Volume 217, number 1, 16-20 FEB 04756 June 1987

Bound electron acceptors of photosystem I

Evidence against the identity of redox center A_1 with phylloquinone

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Received 25 March 1987

Photosystem (PS) I preparations from spinach and from a cyanobacterium contain two molecules of phylloquinone (2-methyl-3-phytyl-1,4-naphthoquinone) per photosystem, only one of which is rapidly destroyed by ultraviolet light. In preparations of the core of PS I, the P700 reaction center, both quinone molecules are rapidly destroyed by UV irradiation. Nearly complete destruction does not alter the shape and size of the EPR spectrum of the electron acceptor A_1, which is believed to be phylloquinone. Photooxidation of P700, the primary electron donor of PS I, is also not affected. The results provide strong evidence against the identity of redox center A_1 with the naphthoquinone.

Photosystem I; P700; Reaction center; Phylloquinone; Electron acceptor; EPR

1. INTRODUCTION

The electron acceptor complex of PS I of oxygenic photosynthesis appears to consist of a chain of redox centers called A_0, A_1, X, A and B. Centers X, A and B are iron-sulfur centers (see [1]); A_0 seems to be a monomer of chlorophyll *a* [2]. EPR and optical spectra of the reduced acceptor A_1 resemble those of a semiquinone [2-5]. Isolated PS I and its core, RC I, contain two molecules of phylloquinone (2-methyl-3-phytyl-1,4-naphthoquinone) as the only quinone present in significant amounts [6-8,10], suggesting that center A_1 may be phylloquinone.

It has not yet been established whether center A_1 and phylloquinone are required for electron transfer in PS I under physiological conditions (see [4]). A functional role of a quinone is challenged by the rather high resistance of the photosystem to UV light [9]. Malkin [10] recently reported that extraction of PS I with organic solvents under mild conditions removes only one of the phylloquinones, without effects on the electron transfer reactions assayed.

To test the proposal [4,5,8,11] that acceptor A_1 is phylloquinone, we looked for conditions allowing destruction of the quinone in situ by near-UV light (cf. [12]). It is found that rapid and complete destruction occurs in P700 reaction centers, highly resolved, photochemically active particles derived from PS I by mild SDS treatment [13]. Irradiated particles show unchanged EPR spectra of acceptor A_1. Light-induced electron transfer also seems to be not affected significantly.

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Abbreviations: DPPH, 1,1-diphenyl-2-picrylhydrazyl radical; P700, primary electron donor of photosystem I; PS, photosystem; RC I, P700 reaction center of PS I

2. MATERIALS AND METHODS

2.1. *Preparations*

PS I was isolated from spinach (*Spinacia oleracea* L.) and from the cyanobacterium *Ana-*

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00145793/87/$3.50

baena variabilis (American Type Culture Collection 29413) with Triton X-100 as in [8]. The P700 reaction centers were derived from PS I by treatment with SDS followed by sucrose density gradient centrifugation [8]. Plastocyanin from spinach and cytochrome *c*-553 from *Anabaena* were isolated as in [14,15].

2.2. *UV treatment*

Samples of 5 ml were irradiated with slow stirring at a concentration corresponding to 0.1 mg Chl/ml in 50 mM Tris-HCl (pH 8.0), 10 mM NaCl, 0.4 M sucrose at 0°C with light from a high-pressure Hg lamp (model St 41, Zeiss) through a UG 1 filter (1 mm thickness, Schott). The light consisted mainly of the 302, 313, 334 and 366 nm lines with calculated relative intensities of 8:33:7:100. Controls were treated identically, but kept in darkness.

2.3. *EPR spectroscopy*

Irradiated and control samples were concentrated in dialysis tubes with polyethylene glycol 2000 (Serva), dialyzed overnight against 5 mM Tris-HCl (pH 8.0), 0.1% Triton X-100 and then against this buffer containing 60% glycerol for 4 h. The following procedure ('phototrapping') is similar to that in [2,5]. After addition of glycine-NaOH (pH 10) to 0.1 M and $Na_2S_2O_4$ to 10 mM, the samples (about 0.5 mg Chl/ml) were bubbled with Ar for 30 min, illuminated under Ar in an EPR tube at 0°C for 5 min with light from a 250 W slide projector spaced 30 cm apart, and after 2 min in darkness frozen in liquid N_2. Baselines were recorded, and the samples then illuminated for 5 min at −78°C (dry ice/acetone bath, light source as before) followed by EPR spectroscopy in liquid N_2. This illumination protocol was repeated several times until the EPR signal of reduced center A_1 was maximal. The X-band EPR spectrometer was a Bruker ER 220 D with a double resonance cavity. DPPH served as a standard.

2.4. *Assays*

Photoreduction of benzyl viologen was followed via the coupled O_2 uptake in a Clark-type oxygen electrode. The basic reaction mixture contained 10 mM sodium phosphate (pH 7.2), 10 mM sodium ascorbate, 0.1 mM 2,3,5,6-tetramethyl-*p*-phenylendediamine dihydrochloride, 0.5 mM benzyl viologen and control or UV-treated samples with 6.7 μg Chl/ml. Cytochrome *c*-553 at 3 μM was added as electron donor for the P700 reaction centers from *Anabaena*. For samples from spinach, the reaction mixture was supplemented with 5 μM plastocyanin and 5 mM $MgCl_2$. Illumination was with red light (> 640 nm) of an intensity of about 300 W/m²; $T = 25$°C.

Photooxidation of P700 was followed at 701 minus 725 nm, bandpass 3.5 nm, in an Aminco DW-2 spectrophotometer at 20°C. Assay mixtures

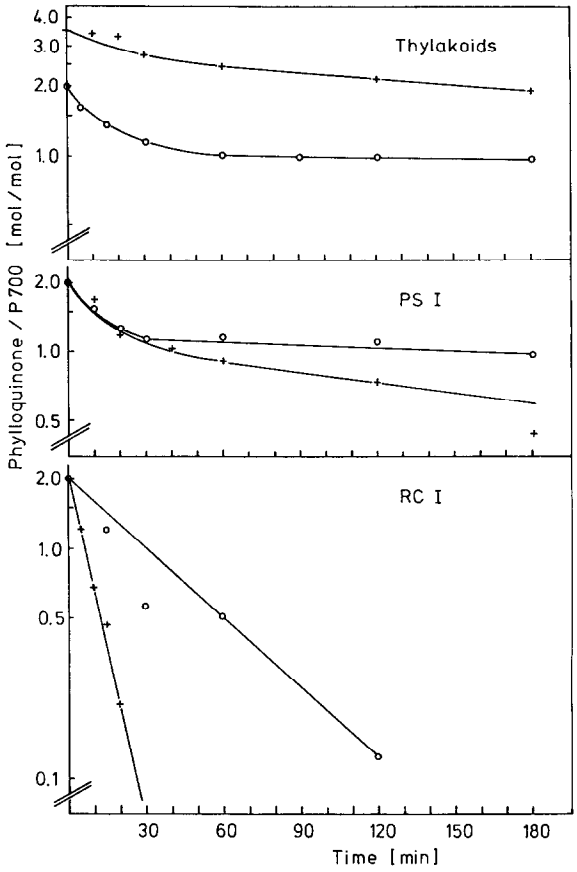

Fig.1. Kinetics of destruction, by near-UV light, of phylloquinone in thylakoids, PS I preparations and in P700 reaction centers from spinach (○—○) and from *A. variabilis* (+—+). Details are given in section 2. The experiment with P700 reaction centers from spinach was performed at a lower intensity of UV light and cannot be compared quantitatively with the other kinetics. P700 was estimated by redox difference spectroscopy as in [8].

contained 50 mM sodium phosphate (pH 7.2), 1 mM sodium ascorbate and samples with 10 μg Chl/ml. Assays under anaerobic conditions were run under N_2 in the presence of 0.1 M glucose, 10 U/ml of glucose oxidase and 650 U/ml of catalase. Samples were illuminated with blue light (Schott BG 28 and Corning 4-96 filters) of 23 W/m^2, the photomultiplier being protected by slits and an interference filter peaking at 712 nm (half-bandwidth 15 nm, Schott). The extinction coefficients of Hiyama and Ke [16] were used.

Chlorophyll was quantified as in [17] for *Anabaena* and as in [18] for spinach, and phylloquinone as in [8].

3. RESULTS AND DISCUSSION

Fig.1. shows the kinetics of destruction, by UV light of 300–370 nm, of phylloquinone in thylakoids, PS I preparations and P700 reaction centers. In thylakoids, about 1 phylloquinone per PS I is destroyed rapidly, the remaining quinone being quite resistant. Very similar biphasic kinetics have been reported by Lichtenthaler and Tevini [19] for spinach thylakoids for light of 254 nm. Since this short-wavelength UV light should be absorbed equally by both oxidized and reduced forms of the naphthoquinone (see spectra in [20]), it is unlikely that the biphasic kinetics are due to dif-

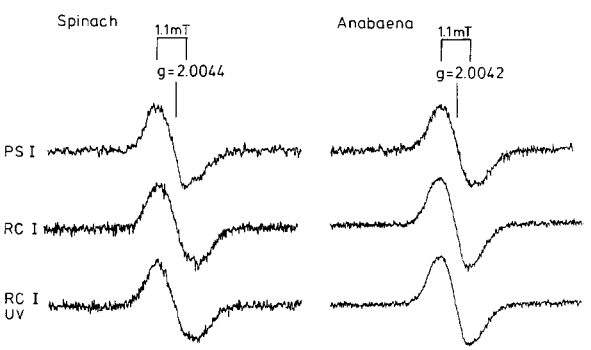

Fig.2. EPR spectra of the electron acceptor A_1 reduced by photoaccumulation in PS I preparations and P700 reaction centers (RC I) from spinach and *A. variabilis*. The samples designated RC I UV were treated with near-UV light and contained 0.15 phylloquinone/P700 (spinach) and 0.10 phylloquinone/P700 (*Anabaena*). P700 was determined by redox difference spectroscopy as in [8]. First derivative spectra are shown. Conditions for EPR spectroscopy: microwave power, 63 μW; microwave frequency for the individual samples, 9.46–9.48 GHz; field modulation frequency, 100 kHz; modulation amplitude, 0.2 mT; scan range, 10.0 mT; field center, 338.0 mT; instrument gain, 1×10^6; $T = 77$ K. The g factor of each spectrum was determined in a double resonance cavity with DPPH as standard. It was checked that the standard did not 'spill over'.

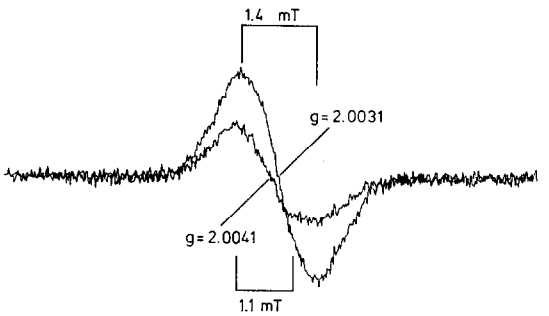

Fig.3. EPR spectra of a UV-treated P700 reaction center preparation from spinach, preilluminated at −78°C for 15 min ($g = 2.0041$) and additionally at −44°C for 5 min ($g = 2.0031$). The first illumination yields a spectrum typical for A_1, the second illumination the more symmetric spectrum of centers A_1 plus A_0. The ratio of phylloquinone/P700 was 0.12. EPR conditions were as in fig.2. A temperature of −44°C was maintained by a slush of ethyl acetoacetate.

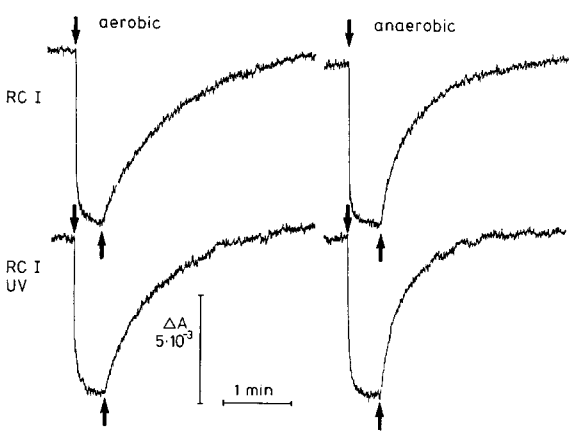

Fig.4. Kinetics of photooxidation in control and UV-irradiated samples of P700 reaction centers from spinach measured at 701 minus 725 nm. The UV-treated sample contained 0.15 phylloquinone/P700. ↓, actinic light on; ↑, actinic light off. Similar results were obtained for *Anabaena*. Details are described in section 2.

ferent redox states of the quinone molecules in the membranes. The cause of UV resistance is unknown. Resistance may be conferred by resonance transfer of excitation energy to an associated pigment. Biphasic kinetics are retained in the PS I preparations from both *Anabaena* and spinach. This is taken as evidence that the naphthoquinone is not just accidentally co-purifying with the hydrophobic photosystem. Destruction of the UV-sensitive naphthoquinone did not change the EPR spectra of acceptor A_1 reduced by phototrapping (not shown).

The P700 reaction centers also contain 2 phylloquinones per P700 [8]. In these particles, which are devoid of the low-M_r polypeptides of PS I and of the iron-sulfur centrs [8,13,21], both quinone molecules are destroyed in a monophasic fashion (fig.1). The difference in kinetics for RC I from *Anabaena* and from spinach (fig.1) is due to different intensities of the UV light (deterioration of the Hg lamp used), and probably does not reflect properties of the preparations. The shape of the EPR spectra of center A_1 is somewhat changed when compared to PS I (fig.2). This may reflect a change in the environment of center A_1. The basic features of the X-band spectra, which are characterized by g factors of 2.004–2.005, a peak-to-peak width of 1.0–1.1 mT and an asymmetric line shape [2,3,5,22], are retained however, and are certainly different from the symmetric EPR spectra of centers A_0 plus A_1 (g factor about 2.0025–2.003, peak-to-peak width 1.35 mT [2,5,22], cf. fig.3). Nearly complete destruction of both phylloquinones in RC I has little effect on the size and shape of the EPR spectra (figs 2,3). Center A_1 thus appears not to be phylloquinone. Also unaffected is the photooxidation of P700 under both aerobic and anaerobic conditions (fig.4) and the PS I-dependent photoreduction of benzyl viologen (not shown). Thus the stable charge separation is not impaired by the UV irradiation.

The nature of center A_1 has been deduced from EPR spectra, optical difference spectra in the UV and from chemical analysis [2–5,8,11]. These methods do not identify center A_1 unequivocally as phylloquinone. The present results provide evidence against such an identification. However, a weakness of our approach is that the products of destruction of phylloquinone by UV light in solution (and in the photosystem) are not known precisely [23], i.e. the altered naphthoquinone may escape analytical detection but still fulfill its role as center A_1. In view of the successful application of destruction of naphthoquinones by near-UV light in other systems, including their functional reconstitution [24–26] and of the drastic effect of 360 nm light on their absorption spectra [24], this possibility may be considered as remote. An alternative approach would be complete extraction of the quinone, a way which may be hampered by the reported [10] resistance of one of the phylloquinones to extraction.

ACKNOWLEDGEMENTS

We are indebted to Professor G. Hauska for discussion and to Professor A. Müller-Broich (both at Regensburg) for access to his EPR facilities. This work was supported by the Deutsche Forschungsgemeinschaft (SFB 43).

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