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Electron transfer reactions in photosystem I following vitamin K_1 depletion by ultraviolet irradiation

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Photosystem I preparations were irradiated with UV to destroy vitamin K_1 in situ. The depletion of vitamin K_1 resulted in inactivation of $NADP^+$ photoreduction and introduction of a ~220 ms component in the flash generated $P700^+$ re-reduction at room temperature. The photoreduction of the terminal FeS centers FA and FB in control and vitamin K_1-depleted preparations at 7 K were comparable. The data confirm that vitamin K_1 is functionally implicated in primary electron transfer reactions in PS I at physiological temperature, and that the anomalous results at cryogenic temperature may be explicable in terms of a by-pass of the vitamin K_1 acceptor site or heterogeneity introduced into the photosystem by quinone removal.

Photosystem I; Primary reaction; Vitamin K_1; Ultraviolet irradiation; EPR; Optical transient

1. INTRODUCTION

The primary charge separation in PS I results in the formation of the radical pair $P700^+A_0^-$. Subsequent rapid electron transfer reactions occur through secondary redox centers referred to as A_1, Fx and FA/FB. The terminal acceptors FA/FB have been identified as two 4Fe:4S clusters associated with an 8.9 kDa polypeptide localized on the stromal domain of the PS I core to interface with the enzymatic apparatus for $NADP^+$ photoreduction. Fx is more electronegative than FA/FB and is most likely another 4Fe:4S cluster, but is associated with the PS I core which comprises the 82 and 83 kDa polypeptides of the PS I reaction center (see [1] for review).

The identity of the acceptor A_1, the postulated donor to Fx, is presently controversial. A_1 was provisionally identified as vitamin K_1 based upon a consideration of its EPR signal [2,3] and, more recently, a comparison of the electron spin polarized flash-induced EPR K-band spectrum of $P700^+A_1^-$ with that of $P870^+Q^-$ in Fe-depleted bacterial reaction centers [4]. Direct support for the participation of vitamin K_1 was provided by Brettel et al. [5] following a detailed examination of optical flash transients in UV at cryogenic temperature. Subsequently positive identification of the junctional involvement of vitamin K_1 was obtained by solvent extraction of vitamin K_1 and the specific reconstitution of room temperature biochemical activities of PS I using exogenous phylloquinone [6]. These reconstitution studies have recently been confirmed using an ether extracted preparation of PS I from spinach [7].

Examination of the solvent-extracted PS I preparations that were totally depleted of vitamin K_1 at cryogenic temperature led to anomalous results in that the centers FA/FB were found to be phototrapped with high efficiency [8] and the EPR signal corresponding to A_1 was still observed (Setif, P., personal communication). Additional controversy regarding the participation of vitamin K_1

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Abbreviations: DPIP, dichlorophenolindophenol; P700, primary electron donor of photosystem I; A_0, primary electron acceptor of photosystem I; Chl, chlorophyll

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in PS I electron transfer came from studies of preparations of PS I that were depleted of vitamin K_1 by UV irradiation to destroy the phylloquinone in situ. Palace et al. [9] reported that the kinetics of the room temperature charge recombination between $P700^+$ and the reduced terminal FeS centers was not affected following vitamin K_1 destruction suggesting the non-participation of vitamin K_1 in the electron transfer sequence. Zeigler et al. [10] also showed that depletion of vitamin K_1 by irradiation treatment of PS I did not alter the EPR spectrum of A_1 and concluded that the signal ascribed to the acceptor A_1 does not originate from vitamin K_1.

In efforts to resolve these discrepancies we have reinvestigated the effect of UV irradiation on the electron transfer activities of PS I at physiological temperature and included a study of the behavior of FeS redox centers at 7 K on the same preparations. The data are consistent with the requirement for vitamin K_1 for the photoreduction of $NADP^+$ and that it is an electron transfer component earlier than Fx in confirmation of [6]. We also confirm and extend the optical flash transient studies of $P700^+$ recombination reactions at room temperature [9] and EPR studies of reduced FeS centers at cryogenic temperature [8,10].

2. MATERIALS AND METHODS

2.1. *Preparations*

Spinach D-144 preparations were isolated according to Anderson and Boardman [11] and stored at −80°C. The PS I preparations had an antenna size of ~290 Chl *a,b*/P700 and a Chl *a/b* ratio of 4.38 ± 0.29.

2.2. *UV irradiation*

The UV irradiation of PS I was conducted at 2°C in a Pyrex thermostatted chamber at a Chl concentration of 10 μg/ml. The light source, Blak Ray model B100A (Ultraviolet Products, San Gabriel, CA) provided wavelengths of 300–400 nm at an intensity of 50 $W \cdot m^{-2}$. These conditions were similar to those used by Palace et al. [9].

2.3. *Biochemical assays*

Vitamin K_1 was extracted and analyzed using reversed-phase HPLC [6] except that the eluant was methylene chloride/methanol (10:90). $NADP^+$ photoreduction was determined spectrophotometrically at 340 nm using supplementary ferredoxin and ferredoxin-$NADP^+$-reductase (Sigma Chemical Co., St Louis, MO) and 20 mM ascorbate and 100 μM DPIP as donor. The reactions were conducted at light saturation but they were most probably limited by the absence of plastocyanin as physiological donor.

2.4. *Optical flash transients*

Flash-induced absorbance transients were detected at both 700 and 820 nm using a PIN 10D silicon photodiode (United Detector Technology, Hawthorne, CA). The detector output was amplified (model 113 preamp, EG&G/PARC, Princeton, NJ) and the transients were averaged using a 125 MHz digital oscilloscope (model 9400, LeCroy, Chestnut Ridge, NY). Saturating activation flashes were provided by a Q-switched, frequency doubled Nd:YAG laser (model DCR 11, Spectra Physics, Mountain View, CA). The flashes were 532 nm and 8–9 ns (full width at half height).

2.5. *EPR spectroscopy*

EPR experiments were carried out using an X-band spectrometer (Varian Associates, Palo Alto, CA) employing an E102-E bridge, with field modulation frequency, 100 kHz; field modulation amplitude, 16 G; lock-in sensitivity, 100 mV; sweep time, 10 min; time constant, 1 s; microwave frequency, 9.055 GHz, and microwave power, 50 mW. The temperature was maintained between 7–10 K using a helium flow cryostat (Air Products, Allentown, PA).

3. RESULTS

The spinach D-144 PS I preparations were analyzed and found to contain 2.12 ± 0.38 mol vitamin K_1/mol P700. Upon irradiation using wavelengths in the range 300–400 nm the vitamin K_1 was totally depleted after 2–2.5 h. Fig.1 shows the flash-induced absorbance signal at 700 nm due to $P700^+$. The decay halftime for the optical transient in the control preparation was ~ 30 ms and can be assumed to be due to the recombination between $P700^+$ and the reduced terminal acceptors FA/FB [1]. After vitamin K_1 depletion (lower trace), the optical transient still showed a slow recombination in confirmation of [9] but the decay halftime was longer. Fig.2 (upper panel) shows that, during the irradiation treatment, the decay halftime of the transient increased to ~ 60 ms, and upon more detailed analysis, we found that this was due to the introduction of a slower decay component with a halftime of approx. 220 ms. This contrasts with Palace et al. [9] who reported that the vitamin K_1 depletion did not modify the P700 recombination kinetics.

Fig.2 (lower panel) shows that the inactivation of $NADP^+$ photoreduction also closely followed the depletion of vitamin K_1 during irradiation treatment. These results confirm those observed following solvent extraction of *Synechocystis* PS I [6], and correlate the loss of physiological electron transfer function with vitamin K_1 removal.

The behavior of the terminal FeS redox centers

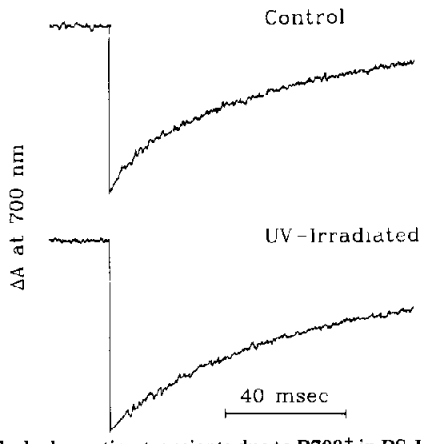

Fig.1. Flash absorption transients due to $P700^+$ in PS I (control) and following vitamin K_1 depletion (UV-irradiated). The transient amplitudes (ΔA) for the control and UV-irradiated samples were 1.66×10^{-3} and 1.72×10^{-3}, respectively.

FA/FB was investigated via EPR at cryogenic temperature for control and irradiated samples. The samples were dark-adapted at room temperature in the presence of ascorbate and DPIP and cooled in the dark to ~ 7 K. After recording a dark baseline, which was used for the correction of subsequent spectra, the samples were illuminated for 15 s and then, following a short dark period, the spectra were recorded in darkness to show irreversibly reduced redox centers (phototrapped). The upper panel of Fig.3 shows

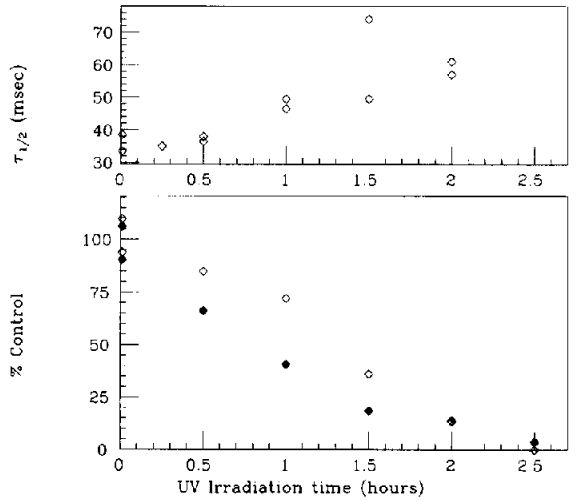

Fig.2. (Upper panel) The effect of UV irradiation on the halftime of the $P700^+$ recovery following flash activation at room temperature. (Lower panel) The effect of UV irradiation on vitamin K_1 concentration (—◆—) and $NADP^+$ photoreduction (—◇—).

that the FeS center FA was phototrapped in both the control and the sample depleted of vitamin K_1 (UV irradiated). The samples were then illuminated for several minutes, and then EPR spectra were determined in continuous light. In continuous light (lower panel of Fig.3) center FB was resolved as well as center FA for both samples. In both the phototrapped and continuous light treatments, the EPR intensities arising from the FA/FB centers in control and UV-irradiated samples were com-

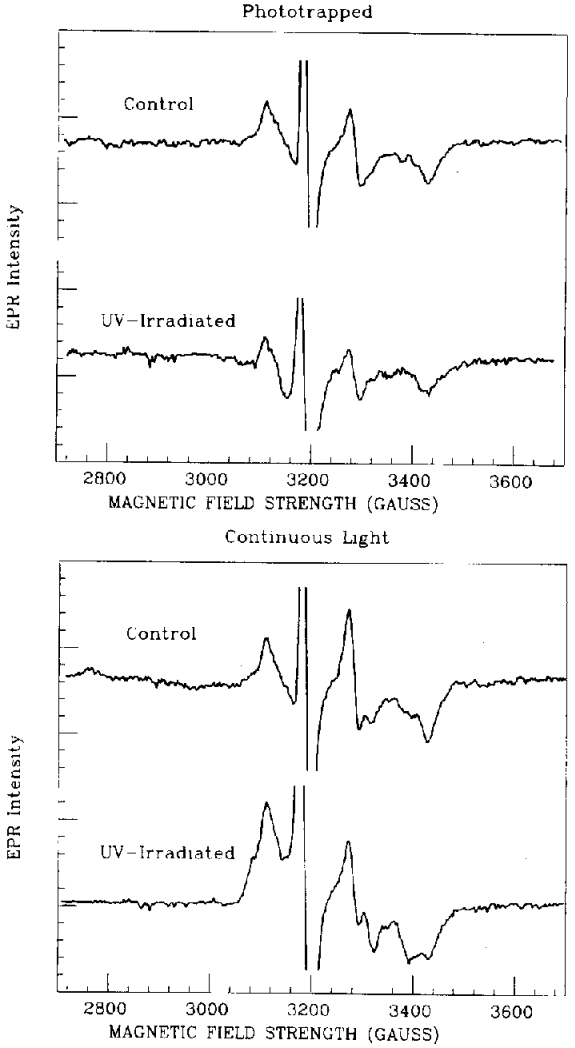

Fig.3. EPR spectra obtained between 7 and 10 K of control and UV-irradiated spinach PS I preparations. (Upper panel) Samples dark adapted at room temperature, cooled in darkness and then illuminated for 15 s. The spectra show redox centers that were phototrapped. (Lower panel) Spectra acquired during continuous illumination of the samples.

parable indicating that the terminal FeS clusters were photoreduced at cryogenic temperature in PS I preparations depleted of vitamin K_1.

4. DISCUSSION

The data presented above on the electron transfer activities of PS I preparations depleted of vitamin K_1 by UV destruction in situ are similar to those reported previously for preparations treated with organic solvents to extract the vitamin K_1 [6,8]. In both protocols for vitamin K_1 removal, we observed the loss of $NADP^+$ photoreduction at physiological temperature [6], but photoreduction of the centers FA and FB at cryogenic temperature was not impaired [8].

An important difference shown in this study was the room temperature flash absorbance transient due to the charge recombination between reduced acceptor(s) and $P700^+$. In solvent extracted PS I preparations from *Synechocystis* we showed that the 30 ms charge recombination reaction between $P700^+$ and reduced FeS was replaced by a much faster reaction [6]. This was interpreted as a recombination between partners of the primary biradical $P700^+A_0^-$, and decay of triplet P700 formed from this species. This process has been shown to be reversible upon addition of vitamin K_1 in a reconstitution process that has recently been confirmed and extended using an ether extracted spinach PS I preparation [7]. This behavior contrasts with the UV-irradiated PS I preparations presented here where although we confirm [9] that vitamin K_1 destruction in situ does not lead to the formation of an ultra-fast recombination reaction at the expense of the slow recombination of $P700^+P430^-$, the decay halftime increased during vitamin K_1 depletion in preparations that were also undergoing inactivation of $NADP^+$ photoreduction. The increase in decay halftime was found to be due to the introduction of a much slower recombination pathway at the expense of the 30 ms component showing that, in contrast to [9], the $P700^+$ reduction kinetics are extensively modified following vitamin K_1 removal.

We conclude that the similarity between the physiological electron transfer activities of PS I preparations depleted of vitamin K_1 via in situ destruction with those of solvent extracted preparations [6,7] confirms that vitamin K_1 is a participant in the electron transfer pathway for $NADP^+$ photoreduction. It was previously suggested that the anomalous data obtained at cryogenic temperature was explicable if either a bypass of the vitamin K_1 acceptor site occurred following vitamin K_1 removal, or some other heterogeneity in the pathway had been introduced [6,8]. The flash transient data presented above show that some heterogeneity in electron transfer is also observed at room temperature following vitamin K_1 destruction via UV irradiation.

Finally, we suggest that although the extraction and reconstitution data appear to implicate a functional role for vitamin K_1 on the acceptor side of PS I, vitamin K_1 is most likely not the chemical identity of the EPR signal generally referred to as A_1. Both solvent-extracted and UV-irradiated PS I preparations with no detectable vitamin K_1 continue to show the EPR signal characteristic of A_1 ([10] and Setif, personal communication) and the photoaccumulated EPR spectra in the PS I of cyanobacteria containing protonated and deuterated vitamin K_1 (position 2-methyl group) are identical indicating that the radical does not originate from phylloquinone [12].

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