EFFECT OF PHOTOREDUCTION OF THE PHOTOSYSTEM-II INTERMEDIARY ELECTRON ACCEPTOR (PHEOPHYTIN) ON TRIPLET STATE OF CAROTENOIDS

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1. Introduction

Photochemical reaction in photosystem-II (PSII) reaction centers results in an electron transfer from the primary donor, P680, probably a chlorophyll (chl) dimer, to the primary acceptor, Q, a special form of plastoquinone (reviewed [1,2]). Pheophytin (Ph), with redox potential of −610 mV [7], acts as an intermediary electron acceptor between P680 and Q [3–10]. When Q is reduced beforehand, the state [$P680^{\cdot+} \cdot Ph^{\cdot-}$] formed during photochemical charge separation decays in ~3 ns as determined by measurements of recombination luminescence [5]. Continuous illumination of PSII preparations at $E_h \simeq -450$ mV (when Q is reduced in the dark), however, leads to a reversible photoaccumulation of the long-lived state [$P680 \cdot Ph^{\cdot-}$], probably due to the fast reduction of $P680^{\cdot+}$ ($\leqslant 1$ μs [11–13]) by a secondary electron donor competing with the charge recombination [3–5,8]. Photoreduction of Ph in PSII is accompanied by the appearance of a narrow EPR signal of $Ph^{\cdot-}$ [6,9,10], as well as a broad EPR 'doublet' ascribed to an exchange interaction between $Ph^{\cdot-}$ and a singly reduced plastoquinone coupled to Fe [9,10]. All these observations [3–10] indicate many similarities between PSII reaction centers and those of photosynthetic bacteria [9,10].

In reaction centers of photosynthetic bacteria, when normal photochemistry is blocked under reducing conditions, charge recombination between the photooxidized primary electron donor, $P^{\cdot+}$, and photoreduced intermediary acceptor, $BPh^{\cdot-}$, leads to formation of a triplet state of P and, subsequently, of a carotenoid triplet state (^{3}Car) by triplet energy transfer (in carotenoid containing strains) [14–23]. In green plants, there is also a ^{3}Car [24,25] that can be an effective quencher for chl fluorescence [26,27]. A ^{3}Car is observed in chloroplasts as well as in subchloroplast particles, enriched in PSI or PSII, and in the light-harvesting particles; its formation, however, does not depend on the redox state of PSI or PSII reaction centers and is probably the result of energy transfer from the triplet state of antenna chl (^{3}chl) to Car [26]. We report here a ^{3}Car in PSII preparations which depends on the redox state of Q and Ph and suggest that at least part of the ^{3}Car can result from charge recombination in [$P680^{\cdot+} \cdot Ph^{\cdot-}$].

2. Materials and methods

Spinach chloroplasts, as well as subchloroplast particles TSF-IIa and TSF-II (containing 1 PSII reaction center/30–40 [9,29–31] and 90–120 [10] chl molecules, respectively, and free of P700 [29,30]), were isolated as in [28–30]. The preparations were suspended in 0.05 M Tris (pH 8.0), with 50% glycerol added for measurements at cryogenic temperatures.

Changes in absorbance (ΔA) and fluorescence yield, induced by continuous actinic light from a 1000 W incadescent lamp filtered by 5 cm $CuSO_4$ solution, were measured in a phosphoroscopic photometer, using a measuring beam modulated at 25 kHz and a Dynatrac-3 lock-in amplifier (Ithaco).

Flash-induced ΔA with 1 μs resolution was measured as in [31] in a 1 mm cuvette. The measuring light source was a tungsten–iodine lamp, filtered by a Jobin Yvon monochromator (bandwidth 8 nm). The

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detector was an EMI-9558 photomultiplier shielded by an appropriate interference filter and a sharp cut-off filter transmitting below 560 nm. A 300 ns dye-laser pulse at 640 nm was used for excitation (80% saturating), the flash-induced ΔA being registered in a Tracor-Northern model-1710 signal averager buffered by a Biomation model 805 waveform recorder. Flash-induced ΔA with a resolution of ~3 ns was measured as in [32], using 3 ns ruby-laser pulses (694 nm) for excitation.

3. Results

After addition of dithionite to TSF-IIa fragments ($E_h \simeq -450$ mV), 300 ns dye-laser flashes induce a large A_{515} increase, decaying with $t_{1/2}$ ~7 μs at 295 K (fig.1). Its amplitude is ~5-times greater than in the presence of 50 μM ferricyanide ($E_h \simeq +430$ mV). A similar effect is seen in TSF-II fragments as well as in chloroplasts but the amplitude of ΔA is ~2 and 4-times smaller, respectively (fig.1). The spectrum of ΔA in TSF-IIa fragments is characterized by bleaching of 3-bands in the 400–500 nm region and by development of a broad band with an A_{515} max (fig.2).

Illumination of TSF-IIa by continuous actinic light at $E_h \simeq +430$ mV causes a reversible increase in chl fluorescence yield (fig.3), indicating that Q is oxidized in the dark and reduced by illumination [1–10]. At $E_h \simeq -450$ mV (when Q is reduced in the dark), actinic light induces ΔA_{685} (fig.3) as well as a 2.5-fold decrease in chl fluorescence yield, both changes characteristic of photoreduction of Ph in PSII reaction centers [3–10]. The actinic light also induces ~3.5-fold attenuation of the flash-induced ΔA_{515} (fig.1,3), in parallel with the photoreduction of Ph (fig.3). No change in the $t_{1/2}$ of decay of ΔA was observed as a result of photoreduction of Ph. Continuous illumination of both TSF-II fragments and chloroplasts at $E_h \simeq -450$ mV caused ~3- and ~4-fold decrease, respectively, in chl fluorescence yield, accompanying

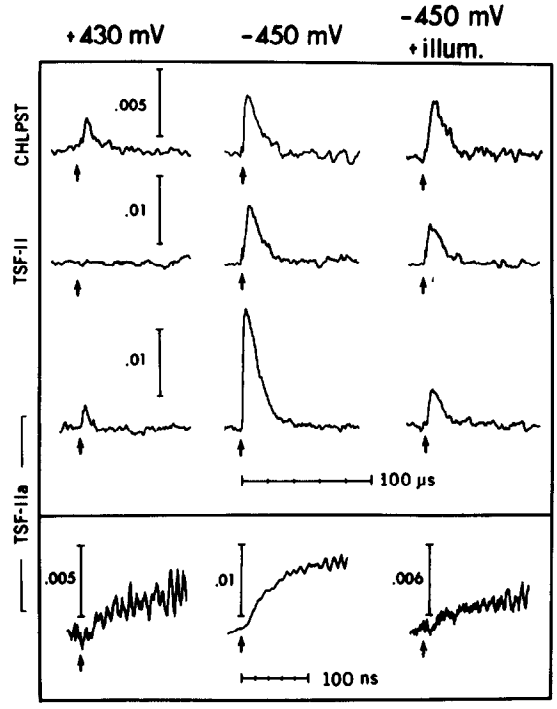

Fig.1. Kinetics of ΔA_{515} induced by laser flashes in chloroplasts, TSF-II, and TSF-IIa fragments, at $E_h \simeq +430$ mV (in the presence of 50 μM ferricyanide) and at $E_h \simeq -450$ mV in the dark and after 1 min illumination; 20°C. Upper: excited by 300 ns laser flash at 640 nm; 65 μg chl/ml; pathlength, 1 mm. Lower: excited by 3 ns laser flash at 694 nm; 15 μg chl/ml; pathlength, 1 cm.

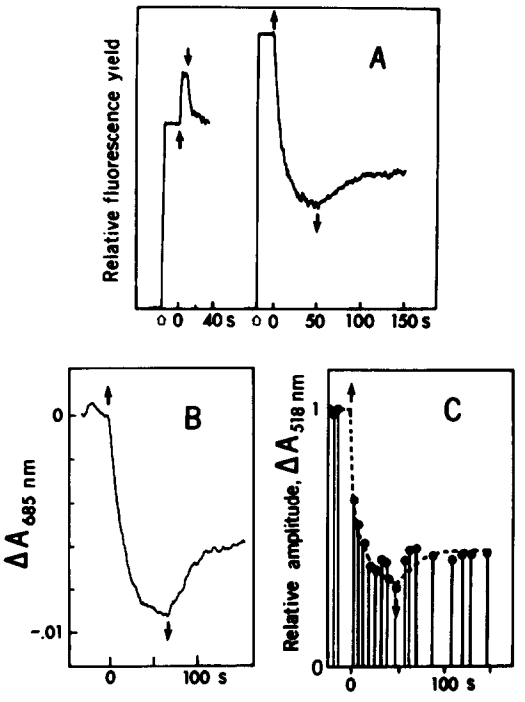

Fig.2. Spectrum of ΔA induced by 300 ns dye-laser flash (at 640 nm) in TSF-IIa fragments at $E_h \simeq -450$ mV. The amplitude of ΔA with decay $t_{1/2}$ of ~7 μs (see fig.1, middle) was used for the plot. Each data point is av. 2–8 repetitive measurements; 65 μg chl/ml; pathlength, 1 mm; 20°C.

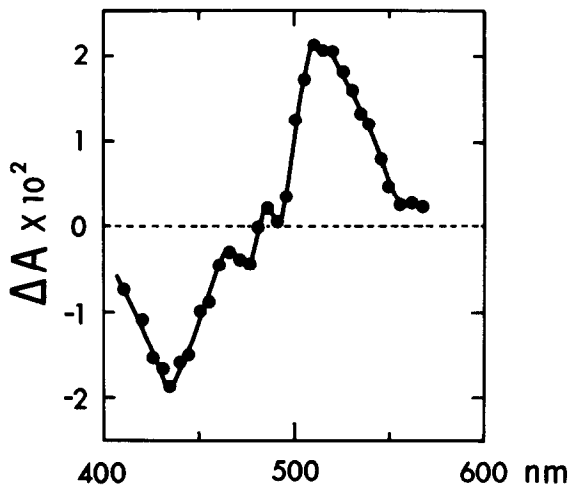

Fig.3. Effect of continuous illumination of TSF-IIa fragments at $E_h \simeq -450$ mV. (A) Right trace, chl fluorescence yield; left trace, same, except sample was poised at +430 mV. (B) ΔA_{685}. (C) Amplitude of the flash-induced ΔA_{515} (see fig.1). The first 3 data points were measured prior to actinic illumination, the remaining data points were measured during and after illumination. The upward hollow arrow in (A) represents probing beam turned on (broadband-blue light; 50–100 ergs/cm² . s); upward and downward arrows represent actinic light on and off (wavelength >600 nm; 5 × 10^5 ergs/cm² . s); 120 μg chl/ml; pathlength, 1 mm; 20°C.

photoreduction of Ph (not shown). However, this effect was accompanied by only a 30% decrease in flash-induced ΔA_{515} in TSF-II and no effect of actinic light was seen in chloroplasts (fig.1).

The decay $t_{1/2}$ of ΔA_{515} in TSF-IIa at $E_h \simeq -450$ mV was not appreciably changed upon lowering the temperature; at 7 and 90 K it remained at 7–8 μs. Phototrapping of Ph in these particles at 295 K (followed immediately by freezing in liquid nitrogen) resulted in ~2.7-fold decrease in flash-induced ΔA_{515}, measured at 7 K.

Measurement of ΔA_{515} with resolution of ~3 ns shows (fig.1, bottom) that its risetime in TSF-IIa at $E_h \simeq -450$ mV is ~33 ns at 295 K. The ΔA is decreased ~4-fold upon either oxidation of Q or photoreduction of Ph (fig.1, bottom).

4. Discussion

The difference spectrum as well as the decay kinetics of the flash-induced ΔA reported here are quite characteristic of ^{3}Car described in chloroplasts and subchloroplast particles [24–27]. Assuming ^{3}Car to have $\epsilon_m^{515} = 10^5\ M^{-1} \cdot cm^{-1}$ [26], 1 ^{3}Car is found in TSF-IIa, TSF-II and chloroplasts per 30–35, 60–80 and 110–130 chl molecules (or ~1, 1.5 and 2/PSII reaction center), respectively. The formation of ^{3}Car occurs in ~33 ns (fig.1), in agreement with the value (<200 ns) reported in [24].

The dye-laser pulses used here excited chl directly but not carotenoids and, according to [26,27], the ^{3}Car formed under these conditions is due to energy transfer from a ^{3}chl to Car. According to [26], formation of triplet states in chloroplasts is not closely related to photosynthetic charge-transfer reactions and instead results from intersystem crossing in singlet excited molecules of antenna chl. These data, however, show that at least part of the ^{3}Car can be related to the functioning of PSII reaction centers. In fact, the extent of photo-induced ^{3}Car increased several-fold upon reduction of Q and decreased markedly upon photoreduction of Ph, the intermediary electron acceptor of PSII. Furthermore, the latter effect is the largest in TSF-IIa fragments, which have the highest concentration of PSII reaction centers. Similar effects have been found earlier in bacterial reaction centers, namely, enhancement of ^{3}Car formation upon reduction of the primary electron acceptor [14–23] and inhibition following photoreduction of the intermediary electron acceptor, BPh [20–22]. In analogy with events in bacterial reaction centers, we suggest that the portion of ^{3}Car which depends on the redox state of Q and Ph arises from a ^{3}chl formed as a result of charge recombination in [$P680^{+} \cdot Ph^{-}$]. That carotenoids are functioning in PSII reaction centers can also be inferred from the requirement for Car in reconstituting both the C550 signal [2] (which is the result of a blue shift of a Ph absorbance band by reduced Q [3,4,33]) and the EPR 'doublet' [9,10], after extraction of Q. The portion of ^{3}Car not affected by photoreduction of Ph (~30% in TSF-IIa and ~70% in TSF-II) is probably formed from a triplet state of antenna chl.

A triplet state of chl arising from radical-pair recombination between oxidized P700 and reduced primary electron acceptor of PSI has been detected by EPR spectroscopy [34]. Appearance of ^{3}Car upon excitation of the PSI reaction centers in addition to the ^{3}Car arising from antenna ^{3}chl may explain the absence of appreciable changes in ^{3}Car formation upon photoreduction of Ph in chloroplasts (fig.2).

Thus, enhancement of ^{3}Car formation under reduction of Q and its subsequent inhibition by photore-

duction of Ph, the intermediary electron acceptor of PSII, show that at least part of the ^{3}Car can result from charge recombination in [$P680^{\cdot+} \cdot Ph^{\cdot-}$].

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