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Hypothesis/review

Photosystem II of green plants: on the possible role of retarded protonic relaxation in water oxidation[1]

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Abstract

Photosystem II (PSII) of green plants and cyanobacteria uses energy of light to oxidize water and to produce oxygen. The available estimates of the oxidizing potential of $P_{680}{}^{+}$, the primary donor of PSII, yield value of about 1.15 V. Two main factors are suggested to add up and engender this high oxidizing potential, namely: (1) the electro*static* influence dominated by Arg-181 of the D2 subunit which elevates the oxidizing potential of $P_{680}{}^{+}$ up to 1 V, some 0.1 V above the E_m value of a hydrogen-bonded chlorophyll *a*; and (2) the *dynamic* component of 0.10–0.15 V due to the experimentally demonstrated retarded protonic relaxation at the P_{680} site. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Photosystem II; Proton transfer; Relaxation; Redox potential; Oxygen evolution; Water oxidation

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Abbreviations: ADRY, reagents, from acceleration of the deactivation reactions of the watersplitting enzyme system Y; BRC, photosynthetic reaction center of non-sulfur purple bacteria; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; OEC, oxygen-evolving complex

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[1] Dedicated to the memory of Vladimir D. Sled'.

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1. Introduction

Photosystem II of green plants and cyanobacteria is a pigment–protein complex that oxidizes water to molecular oxygen (see [1] for a review). Its core is formed by the D1 and D2 polypeptides. The amino acid sequences of both resemble those of the subunits L and M of the photosynthetic reaction centers of purple bacteria (BRC, the crystal structures are available, see [2,3]). Hence, the inner core of PSII has been modeled along BRC with D1 and D2 forming five transmembrane α-helices each [4–8]. The absorption of a light quantum by PSII induces a transmembrane charge separation between the primary donor P_{680}, a chlorophyll *a* moiety, and plastoquinone acceptors. The oxidized P_{680}^+ is reduced in nanoseconds by a unique electron donor – a redox-active tyrosine, Y_Z (D1-Tyr-161). The latter is in turn reduced in micro- to milliseconds by the oxygen evolving complex (OEC) which contains four manganese atoms. Driven by light quanta, the Y_Z-OEC system accumulates sequentially four electron vacancies cycling through states $S_0 \Rightarrow S_1 \Rightarrow S_2 \Rightarrow S_3 \Rightarrow S_4 \rightarrow S_0$ with dioxygen release associated with the spontaneous $S_4 \rightarrow S_0$ transition (see [1,9–12] for reviews).

The nature of P_{680} in PSII is enigmatic. The spectral analysis does not show an excitonically coupled chlorophyll dimer as in the BRC, but rather indicates a presence of several, excitonically weakly coupled pigments (see [13,14]; by analogy with BRC up to four chlorophylls can be involved in a such multimer structure). The positive charge of P_{680}^+ seems to be shared between several pigments at room temperature but to reside on a single pigment at lower temperatures [15,16]. Hereafter we provisionally define a cluster of chlorophyll molecules including P_{680}^+ as a *P_{680} site*.

The cited findings provide only limited help in understanding the high oxidizing potential of P_{680} which has been estimated as ∼1.15 V (reviewed in [1,11]). This is 0.7 V higher than that of P_{700}, a chlorophyll *a* dimer serving as the primary donor of photosystem I. There is a certain consensus that the high oxidizing potential of P_{680}^+ might be due to the excitonical decoupling of the involved chlorophyll *a* molecules and to their hydrogen bonding by the protein [1,11,17]. According to current estimates, the midpoint redox potential (E_m) of an excitonically uncoupled and hydrogen-bonded chlorophyll *a* is expected to be ≤0.9 V [17–19]. The difference between the latter value and the actual oxidizing power of 1.15 V remains unexplained. It is noteworthy that P_{680}^+ does not oxidize other chlorophyll *a* molecules at the P_{680} site instead of Y_Z, although the former are expected: (i) to be closer to P_{680} than Y_Z and (ii) to have lower E_m than the latter (E_m of Y_Z has been estimated as ∼1 V [20,21]). As noted in [17], the absence of such an oxidation indicates that the E_m values of *all* chlorophyll molecules at the P_{680} site are as high as those of the P_{680}^+/P_{680} redox couple (or even higher).

A concomitant consideration of two recent sets of data, namely on the proton release from the different states of OEC [22,23], and on the effect of substituting the D2-Arg-180 by uncharged amino acid residues in *Synechocystis* sp. PCC 6803 [24], may give a clue of how the midpoint potentials of up to four differently placed chlorophyll molecules can be elevated by more than 250 mV above their standard values.

2. Hypothesis

The difference between the high oxidizing potential of P_{680}^+ (∼1.15 V) and the redox potential of a hydrogen-bonded chlorophyll *a* (∼0.9 V) is suggested to be contributed by: (1) the electro*static* influence of protein charges dominated by D2-Arg-181 (0.10–0.15 V); and (2) the retarded protonic relaxation at the P_{680} site providing a *dynamic* component of 0.10–0.15 V. These two factors seem to add up more or less independently to yield the high oxidizing power of P_{680}^+. Arguments that support the hypothesis are considered in the following sections.

3. On the electrostatic asymmetry of the photosystem II core

The molecular models of PSII [5–8] show that the P_{680} site is contributed by transmembrane helices C, D and E of D1 and D2 and by the respective connecting CD loops. The only positively charged residue that could be found in this part of D1D2 is D2-

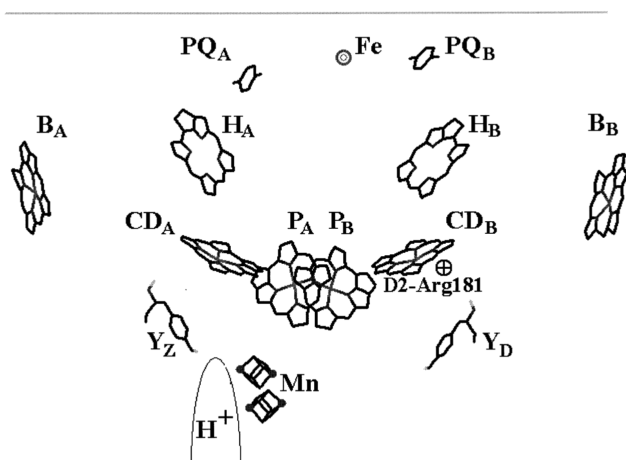

Fig. 1. A hypothetical scheme of the PSII core as inferred from available data (only the cofactors on D1 and D2 are shown). The arrangement of pigments at the P_{680} site follows the preliminary report on the crystal structure of PSII resolved to 6.5 Å [43] and the current molecular models [6–8,14]. Two of four pigments at the P_{680} site, P_A and P_B, correspond to the special pair of BRC, but are more remote from each other to account for lower excitonic coupling; two other, CD_A and CD_B, correspond to the 'voyeur' bacteriochlorophylls of BRC. The positions of Y_Z and Y_D are based on sequence homology with BRC [5,6], EPR data (reviewed in [1]) and electrometric estimates [26,37]. OEC, presented as a 'dimer of Mn-dimers' [44] is shown approximately in the same membrane plane with Y_Z, out of the line connecting Y_Z and P_A [17,22,26]. Y_Z and OEC are shown to be protonically connected with the lumen via the same water-accessible cavity (see the argumentation in [12,22,26]). See [5,8,17,45] for the discussion on the position and function of the accessory antenna chlorophylls (B_A and B_B) which do not have counterparts in the BRC.

Arg-181 (using the higher plant numeration). Several histidine residues are also present; however, their pK values, being neutral in water, tend to decrease in the hydrophobic medium. D2-Arg-181 is a part of a strictly conserved [Phe–Arg–Phe] triplet. Potential pigment-ligating amino acids flanked by two phenylalanine residues may indicate a pigment-binding site [25]. It is noteworthy that the counterpart of [Phe–Arg–Phe] on D1 is the [Phe–Asn–Phe] triplet with a neutral asparagine instead of the positively charged arginine of D2. This electrostatic asymmetry may define the nature of P_{680}.

Fig. 1 shows the working model of the PSII core (in the view of the expected crystal structure of PSII, Fig. 1 just serves the purposes of illustration; see legend for relevant references). The surplus positive potential from D2-Arg-181 will increase the E_m values of all pigments at the P_{680} site with the smallest E_m shift for the most remote P_A and CD_A. Assuming positions of D2-Arg-181 and pigments in line with available molecular models of PSII [5–8] and taking the value of the effective dielectric constant (ε_{eff}) at the P_{680} site of 8–10 [17,26], the E_m shift can be estimated to be 0.07–0.09 V for CD_A. The E_m shift would be increasingly larger for P_A, P_B and CD_B, respectively. If all chlorophyll molecules at the P_{680} site have approximately the same intrinsic E_m, the positive charge of $P_{680}{}^+$ would migrate after the charge separation towards D1 and hence closer to Y_Z staying shared between CD_A and P_A. The $P_{680}{}^+/P_{680}$ redox pair in this case would be contributed by both of them.

It is noteworthy that the substitution of the corresponding D2-Arg-180 in *Synechocystis* sp. PCC 6803 by Ile, Leu or His resulted in a 0.03–0.04 V decrease of the free energy gap between Y_Z and P_{680} (as estimated from the acceleration of the decay kinetics of variable fluorescence in the presence of DCMU [24]). Assuming purely electrostatic grounds for this decrease one can use Coulomb's equation

$$\Delta G_{P_{680},Y_Z} = \delta\phi = \frac{q}{\varepsilon_{eff}}\left(\frac{1}{r_{P_{680}}} - \frac{1}{r_{Y_Z}}\right) \quad (1)$$

where r_{Y_Z} and $r_{P_{680}}$ are the distances from D2-Arg-181 to Y_Z and P_{680} in Å, respectively. The structural models place Y_Z at about 30 Å distance from D2-Arg-181 (in the place of L-Arg-135 of *Rps. viridis* [7,8]). Then the 0.03–0.04 V decrease in the free energy gap between Y_Z and P_{680} would correspond to a center-to-center distance of 15–20 Å between D2-Arg-181 and P_{680}. This also identifies $P_{680}{}^+$ with the pigments that are most remote from the CD helix of D2, i.e. with CD_A and P_A in Fig. 1. Assuming CD_A and P_A to be hydrogen bonded chlorophyll *a* molecules with E_m of about 0.9 V (the bonding might be deduced from the red-shifted absorption spectra of P_{680} components [15,16]), and adding the electrostatic contribution from D2-Arg-181, an estimate of 1.00–1.05 V is obtained for the E_m values of CD_A and P_A. This estimate would correspond to the equilibrium E_m of the $P_{680}{}^+/P_{680}$ redox pair (which is too high to be determined from a potentiometric redox titration). At cryogenic temperatures, the positive charge is expected to localize on a single pigment

with the lowest midpoint potential. Whether this is P_A or CD_A is to be established. This behavior can be well accommodated with the data on temperature-dependent changes of the absorption difference spectra, which indicate that the positive charge of $P_{680}{}^+$ seems to be shared between several pigments at room temperature, but is localized on a single pigment at cryogenic temperatures causing a strong electrochromic shift of the spectrum of neighboring pigment(s) [15,16].

4. On the possible role of retarded protonic relaxation in water oxidation

The oxidizing potential of P_{680} has been estimated as $\sim$1.15 V (see e.g. [21,27]) by combining the experimentally determined kinetic equilibrium constants for the $P_{680}{}^+Y_ZOEC \leftrightarrow P_{680}Y_Z{}^+OEC$ reaction with the midpoint redox potential of Y_Z. Based on kinetic data, such an approach can give only the *operating* potential (hereafter denoted as $E_m{}^{\#}$) of the $P_{680}{}^+/P_{680}$ redox pair but not the *equilibrium* one (E_m). The values of E_m and $E_m{}^{\#}$ may differ: until the surrounding medium has not been completely re-organized to accommodate the surplus positive charge of $P_{680}{}^+$, the $E_m{}^{\#}$ of the latter remains higher that its E_m. Medium relaxation in the case of proteins is stretched in time up to micro- and milliseconds [28–31]. In the time domain of microseconds, the reorganization of the medium in response to a positive charge may cause a deprotonation of some surface protonogenic groups. In the BRC, for example, the oxidation of the primary donor P^+ causes proton release due to the pK shifts of some acid groups which are located close to the BRC/water surface [32]. In chromatophores, this proton release occurs in hundreds of microseconds [33,34] in a rough accordance with an estimate for the dissociation constant k_d of a water-accessible acids on the protein surface [35]:

$$k_d = 2*10^{(10-pK)}\ s^{-1} \quad (2)$$

where pK corresponds approximately to the actual pH.

Contrary to the situation in the BRC, the formation of $P_{680}{}^+$ in PSII is not accompanied by any measurable proton release for, at least, 10 ms. This has been demonstrated: (1) at pH < 5.0, where $P_{680}{}^+$ stays oxidized in a large fraction of PSII after a light flash because of a low equilibrium constant for the electron transfer between Y_Z and $P_{680}{}^+$ [22]; and (2) at neutral pH when the $Y_z{}^+P_{680}{}^+$ state has been generated by illumination of PSII in the $Y_z{}^+P_{680}$ state by a another, closely spaced flash [23].

Thus, the relaxation mode which is contributed by proton release in BRC is severely slowed down at the P_{680} site of PSII. Under the oxygen-evolving conditions $P_{680}{}^+$ is reduced on a time scale of nanoseconds by Y_Z; the residual fraction of $P_{680}{}^+$ can be estimated as $\leq 10^{-3}$ depending on the S-state [21]. As the protonic relaxation at the $P_{680}{}^+$ site occurs only in this residual fraction, the rate of such a relaxation is expected to slow down proportionally. Hence, the life time of the unrelaxed high-potential state of $P_{680}{}^+$ in the oxygen evolving preparations can be estimated as > 10 s. This is comparable with the life time(s) of the S-states in the OEC.

In a sharp contrast with the P_{680} site, the arrival of electron vacancies (coming from P_{680}) at the Y_Z-OEC site causes proton release on a time scale of microseconds (presumably, due to a deprotonation of protonogenic groups facing a water-containing cavity intruding from the lumen, see [12,22,23, 34,36] and Fig. 1). The pH-dependence of the extent of proton release indicates that several groups undergo pK shifts of 1.5–2 pH units [12,34]. This gives an estimate of 0.10–0.15 mV for the energy of protonic relaxation at the Y_Z-OEC site. As P_{680} is embedded just somewhat deeper than Y_Z-OEC relative to the membrane/water interface [26,37], this value may be used as an energy estimate of the potentially possible, but not observable (or dramatically retarded) protonic relaxation at the P_{680} site.

Hence, under oxygen-evolving conditions, the oxidizing *operating* potential of P_{680} seems to remain 0.10–0.15 mV higher than its equilibrium E_m value until the protonic relaxation at the P_{680} site occurs, i.e. at least, for tens of seconds. The subtraction of this contribution from the experimentally estimated oxidizing potential of 1.15 V yields a rather moderate value of 1.00–1.05 V for E_m of P_{680} (cf. above).

The absence of proton release in response to $P_{680}{}^+$ formation indicates: (1) that the solvation penalty is too high for deprotonation of the groups in the nearest vicinity of P_{680}; and (2) that the protonable

groups on the protein/water interface are too far away from P_{680}^{+} to respond. Both effects could be attributed to the capping of the donor side of D1D2 by extrinsic proteins and by lumenal loops of CP43 and CP47 polypeptides and of chlorophyll *a*/*b*-binding proteins (reviewed in [38]). Such a capping not only protects the higher S-states in the OEC from the external reductants, but also increases the span of a low-dielectric medium between P_{680} and the water boundary. Conversely, the damage to this proteineous shield may facilitate the ionic relaxation at the P_{680} site and decrease the $E_m^{\#}$ of P_{680}. This effect may, at least partly, account for: (1) the loss of the oxygen-evolving capacity by PSII upon depletion of the extrinsic polypeptides [38]; (2) the small, varying from preparation to preparation, extent of Y_Z oxidation by P_{680}^{+} in the D1D2/cytochrome b_{559} preparations (see [39,40]; these almost completely stripped preparations are the closest analogs of BRC); and (3) the inhibition of oxygen evolution by ADRY reagents [41,42]. The latter compounds, all being protonophores, may be able to substitute for the retarded ionic component of the medium relaxation at the P_{680} site and to decrease the $E_m^{\#}$ value of P_{680}.

The suggested protonic insulation by CP43, CP47, extrinsic and CAB proteins applies to *all* the chlorophyll molecules at the P_{680} site. Correspondingly, the $E_m^{\#}$ values of *all* of them would be elevated to the same extent due to the retarded proton release.

In conclusion, the high oxidizing potential of P_{680}^{+} of $\sim$1.15 V is suggested to be contributed by the electro*static* influence of protein charges dominated by the positively charged D2-Arg-181 ($\sim$0.10–0.15 V) and by a *dynamic* component ($\geq$0.10–0.15 V) due to the retarded protonic relaxation at the P_{680} site. The transient and fragile nature of the latter component may account, at least to a some extent, for the functional lability of PSII compared to the BRC. The contribution from other factors discussed in [17,22,26], particularly, from: (1) Ca^{2+} which is present in OEC; and (2) a positive charge which is transiently stored in the Mn cluster after the $S_1 \rightarrow S_2$ transition cannot be excluded; however the two factors considered above in detail are already sufficient to quantitatively account for the high oxidizing potential of P_{680}.

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