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Is phylloquinone an obligate electron carrier in photosystem I?

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Comparative quantitative analysis of phylloquinone content and photochemically competent P-700 has been performed on photosystem I particles subjected to photolysis with ultraviolet irradiation. Nonirradiated control particles exhibit a phylloquinone/P-700 stoichiometry of 1.9 ± 0.2. Photolysis of the photosystem I particles induces a progressive depletion of phylloquinone, however, photochemistry as assayed at room temperature by the photooxidation of P-700 is unaffected. These data are not consistent with the assignment of phylloquinone as a functional intermediate at room temperature between P-700 and the iron-sulfur clusters, center A and center B.

Electron carrier A_1; P-700; Photosystem I; Phylloquinone; Signal 1; (Spinach subchloroplast particle)

1. INTRODUCTION

Recent investigations of the photosystem I (PS I) reaction center have detailed a multi-peptide complex comprised of a primary electron donor P-700 and an electron acceptor complex including two transient intermediates, A_0 and A_1, and three membrane-associated iron-sulfur clusters: centers X, A and B [1]. ESR studies have provided considerable insight regarding the thermodynamic properties and stoichiometry of the iron-sulfur acceptors, however the identity and function of the transient electron carriers, A_0 and A_1, are not defined precisely. Both of these early electron acceptors have been postulated to be intermediate between P-700 and the iron-sulfur acceptor complex, with A_0 functioning as the primary electron acceptor. ESR and optical investigations are consistent with the identification of A_0 as a chlorophyll monomer [2–5], however the identity of A_1 is more tenuous. Transient absorption experiments in the ultraviolet have implicated a phylloquinone anion as a participant in the $P\text{-}700^+A_1^-$ recombination reaction at cryogenic temperatures [6]. Similarly, photoaccumulation experiments at 200 K have demonstrated an acceptor species possessing an ESR resonance with $g = 2.0051$ and a peak-to-peak linewidth of 1.05 mT [2,5]. Associated with the accumulation of this signal are absorption changes in the 200–300 nm region, which the authors attributed to a quinone anion [7]. However, these seminal and significant experiments have been performed under non-physiological conditions: high pH, presence of a strong reductant and cryogenic temperatures.

These recent spectroscopic observations are consistent with analytical determinations that associate phylloquinone (2-methyl-3-phytyl-1,4-naphthoquinone) with the PS I reaction center [8–10]. Schoeder and Lockau [8] and more recently Malkin [10] have determined a stoichiometry of 2 phylloquinones per P-700. Additionally, a heterogeneity in binding sites for the two quinones has been reported [10]. That phylloquinone in solution undergoes facile photodegradation during photolysis at wavelengths less than 400 nm has been long established [11]. A similar photolability for this naphthoquinone in PS I has been asserted

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by Lockau et al. [12]. In this regard the photolability of phylloquinone provides the means for a critical test of the role of this quinone in PS I electron transport at room temperature. Here, we demonstrate that electron transport in the PS I reaction center, as monitored by the photooxidation of P-700 under physiological conditions, can occur even in the absence of detectable phylloquinone. These data are not supportive of an obligatory function for phylloquinone in mediating electron transfer between P-700 and the iron-sulfur cluster complex.

2. MATERIALS AND METHODS

Triton (TSF-1) and digitonin (D-144) solubilized PS I particles were prepared as in [13,14]. Transient optical absorption changes for $P\text{-}700^+$ were measured at 820 nm with a purpose-designed, single-beam spectrophotometer [15]. Flash photolysis was performed on a 3 ml cuvette containing PS I particles at 10 μg/ml, 500 μM ascorbate and 100 μM 2,6-dichlorophenolindophenol. ESR spectra were obtained with a Varian E-9 spectrometer interfaced to a PDP11/23 computer. Actinic light for ESR studies was provided by a 1000 W tungsten-halogen source (Oriel).

Quantitation of phylloquinone was performed by modification of published methods for analysis of plastoquinone-9 [16,17]. The PS I sample was oxidized by addition of 200 μM dichlorophenolindophenol and/or 10 mM potassium ferricyanide at each step of quadruplicate extractions into either 8 ml methanol or 6 ml *n*-heptane from 2 ml methanol. Extractions were effected by vortex-mixing the sample for 3 min, followed by centrifugation. The combinex heptane or methanol extract was dried in a rotary evaporator. The resulting residue was redissolved in ~3 ml methylene chloride/*n*-heptane solution (30:70, v/v), loaded onto a silica Sep-Pak (Waters Associates) and eluted with 30 ml of the methylene chloride/heptane mixture. The eluent was evaporated and dissolved in 200 μl of the CH_2Cl_2/heptane (30:70) mixture prior to HPLC analysis. The extraction efficiencies for phylloquinone into heptane (D-144 particles) and into methanol (TSF-1 particles) were determined to be 88.4 ± 16.5 and 76.9 ± 5.3%, respectively.

HPLC assays were performed with a system consisting of a Milton-Roy Constametric II pump, a Valco injection valve (75 μl sample loop), a 2 cm × 2 mm i.d. precolumn (Anspec) packed with 60/80 mesh glass beads, a reversed-phase 5 μm Spherisorb ODS column (25 cm × 4.6 mm i.d., HPLC Technology) and a Dupont UV detector (254 nm). Phylloquinone was eluted isocratically at a flow rate of 1.2 ml/min with a solvent mixture of methanol/methylene chloride/water (91:5:4, v/v). Calibration of the HPLC was achieved with authentic phylloquinone (Sigma). Phylloquinone eluted at a retention time of approx. 8 min and was well separated from other chromatographic peaks. Under the conditions used, α-tocopherol and plastoquinone-9 eluted at 6 and 30 min, respectively.

Ultraviolet photolysis of PS I samples was performed by focussing the filtered output (λ = 300–400 nm) of a 200 W mercury-xenon lamp (Photon Technology International) onto a water-thermostatted sample vessel. During photolysis the sample was maintained at 9°C. With an incident light power at the sample of 3.7 mW/cm^2 photodestruction of phylloquinone (as monitored by HPLC) was complete within 60 min.

3. RESULTS AND DISCUSSION

3.1. *Quantitation of phylloquinone in PS I*

The molar ratios of phylloquinone to P-700 in the TSF-1 and D-144 preparations were determined to be 1.9 ± 0.2 and 2.4 ± 0.2, respectively. These values are in accord with recent assays reported on PS I preparations from spinach [8,10], *Anabaena variabilis* [8] and *Synechococcus* sp. [3].

3.2. *Dependence of the $P\text{-}700^+$ absorption transient on phylloquinone content*

Flash photolysis of a PS I preparation in the absence of an external acceptor yields a transient P-700 absorption whose lifetime is dominated by the back-reaction from the iron-sulfur clusters ($P\text{-}430^-$). Fig.1a displays the transient kinetics of $P\text{-}700^+$ in TSF-1 particles, as monitored at 820 nm; the extent of the absorption change corresponds to ~1 P-700 per 82 chlorophylls. The hypothesis that phylloquinone functions as the secondary electron acceptor (A_1) in PS I electron transport between P-700 and the iron-sulfur

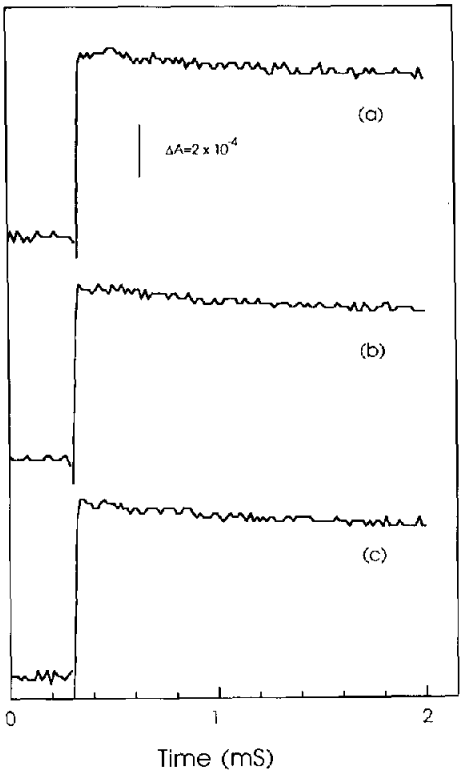

Fig.1. Photooxidation of P-700 in control and UV-irradiated TSF-1 particles. (a) Control sample in Tricine buffer (0.05 M, pH 7.5) containing 100 μM 2,6-dichlorophenolindophenol and 500 μM ascorbic acid. (b) Same as (a) except after UV photolysis to yield a phylloquinone/P-700 ratio of 1.1. (c) Same as (a) except after UV photolysis to remove all detectable phylloquinone. The vertical bar represents an absorbance change of 2×10^{-4} absorbance units. Chlorophyll concentration, 10 μg/ml.

centers would predict the abolition of stable (e.g. a lifetime in excess of ~10 μs at room temperature) P-700 photooxidation in the absence of the quinone, since the prereduction of the iron-sulfur clusters and A_1 results in a $P\text{-}700^+A_0^-$ recombination that occurs in ~30 ns [18]. In contrast to this expectation, fig.1 demonstrates that the extent of the absorption at 820 nm and kinetics of $P\text{-}700^+$ reduction are independent of phylloquinone content. Indeed, the absorption transients are identical (within experimental error) with that of the control after partial (fig.1b, ~1.1 ± 0.2 phylloquinone/P-700) and full destruction of phylloquinone by UV photolysis (fig.1c, no detectable phylloquinone/P-700). The half-time for reduction of $P\text{-}700^+$ (~20 ms) is consistent with the trapping of the donated electron in the center A/B complex [19]. This assertion is supported by the observation that reduction of the PS I sample by dithionite results in the acceleration of $P\text{-}700^+$ decay to 265 μs (not shown). Such a ~250 μs decay is diagnostic at room temperature for the back-reaction between $P\text{-}700^+$ and A_2^- [20]. Note that similar kinetics are observed in the presence of dithionite in both the control and UV-irradiated samples. Hence, phylloquinone appears not to be essential for electron transfer to center X (A_2) or centers A and B.

3.3. *Dependence of ESR signal I on phylloquinone content*

Photooxidized P-700 possesses a characteristic ESR signal with $\Delta H_{pp} \sim 0.72$ mT and a g factor of 2.0025. Observation of the P-700 resonance (signal 1) in PS I particles during continuous illumination at room temperature is diagnostic of trapping of the electron in acceptors incapable of submillisecond recombination with $P\text{-}700^+$. Fig.2a illustrates a typical ESR signal observed in spinach TSF-1 particles during illumination. The intensity of this signal is identical to that observed in TSF-1 particles preoxidized with potassium ferricyanide, thus the photochemically generated signal represents the total complement of P-700 in this sample. Assay of the TSF-1 particle during UV photolysis reveals that the intensity and spectral parameters of signal 1 remain invariant within experimental error (fig.2b,c). Similar observations have been obtained with digitonin PS I particles, hence the invariance of P-700 photochemistry on phylloquinone content is not an artifact of surfactant type.

The independence of P-700 photooxidation, as monitored by either flash photolysis or by steady-state ESR studies, on phylloquinone content is illustrated most graphically in fig.3. This figure represents the composite ESR, flash photolysis and HPLC assays for a variety of TSF-1 and D-144 preparations from spinach. As depicted by fig.3 (and the kinetic studies of fig.1), statistically the phylloquinone composition of the reaction center bears no correlation with the extent of P-700 photooxidation stable in the millisecond domain. These data represent a refinement of the study by

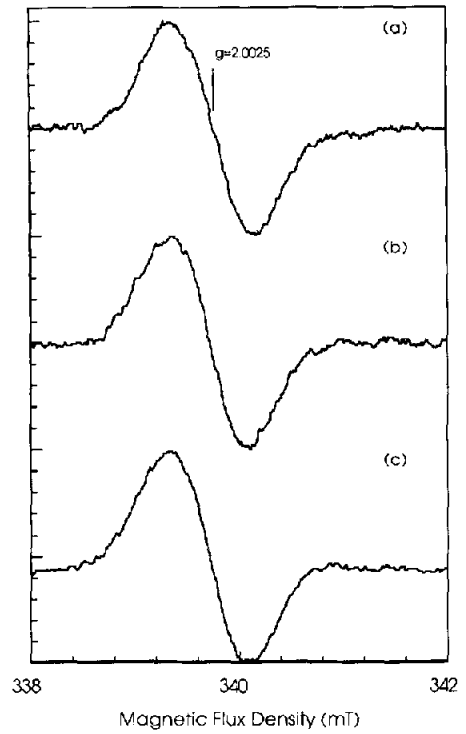

Fig.2. ESR spectra of signal 1 in control and UV-irradiated samples. ESR parameters: microwave power, 20 mW; microwave frequency, 9514 MHz; modulation amplitude, 0.50 mT; scan time, 4 min; time constant, 1 s. (a) Control sample. (b) Irradiated sample containing 1.1 ± 0.2 phylloquinone per P-700. (c) Irradiated sample containing no detectable phylloquinone. Chlorophyll concentration, 400 μg/ml.

Malkin [10], which demonstrated that one of the PS I phylloquinones was preferentially extracted by organic solvents. This readily extractable quinone was determined not to be required for the cryogenic photoreduction of center A. The studies presented here suggest that neither extractable phylloquinone is requisite for electron transfer to centers X, A or B.

The observation that PS I photochemistry, as defined by electron transfer from P-700 to the iron-sulfur clusters, can function in the absence of extractable phylloquinone is inconsistent with the assignment of this component to an obligate role in linear electron transport in the reaction center [6,7]. Our studies cannot be compared directly to the cryogenic studies of A_1 [6,7], in that our kinetic and spectroscopic analyses were performed

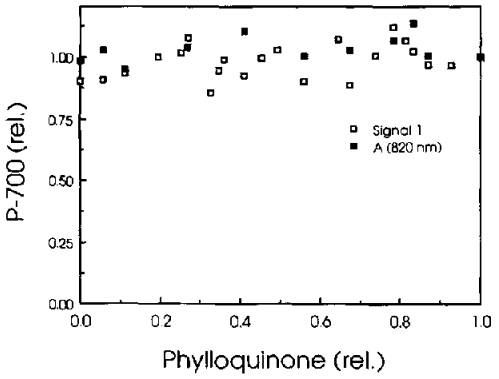

Fig.3. P-700 photooxidation as a function of phylloquinone content in PS I particles. (□) ESR determinations of P-700 photochemistry (signal 1 amplitude). ESR parameters are given in fig.2. (■) Flash photolysis measurements of the $P\text{-}700^+$ transient were obtained as given in fig.1. Phylloquinone concentration is represented relative to that in unirradiated control particles.

without strong reductants and under physiological conditions. The observed reduction of a quinoidal acceptor may result from an artifactual electron transfer enhanced by the redox poise of the sample in an alkaline and cryogenic environment. Thus phylloquinone, if not the secondary electron acceptor in linear electron transport in PS I, might function in non-cyclic transport or in some unknown capacity. Alternatively, the reported absorption changes linking phylloquinone to A_1 might originate in a chromophore covalently linked to reaction center peptide or bound in a hydrophobic environment inaccessible to organic solvents. This hypothesis would preserve the assignment of A_1 to phylloquinone but would require a minimum of three quinones per P-700 in highly purified PS I particles.

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