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REGULAR PAPER

Primary light-energy conversion in tetrameric chlorophyll structure of photosystem II and bacterial reaction centers: II. Femto- and picosecond charge separation in PSII D1/D2/Cyt b559 complex

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Abstract In Part I of the article, a review of recent data on electron-transfer reactions in photosystem II (PSII) and bacterial reaction center (RC) has been presented. In Part II, transient absorption difference spectroscopy with 20-fs resolution was applied to study the primary charge separation in PSII RC (DI/DII/Cyt b 559 complex) excited at 700 nm at 278 K. It was shown that the initial electron-transfer reaction occurs within 0.9 ps with the formation of the charge-separated state $P680^{+}Chl_{D1}^{-}$, which relaxed within 14 ps as indicated by reversible bleaching of 670-nm band that was tentatively assigned to the Chl_{D1} absorption. The subsequent electron transfer from Chl_{D1}^{-} within 14 ps was accompanied by a development of the radical anion band of $Pheo_{D1}$ at 445 nm, attributable to the formation of the secondary radical pair $P680^{+}Pheo_{D1}^{-}$. The key point of this model is that the most blue Q_y transition of Chl_{D1} in RC is allowing an effective stabilization of separated charges. Although an alternative mechanism of charge separation with Chl_{D1}* as a primary electron donor and $Pheo_{D1}$ as a primary acceptor can not be ruled out, it is less consistent with the kinetics and spectra of absorbance changes induced in the PSII RC preparation by femtosecond excitation at 700 nm.

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Keywords Chlorophyll · Pheophytin · Photosystem II · Primary charge separation · Reaction center

Abbreviations

B_A	Bacteriochlorophyll, primary electron acceptor in BRC
BRC	Bacterial reaction center
D1/D2/Cytb559	PSII RC
CD	Circular dichroism
Chl	Chlorophyll *a*
Chl_{D1}	Chl located in D1 protein subunit
ET	Electron transfer
HOMO	Highest occupied molecular orbital
LUMO	Lowest unoccupied molecular orbital
Pheo	Pheophytin *a*
$Pheo_{D1}$	Pheo located in D1 protein subunit
PSII	Photosystem II
Q_A	Primary plastoquinone electron acceptor
RC	Reaction center

Introduction

In Part I of the article, a review of recent data on the mechanism of primary charge separation in bacterial reaction centers (BRC) and photosystem II RC (PSII RC) has been presented. The role of tetrameric (bacterio)chlorophyll structure in efficient charge separation in bacterial and oxygenic photosynthesis was discussed. In Part II, we describe new data on the kinetics and spectra of absorbance changes in isolated PSII RCs (D1/D2/Cyt b559) at 278 K obtained by the pump–probe method with 20-fs time resolution and with excitation centered at 700 nm.

Materials and methods

Preparation

Isolated PSII RCs (D1/D2/Cytb_{559} complexes) were prepared from spinach by the method described in Van Leeuwen et al. (1991). The RC preparations were suspended in a buffer consisting of 20 mM Bis–Tris (pH 6.5), 0.03% (w/v) *n*-dodecyl-β-D-maltoside, and 200 mM sucrose. The optical density at the Q_y absorption maximum (675.5 nm) was adjusted to about 1.0/mm. The measurements were done at 278 K under anaerobic conditions.

Femtosecond laser photolysis setup

Transient absorption spectra were measured by the femtosecond pump-supercontinuum probe setup. The output of a Ti:sapphire oscillator (800 nm, 80 MHz, 80 fs,«Tsunami»,«Spectra-Physics», USA) was amplified by a regenerative amplifier system («Spitfire»,«Spectra-Physics», USA) up to 1 mJ/pulse at the repetion rate of 1 KHz. The amplified pulses were splitted into two beams. One of the beam was directed in a noncollinearly phase-matched optical parametric amplifier. Its output centered at 700 nm was compressed by a pair of quartz prisms. The gauss pulse of 20 fs at 700 nm with the bandwith of ~40 nm (full width at half-maximum) was used as a pump. The spectral profile of the pump pulses gives three times more excitation of PSII RC at 680 nm than at 670 nm. The second beam was focused onto a thin quartz cell with H_2O to generate supercontinuum probe pulses. The pump and probe pulses were time-delayed with respect to each other by means of a computer-controlled delay stage. They were then attenuated, recombined, and focused onto the sample cell. The pump and probe light spots have the diameters of 200 and 120 μm, respectively. The pump pulse energy was attenuated at 100 nJ to get optimal excitation on a linear part of the light curve. The pump pulse operation frequency was 50 Hz, low enough to exclude permanent bleaching of the sample due to photochemical processes in RC. The relative polarizations of the pump and the probe beams were adjusted to 54.7° (magic angle). After the sample, the supercontinuum was dispersed by a polychromator («Acton SP-300») and detected by a CCD camera («Roper Scientific SPEC-10»). Transient spectra of absorbance changes ΔA (t, λ) were recorded over the ranges of 400 to 740 nm. The measured spectra were corrected for group delay dispersion in the supercontinuum using the procedure described previously (Ushakov et al. 2004). Experiments were carried out at 278 K in a 0.5-mm path length flow optical cell. Together with operation frequency, the circulation rate in the flow cell was fast enough to avoid multiple excitation of the same sample volume.

Results

Figure 1 shows the spectra of absorbance changes (ΔA) at 278 K obtained with isolated PSII RCs in the range of 400 to 710 nm at various delays (between 0.1 and 28.5 ps) relative to the 20-fs excitation pulse centered at 700 nm. The main changes are related to the bleaching of Soret and Q_Y bands of Chl/Pheo molecules at ~430 and 682 nm, respectively, including stimulated emission from those molecules, which gives more intensive bleaching in red than in blue. In agreement with the previous measurements (Shkuropatov et al. 1999) indicating that both Pheo molecules contribute to the most red absorption in the RC around 680 nm, the bleaching of the Q_x band of Pheo at about 545 nm is observed at early delay times (0.1–0.2 ps) and persists up to 28.5 ps, the longest delay time employed in this work. The amplitude of the 545-nm bleaching is almost constant within this time period. This observation suggests that the excited state of the RC includes partially $Pheo^*_{D1,D2}$ that is eventually converted to the charge-separated state $P680^+Pheo_{D1}^-$ with similar bleaching at 545 nm. It should be noted that the bleaching of Pheo narrow band at 515 nm is increased, but that at 420 nm is relaxed in ps-time domain showing a transformation of $(Pheo_{D1}/Pheo_{D2})^*$ to $(Pheo_{D1})^-$.

Inset of Fig. 1 shows that kinetics of ΔA at 665 nm includes fast (completed within 2.5 ps) bleaching with subsequent relaxation with time constant of 13.3 ps. As it is shown later, these absorbance changes are related to the primary electron acceptor (Chl670) reduction and its reoxidation is due to further electron transfer to $Pheo_{D1}$.

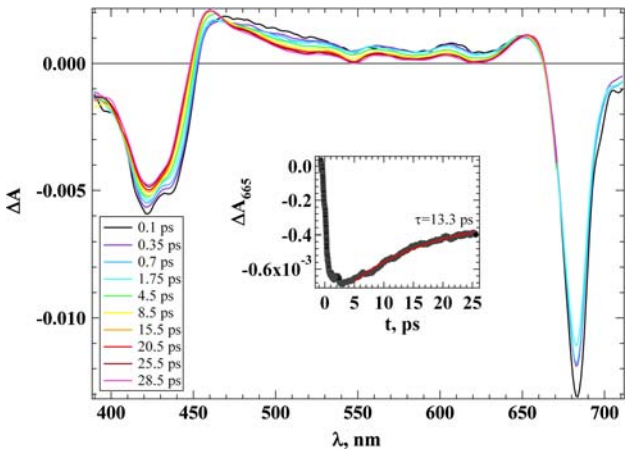

Fig. 1 Difference absorption spectra of D1/D2/Cytb559 complex excited by 20-fs pulses centered at 700 nm with energy of 100 nJ at 278 K. The spectra measured at different delays is indicated in inset A. Inset B shows the kinetics of ΔA at 665 nm revealed directly from the experiment. Fast bleaching at 665 nm is completed to ~2.5 ps and followed by the relaxation with time constant of 13.3 ps

Most profound changes of ΔA spectra are observed in the spectral region of 410 to 470 nm (Fig. 1) suggesting the fs- and ps-formation of the radical anion bands of Chl and/or Pheo, which are known to absorb near 450 nm (Fujita et al. 1978). In order to reveal the dynamics of this process, the ΔA spectrum of the RC excited state measured at the earliest delay of about 0.1–0.15 fs was subtracted from the spectra taken at later delays to get the spectra of $\Delta\Delta A$. This was done suggesting that the bleaching of the Soret band is similar for (Chl/Pheo)* and $(Chl/Pheo)^-$. Then, the difference in $\Delta\Delta A$ near 450 nm is mostly related to the radical anion bands of $(Chl/Pheo)^-$. The result of the subtraction is shown in Fig. 2 for the range of 405 to 585 nm. The appearance of the 445-nm radical anion band very similar to the radical anion band (see Fujita et al. 1978) can be observed already at the delays shorter than 1 ps. It is followed by a development approximately within $\geq$30 ps. The kinetics of this development (Fig. 2, inset B) was approximated by the exponential components with the time constants of 1.4 ± 0.2 ps (amplitude $\sim$26%) and 14.7 ± 5 ps ($\sim$74%). The indicated amplitudes (in parentheses) were changed up to $\sim$50% for both the components after improving resolution.

Apparently, the development of the radical anion band at 445 nm should be accompanied by a decay of the excited state of RC. The broad, negative $\Delta\Delta A$ band in the region of 470–580 nm centered at $\sim$510 nm (Fig. 2), which is developed simultaneously with excitation of the RC and then is decreased within fs and ps time domains (Fig. 2), can be used as an indicator for the formation and decay of the excited state of RC.

Figure 2, inset C, shows that the kinetics of the decay at 510 nm can be described by two exponential components

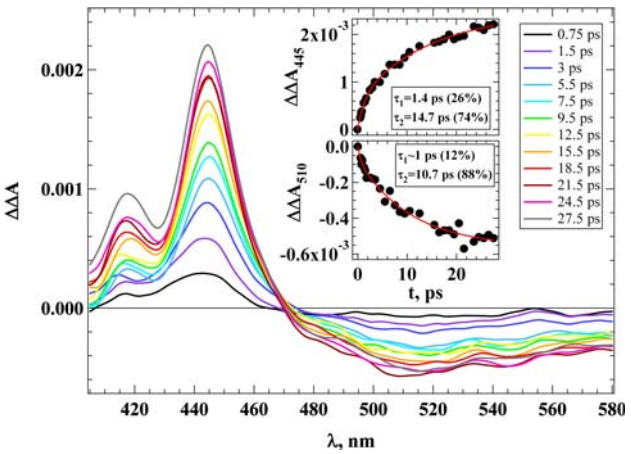

Fig. 2 Data of Fig. 1 were used to calculate the difference–difference absorption spectra in the range of 415 to 580 nm, which are obtained as a result of the subtraction of the spectrum in time domain of 0.1–0.15 ps from the spectra at later delays indicated in inset A. Insets B and C show the kinetics of $\Delta\Delta A$ at 445 and 510 nm, respectively. Two kinetic components of $\Delta\Delta A$ are revealed with time constants of 1–1.4 and 10–15 ps

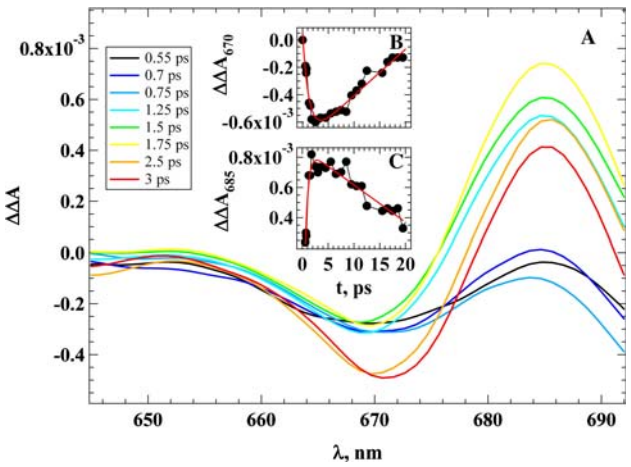

Fig. 3 Data of Fig. 1 were used to calculate the difference–difference absorption spectra in the range of 645 to 692 nm, which are obtained as a result of the subtraction of the spectrum in time domain of 0.1–0.15 ps from the spectra at later delays indicated in inset A. Insets B and C show the kinetics of $\Delta\Delta A$ at 670 and 685 nm, respectively. Two kinetic components of $\Delta\Delta A$ are revealed with time constants of $\sim$1 and $\sim$14 ps

with time constants of 0.6 ± 0.6 ps ($\sim$12%) and 10.7 ± 2 ps ($\sim$88%), respectively, which are similar to those observed for the kinetics of $\Delta\Delta A_{445}$ within the experimental error.

The nature of the two kinetic components observed for $\Delta\Delta A_{445}$ and $\Delta\Delta A_{510}$ can be further clarified by the measurements of $\Delta\Delta A$ in the region of the Q_y absorption of the RC pigments. For this purpose, Fig. 3 shows the calculated difference spectra ($\Delta\Delta A$) for different delays that are similar to those depicted in Fig. 2, but are extended to the range of 645 to 740 nm. Here, fs- and ps-dynamics show the main spectral features including bleaching at 670 nm and development centered at 685 nm, close to the spectral position of the main absorption bleaching in RC upon the 700-nm excitation (Fig. 1). The amplitudes of the changes around 670 and 685 nm measured as differences

$$\Delta\Delta A_{670} = \Delta A_{670} - (\Delta A_{645} + \Delta A_{685})/2 \text{ and}$$
$$\Delta\Delta A_{685} = \Delta A_{685} - (\Delta A_{670} + \Delta A_{700})/2$$

were used as an indicator for kinetic bleaching and development. Figure 3 insets show the kinetics of $\Delta\Delta A_{670}$ and $\Delta\Delta A_{685}$. Both kinetics were approximated by the two exponential components with opposite signs and time constants of $\sim$1 and $\sim$15 ps.

Discussion

Although the DI/DII/Cyt b559 RC complex binds a minimum number of cofactors (six Chls and two Pheos), the dynamics of energy transfer between chromophores and the kinetics and mechanism of primary charge separation in

RC are not yet well understood. The controversy (see Part I of this article) is, to great extent, caused by a significant overlap in the Q_y spectral region together with several difficulties in distinguishing excited states from charge-separated states. Also, rapid equilibration among excited states, between excited states and charge-separated states, and among charge-separated states seems to be observed in the PSII RC that makes it difficult to determine the intrinsic rate constants for charge separation (see, for example, Germano et al. 2004; Groot et al. 2005).

Van Brederode et al. (1997, 1999) were the first to show that direct excitation of the accessory BChl B_A in RCs of purple bacteria can lead to primary charge separation with time constant shorter than 1 ps. Apparently, this reaction should have a low quantum yield in native bacterial RCs because it is in competition with very fast ($\sim$100 fs) energy transfer from B_A* to P870 (Vulto et al. 1997), or it might be related to two-photon process. This finding has triggered, however, a discussion on the nature of the primary electron donor in PSII RCs (Van Brederode et al. 1999; Dekker and van Grondelle 2000). Recently, the data have been presented suggesting that the lowest excited state in the PSII RC was not necessarily localized on the "special pair" P680* and could be on Chl_{D1}* (Prokhorenko and Holzwarth 2000; Diner et al. 2001; Diner and Rappaport 2002; Barter et al. 2003; Germano et al. 2004; Groot et al. 2005; Holzwarth et al. 2006; Pawlowicz et al. 2007; Raszewski and Renger 2008; Raszewski et al. 2008). However, for example, the photon echo data (Prokhorenko and Holzwarth 2000) are mostly based on excitonic calculations including the site energies which are not well defined yet. It is not yet clear at present, whether a single mechanism is realized in PSII RCs or both Chl_{D1}* and P680* can initiate charge separation. In this respect, it is interesting that a large variation in the initial rates of charge separation has been reported for PSII RC, depending on the experimental conditions such as the wavelengths of excitation and temperature (see Germano et al. 2004 and the references therein).

It is well known that the Q_y absorption of all the eight pigments in the PSII RC appears as a single band centered at 675–676 nm (675.5 nm in our preparation) at room temperature, which is only partially resolved at cryogenic temperatures, displaying overlapping bands around 670 and 680 nm, with a shoulder at about 684 nm. The Pheo Q_x transition is well resolved from that of the Chls, peaking at about 542–543 nm at room and low temperatures. The main purpose of femtosecond pump light pulses at 700 nm employed in this work was to minimize the excitation of the pigments absorbing at 670 nm. As indicated in Materials and methods, these pulses give three times more excitation at 680 nm than at 670 nm in RC.

Figure 1 shows that the absorption difference spectra obtained at early delay times (0.1–0.2 ps) display the Pheo bleaching at $\sim$545 nm, about the same wavelength as the Q_x maximum in the ground state absorption (not shown). The negative band at approximately 580 nm attributable to the bleaching of the Chl Q_x absorption band is also seen in the difference spectra. Between 470 and 620 nm, the bleaching of the Pheo and Chl Q_x transitions is superimposed on a broad development due to excited state absorption of the pigments. In the Q_y region, the early time difference spectra (Fig. 1) show an asymmetric negative band that is red-shifted (to 682 nm) with respect to the main ground state absorption band peaking at 675.5 nm. This 682-nm band represents the ground state bleaching/stimulated emission contributions from the Pheo and Chl molecules excited by the 700-nm pulses. A broad, positive signal of relatively small amplitude between 620 and 660 nm results from the excited state absorption of those molecules.

The low temperature experiments performed with Pheo-modified D1/D2/Cytb559 RC complexes have shown that the central pigments of RC, two Pheos, and four Chls seem to absorb in the 676–685-nm range (Germano et al. 2001). Delocalized excited states were detected in the active cofactor branch, while the inactive branch Pheo and the nearby Chl mainly contributed to localized transitions at 676 and 680 nm, respectively (Germano et al. 2001). At least two distant chlorophylls Chl_{Z1} and Chl_{Z2}, which transfer excitation energy to the 680-nm pigment pool contribute to the PSII RC absorption band around 670 nm. With this assignment, the pump pulse centered at 700 nm could potentially excite several chromophores, with some selection for transitions in the active cofactor branch. It is therefore reasonable to assume that at least P680 and $Pheo_{D1}$ are the cofactors excited at 700 nm. The Q_y transition of Chl_{D1} is also a possible candidate for excitation with the 700-nm flash (Germano et al. 2001); however, the sum of the data obtained in this report seems to be better consistent with its spectral position at 670 nm (see below).

Our interpretation of the results of measurements in PSII RC excited by 20-fs pulses centered at 700 nm and presented in Figs. 1, 2, and 3 is as follows. Amplitude of the bleaching of Pheos band (at 545 nm) simultaneously with the excitation is similar to that induced by the formation of $Pheo^-$ at $\geq$30 ps delay (Fig. 1). It means that the contribution of $Pheo_{D1}$* (and possibly $Pheo_{D2}$*) to the bleaching at 545 nm in excited RC is almost equal to the contribution of $Pheo_{D1}^-$ bleaching at 545 nm connected to radical pair formation. If one suggests that the extinction coefficients are equal for the two bleachings, it would mean that Pheos* are converted to $Pheo_{D1}^-$ when RC are excited at 700 nm. One can find that the ratio of the bleachings at 420/545 nm equal to $\sim$13 in the excited state is slightly greater than the 420/545 nm ratio ($\sim$8) in the photoaccumulation of $Pheo_{D1}^-$ (Klimov et al. 1977, 1980). Furthermore, the

bleaching at 435 nm is observed in the excited state of RC and not in the photoaccumulation of $Pheo^-$. It means that in RC* excited by 700-nm pulses, the bleachings include some other pigments, probably P680, having an absorption at 435 nm (Doring et al. 1967, 1969). This is consistent with the 682-nm bleaching that includes the stimulated emission as well.

There are two possible explanations for the results presented in Figs. 2 and 3: (1) the current model of charge separation in PSII RC (see above and Part I of this article) suggesting that the excited state Chl_{D1}* transfers an electron to $Pheo_{D1}$ with the formation of the first radical pair $Chl_{D1}{}^+Pheo_{D1}{}^-$; (2) the BRC-like model suggesting that an electron is transferred from P680* to $Pheo_{D1}$ possibly via Chl_{D1}. In both cases, an electron from $Pheo_{D1}{}^-$ is transferred to Q_A within 200 ps (Nuijs et al. 1986). Further, we shall denote these models as Model 1 and Model 2, respectively. Some of the results presented here are consistent with both models, but some are not. Model 1 suggests that the radical anion band of $Pheo^-$ at 445 nm (Fujita et al. 1978) should be developed with fastest time constant as well as the bleaching of Chl_{D1} bands due to the formation of $Chl_{D1}{}^+$. Only after the completion of this fast reaction, an electron is transferred from P680 to $Chl_{D1}{}^+$ with a slower time constant. According to Model 2, the fast reaction reflects the formation of $P680^+$ $Chl_{D1}{}^-$ while slow one corresponds to the electron transfer from $Chl_{D1}{}^-$ to $Pheo_{D1}$.

Indeed, according to the described results, there are two kinetic components with average time constants of 0.9 and 14 ps. The subtraction of the spectrum of ΔA, measured at 0.1–0.15 ps, from the spectra at later delays reveals some spectral features (Fig. 2, 3), which show the decay of the excited RC measured as a decay of broad band of RC* centered at 510 nm (Fig. 2, inset C) accompanied by the formation of the anion radical band at 445 nm (Fig. 2, inset B) having two components with the average time constants of 0.9 and 14 ps. These results should be discussed according to the assumptions about two mechanisms of the charge separation presented in Models 1 and 2. According to Model 1, the radical anion band of $Pheo_{D1}{}^-$ at 445 nm cannot be developed in slow kinetics if we do not suggest some special influence of $Chl_{D1}{}^+$ on the spectral band of $Pheo_{D1}{}^-$, which disappears when an electron is transferred from P680 to $Chl_{D1}{}^+$. In agreement with Model 2, the $Pheo_{D1}{}^-$ band at 445 nm should be developed in fast and slow kinetics as it can be seen from Fig. 2, inset B.

According to Model 1, the excited state RC* with predominant excitation of Chl_{D1} should disappear synchronously with the formation of the state $Chl_{D1}{}^+Pheo_{D1}{}^-$ in contrast to fast and slow component kinetics of the RC* broad band observed at 510 nm (Fig. 2, inset C). This contradiction can be avoided if one suggests that the excitation of the red band of RC with bleaching at 682 nm (Fig. 1) do not include the excitation of Chl_{D1}. In this case, a new contradiction appears: the Chl_{D1} cannot be a trap for excited RC (at least by fs pulses centered at 700 nm). Model 2 suggests that P680 is excited by fs broad band centered at 700 nm together with the excitation of Pheo molecule(s) in some RC. Then P680* transfers an electron to Chl_{D1} and then to $Pheo_{D1}$ like in BRC. Limited contribution ($\sim$26%, which can be increased up to 50% after better resolution) of fast component to ΔA_{445} can be explained by a smaller extinction coefficient for $Chl_{D1}{}^-$ due to the overlapping of the bleaching and appearing bands or by $\sim$50% mixing of the states P680* and $P680^+Chl_{D1}{}^-$.

The spectra presented in Fig. 3 can be considered from the point of view of dynamic hole burning. This suggestion is unlike since the sub-ps relaxation of bleached 670-nm band should be observed but that is not the case. According to Model 2, the bleaching at 670 nm seen in Fig. 3 can be an indication of the involvement of Chl-670 in the charge separation process. Kinetics of the bleaching at 670 nm as well as of the development at 685 nm includes two components with similar time constants mentioned above, but with opposite directions: fast component (0.9 ps) shows the bleaching at 670 nm and development at 685 nm, while slow component (14 ps) shows the relaxations of both processes. The bleaching at 670 nm can be interpreted as a photoreduction of Chl_{D1} due to the primary charge separation between P680* and Chl_{D1}. Since this process is a conversion of RC* to the charge separated state, it should be accompanied by a loss of stimulated emission at 685 nm which can be observed as positive $\Delta\Delta A$ at 685 nm with similar kinetics (Fig. 3, inset C). Further appearance of bleaching at 685 nm (14 ps) could be due to slower electron transfer from $Chl_{D1}{}^-$ to $Pheo_{D1}$. Furthermore, the photoreduction of Chl_{D1} is accompanied by similar amplitude development at 445 nm (Fig. 2) at earlier delays, as it should be observed according to the reduction of Chl (Fujita et al. 1978). Since the band bleaching at 670 nm was observed always when $Pheo^-$ in PSII was photoaccumulated (Klimov et al. 1977, 1980), it can be suggested that this bleaching is a result of the quantum mechanical exchange interaction between Chl_{D1} and $Pheo_{D1}{}^-$. In fact, the bleaching of the 670-nm band is clearly observed even at 77 K (Shuvalov et al. 1989). The fast ($\sim$0.9 ps) bleaching and slow ($\sim$14 ps) relaxation of ΔA at 665 nm and $\Delta\Delta A$ at 670 nm (Figs. 1, 3) are not consistent with a role of that bleaching as a simple indicator of Pheo reduction since Pheo is constantly reduced in ps time domain (Fig. 2). If Chl_{D1} absorbs at 670 nm, it is clear that Chl_{D1} cannot be a trap for excitation energy in contrast to Model 1. Then the excitation centered at 700 nm allows observing fs kinetics of photoreduction of

Chl_{D1} and ps reoxidation of Chl_{D1}^- by further ET to $Pheo_{D1}$.

The Model 2 is consistent with data on CD and LD measurements in PSII RC at low temperature. At 6 K, the CD spectrum of PSII RC includes two positive bands at 680 and 673 nm and a negative band at 667 nm (Germano et al. 2001). The bands at 680 and 667 nm seem to represent the excitonic interaction in the dimer P680 with splitting energy of $\sim$300 cm^{-1} in good agreement with the calculated coupling energy of $\sim$150 cm^{-1} (Raszewski et al. 2005, 2008). This conservative CD spectrum is not distorted by the reduction of $Pheo_{D1}$ except a few nm blue shift of P680 bands observed in CD (Tetenkin et al. 1989) and in absorbance changes spectra (Ganago et al. 1982). Therefore, the positive CD band at 673 nm can reflect a rotation strength of the transition of Chl_{D1} which is similar to that of B_A in BRC at 800 nm. In both cases, some magnetic dipole moment of the transitions appears to be observed in non-conservative CD spectra at 673 nm (PSII RC) and 800 nm (BRC). Meaning of that for an electron acceptor molecule should be clarified. Linear dichroism measurements at 6 K show (Germano et al. 2001) that both transitions at 673 and 680 nm have positive polarization. This polarization is increased (upto approximately +0.25) after replacement of $Pheo_{D1,D2}$ with 13′-OH-Pheo showing that transitions belong to $Chl_{D1/D2}$ and P680, respectively. In addition, the replacement of Pheo's leads to the decrease of the negative polarization in the 680-nm region caused by the disappearance of Pheo's transitions at 680 nm with perpendicular orientation (Germano et al. 2001). Interestingly, the oxidation of P870 in BRC is accompanied by a blue shift of the primary electron acceptor B_A band at 800 nm. Similar blue shift of the band is observed at 670 nm, when P680 is oxidized at 77 K (Shuvalov et al. 1989). Another interesting feature is revealed under the photoreduction of $Pheo_{D1}$ at 77 K accompanied by the bleaching of the 668-nm band ($\sim$670 nm under correction taking into account a development at 675 nm), which is similar to the bleaching of the B_A band at 800 nm under photoreduction of $BPheo_A$ in BRC (Shuvalov and Klimov 1976). Furthermore, the transitions of $Chlz_{D1,D2}$ should have a negative polarization according to their orientation in X-ray model (Ferreira et al. 2004) which is consistent with the negative band at 668 nm in LD spectra independent of the presence of $Pheo_{D1,D2}$ in RCII (Germano et al. 2001). Thus, one can conclude that the transition at 673 nm has a positive polarization in LD and a positive rotation strength in CD, and these features are not distorted by the replacement of $Pheo_{D1,D2}$ with 13′-OH-Pheo. These features show evidently that the transition at 673 nm belongs to $Chl_{D1/D2}$. Taking into account that wavelength at 673 nm is shifted to the red by $\sim$3 nm by the presence of negative bands at 668 nm in LD spectra (due to $Chlz_{D1,D2}$) and at 667 nm in CD spectra (probably, due to short wavelength exciton transition of P680), the $Chl_{D1/D2}$ transition is located around 670 nm. Participation of the Chl-670 in the electron transfer shows that it is Chl_{D1} (Fig. 3).

Let us consider the energies of the pigment HOMO and LUMO orbitals participating in the electron transfer in PSII RC and BRC. In BRC, most red Q_Y absorption belongs to P870 (870–11,494 cm^{-1}), intermediate to B_A (800–12,500 cm^{-1}), and most blue absorption belongs to H_A (760–13,158 cm^{-1}). Energy difference $\Delta E_{BP} = 1{,}006$ cm^{-1} and $\Delta E_{HB} = 658$ cm^{-1}. If energy of P* is above LUMO of B by $\sim$450 cm^{-1} (Shuvalov and Yakovlev 1998; Nowak et al. 1998), then the energy of HOMO of P^+ is above that of B^- by 1,450 cm^{-1}. When an electron is transferred from P* to B_A, the back reaction from B_A^- to LUMO of P^+ has a barrier of 450 cm^{-1} and from original HOMO of B^- to HOMO of P^+ has a barrier of 1,450 cm^{-1}. These barriers are enough to block the back reactions at room temperature. If energy of B* is above LUMO of H by $\sim$1,500 cm^{-1} then the energy of HOMO of B^+ is above that of original H^- by 2,158 cm^{-1}. When an electron is transferred from B* to H, the back reaction from H^- to LUMO of B^+ has a barrier of 1,500 cm^{-1} and from HOMO of H^- to original HOMO of B^+ has a barrier of 2,158 cm^{-1}. These barriers are again enough to block the back reactions at room temperature.

If in PSII RC most red absorption belongs to $Pheo_{D1}$ and P680 (680–14,706 cm^{-1}) and most blue to Chl_{D1} (670–14,925 cm^{-1}), the energy difference is $\Delta E_{CP} = \Delta E_{CH} = 220$ cm^{-1}. If energy of P* is above LUMO of Chl by $\sim$300 cm^{-1}, then the energy of HOMO of P^+ is above that of Chl^- by 520 cm^{-1}. According to Model 2, if an electron is transferred from P* to Chl_{D1} then the back reaction from Chl^- to LUMO of P^+ has a barrier of 300 cm^{-1} and from original HOMO of Chl^- to HOMO of P^+ has a barrier of 520 cm^{-1}. These barriers are good enough to block the back reactions at room temperature. Further, electron transfer to $Pheo_{D1}$ (−0.64 V, Rutherford et al. 1981)) stabilizes the separated charges for several nanoseconds. In the case when all P680, Chl_{D1}, and $Pheo_{D1}$ have transitions near 680 nm then $|\Delta E|$ for the electron transfer is equal to ΔE for the back transfer between primary electron donor and acceptor (P680* and Chl_{D1}, or Chl_{D1}* and $Pheo_{D1}$) allowing exchange interaction without the stabilization of separated charges. Considerable part of the energy should disappear as fluorescence or dark dissipation in this case.

Thus, in agreement with the charge separation in BRC, the efficient charge separation in PSII RC is consistent with the most blue transition for Q_Y of Chl_{D1} at 670 nm which is revealed from Fig. 3. In other words, the primary charge separation occurs in tetrameric Chl complex of PSII RC between P680* and Chl_{D1} ($\sim$0.9 ps) and then an electron is transferred to $Pheo_{D1}$ ($\sim$14 ps) like in BRC except some

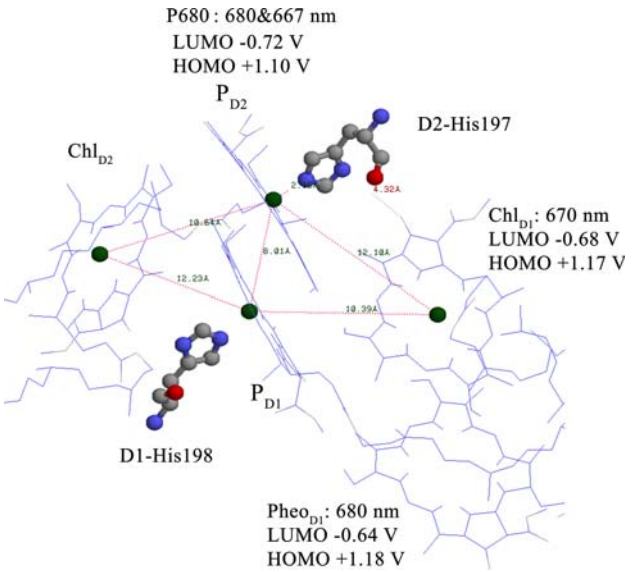

Fig. 4 Arrangement of pigments in PSII RC is found using X-ray analysis by Loll et al. (2005). According to Model 2, P_{D1} and P_{D2} form a dimer, the primary electron donor P680, with excitonic transitions at 680 and 667 nm in PSII RC preparation. Chl_{D1} is a primary electron acceptor with the site energy around 670 nm. $Pheo_{D1}$ is a secondary electron acceptor with site energy around 680 nm and a redox potential of −0.64 V (Rutherford et al. 1981). Using this value and $-\Delta E$ of ∼0.038 V for each step of electron transfer, the redox potentials for HOMO and LUMO orbitals of the electron donor and acceptors were found and indicated in Fig. 4. According to Model 2, the electron transfer from P680* to Chl_{D1} occurs within 0.9 ps and from Chl_{D1}^- to $Pheo_{D1}$ within 14 ps in PSII RC. According to Model 1, Chl_{D1} is a primary electron donor, $Pheo_{D1}$ is a primary electron acceptor, and P680 is a secondary electron donor

difference in time constants (see part I and below). So, the main conclusion from this discussion is consistent with the mechanism of Model 2. The key point of this Model 2 is that the most blue Q_y transition of Chl_{D1} at 670 nm in RC is allowing the effective stabilization of separated charges.

In conclusion, although the results of the femto- pico-second measurements presented in this report cannot rule out Model 1, they seem to be better consistent with the Model 2 for measurements of PSII RC preparation. (It should be noted that according to the recent data of Okubo et al. (2007), the structure of P680 might be perturbed in the PSII RC preparation.) The Model 2 is very similar to that for BRC suggesting that the sequential electron transfer

$$P680^* \rightarrow P680^+Chl_{D1}^- \rightarrow P680^+Pheo_{D1}^-$$

in PSII RC is realized upon excitation at 700 nm with the time constants for the initial and secondary reactions of 0.9 and 14 ps, respectively. Interestingly, the time constants are different from those observed for purple bacterial RC in which the electron transfer rate from P870* to B_A is slower (∼3 ps), but from B_A to H_A is faster (∼1 ps). From recent X-ray analysis, it is known (Fig. 4, Loll et al. 2005) that the center-to-center distances between P_{D1} and P_{D2} is 8.01 Å, between P_{D1} and Chl_{D1} is 10.2 Å, between Chl_{D1} and $Pheo_{D1}$ is 10.6 Å, between P_{D1} and $Pheo_{D1}$ is about 17 Å, and between P_{D2} and $Pheo_{D1}$ is about 20 Å. From this picture, it follows that P_{D1} and P_{D2} are in closest distance, $Chl_{D1,D2}$ are closest pigments to dimer P680, and Chl_{D1} is located between P680 and $Pheo_{D1}$ like a location of B_A between P870 and $BPheo_A$ in BRC structure. Thus, the Model 2 is consistent with the dimer nature of P680 having maximal calculated coupling (158 cm^{-1}) in transition monopole approximation (Raszewski et al. 2005, 2008). Model 2 has an advantage with respect to primary charge separation occurring in excited dimer P680 since it has a parallel arrangement of macrocycles of Chls P_{D1} and P_{D2} (Ferreira et al. 2004; Loll et al. 2005) important for the formation of excimer or exciplex with charge transfer character (Terenin 1967; Beens and Weller 1975; Frese et al. 2003; Hughes et al. 2006). Study of PSII RC and BRC similarities is in progress with respect to the charge separation starting in the dimers P680 and P870 (see also part I).

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