Rapid Reports

Effect of Charge Distribution over a Chlorophyll Dimer on the Redox Potential of P680 in Photosystem II As Studied by Density Functional Theory Calculations†

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ABSTRACT: The effect of charge distribution over a chlorophyll dimer on the redox potential of P680 in photosystem II was studied by density functional theory calculations using the P680 coordinates in the X-ray structure. From the calculated ionization potentials of the dimer and the monomeric constituents, the decrease in the redox potential by charge delocalization over the dimer was estimated to be ~140 mV. Such charge delocalization was previously observed in the isolated D1−D2−Cyt b_{559} complexes, whereas the charge was primarily localized on P_{D1} in the core complexes. The calculated potential decrease of ~140 mV can explain the inhibition of Y_Z oxidation in the former complexes and in turn implies that the charge localization on P_{D1} upon formation of the core complex increases the P680 potential to the level necessary for water oxidation.

Photosystem II (PSII)[1] is a multisubunit protein complex embedded in thylakoid membranes of plants and cyanobacteria. It has a unique and important function of light-driven water oxidation that results in the release of molecular oxygen to the atmosphere. Light illumination induces the primary charge separation between the Chl electron donor P680 and the Pheo electron acceptor to form the $P680^+Pheo^-$ radical pair (*1*). The electron is then transferred to the quinone electron acceptors, Q_A and Q_B, while $P680^+$ oxidizes the Mn cluster, the catalytic site of water oxidation, via the redox-active tyrosine Y_Z (D1-Tyr161). Recently determined X-ray crystallographic structures of cyanobacterial PSII core complexes (*2, 3*) showed that P680 has a dimeric structure with two Chl molecules ligated by D1-His198 and D2-His197.

Accomplishment of water oxidation in PSII requires not only the Mn cluster as a catalytic site but also the extremely high oxidation power of $P680^+$, because the redox potential (E_{ox}) of the water oxidation reaction is quite high (E_{ox} = 880 mV at pH 6.0). In fact, the E_{ox} of P680 has been estimated to be as high as 1100−1300 mV (*4–7*). This value is much higher than the E_{ox} of P870 [~500 mV (*8*)] in bacterial reaction centers, from which PSII is thought to have developed 2.5−2.6 billion yeas ago. The mechanism for realizing such a high E_{ox} of P680 has been extensively argued (*1*), and several factors such as a higher E_{ox} of Chl *a* than BChl *a* (*9*), a low-dielectric environment at the binding pocket (*10*), electrostatic interactions with other cofactors and proteins (*11, 12*), and localization of a positive charge on one Chl (*13*) have been proposed.

As for the latter factor of charge localization, Diner et al. (*14*) reported from site-directed mutagenesis studies that the charge on $P680^+$ is primarily localized on P_{D1} in the cyanobacterial PSII core complexes. Recently, Okubo et al. (*15*) also showed using FTIR difference spectroscopy that the positive charge is mostly localized on one Chl in the PSII core and membrane preparations. However, they observed that the electronic structure of $P680^+$ is largely perturbed in isolated RC complexes (D1−D2−Cyt b_{559} complexes), and the charge is significantly delocalized over the dimer. From this observation, it was suggested that inhibition of oxidation of Y_Z in the RC complex may be caused by the decrease in the E_{ox} of P680 induced by charge delocalization (*15*).

Although it has been generally predicted that charge localization increases the E_{ox} of P680 (*13, 15*), the extent of this effect has not been estimated quantitatively. In this study, we have investigated the effect of charge distribution over the Chl dimer on the E_{ox} of P680 using density functional theory (DFT) calculations. The results provided insight into the mechanism by which P680 gains the extremely high E_{ox} necessary for water oxidation.

The atomic coordinates of P680 except for hydrogen atoms were taken from the X-ray crystallographic structure at 3.0 Å resolution of the PSII core complexes from *Thermosynechococcus elongatus* by Loll et al. (PDB entry 2axt) (*3*), and the 17-propionic ester groups were replaced with ethyl groups. The coordinates of hydrogen atoms were determined by geometry optimization at the B3LYP/6-31G(d) level (for a detailed computational method, see the Supporting Information). The thus obtained P680 structure is shown in Figure 1. Using this geometry, the electronic energies of the Chl

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[1] Abbreviations: BChl, bacteriochlorophyll *a*; Bphe, bacteriopheophytin *a*; Chl, chlorophyll *a*; DFT, density functional theory; E_{ox}, redox potential of a one-electron oxidation reaction; FTIR, Fourier transform infrared; IP, ionization potential; P680, special pair chlorophyll of photosystem II; Pheo, pheophytin *a*; PSII, photosystem II; RC, D1−D2−Cyt b_{559} complex; Y_Z, redox-active tyrosine on the D1 protein.

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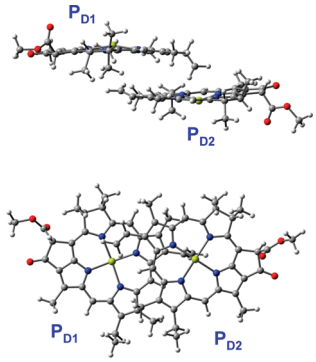

FIGURE 1: Structure of P680 with top and side views used for energy calculations. Atom coordinates except for hydrogen were adopted from the X-ray crystallographic structure of the PSII core complexes from *T. elongatus* by Loll et al. (PDB entry 2axt), and the 17-propionic ester groups were replaced with ethyl groups. The coordinates of hydrogen atoms were determined by geometry optimization at the B3LYP/6-31G(d) level.

Table 1: Calculated Electronic Energies and Ionization Potentials (electronvolts) of a Chl Dimer and Monomeric Constituents, P_{D1} and P_{D2}, of P680

	energy (eV)			
	neutral	cation	ΔIP (eV)	ΔIP_{m-d}[a]
dimer	−104604.691	−104599.162	+5.529	—
P_{D1}	−52302.529	−52296.826	+5.703	+0.174
P_{D2}	−52302.873	−52297.235	+5.638	+0.109

[a] IP(monomer) − IP(dimer).

dimer and its monomeric constituents, P_{D1} and P_{D2}, in the neutral and cationic states were calculated at the B3LYP/6-311+G(d) level at $\epsilon = 2.2$.

The charge distribution estimated by natural population analysis showed that the positive charge on $P680^+$ is significantly delocalized over the two Chl molecules with a slightly biased distribution on P_{D2} (0.46:0.54 P_{D1}:P_{D2}). The recent DFT calculations for bacterial $P870^+$ (*16*) using the geometry of the X-ray structure of the *Rhodobacter sphaeroides* reaction center (*17*) showed that the charge is more distributed to P_L due to different orientations of the 13^2-methyl ester groups between P_L and P_M. The X-ray structure of P680 did not exhibit such a large difference in the orientation of the methyl ester groups (Figure 1).

The significant charge delocalization on $P680^+$ estimated above for the P680 X-ray structure of the *T. elongatus* core complexes is in contrast to the experimental observation that the charge is mostly localized on one Chl, probably P_{D1} in the core and membrane preparations (*14, 15*), including the core complexes from the same bacterium (*15*). However, the calculation result is in agreement with the observation for the isolated RC complexes showing significant charge delocalization over the dimer (*15*). This indicates that the charge localization on P_{D1} in the core complex does not originate from the geometry of the Chl dimer itself, but rather from the asymmetric protein environment induced by subunits in the core complex other than the D1 and D2 proteins. It is noted that Diner et al. (*14*) showed that the $P680^+$−P680 spectra in the visible region were invariant on the time scale of 10 ns to 1 μs and hence suggested that the cation location was fully equilibrated in less than 10 ns. Thus, the charge localization on P_{D1} estimated at 1 μs (*14*) or by accumulation under continuous illumination (*15*) in the core or membrane preparations is not ascribed to unrelaxed charge distribution.

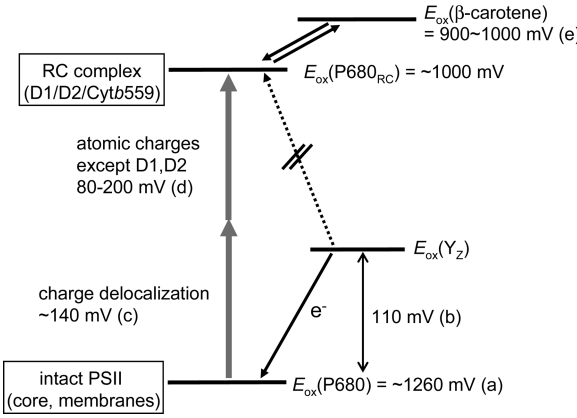

FIGURE 2: Relationship of the redox potentials of P680 in intact PSII and the RC complexes with those of Y_Z and β-carotene. The data are adopted from the experimental or theoretical estimations by (a) Rappaport et al. (*5*), (b) Metz et al. (*20*), (c) this work, (d) Ishikita et al. (*11, 12*), and (e) Ishikita et al. (*23*).

The ionization potentials (IPs) of the Chl dimer and the monomeric constituents, P_{D1} and P_{D2}, were estimated from the calculated electronic energies in the neutral and cationic states (Table 1). The IPs of P_{D1} and P_{D2} were higher than the IP of the dimer by 174 and 109 meV, respectively. It is noted that the similar values of 149 and 121 meV for P_{D1} and P_{D2}, respectively, were obtained when the X-ray structure at 3.5 Å resolution by Ferreira et al. (*2*) was used as a P680 structure, although the Mg−Mg distance was slightly longer (8.2 Å) in this structure than that of the structure by Loll et al. (8.0 Å) (*3*). This suggests that the slight change in the Chl−Chl distance does not much affect the IP of the dimer. From these results, the IP difference of 140 meV between the dimer and the monomer was estimated as an average of the IP differences obtained from the two X-ray structures. This IP difference represents the difference in the E_{ox} of P680 between the state in which the charge is delocalized over the dimer and the state in which it is localized on either Chl molecule. Thus, the E_{ox} of P680 is expected to increase by ~140 mV upon charge localization on one Chl molecule.

Charge localization on one Chl in a dimer was previously realized in the BChl−Bpheo heterodimer of P870 in the HL(M202) and HL(L173) mutants of *Rb. sphaeroides* (*18, 19*). The E_{ox} of P870 (~500 mV) of wild-type P870 was increased by 130−180 mV with these mutations (*18, 19*). The good agreement of this experimental values for bacterial P870 with the calculated values for P680 (~140 mV) indicates that the latter theoretical value is an appropriate estimation as the effect of charge distribution over the Chl dimer on its E_{ox}.

The value of ~140 mV as an E_{ox} decrease of P680 due to charge delocalization supports the previous proposal by Okubo et al. (*15*) that the E_{ox} of P680 is lowered to the level of the E_{ox} of Y_Z upon isolation of the RC complexes and that this is a main cause for the inhibition of Y_Z oxidation in the latter preparation; the E_{ox} of Y_Z has been estimated to be lower by ~110 mV (*20*) than that of P680 in intact PSII [E_{ox} ~ 1260 mV according to the recent estimation by Rappaport et al. (*5*)]. In addition, Ishikita et al. proposed by theoretical calculations that the atomic charges and dielectric volumes of the protein subunits except for D1 and D2 contribute to the increase in the E_{ox} values of P_{D1} and P_{D2} by 80−90 mV (*11*) using the 3.2 Å resolution X-ray structure (*21*) and by 170−200 mV (*12*) using the 3.0 Å structure (*3*). Thus, the E_{ox} of P680 in the RC

complexes is lowered to ~1000 mV by the additive effects of charge delocalization and the loss of atomic charges of the stripped subunits (Figure 2). This level of the E_{ox} is consistent with the fact that at cryogenic temperatures $P680^+$ in the RC complex is stabilized in equilibrium with the radical cation of β-carotene (*22*), the E_{ox} of which has been estimated to be 900−1000 mV (*23*) (Figure 2). Also, this E_{ox} of P680 is sufficiently low to explain the complete loss of Y_Z oxidation in the RC complexes.

These results in turn imply that significant charge localization on P_{D1} in $P680^+$ in the core complex increases the E_{ox} of P680 to the level that enables water oxidation. The mechanism for inducing such asymmetric charge distribution upon core complex formation is unknown at present. One of the possible causes for this asymmetry is atomic charges of the CP43, CP47, and small subunits around D1 and D2 proteins. The theoretical estimation by Ishikita et al. (*12*) in fact showed that the atomic charges of the protein subunits except for D1 and D2 induced an upshift in the E_{ox} of P_{D2} larger by 30 mV than that of P_{D1} (203 and 174 mV upshifts for P_{D1} and P_{D2}, respectively) when using the 3.0 Å resolution X-ray structure, although their earlier study using the 3.2 Å structure showed comparable upshifts (85 and 82 mV upshifts for P_{D1} and P_{D2}, respectively) (*11*). It is also possible that the interaction of a specific subunit with the D1 or D2 protein contributes to the asymmetric charge distribution. In this respect, the previous observation by Hoshida et al. (*24*) that the binding of the PsbL protein is required to restore the ability of Y_Z oxidation in the D1−D2−CP47−Cyt b_{559}−PsbI−PsbW complex is intriguing. The orientations of the His ligands (D1-His198 and D2-His197) could be changed by the interactions with subunits other than D1 and D2 to affect the charge distribution. Indeed, Diner et al. (*14*) showed that site-directed mutations at D1-H198 in *Synechocystis* PCC6803 shifted the redox potential of P680, although such changes were not observed in the similar mutants of *T. elongatus* in the recent study by Sugiura et al. (*25*).

SUPPORTING INFORMATION AVAILABLE

Details of the computational method. This material is available free of charge via the Internet at http://pubs.acs.org.

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