Supporting Information

Saito et al. 10.1073/pnas.1300817110

SI Text

Tyrosine D at High pH. At pH values higher than pH 7.6, tyrosine D (TyrD; D2-Tyr160) becomes extremely rapid as an electron donor (1), outcompeting tyrosine Z (TyrZ; D1-Tyr161) in the Mn-depleted system and becoming easily oxidizable at 4 K and below (i.e., with no energy barriers) (2, 3). In many ways then, TyrD becomes more TyrZ-like at higher pH. How do these observations fit with the model presented here for TyrD function at pH 6.5? Without a specific crystallographic model of Photosystem II (PSII) at higher pH, modeling, calculations, and conclusions will inevitably be less reliable. Nevertheless, it is worth investigating if the model for the redox mechanism of TyrD proposed in this paper can provide insights for understanding the remarkable change in the properties of TyrD at higher pH.

Here, we have pointed out the key structural features that determine the slow TyrD kinetics, whereas those responsible for the fast TyrZ kinetics were discussed earlier (2). For TyrZ, there is a short, activation-less H-bond from TyrZ to D1-His190. This forms part of the H-bonding chain running from two water molecules near the Mn_4Ca cluster via TyrZ toward the aqueous medium: $(HOH)_2$··›TyrZ-OH··›N_ε-His-N_δH··›O=Asn. The waters play a role in tuning the H-bond (2). On oxidation, the phenol proton of TyrZ is rapidly transferred to D1-His190, and this is probably the start of a proton transfer chain (1). For TyrD, an Arg (D2-Arg294), rather than an Asn (D1-Asn298), acts as the H-bond partner to D2-His189 on the side distal to the TyrD. This results in a change in the H-bond direction of the system, the absence of the activation-less Tyr-His H-bond, and the presence of water acting as an H-bond acceptor from TyrD. The fact that the water molecule is isolated and must move on protonation makes oxidation of TyrD slow. At high pH, the increased rate of electron transfer and the activation-less oxidation of TyrD at cryogenic temperatures suggest the occurrence of a situation similar to that seen for TyrZ (i.e., reversal of the direction of the H-bond chain and the appearance of a short, activation-less H-bond associated with deprotonation of the tyrosine on oxidation). These are the features we looked for in our modeling.

Taking the H-bonding model at pH 6.5 as described and the crystal structure at pH 6.5 as a starting state, the simplest approach was to investigate the influence of deprotonation in the extended H-bonding network around TyrD. The most obvious candidate for deprotonation is $[H_2O‹··\mathbf{H}$-TyrD-O‹··HN_ε-His-$N_\delta]^0$ where the key protons are indicated in bold face (Table 1), resulting in the formation of the [TyrD-OH··›N_ε-His-$N_\delta]^-$ state (Table S1). Here, the TyrD no longer provides an H-bond to water; instead, it donates an H-bond to His, which represents a reversal of the H-bond direction. Remarkably, the calculated distance between phenolic O of TyrD and the nearest N of D2-His189 (O_{TyrD}−$N_{\varepsilon,His189}$) was ∼2.5 Å (Table S1), which is significantly shorter with respect to 2.7 Å at pH 6.5 in the PSII crystal structure (4). The energy curve for the H-bond between TyrD and D2-His189 in this state resembles that of a barrier-less, single-well H-bond (Fig. S2, blue curve), similar to that previously observed for the H-bond between TyrZ and D1-His190 (5). This state may be best expressed as having a symmetrical H bond, $[TyrD\text{-}O··H··N_\varepsilon\text{-}His\text{-}N_\delta]^-$. This shorter H-bond between TyrD and D2-His189 may represent the situation observed by Hienerwadel et al. (6) at high pH. It is also consistent with the much faster TyrD oxidation rate and the ability to undergo oxidation at liquid helium temperature at pH values above pH 7.6 (2). Because the H-atom remains delocalized between TyrD and D2-His189 in the barrier-less potential well of the $[TyrD\text{-}O··H··N_\varepsilon\text{-}His\text{-}N_\delta]^-$ state, this model does not contradict the conclusion from FTIR that TyrD-OH remains protonated above pH 7.5 (3).

This simple model (deprotonation of $[H_2O‹··\mathbf{H}$-TyrD-O‹··HN_ε-His-$N_\delta]^0$) seems to result in a state that is consistent with the redox properties of TyrD seen at high pH. Without further experimental support, however, this model is somewhat tentative. It is, of course, possible to extend this model further, considering a somewhat unorthodox role for D2-Arg294 as a proton acceptor and then determining how this affects its potential ion-pair partner, CP47-Glu364, and so on down the putative proton chain. It is also possible to investigate alternative, less structurally conservative models, such as a small conformation change in which D2-Arg294 is replaced by D2-Asn292 (which is only 4 Å away), making a TyrZ-like H-bonding motif. These models are more speculative, however, and should await more experimental results. At present, we can conclude that the mechanistic model for TyrD at pH 6.5 presented in the main text needs only very minor and conservative changes (e.g., those calculated in Fig. S2) to obtain a situation that explains the fast kinetics seen for TyrD seen above pH 7.5.

Given that the kinetics of electron transfer from TyrZ in the functional system can be nearly an order of magnitude faster than the fastest TyrD kinetics, it may be that the short TyrZ H-bond in intact PSII is better tuned than that in TyrD at high pH, and that may be due to an influence of the water cluster in TyrZ (2). However, the fact that nearly all the centers are able to undergo oxidation at 4 K with the high-pH–treated TyrD (2, 3), whereas only a small fraction can do this with TyrZ even in the best conditions (7, 8), indicates that the H-bond, TyrD-OH··›N_ε-His, is more homogeneous than that of its TyrZ counterpart. This may be attributed to the dynamics of the bonding of the water cluster to TyrZ.

TyrD in the Sr-Substituted PSII. In the Sr-substituted PSII crystal structure, in the region of TyrD, proximal H_2O (H_2O_{prox}) was absent but distal H_2O (H_2O_{dist}) was present in all centers (9). The most straightforward interpretation is that the sample had seen enough light before freezing to oxidize the TyrD, forming $TyrD^\bullet$ in all centers. If this is the case, the absence of H_2O_{prox} would not be associated with the substitution of Ca with Sr in the Mn_4Ca cluster but would, instead, be related to the specific illumination history of the sample before freezing. Similarly the observation that in the native PSII, the proportion of centers having H_2O_{dist} is larger than that with H_2O_{prox} (4) would be an indication that this sample saw less light or longer dark adaptation before freezing, which, again, is a property specific to the pretreatment of the sample.

1. Faller P, et al. (2001) Rapid formation of the stable tyrosyl radical in photosystem II. *Proc Natl Acad Sci USA* 98(25):14368–14373.
2. Faller P, Rutherford AW, Debus RJ (2002) Tyrosine D oxidation at cryogenic temperature in photosystem II. *Biochemistry* 41(43):12914–12920.
3. Faller P, Goussias C, Rutherford AW, Un S (2003) Resolving intermediates in biological proton-coupled electron transfer: A tyrosyl radical prior to proton movement. *Proc Natl Acad Sci USA* 100(15):8732–8735.
4. Umena Y, Kawakami K, Shen J-R, Kamiya N (2011) Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 Å. *Nature* 473(7345):55–60.
5. Saito K, Shen J-R, Ishida T, Ishikita H (2011) Short hydrogen bond between redox-active tyrosine Y_Z and D1-His190 in the photosystem II crystal structure. *Biochemistry* 50(45): 9836–9844.
6. Hienerwadel R, Diner BA, Berthomieu C (2008) Molecular origin of the pH dependence of tyrosine D oxidation kinetics and radical stability in photosystem II. *Biochim Biophys Acta* 1777(6):525–531.
7. Zhang C, Styring S (2003) Formation of split electron paramagnetic resonance signals in photosystem II suggests that $tyrosine_Z$ can be photooxidized at 5 K in the S0 and S1 states of the oxygen-evolving complex. *Biochemistry* 42(26):8066–8076.

8. Zhang C, Boussac A, Rutherford AW (2004) Low-temperature electron transfer in photosystem II: A tyrosyl radical and semiquinone charge pair. *Biochemistry* 43(43): 13787–13795.

9. Koua FH, Umena Y, Kawakami K, Shen JR (2013) Structure of Sr-substituted photosystem II at 2.1 Å resolution and its implications in the mechanism of water oxidation. *Proc Natl Acad Sci USA* 110(10):3889–3894.

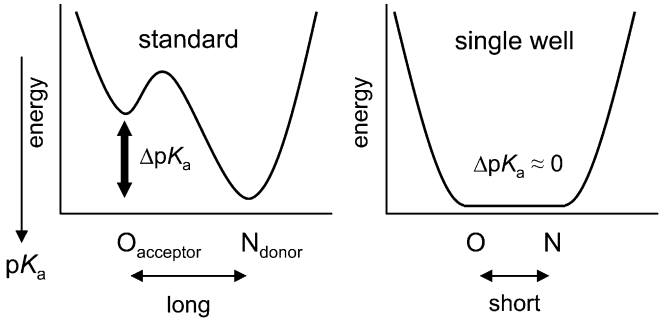

Fig. S1. Overview of potential-energy profiles: standard H bonds (asymmetrical double-well), typically with an O_{donor}–$O_{acceptor}$ distance >~2.6 Å (*Left*), and single-well (ionic) H-bonds, typically with an O_{donor}–$O_{acceptor}$ distance of <~2.5 Å (*Right*). The corresponding O–N distances are generally greater than O–O distances. ΔpK_a (thick vertical arrow) indicates the pK_a difference between H-bond donor and acceptor moieties.

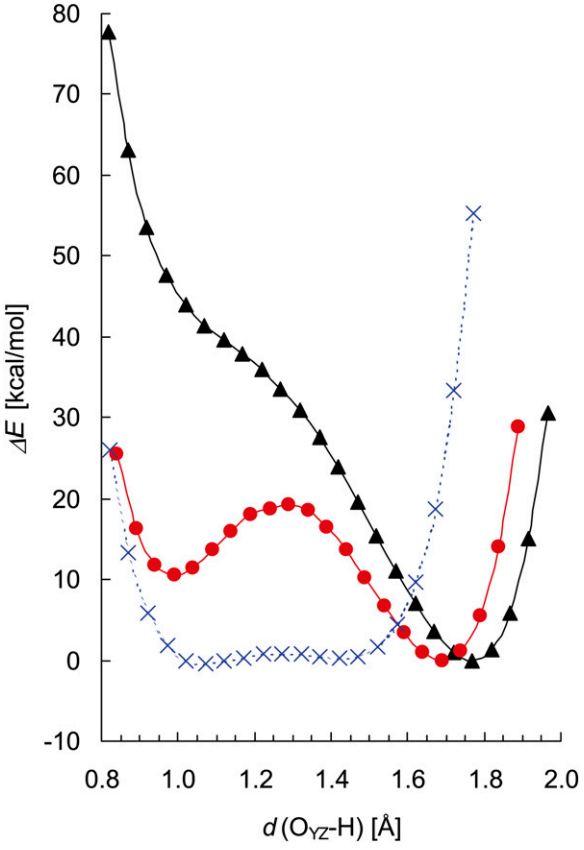

Fig. S2. Potential-energy profiles along the proton transfer coordinate for an H-bond donor-acceptor pair, TyrD and D2-His189. The $[TyrD\text{-}O\cdot\cdot\mathbf{H}\cdot\cdot N_{\varepsilon}\text{-}His\text{-}N_{\delta}]^{-}$ state, which represents the deprotonated form of the $[H_2O_{prox}\cdots\mathbf{H}\text{-}TyrD\text{-}O\cdots HN_{\varepsilon}\text{-}His\text{-}N_{\delta}]^{0}$ state observed at high pH is shown (blue cross curve). The $([H_2O_{prox}\cdots H\text{-}TyrD\text{-}O\cdots\mathbf{H}N_{\varepsilon}\text{-}His\text{-}N_{\delta}])$ (black triangle curve, at low pH) and $([TyrD\text{-}O^{\bullet}\cdots\mathbf{H}N_{\varepsilon}\text{-}His\text{-}N_{\delta}]\leftrightarrow[TyrD\text{-}O\mathbf{H}\cdots N_{\varepsilon}\text{-}His\text{-}N_{\delta}^{\bullet}])$ (red circle curve, at low pH) states are also shown (Fig. 2).

Table S1. H-bond distances for TyrD in QM/MM optimized geometries in the PSII protein environment (measured in angstroms)

Redox/protonation state	O_{TyrD}–$N_{\varepsilon,His}$	O_{TyrD}–H	H–$N_{\varepsilon,His}$	O_{TyrD}–O_{H2O}	$N_{\delta,His}$–N_{Arg}
Original (1.9-Å structure)	2.74	—	—	Distal, 4.30; proximal, 2.73	2.81
High pH (Fig. S2, blue curve)					
[TyrD-O··**H**··N_{ε}-His-N_{δ}]$^{-}$ (1)	2.56	1.07	1.49	4.20	2.80
[TyrD-O··H··N_{ε}-His-N_{δ}]$^{-}$ (2)	2.53	1.38	1.15	4.24	2.84

Arg, D2-Arg294; His, D2-His189; O_{TyrD}, phenolic O of TyrD; —, not applicable.

Other Supporting Information Files

Dataset S1 (PDB)