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Spectroscopic Evidence for a Redox-Controlled Proton Gate at Tyrosine D in Photosystem II

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Supporting Information

ABSTRACT: Tyrosine D (TyrD) is one of two well-studied redox active tyrosines in Photosystem II. TyrD shows redox kinetics much slower than that of its homologue, TyrZ, and is normally present as a stable deprotonated radical ($TyrD^{\bullet}$). We have used time-resolved continuous wave electron paramagnetic resonance and electron spin echo envelope modulation spectroscopy to show that deuterium exchangeable protons can access TyrD on a time scale that is much faster (50−100 times) than that previously observed. The time of H/D exchange is strongly dependent on the redox state of TyrD. This finding can be related to a change in position of a water molecule close to TyrD.

The water-oxidizing enzyme Photosystem II (PSII) has two redox active tyrosines, TyrZ and TyrD. Although symmetrically placed in the two core subunits D1 and D2,[1] and displaying similar spectroscopic characteristics as determined by electron paramagnetic resonance (EPR), TyrZ and TyrD are distinct in their kinetic behavior.[2,3] Both tyrosines are oxidized in proton-coupled reactions. TyrZ, placed close to the $CaMn_4$ cluster, is involved in fast electron transfer chemistry during water oxidation. TyrD displays much slower kinetics and stays in the oxidized, deprotonated state ($TyrD^{\bullet}$) during catalysis. Its slow oxidation kinetics, along with the extreme stability of the radical state, has generally been explained by the position of TyrD in the protein. The cavity around TyrD is more shielded and hydrophobic than that around TyrZ.[1] This has been further substantiated by very slow exchange of protons around TyrD, and it has long been known that it takes many hours to fully deuterate the environment of TyrD.[4] However, in this report, we show that TyrD is not as isolated as previously thought.

TyrD can be oxidized (via P_{680}^{+}) through charge equilibrium with the $CaMn_4$ cluster.[5−7] A dark-adapted PSII sample with TyrD reduced (Figure 1A) was given one laser flash. The flash advances the $CaMn_4$ cluster one step in the catalytic cycle, producing the S_2 state. The S_2 state then oxidizes TyrD, returning the $CaMn_4$ cluster to the S_1 state ($S_2TyrD^{red} \rightarrow S_1TyrD^{ox}$).[5] The signal after the flash represents ∼80% of the maximal inducible radical (Figure 1A). The rate of this reaction allows the oxidation of TyrD to be followed by time-resolved X-band continuous wave EPR (CW-EPR) (Figure 1B, blue circles). This is a direct way of probing both the kinetics and the access of solvent to TyrD.[5,8] As described by Vass and Styring,[5] the kinetics can be fit with a biexponential function giving two rate constants (1.65 and 0.14 s^{-1} at pH 6.3) that are characteristic for the pH of the sample.

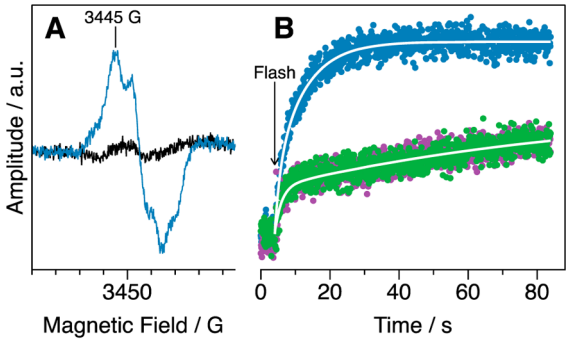

Figure 1. (A) CW-EPR spectra of $TyrD^{\bullet}$ recorded at room temperature in dark before the laser flash (black) and ∼80 s after the laser flash (blue) in a sample at pH 6.3. (B) Kinetics of induction of TyrD oxidation after one saturating laser flash in a sample at pH 6.3 (blue) or a sample at pD 6.3 incubated in deuterated buffer for either 3 h (green) or 1.5 min (purple). The amplitude was recorded at 3445 G. The solid lines represent fittings of the data points using a biexponential rise function.

When the kinetics of TyrD oxidation was recorded after the sample had been incubated for 3 h in deuterated buffer at pD 6.3 (Figure 1B, green circles), the amplitude achieved was smaller (see the Supporting Information for a discussion of this effect) and the rate constants were much lower (0.51 and 0.015 s^{-1}). The change in kinetics clearly shows that the oxidation of TyrD is affected by the presence of deuterium. Remarkably, when the same kinetics was recorded after H/D exchange for only 1.5 min (purple circles), which is the lower limit of our sample handling procedure, we observed identical kinetics as after exchange for 3 h. Thus, as it seems, the proton(s) affecting the rate of TyrD oxidation can be replaced within 1.5 min.

This fast H/D exchange is surprising and contradicts many previous observations reported in the literature. In an early electron nuclear double resonance (ENDOR) experiment by Babcock and co-workers,[4] they concluded that complete H/D exchange at TyrD took many hours. After this, more refined experiments using ENDOR and electron spin echo envelope

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modulation (ESEEM) spectroscopy confirmed the slow exchange around TyrD and a contrasting much faster exchange around TyrZ;[9−12] Diner et al.[9] measured the half-time of exchange to be 9 h for TyrD, while it was less than 2 min for TyrZ.

Hoff and co-workers[13] showed that a two-pulse ESEEM experiment can be used to detect exchangeable protons close to TyrD (within ∼3 Å). In the frequency domain spectrum, this was indicated by the appearance of a peak at 2.6 MHz[13] (see also ref 9). Therefore, we conducted a two-pulse ESEEM experiment using the same type of sample as for the kinetic measurements, i.e., in which TyrD was reduced during the buffer exchange. After H/D exchange for 3 h, TyrD was reoxidized by a short illumination and the ESEEM spectra were recorded. A distinct peak at 2.5 MHz was observed in the frequency domain (Figure 2A, green trace) recorded at 9.70

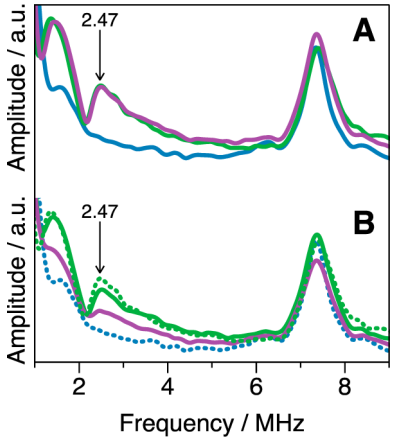


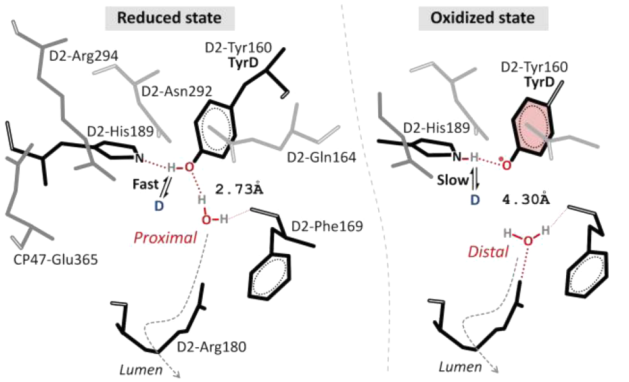
Figure 2. Frequency domain two-pulse ESEEM spectra of TyrD•. Spectra recorded for samples in which deuterium exchange was conducted when TyrD was in its reduced (A) or oxidized (B) state. Samples were incubated at pD 6.3 for either 3 h (green) or 1.5 min (purple). The figure also shows a spectrum recorded in a buffer at pH 6.3 (blue). For comparison, spectra from panel A are shown as dotted lines in panel B.

GHz. We assign this to be the same peak observed in ref 13, the small difference reflecting the frequency used in the experiment. No peak was observed at this position when measuring a sample at pH 6.3 (blue trace), which clearly shows that the peak originates from deuterium-exchanged protons. Peaks are also observed at 1.6 and 7.3 MHz. The 1.6 MHz peak has been assigned to a β-methylene proton(s) on TyrD,[14] whereas the 7.3 MHz peak originates from the proton matrix in the bulk. When the ESEEM spectrum was recoded for an identical sample but after buffer exchange for only 1.5 min, the amplitude of the 2.5 MHz peak (Figure 2A, purple trace) was almost identical to that after exchange for 3 h (green trace). Obviously, we observe complete H/D exchange in <2 min both in the kinetic experiment and in the ESEEM experiment.

What then is the reason for the apparent discrepancy between the fast H/D exchange observed here and the slower exchange reported previously? In our kinetic measurements, TyrD was, and had to be, reduced during the exchange. This is not necessary in the ESEEM experiments, and in fact, in all previous investigations TyrD was always oxidized during the exchange procedure.[4,9,11−13] We therefore also made the same measurements as in Figure 2A, but buffer exchange was conducted in the presence of oxidized TyrD (Figure 2B). After incubation for 3 h, the 2.5 MHz peak was smaller than the 2.5 MHz peak in Figure 2A. This indicates that 3 h is not enough to reach complete H/D exchange when TyrD is oxidized, which is similar to earlier reports (see the Supporting Information). After the shorter 1.5 min incubation time (purple trace), the peak at 2.5 MHz was almost absent. Thus, it is evident that the exchange time very much depends on whether TyrD is in its reduced or oxidized state when the protons are replaced. When TyrD is reduced, the exchange is fast, taking place on a time scale of seconds to minutes.

How can the time of replacing the exchangeable proton depend on the redox state of TyrD? In the 1.9 Å resolution structure, a water molecule, which can occupy two different positions (Scheme 1), is able to hydrogen bond to TyrD,[1]

Scheme 1. Environment around TyrD[1] in Its Reduced and Oxidized States



serving as an alternative hydrogen bond partner in addition to D1-His189.[15−17] This water can, in its more distal position, also bind to D2-Arg180 (Scheme 1). This connects TyrD to a hydrogen bond network that extends all the way to the luminal surface of PSII, making the transport of H/D possible.[1,15] From our data, it seems that when TyrD is in its oxidized state this pathway will communicate deuterons slowly and when the tyrosine is reduced the communication will be fast. Interestingly, in a recent quantum mechanics/molecular mechanics (QM/MM) analysis of the 1.9 Å resolution structure,[15] the redox state of TyrD was found to govern the occupancy of the water molecule at the two positions. When TyrD was reduced, the water molecule was within hydrogen bonding distance of the phenolic oxygen. When TyrD was oxidized, the water molecule moved to the distal position. The water is then 4.3 Å from TyrD[1] and can no longer hydrogen bond (Scheme 1).

We believe that the movement of the water molecule can be reconciled with our present data, assuming that the 2.5 MHz peak belongs to the phenolic proton of TyrD hydrogen bonded to D2-His189.[9] As the ESEEM spectra always were recorded when TyrD was oxidized, the water is located in the distal position according to QM/MM analysis.[15] The longer distance to TyrD• makes the water molecule a less likely candidate for the deuterium coupling observed by ESEEM. This is also in line with the original assignment of the "kinetic" (and exchangeable) proton; Vass and Styring[5] found a pK of 7.3 for the pH-dependent rate constant of the slower kinetic component in the biexponential oxidation of TyrD, which is of the magnitude where a histidine would titrate. It has also been reported that the H/D exchangeable proton at TyrD is no longer seen in

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ENDOR experiments when D2-His189 is replaced with Gln.[10,18]

The potential energy barrier that separates the two water positions (see discussion in ref 15) is a likely reason for redox-dependent exchange. When TyrD is reduced, its bound water can mediate deuterium exchange in the hydrogen bond to D2-His189 (Scheme 1). According to the potential energy profile presented in ref 15, the water can still move between the two positions, connecting TyrD to the pathway at D2-Arg180, although the energy is lower at the proximal position when TyrD is reduced.

When TyrD is oxidized, the water is far away and the pathway is broken at TyrD (Scheme 1). It is energetically more unfavorable for the water to move between the two positions when TyrD is oxidized.[15] Thus, the water is less likely to occupy the position close to TyrD$^{\bullet}$, making deuterium exchange less probable (maybe even impossible without concomitant reduction) and slow. In fact, Saito et al.[15] suggested that the necessary movement of the water upon reduction explains the great stability of TyrD$^{\bullet}$.

It is not a given that this "redox gate" has any physiological significance, but in its radical state, TyrD cannot compete with TyrZ in the donation of an electron to P_{680}^{+} in the functional enzyme. The long lifetime of TyrD$^{\bullet}$ is surely important for an efficient turnover in the contemporary PSII. In contrast, the transient nature of TyrZ implies that this residue mostly exists in its reduced state and the H/D exchange rates for TyrZ reported are similar to the fast rates observed here.[9] Thus, the two tyrosines seem to exchange on a similar time scale when they are reduced.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, additional notes, and instrumental settings. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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