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Primary charge separation in closed Photosystem II with a lifetime of 11 ns. Flash-absorption spectroscopy with O_2-evolving Photosystem II complexes from *Synechococcus*

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Absorption changes induced by picosecond laser flashes have been studied in purified O_2-evolving Photosystem II complexes from *Synechococcus* sp. If the first quinone acceptor, Q_A, is reduced before the flash, we observe a rapid phase with approx. 1 ns lifetime which reflects the decay of excited singlet states of antenna pigments, followed by a clearly separated phase of approx. 11 ns. Based on measurements made between 420 nm and 820 nm the 11 ns phase is attributed to the decay of the radical pair, chlorophyll a_{II}^{+} pheophytin a^{-} (Chl a_{II}^{+} Pheo^{-}). It decays by charge recombination to the ground state and a small fraction (approx. 20%) to the triplet state ^{3}Chl a_{II} Pheo via ^{3}(Chl a_{II}^{+} Pheo^{-}). From the absence of a significant fluorescence decay component with approx. 11 ns lifetime we conclude that the observed radical-pair recombination does not give rise to regeneration of the excited singlet state of Chl a_{II} and cannot account for the variable fluorescence. The yield of radical-pair formation is estimated to be approx. 60% ± 15% of the yield of Chl a_{II}^{+} Q_A^{-} formation in open reaction centers. The data can be explained by a model which is based on the assumption that the radical pair initially generated in closed reaction centers has a higher free energy relative to that in open centers due to the negatively charged Q_A, but relaxes rapidly to an energetically more favorable state with 11 ns lifetime. The model predicts that the yield of radical pair formation increases with decreasing antenna size. The triplet state of carotenoids, ^{3}Car, was monitored at 515 nm in closed Photosystem II. ^{3}Car was formed with low yield compared to the yield of radical-pair formation. Its rise time of about 25 ns is attributed to triplet energy transfer from chlorophyll triplets in the antenna.

Introduction

Absorption of light by photosynthetic organisms raises an antenna pigment to an excited singlet state. The excitation energy is transferred on a picosecond time scale to a special pigment, P, the primary electron donor of the reaction center. In Photosystem II (PS II) this primary donor is a special chlorophyll *a*, called Chl a_{II} or P-680. From the lowest excited singlet state of Chl a_{II} an electron-transfer reaction leads to the oxidation of Chl a_{II} and the reduction of the first stable acceptor, a special plastoquinone, Q_A.

There is strong evidence that a pheophytin *a* serves as intermediary acceptor, called I, located between Chl a_{II} and Q_A. This was at first suggested because of the observation that strong continuous illumination at low redox potential, i.e.,

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Abbreviations: Car, carotenoid; Chl, chlorophyll; I, intermediary electron acceptor; Mes, 4-morpholineethanesulphonic acid; P, primary electron donor; Pheo, pheophytin *a*; PS II, Photosystem II; Q_A, primary quinone acceptor; Tris, 2-amino-2-hydroxymethylpropane-1,3-diol.

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Q_A was chemically reduced, caused the accumulation of the pheophytin radical anion in PS II, although with a low quantum yield [1]. The radical anion has been characterized by EPR signals [2] and its differential absorption spectrum [1,2].

The low quantum yield has been explained by the competition between a fast recombination of the radical pair, Chl a_{II}^{+} $Pheo^{-}$, and electron donation to Chl a_{II}^{+} by a secondary donor [2]. Additional evidence for radical pair formation in closed PS II came from the observation of a light-induced spin polarized triplet state of Chl a_{II} which is characteristic for the radical pair mechanism [3]. Recently, picosecond measurements have directly shown that pheophytin is an intermediary electron acceptor between Chl a_{II} and Q_A [4,5]. Absorption changes with a time constant of 250–500 ps were attributed to the reoxidation of $pheophytin^{-}$ [4,5]. Most probably $pheophytin^{-}$ transfers its electron directly to Q_A, as reduction of Q_A has been shown to occur within less than 1 ns [6]. Double-flash experiments led to the conclusion that there is no stable electron acceptor accessible in PS II as long as Q_A is reduced [7].

It has been known for a long time that reduction of Q_A (state PIQ_A^{-}, also called closed center) causes an approx. 3-fold increase of the fluorescence yield, compared to open centers (state PIQ_A). In connection with the discovery of the intermediary acceptor I it has been suggested that the additional or variable fluorescence is actually recombination luminescence [1]. According to this suggestion, excitation of closed PS II centers leads to the state $P^{+}I^{-}Q_A^{-}$, which recombines to PIQ_A^{-}. One decay pathway is the back reaction, $P^{+}I^{-} \rightarrow P^{*}I$, regenerating the excited singlet state of the primary donor, P^{*}, which may equilibrate with the antenna chlorophylls. The decay of the excited singlet state generated by back reaction should cause the variable fluorescence. Its decay kinetics should be approx. the same as that of the radical pair.

The proposal was supported by measurements of fluorescence decay kinetics and flash-induced absorption changes in spinach chloroplast fragments enriched with PS II [8]. After prior reduction of Q_A, absorption changes with about 4 ns lifetime were attributed to Chl a_{II}^{+} $Pheo^{-}$ decay and a fluorescence decay component with 4.3 ns lifetime was found [8]. Picosecond absorption change measurements have been subsequently reported, indicating radical-pair decay with a lifetime of 2 ns, if Q_A is reduced [4]. Single photon-counting measurements with refined time resolution showed that the slowest significant fluorescence decay phase has a lifetime of only 1.2–2.3 ns (for review see Ref. 9). If the back reaction generating Chl a_{II}^{*} requires energy, the yield of variable fluorescence should decrease upon lowering of the temperature. Experimental results concerning this point are controversial [10,11].

The suggestion that the variable fluorescence originates from radical pair recombination has been contradicted in subsequent reports. The fluorescence of algae mutants lacking PS II reaction center proteins shows a long-lived decay (approx. 2 ns) similar to that in closed PS II of the wild type, indicating that charge recombination is not essential to produce such a long-lived component [12]. Furthermore, it was reported that the fastest fluorescence decay phase ($\tau \approx 200$ ps), which predominates for open reaction centers, decreases upon reduction of Q_A [13]. If the primary radical pair formation occurs also in reaction centers with Q_A in the reduced state, one would expect that the fastest phase, which is correlated to the trapping of the excitation in the reaction center, remains approximately unchanged upon reduction of Q_A [13].

From these findings it was proposed [14] that the efficiency of the radical pair formation is decreased, if Q_A is in its reduced state. It results a less effective trapping by the primary photochemical charge separation and a longer lifetime of the excitation in the antenna giving rise to the variable fluorescence [14]. Picosecond absorption change measurements performed by the same authors were taken as evidence that the yield of radical pair formation is low upon reduction of Q_A [5]. According to their interpretation, the lifetime of the radical pair should coincide with the time constant of the long-lived fluorescence decay phase ($\tau = 1.3$ ns) [5].

In the present work we reinvestigated the reaction sequence in closed PS II by flash absorption spectroscopy. Due to the use of purified O_2-evolving PS II complexes from *Synechococcus* and improvement of our measuring equipment, flash-induced absorption changes could be measured in a wide spectral range with a time resolution of 1 ns. We found a prominent decay phase with a lifetime of 11 ns which can be clearly attributed to charge recombination in the radical pair, Chl a_{II}^{+} Pheo^{-}. This recombination is not the origin of the variable fluorescence. The results of this work have been presented already at the "51. Physiker Tagung", Berlin, March 30–April 3, 1987.

Materials and Methods

The thermophilic cyanobacterium *Synechococcus* sp. was grown as described by Rögner et al. [15]. Oxygen-evolving PS II particles were extracted, in principle, according to the methods described by Schatz and Witt [16]. The crude extract was further purified by sucrose density-gradient centrifugation as described by Rögner et al. [15] in order to remove most of the phycobiline pigments. The PS II preparations were finally obtained in $2 \cdot 10^{-2}$ M Mes/NaOH (pH 6.5); 10^{-2} M $MgCl_2$, $2 \cdot 10^{-2}$ M $CaCl_2$ and about 1 M sucrose and stored at $-80\,°C$. The PS II complexes are characterized by a PS II/PS I ratio of more than 20, a Chl/P-680 ratio of about 60, and O_2-flash yield of about $2.5 \cdot 10^{-3}$ O_2 per Chl and flash, corresponding to 100 Chl per Chl a_{II} active in O_2 evolution. Deviations in different preparations were within ± 30% of these values. For Tris-treatment, the crude extract was diluted with 2.4 M Tris/HCl (pH 8.3) solution in a ratio of 2 : 1 (v/v) and incubated for 15 min in daylight at room temperature. For purification a sucrose gradient centrifugation (20–40% sucrose in 10^{-2} M Tris/HCl (pH 8.0), 10^{-2} M $MgCl_2$ and $2 \cdot 10^{-2}$ M $CaCl_2$) was performed. The purified Tris-treated extract was dialyzed against the reaction medium (see below) and stored at $-20\,°C$ before further use.

For the measurement of flash-induced absorption changes the stock solutions were diluted with the reaction medium containing $5 \cdot 10^{-2}$ Mes/NaOH (pH 5.5–7.0), 10^{-2} M $MgCl_2$, $2 \cdot 10^{-3}$ M KH_2PO_4 and 0.5 M mannitol. Potassium ferricyanide (10^{-3} M) and phenyl-*p*-benzoquinone (10^{-4} M) were used as electron acceptors. For measurements with the first stable quinone acceptor in the reduced state, reduction was achieved either by addition of $Na_2S_2O_4$ (approx. 1 mg/ml) or by light in the absence of electron acceptors. All measurements were performed at room temperature.

For measurements of absorption changes at 824 nm and 780 nm laser diodes (TXTF 8300 from AEG Telefunken and CQL 16 from Philips) were used as measuring light source. The light passed through the sample and an interference filter. It was detected by a photodiode (Lasermetrics Series 3117 high speed photodetector). The signals were amplified (amplifier TV 83/10408C from Telemeter), digitized (Tektronix 7912 AD with plug-in 7B10 and 7A29) and transmitted to an Apple II microcomputer. The electrical bandwidth was 1 kHz to 350 MHz.

Absorption changes between 610 and 710 nm were measured using the apparatus which has been described previously [17], with the following modifications. The measuring light beam was pulsed by an optical modulator (LM0202P5W from Gsänger) driven by a video amplifier (from d.c. to 6 MHz, Model VLA30 from Electro-Optic Developments) to which squarewave pulses from a Wavetek pulse generator (Model 166) were applied (pulse duration, 40 μs; modulation of the light intensity was by a ratio of about 200 : 1). The same detection system as described above was used, except that a d.c. current was fed into the load resistor in order to balance the photocurrent during the measuring light pulse. The electrical bandwidth was from d.c. to 350 MHz. Part of the measurements were performed with continuous measuring light in the absence of electron acceptors. Under these conditions Q_A was kept in the reduced state by the measuring light.

For measurements between 410 nm and 490 nm a xenon flash lamp (FXP-850 from EG&G) was used as measuring light source. The lamp discharged an energy of about 25 J. A home-built pulse-shaping network provides a flash with 60 μs FWHM. The measuring beam passed through an interference filter, the sample and interference- and color-glass filter. The measuring light was focused and detected by a photodiode (FND-100Q). The photocurrent in the plateau of the xenon flash was balanced by a d.c. off-set current fed to the load resistor. Further signal processing was as described above. The electrical bandwidth was from d.c. to 250 MHz.

The formation and decay of the carotenoid triplet was monitored at 514.5 nm using a cw-Ar ion laser (Coherent Innova 90/5) equipped with an etalon unit. The set-up regarding the measuring beam was similar to the one described for measurements in the 680 nm region. By the restriction of the solid angle between the sample and the photodiode and a protecting narrow band interference filter, scattered exciting light (532 nm) did not interfere with the measurement of absorption changes at 514.5 nm. The detection system was the same as described for 824 nm.

For the measurements described so far, the sample was excited at 532 nm by a frequency-doubled Nd/YAG laser (Model 471D with an acousto-optic mode locker from Quantel). The excitation pulse had a pulse-width of approx. 35 ps and a pulse energy of 3–5 mJ.

For measurements of the spectrum of carotenoid triplet minus carotenoid ground state in the wavelength region between 460 nm and 570 nm, the sample was excited at about 640 nm by a dye laser (Lambda Physics) with $6 \cdot 10^{-4}$ M DCM (Exciton) solved in methanol as a dye. The dye was pumped by 3 ns (FWHM) laser flashes at 532 nm from a frequency-doubled Nd/YAG laser (YG 441 from Quantel). That gave pulses lasting about 3 ns with a pulse energy of about 20 mJ. For measurements in the nanosecond time range the apparatus with the Xe-flash lamp was used (see above), except that the signals were digitized by a Biomation 6500 transient recorder with 2 ns/point and stored or averaged by a Nicolet 1170 signal averager. The electrical bandwidth was from d.c. to 100 MHz. For measurements of the decay of the carotenoid triplet a Tungsten lamp was used as a measuring light source. The measuring light was detected by a photodiode (SGD 444 from EG&G) loaded with 500 Ω. The signals were amplified (Tektronix AM 502) and digitized by the Biomation 6500 transient recorder with 50 ns per point. The electrical bandwidth was 2 Hz to 1 MHz.

The signals were transmitted to an Apple II microcomputer. Data analysis was carried out by reiterative convolution [18,19]. The instrumental response function was obtained by monitoring scattered excitation light. The signals were deconvoluted into a sum of two exponentials and a constant. The fitting procedure minimizes the sum of the unweighted least squares.

Results

Fig. 1 shows the time-course of the absorption changes at 680 nm in PS II particles from *Synechococcus* sp. with Q_A in the oxidized (Fig. 1 top) and in the reduced state (Fig. 1 bottom) measured under otherwise identical conditions. The inset shows the instrumental response to the picosecond excitation laser flash. In reaction centers with oxidized Q_A (Fig. 1 top), excitation results in a

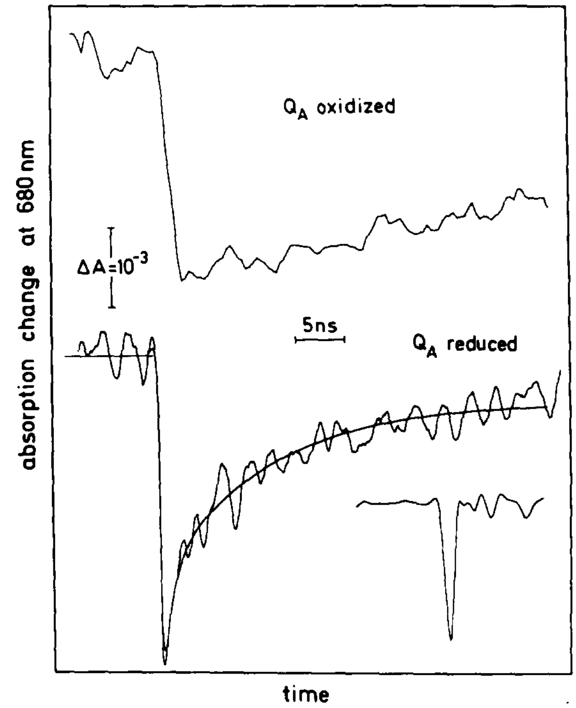


Fig. 1. Time-course of flash-induced absorption changes at 680 nm in O_2-evolving PS II complexes from *Synechococcus* with Q_A in the oxidized (top) and in the reduced (bottom) state. The inset in the bottom right corner shows the instrumental response to the picosecond excitation flash obtained by monitoring scattered excitation light at 532 nm. Excitation by non-saturating laser flashes (35 ps FWHM, 532 nm, repetition rate 10 Hz), optical pathlength of the pulsed measuring light, 1 cm, $5 \cdot 10^{-6}$ M Chl, 256 averages; top: 10^{-4} M phenyl-*p*-benzoquinone and 10^{-3} M $K_3(Fe(CN)_6)$ added as electron acceptors; bottom: Q_A reduced by addition of dithionite. The solid line represents the best fit according to Eqn. 1. The fit parameters are given in the text.

stable charge separation. The absorption decrease at 680 nm reflects the photooxidation of Chl a_{II}. The rise-time is determined by the resolution of the apparatus ($t_{10/90} \approx 900$ ps). The decay is due to the re-reduction of Chl a_{II}^{+} by the immediate donor in O_2-evolving PS II particles under repetitive flash excitation [17]. With Q_A in the reduced state, the recovery of the flash-induced absorption decrease is accelerated. A very rapid phase is followed by a prominent phase with about 11 ns lifetime. Absorption changes with slower decay kinetics are rather small. For a quantitative analysis of the decay kinetics the signal was deconvoluted using the convolution function

$$\Delta A(t) = \int_0^t I(t')\left\{\Delta A_1 \exp\left(-\frac{t-t'}{\tau_1}\right) + \Delta A_2 \exp\left(-\frac{t-t'}{\tau_2}\right) + \Delta A_3\right\} \mathrm{d}t' \qquad (1)$$

where $I(t')$ presents the instrumental response function (see Fig. 1, inset). The five parameters ($\Delta A_i (i = 1\text{–}3)$, τ_1, τ_2) and the zero time of $I(t')$ with respect to the signal were adjusted. The fit yielded the following parameters $\Delta A_1 = -2.1 \cdot 10^{-3}$, $\tau_1 = 923$ ps, $\Delta A_2 = -2.2 \cdot 10^{-3}$, $\tau_2 = 11.4$ ns, $\Delta A_3 = -5.4 \cdot 10^{-4}$. The very rapid phase adapted by the fastest exponential reflects the instrumental response to not-resolved components with lifetimes shorter than 2 ns. The decay of the excited singlet state of chlorophyll *a* contributes to this decay phase (not shown). The lifetime, τ_1, obtained by the fitting procedure varied between 600 ps and 1.7 ns for different measurements. This variation may be due to differences in the preparations and in the excitation energy.

For the slower phase the fits yielded lifetimes of $\tau_2 = 11$ ns $\pm$ 3 ns. Because of this comparatively long lifetime, excited singlet states can be excluded as the origin of this decay phase. At 680 nm the amplitude of the 11 ns phase is about 70% $\pm$ 15% of the absorption change observed for the formation of $P^+Q_A^-$ under normal conditions (Q_A oxidized before the flash) (see Fig.1, top). If the sample was illuminated in the presence of dithionite and hydroquinone by strong continuous light, the flash-induced 11 ns phase disappeared almost completely (Fig. 2, upper trace). Turning off the light and leaving the sample in the dark for several minutes, we again observed the 11 ns phase with the same magnitude as prior to the strong background illumination (Fig. 2, lower trace).

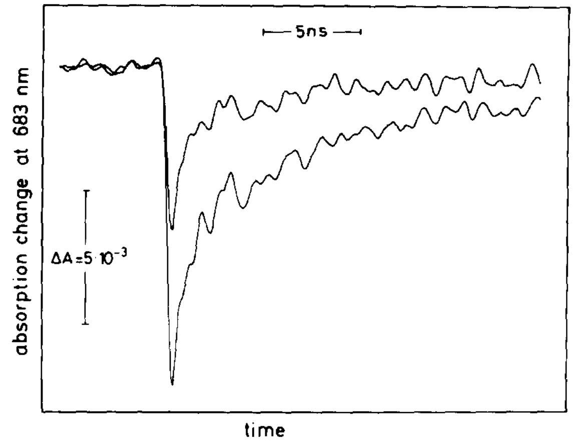


Fig. 2. Time-course of flash-induced absorption changes at 683 nm in the presence of dithionite and 10^{-3} M hydroquinone during and 10 min after strong continuous illumination (upper and lower trace); $13.5 \cdot 10^{-6}$ M Chl, 256 averages; other conditions as in Fig. 1.

For an identification of the 11 ns phase, two possibilities suggest themselves. (I) Triplet energy transfer: chlorophyll triplet is formed by intersystem crossing from the excited singlet state. The quantum efficiency for triplet formation is increased similar to the fluorescence yield upon reduction of Q_A. The chlorophyll triplet decays by triplet energy transfer to a carotenoid ('triplet valve' [20]), preventing the production of singlet oxygen via the reaction $^3\mathrm{Chl} + O_2 \rightarrow \mathrm{Chl} + O_2\,(^1\Delta g)$. The 11 ns phase could be attributed to the reaction $^3\mathrm{Chl} + \mathrm{Car} \rightarrow \mathrm{Chl} + {}^3\mathrm{Car}$. (II) Radical pair decay: if Q_A is reduced prior to the flash, a charge separation takes place between Chl a_{II} and pheophytin, the intermediary acceptor between Chl a_{II} and Q_A. The radical pair, Chl a_{II}^{+} Pheo$^-$, recombines with a lifetime of 11 ns. For a decision, we measured (a) the spectrum of the 11 ns phase and (b) the yield and kinetics of carotenoid triplet formation at 515 nm.

Spectral characterization of the 11 ns phase

Fig. 3 shows the spectrum of the absorption changes that accompany the photoreaction with a

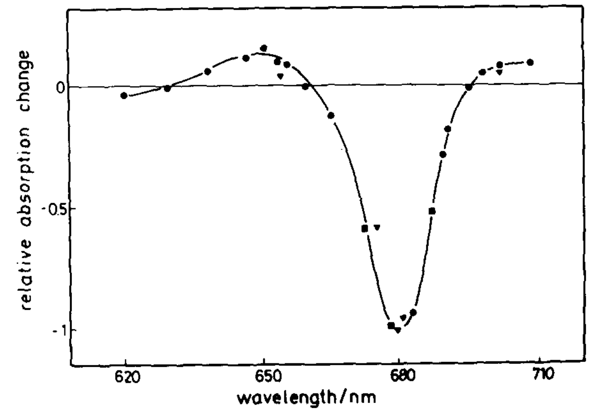


Fig. 3. Spectrum of absorption changes with the lifetime of 11 ns induced by picosecond laser flashes. Q_A was reduced by the measuring light and the excitation flashes (10 Hz) in the absence of electron acceptors. Different symbols represent different sets of experiments; $13.5 \cdot 10^{-6}$ M Chl (■, ▼) and $36 \cdot 10^{-6}$ M Chl (●); 256 averages. In each set of experiments the data are normalized to 680 nm.

lifetime of approx. 11 ns between 620 nm and 710 nm. It is characterized by the strong bleaching around 680 nm, an absorption increase between 630 nm and 660 nm and the development of a broad absorption band above 696 nm extending to the near-infra-red (see below).

The small absorption change remaining after 40 ns (see Fig. 1 bottom) which is about equal to the fitting parameter ΔA_3 shows the spectrum depicted in Fig. 4. The spectrum is characterized by a bleaching with a maximum around 680 nm and by a small absorption increase above 696 nm. The ratio $\Delta A_3/\Delta A_2$ at 680 nm is typically 0.2.

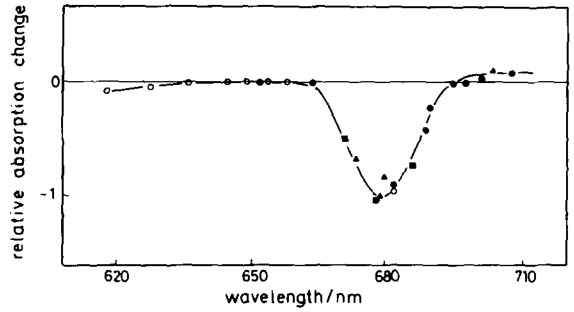


Fig. 4. Spectrum of flash-induced absorption changes remaining at t = 35 ns if Q_A is in the reduced state. Different symbols refer to different sets of experiments; $13.5 \cdot 10^{-6}$ M Chl (■, ▲) and $36 \cdot 10^{-6}$ M Chl (●, ○). Other details as in Fig. 3.

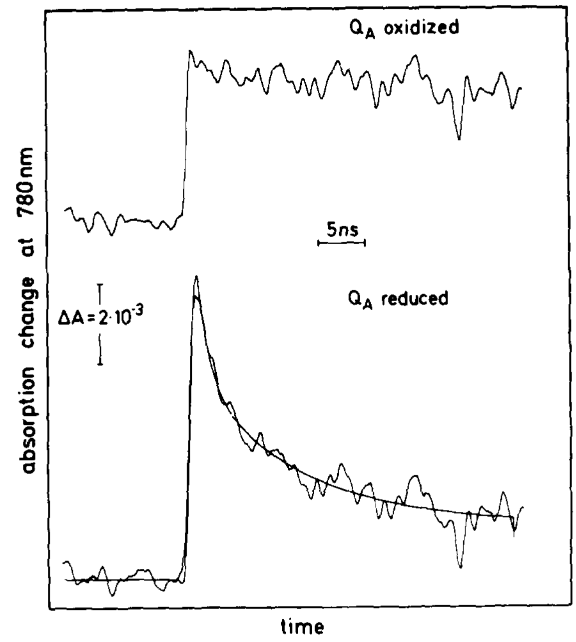


Fig. 5. Time-course of absorption changes at 780 nm induced by picosecond laser flashes in O_2-evolving PS II complexes with Q_A in the oxidized (top) and in the reduced state (bottom), optical pathlength 5 cm; $20.5 \cdot 10^{-6}$ M Chl, 256 averages, repetition rate 10 Hz; top: in the presence of electron acceptors as in Fig. 1, top; bottom: Q_A reduced by dithionite. The solid line represents the best fit according to Eqn. 1. The parameters are given in the text.

In the near-infrared we measured the absorption changes at 780 nm and 824 nm. Fig. 5 shows the time-course of the absorption change at 780 nm with Q_A in the oxidized (Fig. 5, top) and in the reduced state (Fig. 5, bottom). Upon reduction of Q_A, the decay with an approx. lifetime of 11 ns is again observed. The best fit parameters are: $\Delta A_1 = 4.6 \cdot 10^{-3}$, $\tau_1 = 1.24$ ns, $\Delta A_2 = 3.9 \cdot 10^{-3}$, $\tau_2 = 11.0$ ns, $\Delta A_3 = 1.35 \cdot 10^{-3}$. With Q_A in the oxidized state, the absorption change due to the 'stable' formation of Chl a_{II}^{+} Q_A^{-} was $3.7 \cdot 10^{-3}$ (see Fig. 5, top).

The absorption change transients detected at 824 nm are very similar to those at 780 nm (not shown). The fit yielded $\Delta A_1 = 3.1 \cdot 10^{-3}$, $\tau_1 = 1.69$ ns, $\Delta A_2 = 2.9 \cdot 10^{-3}$, $\tau_2 = 10.8$ ns and $\Delta A_3 = 9.5 \cdot 10^{-4}$. With Q_A in the oxidized state, ΔA was $3.3 \cdot 10^{-3}$. A direct comparison of the amplitudes at 824 nm and 780 nm is not possible because the excitation energies were different.

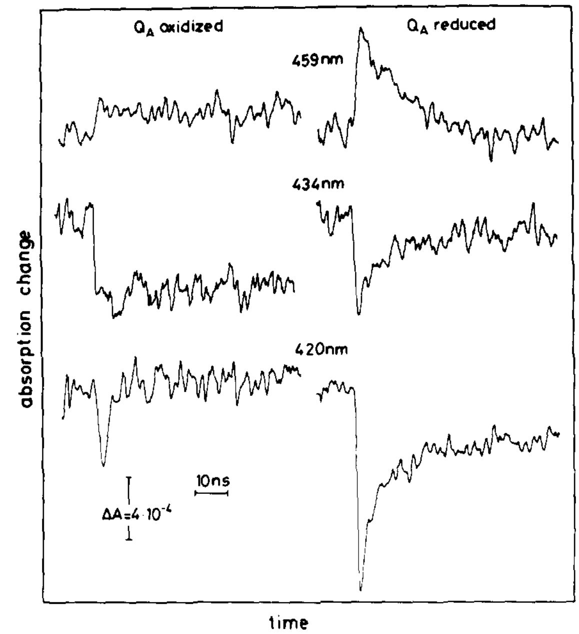


Fig. 6. Time-course of absorption changes at 459 nm (top), 434 nm (middle) and 420 nm (bottom) in Tris-treated PS II complexes induced by picosecond laser flashes with Q_A in the oxidized (left) and in the reduced state (right). Optical pathlength 1 cm; $8.6 \cdot 10^{-6}$ M Chl, repetition rate 1 Hz, 64 averages; left: in the presence of electron acceptors as in Fig. 1; right: Q_A reduced by dithionite.

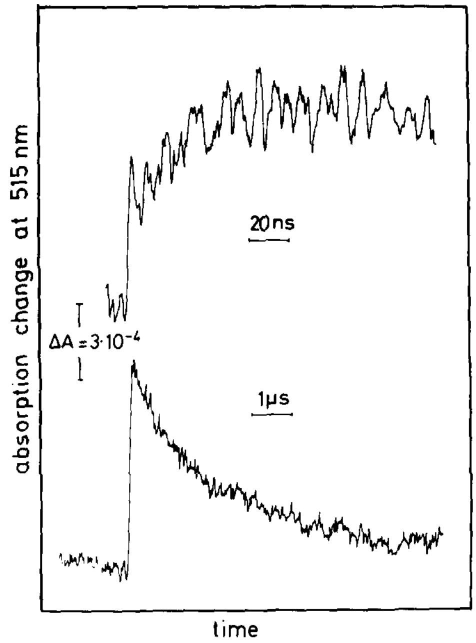


Fig. 7. Time-course of absorption changes at 515 nm in O_2-evolving PS II complexes induced by laser flashes at 532 nm. Q_A was reduced by light. Optical path, 1 cm; $15 \cdot 10^{-6}$ M Chl, repetition rate, 10 Hz; top: 256 averages; bottom: 1024 averages.

Fig. 6 shows the absorption changes at three characteristic wavelengths in the blue wavelength region. Under normal conditions,i.e., Q_A oxidized, the absorption changes can be attributed to the formation of Chl $a_{II}^{+}Q_A^{-}$. The maximum of the bleaching is observed at 434 nm; at 420 nm and 459 nm a small absorption increase is found (Fig. 6 left). Upon reduction of Q_A at each of the three wavelengths, the 11 ns phase appears (Fig. 6, right). The initial amplitude of this phase corresponds to an absorption decrease of approx. $-5.9 \cdot 10^{-4}$ at 420 nm, of approx. $-4 \cdot 10^{-4}$ at 434 nm and to an absorption increase of approx. $5.8 \cdot 10^{-4}$ at 459 nm. For Fig. 6 Tris-treated PS II particles from *Synechococcus* were used. Very similar results have been obtained with PS II particles active in O_2 evolution (not shown).

Yield and kinetics of carotenoid triplet formation

The object of the experiments described in the following was to measure the yield and kinetics of carotenoid triplet formation in order to examine whether the 11 ns phase can be explained by triplet energy transfer from chlorophyll to carotenoid.

Fig. 7 shows the time-course of the absorption change at 515 nm in PS II particles from *Synechococcus* under conditions of reduced Q_A. Fig. 7, top, shows the kinetics in the time range of up to 140 ns. The time-course can be understood by the following assumption. The fast rise reflects the formation of the chlorophyll *a* singlet state. Its decay and the formation of the chlorophyll triplet by intersystem crossing is followed by the triplet energy transfer from chlorophyll to carotenoids with a time constant of about 25 ns. The triplet state of carotenoid has a higher differential absorption at 515 nm than that of chlorophyll *a*. This gives rise to the observed absorption increase with $\tau \approx 25$ ns.

The kinetics of the absorption changes at 515 nm on a microsecond time scale are shown in Fig. 7, bottom. The signal decays nearly monoexponentially with a lifetime of approx. 2.0 μs in a

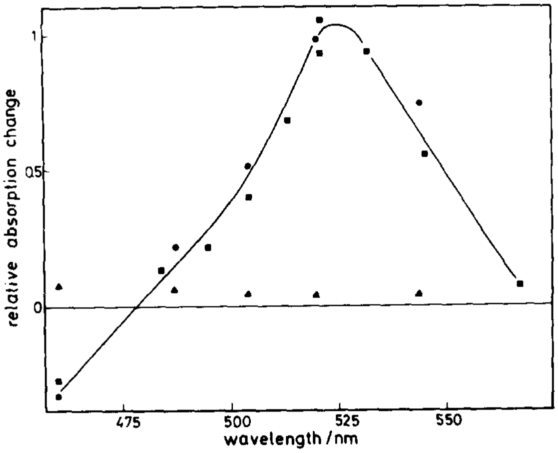


Fig. 8. Spectrum of absorption changes induced by 3-ns laser flashes at about 640 nm in Tris-treated PS II complexes. Q_A was reduced by dithionite. ■, absorption change 150 ns after the flash; ●, absorption changes with 8.5 μs lifetime; and ▲, absorption changes 35 μs after the flash; $8 \cdot 10^{-6}$ M Chl, 64 averages, repetition rate 1 Hz.

aerated sample. Upon removal of dissolved O_2 by addition of dithionite, the lifetime became approx. 8.5 μs (not shown), which is quite characteristic for ^{3}Car decay. Under both conditions (aerated and O_2-depleted), a rather small absorption change with lifetime of approx. 30 μs or more was also observed. Fig. 8 shows the amplitude of the 8.5 μs phase (●) and the absorption change taken 150 ns after the flash (■) as a function of the wavelength. The spectrum closely resembles the spectrum reported earlier for Car-triplet formation in chloroplasts [20]. The small absorption changes remaining 35 μs afer the flash in an O_2-depleted sample are also depicted in Fig. 8 (▲). These values were not significantly changed by the presence of O_2 (not shown).

For a direct comparison, the absorption changes were measured at 515 nm and 680 nm in several preparations under identical excitation conditions and using identical samples at both wavelengths. Assuming a differential extinction coefficient of $75\,000\ M^{-1} \cdot cm^{-1}$ for the formation of ^{3}Car (based on the spectrum in Fig. 8 assuming $\Delta\varepsilon \approx 100\,000\ M^{-1} \cdot cm^{-1}$ for the maximum of the ^{3}Car/Car difference spectrum [21]), the yield was one carotenoid triplet per 1500 chlorophyll *a*. On the other hand, if we attribute the complete 11 ns phase at 680 nm to the decay of chlorophyll triplets by energy transfer to carotenoids and assuming a $\Delta\varepsilon \leqslant 65\,000\ M^{-1} \cdot cm^{-1}$ for the decay of chlorophyll triplet, the amplitude of the 11 ns phase ($\Delta A_2 = 4.5 \cdot 10^{-3}$ under excitation conditions as in Fig. 7) would correspond to one ^{3}Chl per 217 or less chlorophylls.

Therefore, we conclude that the 11 ns phase does not result from triplet energy transfer. Firstly, the time constant of the triplet energy transfer was measured at 515 nm to be approx. 25 ns, i.e., significantly slower than the lifetime of the decay observed at 680 nm (approx. 11 ns). Secondly, the carotenoid triplet yield is by nearly one order of magnitude too small in order to explain the amplitude of the 11 ns phase at 680 nm by the reaction $^3\text{Chl} + \text{Car} \rightarrow \text{Chl} + {}^3\text{Car}$.

Discussion

In the present work we investigated flash-induced absorption changes in PS II particles from *Synechococcus*, when the first stable electron acceptor, Q_A, is reduced. Evidence that no stable charge separation is possible with Q_A in the reduced state was obtained earlier by analysis of double-flash experiments [7]. With an improved time resolution of $t_{10/90} \approx 900$ ps in this work, we were able to resolve the kinetics of the fast absorption change transients at different wavelengths appearing upon reduction of Q_A. Purified oxygen-evolving and Tris-treated PS II particles have been used with about 60 Chl *a*/PS II. The phycobiline proteins have been almost completely removed by purification steps [15].

With Q_A in the oxidized state, a stable charge separation occurs after absorption of light (see, e.g., Fig. 1, top). Following the rapid formation of Chl a_{II}^+ Q_A^-, a secondary donor transfers an electron to Chl a_{II}^+. The fastest half-life time of the multiphasic Chl a_{II}^+ reduction is about 20 ns (Fig. 1, top, Refs. 22 and 23). The reduced Q_A is stable for about 10^{-4} s (for a review, see Ref. 24). After prior reduction of Q_A by light or chemically by dithionite, the kinetics of the flash-induced absorption changes are drastically altered (see Figs. 1, 5 and 6). We observed a rapid transient which reflects the instrumental response to not-resolved components with lifetimes shorter than about 2 ns followed by a prominent decay phase of approx. 11 ns lifetime; absorption changes with slower decay kinetics are rather small.

We are taking into account that the following species are possibly involved in the photoreactions of PS II under conditions of reduced Q_A:

- the lowest excited singlet states of antenna chlorophylls (Chl*) and of Chl a_{II} (P^*)
- the radical pair (P^+I^-)
- the triplet state of antenna chlorophylls, ^{3}Chl, formed by intersystem crossing from Chl*
- the triplet state of Chl a_{II} (^{3}P) formed by charge recombination in those radical pairs which have evolved from the singlet radical pair $^1(P^+I^-)$ to the triplet radical pair state $^3(P^+I^-)$
- the triplet state of carotenoids, ^{3}Car, formed by triplet energy transfer from ^{3}Chl and/or ^{3}P.

The rapid transients observed in this work ($\tau <$ 2 ns) can be attributed to a superposition of (a) formation and decay of excited singlet states, (b) formation of the radical pair, and (c) formation of triplet states by intersystem crossing.

The deconvolution yielded a lifetime of about 1100 ± 500 ps for the rapid decay, using one exponential for this phase. From fluorescence measurements it is known that Chl* decays at least biphasically with lifetimes of 250 ps and 1.4 ns if Q_A is reduced [5]. The spectrum (not shown) of the rapid transient observed in this work shows a strong bleaching around 680–683 nm with a tail extending to about 710 nm. We attribute the apparent bleaching above 700 nm to the stimulated emission from the excited singlet states. An absorption decrease on the long-wavelength side of the Q_y absorption band of Chl was also observed in Ref. 4. This may indicate a significant contribution of the excited singlet state to the difference spectrum which was attributed to the reduction of pheophytin in Ref. 4.

The decay phase with a lifetime of approx. 11 ns under conditions of reduced Q_A could be well resolved in this work. Based on the following considerations, we assume that this phase in essence reflects the decay of the radical pair, P^+I^-, by charge recombination:

The long lifetime excludes excited singlet states as origin of this decay phase. The decay kinetics of Chl* have been well characterized by fluorescence decay measurements [9]. None of the studies revealed a significant component with a lifetime longer than 4 ns upon reduction of Q_A.

An identification of this phase with triplet energy transfer from ^{3}Chl to carotenoid has been carefully excluded by the measurements of formation of ^{3}Car at 515 nm (see Results). The rise kinetics and the yield of ^{3}Car do not fit the 11 ns decay phase observed at 680 nm. Furthermore, the difference spectrum of this phase in the red wavelength region, particularly the absorption increase around 650 nm, contradicts an assignment of the 11-ns phase to the ^{3}Chl decay.

When the sample was illuminated with strong continuous light in the presence of dithionite and an artificial donor, we observed that the 11 ns phase disappeared reversibly (Fig. 2). It is generally accepted that under these conditions the radical anion of pheophytin is accumulated [1] so that the radical pair, P^+I^-, can no longer be formed.

The spectrum of the 11 ns phase shows features that reflect most probably the contribution from the pheophytin-anion formation: the absorption increase between 630 and 660 nm (see Fig. 3), the absorption decrease around 420 nm, and the stronger absorption increase around 459 nm compared to Chl a_{II}^+ Q_A^- (see Fig. 6).

It is remarkable that the maximum of the strong bleaching around 680 nm for the 11 ns phase, i.e., the formation of Chl a_{II}^+ Pheo$^-$ (see Fig. 3), appears to be not significantly shifted compared to the Chl a_{II}^+/Chl a_{II} difference spectrum [17]; although the difference spectrum for photo-accumulation of Pheo$^-$ in PS II shows a maximum bleaching at 685 nm [2]. The latter spectrum has been explained as a superposition of two components: (1) the difference spectrum for Pheo$^-$/Pheo in vitro (red shifted in vivo by 17 nm); and (2) an electrochromic difference spectrum due to a blue shift of a chlorophyll absorption band at 680 nm caused by Pheo$^-$ [25]. The comparison of the measured spectrum of the 11 ns phase (Fig. 3) with the sum of the difference spectra for Chl a_{II}^+ and Pheo$^-$ formation indicates that the spectrum of the 11 ns phase does not contain the electrochromic shift of a chlorophyll absorption band centered at 680 nm. Maybe it is the absorption band of Chl a_{II} itself, which is shifted by Pheo$^-$, so that the spectrum for the formation of Chl a_{II}^+Pheo$^-$ cannot contain this contribution. The opposite conclusion was reported by Nuijs et al. [4], but their spectra indicate contributions from singlet excited chlorophylls (see above).

In this work we observed a lifetime of about 11 ns (half-life time, approx. 7.5 ns) for the radical pair, Chl a_{II}^{+} Pheo^{-}, if the electron transfer from Pheo^{-} to $\mathrm{Q_A}$ is blocked. The long lifetime differs significantly from results reported earlier ($\tau \approx 4$ ns [8]; $\tau \approx 2$ ns [4]; $\tau \approx 1.3$ ns [5]). In the first place, the contradictions may result from the different PS II preparations used. Secondly, the different experimental set-ups may be the reason. In the work of Shuvalov et al. [8] the absorption changes were measured with a time resolution of approx. 3 ns, i.e., their 4-ns component reflects the instrumental response to absorption changes caused by singlet-excited chlorophyll (lifetime of the main components is approx. 2 ns) and by the radical pair. This may give rise to an underestimation of the radical pair lifetime. In the ps-absorption change measurements the time-course was followed only up to 2 ns [4,5]. This makes locating of a phase with 11 ns lifetime difficult.

Absorption changes with slower decay kinetics ($\tau \gtrsim 25$ ns) observed under conditions of reduced $\mathrm{Q_A}$ are attributed to triplet states. Triplet energy transfer from ^{3}Chl to ^{3}Car gives rise to an absorption increase at 515 nm with $\tau \simeq 25$ ns (see Fig. 7, top). The contribution of this reaction to absorption changes in the blue, red and near-infrared spectral regions was found to be rather small compared to absorption changes caused by $\mathrm{P^+I^-}$ formation in accordance with the relatively low yield of ^{3}Car (approx. 1 ^{3}Car/1500 Chl under excitation conditions, where approx. 1 $\mathrm{P^+Q^-}$ per 180 Chl is generated in open reaction centers). Therefore, the 25-ns phase could not be separated by the fitting procedure which was restricted to two exponentials (see Eqn. 1). Measurements on a longer time-scale in the red-wavelength region (not shown) indicate that the fitting parameter ΔA_3 (see Eqn. 1) is mainly due to a component which is longer lived compared to $\tau \approx 25$ ns. The spectrum of ΔA_3 shows a maximum bleaching at 680 nm (see Fig. 4). This suggests ^{3}P as the origin of the long-lived component, because ^{3}Chl should have the maximum bleaching at about 673 nm.

The yield of ^{3}P generated by the flash after prior reduction of $\mathrm{Q_A}$ is estimated to be 10%–15% of the yield of $\mathrm{P^+Q_A^-}$ formation in open reaction centers. This value was calculated from the ratio of ΔA ($t \approx 100$ ns) in closed reaction centers to ΔA attributed to $\mathrm{P^+Q_A^-}$ formation in open reaction centers at 680 nm, assuming that the differential extinction coefficients of $\mathrm{P^+/P}$ and $^3\mathrm{P/P}$ are approx. the same (or at 780 nm with $\Delta\varepsilon\ (\mathrm{P^+/P}) \approx 4500\ \mathrm{M^{-1} \cdot cm^{-1}}$ [26] and $\Delta\varepsilon(^3\mathrm{Chl}\ a/\mathrm{Chl}\ a) \approx 6500\ \mathrm{M^{-1} \cdot cm^{-1}}$ [26], assuming that ^{3}P and ^{3}Chl have about the same $\Delta\varepsilon$).

Concerning the question whether ^{3}P decays by triplet energy transfer to carotenoid, it is of interest that in the green spectral region (see Figs. 7 and 8) some absorption change remains after the decay of ^{3}Car. Its wavelength dependence and amplitude would be consistent with the assumption that these long-lived ($\tau > 30$ µs) absorption changes are due to ^{3}P. Antenna chlorophyll triplets can be excluded as their origin because the long lifetime was also observed in aerated samples. Therefore, we assume that – in contrast to antenna chlorophyll triplets – ^{3}P does not decay by triplet energy transfer to carotenoids and that ^{3}P is not quenched by dissolved oxygen.

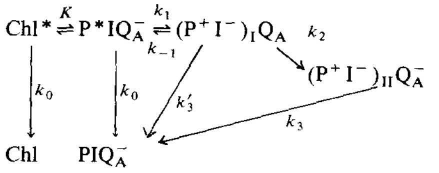
The yield of the radical pair formed in the presence of reduced $\mathrm{Q_A}$ can be estimated as follows. We compare the absorption change decaying with 11 ns, ΔA_2, in closed reaction centers with the absorption change attributed to $\mathrm{P^+Q_A^-}$-formation in open reaction centers, $\Delta A_{\mathrm{P^+Q_A^-}}$. Assuming that ^{3}P is formed as a result of radical pair recombination, the 11 ns phase is a superposition of $\mathrm{P^+I^-}$ decay and ^{3}P formation. It follows

$$\frac{\Delta A_2}{\Delta A_{\mathrm{P^+Q_A^-}}} = \frac{Y_{\mathrm{P^+I^-}} \Delta\varepsilon(\mathrm{P^+\,I^-/PI}) - Y_{^3\mathrm{P}} \Delta\varepsilon(^3\mathrm{P/P})}{Y_{\mathrm{P^+Q^-}} \Delta\varepsilon(\mathrm{P^+\,Q_A^-/PQ_A})} \tag{2}$$

with $Y_{^3\mathrm{P}}$ = yield of ^{3}P; $Y_{\mathrm{P^+I^-}}$ = yield of $\mathrm{P^+I^-}$; and $Y_{\mathrm{P^+Q_A^-}}$ = yield of $\mathrm{P^+Q_A^-}$ in open reaction centers. Using $\Delta\varepsilon$ values from Refs. 2, 17 and 25–29 and $Y_{^3\mathrm{P}}/Y_{\mathrm{P^+Q_A^-}} = 0.1$–$0.15$ (see above) we obtain for $Y_{\mathrm{P^+I^-}}/Y_{\mathrm{P^+Q_A^-}} = 0.60 \pm 0.15$. For example, at 680 nm it follows $Y_{\mathrm{P^+I^-}}/Y_{\mathrm{P^+Q_A^-}} = 0.55$ with $\Delta A_2/\Delta A_{\mathrm{P^+Q_A^-}} = 0.7$, $\Delta\varepsilon(\mathrm{P^+I^-/PI}) = 97\,000\ \mathrm{M^{-1} \cdot cm^{-1}}$, assuming that the contribution of $\mathrm{I^-}$ in the red-wavelength region is similar to pheophytin in vitro with the maximum bleaching shifted to 680 nm, $\Delta\varepsilon(\mathrm{P^+/P}) = 65\,000\ \mathrm{M^{-1} \cdot cm^{-1}}$ [17]. The uncertainty in the estimation of $Y_{\mathrm{P^+I^-}}$ is substantial because of the not exactly known differential extinction coefficients. Furthermore, the value of $Y_{P^+I^-}$ estimated in this way may depend slightly on the excitation energy due to a different extent of singlet annihilation in open and closed reaction centers.

Conclusions with regard to an interpretation of the variable fluorescence may be drawn from our finding that the radical pair lives about 11 ns upon reduction of Q_A. The fluorescence decay in open reaction centers is described by a fast (50–150 ps), a middle (400–750 ps) and a minor slow component (1.3–2.0 ns) [9]. There appears to be agreement that the increase in fluorescence yield upon reduction of Q_A is mainly due to a strong 1.3–2.0 ns phase. For instance, Holzwarth et al. [5] studied the fluorescence decay in PS II particles from *Synechococcus* similar to those used in this work and reported the following data for the lifetimes and relative initial amplitudes: 60–80 ps (75–80%), 400–450 ps (15–20%), 1.7–1.8 ns (5%) in open PS II centers and 240–260 ps (40%), 1.3–1.4 ns (50%) and 3.5–4.5 ns (10%) in closed PS II centers. The minor contributions (up to 10%) by the longest-lived components under both conditions are attributed to impurities and/or loosely bound chlorophyll in their preparations [5]. In order to examine whether a long-lived component ($\tau \approx 11$ ns) appears in the purified PS II particles used in this work, we measured the fluorescence decay with the same set-up as described for the detection of absorption changes. The relative initial amplitude of such a long-lived component was estimated to be smaller than 5% in the presence of reduced Q_A (unpublished results). We conclude that a fluorescence decay component originating from charge recombination via the excited singlet state with a lifetime similar to that found for the radical pair does not contribute significantly to the variable fluorescence.

The simplified kinetic Scheme I can account for; (a) the reported biphasic fluorescence decay ($\tau = 250$ ps (44%) and $\tau = 1.35$ ns (56%)); (b) the observed yield of radical pair formation ($Y_{P^+I^-} \approx 0.6\ Y_{P^+Q_A^-}$); and (c) the 11-ns lifetime of the radical pair if Q_A is in the reduced state. The basic assumption is that the radical pair initially generated by the flash, $(P^+I^-)_I$, has a higher free energy relative to P^+I^- in open reaction centers relax to an energetically more favorable conformation, $(P^+I^-)_{II}$. This relaxation may involve a reorganization of the surrounding protein following charge separation. The rate constant for the decay of the excited singlet state, k_0, is the sum of the rate constants for radiative (k_f) and non-radiative pathways (k_d), including intersystem crossing to ^{3}Chl *a*. Furthermore, we assume fast equilibration of the excitation between the antenna chlorophylls and Chl a_{II} (≡P). The recombination of $(P^+I^-)_{II}$ via the excited singlet state is negligible, because an 11-ns fluorescence decay component was not observed.



Scheme I.

For the sake of simplicity the spin dephasing of $^1(P^+I^-)_{II}$ to $^3(P^+I^-)_{II}$ and the following decay to ^{3}P is not depicted in the scheme and the rate constant for the recombination of $(P^+I^-)_I$ is assumed to be equal to the overall decay constant of $(P^+I^-)_{II}$ ($k_3' = k_3$). The time course of the fluorescence intensity is given by:

$$F(t) = k_f[A^*]_0\left(\frac{k_{-1}+k_s-L_1}{L_2-L_1}\,e^{-L_1t} + \frac{k_{-1}+k_s-L_2}{L_1-L_2}\,e^{-L_2t}\right) \quad (3)$$

with

$$k_s = k_2 + k_3$$

$$[A^*]_0 = [Chl^*](t=0) + [P^*](t=0)$$

$$L_{1,2} = \frac{A \pm \sqrt{A^2-4B}}{2}$$

$$A = k_0 + k_s + \bar{k}_1 + k_{-1}$$

$$B = k_0k_{-1} + k_0k_s + \bar{k}_1k_s$$

$$\bar{k}_1 = k_1\frac{K}{1+K}$$

and the time-course of the radical pair is given by

$$[\mathrm{P^+ I^-}]_{\mathrm{I}}(t) = \frac{\bar{k}_1[\mathrm{A^*}]_0}{L_2 - L_1}(\mathrm{e}^{-L_1 t} - \mathrm{e}^{-L_2 t}) \tag{4}$$

$$[\mathrm{P^+ I^-}]_{\mathrm{II}}(t) = [\mathrm{A^*}]_0\left(\frac{k_2\bar{k}_1}{(L_2 - L_1)(k_3 - L_1)}\,\mathrm{e}^{-L_1 t} + \frac{k_2\bar{k}_1}{(L_1 - L_2)(k_3 - L_2)}\,\mathrm{e}^{-L_2 t} + \frac{k_2\bar{k}_1}{(L_1 - k_3)(L_2 - k_3)}\,\mathrm{e}^{-k_3 t} \right) \tag{5}$$

According to the proposed scheme, the $(\mathrm{P^+I^-})_{\mathrm{II}}$ decay accounts for the observed 11-ns phase with the extrapolated initial amplitude

$$Y_{\mathrm{P^+I^-}} = \frac{k_1\bar{k}_1}{(L_1 - k_3)(L_2 - k_3)}\,. \tag{6}$$

A good description of the experimental findings is obtained with the following values: $1/k_0 = 2.0$ ns, $1/\bar{k}_1 = 592$ ps, $1/k_{-1} = 644$ ps; $1/k_2 = 1.1$ ns and $1/k_3 = 11$ ns. It results for the fluorescence decay: $\tau_1 = 250$ ps (44%) and $\tau_2 = 1.35$ ns (56%); for the $(\mathrm{P^+I^-})_{\mathrm{II}}$ decay: $Y_{\mathrm{P^+I^-}} = 0.60$ and $\tau = 11$ ns. For open reaction centers the same formalism has been used with k_2, being the rate constant for $\mathrm{P^+I^-Q_A \rightarrow P^+IQ_A^-}$ and neglecting $\mathrm{P^+I^-}$ and $\mathrm{P^+Q^-}$ recombination ($k_3 = 0$). We use the values $1/k_0 = 2.0$ ns, $1/\bar{k}_1 = 86$ ps, $1/k_{-1} = 542$ ps and $1/k_2 = 370$ ps; and obtain $\tau_1 = 70$ ps (81%) and $\tau_2 = 425$ ps (19%) for the fluorescence decay and ($Y_{\mathrm{P^+Q_A^-}} = 0.93$ for the yield of $\mathrm{P^+IQ_A^-}$.

For the difference in standard free energy between $(\mathrm{P^+I^-})_{\mathrm{I}}$ and Chl a^* one obtains according to Scheme I

$$\Delta G^{\mathrm{O}}(\mathrm{Chl^*} \rightarrow (\mathrm{P^+I^-})_{\mathrm{I}}) = -kT \ln\left\{ \frac{\bar{k}_1}{k_{-1}}(K+1) \right\} \tag{7}$$

and between $(\mathrm{P^+I^-})_{\mathrm{I}}$ and P*

$$\Delta G^{\mathrm{O}}(\mathrm{P^*} \rightarrow (\mathrm{P^+I^-})_{\mathrm{I}}) = -kT \ln\left\{ \frac{\bar{k}_1}{k_{-1}}\left(1 + \frac{1}{K}\right) \right\} \tag{8}$$

In a first approximation, neglecting the difference of free energy between Chl a^* and P*; the equilibrium constant $K = [\mathrm{P^*}]/[\mathrm{Chl^*}]$ is approx. $1/N_{\mathrm{A}}$, where N_{A} is the number of antenna chlorophylls per reaction center. With $N_{\mathrm{A}} \approx 60$ we obtain -3 mV in closed and -48 mV in open reaction centers for $\Delta G^{\mathrm{O}}(\mathrm{Chl^*} \rightarrow (\mathrm{P^+I^-})_{\mathrm{I}})$ and -109 mV in closed and -153 mV in open reaction centers for $\Delta G^{\mathrm{O}}(\mathrm{P^*} \rightarrow (\mathrm{P^+I^-})_{\mathrm{I}})$.

According to the kinetic scheme proposed for closed PS II particles, the decay kinetics of the excited states (and hence of the fluorescence) and the yield of radical pair formation depend on the equilibrium constant, K. The smaller the value for K, i.e., the larger the antenna size, the larger will be the fraction of the excited states with a lifetime close to $1/k_0 \approx 2$ ns (or less due to singlet–singlet annihilation) and the lower the yield of radical-pair formation. In flash absorption spectroscopy, this should show up in a rapid transient with $\tau \leqslant 2$ ns mainly due to excited chlorophylls which dominates over the 11 ns phase due to recombination of $(\mathrm{P^+I^-})_{\mathrm{II}}$.

This might have been the case for very recently reported absorption changes at 830 nm in PS II membrane fragments from spinach [30]. The decay of ΔA_{830} occurred mainly via kinetics of 500 ps at the most, under conditions of reduced $\mathrm{Q_A}$ [30]. This observation was interpreted to indicate formation of the radical pair $\mathrm{P^+I^-}$ with a yield of 30–50% and a recombination half-life time of 500 ps or less [30]. Although an interpretation based on measurements at only one particular wavelength is rather uncertain, we consider it more likely that the rapid transient mainly reflects absorption changes due to the excited Chl singlet state, which shows an absorption band in the 800–830 nm region similar to that of $\mathrm{P^+}$ (Refs. 31 and 4; see also Brettel, unpublished results). It is remarkable that the absorption changes under conditions of reduced $\mathrm{Q_A}$ (e.g., in the presence of dithionite) showed additionally a slower component of about 40% of the detected initial amplitude [30]. This component might contain recombination of the radical pair, $\mathrm{P^+I^-}$, but – due to a larger antenna size – formed with a lower yield than that of our PS II complexes.

After the measurements described in this work were finished, absorption changes in a $\mathrm{D_1}$-$\mathrm{D_2}$-cytochrome *b*-559 complex from spinach were reported [32,33]. This complex is assumed to represent the reaction of PS II [34]. Lifetimes of 36 ns

[32] and 46 ns [33] have been found for the radical pair, P^+I^-. The rather long lifetime compared to our results may be related to the absence of Q_A in these complexes. Structural modifications caused by the preparation of the reaction center complex may also account for the relatively long lifetime. The PS II particles used in this work show efficient electron transport from water to Q_A. Therefore, we suppose that our results may be representative of the reactions in native PS II.

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