His190 interacts with both Tyr_Z and the Mn-cluster. The distance between Tyr_Z and the Mn-cluster in His190 bridging model presented here (Fig. 2) is 7 Å, which is consistent with X-ray data [1,2].

The proposal of the binding of His190 to the Mn-cluster was confirmed in the 3.7 Å structure data [2], but not in 3.0–3.5 Å structure data [3–5]. Recently, it has been found that nearly all the Mn ions were reduced to Mn^{II} state under crystallographic study condition due to the radiation damage arising from X-ray [32,33]. Corresponding to the photo-reduction of the Mn-cluster by X-ray, photo-oxidation of the ligands or close environment of the Mn-cluster may occur; consequently, the protons are released to the environment. Therefore, one would suspect that the structure and configuration of the ligands and close environment could be significantly different from that in active PSII. Moreover, the imidazolate group ($pK_a \approx 6$–10, see below discussion) of His190 (see Fig. 2) is one of the most sensitive groups to be protonated around the Mn-cluster. Once it is fully protonated, it may move away from the Mn-cluster. Considering the higher risk of radiation damage in the higher resolution X-ray structure measurement, it would be more likely that the direct binding of His190 to Mn-cluster was lost in the higher resolution structural data.

It is noted that the protonation of ligands and the loss of His binding to metal ion by radiation damage arising from X-ray have also been observed in other enzyme systems [51,52]. For example, in nickel-containing superoxide dismutase, Wuerges, et al. [51] found that the imidazolate of binding His1 at Ni^{III} became fully protonated with two protons on two imidazolic nitrogen atoms, which led to the change of its configuration and move away from Ni ion after X-ray-induced reduction. It should be mentioned that only one metal ion and one electron are involved in the photo-reduction of metal ion in Ni-SOD; while four Mn ions and at least 6 electrons (since the redox state of the four Mn ions is IV, IV, III, III, respectively, in the S_1 state [10–

14]) are involved in PSII. It is reasonable to deduce that more pronounced changes of the ligands might be induced by the radiation damage in PSII than that in Ni-SOD.

Considering the mutagenesis studies [19,20] and radiation damage [32,33] discussed above, a mechanism for the change of His190 in PSII raised by X-ray is proposed (see Fig. 3). In the final step in Fig. 3, the His190 can only form weak or normal hydrogen bond with Tyr_Z due to unmatched pK_a between His190 (pK_a=6) and Tyr_Z (pK_a=10.9), and its binding to the Mn-cluster is lost, which is similar to His1 in Ni-SOD [51].

2.3. Existence of the low barrier hydrogen bond

It is known that the strength of a hydrogen bond depends on its length and linearity, the nature of its microenvironment, and the degree to which the pK_a values of the conjugate acids of the heavy atoms sharing the proton are matched [53–55]. The LBHB is one type of most strong hydrogen bond, in which the overall distance of two heavy (oxygen or nitrogen) atoms is shortened, and the energy barrier for proton transfer is close to the zero point energy level [55–57]. It has been found that the LBHB plays critical role to stabilize intermediates and lower the energy of transition states in a number of enzymatic reactions [55–57], including serine protease, mandelate racemase, aspartic protease [58], etc.

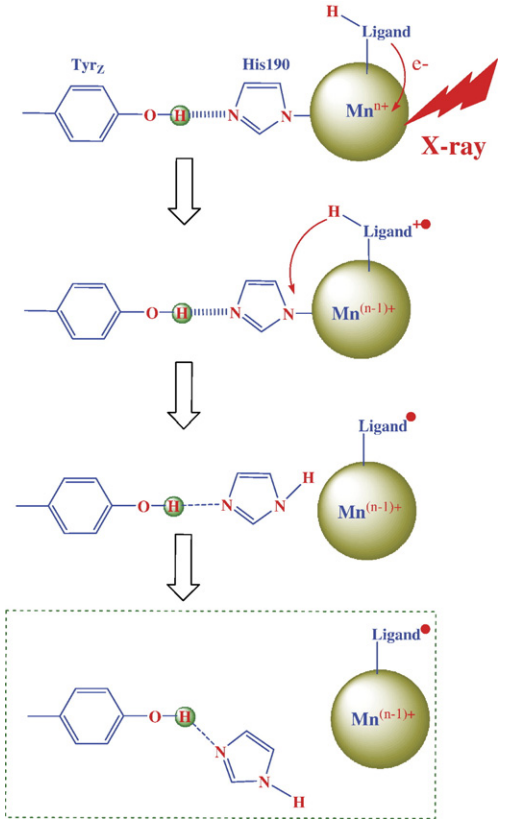

Fig. 3. Possible change of His190 arising from the radiation damage by the X-ray: The thick dashed line indicates the low-barrier hydrogen bond (LBHB); the thin dashed line indicates weak or normal hydrogen bond. For clarity, one Mn ion with one ligand is shown for the Mn-cluster.

Table 1
pK_a values for tyrosine, histidine and imidazole on metal ions

Complexes	pK_a	Ref.
His	6.0	[59]
Tyr	10.9	[59]
$(NH_3)_5Cr^{III}$(ImH)	9.35	[61]
$(CN)_5Fe^{III}$(ImH)	10.93	[60]
$(NH_3)_5Co^{III}$(ImH)	9.99	[62]
$(NH_3)_5Rh^{III}$(ImH)	9.97	[62]

It has been suggested that there could exist a LBHB between Tyr_Z and His190 based on the low temperature EPR studies of Tyr_Z oxidation and the optical study of P^+_{680}. reduction [34,35,42,43]. However, it is not obvious how such strong hydrogen bond can be formed. The formation of LBHB requires the matched pK_as ($\Delta pK_a \approx 0$) of both proton donor and proton acceptor [55–57]. However, the pK_as of Tyr and His are 10.9 and 6 [59], respectively, and the difference of their pK_a (ΔpK_a) is 4–5. Therefore, it is unlikely to form such strong hydrogen bond between the typical type of Tyr and His. Alternately, it is known that the pK_a of His is around 10 when the imidazole group ligates to metal ion (such as Cr^{III}, Fe^{III}, Co^{III}, Rh^{III}, etc.) [60–62] (see Table 1). If it holds true for Mn^{III} in PSII, the pK_a of Tyr_Z and the binding His190 will match very well, which provides the possibility for LBHB formation under this condition.

Since the energy barrier for the proton transfer in LBHB is significantly lower than that in normal or weak hydrogen bonds [55–57], the presence of LBHB between Tyr_Z and His190 provides a good explanation for the observation of Tyr_Z oxidation at cryogenic temperature [34–41]. Apparently, the pK_a of this binding His is strongly dependent on the redox state of metal ion. One would expect that the pK_a decreases when the redox state of Mn ion changes from +3 to +4, and increase when the redox state of Mn ion changes from +3 to +2. In PSII, the redox states for four Mn ions are assigned as (III, III, III, IV) [10–14,63], (III, III, IV, IV) [10–14] and (III, IV, IV, IV) [10–14] to the S_0, S_1 and S_2 states, respectively. It was suggested that the Mn (II) existed in the S_0 state based on the CW EPR studies [64,65]. However, recently ^{55}Mn pulse ENDOR study reported by Kulik et al. shows that the oxidation state for S_0 state is (III, III, III, IV)[63]. Because the Tyr_Z can be oxidized at cryogenic temperature in all these states [34–41], the Mn ion bound to His190 is most likely in Mn^{III} state. For the S_3 state, the redox states of four Mn ions may be in (IV, IV, IV, IV) state [11] or in (III, IV, IV, IV) with one cation radical on ligand [10], one would expect that the pK_a of His190 might decrease. Correspondingly, the strength of the hydrogen bond decreases due to unmatched pK_a of Tyr_Z and His190 under this condition. This is consistent with the observation that the Tyr_Z oxidation becomes difficult in higher oxidized state (such as, S_3 state) in active PSII [40]. In addition, the existence of strong hydrogen bond is readily reconciled with the reports of very low kinetic H/D isotope effect [66–68] of Tyr_Z oxidation.

Here, it should be mentioned that the above assignment for the ligated Mn ion to His190 is not absolutely necessary due to

the electronic delocalization of the whole Mn-cluster. Therefore, it is also possible that the His190 binds to Ca ion, which is associated with three or four Mn ions through three μ-O bridges as predicted by Zhang, et al.[69] and revealed by the X-ray data [3]. Finally, it should be pointed out that the strong hydrogen bond between the OH group of Tyr_Z (as proton donor) and His (as proton acceptor) described above should change to weak hydrogen bond between the NH group of His 190 (as proton donor) and the Tyr_Z radical (as proton acceptor) upon the oxidation of Tyr_Z due to the unmatched pK_a of $Tyr_Z^{+\cdot}$ ($pK_a = -2$) [70] and the binding His190 ($pK_a = 10$) on Mn-cluster.

2.4. Function of the proton or positive charge formed after TyrZ oxidation

Since the Tyr_Z is in protonated state, one proton is released upon its oxidation due to the low pK_a of $Tyr_Z^{+\cdot}$ ($pK_a = -2$) [70]. One base takes the proton, which is no doubt His190. However, there is a question about where the proton or positive charge goes after that. One suggestion was that the proton was released quickly to bulk upon Tyr_Z oxidation [29,70–72]. Second opinion [73,74] was that the proton or positive charge stayed on His190 alone, and returned to Tyr_Z upon its reduction. Furthermore, there was suggestion that proton was somewhat delocalized within a hydrogen bond network in the protein [75,76]. In all these models, the positive charge is just a by-product after Tyr_Z oxidation without any obvious function for water oxidation.

In contrast, the His190 bridging model described here suggests that the movement of proton to His190 upon Tyr_Z oxidation leads to the delocalization of positive charge on the Mn-cluster, which has advantage to be further used to accelerate the proton release from the H_2O substrate in Mn-cluster. Therefore, the positive charge may contribute directly to the water oxidation. Generally, the different redox state Mn ion has different ability to stabilize the positive charge, namely, the lower redox state has higher stabilization energy for the positive charge. The higher stabilization energy would supply higher driving force for Tyr_Z oxidation. Therefore, the His190 bridging model clearly predicts that the oxidation of Tyr_Z is easier at lower redox states of the Mn-cluster (S_0 and S_1 states), but more difficult at higher redox states (S_2 and S_3 states). In addition, comparing with His residue alone, the His–Mn-cluster motif certainly has larger stabilization energy for the positive charge. Accordingly, the oxidation of Tyr_Z should be much easier than that of Tyr_D in this His190 bridging model. All these predictions are consistent with experimental observations for the S-state dependence of Tyr_Z oxidation [6–9,77,78] and the difference between Tyr_Z and Tyr_D [18,31], which cannot be readily explained by most previous models.

The positive charge delocalized on Mn-cluster will accelerate the proton release from substrate water molecules to the bulk through proton channel. Obviously, the proton release step should occur after the Tyr_Z oxidation in the His190 bridging model but before the $Tyr_Z^{\cdot}$ reduction (see below discussion). All these predictions are consistent with proton release measurement [46,79].

2.5. Mechanism for $TyrZ^{\cdot}$ radical reduction

Compared with the concerted mechanism for Tyr_Z oxidation wherein the electron from Tyr_Z to $P_{680}^{+\cdot}$ and the proton from the phenolic oxygen of Tyr_Z to nitrogen of the imidazole of His190 are strongly coupled [47,80], the mechanism for $Tyr_Z^{\cdot}$ reduction is much controversy [28,29,70–74]. In the original hydrogen abstraction model [28,29], the concerted mechanism proposed that $Tyr_Z^{\cdot}$ radical directly abstracted hydrogen atom from substrate water bound to Mn ions; whereas, some other models suggested that $Tyr_Z^{\cdot}$ was reduced via the electron and proton transfer in a consecutive way (at least in the low S-states) [71,72].

In the His190 bridging model shown in Fig. 2, the H-atom shifts from the imidazolic nitrogen atom of His190 to the phenolic oxygen of $Tyr_Z^{\cdot}$, leading to the reduction of $Tyr_Z^{\cdot}$ and oxidation of the Mn-cluster. Thus, the electron and proton transfer are coupled upon the reduction of $Tyr_Z^{\cdot}$. Similar to the original hydrogen abstract mechanism [28,29], the overall charge is in neutral or nearly so in this model, which has advantage to keep low activation energy in low dielectric constant environment. Meanwhile, the recovery of LBHB from weak hydrogen bond may help to overcome the high activation energy for the hydrogen atom transfer mechanism. It is suggested that the formation of LBHB can supply more than 10 to 20 kcal/mol energy [55–57], thus it facilitates hydrogen atom transfer reaction above. Similar effect has been proposed for many other enzyme reactions [55–57].

Finally, it should be mentioned that Tyr_Z oxidation and $Tyr_Z^{\cdot}$ reduction follow the same rule as shown in Fig. 2 to accomplish the turnover of Tyr_Z in all S-state transitions during water oxidation; while the proton release into bulk in different S_i-state transitions would be varied due to different electrostatic effects of the periphery and the Mn-cluster [46].

3. Conclusion

Here a new model of His190 bridging between Tyr_Z and Mn-cluster is proposed, which predicts the existence of the LBHB between Tyr_Z and His190. The LBHB plays a key role to decrease the activation energy for both Tyr_Z oxidation and $Tyr_Z^{\cdot}$ reduction during photosynthetic water oxidation, which results in the strong coupling of the electron transfer and proton transfer processes. The positive charge formed after Tyr_Z oxidation is delocalized on the Mn-cluster, which may play an important functional role for water oxidation to accelerate the proton release from substrate water on Mn-cluster. This model readily reconciles the S-state dependence of Tyr_Z oxidation and $Tyr_Z^{\cdot}$ reduction, proton release, isotopic effect and recent EPR studies. It also provides explanation for the dramatically different behavior between Tyr_Z and Tyr_D in active PSII. This model predicts that His190 is bonding to the Mn or Ca^{2+} ion of the Mn-cluster, which has not been observed by recent structure data. It is probably mainly due to the radiation damage of the Mn-cluster and its environment by the X-ray, in which the imidazolate group of His190 could be protonated, leading to the loss of its binding to Mn-cluster and the strong hydrogen bond

with Tyr_Z. This type of change arising from radiation damage has already been confirmed in other enzyme systems [51].

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