Perturbation of the Structure of P680 and the Charge Distribution on Its Radical Cation in Isolated Reaction Center Complexes of Photosystem II as Revealed by Fourier Transform Infrared Spectroscopy†

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Received January 25, 2007; Revised Manuscript Received February 23, 2007

ABSTRACT: The structure and the electronic properties of P680 and its radical cation in photosystem II (PSII) were studied by means of Fourier transform infrared spectroscopy (FTIR). Light-induced $P680^+$/P680 FTIR difference spectra in the mid- and near-IR regions were measured using PSII membranes from spinach, core complexes from *Thermosynechococcus elongatus*, and reaction center (RC) complexes (D1-D2-Cyt*b*559) from spinach. The spectral features of the former two preparations were very similar, indicating that the structures of P680 and its radical cation are virtually identical between membranes and cores and between plants and cyanobacteria. In sharp contrast, the spectrum of the RC complexes exhibited significantly different features. A positive doublet at ∼1724 and ∼1710 cm^{-1} due to the 13^1-keto C═O stretches of $P680^+$ in the membrane and core preparations were changed to a prominent single peak at 1712 cm^{-1} in the RC complexes. This observation was interpreted to indicate that a positive charge on $P680^+$ was extensively delocalized over the chlorophyll dimer in RC, whereas it was mostly localized on one chlorophyll molecule (70−80%) in intact P680. The significant change in the electronic structure of $P680^+$ in RC was supported by a dramatic change in the characteristics of a broad intervalence band in the near-IR region and relatively large shifts of chlorin ring bands. It is proposed that the extensive charge delocalization in $P680^+$ mainly causes the decrease in the redox potential of $P680^+$/P680 in isolated RC complexes. This potential decrease explains the well-known phenomenon that Y_Z is not oxidized by $P680^+$ in RC complexes.

Photosystem II (PSII)[1] is a multisubunit protein complex involved in thylakoid membranes of plants and cyanobacteria. It has a unique and important function of light-induced water oxidation that results in the release of molecular oxygen to the atmosphere. All the redox components in the major electron-transfer pathway are bound to the D1 and D2 proteins (*1*), which makes a core complex together with CP43 and CP47 that harbor core antenna pigments (*2*) and about 20 smaller proteins (*3*). In plants, the core complex is further surrounded by light-harvesting complexes such as LHCII (*4*). Light absorption triggers the formation of the $P680^+Pheo^-$ charge separated state (*5*). The electron is then transferred to plastoquinone molecules, Q_A and Q_B, while $P680^+$ oxidizes the tyrosine residue Y_Z (D1-Tyr161) and then the Mn cluster, which is the catalytic site of water oxidation. Recent X-ray crystallographic structures of PSII core complexes from thermophilic cyanobacteria *Thermosynechococcus elongatus* (*6*, *7*) and *Thermosynechococcus vulcanus* (*8*) exhibited that P680 has a dimeric structure of two Chl molecules that are ligated by D1-His198 and D2-His197.

To oxidize water, $P680^+$ has an extremely high redox potential of about +1.2 V (*9*, *10*). Since primary donors of other types of photosynthetic RC proteins such as P870 of purple bacteria and P700 of photosystem I have much lower potentials of ∼+0.5 V (*11*, *12*), and isolated Chl *a* in organic solvents also showed lower potentials of +0.7−0.9 V (*13*), the mechanism to have such a high redox potential of P680 has been a subject of extensive research (*5*, *14*). Several factors for the high potential have been argued, such as electrostatic interactions with other cofactors and proteins (*15*), a low dielectric environment at the binding pocket (*16*), localization of a positive charge on one Chl (*17*), and a higher oxidation potential of Chl *a* in comparison with Bchl *a* (*18*). How these factors were modified during the evolution of PSII from bacterial RC to gain the high potential of P680 is

† This study was supported by Grants-in-Aid for Scientific Research (17GS0314 and 18570145) from the Ministry of Education, Science, Sports, Culture and Technology, and by Special Research Project "NanoScience" at the University of Tsukuba.

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[1] Abbreviations: Chl, chlorophyll; Chl_Z, the redox-active accessory chlorophyll in photosystem II; Cyt*b*559, cytochrome *b*559; DM, *n*-dodecyl-*β*-D-maltoside; FTIR, Fourier transform infrared; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; IR, infrared; Mes, 2-(*N*-morpholino)ethanesulfonic acid; PSII, photosystem II; P680, the special pair chlorophyll of photosystem II; RC, reaction center; SiMo, silicomolybdate; Y_Z, redox-active tyrosine on the D1 protein.

10.1021/bi700157n CCC: $37.00
Published on Web 03/20/2007

an important problem, because this evolution, which took place ~2.5 billion years ago (*19*, *20*), was a turning point for generation of aerobic atmosphere and subsequent prosperity of life on earth.

The isolated RC complex of PSII, which consists of the D1, D2, Cyt*b*559, and psbI proteins, is a minimum unit that retains the primary charge separation and corresponds to the RC complex of purple bacteria comprising the L, M, and H subunits (*1*, *21*). The D1 and D2 proteins have sequence homologies to the L and M proteins, respectively, suggestive of their evolutionary relationships. An intriguing question is whether only the sequences of the D1 and D2 modified from the L and M determine the P680 property or other proteins forming a core complex are required to express the P680 function. To answer this question, the structure and the property of P680 should be carefully compared between the RC complexes and more intact core or membrane preparations.

In this study, we have studied the electronic and vibrational structures of P680 and its radical cation in different PSII preparations using Fourier transform infrared spectroscopy (FTIR). Light-induced $P680^+$/P680 FTIR difference spectra in the mid- and near-IR regions were compared among the PSII RC complexes, core complexes, and membranes from either spinach or *T. elongatus*. We found that the structure of P680 and the charge distribution on $P680^+$ were significantly altered in the RC complexes in comparison with the core and membrane preparations. The effect of such changes on the redox potential of P680 is discussed in relevance to the fact that Y_Z is not oxidizable by $P680^+$ in RC.

MATERIALS AND METHODS

PSII membranes were prepared from spinach following the method reported previously (*22*). Q_A as well as the Mn cluster was depleted by treatment with 100 mM sodium dithionite and 30 μM benzyl viologen (*23*) followed by dark incubation for 18 h at 4 °C. PSII core complexes of *T. elongatus*, in which the carboxyl terminus of the CP43 subunit was genetically His-tagged, were purified as previously described (*24*). Mn depletion from the core complexes was performed by NH_2OH treatment (10 mM) for 1 h at room temperature (*24*). PSII RC complexes (D1-D2-Cyt*b*559) were isolated from spinach as described in ref *25*, followed by replacement of the original detergent (Triton X-100) with 0.2% DM.

Samples for FTIR measurements were prepared as follows. The Q_A-depleted PSII membranes from spinach were suspended in a pH 6.5 Mes buffer (40 mM Mes, 400 mM sucrose, and 20 mM NaCl) in the presence of 100 mM potassium ferricyanide and 0.9 mM SiMo and centrifuged for 30 min at 150000*g*. The resultant pellet was sandwiched between a pair of BaF_2 plates (13 mm in diameter). An aliquot (8 μL) of a suspension of the Mn-depleted PSII core complexes of *T. elongatus* (~4.5 mg Chl/mL) in a pH 6.0 Mes buffer (10 mM Mes, 5 mM NaCl, and 0.06% DM) was mixed with 1 μL of 500 mM potassium ferricyanide and 1 μL of 10 mM SiMo, and loaded on a BaF_2 plate. The sample was then lightly dried under N_2 gas flow and covered with another BaF_2 plate with 0.7 μL of water. The RC complexes of spinach (1.4 mg of Chl/mL; 10 μL) in a pH 7.5 Hepes buffer (10 mM Hepes, and 2 mM DM) mixed with 1 μL of 100 mM potassium ferricyanide and 0.2 μL of 6 mM SiMo were loaded on a BaF_2 plate, dried, and covered with another BaF_2 plate with 0.7 μL of water in a similar manner. The sample temperature was adjusted to 265 K for membrane and core samples and 230 K for RC complexes in a liquid-N_2 cryostat (Oxford, model DN1704) using a temperature controller (Oxford, model ITC-5).

$P680^+$/P680 FTIR difference spectra were recorded on a Bruker IFS-66/S spectrophotometer equipped with an MCT detector (D313-L) (*26*). For near-IR measurements, a KBr beam splitter and a globar light source for mid-IR measurements were replaced with a CaF_2 beam splitter (T401/6) and a tungsten light source (Q403/6), respectively. In addition, Ge filters (OCLI or Topcon) to transmit the 3800−600 cm^{-1} region placed at the front and back of the sample in the IR beam path were replaced with those to transmit the 9000−1000 cm^{-1} region in near-IR measurements. Scans under dark (1 s) and during illumination (1 s) were repeated 5000 times for the membrane and RC samples and 1000 times for the core sample, and averaged single-beam spectra were used to calculate light-minus-dark difference spectra. Light illumination was performed with continuous red light (~16 mW/cm^2 at the sample point) from a halogen lamp (Hoya-Schott HL150) equipped with a red cutoff filter (>600 nm) for mid-IR measurements and a continuous beam at 661 nm (~5.4 mW/cm^2 at the sample point) from a diode laser (L4660S-90-TE, Micro Laser Systems, CA) for near-IR measurements. The spectral resolution was 4 cm^{-1}.

RESULTS

Figure 1 shows the light-induced $P680^+$/P680 FTIR difference spectra in the mid-IR region (1800−1100 cm^{-1}) of PSII membranes of spinach (a) and PSII core complexes of *T. elongatus* (b) measured at 265 K, and PSII RC (D1-D1-Cyt*b*559) complexes of spinach (c) at 230 K. These temperatures were chosen to maximize the P680 signals with the least contamination of signals from other redox cofactors. It is noted that the spectra of the three samples measured at 250 K were very similar to those in Figure 1. The mixture of ferricyanide and SiMo was used as electron acceptors. The membrane preparation was depleted of Q_A to remove Q_A^-/Q_A signals, which contaminated the spectrum when Q_A-intact PSII membranes were used (data not shown). However, modification of P680 signals by Q_A depletion was not observed. Such Q_A depletion was not necessary for the core sample, probably because electron abstraction from Pheo or Q_A by the exogenous electron acceptors (SiMo and ferricyanide) was fast enough in the core complexes. The spectrum of the RC complex of spinach was very similar to the previous spectra obtained using SiMo by Breton et al. (*27*) or ferricyanide by our group (*28*). In the latter study, we needed to bleach carotenoid beforehand because, with only ferricyanide, the relaxation of $P680^+$ was relatively slow and it was in equilibrium with a carotenoid radical cation. However, addition of SiMo accelerated the $P680^+$ relaxation (<2 s at 230 K) and thus repetitive measurements of a light (1 s)−dark (1 s) cycle provided a $P680^+$/P680 spectrum without carotenoid signals, which are typically seen as a strong positive band at ~1440 cm^{-1} (*28*).

The $P680^+$/P680 spectra in the mid-IR region contain the chlorin ring vibrations (<1620 cm^{-1}) and the 13^1-keto C=

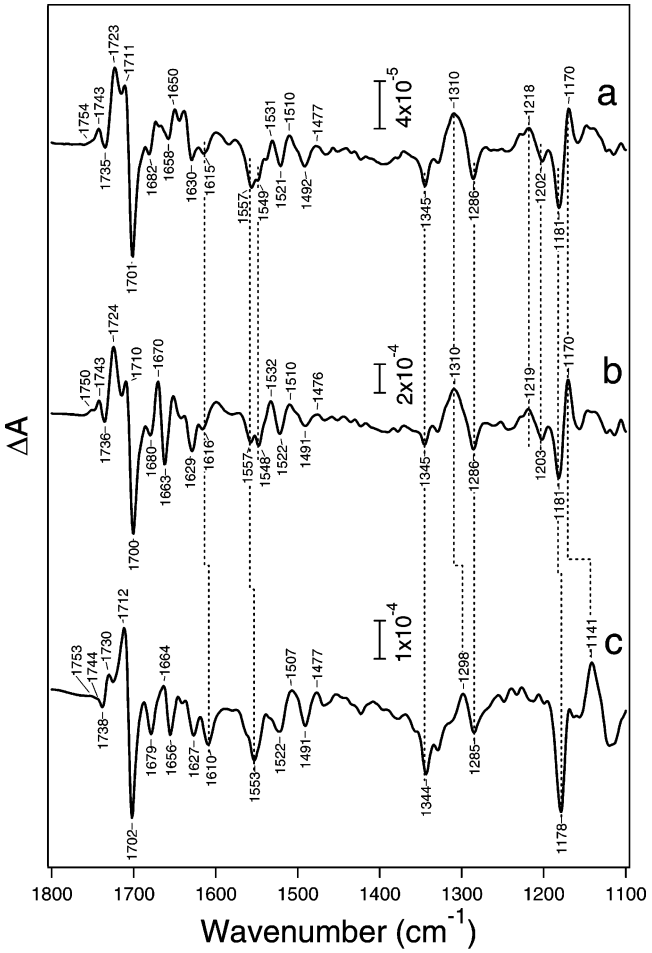

FIGURE 1: Light-induced $P680^+$/P680 FTIR difference spectra in the mid-IR region of (a) PSII membranes of spinach, (b) PSII core complexes of *T. elongatus*, and (c) PSII RC (D1-D2-Cyt*b*559) complexes of spinach.

O (1730−1690 cm^{-1}) and 13^2-ester C═O (1760−1730 cm^{-1}) stretching vibrations of Chl (*29*−*33*). Bands at 1690 −1620 cm^{-1} mostly arise from the amide I vibrations of polypeptide backbones (*34*), which can differ among three preparations due to different sequences and protein compositions.

Because the chlorin ring modes result from complex couplings of the CC and CN stretching and the CH bending vibrations, they can be used as "fingerprints" to detect changes in structures and interactions of a Chl molecule in proteins (*29*−*33*). The observed chlorin ring bands in 1620−1100 cm^{-1} were virtually identical between the PSII membranes of spinach and the core complexes of *T. elongatus* (Figure 1a,b). In contrast, band features in the RC complexes (Figure 1c) were significantly different. Positive peaks at 1310 and 1170 cm^{-1} due to $P680^+$ in the membrane and core preparations downshifted by 12 and 29 cm^{-1}, respectively, in RC to provide peaks at 1298 and 1141 cm^{-1}, and the peak at ∼1219 cm^{-1} in the former two preparations lost the intensity in RC. As for the negative peaks that belong to neutral P680, those at ∼1615, 1557, 1345, 1286, and 1181 cm^{-1} in the membranes and cores slightly (by 1−6 cm^{-1}) downshifted to 1610, 1553, 1344, 1285, and 1178 cm^{-1}, respectively, in RC. In addition, the 1344 and 1178 cm^{-1} peaks in RC exhibited much stronger intensities than the corresponding peaks in the membranes and cores, while the peaks at ∼1549 and ∼1202 cm^{-1} diminished in RC.

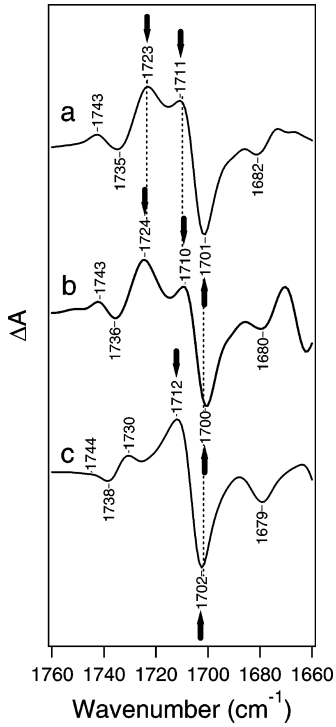

FIGURE 2: An expanded view of the 13^1-keto C═O stretching region of the $P680^+$/P680 FTIR spectra of (a) PSII membranes of spinach, (b) PSII core complexes of *T. elongatus*, and (c) PSII RC complexes of spinach. Major 13^1-keto C═O peaks are indicated by arrows.

An expanded view of the 13^1-keto C═O region is presented in Figure 2. All three samples showed similar prominent negative peaks at 1702−1700 cm^{-1}. These frequencies indicate that the 13^1-keto C═O groups of neutral P680 are free from a hydrogen bond (*35*−*37*) being consistent with the X-ray structures of PSII core complexes (*6*−*8*). Most probably, both of the 13^1-keto C═O groups of the two Chl molecules have similar frequencies at ∼1700 cm^{-1}, because no other negative peak with a comparable intensity was found in the free 13^1-keto C═O region [1710−1680 cm^{-1} (*35*−*37*)]. A minor peak at 1682 cm^{-1} in membrane and core preparations (Figure 2a,b) cannot arise from P680, because the weak intensity should originate from a small frequency shift, which always gives a differential signal, but a corresponding positive peak at a higher frequency (because of cation formation; see below) was not found in the spectra. Also, this peak position represents a highly polar environment or a weak hydrogen bond interaction (*35*−*37*), which is inconsistent with the protein environment detected by X-ray crystallography. The similar peak at 1679 cm^{-1} in RC showed a slightly larger intensity, and was more prominent in previous $P680^+$/P680 spectra of RC complexes (*27*, *28*, *38*); however, it may arise from an amide I band with some contribution from a Chl_Z signal. The similar 13^1-keto C═O frequencies of neutral P680 in the three PSII preparations indicate that the protein environments around the 13^1-keto C═O groups of P680 are virtually unchanged by isolation of core and RC complexes.

Upon oxidation of a Chl molecule, the 13^1-keto C═O frequency upshifts and a positive band appears on the higher-frequency side of the neutral Chl band in a cation-minus-neutral difference spectrum (*39*). In the membranes and core samples, a similar doublet signal due to $P680^+$ was observed

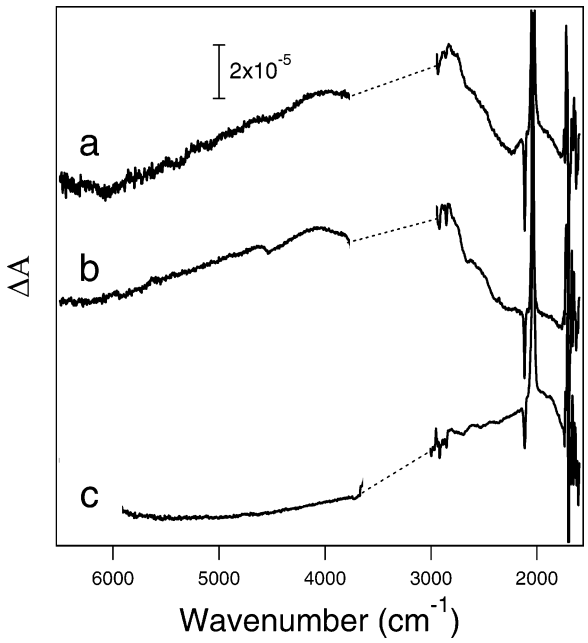

FIGURE 3: P680$^+$/P680 difference spectra in the near-IR region of (a) PSII membranes of spinach, (b) PSII core complexes of *T. elongatus*, and (c) PSII RC complexes of spinach. Spectra were normalized at the intensities of the 13^1-keto C═O signals. Peaks at ∼2116/2038 cm^{-1} are due to ferricyanide/ferrocyanide. The region in 3700−3000 cm^{-1} was saturated with strong water OH absorption.

at 1724−3 and 1711−0 cm^{-1} (Figure 2a,b). In contrast, the band feature in the RC complexes was significantly different and a single prominent band appeared at 1712 cm^{-1} (Figure 2c). The minor peak at 1730 cm^{-1} might also arise from the 13^1-keto C═O rather than the 13^2-ester C═O, because a negative 13^2-ester C═O peak due to neutral P680 was observed at 1738 cm^{-1} accompanied with a positive feature of P680$^+$ at 1753−1744 cm^{-1}.

Figure 3 shows the near-IR region of the P680$^+$/P680 difference spectra of the three PSII preparations. In this region, a broad intervalence band appears (*40*−*45*), indicative of some degree of charge delocalization on a Chl dimer in its radical cation (*40*, *46*−*48*). Note that the region in 3700−3000 cm^{-1} was saturated with strong water OH absorption and peaks at ∼2116/2038 cm^{-1} are due to ferricyanide/ferrocyanide. The PSII membranes of spinach (a) and the core complexes of *T. elongatus* (b) showed a similar feature of a very broad band with a maximum at around 3000 cm^{-1}, whereas the RC complexes of spinach showed a narrower band with a maximum at around 2000 cm^{-1} (c). The latter band feature in the RC complex was identical to that reported previously (*28*, *41*). It should be noted that the minor positive feature at ∼2000 cm^{-1} in the membrane sample is largely attributed to a heat effect on a broad water band at ∼2200 cm^{-1} (combination band). The heat effect was more pronounced in the membrane preparation than the core complexes and RC because of the larger number of Chl molecules per center. It is possible that a real shoulder band also contributes to the feature around this frequency, analogously to the ∼2200 cm^{-1} shoulder in the intervalence band of bacterial P$^+$ (*40*), but its identification needs further careful experiments.

DISCUSSION

The PSII membranes of spinach and the PSII core complexes of *T. elongatus* showed very similar P680$^+$/P680 FTIR difference spectra in both the mid-IR and near-IR regions. Spectral features of chlorin ring vibrations in 1620−1100 cm^{-1} (Figure 1), 13^1-keto C═O bands in 1730−1700 cm^{-1} (Figure 2), and a broad intervalence band with a maximum at ∼3000 cm^{-1} (Figure 3) were virtually identical between these two preparations. This observation indicates that the structure of P680 and the charge distribution on P680$^+$ are unchanged between membrane and core preparations and between higher plants and cyanobacteria.

In sharp contrast, spectral features of isolated RC complexes were significantly different from those of membrane and core preparations. A prominent peak was observed at 1712 cm^{-1} in the 13^1-keto C═O region of P680$^+$ instead of a doublet signal at ∼1724 and ∼1710 cm^{-1} in the cores and membranes (Figure 2c), and the near-IR intervalence band was largely (by ∼1000 cm^{-1}) downshifted to 2000 cm^{-1} with a narrower width (Figure 3c). In addition, the chlorin ring bands of P680$^+$ at 1310 and 1170 cm^{-1} largely downshifted to 1298 and 1141 cm^{-1} (Figure 1). As for the negative bands of neutral P680, most of the peaks of chlorin vibrations slightly (1−6 cm^{-1}) downshifted. In particular, the 6−4 cm^{-1} downshift of the 1616−5 and 1557 cm^{-1} bands, which reflect the core size and hence are typical markers of the coordination number (*31*), may indicate that the core size became larger probably due to the slight movement of the His ligand toward the ring plane. In addition, the peaks at 1344 and 1178 cm^{-1} were intensified. Such intensification of the chlorin ring bands of neutral Chl are unusual and have not been seen in the Chl *a*$^+$/Chl *a* spectrum in an organic solvent and the P700$^+$/P700 spectrum of PS I (*39*). This observation has been attributed to the distortion of the chlorin ring of P680 (*28*). It is noted, however, that these structural perturbations are not to the extent that the conjugation to the 13^1-keto C═O bond is altered, because the peak position of the 13^1-keto C═O of neutral P680 (1702−1700 cm^{-1}) was virtually unchanged in RC (Figure 2).

The upshift of a 13^1-keto C═O frequency upon cation formation can be used for approximate estimation of the degree of charge delocalization on a Chl dimer at the time scale of IR absorption (sub-ps) (*49*, *50*). The schematic picture of the relationship between frequency shifts and charge delocalization is presented in Figure 4 under the assumption that two Chl molecules have the same 13^1-keto C═O frequencies in the neutral state and the same upshifted frequencies in the cation state. In a monomer Chl, the upshift is 25−32 cm^{-1} as previously demonstrated in the Chl *a*$^+$/Chl *a* spectra in solutions (*39*, *51*) and the Chl$_Z^+$/Chl$_Z$ spectrum in spinach PSII membranes (*52*, *53*) (Table 1; Figure 4a). In a Chl dimer, complete charge localization on one Chl molecule will provide the same frequency shift as a monomer Chl and shows only a single band (Figure 4b). When a positive charge is partially shared with the other Chl molecule, the C═O frequency of the Chl with dominant charge slightly downshifts from that of the complete localization, while the frequency of the other Chl with minor charge slightly upshifts from that of neutral Chl (Figure 4c). Thus, in this case, two positive peaks appear in a difference spectrum. Furthermore, in a Chl dimer with complete charge

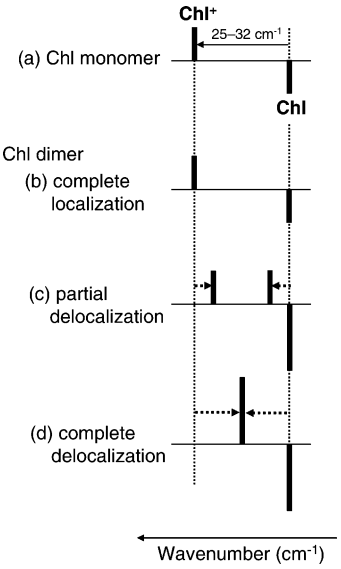

FIGURE 4: Schematic diagram of upshifts of the 13^1-keto C═O band of Chl upon cation formation in monomeric (a) and dimeric (b−d) forms. Bands of neutral and cationic Chl are expressed by negative and positive bars, respectively, that are comparable to FTIR difference spectra. In the dimer, both neutral Chl molecules were assumed to have the same C═O frequency. Three cases for the degree of charge delocalization on the dimer in the radical cation were presented: (b) complete localization on one Chl molecule, (c) partial delocalization, and (d) complete delocalization over the two Chl molecules.

Table 1: 13^1-Keto C═O Frequencies (cm^{-1}) of P680 and Its Radical Cation in Different PSII Preparations in Comparison with the Frequencies of Chl_Z and Isolated Chl *a*

	samples	neutral	radical cation	shift
P680	spinach PSII membranes	1701	1723	+22
			<1711	<+10
P680	*T. elongatus* core complexes	1700	1724	+24
			<1710	<+10
P680	spinach RC complexes	1702	1712	+10
Chl_Z[a]	spinach PSII membranes	1684	1714	+30
Chl *a*[b]	in THF	1693	1718	+25
Chl *a*[c]	in CH_2Cl_2	1685	1717	+32

[a] Reference *52*. [b] Reference *39*. [c] Reference *51*.

delocalization, where the charge is shared equally between the two Chl molecules, the C═O frequency is positioned at the middle frequency of neutral and cationic Chls and hence only one band is observed (Figure 4d). Thus, the frequency shift is expected to correlate linearly with the degree of delocalization as long as the relative charge distribution over the atoms of each chlorin ring is unchanged.

According to this scheme, the presence of two positive bands in PSII membranes and core complexes (Figure 2a,b; Table 1) is interpreted as partial charge delocalization on the Chl dimer. The smaller intensity in the lower-frequency band at ~1710 cm^{-1} than the ~1724 cm^{-1} band may imply that the band partly overlaps the negative band of neutral Chl at ~1700 cm^{-1} and hence the real band frequency should be slightly lower than the observed peak frequency (Table 1). Thus, the sum of the upshifts is estimated to be ~30 cm^{-1}, which is in good agreement with the shift in monomer Chl (25−32 cm^{-1}). Assuming a ~30 cm^{-1} upshift for complete charge localization, the observed 22−24 cm^{-1} upshift (Table 1) indicates that 70−80% of a positive charge resides on one Chl and 20−30% on the other Chl in $P680^+$ of membranes and core complexes. This conclusion is consistent with the proposal by Diner et al. (*54*) that the positive charge is mostly localized on the Chl on the D1 side (P_{D1}).

On the other hand, the appearance of a single prominent band at 1712 cm^{-1} in the RC complex (Figure 2c) indicates that the positive charge is extensively delocalized and almost equally shared between the two Chl molecules. The upshift by 10 cm^{-1} is slightly smaller than the expected value of 12−16 cm^{-1} from the shifts of monomeric Chl. The exact reason for the smaller shift is unknown at present, but distortion of the ring structure of P680 in RC (see above) could change the charge distribution within the chlorin ring. Indeed, the altered spin density distribution within the chlorin ring of $P680^+$ has been detected by ^{13}C-solid-state NMR (*55*). We propose that the minor band at 1730 cm^{-1} (Figure 2) is due to the small population of inhomogeneous RC complexes in which the charge is almost completely localized on one Chl in P680 or electron exchange between the two Chl molecules is much slower than the time scale of IR absorption.

The change in the charge distribution on $P680^+$ by isolation of the RC complexes was supported by the significant change in the characteristics of the intervalence band in the near-IR region (Figure 3). This broad electronic band reflects the potential surfaces of a dimeric radical cation (*40*, *46*−*48*), and thus the large shift of the band frequency (by ~1000 cm^{-1}) and the change in the width indicate that the electronic structure of $P680^+$ is significantly perturbed in the RC complex. The decrease in the band frequency suggests a smaller energy gap between the two hole-localized states, $P_{D1}{}^+P_{D2}$ and $P_{D1}P_{D2}{}^+$ (*40*, *46*−*48*), and hence a higher symmetry in the charge distribution on $P680^+$ in agreement with the conclusion from the 13^1-keto C═O bands. Furthermore, the large shifts of the chlorin ring bands of $P680^+$ at 1310 and 1170 cm^{-1} (membranes and cores) to 1298 and 1141 cm^{-1} (RC), respectively (Figure 1), are consistent with the change in the $P680^+$ structure.

The conclusion that the positive charge is extensively delocalized over the dimer in $P680^+$ of RC seems controversial to the previous ENDOR study using RC complexes, which reported that 82% of the unpaired electron spin was located on one Chl molecule in $P680^+$ (*56*). However, it has been suggested that spin distribution can be more asymmetric than charge distribution because of the spin polarization of the low-lying spin-paired π-orbitals (*57*, *58*). A similar discrepancy has been observed in $P700^+$; strong charge delocalization was reported by FTIR (*43*, *59*), whereas rather asymmetric spin distribution has been suggested by ENDOR (*60*).

The previously reported $P680^+$/P680 spectrum (1800−1500 cm^{-1}) of the RC complexes of *Synechocystis* sp. PCC 6803 in the presence of SiMo showed prominent positive peaks at 1724 and 1709 cm^{-1} (*38*). However, a strong negative peak was also observed at 1682 cm^{-1} next to the 13^1-keto C═O peak at 1695 cm^{-1} due to neutral P680. Thus, it could be possible that the spectrum contained a relatively large contribution of Chl signals other than P680. A similar, albeit much less pronounced, characteristic was observed in the $P680^+$/P680 spectrum of the spinach RC in the presence of only SiMo as an electron acceptor (*27*); the peaks at 1724 and 1681 cm^{-1} were slightly more prominent than the

corresponding peaks in the spectrum in Figure 1c. It seems that SiMo has a tendency to accumulate the cations of monomeric Chls during illumination, probably because of fast electron abstraction from Pheo. Measurements of the intervalence band in the near-IR region and the chlorin ring bands in the 1400−1100 cm^{-1} region may be necessary to determine whether the spectrum of the RC of *Synechocystis* 6803 belongs to the spectral type of spinach RC or that of core/membranes. Also, it was reported that the *Synechocystis* RC contains some extra polypeptides (*61*) and thus the protein composition of this complex can be different from that of spinach (D1, D2, Cyt*b*559, and psbI).

The degree of charge delocalization on the dimer is one of the important factors to determine the redox potential of a Chl dimer (*49*, *57*, *58*). Delocalization of a positive charge over a dimer stabilizes the radical cation and hence decreases the redox potential of a P^+/P couple. Indeed, the HL(M202) and HL(L173) mutants of *Rb. sphaeroides*, in which P870 is a Bchl−Bpheo heterodimer and thus the positive charge is completely localized on the Bchl side, showed an increase in the $P870^+/P870$ redox potential by 0.13−0.18 V (*62*−*64*). Thus, the decrease in redox potential by charge delocalization in a Chl dimer is expected to be 0.1−0.2 V. The redox potential of the $P680^+/P680$ couple has been estimated to be ∼+1.2 V (*9*, *10*). We propose that the redox potential of $P680^+$ is decreased to +1.0−1.1 V in RC by charge delocalization over the dimer. This value is comparable to the redox potential of the $Y_Z{\cdot}/Y_Z$ couple (*14*), and thus explains the well-know phenomenon that Y_Z is not oxidized by $P680^+$ in RC complexes (*21*). Further decrease in the P680 potential by ring distortion (see above) or some other reasons and increase in the $Y_Z{\cdot}/Y_Z$ potential by perturbation of hydrogen-bond networks around Y_Z may also contribute to this phenomenon. The relatively low potential of $P680^+/P680$ in RC is consistent with the observation that $P680^+$ is rather stable at cryogenic temperatures in the presence of ferricyanide in equilibrium with a carotenoid radical cation (*28*), whose reduction potential has been estimated to be ∼+1 V (*65*).

The cause for the difference in the charge distribution in $P680^+$ between RC and membranes/cores is unknown at present. According to the X-ray structures of PSII core complexes (*6*−*8*), the molecular interactions of P680 seem rather symmetric; both Chl molecules are ligated by a His side chain (D1-His198 and D2-His197), and the 13^1-keto C═O groups are free from a hydrogen bond, whereas 13^2-ester C═O groups are engaged in a hydrogen bond with a Thr (D1-Thr286) or Ser (D2-Ser282) residue. Thus, it is presumed that the asymmetry of P680 in cores/membranes is produced by complex formation with other proteins such as CP43, CP47, and some specific small subunits, and possibly with lipids. Indeed, Hoshida et al. (*66*) reported that binding of the psbL protein is required to restore the Y_Z oxidation in the D1/D2/CP47/Cyt*b*559/psbI/psbW complex and that the Phe31−Phe36 region, which seems to interact with the helical part of the D2 subunit facing P_{D2} in the X-ray structures (*6*, *7*), is essential to this function. The fact that the very high redox potential of $P680^+/P680$ is determined not only by the D1 and D2 subunits but requires the assembly of other proteins is an important aspect in consideration of PSII evolution from bacterial RC. Finally, the results of this study suggest that when isolated RC complexes are used for the study of P680, it should be kept in mind that the structure and electronic properties of P680 are rather modified in comparison with P680 in more intact PSII preparations.

ACKNOWLEDGMENT

The authors thank Mr. Yuichi Kitajima for the technical assistance of FTIR measurements and Professor Mamoru Mimuro for his kind support.

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BI700157N