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The role of TyrD in the electron transfer kinetics in Photosystem II

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ABSTRACT

Redox-active tyrosine (Tyr) D is indirectly involved in controlling the primary electron transfer in PSII. The presence of the oxidized TyrD renders $P680^+$ more oxidizing by localizing the charge more on P_{D1} and thus facilitates trapping of the excitation energy in PSII. We also conclude that the mechanism of the primary charge separation and stabilization is altered upon Q_A reduction.

1. Introduction

Photosystem II (PSII) functions as light-driven water-oxidizing enzyme in the thylakoid membranes of cyanobacteria and higher plants [1]. The structure of PSII was recently determined at high 3 Å resolution [2]. The X-ray structure provided the basis for a model of the water-splitting site, the Mn_4Ca cluster [3]. In the cyanobacterial PSII photons are absorbed by the chlorophyll (Chl) pigments in one of the core antennae, CP43 and CP47 (here CP stands for chlorophyll protein), and delivered to the reaction center (RC) for subsequent charge separation (CS) and transport of an electron across the membrane. The electron moves along the active cofactor branch, consisting of chlorophyll P_{D1}, accessory chlorophyll Chl_{accD1}, pheophytin $Pheo_{D1}$, and two quinone molecules, Q_A and Q_B (Fig. 1). The "inactive", quasi-symmetric branch is composed of chlorophylls P_{D2}, Chl_{accD2}, and $Pheo_{D2}$. The pseudo-C2 symmetry organization of the cofactors in the RC comprises also two peripheral chlorophylls, Chl_{ZD1} and Chl_{ZD2}, the two redox-active tyrosines, TyrZ and TyrD, and the Mn_4Ca cluster. PSII is characterized by an extraordinary high oxidative power, the highest found in nature, which is large enough to drive the water oxidation. Splitting of water is achieved in an oxidation–reduction chain that involves P_{D1}/TyrZ, TyrZ/(Mn_4Ca cluster), and finally (Mn_4Ca cluster)/H_2O redox reactions. Four such light-driven cycles are necessary to split water and release oxygen to the lumenal side of the membrane (review articles on the light-driven reactions in PSII [4], and others in the same volume).

The redox-active TyrZ is the intermediate compound in the electron transfer from the Mn_4Ca cluster to $P680^+$. Its counterpart TyrD is not directly involved in the water oxidation, even though it shares with TyrZ similar spectroscopic features. The TyrD radical is much more stable, and its actual role(s) in PSII structure is still not completely clear (for a review on tyrosine D see [5]). Due to the strong analogy between the active and inactive branches in PSII RC, it was postulated that the PSII ancestor was a homodimer enzyme, using both branches for charge separation and perhaps even oxidation of water [6].

It seems to be well established that PSII functions at physiological conditions with TyrD in its oxidized state, given that upon the onset of illumination TyrD gets oxidized by the charge equivalents accumulated in S_2/S_3 states of the manganese cluster. Concomitant with the oxidation of the Tyr molecule a proton transfer step between the Tyr radical and the neutral neighboring histidine D2-His189 occurs [7]. As a consequence, TyrD, as well as TyrZ, are neutral radicals in their oxidized states [8]. This is confirmed by FTIR experiments on the Mn-depleted PSII cores [9] which show that TyrD in the reduced state is likely to be protonated and to form a H-bond to the imidazole ring of a histidine, whereas other measurements suggested that TyrD in the oxidized form is deprotonated [10–12]. This is the general idea of a

Abbreviations: Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PSII, Photosystem II; RC, reaction center; DAS, decay-associated (emission) spectrum; Pheo, pheophytin; RP, radical pair; Tyr, tyrosine; β-DM, *n*-dodecyl-β-D-maltoside; MES, 2[*N*-morpholino]ethanesulfonic acid; SAES, species-associated (emission) spectrum

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proton rocking motion model proposed by Babcock et al. in the late 1980s [8]. Since the protein environment in the vicinity of TyrD has hydrophobic character and the Tyr site is not easily accessible to the lumen, the proton released upon TyrD oxidation stays in its proximity, interacting via the hydrogen bond with D2-His189. The TyrD radical is stable for a long time, for minutes to hours, when compared with its more "glamorous sister", TyrZ [5]. However, the recent study of Hienerwadel et al. [13] introduces doubts as to the role of the histidine residue in the hydrogen-bonding interactions with TyrD. Nevertheless, TyrD is characterized by lower redox potential than TyrZ (0.75 V *vs.* 0.95–1.1 V) and much slower electron donation to $P680^+$ [14]. However, in PSII lacking TyrZ it is able to donate the electrons to $P680^+$ as fast as TyrZ under physiological conditions [15].

At present the purpose of TyrD in the PSII reaction center is not entirely clear. The following redox and electrostatic functions of TyrD have been postulated: 1) it stabilizes higher valence states of the manganese cluster, which might be important during the photo-assembly of the manganese cluster [16]; 2) TyrD radical increases the potential energy of $P680^+$; 3) $TyrD^{\cdot}(H^+)$ affects the donor side redox activity [17], possibly via the influence on the hydrogen-bond network in the vicinity of TyrZ [18]; 4) after the first turnover it affects the distribution of the positive charge on the Chl pair $(P_{D1}P_{D2})^+$ and thus both accelerates the TyrZ oxidation (*i.e.* shifts the charge towards P_{D1}) and directs the photochemical reactions to the D1 protein side [19].

Despite being designed to study specifically the features of TyrZ, the TyrD-less mutant provides an excellent opportunity to investigate the puzzling functions of TyrD and the possible influence on the early electron transfer steps. The point mutation was introduced in the D2 protein of *Chlamydomonas* [18], *Synechocystis* [20], and finally *T. elongatus* [21]. In view of the fact that the structural data are available for the cyanobacterial system, it is very suitable to study the latter system. In the generated mutant TyrD is substituted with redox-inactive phenylalanine (for details see [21]).

In this work we have studied the influence of TyrD on the early electron transfer in the intact, oxygen-evolving cyanobacterial PSII core complexes. Fast time-resolved fluorescence experiments were designed to investigate the effect of the tyrosine radical on the $P680^+$ redox potential and the charge distribution on the $(P_{D1}P_{D2})^+$ pair, and consequently the charge separation and electron transfer kinetics in the RC and in particular the effects on the energetics of the intermediates.

The electron transfer kinetics has been studied in detail in PSII WT preparations with open and closed RCs [22–24] (for review on the energy/electron transfer processes see [4]). If the assumption of the influence of $TyrD^{\cdot}(H^+)$ on the charge distribution on $(P_{D1}P_{D2})^+$ and the redox potential of $P680^+$ is correct, then we should be able to: 1) observe differences in the electron transfer reactions (rate constants and/or lifetimes and free energy differences of intermediates) between the WT and the TyrD-less preparation; and 2) possibly validate also further our previous findings on the switch in the CS and electron transfer mechanism in PSII with reduced Q_A [24].

2. Materials and methods

2.1. Samples

Time-resolved fluorescence measurements were performed on PSII core complexes (dimeric form) prepared from *Thermosynechococcus elongatus* WT′ (attached His-tag on C-terminus of CP43, knockout psbD2) and the D2-Y160F mutant (called TyrD-less, knockout psbD2) [21]. *T. elongatus* cells were cultivated in DTN medium under continuous light (~80 μmol photons/m^2/s), and PSII core complexes were purified as described in [25]. The D2-Y160F mutation was introduced into *psbD1* after *psbD2* was deleted. Both preparations exhibited high activity, with the oxygen evolution ranging from 3600 μmol O_2/(mg Chl*h) to 4200 μmol O_2/(mg Chl*h). For the fluorescence kinetics experiment, isolated PSII complexes were diluted in 40 mM MES buffer, pH 6.5 (Serva, Heidelberg, Germany) containing 15 mM $MgCl_2$ (Roth, Karlsruhe, Germany), 15 mM $CaCl_2$ (Merck, Darmstadt, Germany), 1 M Betaine (Sigma, Taufkirchen, Germany), 0.03% β-DM (Glycon, Luckenwalde, Germany). The final concentration was adjusted to be below OD_{663} = 0.3 cm^{-1}. To keep the RCs in the open

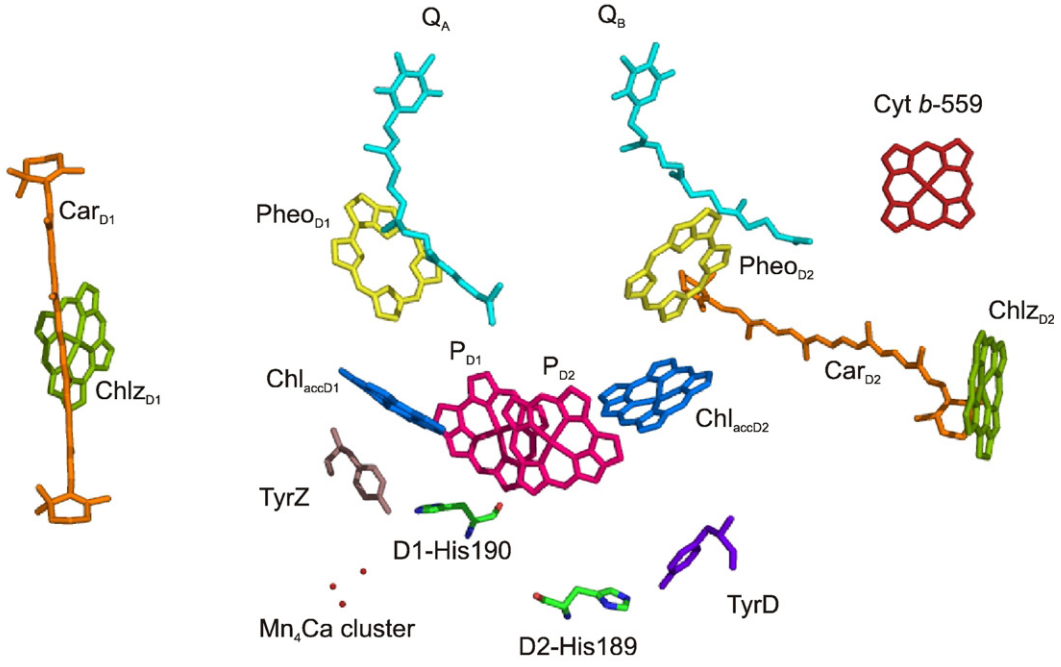

Fig. 1. PSII RC cofactors based on the *2AXT.pdb* entry [2]. The protein matrix is not shown for clear view. The cofactors form two branches in a pseudo-C2 symmetrical order: P_{D1} and P_{D2} (magenta), accessory chlorophylls Chl_{accD1} and Chl_{accD2} (blue), pheophytins $Pheo_{D1}$ and $Pheo_{D2}$ (yellow), quinones Q_A and Q_B (cyan), peripheral chlorophylls $Chlz_{D1}$ and $Chlz_{D2}$ (green), β-carotenes Car_{D1} and Car_{D2} (orange), tyrosines TyrZ (grey) and TyrD (violet), histidine residuals D1-His190 and D2-His189, Mn_4Ca cluster (red dots) and cytochrome Cyt *b*-559 (dark red).