NANOSECOND FLUORESCENCE AND ABSORBANCE CHANGES IN PHOTOSYSTEM II AT LOW REDOX POTENTIAL

Pheophytin as an intermediary electron acceptor

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1. Introduction

The electron-acceptor system in photosystem II (PS II) reaction centers of green plants is similar to that in bacterial reaction centers. In both kinds of reaction centers, a quinone (Q) acts as an early electron acceptor (ubiquinone or menaquinone in bacterial [1] and plastoquinone in PS II [2,3]). If the quinone is in the reduced form, continuous illumination causes the accumulation of a bacteriopheophytin (BPh) radical anion ($BPh^{\cdot -}$) in bacterial reaction centers [4–6] and of a pheophytin (Ph) radical anion ($Ph^{\cdot -}$) in PS II [7–13]. The radical anions of BPh [5,14,15] and of Ph [12,13] participate in an exchange interaction with the radical anion of the quinone, coupled to a non-heme Fe atom, indicating that the distance between (B)Ph and Q is very short. The reduction of the quinone increases the formation of pigment triplet states in both bacteria [4,16] and PS II [29]. This is inhibited upon photoaccumulation of $BPH^{\cdot -}$ or $Ph^{\cdot -}$. Reduction of Q also is accompanied by the appearance of ns delayed fluorescence, which decreases during photoaccumulation of $BPh^{\cdot -}$ [4] or $Ph^{\cdot -}$ [9]. The delayed fluorescence has a characteristic magnetic field dependence in bacterial reaction centers [17–19] and in PS II [18] and an activation energy of 0.04–0.12 eV [4,9].

When the quinone in bacterial reaction centers is reduced, flash excitation generates a state 'P^F', which has a lifetime of ~10 ns [16,20]. The transient state is probably the ion-radical pair [$P870^{\cdot +}$ $BPh^{\cdot -}$] [4–6]. Picosecond measurements have shown that BPh is an intermediate electron acceptor between the primary electron donor, P870 and Q [21–24]. It has been suggested [7–13] that Ph (with E_m −610 mV [10]) acts similarly in PS II as an intermediary electron carrier between P680 and Q. Ph is found in PS II preparations in about equimolar concentration with P680 [11,12]. However, the [$P680^{\cdot +}Ph^{\cdot -}$] ion-radical pair had been not detected.

Direct measurements of the formation of an ion-radical pair in PS II reaction centers are described here. At low redox potentials, ΔA with lifetime of ~4 ns are observed in PS II. The spectrum of the ΔA is consistent with the sum of the spectra for the formation of the cation radical of the primary electron donor, $P680^{\cdot +}$, and of the anion radical, $Ph^{\cdot -}$. The ΔA are accompanied by delayed fluorescence with about the same lifetime. Both optical signals decrease when the reaction centers are driven into the state [P680 $Ph^{\cdot -}$] by continuous illumination at low redox potentials.

2. Materials and methods

Subchloroplast fragments (TSF II and TSF IIa) enriched in PS II reaction centers were fractionated from spinach chloroplasts following Triton treatment [25]. TSF IIa contains 1 P680/30–40 chl molecules and is free of P700 [25–27]. The fragments were suspended in 0.05 M Tris–HCl (pH 7.8). All measurements were made at 20°C in a 1 cm cuvette with 90° excitation geometry.

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Absorbance changes (ΔA) were measured with time resolution of ~3 ns essentially as in [24] except that a Tektronix R7912 transient digitizer was used for signal acquisition and averaging. Excitation was supplied by non-saturating 3 ns ruby laser pulses at 694.3 nm. A flash lamp with a pulsewidth of ~30 μs provided the measuring light.

An Ortec single-photon counting apparatus with a resolution of ~0.2 ns was used for fluorescence measurements. The excitation light source was a Spectra-Physics cavity-dumped, mode-locked rhodamine-6G dye laser, pumped synchronously by an argon laser. The dye laser pulses were at 590 nm, and had a width of 20–30 ps and a repetition frequency of 0.8 MHz. The laser intensity was attenuated to give a counting frequency of ~7 kHz for the fluorescence photons. Fluorescence was measured at 685 nm with an RCA C31034 photomultiplier. The fluorescence decay kinetics were deconvoluted by a computer, using a non-linear least-squares program.

3. Results and discussion

In agreement with [9,28], the reduction of plastoquinone in TSF–II particles induces the appearance of fluorescence (recombination luminescence [9]) with a lifetime of 4.3 ns (fig.1C). The ratio of the quantum yield of prompt fluorescence (lifetime ~1 ns) to that of the delayed fluorescence is ~2.5. The delayed fluorescence is not observed in the presence of 50 μM ferricyanide (fig.1A), or after reduction of Ph by continuous illumination in the presence of dithionite (fig.1B).

The delayed fluorescence at low redox potentials is accompanied by ΔA with a risetime of <1 ns and a lifetime of ~4 ns (fig.2C). These ΔA are not seen in the presence of ferricyanide or after photoreduction of Ph (fig.2A,B). The short-lived ΔA are superimposed on longer-lived ΔA that probably are due to triplet states of chlorophyll (chl) and carotenoids. Formation of the chl triplet state gives an $A_{410-430}$ decrease; the bleaching occurs within a few ns and decays with a lifetime of ~33 ns (fig.2C). The carotenoid triplet state gives an A_{515} increase with a risetime of ~33 ns (fig.2D). The ΔA due to the chl triplet state are not dependent on the redox state of Ph (fig.2B). This suggests that the chl triplet state is formed in antenna chl molecules, rather than in reaction centers. The carotenoid triplet state presumably forms by energy transfer from the chl triplet.

The spectrum of the ΔA with lifetime of ~4 ns

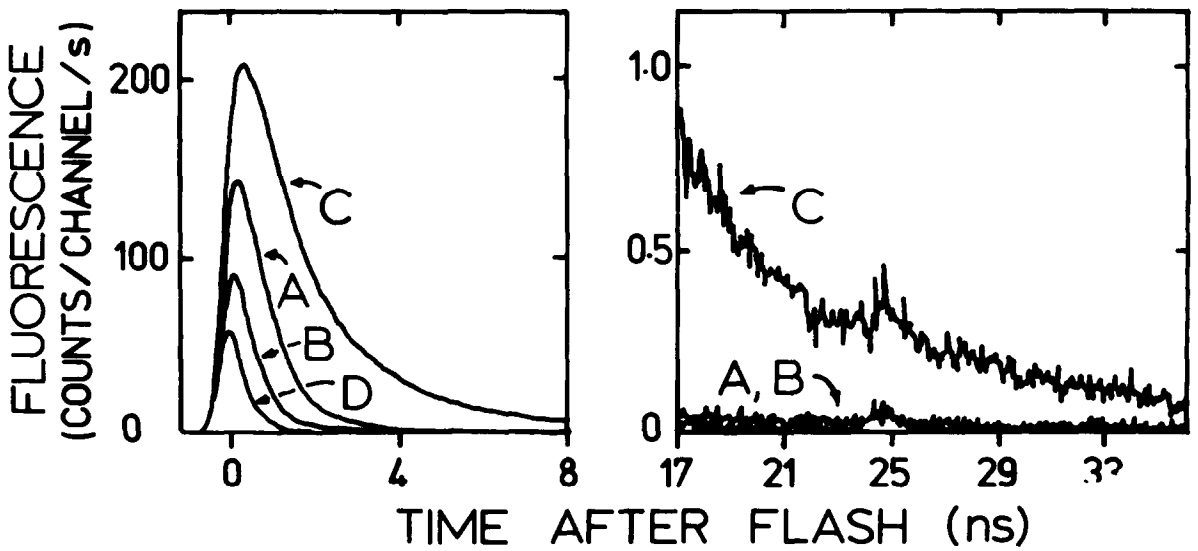

Fig.1. Kinetics of fluorescence from TSF II (15 μg chl/ml) at 20°C, measured by single-photon counting with excitation pulses at 590 nm (20–30 ps duration). The channel width was 79 ps. (A) In the presence of 50 μM ferricyanide; prompt fluorescence lifetime is 0.42 ns. (B) At E_h ~–450 mV (obtained by the addition of $Na_2S_2O_4$), ~60 s after continuous illumination with white light (~10^5 ergs/cm^2 . s) for 15 s; prompt fluorescence lifetime is 0.18 ns (C) At ~–450 mV in the dark; prompt fluorescence lifetime is 1.06 ns; delayed fluorescence lifetime is 4.3 ns. (D) Excitation pulse, measured by single photon counting.

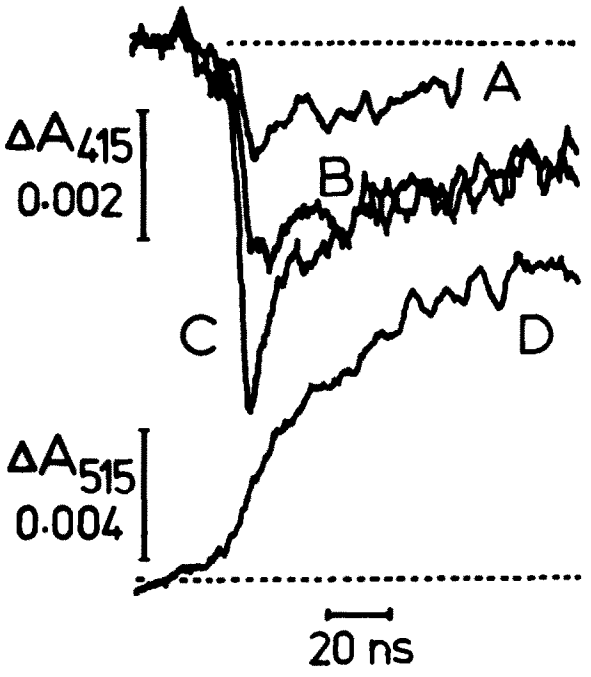

Fig.2. ΔA_{415} and ΔA_{515} of TSF IIa fragments induced by 3 ns laser pulses at 694.3. (A) In the presence of 50 μM ferricyanide. (B) At ~−450 mV after continuous illumination. (C,D) At ~−450 mV in the dark. (A–C) 15 μg chl/ml; (D) 45 μg chl/ml. Kinetics were averaged from 5–15 measurements.

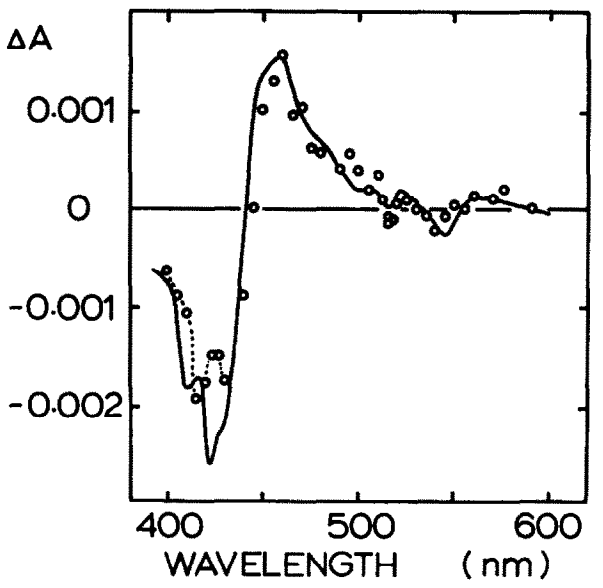

Fig.3. Spectrum of TSF IIa ΔA with lifetime of ~4 ns at ~−450 mV. Amplitude of ΔA was normalized for 15 μg chl/ml. Solid curve shows the sum of spectra for $P680^{+}$ and Ph^{-} formation, normalized at their red maximal bleachings (spectra from [3] and [12], respectively.)

(fig.3,o) includes A_{415}, A_{430}, A_{515} and A_{540} decreases and $A_{440-500}$ and A_{570} increases, with a maximum near 460 nm. The bleaching at 415, 515 and 540 nm and the development of an at A_{460} band are characteristic of $Ph^{\cdot-}$ formation in PS II [7–12]. The bleaching at 430 nm and $A_{460-580}$ increases region are characteristic of $P680^{+}$ formation [3,11,27]. The solid curve in fig.3 shows the sum of the spectra for $P680^{+}$ and $Ph^{\cdot-}$ formation, taken from [3] and [12], respectively.

Fig.3 shows reasonable coincidence of the sum of the $P680^{+}$ and $Ph^{\cdot-}$ spectra with the measured spectrum of the ΔA with lifetime of ~4 ns. When Q is in the reduced state, light absorption evidently induces charge separation between P680 and Ph, leading to the formation of the ion-radical pair $[P680^{+}\ Ph^{\cdot-}]$ in <1 ns. The recombination of this pair occurs in ~4 ns, and is accompanied by delayed fluorescence with about the same lifetime (fig.1C). The dependence of both the ΔA and the delayed fluorescence on the redox state of Q and Ph (fig.1,2) supports the assignment of the ΔA to the formation of the ion-radical pair $[P680^{+}\ Ph^{\cdot-}]$. The lifetime of the electron transfer from $[P680^{+}\ Ph^{\cdot-}]$ to Q appears to be <400 ps since the ΔA and delayed fluorescence due to the state $[P680^{+}\ Ph^{\cdot-}]$ are decreased by factor of ⩾10 when Q is in the oxidized form (fig.1,2).

These experiments thus strengthen the idea [7–13] that charge separation between P680 and plastoquinone in PS II involves Ph as an intermediate electron acceptor, as shown in scheme 1.

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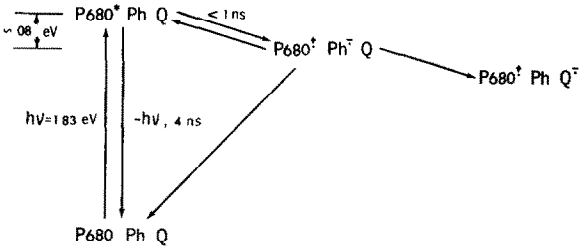

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