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Examination of fluorescence lifetime and radical-pair decay in Photosystem II membrane fragments from spinach

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Absorption changes at 830 nm, ΔA_{830}, induced by repetitive (1 Hz) strong actinic 35 ps laser pulses ($\lambda = 532$ nm; $E = 4 \cdot 10^{15}$ photons/cm^2 per pulse) were detected in oxygen-evolving Photosystem (PS) II membrane fragments with a time resolution of about 500 ps. The following results were obtained: (a) in the presence of 2 mM $K_3(Fe(CN)_6)$ the relaxation of ΔA_{830} is dominated by the multiphasic P-680$^+$-reduction kinetics (ns and μs range) and the fast I^- reoxidation, (b) if the reaction centers are kept largely in the reduced state P-680IQ_A^-, the initial amplitude of ΔA_{830} remains almost unaffected, but the decay mainly occurs via a kinetics of at most 500 ps, (c) if the reaction centers are transformed into the state P-680I$^-$ Q_A^- by strong illumination in the presence of $Na_2S_2O_4$, then the initial amplitude of ΔA_{830} becomes drastically diminished, (d) after a few cycles of strong continuous actinic light and dark recovery (4 min) ΔA_{830} is dominated by a 3 ns relaxation kinetics. Comparative measurements of the fluorescence decay after excitation with weak 620 nm pulses ($E = 2 \cdot 10^{10}$ photons/cm^2 per pulse) exhibited the following dependencies of the kinetics of the redox state of the reaction center: up to 200 ps kinetics dominate for the states P-680IQ_A and for P-680I$^-$ Q_A^-, whereas for the state P-680IQ_A^- the fluorescence relaxes predominantly via 1.3 ns kinetics. The analysis of these results led to the following conclusions. (i) The PS II reaction center provides a rather shallow trap for excitons. (ii) The fluorescence emission in the state P-680IQ_A^- is probably not a recombination fast delayed light emission but represents a prompt fluorescence of the antenna. (iii) If the yield of flash-induced radical pair formation in reaction centers of the state P-680IQ_A^- is significant (at least 25%), then the recombination reaction P-680$^+$I$^-$$Q_A^\cdot$ → P-680I$Q_A^\cdot$ takes place with $t_{1/2} \leqslant 500$ ps.

Introduction

The transformation of solar radiation into electrochemical free energy takes place in the reaction complexes of photosynthesizing organism. The overall process implies different types of reactions, like exciton migration, exciton trapping, primary radical pair formation and subsequent stabilization of the charge separation (for recent review, see Refs. 1, 2). Information about the reaction

Abbreviations: PS II, Photosystem II; Chl, chlorophyll; P-680, primary electron donor of PS II; Mes, 4-morpholineethanesulfonic acid; Pheo, pheophytin.

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mechanism of these processes can be obtained by measurements of time-resolved fluorescence relaxation kinetics, reflecting the overall exciton decay, and by detecting transient absorption changes which monitor the very fast primary electron transfer events from the photoreactive pigment to its associated acceptor components. Among different types of reaction centers (purple bacteria, green bacteria and PS I and PS II of oxygen-evolving organisms) the complex of PS II is of special interest because its turnover provides the oxidizing redox equivalents for water oxidation to molecular dioxygen (for review, see Ref. 3). The reaction sequence of this complex can be summarized by

$$\text{P-680 I } Q_A \overset{\langle\epsilon\rangle}{\rightleftharpoons} {}^1\text{P-680}^* \text{IQ}_A \rightleftharpoons \text{P680}^+\text{I}^-\text{Q}_A \rightleftharpoons \text{P-680}^+\text{IQ}_A^- \quad (1)$$

where P-680 refers to the photoactive chlorophyll *a*, I to the intermediary redox carrier(s) and Q_A to the primary quinone acceptor, $\langle\epsilon\rangle$ symbolizes a singlet exciton in the antenna. A charge separation sufficiently stable for efficient water oxidation can be achieved only by electron transfer from I^- to Q_A [4]. If Q_A^- stays reduced the radical pair $\text{P-680}^+\text{I}^-$ rapidly decays; i.e., PS II reaction centers in the state P-680IQ_A^- are functionally blocked for photosynthesis. If the reaction centers are open for photochemical exciton trapping, the quantum yield of fluorescence emission attains a minimum level, whereas reaction centers in the state P-680IQ_A^- give rise to maximum yield. On the other hand, other redox states of functionally blocked reaction centers, e.g., $\text{P-680}^+\text{IQ}_A^-$ or $\text{P-680I}^-\text{Q}_A^-$ have been shown to act as nonphotochemical quenchers of singlet excitons (for the latest reviews, see Ref. 5).

With respect to exciton trapping two questions are of mechanistic relevance: (a) What is the relative depth of the reaction center trap compared with the energy levels of the antenna pigments? Two extreme situations have to be considered. If the trap acts as an irreversible sink the overall process of exciton decay is limited by exciton migration, whereas in the case of a rather shallow trap the reaction center processes determine the photochemical exciton capture. (b) What is the origin of the slow component ($t_{1/2} = 1$–2 ns) of the fluorescence decay that increases due to the transition of P-680IQ_A into the state P680IQ_A^-? This emission could be due to either an increase of prompt fluorescence yield or a fast delayed fluorescence caused by exciton formation through a rapid radical pair recombination of the state $\text{P-680}^+\text{I}^-\text{Q}_A^-$, as originally proposed by Klimov and co-workers [6,7]. In this paper, the abovementioned problems were analyzed by comparative measurements of laser flash-induced absorption changes at 830 nm, reflecting the turnover of the reaction center and of fluorescence decay kinetics as a function of different redox states of the trap.

Parts of this work have been presented at the 50th Physikertagung, Heidelberg, 1986.

Materials and Methods

Oxygen-evolving PS II fragments were prepared from spinach according to the procedure described in Ref. 8, with modifications as described in Ref. 9. Measurements of 830 nm absorption changes were performed with a single beam flash photometer as described in principle in Ref. 10. The measuring light was provided by a laser diode (Sharp LTO 15 MD, $\lambda = 828$ nm). Using a microscope objective lens the beam was focussed through the cuvette (4 cm optical pathlength) into a 1 mm aperture which was located in front of the photodetector (Silicon Avalanche Photodiode AR-S5, Antel). In order to suppress a flash artefact, the distance between the cuvette and the aperture was about 1 m. The photodiode was protected against fluorescence by a 830 nm interference filter and coupled via a 1 GHz amplifier (Telemeter TV 83/10408 C) to a Tektronix 7912 digitizer. 128 signals were averaged and stored on floppy disk. The samples were excited at 532 nm with 35-ps pulses (repetition rate, 1–2 Hz) from a frequency-doubled modelocked Nd-YAG laser at a maximum photon density of $4 \cdot 10^{15}$ $h\nu/\text{cm}^2$ per pulse. The time resolution of this equipment is illustrated in Fig. 1.

Fluorescence measurements were performed with the following set-up. The samples were excited with a cavity-dumped ps dye laser (Spectra Physics, Argon laser type 171; mode locker type 342; dye laser type 375 and cavity dumper type 344 with a dumping driver type 454). The pulses ($\lambda = 620$ nm, 15 ps) passed through neutral den-

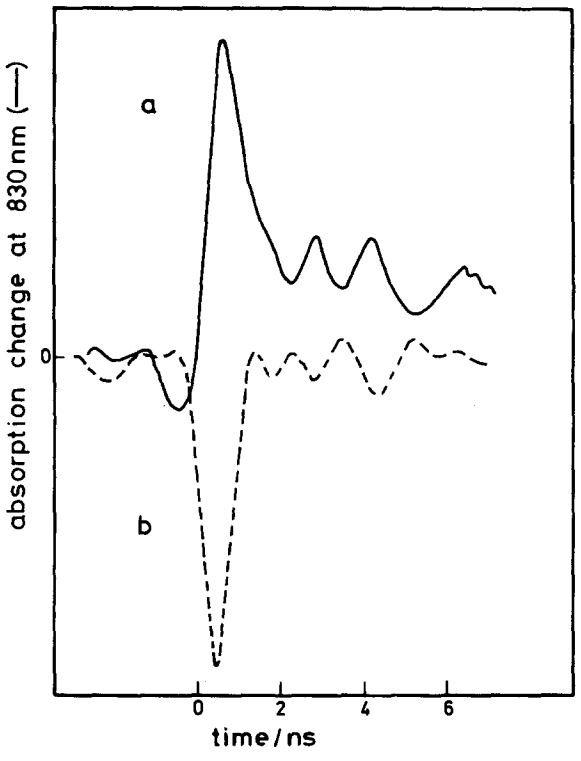

Fig. 1. Laser flash-induced absorption changes at 830 nm as a function of time in PS II particles without the acceptor $K_3(Fe(CN)_6)$ (a), and the time course of 35 ps laser pulses as detected by our measuring device (b). The observed broadening is mainly due to the electric bandwidth (700 MHz) of the amplifier of the digitizer.

sity filters, giving an actinic photon density of $2 \cdot 10^{10}$ $h\nu/cm^2$ per pulse at a repetition rate of 8 kHz. The fluorescence emitted from the sample, passing a red cut-off filter RG 665 (Schott), was focussed on the detector (avalanche photodiode ADP, Telefunken type BPW 28). The signals were amplified and recorded via a sampling device (Tektronix, Type 7 S 12) with an S 6 sampling head. The time resolution was limited by the 3 GHz bandwidth of the amplifier (B&H Electronics, model DC 3002 A). The sample suspensions contained: PS II particles (100 μg chlorophyll/ml), 20 mM Mes/NaOH (pH 6.5); 0.06% sulfobetaine (SB 12) was added to reduce particle scattering. It was checked that at this low concentration sulfobetaine did not significantly affect the oxygen evolution capacity and the fluorescence decay kinetics (data not shown). Further additions as described in the legends to the figures.

Results

Fig. 2 shows typical traces of laser flash-induced absorption changes at 830 nm in PS II particles with a maximum time resolution of about 500 ps. In this case the kinetics of the radical pair formation $P\text{-}680^+I^-$ cannot be resolved. Furthermore, most of the Chl* *a*-singlet states formed in the antenna escaped our detection because of their fast decay [11]. Accordingly, the relaxation kinetics of the signals should predominantly reflect the reduction of $P\text{-}680^+$ and the reoxidation of I^-. The intermediate I was shown to be pheophytin [12]. The curves of Fig. 2 (top) were obtained in sample containing 2 mM $K_3(Fe(CN)_6)$ as exogenous electron acceptor. In this case the functionally competent redox state $P\text{-}680IQ_A$ becomes restored between the flashes. The relaxation kinetics recorded at two different time scales exhibit a multiphasic pattern. The fast kinetics with a half-lifetime of < 500 ps probably reflects the reoxidation of I^- by Q_A, which was recently determined to be 200–400 ps [11,13].

The extent of these kinetics is not fully resolved, due to the limited time resolution (vide supra). The slower kinetics in the ns and μs range indicate the reduction of $P\text{-}680^+$ that was found to occur in this time domain under repetitive flash excitation in samples with intact water-oxidizing enzyme system [14,15]. These kinetics are more clearly resolved at a 500 ns sweep (right side of Fig. 2). The trace recorded with a 500 ns sweep reveals a markedly reduced amplitude of the fast kinetics. This illustrates the effect of data suppression in the limit of the time resolution of our digitizer at this recording sweep. The ns components are transformed into μs kinetics after incubation with hydroxylamine (data not shown), as expected from previous studies for the $P\text{-}680^+$-reduction [10,16].

A different relaxation pattern is expected to arise in samples without exogenous electron acceptors (see Fig. 1(a) and Fig. 2, bottom, without $K_3(Fe(CN)_6)$). Under repetitive flash excitation at 2 Hz a significant fraction of the reaction centers attain the redox state $P\text{-}680IQ_A^-$ because of the

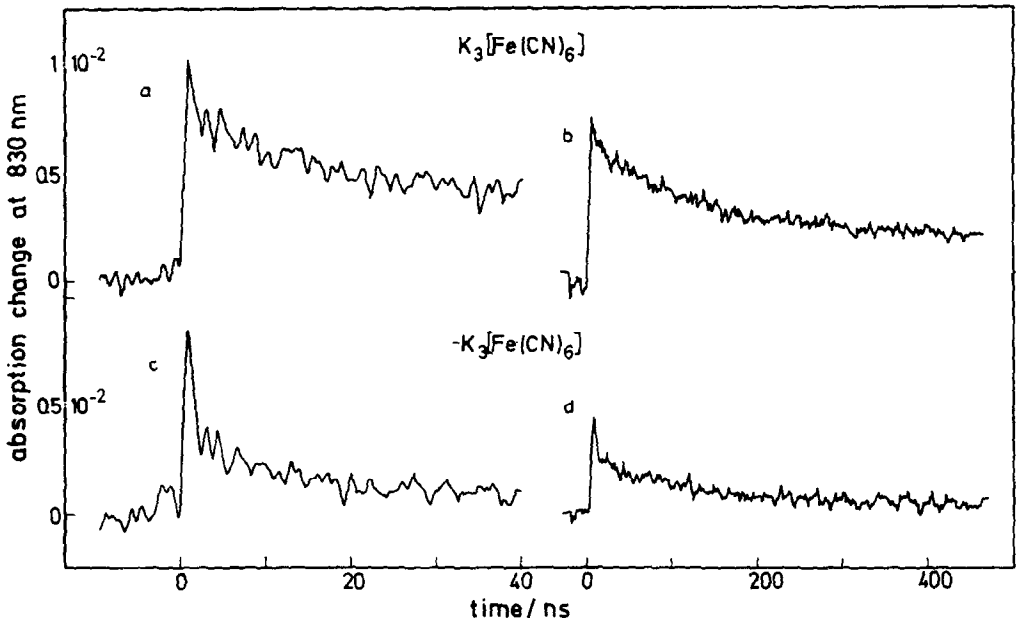

Fig. 2. Absorption changes at 830 nm as a function of time in PS II membrane fragments in the presence (2 mM) and absence of $K_3(Fe(CN)_6)$. Experimental conditions as described in Materials and Methods.

restricted reoxidation of Q_A^-. Accordingly, a smaller part of the relaxation kinetics should reflect the pattern of the reaction centers still remaining open under these conditions, but the overall decay should be dominated by the radical pair recombination kinetics in the state $P\text{-}680^+I^-Q_A^-$, provided that the yield of formation of the state $P\text{-}680^+I^-Q_A^-$ is significant (vide infra). The reaction $P\text{-}680^+I^-Q_A^- \rightarrow P\text{-}680IQ_A$ has been reported to be characterized by a half-lifetime of 1–4 ns [7,11,13]. Surprisingly, virtually no relaxation kinetics of this type are observed (see Fig. 1(a)).

In the experiments presented at the bottom of Fig. 2 a large fraction of the reaction centers was photochemically transformed into the redox state $P\text{-}680IQ_A^-$. Another possibility to attain this state is a chemical reduction of Q_A by $Na_2S_2O_4$. Under these conditions, basically the same pattern was obtained as in the case of omitting an exogenous acceptor, except for a slightly smaller initial amplitude (compare Fig. 3, signal a , with Fig. 2, bottom). Markedly different absorption changes are anticipated to arise if the samples in the presence of $Na_2S_2O_4$ are illuminated by strong continuous background light. This treatment causes the photoaccumulation of the redox state $P\text{-}680I^-Q_A^-$ [6,7]. Therefore, the radical pair formation $P\text{-}680^+I^-$ is prevented and the flash-induced absorption changes should disappear. The data shown in Fig. 3, signal b, indicate a large decrease of the signal amplitude. Significant photoaccumulation of $P\text{-}680\ I^-Q_A^-$ was found to occur only at a sufficiently low ambient redox potential [17,31]. Illumination of samples in the absence of $Na_2S_2O_4$ and exogenous acceptors should lead to accumulation of $P\text{-}680IQ_A^-$ only, but not of $P\text{-}680I^-Q_A^-$. This idea is supported by the finding that strong background illumination in the absence of $Na_2S_2O_4$ hardly affects the amplitude and kinetics of the 830 nm absorption changes (*data not* shown). In the dark after continuous illumination the photoaccumulated state $P\text{-}680I^-Q_A^-$ slowly reoxidizes to $P\text{-}680IQ_A^-$, because at neutral pH $Na_2S_2O_4$ is thermodynamically unable to keep I^- in its reduced state.

Therefore, after sufficient dark incubation the state $P\text{-}680IQ_A^-$ should recover and the flash-induced 830 nm absorption changes reappear. This is experimentally confirmed, as shown in Fig. 3, curve c. However, careful inspection reveals a marked change of the relaxation kinetics, as

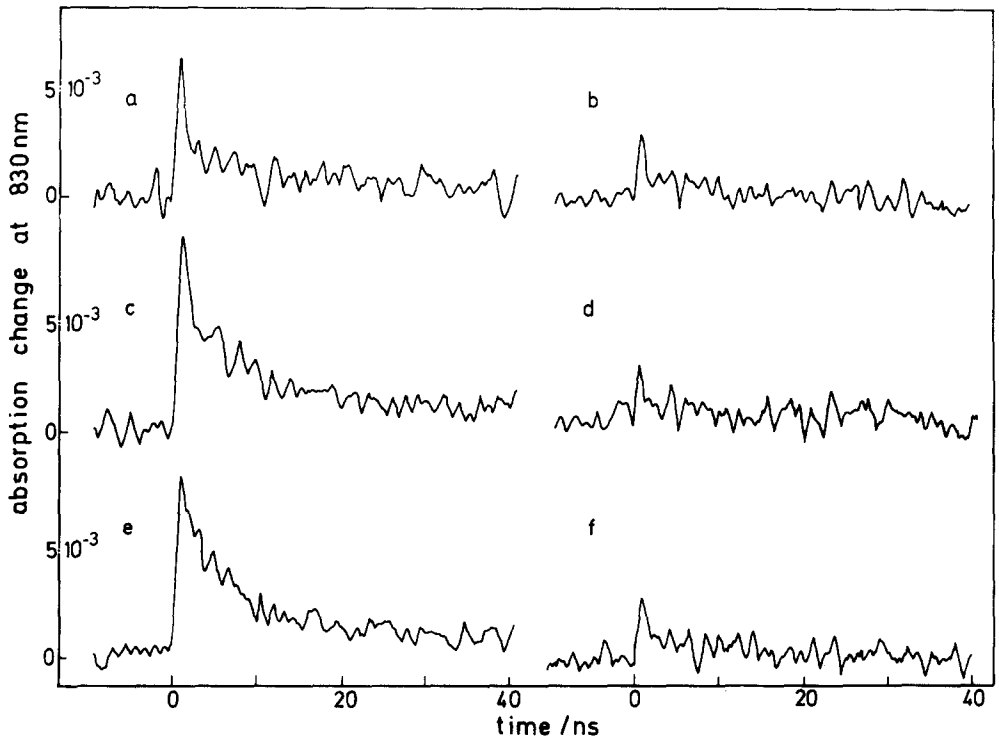

Fig. 3. Absorption changes at 830 nm as a function of time in PS II membrane fragments in the presence of 2 mg/ml dithionite. (a) Control without background illumination; (b) under strong background illumination (the measurements started 2 min after switching on the background light); (c) sample of (b) dark-adapted for 4 min before starting the measurement; (d) sample of (c) under same background illumination as in (b); (e) sample of experiment (d) dark-adapted for 4 min before starting the measurement; (f) sample (a) under further strong background light as in (b). Experimental conditions as described in Materials and Methods.

reflected by an increase of a component with a half-lifetime of 2.7 ns at the expense of the very fast kinetics (compare Fig. 3, curvea a and c). This phenomenon becomes even more pronounced after a second light/dark cycle, as is seen by the traces depicted in Fig. 3, curve e. In the pretreated samples the 830 nm absorption changes decay with half-lifetimes of 2.7 (70%) and 15 ns (30%). This result indicates that during the light/dark cycles in the presence of $Na_2S_2O_4$ irreversible modifications arise in the reaction center complex.

The redox state of the reaction center complex crucially affects the exciton trapping kinetics, as reflected by the fluorescence decay. Accordingly, fluorescence lifetime measurements were performed with the equipment described in Materials and Methods. In the presence of $K_3(Fe(CN)_6)$ a fast fluorescence decay is observed (see Fig. 4, top), as expected for systems with open reaction centers (for a recent review, see Ref. 18). In the absence of exogenous electron acceptors the reaction centers attain predominantly the redox state P-680IQ_A^-, which is characterized by a high fluorescence yield. Under these conditions most of the flash-induced fluorescence (70% of the total extent) exhibits a decay kinetics with a half-lifetime of 1.3 ns (see Fig. 4, bottom), in correspondence with reports in the literature (for a recent review, see Ref. 18). Almost the same 1.3 ns fluorescence decay kinetics is obtained if the state P-680IQ_A^- is formed by addition of $Na_2S_2O_4$ without strong background illumination, as is shown in Fig. 5(a).

However, after illumination of the sample in the presence of $Na_2S_2O_4$ the fluorescence decays for the most part with a half-lifetime of less then 200 ps (see Fig. 5(b)). The apparent decay kinetics in Fig. 5(b) are limited by the time resolution of our equipment (150 ps at most). This result is consistent with a large quenching of the steady-state fluorescence quantum yield, as previously reported [6,7]. After a sufficiently long dark in-

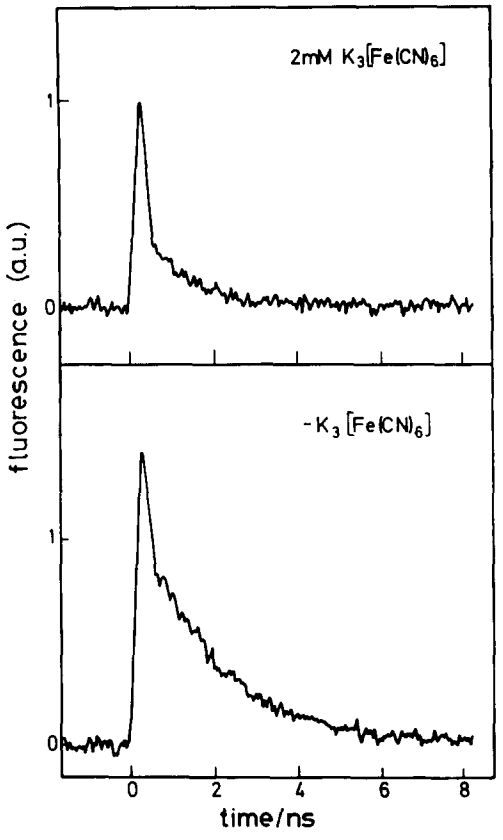

Fig. 4. Laser flash-induced fluorescence as a function of time in PS II membrane fragments in the presence (2 mM) and absence of $K_3(Fe(CN)_6)$. Experimental conditions as described in Materials and Methods.

cubation, the steady-state fluorescence largely recovers in many preparations. The restoration degree of the fluorescence is strongly dependent on the type of the preparation [6,19,31] and seems also to depend on the redox potential [6]. The data depicted in Fig. 5(c) indicate that only a partial restoration of the slow (1–2 ns) fluorescence decay can be achieved. This phenomenon probably implies two effects: (a) an incomplete recovery of the redox state P-680IQ_A^- in the dark incubation after illumination and (b) an enhanced exciton trapping in reaction centers that are modified during light/dark cycles in the presence of $Na_2S_2O_4$. The idea of an incomplete recovery is confirmed by steady-state fluorescence measurements [31]. The possibility of irreversible modification of the reaction center complex will be more extensively outlined in the discussion.

A comparison of Fig. 1(a) with Fig. 4, bottom, shows that under conditions where the reaction centers predominantly attain the state P-680IQ_A^- in the dark time between the flashes, the half-lifetime of the prominent kinetics of the fluorescence decay exceeds that of the 830 nm absorption changes ($t_{1/2} \leqslant 500$ ps).

At first glance, the results seem to be highly contradictory. However, it has to be emphasized that the photon density of the excitation pulses for the measurements of the 830 nm absorption changes was higher than those of the fluorescence decay measurements, by five orders of magnitude. Therefore, a completely different exciton population was created in the antenna in both types of experiments and also the redox state of the reaction centers is differently affected by the two types of excitation pulses. The pulses used for the above-mentioned fluorescence measurements transform only a negligibly small fraction of the reaction centers from state P-680IQ_A^- into P-680$^+I^-Q_A^-$. On the other hand, the high photon density pulses inducing the 830 nm absorption changes transform the reaction centers synchronously from state P-680IQ_A^- to P-680$^+I^-Q_A^-$ at the maximum yield that can be achieved in reaction centers with reduced Q_A^-. In order to test the influence of different photon densities of the laser pulses, the fluorescence decay kinetics were measured under the same excitation conditions as used for inducing the 830 nm absorption changes. Furthermore, fluorescence decay kinetics were also detected at highly attenuated photon densities of the pulses. The results obtained are depicted in Fig. 6. A comparison of the traces at the top of Fig. 6 with those at the bottom shows that the fluorescence lifetime significantly depends on the pulse energy if the reaction centers are in the state P-680IQ_A^-, whereas much less differences are seen if the state P-680IQ_A becomes regenerated between the flashes due to the presence of $K_3(Fe(CN)_6)$.

The observation of a faster fluorescence decay after excitation with high photon density pulses of samples containing reaction centers kept in state P-680IQ_A^- before the flash is in line with recent fluorescence measurements under double flash group excitation [20].

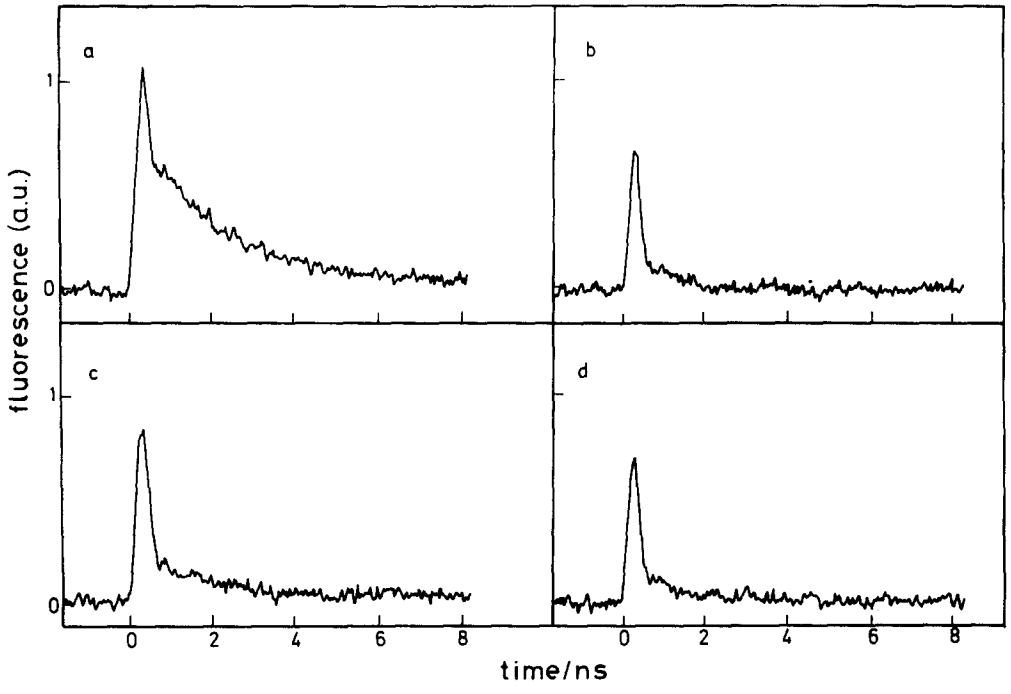

Fig. 5. Laser flash-induced fluorescence as a function of time in PS II membrane fragments in the presence of 2 mg/ml dithionite. In the absence (left) and presence (right) of strong background light (a) without preillumination; (b) under strong background illumination (the measurement started 2 min after switching on the background light). (c) Sample of (b) dark-adapted for 4 min before starting the measurement; (d) second preillumination. Experimental conditions as described in Materials and Methods.

Discussion

Two results of the present study are mechanistically very interesting: (a) The absence of a significant 1–2 ns relaxation kinetics of flash-induced 830 nm absorption changes under conditions where the radical pair recombination $P\text{-}680^+I^-Q_A^- \rightarrow P\text{-}680IQ_A^-$ is expected to take place at its maximum extent. This result seems to contradict previously reported data for the time course of this reaction [7,11,13]. (b) The modification of ΔA_{830} relaxation kinetics after actinic light/dark cycles in the presence of $Na_2S_2O_4$.

The former effect relates to the maximum yield and decay kinetics of the radical pair $P\text{-}680^+I^-Q_A^-$, whereas the latter phenomenon might indicate functional changes of the reaction center complex.

Maximum yield and radical pair decay of $P\text{-}680^+I^-Q_A^-$

In order to clarify this crucial point, at first our experimental conditions have to be shown to permit a correct detection of the amplitude and the time course of $P\text{-}680^+(t)$ and $I^-(t)$. For kinetic reasons this problem can be checked most easily for samples with open reaction centers. In this case, the well-known multiphasic reduction kinetics of $P\text{-}680^+$ [14,15] are observed (see Fig. 2, top). Using a difference extinction coefficient $\Delta\epsilon_{830}$-$(P\text{-}680^+/P\text{-}680) = 6000\ M^{-1}$ [21], the amplitude of ΔA_{830} measured at 2 ns after the flash in Fig. 2(a) corresponds with the formation of $P\text{-}680^+$ in almost all reaction centers (values of 80–100% were obtained). In the presence of 1 mM NH_2OH relaxation kinetics with $t_{1/2} \geq 1$ ns are completely transformed into μs kinetics without change of the amplitude (data not shown). This shows that the $\Delta A_{830}(t)$ value at $t > 2$ ns in Fig. 2(a) almost entirely reflects the reduction of $P\text{-}680^+$. Based on in vitro measurements [12], a similar contribution to $\Delta A_{830}(t)$ was expected for the turnover of I (Pheo) at $t \leq 1$ ns. In this time domain the relaxa-

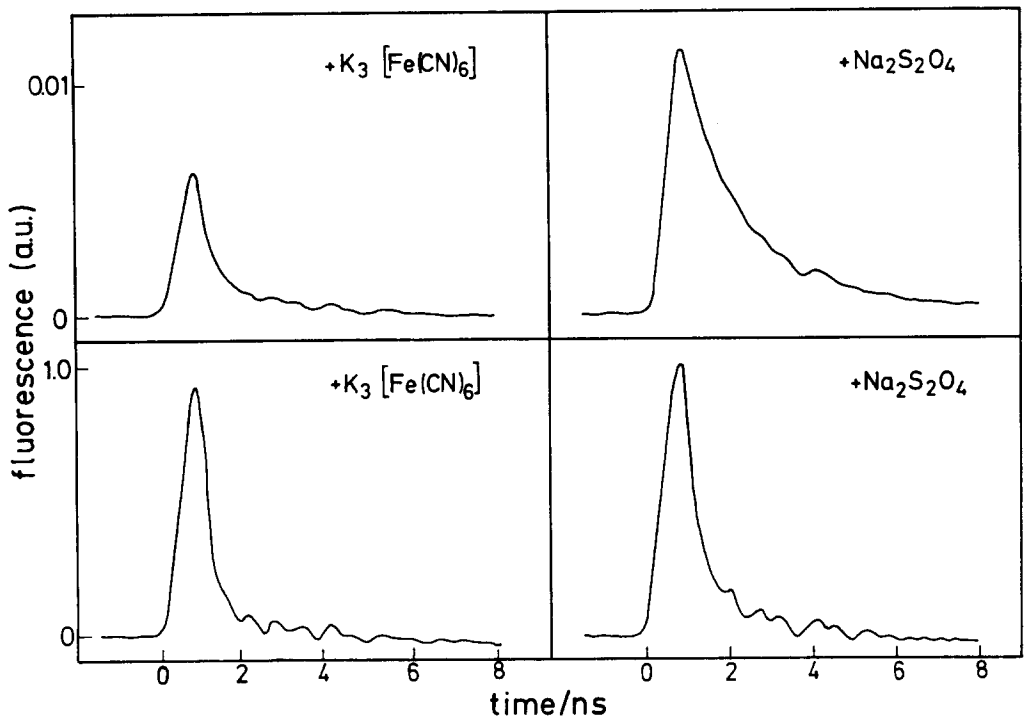

Fig. 6. Laser flash-induced fluorescence as a function of time in PS II membrane fragments in the presence of 2 mM $K_3(Fe(CN)_6)$ (left) and 2 mg/ml $Na_2S_2O_4$ (right). The experimental set-up is different from that used in Figs. 4 and 5. For excitation and monitoring of the fluorescence traces the same equipment was used as in Figs. 2 and 3, except for switching off the 830 nm measuring beam. The 830 nm interference filter in front of the photodiode was replaced by a 690 nm filter. In the top traces the photon density of the exciting pulses was attenuated by a factor of 500 using neutral density filter, in the bottom traces the neutral density filters were put in front of the photodetector in order to reduce the fluorescence intensity.

tion of $\Delta A_{830}(t)$ reflects the reoxidation of I^- with half-lifetimes of 200–300 ps [11,13]. Fig. 2(a) reveals a comparatively small amplitude of these kinetics. This phenomenon could be explained either by incomplete detection of $I^-(t)$ due to limited time resolution or by a relation $\Delta\epsilon_{830}(I^-/I) \leqslant 0.4 \cdot \Delta\epsilon_{830}(P\text{-}680^+/P\text{-}680)$. Because of the limited time resolution of our equipment (see Fig. 1) we favor the former idea i.e., the turnover of I (Pheo) is not completely resolved, and therefore the amplitude appears to be diminished due to the time resolution of our equipment. This implies the absence of contributions from excited chlorophyll singlets. Based on these considerations, we conclude that ΔA_{830} properly detects $P\text{-}680^+$ and I^- at times $t \geqslant 300$ ps. Therefore, a radical pair combination of the type $P\text{-}680^+I^-Q_A^- \rightarrow P\text{-}680IQ_A$ can be completely (i.e., within the experimental errors of 10–20%) resolved by our device provided that the half-lifetime of this process is $t_{1/2} \geqslant 1$ ns. An inspection of the data depicted in Figs. 2(c) and 3(a) clearly shows that kinetics with $t_{1/2} = 1$–2 ns only marginally contribute to the overall decay, which is dominated by an apparent half-lifetime of $t_{1/2} \approx 500$ ps. This relaxation is only slightly slower than the decay of the signal detected by our equipment when the photodiode was directly illuminated with highly attenuated 35 ps laser pulses (Fig. 1(a)). Therefore, $t_{1/2} \approx 500$ ps is the upper limit of the real decay time of ΔA_{830} in samples with Q_A^- kept reduced.

The results of Figs. 2(c) and 3(a) can be interpreted in two alternative ways: (i) $\Delta A_{830}(t)$ could reflect the radical pair recombination ($P\text{-}680^+I^-Q_A^- \rightarrow P\text{-}680IQ_A^-$) with a decay dominated by a very fast relaxation kinetics ($t_{1/2}$

$\leqslant$ 500 ps), or (ii) the yield of radical pair formation is very low and $\Delta A_{830}(t)$ indicates mainly the decay of chlorophyll singlets (triplets are excluded for kinetic reasons). The absorption changes at 830 nm in Figs. 2(c) and 3(a) are interpreted to indicate the radical pair formation for two reasons: (a) The absorption changes largely decrease in their amplitudes if the radical pair formation is suppressed by photoaccumulation of the state P-680$I^-Q_A^-$ (see fig. 3(b) and (f)). The small remaining amplitude could be due to incomplete transformation into the state P-680$I^-Q_A^-$ or a possible reaction with the second pheophytin that exists in PS II [22]. Further experiments are required to clarify this point. (b) The absorption changes in Figs. 2(c) and 3(a) are probably not due to singlet states because of their absence in Fig. 2(a) (vide supra) and of the only marginal effect of the redox state of Q_A on the initial amplitude of the fast detectable fluorescence at higher laser pulse energies (see Fig. 6, bottom).

The above assignment is supported by recent measurements of the integral fluorescence quantum yield under double flash group excitation. In this case the time correlation between the two flashes declines with $t_{1/2} = 400 \pm 100$ ps if the reaction centers are in the state P-680IQ_A^- before the double flash excitation. This phenomenon can be explained by the disappearance of P-680$^+I^-Q_A^-$ with $t_{1/2} = 300$–500 ps, as discussed in Ref. 20.

The data in figs. 2(c) and 3(a) also permit the calculation of the upper limit of the yield of radical pair formation under conditions of Q_A^- staying reduced. For the sake of simplicity, $\Delta\epsilon_{830}$(P-680$^+$/P-680) and $\Delta\epsilon_{830}(I^-/I)$ are taken to be practically the same. The extent of the relaxation kinetics of ΔA_{830} in Figs. 2(c) and 3(a) resembles that of the comparatively slow decay in Fig. 2(a), which is ascribed to P-680$^+$ reduction. Accordingly, the maximum yield of the radical pair (P-680$^+I^-$) formation in reaction centers with Q_A^- kept reduced is estimated to be 30–50% and the recombination takes place with $t_{1/2} \leqslant 500$ ps (vide supra). The calculated maximum yield closely fits with recently published results [13]. However, the half-lifetime of at most 500 ps is significantly shorter than previous data in the literature [7,11,13]. The originally presented value of approx. 4 ns [7] was probably affected by the limited time resolution of the equipment used. At the present time we cannot easily explain the inconsistency with the data ($t_{1/2} \approx 2$ ns) of Ref. 11, because the experiments were performed with sufficiently high time resolution and under comparable excitation conditions. Supposing the difference of the P-680$^+I^-Q_A^-$ half-lifetimes reported in Ref. 13 and in this study (1 ns vs at most 500 ps) to be really significant, then the explanation of this phenomenon requires a more detailed discussion. The essential difference of the experimental protocol is the energy of the actinic laser pulses. A significant effect of singlet-singlet annihilation processes has to be considered for the interpretation of the present results, but it is negligibly small in Ref. 13. This phenomenon will be briefly discussed within the pattern of exciton trapping and transformation at the PS II reaction centers.

Mechanism of exciton transformation at PS II

A simplified scheme of the reaction pattern is depicted in Fig. 7. A possible effect of exciton density in the antenna on the P-680$^+I^-Q_A^-$ lifetime can be explained by two assumptions: (i) the reaction centers act as shallow traps, and (ii) the recombination described by k* is not the rate-limiting step for the P-680$^+I^-Q_A^-$ decay after excitation with low photon density pulses.

In spite of the lack of direct experimental evidence, theoretical studies favor the shallow trap model of PS II reaction centers [23,24]. Based on these considerations and taking into account the data of Ref. 20, a half-lifetime of at most 500 ps appears to be a reasonable value for the state P-680$^+I^-Q_A^-$, corresponding with a rate constant $k^* \geqslant 1.5 \cdot 10^9\ s^{-1}$. This value raises another mechanistically important question. In order to account for the high quantum yield of the charge separation in open reaction centers [25], the radical pair recombination in the state P-680$^+I^-Q_A$ should be markedly slower than the reoxidation of I^- by Q_A, which was found to be characterized by $k_1 = (2\text{–}4) \cdot 10^9\ s^{-1}$ [11,13]. Therefore, k^* is expected to be at most $3 \cdot 10^8\ s^{-1}$ for Q_A staying oxidized. The conclusion about a strong effect of the redox state of Q_A on the rate of radical recombination (k^*) appears to be supported by two findings: (a) In isolated subcomplexes lacking Q_A the half-life time of the reaction P-680$^+I^- \rightarrow$ P-680 I was re-

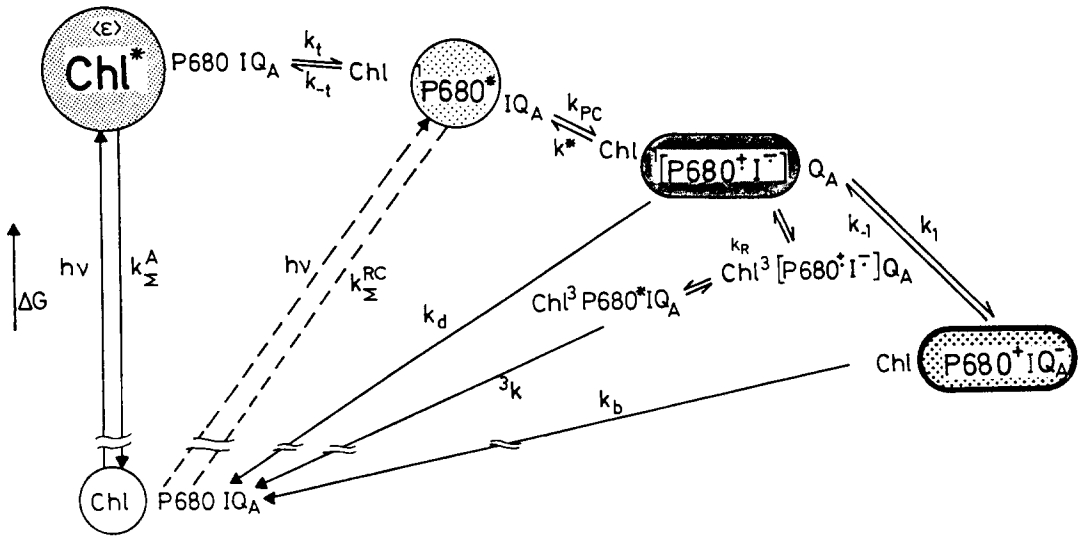

Fig. 7. Simplified scheme of the mechanism of exciton trapping and transformation at the PS II reaction center. Chl* symbolizes a chlorophyll molecule in the core antenna that directly transfers the exciton to P-680, $\langle\epsilon\rangle$ represents an exciton generated by light absorption. The rate constants describe the following processes: exciton transfer in the forward (k_t) and backward direction (k_{-t}), primary radical pair formation (k_{pc}) and its back reaction to P-680* (k^*), the sum of decay processes in the antenna (k_Σ^A), and the reaction center (k_Σ^{RC}), respectively, dissipative reactions of the primary radical pair leading to ground (k_d) or triplet state (k_R) of P-680, stabilization (k_1) and decay of the charge separation (k_{-1}, k_b). The details of exciton migration within the antenna are not explicitly considered, but depending on the depth of the trap the rate constants for the individual transfer steps between antenna pigments can markedly differ from k_t and k_{-t}. The free energy scale is interrupted because the energy difference between Chl* and Chl (1.83 eV) largely exceeds that between Chl* and P-680$^+$IQ$_A^-$ (0.5–0.6 eV).

ported to be 36 ns [26], corresponding with $k^* \approx 2 \cdot 10^7\,s^{-1}$, (b) Q_A^- was previously shown to exert a strong electrostatic effect on I, as reflected by the electrochromic C550 band shift [27]. Therefore, the redox potential of I/I^- could be markedly shifted due to the electrical potential caused by Q_A^-. This shift could imply drastic changes of the rate constant k^* [28,29].

Modification of the reaction pattern by actinic light / dark cycles in the presence of $Na_2S_2O_4$

A very interesting phenomenon was observed after repeated light/dark cycles leading to Pheo$^-$ photoaccumulation and its dark reversal. After two cycles remarkably slower decay kinetics were observed with half-lifetimes of 2.7 (70%) and 15 ns (30%). Concomitantly, the fluorescence decay was shown to be faster after two light/dark cycles compared with the dark control in the state P-680IQ$_A^-$ (see Fig. 5(c)). Therefore, it seems likely that after light/dark treatment in the presence of $Na_2S_2O_4$ the reaction centers undergo irreversible changes. Three alternative types of mechanistic modifications have to be considered to explain this phenomenon: (a) the yield of radical pair formation drastically increases while the decay kinetics remain unaffected. This effect appears to be unlikely if our assignment of the 830 nm absorption changes in Figs. 2(c) and 3(a) to a radical pair recombination is correct. (b) The yield is only marginally affected but the radical pair decay kinetics markedly slow down. This idea is in line with previous findings [19] of a faster photoaccumulation of state P-680 Pheo$^-$Q$_A^-$ after light/dark treatment in the presence of $Na_2S_2O_4$. (c) The signal in Fig. 3(e) could mainly due to a triplet state. In normal reaction centers of the state P-680IQ$_A^-$ the light-induced triplet yield is rather low even at liquid helium temperature, in contrast to the situation in bacterial reaction centers [30]. After a few light/dark cycles in the presence of $Na_2S_2O_4$ the reaction centers could become modified in a way that leads to a drastically increased triplet yield via enhanced efficiency of the radical

pair recombination. However, it has to be emphasized that a 3 ns half-lifetime is rather short for the triplet state. Therefore, further experiments are required to clarify the underlying mechanism of the modifications in the PS II reaction center after light/dark cycles in the presence of $Na_2S_2O_4$.

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References

1 Pearlstein, R.M. (1982) in Photosynthesis, Vol. 1, Energy Conversion by Plants and Bacteria (Govindjee, ed.), pp. 293–330, Academic Press, New York
2 Diner, B.A. (1986) in Encyclopedia of Plant Physiology, New Series, Photosynthesis 3 (Staehelin, L.A. and Arntzen, C.J., eds.), pp. 422–436, Springer, Berlin
3 Renger, G. and Govindjee (1985) Photosynth. Res. 6, 33–55
4 Renger, G. and Eckert, H.J. (1980) Bioelectrochem. Bioenerg. 7, 101–124
5 Govindjee, Amesz, J. and Fork, D.C. (eds.) (1986) Light Emission by Plants and Bacteria, Academic Press, New York
6 Klimov, V.V., Klevanik, A.V. and Shuvalov, V.A. (1977), FEBS Lett. 82, 183–186
7 Shuvalov, V.A., Klimov, V.V., Dolan, E., Parson, W.W. and Ke, B. (1980) FEBS Lett. 118, 279–282
8 Berthold, D.A., Babcock, G.T. and Yocum, C.F. (1981) FEBS Lett. 167, 127–130
9 Völker, M., Ono, T., Inoue, Y. and Renger, G. (1985) Biochim. Biophys. Acta 806, 25–34
10 Van Best, J.A. and Mathis, P. (1978) Biochim. Biophys. Acta 503, 178–188
11 Nijs, A.M., van Gorkom, H.J., Plijter, J.J. and Duysens, L.N.M. (1986) Biochim. Biophys. Acta 848, 167–175
12 Fajer, J., Davis, M.S., Forman, A., Klimov, V.V., Dolan, E. and Ke, B. (1980) J. Am. Chem. Soc. 102, 7143–7145
13 Holzwarth, A.R., Brock, H. and Schatz, G.H. (1987) in Progress in Photosynthesis Research (Biggins, J., ed.), Vol. I, pp. 61–65, Martinus Nijhoff, Dordrecht
14 Renger, G., Eckert, H.J. and Weiss, W. (1983) in The Oxygen Evolving System in Photosynthesis (Inoue, Y., Crofts, A.R., Govindjee, Murata, N., Renger, G. and Satoh, K., eds.), pp. 73–82, Academic Press Japan, Tokyo
15 Brettel, K. and Witt, H.T. (1983) Photobiochem. Photobiophys. 6, 253–260
16 Völker, M., Eckert, H.J. and Renger, G. (1987) Biochim. Biophys. Acta 890, 66–76
17 Rutherford, A.W. and Mathis, P. (1983) FEBS Lett. 154, 328–334
18 Holzwarth, A.R. (1985) Photochem. Photobiol. 43, 707–725
19 Renger, G., Koike, Y., Yuasa, M. and Inoue, Y. (1983) FEBS Lett. 163, 89–93
20 Dobek, A., Deprez, J., Geacintov, N.E., Paillotin, G. and Breton, J. (1985) Biochim. Biophys. Acta 806, 81–92
21 Haveman, J. and Mathis, P. (1976) Biochim. Biophys. Acta 440, 346–355
22 Murata, N., Araki, S., Fujita, Y., Suzuki, K., Kuwabara, T. and Mathis, P. (1986) Photosynth. Res. 9, 63–70
23 Shipman, L.L. (1980) Photochem. Photobiol. 43, 157–167
24 Paillotin, G. (1976) J. Theor. Biol. 58, 219–235
25 Sun, A.K.S. and Sauer, K. (1971) Biochim. Biophys. Acta 234, 399–414
26 Danielius, R.V., Satoh, K. Von Kan, P.J.M. Plijter, J.J., Nuijs, A.M. and Van Gorkom, H. (1987) FEBS Lett. 213, 241–244
27 Van Gorkom, H.J. (1974) Biochim. Biophys. Acta 347, 439–442
28 De Vault, D. (1980) Q. Rev. Biophys. 13, 387–564
29 Marcus, R.A. and Sutin, N. (1986) Biochim. Biophys. Acta 811, 265–322
30 Rutherford, A.W. (1986) Biochem. Soc. Trans. 14, 15–17
31 Rengex, G. and Kayed, A. (1987) Biochim. Biophys. Acta, Vol. 894, in the press