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Formation and decay of P680 $(P_{D1}–P_{D2})^{+}Pheo_{D1}^{-}$ radical ion pair in photosystem II core complexes☆

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ABSTRACT

Under physiological conditions (278 K) femtosecond pump-probe laser spectroscopy with 20-fs time resolution was applied to study primary charge separation in spinach photosystem II (PSII) core complexes excited at 710 nm. It was shown that initial formation of anion radical band of pheophytin molecule ($Pheo^{-}$) at 460 nm is observed with rise time of ~11 ps. The kinetics of the observed rise was ascribed to charge separation between Chl (chlorophyll *a*) dimer, primary electron donor in PSII (P680*) and Pheo located in D1 protein subunit ($Pheo_{D1}$) absorbing at 420 nm, 545 nm and 680 nm with formation of the ion-radical pair $P680^{+}Pheo_{D1}^{-}$. The subsequent electron transfer from $Pheo_{D1}^{-}$ to primary plastoquinone electron acceptor (Q_A) was accompanied by relaxation of the 460-nm band and occurred within ~250 ps in good agreement with previous measurements in Photosystem II-enriched particles and bacterial reaction centers. The subtraction of the $P680^{+}$ spectrum measured at 455 ps delay from the spectra at 23 ps or 44 ps delay reveals the spectrum of $Pheo_{D1}^{-}$, which is very similar to that measured earlier by accumulation method. The spectrum of $Pheo_{D1}^{-}$ formation includes a bleaching (or red shift) of the 670 nm band indicating that Chl-670 is close to $Pheo_{D1}$. According to previous measurements in the femtosecond–picosecond time range this Chl-670 was ascribed to Chl_{D1} [Shelaev, Gostev, Vishnev, Shkuropatov, Ptushenko, Mamedov, Sarkisov, Nadtochenko, Semenov and Shuvalov, J. Photochemistry and Photobiology, B: Biology 104 (2011) 45–50]. Stimulated emission at 685 nm was found to have two decaying components with time constants of ~1 ps and ~14 ps. These components appear to reflect formation of $P680^{+}Chl_{D1}^{-}$ and $P680^{+}Pheo_{D1}^{-}$, respectively, as found earlier. This article is part of a Special Issue entitled: Photosynthesis Research for Sustainability: Keys to Produce Clean Energy.

1. Introduction

A review of photosystem II (PSII) structure and function was presented in many papers (see [1] for references).

Pigment–protein complex of PSII functions at physiological conditions as a light-dependent water:plastoquinone oxidoreductase in the thylakoid membranes of cyanobacteria, green algae and higher plants. The electron density map of dimeric PSII core complex from the cyanobacterium *Thermosynechococcus elongatus* has recently been resolved to a resolution of 1.9 Å [2]. Each core PSII complex contains reaction center (RC) incorporated in D1/D2 proteins, the α and β subunits of cyt b559, two integral antenna proteins, CP43 and CP47, which carry 13 and 16 chlorophyll *a* (Chl) molecules , respectively. The RC D1/D2 proteins are located approximately symmetrically with respect to transmembrane region, which is very similar to the arrangement of the L/M subunits in bacterial RC (BRC) [3,4]. 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 [5,6]. As in the BRC, electron transfer in PSII is known to proceed only along D1 branch with the formation of $P680^{+}Pheo_{D1}^{-}$ and then $P680^{+}Q_A^{-}$ [7–11].

In accordance with generally accepted notions, in BRC the initial species which accepts excitation energy from antenna is the bacteriochlorophyll dimer P870 transforming it to the energy of charge separated state $P870^{+}B_A^{-}$. However it was suggested that excitation of the accessory BChl B_A in the YM210W mutant RC led to a significant amount of $P^{+}B_A^{-}$ formation in less than 1 ps, without involvement of P*. Then $P^{+}B_A^{-}$ decays into $P^{+}H_A^{-}$ on a few ps timescale similar to WT BRC [12]. However this suggestion seems unlikely because B_A* should transfer excitation energy to P870 within 100 fs [13]. The formation of $P^{+}B_A^{-}$ without involvement of P* would occur with quantum yield < 3%. To

Abbreviations: RC, reaction center; BRC, bacterial reaction center; B_A, bacteriochlorophyll, primary electron acceptor in BRC; PSII, photosystem II; D1/D2/Cytb559, PSII RC; Chl, chlorophyll *a*; P680, Chl dimer, primary electron donor in PSII; Chl_{D1}, Chl located in D1 protein subunit; ET, electron transfer; Pheo, pheophytin *a*; $Pheo_{D1}$, Pheo located in D1 protein subunit; Q_A, primary plastoquinone electron acceptor

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overcome this discrepancy, one might assume that the vibronic energy of P870* (P870^{*vib}) released as an energy difference between B_A* and P* promotes the electron transfer between P870^{*vib} and B_A in the YM210W mutant. This process can be considered as a modification of normal electron transfer pathway in BRC.

The consideration of accessory (B)Chl* as a primary electron donor not only for BRC but also for PSII RC causes a number of problems (see [1] for discussion) and requires convincing proof which is currently not available.

However, according to the current consensus based on the mentioned YM210W BRC experiments, the accessory Chl on the D1 branch (Chl_{D1}) is considered as a true primary electron donor in the PSII. In the framework of this hypothesis the electron transfer starts with the formation of the pair $Chl^{+}_{D1}Pheo^{-}_{D1}$ both at cryogenic temperatures and at physiological conditions [14–18]. This suggestion was supported by visible/midinfrared pump-probe experiments, which have shown the initial formation of the radical pair $Chl^{+}_{D1}Pheo^{-}_{D1}$ in a significant fraction of the PSII RCs on a sub-picosecond timescale ([14,17] and references therein). The formation of P^{+}_{D1} was observed only after 5–6 ps followed by radical pair relaxation. It is possible that the observed multiexponential kinetics of the charge separation is at least partially due to the small energy differences between most of the excited and charge separated states of the PSII RC. Later [15,16] it was suggested that the primary electron transfer towards Phe_{D1} can in principle start from P_{D2}, Chl_{D1}, or P_{D1} producing different first charge-separated configurations $P^{+}_{D2}P^{-}_{D1}$, $Chl^{+}_{D1}Phe^{-}_{D1}$, or $P^{+}_{D1}Chl^{-}_{D1}$, respectively.

On the other hand, it was shown that in PSII RCs [19] and PSII core complexes [1] excited by 20-fs pulses centered at 700–710 nm the initial electron transfer reaction took place within ~1 ps from P680* with formation of the $P680^{+}Chl^{-}_{DI}$ charge-separated state, as indicated by ~1 ps bleaching and ~14 ps relaxation of the 670-nm band that was tentatively assigned to the Chl_{D1} absorption. The relatively long rise (~1 ps) and decay (~13 ps) times of Chl_{D1} bleaching are not consistent with two-photon processes, Raman scattering or other optical effects. The subsequent electron transfer from Chl^{-}_{D1} occurred within ~13 ps and was accompanied by a development of the radical anion band of $Pheo_{D1}$ in the blue region, assigned to formation of the secondary radical pair $P680^{+}Pheo^{-}_{D1}$. According to this model, the energy transfer from P680* to Chl_{D1} (Chl-670) is suppressed which allows an effective primary charge separation and stabilization of separated charges.

In the earlier papers describing the photochemical accumulation of $Pheo^{-}$ in PSII at room and low temperatures [10,11] it was claimed that this reaction can be related to the primary charge separation in RC with the formation of $P680^{+}Pheo^{-}$ by analogy with bacterial RCs (see below). The formation of $P680^{+}Pheo^{-}$ should be proved by fast (fs/ps/ns) transient absorption spectroscopy used in a number of papers [19–23], but it still requires more detailed analysis of results with high signal/noise ratio. The formation of $P680^{+}Chl^{-}_{D1}$ (Chl_{D1} according to [1] is Chl-670) should be supported by a number of approaches since this problem is critical for elucidation of the mechanism of the primary charge separation. In addition, important properties concerning kinetics and amplitude of anion radical band of $Pheo^{-}$ near 460 nm should be clarified.

In this paper, the spectrum of $Pheo^{-}$ formation in the picosecond time domain (as well as accumulation of $Pheo^{-}$ in the second time domain [10,11]) was analyzed with respect to the 670-nm bleaching (or redshift) upon $Pheo^{-}$ formation. Important properties concerning kinetics and amplitude of anion radical band of $Pheo^{-}$ near 460 nm were clarified.

The questions concerning the nature of pairs $P680^{+}Pheo^{-}_{DI}$ and $P680^{+}Chl^{-}_{DI}$ are: whether both pairs have ion-radical character, whether these pairs serve as excited state quenchers and whether the spectra ascribed to the ion-radical pairs comprise the contribution from excited states?

To answer these questions the fs/ps measurements of differential absorption changes at different delays were performed at 278 K with excitation of PSII core complexes by 20 fs pulses at 710 nm.

2. Materials and methods

2.1. Preparation

Oxygen-evolving core complexes of PSII were isolated from spinach by the method described in [24]. The purified preparations were suspended in a buffer consisting of 20 mM Bis–Tris (pH 6.5), 20 mM $MgCl_2$, 5 mM $CaCl_2$, 75 mM $MgSO_4$, 0.03% (w/v) *n*-dodecyl-β-D-maltoside and 400 mM sucrose. The light-saturated oxygen evolution rate measured with a Clark-type electrode (Hansatech) at 297 K was typically 1000–1300 μmol O_2 (mg of Chl)$^{-1}$ h^{-1} with 1 mM $K_3Fe(CN)_6$ and 250 μM 2,5 dichloro-*p*-benzoquinone (DCBQ) as electron acceptors. The core complex absorption was close to ~2 OD at the Q_y band maximum 675 nm in femtosecond measurements. The measurements were done at 278 K. As mentioned earlier [1] absorption spectra measurements have shown that at 90 K a shoulder around 710–730 nm is observed for PSII core complexes in contrast to isolated PSII RCs.

2.2. Femtosecond laser photolysis setup

Transient absorption spectra were measured by the femtosecond pump to 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) at a repetition rate of 1 kHz as described earlier [1]. The amplified pulses were splitted into two beams. One of the beams was directed into a noncollinearly phase-matched optical parametric amplifier. Its output centered at 710 nm was compressed by a pair of quartz prisms. The gauss pulse of 20 fs at 710 nm with the bandwith of ~40 nm (full width at half-maximum) was used as a pump. 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 using a computer-controlled delay stage. They were then attenuated, recombined, and focused onto the sample cell. The pump and probe light spots had the diameters of 300 and 120 μm, respectively. The pump pulse energy was attenuated at 50 nJ to get optimal excitation on a linear part of the light curve. Experiments were carried out at 278 K in 0.5-mm path length flow optical cell. Frequency control of laser pulses was produced by regular device synchronization and control amplifier SDG II Spitfire 9132, manufactured by Spectra-Physics (USA). The device allowed to change the pulse repetition frequency of the amplifier output from 0 to 1000 Hz.

The pump pulse operation frequency was 50 Hz, which is sufficiently low to exclude permanent bleaching of the sample due to photochemical processes in RC. Together with operation frequency the circulation rate in the flow cell was fast enough to avoid multiple excitation of the same sample volume. The relative polarizations of pump and probe beams were adjusted to 54.7° (magic angle) or in parallel and perpendicular polarizations, where indicated. After the sample, the supercontinuum was dispersed by a polychromator (Acton SP-300) and detected by CCD camera (Roper Scientific SPEC-10). Transient spectra of absorbance changes ΔA (t, λ) were recorded over the range of 400–740 nm. The measured spectra were corrected for group delay dispersion in the supercontinuum using the procedure described previously [25]. Anisotropy of differential absorption ΔA (λ, t) was determined as $P = (\Delta A - \Delta A\perp) / (\Delta A + 2\Delta A\perp)$.

3. Results

The difference absorption spectra (ΔA) obtained with isolated spinach PSII core complexes at 278 K in the range of 400–725 nm at various delays in ps time domain (from 44 ps to 455 ps) are shown in Fig. 1. The spectra include the Chl/Pheo bleaching around 420–450 nm, at 545 nm and at 685 nm and the developments of Chl/Pheo anion radical bands at 460 and 660 nm. These latter two bands decreased from 44 ps to 455 ps indicating the electron transfer from $Pheo^{-}$ to Q_A. The electron transfer

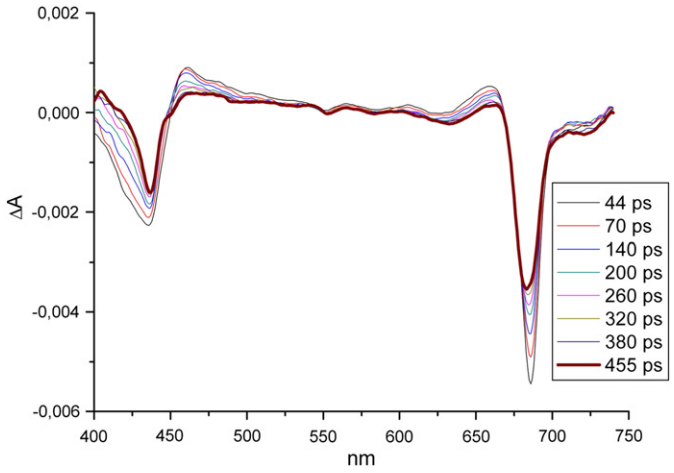

Fig. 1. Differential absorbance changes ΔA in PSII core complexes at 278 K excited by 20-fs pulses at 710 nm at different delays indicated on the inset. The spectral region around 545–550 nm represents a sum of C550 (the blue shift of $Pheo_{D1}$ absorption caused by Q_A reduction) and of the bleaching of $Pheo_{D1}$ band due to its photoreduction.

to Q_A should be completed within 455 ps [21]. It means that the transient spectrum at 455 ps delay corresponds to $P680^+$ formation and this spectrum can be considered as spectral feature of $P680^+$ differential spectrum (the difference absorption spectrum for Q_A^- formation does not have remarkable features in visible range).

Fig. 2 shows the difference between the transient spectra measured at 23 ps or 44 ps and the spectrum measured at 455 ps, which is ascribed to $P680^+$ difference spectrum. Since during this time range the only electron transfer event is the formation of $P680^+Q_A^-$, this ΔΔA spectrum can be assigned to the $Pheo^-$ formation and has the bleaching at 420 nm, 450 nm, 545 nm, 671 nm and 685 nm and the developments at 460 nm and 660 nm. This spectrum is similar to the spectra previously obtained by accumulation methods [10,11] and therefore proves that charge separation between $P680^*$ and Pheo is accompanied by the entire transfer of electron density from $P680^*$ to Pheo in ps time domain. No additional electron acceptors were observed between Pheo and Q_A in the time domain between 23 ps and 44 ps. This also proves that the accumulation method applied previously for the study of PSI RC [26], bacterial RC [27] and PSII RC [10,11] demonstrates the photochemical reactions in RCs.

The more detailed analysis of the spectra presented in Fig. 2 also shows additional bleaching at 670 nm which is assigned to Chl-670 [1]. This feature indicates the close position of the $Pheo_{D1}$ to Chl-670 molecule, which was suggested to play a role of the primary electron acceptor Chl_{D1} functioning between $P680^*$ and $Pheo_{D1}$ [1]. The bleaching at 450 nm probably reflects the relaxation of the Chl excited state, in which spectrum remarkably differs in the blue region from that of $P680^+$.

The kinetics of the $Pheo^-$ band at 460 nm in fs/ps time domain (see Fig. 1 for ps delay) can be plotted for estimation of the lifetimes of rise and decay of the $Pheo^-$ band (or electron transfer from $Pheo^-$ to Q_A). The kinetics plotted in Fig. 3 suggests that anion radical $Pheo^-$ appears within 11 ± 2 ps and decays within 250 ± 55 ps in agreement with earlier measurements [22]. The rise time ascribed to the appearance of $Pheo^-$ indicates that no faster photoreaction involving Pheo is observed which is not consistent with the suggestion claimed in [14–18]. However the flash-induced spectral changes ascribed to photoreduction of Chl-670 (Chl_{D1}^-) were observed within ~1 ps [1]. The spectral features typical to Chl_{D1}^- decayed with lifetime of ~13 ps which is in good correlation with the appearance of $Pheo^-$ (lifetime 11 ± 2 ps, Fig. 3).

Another possibility for detection of $Pheo^-$ formation is revealed from the analysis of the stimulated emission decay near 685 nm. Fig. 4 shows that the stimulated emission, measured with parallel orientation of electric vectors of the excitation and measuring beams, has at least two kinetic phases of decay with lifetimes of 1 ± 0.2 ps and 13 ± 2 ps (initial anisotropy is ~0.18). The lifetime of the first component is consistent with the formation of $P680^+Chl_{D1}^-$ [1] and the second one — with the formation of $P680^+Pheo^-$ (Fig. 3). This result shows that the first ion-radical pair is a quencher of the excited state of pigments. This observation proves that charge separation within ~1 ps in PSII RC does not involve electron transfer to Pheo. The kinetics of stimulated emission decay registered with perpendicular orientation of beams shows the appearance of new component with remarkably less positive polarization (anisotropy is ~0.05) than the first component with lifetime of ~1 ps observed at parallel orientation. The appearance of this component was accompanied by absolute increase of stimulated emission amplitude with perpendicular orientation which is quite unusual for decaying emission. Since about 30% of the stimulated emission decays with significantly longer lifetime of ~13 ps, we suggest that the formation of $P680^+Chl_{D1}^-$ state can be considered as a mixture of the charge transfer $P680^+Chl_{D1}^-$ and excited P680* states. The mixture of states can be presented as: $P680^{(1-\delta)*}$ ($P680^{\delta+}Chl_{D1}^{\delta-}$) where δ is ~0.5. This mixed state can be observed as stimulated emission at

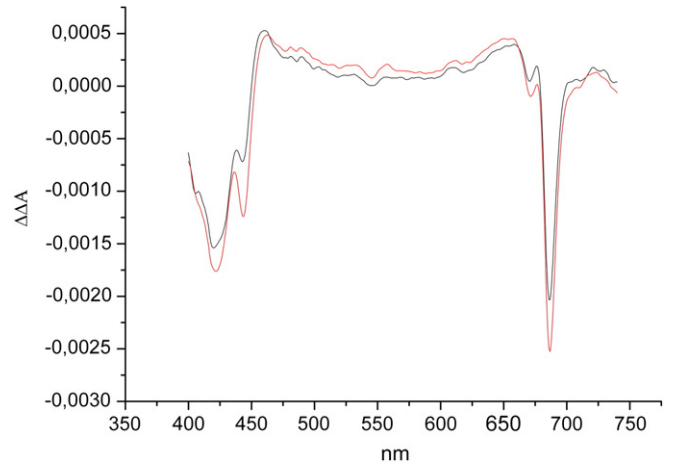

Fig. 2. Difference ΔΔA spectra, obtained by subtraction of ΔA spectrum at 455 ps delay ($P680^+$ spectrum) from ΔA spectra at 23 ps (red) and 44 ps (black) delays. The bleaching at 545 nm reflects formation of the $Pheo^-$. The subtraction procedure eliminates the blue shift of $Pheo_{D1}$ absorption caused by Q_A reduction (observed in Fig. 1). For other experimental conditions see legend in Fig. 1.

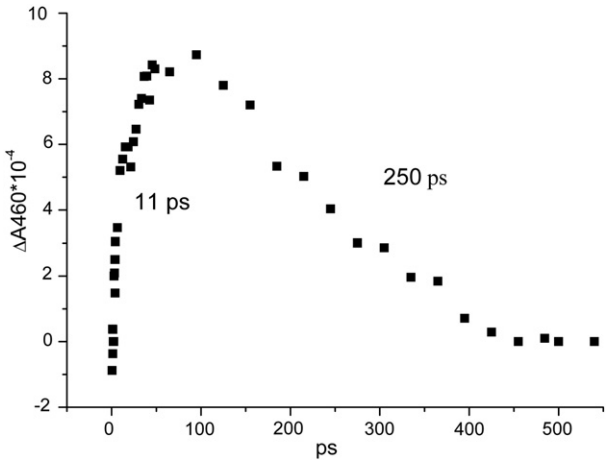

Fig. 3. Kinetics of absorbance changes ΔA in PSII core complexes at 460 nm. The rise time of the formation of the $Pheo^-$ band at 460 nm is 11 ± 3 ps. The decay of the $Pheo^-$ has a lifetime of 250 ± 50 ps.

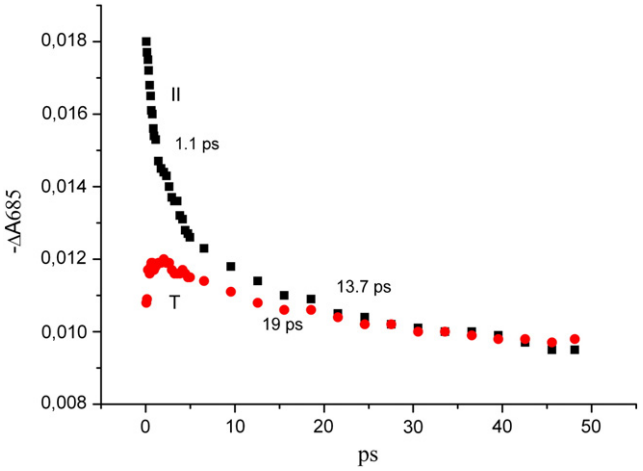

Fig. 4. Kinetics of absorbance changes −ΔA in PSII core complexes at 685 nm. The kinetics represents a sum of $P680^+$ formation and stimulated emission from P680* and/or $Pheo_{D1}$*. The rise time of the bleaching at 685 nm is less than 20 fs for both parallel and perpendicular orientations of electric dipole moments for excitation and measuring beams. The decay kinetics of the band for parallel polarization has two components with lifetimes of 1.1 ± 0.3 ps and 14 ± 3 ps. For perpendicular polarization the kinetics is characterized by small positive (lifetime ~2.5 ps) and negative (lifetime 19 ± 4 ps) components.

~685 nm, which decays due to further electron transfer from $Chl_{D1}^{\delta-}$ to Pheo within ~13 ps. However it cannot be ruled out that the two-component kinetics of the stimulated emission decay is due to the existence of two populations of PSII RCs decaying with different lifetimes (see [28] for further discussion).

4. Discussion and conclusion

From the data presented earlier [1] and here we can conclude that:

1. The formation of anion-radical of Pheo ($Pheo^-$) is accompanied by the appearance of band at 460 nm within 11 ± 3 ps (Fig. 3). This is consistent with the disappearance of the state $P680^+Chl_{D1}^-$ with similar lifetime reflecting the electron transfer from Chl_{D1}^- to Pheo [1].
2. The $P680^+Pheo_{D1}^-$ state disappears within 250 ± 50 ps due to electron transfer from $Pheo_{D1}^-$ to Q_A. The formation of $P680^+Pheo_{D1}^-$ can be confirmed by the subtraction of the spectrum of state $P680^+$ observed at 455 ps delay from the spectra of $P680^+Pheo_{D1}^-$ state observed at 23 ps or 44 ps delay. The obtained spectra are very similar to the spectrum of $Pheo_{D1}^-$ observed previously by the accumulation method [10,11]. The bleaching bands and pigment spectral shifts are independent for the formations of $P680^+$ and $Pheo^-$, which allows observing the additive sum of its individual features in ΔA spectra at 23 ps and 44 ps delays. This situation is different for bacterial RCs, where intensive electrochromic shift of the B_A band at 800 nm is observed upon formation of P^+ and $BPheo^-$ ions [27].
3. The bleaching (or red shift) of the 670-nm band in the spectrum of $Pheo^-$ formation obtained by fs/ps measurements (here) or accumulation method for room and low temperatures [10,11,29] indicates significant interaction and close arrangement of $Pheo_{D1}$ and Chl-670 molecules.
4. The bleaching of the 670-nm band (675 nm at low temperature [1]) in the spectra of $P680^+$ formation measured in PSII RC and core complexes [1,19] indicates the nearby location of P680 and Chl-670 molecules.
5. Taking into account the last two observations we can conclude that Chl-670 is located in the vicinity of both P680 and $Pheo_{D1}$. The bleaching of 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 expression for dipole strength (D) of the excitonic band in aggregate [30], 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:

$$D_A = B - Cv_\alpha v_\beta / \left(v_\beta{}^2 - v_\alpha{}^2\right), \quad (1)$$

where ν_α and ν_β are frequencies of the transitions in two interacting molecules α and β, D and B are positive, and C is suggested to be positive constant. If ν_α is frequency for the 670-nm transition in Chl_{D1} and ν_β for 680-nm transition in P680 (or $Pheo_{D1}$), then the D value increases for 670-nm transition and decreases for 680-nm due to excitonic interaction in the aggregate. If the interaction is broken by photochemistry in PSII RC, then D_A drops to a value characteristic of the lack of interaction between 670- and 680-nm transitions.
Since both oxidation of P680 and reduction of $Pheo_{D1}$ were accompanied by the decrease of the 670-nm transition amplitude, we conclude that Chl-670 is located between P680 and $Pheo_{D1}$ and may play a role of the intermediary electron carrier Chl_{D1} between P680* and $Pheo_{D1}$ as was shown earlier [1].
6. The decay kinetics of the stimulated emission at ~685 nm (Fig. 4) indicates the existence of at least two emitting centers. The first center with remarkable positive polarization (~0.18) appears to reflect the emission from the excited states of P680* and/or $Pheo_{D1}$*. This emission decays with a lifetime of ~1 ps due to electron transfer from P680* to Chl_{D1} with the formation of mixed state $P680^{(1-\delta)*}$ ($P680^{\delta+}$-$Chl_{D1}^{\delta-}$) (see [1]). This latter state (second center of emission) emits light at 685 nm with smaller positive polarization (~0.05). The decrease of the component with parallel polarization is accompanied by the simultaneous increase of the same component with perpendicular polarization $-\Delta A_{685}$ (Fig. 4). This evidently shows the formation of the new emitting center at 685 nm which is probably caused by the formation of the state $P680^{(1-\delta)*}$ ($P680^{\delta+}Chl_{D1}^{\delta-}$) where δ is about 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 [1].

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