On the function of two vitamin K_1 molecules in the PS I electron acceptor complex

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Various PS I preparations from higher plants and cyanobacteria are found to contain vitamin K_1, or phylloquinone, in a stoichiometry of 2 vitamin K_1/P700. Extractions of lyophilized PS I preparations with organic solvents led to removal of one molecule of vitamin K_1 but conditions have not been established for the removal of the second quinone. The quinone-depleted PS I complex ($\sim$1 vitamin K_1/P700) was fully active in transferring electrons at both physiological and cryogenic temperatures, indicating one vitamin K_1 molecule was not required for these activities.

Phylloquinone *Fe-S center* *P700* *Photosystem I*

1. INTRODUCTION

The PS I primary electron acceptor complex is known to contain multiple bound electron acceptors, the best studied of which are iron-sulfur centers A, B and X [1,2]. In addition to these centers, recent evidence has identified two early electron acceptors, denoted A_0 and A_1, that precede the iron-sulfur centers [3,5]. While spectral evidence suggests A_0 may be a monomeric form of chlorophyll [6,7], the chemical identity of A_1 is less well defined. Several recent reports [8,9], as well as earlier work [10,11], have identified vitamin K_1 or phylloquinone in the PS I complex and studies of absorbance changes in the ultraviolet region have provided evidence that vitamin K_1 can be photoreduced to the semiquinone state and that reduced vitamin K_1 participates in a back-reaction with $P700^+$ that has been previously shown to involve the A_1 acceptor [12]. Thus, a rapidly growing body of work is emerging that indicates a role for vitamin K_1 as an early PS I electron acceptor.

Here, the stoichiometry of vitamin K_1 in various PS I preparations is found to be 2 molecules of vitamin K_1 per P700, in agreement with the recent report of Schoeder and Lockau [9]. However, a heterogeneity of vitamin K_1 binding has been identified in experiments on the organic solvent extraction of the quinone from PS I. One quinone molecule can be readily removed and its removal does not affect PS I activity measured at either physiological or cryogenic temperatures. Attempts to remove the second quinone have not yet been successful, indicating a tight association with the PS I reaction center complex.

2. MATERIALS AND METHODS

A PS I complex, containing 200 Chl/P700, was prepared from spinach thylakoid membranes as described [13,14]. For the extraction of vitamin K_1, the PS I-200 complex from the sucrose gradient ($\sim$2–3 mg Chl) was diluted with distilled water to a volume of 25 ml and centrifuged for 1 h at 60 000 rpm in a Spinco Ti60 rotor. The pelleted PS I complex was resuspended in a small amount of distilled water and lyophilized to dryness for 20 h. The dried material was suspended in the desired solvent and homogenized with a Teflon homogenizer to insure a uniform suspension. Extractions were carried out for varying times at 25°C in the dark with stirring. The solvent was removed by centrifugation at 40 000 $\times$ g for 10 min, the pellet dried

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under N_2 and then resuspended in a solution containing 0.3 M sucrose, 50 mM Tris-HCl (pH 7.8), 20 mM NaCl, 5 mM $MgCl_2$ and 0.1% Triton. For successful extractions, it was essential that no water contaminated the sample during the above-described procedures.

P700 was assayed using a photochemical method with steady-state actinic light in an Aminco DW-2 spectrophotometer [14]. Wavelengths used were 435–444 nm and concentrations based on an extinction coefficient of 44 $mM^{-1} \cdot cm^{-1}$ at these wavelengths [15]. Vitamin K_1 was measured after total extraction from the complex by the method of Redfearn and Friend [16]. The final ethanol solution was assayed using an LKB HPLC apparatus with a Kratos detector. An LKB Lichrosorb RB-18 (5 μM) reversed-phase column was used with a mobile phase of methanol/isopropanol (80:20, v/v). The flow rate was 1.0 ml/min. A standard vitamin K_1 sample (Sigma) showed a retention time of 12 min with this system. Vitamin K_1 was monitored at 270 nm.

EPR spectra were recorded at 15–20 K as in [17]. Light minus dark spectra were obtained by computer subtraction. Assays of PS I-dependent O_2 uptake were done with reduced DCPIP and plastocyanin as electron donor and methyl viologen as the electron acceptor using procedures similar to those in [17].

3. RESULTS

3.1. *Vitamin K_1 content of PS I*

In agreement with recent results of Takahashi et al. [8] and Schoeder and Lockau [9], analyses of several different spinach PS I preparations gave a ratio of vitamin K_1/P700 of approx. 2.0. The PS I-200 preparation for most of the present studies had a ratio of 1.9–2.2 for 5 different preparations while an antenna-depleted PS I complex (PS I-100) [18] had a ratio of 2.0. A highly enriched PS I complex (Chl/P700 = 50) prepared with LDAO using DEAE-cellulose chromatography [19] had a ratio of vitamin K_1/P700 = 1.8. Finally, a PS I preparation from a cyanobacterium containing 130 Chl/P700 [20] was found to have a ratio of vitamin K_1/P700 = 1.8. These values clearly indicate a general stoichiometry of 2 molecules of vitamin K_1 per P700 in a variety of PS I complexes isolated from different organisms with different detergents.

3.2. *Effect of organic solvent extraction on vitamin K_1 in PS I*

The results shown in table 1 summarize a series of experiments attempting to extract vitamin K_1 with organic solvents from a lyophilized PS I-200 complex. Initially, hexane or petroleum ether were used as the solvent and while the native complex contained 2 molecules of vitamin K_1 per P700, complexes extracted with these solvents for up to 4 h at room temperature showed the loss of only one molecule of vitamin K_1. In an attempt to use stronger extraction conditions, studies with hexane plus 0.05% methanol were done, but these extractions only led to the removal of one molecule of vitamin K_1 as well. Higher amounts of methanol along with hexane led to solubilization of substantial amounts of chlorophyll with the PS I preparations used in this work.

Table 1

Effect of organic solvent extraction on P700 and vitamin K_1 of PS I

Treatment	P700 (nmol/mg Chl)	Vitamin K_1	Vitamin K_1/P700
Control PS I-200	6.1	11.8	1.9
Hexane-extracted PS I-200			
1 h	5.7	5.3	1.1
2 h	5.3	5.3	1.0
4 h	4.9	4.7	1.1
Hexane + methanol (0.05%) extracted PS I-200			
30 min	4.9	4.2	1.2
1 h	4.9	4.8	1.1

3.3. *Photochemical activities in vitamin K_1-deficient PS I preparations*

Various photochemical assays were done with the control PS I-200 preparation, which contained 2 vitamin K_1 per P700, and the organic-solvent extracted preparation that contained 1 vitamin K_1 per P700.

Both preparations showed P700 photooxidation activity at physiological temperatures and, as shown in fig.1A, both were able to catalyze a light-dependent uptake of O_2 with reduced DCPIP and plastocyanin as the electron donor. In this case, comparable activities were observed as both preparations had oxygen consumption activities of approx. 500 μM O_2 consumed/mg Chl per h.

Photochemical activity of the PS I reaction center was also determined at cryogenic temperatures. In this case, the photoreduction of iron-sulfur center A at 15 K was measured. The photoreduction of this center is characterized by EPR *g* values of 2.05, 1.94 and 1.86 (fig. 1B) and both the control preparation (2 vitamin K_1/P700) and the organic-solvent extracted PS I complex (1 vitamin K_1/P700) showed center A photoreduction; the extent of center A photoreduction per mg chlorophyll was comparable. Thus, there was no apparent difference in PS I activity at physiologial and cryogenic temperatures between a control PS I complex and a complex containing a single vitamin K_1 molecule.

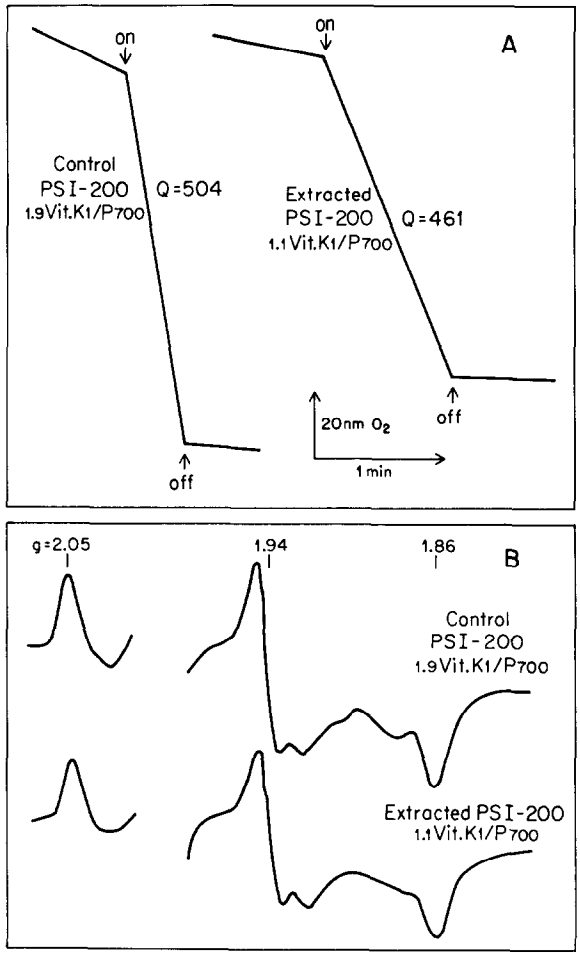

Fig.1. PS I reactions in control and organic-solvent extracted PS I complexes. The control PS I-200 complex contained 1.9 vitamin K_1/P700 and the organic-solvent extracted complex contained 1.1 vitamin K_1/P700. (A) Electron transfer from reduced DCPIP and plastocyanin to methyl viologen measured as O_2 uptake. Reaction mixtures of 2.0 ml contained 20 mM Hepes buffer (pH 7.5), 5 mM $MgCl_2$, 15 mM NaCl, 5 mM ascorbate, 0.2 mM DCPIP, 2 μM spinach plastocyanin, 200 μM methyl viologen, and 12.5 μg chlorophyll. Samples were illuminated with saturating orange light. (B) Photoreduction of iron-sulfur center A at cryogenic temperature. Control (0.85 mg Chl/ml) and organic-solvent extracted PS I-200 (0.60 mg Chl/ml) were incubated with 2 mM ascorbate prior to freezing to 77 K. EPR spectra were recorded in the dark at 15 K and then after illumination for 30 s with red light. Light minus dark spectra were obtained by subtraction of appropriate spectra. EPR conditions: field, 3400 ± 250 G; microwave power, 10 mW; modulation amplitude, 12.5 G; temperature, 17 K.

4. DISCUSSION

In many respects, the properties of vitamin K_1, or phylloquinone, in the PS I electron acceptor complex are reminiscent of the quinones in bacterial reaction centers and the PS II complex [21]. In the latter two cases, two quinone molecules, denoted Q_A and Q_B, are present per reaction center and one of these is more tightly bound than the second. It has been previously shown that extraction with non-polar solvents led to a removal of Q_B but that the tighter binding of Q_A necessitated the inclusion of ~0.05% methanol with the non-polar solvent for extraction of Q_A [22-24].

Here, a difference in binding between the two PS I vitamin K_1 molecules has been observed in the spinach PS I complex. One molecule appears more loosely bound as it can be extracted by a short

treatment with non-polar solvent, and, in this regard, this extractability pattern resembles that of Q_B. However, the binding of the second vitamin K_1 is more tenacious and conditions have not yet been established for the reversible removal of this quinone. Conditions comparable to those used for Q_A extraction from bacterial reaction centers [22] and PS II [23,24] did not remove this molecule. This behavior would be consistent with an extremely hydrophobic environment for this quinone molecule that could be related to its presumed function at low redox potentials.

While it is not yet possible to define a precise role for the tightly bound quinone molecule on the basis of this work, no requirement for the second quinone in PS I electron transfer reactions at either physiological or cryogenic temperatures was observed. In this regard, the analogy with other reaction centers breaks down as electron transfer from Q_A to Q_B is known to occur in each system. It is clear from these studies that electron transfer between the vitamin K_1 molecules does not appear to be required for center A photoreduction or for electron transfer to methyl viologen. Therefore, a role for one tightly bound quinone molecule as the early electron acceptor, A_1, in the PS I complex is an attractive model, but the second quinone molecule has no definable function in PS I function at this time.

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REFERENCES

[1] Malkin, R. (1982) Annu. Rev. Plant Physiol. 33, 455-479.
[2] Rutherford, A.W. and Heathcote, P. (1985) Photosynth. Res. 6, 295-316.
[3] Bonnerjea, J. and Evans, M.C.W. (1982) FEBS Lett. 148, 313-316.
[4] Gast, P. Swarthoff, T., Ebskamp, F.C.R. and Hoff, A.J. (1983) Biochim. Biophys. Acta 722, 163-175.
[5] Thurnauer, M.C. and Gast, P. (1985) Photobiochem. Photobiophys. 9, 29-38.
[6] Heathcote, P. and Evans, M.C.W. (1981) in: Photosynthesis (Akoyunoglou, G. ed.) vol.2, pp. 665-675, Balaban, Philadelphia.
[7] Fajer, J. Davis, M.S., Forman, A., Klimov, V.V., Dolan, E. and Ke, B. (1980) J. Am. Chem. Soc. 102, 7143-7145.
[8] Takahashi, Y., Hirota, K. and Satoh, S. (1985) Photosynth. Res. 6, 183-192.
[9] Schoeder, H.-U. and Lockau, W. (1986) FEBS Lett. 199, 23-27.
[10] Interschick-Niebler, E. and Lichtenthaler, H. (1981) Z. Naturforsch. 36c, 276-283.
[11] Thornber, J.P., Alberte, R.S., Hunter, F.A., Shiozawa, J.A. and Kan, K.-S. (1977) Brookhaven Symp. Biol. 28, 132-148.
[12] Brettel, K., Setif, P. and Mathis, P. (1986) Abstr. VII Int. Congr. Photosynth., pp.304-389.
[13] Mullet, J.E., Burke, J.J. and Arntzen, C.J. (1980) Plant Physiol. 65, 814-822.
[14] Lam, E., Ortiz, W., Mayfield, S. and Malkin, R. (1984) Plant Physiol. 74, 650-655.
[15] Hiyama, T. and Ke, B. (1972) Biochim. Biophys. Acta 267, 160-171.
[16] Redfearn, E.R. and Friend, J. (1960) Phytochemistry 1, 147-151.
[17] Malkin, R. (1984) Biochim. Biophys. Acta 764, 63-69.
[18] Ortiz, W., Lam, E., Ghirardi, M. and Malkin, R. (1984) Biochim. Biophys. Acta 766, 505-509.
[19] Malkin, R. (1975) Arch. Biochem. Biophys. 169, 77-83.
[20] Lundell, D.J., Glazer, A., Melis, A. and Malkin R. (1985) J. Biol. Chem. 260, 646-654.
[21] Okamura, M.Y., Feher, G. and Nelson, N. (1982) in: Photosynthesis: Energy Conversion by Plants and Bacteria (Govindjee, ed.) vol.I, pp.196-272, Academic Press, New York.
[22] Cogdell, R.J., Brune, D.C. and Clayton, R.K. (1974) FEBS Lett. 45, 344-347.
[23] Knaff, D.B., Malkin, R., Myron, J.C. and Stoller, M. (1977) Biochim. Biophys. Acta 459, 402-411.
[24] Klimov, V.V., Dolan, E., Show, E.R. and Ke, B. (1980) Proc. Natl. Acad. Sci. USA 77, 7227-7231.