

REVIEW

Primary Radical Ion Pairs in Photosystem II Core Complexes

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Abstract—Ultrafast absorption spectroscopy with 20-fs resolution was applied to study primary charge separation in spinach photosystem II (PSII) reaction center (RC) and PSII core complex (RC complex with integral antenna) upon excitation at maximum wavelength 700-710 nm at 278 K. It was found that the initial charge separation between P680* and Chl_{D1} (Chl-670) takes place with a time constant of ~1 ps with the formation of the primary charge-separated state P680* with an admixture of: P680^{δ+}(1-^δ) (P680^{δ+}Chl_{D1}^{δ-}), where $\delta \sim 0.5$. The subsequent electron transfer from P680^{δ+}Chl_{D1}^{δ-} to pheophytin (Pheo) occurs within 13 ps and is accompanied by a relaxation of the absorption band at 670 nm (Chl_{D1}^{δ-}) and bleaching of the Pheo_{D1} bands at 420, 545, and 680 nm with development of the Pheo⁻ band at 460 nm. Further electron transfer to Q_A occurs within 250 ps in accordance with earlier data. The spectra of P680⁺ and Pheo⁻ formation include a bleaching band at 670 nm; this indicates that Chl-670 is an intermediate between P680 and Pheo. Stimulated emission kinetics at 685 nm demonstrate the existence of two decaying components with time constants of ~1 and ~13 ps due to the formation of P680^{δ+}Chl_{D1}^{δ-} and P680⁺Pheo_{D1}⁻, respectively.

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The present work is dedicated to the memory of the great biophysicist Academician A. A. Krasnovsky and presents an overview of our recent work on femtosecond (fs) measurements of the primary charge separation in reaction centers (RCs) of pigment–protein complex of photosystem II (PSII) under physiological conditions [1-3].

PSII is the light-driven H₂O:plastoquinone-oxidoreductase located in thylakoid membranes of cyanobacteria, green algae, and higher plants. PSII is the main source of the oxygen on Earth, and is also involved in the

formation of the primary biomass in the biosphere. The electron density map of dimeric PSII core complex from the cyanobacterium *Thermosynechococcus elongatus* has recently been solved to a resolution of 2.9-1.9 Å [4, 5]. Each monomer of PSII core complex contains RC D1 and D2 proteins, α - and β -subunits of cyt b559, two integral antenna proteins – CP43 and CP47, which carry 13 and 16 chlorophyll *a* molecules (Chl), respectively, as well as three extrinsic proteins – 33 kDa (PsbO), 17 kDa (PsbV, cyt c550), and 12 kDa (PsbU). Peripheral proteins are required for maintaining the stability and function of the oxygen-evolving complex.

The RC D1/D2 proteins are located approximately symmetrically with respect to the transmembrane region, which is very similar to the arrangement of the L/M subunits in bacterial RC (BRC) [6, 7]. Four Chls (special pair chlorophyll molecules P_{D1} and P_{D2}, denoted as P680, and two accessory chlorophylls Chl_{D1} and Chl_{D2}, in BRC denoted as B_{A,B}), two pheophytins (Pheo_{D1} and Pheo_{D2}, in BRC denoted as H_{A,B}), and two plastoquinones (Q_A and Q_B) are arranged in two symmetrical branches A and

Abbreviations: B_A, bacteriochlorophyll primary electron acceptor in BRC; BRC, bacterial reaction center; Chl, chlorophyll *a*; Chl_{D1} and Chl_{D2}, monomeric chlorophylls located between P680 and pheophytin in D1 and D2 subunits, respectively; D1/D2/cyt b559, PSII RC; P680, special chlorophyll pair in PSII RC; P_{D1} and P_{D2}, chlorophyll molecules in D1 and D2 subunits and forming P680; Pheo, pheophytin; Pheo_{D1}, Pheo located in D1 protein subunit; PSII, photosystem II; PSII core complex, PSII complex with integral antenna; Q_A, plastoquinone primary electron acceptor; RC, reaction center.

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B. As in the BRC, electron transfer in PSII is known [8–11] to proceed only along the D1 branch forming $P680^+Pheo^-$ and then $P680^+Q_A^-$.

It should be noted that P_{D1} and P_{D2} are located close to Chl_{D1} and Chl_{D2} ; the distance between the central Mg atoms of P_{D1} and Chl_{D1} and that between P_{D2} and Chl_{D2} are 10.2–10.4 Å, respectively [12, 13]. The head groups of P_{D1} and P_{D2} are in direct van der Waals contact; the Mg–Mg distance is 8.2 Å [12] or 7.6 Å [13]. Although dimeric “special pair” of P_{D1} and P_{D2} with parallel orientation of the macrocycles has weaker coupling than that in the BRC special pair [14], the interaction within P680 is stronger than between P680 and Chl_{D1} molecules.

According to the X-ray structure of PSII crystals, the porphyrin ring planes of P_{D1} and Chl_{D1} , P_{D2} and Chl_{D2} , Chl_{D1} and $Pheo_{D1}$, and Chl_{D2} and $Pheo_{D2}$ are not parallel. Thus, one can assume that the formation of excimer or exciplex between parallel macrocycles [15] can be observed only for P680, but not for Chl_{D1} , $Pheo_{D1}$ and the other molecules. Recent spectral measurements in photosystem I (PSI) complexes induced by 20-fs pulses at 720 nm have shown [16] that the excimer is initially formed within P700, which has parallel orientation of Chl macrocycles, while primary charge separation occurs in aggregate consisting of six molecules of RC Chls forming the primary donor P700 and the primary electron acceptor A_0 [16].

In the study of PSII RC, a key issue is to determine the frequency of the spectral transitions in each of the four chlorophyll molecules and two molecules of pheophytin, as well as to identify the primary charge separated state. According to recent measurements in isolated PSII RCs using 20-fs laser pulses with a maximum at 700 nm (278 K) [1], the light-induced charge separation is initiated within the four excitonically coupled Chl molecules that form the photoactive core of the PSII complex. Primary charge separation with formation of $P680^+Chl_{D1}^-$ (with Chl_{D1} absorbing at 670 nm) is similar to the formation of the radical pair $P870^+B_A^-$ in BRC [17, 18]. The formation of $P680^+Chl_{D1}^-$ is observed within ~1 ps, but the subsequent transfer of an electron to Pheo occurs within ~14 ps.

Although studies concerning the very early light-induced steps in PSII complexes were initiated over three decades ago, there is still considerable debate about the nature of the primary electron donor under physiological conditions, i.e. it is unclear what the initial electron transfer is: whether it starts from excited special pair P680, from accessory Chl_{D1} , or both cofactors are involved in primary charge separation. It should be noted that in all cases transfer of an electron from $Pheo^-$ to Q_A occurs within ~200 ps [19, 20].

Based on some recent publications, the accessory Chl_{D1} is the primary electron donor in PSII RC; as such, the primary charge separation is due to $Chl_{D1}^+Pheo^-$ formation both at cryogenic temperatures and under physiological conditions [21–32]. However, as mentioned

before, the results obtained in PSII complexes excited by 20-fs pulses with a maximum wavelength 700–710 nm indicate that $P680^+Chl_{D1}^-$ is the primary ion-radical pair [1, 2]. On the basis of experimental studies performed in isolated PSII RC by kinetic absorption spectroscopy under various excitation conditions at 77 K [33] and the quantitative modeling of the kinetics of absorption changes [34], the possibility of the existence of alternative pathways for charge separation in PSII, i.e. where P680 or Chl_{D1} play the role of primary electron donor, was considered [33, 34]. Analysis of the kinetics obtained for PSII RC by 2D spectroscopy showed that involvement of the two electron transfer pathways allowed to obtain better agreement with experiment [35].

Recently we showed [1] that the spectroscopic data derived from PSII RC at low temperature (6 K) [36] indicate that the Q_y absorption band near 670 nm can be attributed to $Chl_{D1/D2}$, which is characterized by a positive polarization in the spectra of circular and linear dichroism. Thus, the spectral form of Chl-670 corresponds to Chl_{D1} [1]. In a recent paper we have presented new experimental data for spinach PSII core complexes using 20-fs photolysis (pump–probe) at 710 nm [2]. These data support our previous results [1] derived from isolated PSII RCs and provide evidence that at physiological temperatures and conditions used by us (20-fs excitation with a maximum at 700 nm, 278 K), the primary electron donor and acceptor are $P680^*$ and Chl_{D1} , respectively.

It was previously shown that illumination of oxygen-evolving PSII core complexes at 1.7 K, as well as spinach leaves at 293 K, resulted in the reduction of Q_A , the action spectrum of which has a band in the 710–730 nm region [37, 38]. Therefore, for the excitation of the sample it was possible to use femtosecond pulses with a peak wavelength at 710 nm.

KINETICS OF PRIMARY REACTIONS OF ELECTRON TRANSPORT IN PSII RC

Differential spectra of absorbance changes (ΔA) at 278 K derived from isolated PSII RCs in the range of 400–710 nm at various delays (from 0.1 to 28.5 ps) were determined upon excitation of samples with 20-fs laser with maximum wavelength at 700 nm [1]. The main changes were related to the bleaching of the Soret and Q_y bands of Chl and Pheo molecules at ~430 and 682 nm, respectively, including stimulated emission from these molecules in the red region of spectra. In agreement with previous measurements [9] indicating that both Pheo molecules contribute to the most long wavelength absorption bands of absorption in RC, the bleaching of the Q_x band of Pheo at 545 nm is observed at early delay times (0.1–28 ps). 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 $\text{Pheo}_{\text{D1,D2}}^*$ that is eventually converted to the charge-separated state $\text{P680}^+\text{Pheo}_{\text{D1}}^-$ with similar bleaching at 545 nm.

The kinetics of ΔA at 665 nm includes a fast (completed within 2.5 ps) bleaching with subsequent relaxation with the time constant of 13.3 ps. As will be shown below, these absorbance changes are related to the reduction of the primary electron acceptor (Chl-670, identified as Chl_{D1}) and its subsequent oxidation by further electron transfer to Pheo_{D1} .

Significant changes of ΔA spectra in the range 410–470 nm are observed and give evidence in favor of fs and ps formation of the radical anion bands of Chl^- and/or Pheo^- , which absorb near 450 nm [39]. To reveal the dynamics of this process, the ΔA spectrum of the RC excited state measured at the earliest delay (0.1–0.15 fs) was subtracted from the spectra taken at later delays; as a result, double differential spectra $\Delta\Delta A$ were obtained. It

has been suggested that the bleaching of the Soret band is similar for $(\text{Chl}/\text{Pheo})^*$ and $(\text{Chl}/\text{Pheo})^-$. Therefore, the difference $\Delta\Delta A$ should be mainly related to radical anion $(\text{Chl}/\text{Pheo})^-$ formation. The appearance in the $\Delta\Delta A$ spectra for PSII RC of the band at 445 nm related to radical anion formation [39] occurs at time delays shorter than 1 ps, followed by a further increase in absorption with a characteristic time of 14 ps [1].

PRIMARY AND SECONDARY ION-RADICAL PAIR FORMATION IN PSII CORE COMPLEXES

As shown earlier, the oxidation of the primary electron donor P680 (684 nm) at low temperature (77 K) was observed in isolated PSII RC complexes in the presence of external electron acceptor SiMo [2] (Fig. 1a). At the same time, the oxidation of Chl-674 (possibly along with P680) with a bleaching at 674 nm upon formation of the

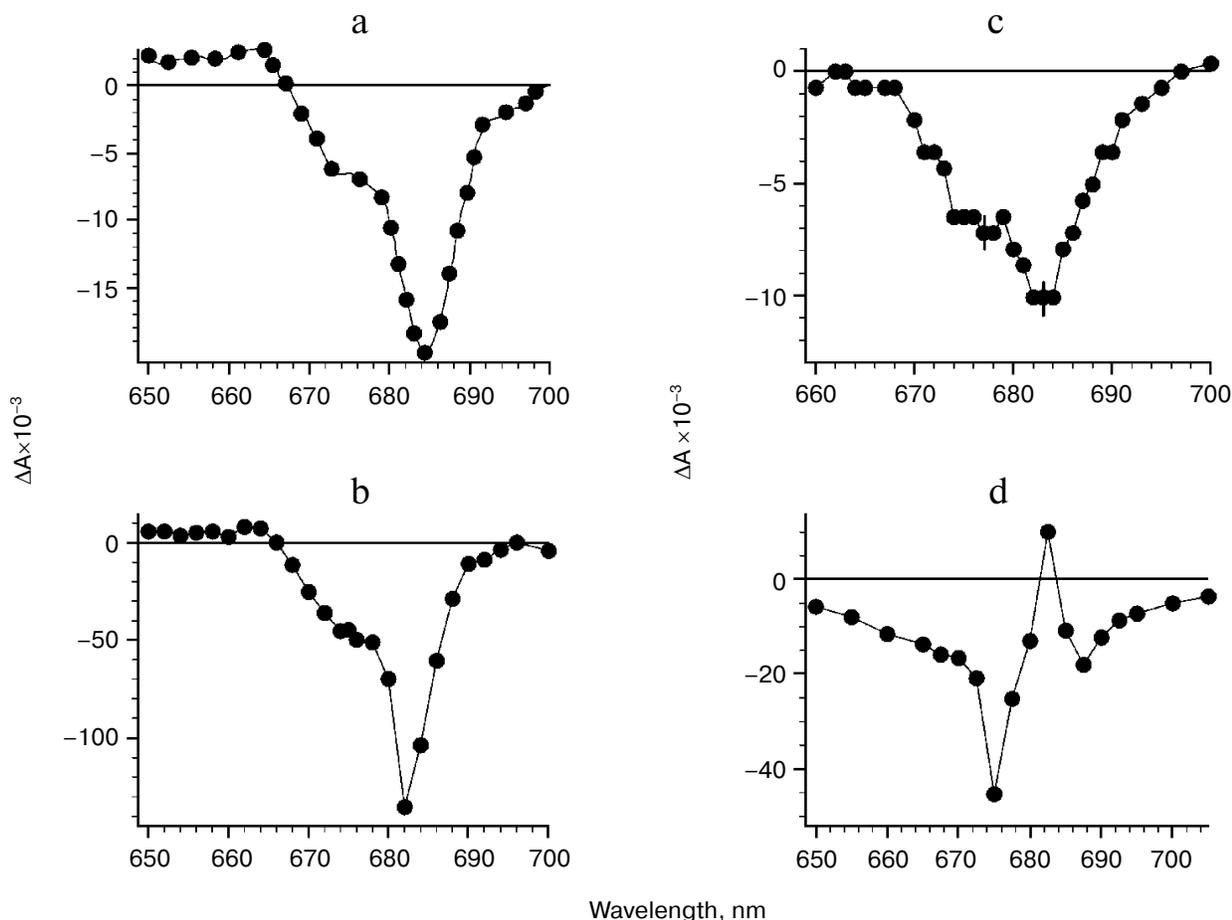


Fig. 1. a) Spectrum of irreversible difference absorbance changes ΔA (light-minus-dark) in PSII RC in the presence of SiMo (0.1 mM) excited by red light ($\lambda > 600$ nm) at 77 K. b) Spectrum of reversible ΔA (time constant is a few seconds) measured with a phosphorescopic set-up in PSII RC in the presence of DCBQ (1 mM) and SiMo (0.1 mM) with the same excitation as in (a) but at 90 K. c) Spectrum of reversible ΔA (time constant of a few msec) measured with the phosphorescope in PSII RC in the presence of DPQ (1 mM) and SiMo (0.1 mM) at 90 K. d) Spectrum of reversible ΔA (time constant of a few msec) in PSII core complexes in the presence of SiMo (0.1 mM) at 90 K.

state $\text{Chl}_{\text{D1}}^+\text{Q}_{\text{A}}^-$ in PSII core complex was observed (Fig. 1d). Note that earlier a bleaching at 674 nm was attributed to P_{D1} form [23, 30–32, 40–42]. However, analysis of the results obtained upon excitation of isolated RCs and PSII core complexes with 20-fs laser pulses shows that Chl-670 with bleaching and relaxation within ~ 1 and ~ 14 ps, respectively, is evidently an intermediary electron acceptor between P680 and Pheo at 278 K and does not seem to be a part of P680 (P_{D1} or P_{D2}) [1–3].

In favor of the participation of Chl_{D1} (Chl-670) in electron transfer between P680 and Pheo indicate data concerning redox potential changes of $\text{P680}/\text{P680}^+$ and $\text{Chl}_{\text{D1}}/\text{Chl}_{\text{D1}}^+$ induced by the formation of Q_{A}^- at low temperature in PSII core complexes [1–3]. Assuming fully “frozen” atomic polarizability of protein and solution medium (corresponding to dielectric permittivities (ϵ) for protein 2.5 and for medium 1.84) at 100 K, we obtained a 44 meV negative shift of midpoint redox potential (E_{m}) of $\text{Chl}_{\text{D1}}/\text{Chl}_{\text{D1}}^+$ with respect to that of $\text{P680}/\text{P680}^+$ induced

by the field of Q_{A}^- . This shift is due to the steric position of both donors with respect to Q_{A}^- and does not exceed 8–11 meV at room temperature (corresponding to ϵ for protein 4–6 and ϵ for medium 81). Taking into account an energy difference of 27 meV between quanta at 674 and 684 nm and close position of LUMO orbitals of P680 and Chl_{D1} , the redox potential of $\text{Chl}_{\text{D1}}/\text{Chl}_{\text{D1}}^+$ at low temperature in PSII core complex can become more negative than that of $\text{P680}/\text{P680}^+$ (17 meV). Note that such effect is observed upon formation of the $\text{Chl}_{\text{D1}}^+\text{Q}_{\text{A}}^-$ state with subsequent recombination in the millisecond time domain in PSII core complex. As a result, in PSII core complex the Chl_{D1} might function as terminal electron donor at low temperature with bleaching at 674 nm along with the P680 bleaching at 684 nm.

Figure 1a shows differential (light minus dark) absorption spectra derived from isolated PSII RC in the presence of silicomolybdate (SiMo) as an external electron acceptor at 77 K. The figure shows that under irreversible charge separation the P680 may function as an electron donor (bleaching at 684 nm according to Shuvalov et al. [43]). A bleaching and slight blue shift of the band around 670 nm, probably due to Chl_{D1} oxidation, is observed.

Figure 1 (b and c) shows PSII RC spectra in the presence of SiMo at a higher concentration of dichlorobenzoquinone (DCBQ) (1 mM) (Fig. 1b) or decylplastoquinone (DPQ) (Fig. 1c).

When DCBQ is added before freezing, the bleaching becomes mostly reversible (recombination time in the range of seconds), and the spectrum of ΔA demonstrates the bleaching centered at 684 nm with a shoulder at 674 nm (Fig. 1b). Upon addition of DPQ the recombination time of $\text{Chl}_{\text{D1}}^+\text{Q}_{\text{A}}^-$ is decreased to several milliseconds. This leads to the decrease in the amplitude of absorbance changes because the phosphorescopic setup has limited resolution in the millisecond time domain (Fig. 1c). In this case the more pronounced bleaching at 674 nm is observed due to the closer position of DPQ to the putative Q_{A} site in the RC complex (Fig. 1c). The spectrum in Fig. 1c depicts two bleachings at 684 and 674 nm with the amplitude at 684 nm much smaller than that in Fig. 1b. The spectrum of reversible absorption changes (relaxation time of several milliseconds) in PSII core complexes in the presence of SiMo at 90 K is shown in Fig. 1d. The spectrum shows that reversible millisecond recombination for the $(\text{P680}-\text{Chl}_{\text{D1}})^+\text{Q}_{\text{A}}^-$ state is accompanied by bleaching at 674 nm and blue shift (and bleaching) of the 684 nm band. Similar data were described in [23, 30–32, 40–42] using an assumption about the oxidation of P_{D1} and reduction of Q_{A} . However, we assume that the resulting spectrum can be attributed to the state $(\text{P680}-\text{Chl}_{\text{D1}})^+\text{Q}_{\text{A}}^-$ [1–3] in which the positive charge is distributed between P680 and Chl_{D1} .

Figures 2–4 present data of femtosecond experiments with spinach PSII core complexes. To minimize the exci-

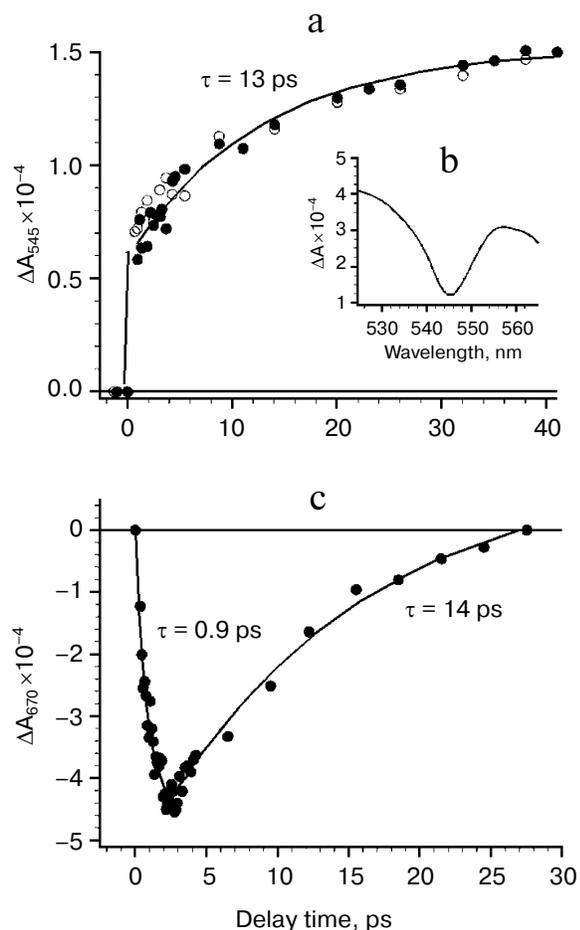


Fig. 2. a) Kinetics of absorbance changes ΔA at 545 nm in PSII core complex excited by 20-fs pulses at 710 nm. b) The spectrum of ΔA in the 525–565 nm region measured at 33 ps delay and representing the bleaching of the Pheo absorbance band at 545 nm. c) ΔA kinetic at 670 nm; conditions as in Fig. 2a.

tation of antenna Chls, 20-fs pulses centered at 710 nm and energy of 50 nJ were used.

Differential spectra of absorption changes (ΔA) at 278 K in the spectral range 400–725 nm at various delay times (between 0.3 and 455 ps) reveal some important features. These features are clearly evident when considering subtraction of absorption changes measured at 0.15 ps from ΔA registered at later delay times. Figure 2a shows kinetics of absorption changes induced by femtosecond pulses at the 545 nm band reflecting the formation of excited state (unresolved fast time component <20 fs) and the Pheo anion-radical (characteristic time $\tau = 13$ ps). The inset (Fig. 2b) shows the differential spectrum at 33 ps delay caused by bleaching of the Q_X band of Pheo_{D1}. Figure 2c shows the kinetics of absorption changes at 670 nm due to the formation and disappearance of the radical anion Chl_{D1}⁻. It is seen that the formation of Chl_{D1}⁻ occurs within 0.9 ps, and the kinetics of its decay (14 ps) coincides with the kinetics of Pheo⁻ formation (Fig. 2a).

Figure 3 shows kinetics of absorption changes at 460 nm due to the formation ($\tau \sim 11$ ps) and disappearance ($\tau \sim 250$ ps) of Pheo_{D1}⁻ anion-radical. Note that the oxidation kinetics of Chl_{D1}⁻ (Fig. 2c, $\tau \sim 14$ ps) agrees quite well with the kinetics of Pheo_{D1} reduction (Fig. 2a, $\tau \sim 13$ ps and Fig. 3, $\tau \sim 11$ ps). This indicates that electron transfer from monomeric Chl_{D1} to Pheo_{D1} occurs with a characteristic time of 11–14 ps. The kinetics of formation of Pheo⁻ is more than an order of magnitude slower than the oxidation kinetics of P680 and Chl_{D1}⁻ formation ($\tau = 1$ ps). Thus, these data indicate that the primary electron acceptor, at least under the experimental conditions used, is Chl_{D1}, while Pheo_{D1} plays the role of the secondary electron acceptor.

Figure 4 shows that the stimulated emission at wavelength 685 nm measured with a parallel orientation of the

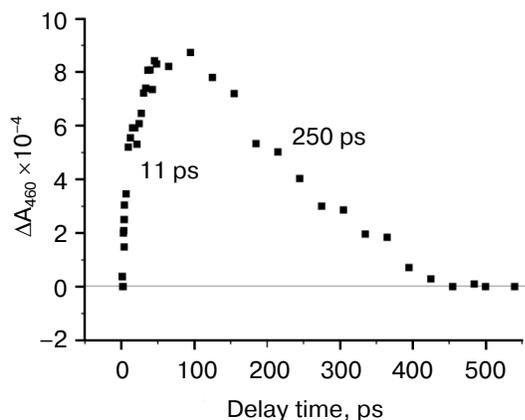


Fig. 3. Kinetics of absorbance changes ΔA at 460 nm in PSII core complexes (conditions as in Fig. 2a). The rise time of the formation of the 460-nm band (Pheo⁻) is within 11 ± 3 ps, and its relaxation time is 250 ± 50 ps.

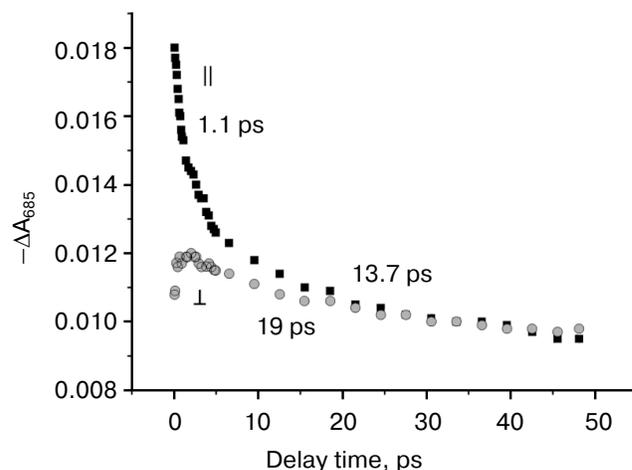


Fig. 4. Kinetics of absorbance changes ΔA at 685 nm (stimulated emission) in PSII core complexes (conditions as in Fig. 2a). The rise time of the formation of the 685-nm band is 20 fs for both parallel and perpendicular orientation of electric dipole moments for excitation and measuring beams. The decay of the band for parallel polarization has two components with lifetimes of ~ 1 and ~ 13.7 ps. For perpendicular polarization the band at 685 nm has two components with approximately the same lifetimes, but the first one increases and the second decays.

electrical vectors of the exciting and probing pulses has at least two decay kinetic phases with lifetimes $\tau_1 \sim 1$ ps and $\tau_2 \sim 14$ ps (initial anisotropy ~ 0.25). The τ_1 component coincides with the kinetics of formation of $P680^+Chl_{D1}^-$ [1–3], and the τ_2 component coincides with formation of $P680^+Pheo_{D1}^-$ (Figs. 2c and 3). This result shows that the primary ion-radical pair $P680^+Chl_{D1}^-$ is a quencher of the excited states of the pigments. Thus, under these experimental conditions, the electron transfer reaction to Pheo is not involved in the process of primary charge separation in PSII RC. The decay kinetics of stimulated emission detected with perpendicular orientation of the electric vectors of the exciting and probing pulses demonstrates the appearance of a new component with a significantly lower polarization (anisotropy is 0.08) than that of the first component with lifetime of ~ 1 ps observed in the parallel orientation. The emergence of this new component was accompanied by an absolute increase in amplitude with perpendicular orientation, which is quite unusual for stimulated emission decay. Since about 30% of the stimulated emission decays over a longer time ($\tau_2 \sim 14$ ps), we have assumed that the formation of the state $P680^+Chl_{D1}^-$ can be considered as a mixture of states with charge transfer $P680^+Chl_{D1}^-$ and excited state $P680^*$. Mixture of states can be represented as $P680^{(1-\delta)*}(P680^{\delta+}Chl_{D1}^{\delta-})$, where $\delta \sim 0.5$. This mixed state is observed as a stimulated emission at ~ 685 nm with a low positive polarization, which decays by further electron transfer from $Chl_{D1}^{\delta-}$ to Pheo within ~ 14 ps. Nevertheless, it is possible that the two-component

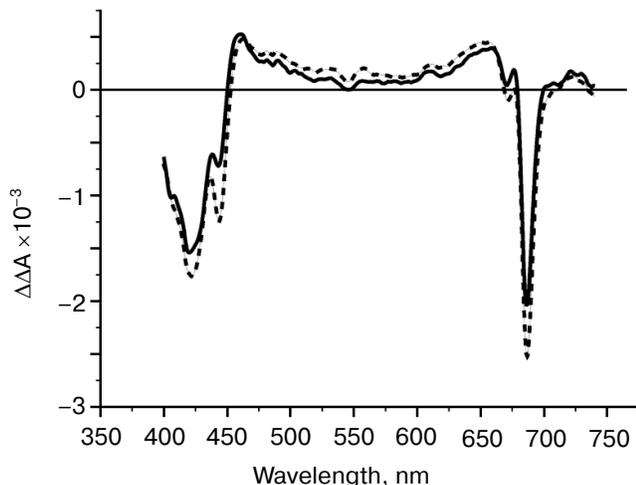


Fig. 5. Difference spectrum of irreversible absorbance changes $\Delta\Delta A$ (light-minus-dark) in PSII core complexes at 278 K excited by 20-fs pulses at 710 nm obtained by subtraction of the spectrum ΔA at 445 ps delay (P680⁺ spectrum) from spectra ΔA at 23 and 44 ps delays. The bleaching at 545 nm reflects the kinetics of photoreduction of Pheo.

decay kinetics of stimulated emission is due to the existence of two populations of PSII RC decaying with different lifetimes. However, increasing of the perpendicular component with delay time is not consistent with this interpretation.

As shown in Fig. 3, the band of the Pheo anion-radical at 460 nm completely disappeared at ~ 450 ps delay, indicating the full electron transfer from Pheo⁻ to Q_A. It is known that electron transfer to Q_A must be completed within 450 ps [19]. Thus, the transient spectrum at a delay of 445 ps corresponds to the formation of P680⁺, because the differential absorption spectrum of Q_A⁻ does not appear in the visible spectrum.

Figure 5 shows the difference between the transient spectra measured at 23 or 44 ps and the spectrum measured at 455 ps, which is ascribed to the P680⁺ difference spectrum. Since during this time range the only electron transfer event is the formation of P680⁺Q_A⁻, this $\Delta\Delta A$ spectrum can be assigned to Pheo⁻ formation and has bleaching at 420, 545, 671, and 685 nm and developments at 460 and 660 nm. The spectrum is similar to those previously obtained by accumulation methods [11, 44], and it therefore proves that charge separation between P680* and Pheo is accompanied by the entire transfer of electron density from P680* to Pheo via Chl_{D1} in the picosecond time domain. This result also confirms that the accumulation method applied previously for the study of PSI [45], BRC [46], and PSII RC [11, 44] demonstrates the photochemical reactions in RCs.

The spectrum of the formation of Pheo_{D1}⁻ presented in Fig. 5 also shows an additional bleaching at 670 nm, which was assigned to Chl-670 [1-3]. This feature indi-

cates the close position of the Pheo_{D1} to the Chl-670 molecule, which as we earlier suggested can play a role of the primary electron acceptor Chl_{D1} functioning between P680* and Pheo_{D1} [1-3].

DISCUSSION

Data presented in this work show that the primary charge separation in RC and in PSII core complexes induced by 20-fs pulses with peak wavelength of 700-710 nm (278 K) is due to the formation of the state P680⁺Chl_{D1}⁻. In so doing, the time constant for the formation of P680⁺Chl_{D1}⁻ is ~ 0.8 ps, which is revealed from the Chl-670 rise time and delay of stimulated emission at 685 nm. The decay time of the state P680⁺Chl_{D1}⁻ is ~ 13 ps measured from Chl-670 decay, from the formation of the Pheo⁻ anion radical band at 460 nm, the bleaching at 545 nm, and from stimulated emission at 685 nm.

The formation of the state P680⁺Pheo_{D1}⁻ in the range of 20-40 ps can be confirmed by the subtraction of the spectrum of state P680⁺ observed at 445 ps delay from the spectra of P680⁺Pheo_{D1}⁻ measured at 23 and 44 ps (Fig. 5). The resulting spectra are very similar to the spectrum of Pheo_{D1}⁻ observed previously by the accumulation method [11, 44].

The bleaching bands and pigment spectral shifts are independent for formations of P680⁺ and Pheo⁻, which allows observing the additive sum of its individual features in ΔA spectra at 23 and 44 ps delays. This is in contrast to the situation observed for bacterial RCs where intensive electrochromic shift of the B_A band at 800 nm is observed in varying degrees for formation of both P⁺ and BPheo⁻ ions [46]. The P680⁺Pheo_{D1}⁻ state disappears within 250 ± 50 ps due to electron transfer from Pheo_{D1}⁻ to Q_A. The bleaching (or red shift) of the 670-nm band in the spectrum of the Pheo⁻ formation obtained from fs/ps measurements [1-3] or the accumulation method at room and low temperatures [11, 43, 44] indicates significant interaction and close arrangement of Pheo_{D1} and Chl-670 molecules. The bleaching of the 670-nm band (675 nm at low temperature [2]) in PSII RC and core complexes [1, 2] also indicates the nearby location of the P680 and Chl-670 molecules.

Taking into account the last two observations, we conclude that Chl-670 is located in the vicinity of both P680 and Pheo_{D1}. The bleaching of the Chl-670 band in both cases can be due to disappearance of excitonic interaction between Chl-670 and P680 or Pheo_{D1} when two latter electron carriers are oxidized or reduced, respectively. According to the expression for dipole strength (*D*) of the excitonic band in aggregate [47], the *D* value for the transition A in aggregate (which is close to transition α in monomer) has a sign depending on the following expression:

$$D_A = B - C v_\alpha v_\beta / (v_\beta^2 - v_\alpha^2),$$

where v_α and v_β are frequencies of the transitions in two interacting molecules α and β , D and B are positive, and C is suggested to be a positive constant. If v_α is frequency for the 670-nm transition in Chl_{D1} and v_β is for the 680-nm transition in P680 (or Pheo_{D1}), then the D value increases for the 670-nm transition and decreases for 680-nm due to excitonic interaction in the aggregate. If interaction is broken by photochemistry in PSII RC, D_A is decreased to the value characteristic of the absence of interaction between 670- and 680-nm transitions. Since both oxidation of P680 and reduction of Pheo_{D1} were accompanied by decrease in the 670-nm transition amplitude, we conclude that Chl_{D1} is located between P680 and Pheo_{D1} and may play the role of the intermediary electron carrier between P680* and Pheo_{D1} as suggested earlier [1-3]. The decay kinetics of the stimulated emission at ~ 685 nm (Fig. 4) indicates that at least two emitting centers are observed. The first center with maximal positive polarization (~ 0.25) appears to reflect the emission from the excited states of P680* and/or $\text{Pheo}_{\text{D1}}^*$. This emission decays with a lifetime of ~ 1 ps due to electron transfer from P680* to Chl_{D1} with the formation of mixed state $\text{P680}^{(1-\delta)*}(\text{P680}^{\delta+}\text{Chl}_{\text{D1}}^{\delta-})$. This latter state (second center of emission) emits light at 685 nm with smaller positive polarization (~ 0.08). The decrease in the component with parallel polarization is accompanied by an increase in the same component with perpendicular polarization $-\Delta A_{685}$ (Fig. 4). This evidently shows the formation of a new emitting center at 685 nm, which is probably caused by the formation of the state $\text{P680}^{(1-\delta)*}(\text{P680}^{\delta+}\text{Chl}_{\text{D1}}^{\delta-})$, where $\delta \sim 0.5$. The latter state decays due to further electron transfer to Pheo_{D1} within ~ 13 ps as observed by fs/ps measurements showing Pheo^- formation.

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