Phylloquinone copurifies with the large subunit of photosystem I

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Photosystem I preparations from the cyanobacterium *Anabaena variabilis* and from spinach were analyzed for their quinone content. The dominant quinone was identified as phylloquinone. It was present at a ratio of roughly 2 per P700 (the primary electron donor of photosystem I) both in particles prepared with Triton X-100 and on the large, P700-carrying subunit derived from these particles by treatment with SDS. The results suggest a function of phylloquinone in the reaction center.

Cyanobacterium (Spinach) Photosystem I Phylloquinone HPLC

1. INTRODUCTION

Oxygen-evolving organisms contain, in their thylakoid membranes, plastoquinones, tocopherylquinones and naphthoquinones. At present, functions in photosynthetic electron transport can only be assigned to the plastoquinones, namely on the electron donor and acceptor sides of photosystem (PS) II, between PS II and the cytochrome b_6/f complex (the plastoquinone pool) and as part of the latter complex (review [1]).

Recent spectroscopic evidence suggests that a quinone may play a role in the electron acceptor complex of PS I [2,3]. Membrane fragmentation studies have already indicated an association of quinones with that photosystem: Thornber's group detected a quinone which they could not identify in a PS I particle from a cyanobacterium [4] and phylloquinone in a preparation from tobacco which also contained cytochromes b_6 and f [5]. Lichtenthaler and co-workers [6,7] reported an enrichment of phylloquinone in light digitonin particles (which are enriched in PS I) from chloroplasts; they further observed that 30% of the total thylakoid content of this naphthoquinone comigrated with the chlorophyll-binding protein I (CP I) in SDS gel electrophoresis under mildly dissociating conditions.

Here we show that about two molecules of phylloquinone per P700 (the primary electron donor of PS I) are retained on highly resolved PS I particles isolated from thylakoids of a cyanobacterium and of spinach with the detergent Triton X-100. This accounts for 60% (cyanobacterium) to virtually all (spinach) of the phylloquinone complement of the parent membranes. The quinone is retained on the large chlorophyll-protein subunit (the P700 reaction center) derived from the PS I particles by treatment with SDS. The results suggest that phylloquinone is the quinone which has been proposed to function as the intermediate electron acceptor A_1 [2,3].

2. MATERIALS AND METHODS

All preparations were carried out in dim light. PS I particles were isolated from thylakoid membranes of the cyanobacterium *Anabaena variabilis* ATCC 29413 and from spinach (*Spinacia oleracea* L.) as in [8]. Briefly, washed membranes contain-

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ing 1 mg chlorophyll (chl)/ml were solubilized with 1% (w/v) Triton X-100 and PS I isolated in Tris or phosphate buffers containing 0.1% Triton X-100 by repeated chromatography on columns of DEAE-cellulose, by ammonium sulfate precipitation and sucrose density gradient centrifugation. The particles contained 70–90 chl/P700, the yield being 30%. The P700 reaction center (large chlorophyll-protein subunit) was derived from the PS I particles by treatment with 0.5% (w/v) SDS followed by sucrose density gradient centrifugation in 0.1% Triton X-100 [9,10]. The P700 content was determined by photooxidation [11] in reaction mixtures containing 1 mM sodium ascorbate first in the absence and then in the presence of 0.05 mM *N,N,N',N'*-tetramethyl-*p*-phenylenediamine to ensure complete reduction. The extinction coefficients used were those of Hiyama and Ke [12]: 70 $mM^{-1} \cdot cm^{-1}$ for *Anabaena* and 64 $mM^{-1} \cdot cm^{-1}$ for spinach.

Total lipids were extracted according to Bligh and Dyer [13] after oxidation of the material with a few grains of ferricyanide and applied to precoated TLC plates (silica gel 60 F_{254}; Merck, Darmstadt). The plates were developed with benzene and inspected under light of 254 nm [14]. The spot with an R_f value of about 0.5, which contained both phylloquinone and plastoquinone-9 (see [14]), was scraped off the plates and eluted with ethanol. After centrifugation, the ethanolic extracts were analyzed by HPLC using an octyl =Si 100 Polyol 0.005 mm column (size 4.6 × 250 mm; Serva, Heidelberg). The column was eluted at a flow rate of 1 ml/min first with methanol:H_2O (95:5, v/v). After 12 min, the eluent was switched within 2 min to 100% methanol and the elution continued. Elution of prenylquinones was monitored at 260 nm (model 2151 variable wavelength monitor; LKB, Sweden) and the peaks integrated (Shimadzu C-R3A Chromatopac, Kyoto). The HPLC apparatus was calibrated with chromatographically pure phylloquinone (vitamin K_1; Sigma, St. Louis, MO) and plastoquinone-9 (a gift from Dr G. Hauska, Regensburg) carried through the procedure. The extinction coefficients used for stock solutions were 14.9 $mM^{-1} \cdot cm^{-1}$ for oxidized phylloquinone at 270 nm [15] and 15 $mM^{-1} \cdot cm^{-1}$ between the oxidized and reduced forms of plastoquinone-9 at 255 nm [16]. Chlorophylls were determined, and SDS-polyacrylamide gel electrophoresis was carried out by standard methods cited in [11,17].

3. RESULTS AND DISCUSSION

3.1. *Qualitative analysis*

Fig.1 shows HPLC traces of the quinone analysis of membranes, PS I and its large, P700-carrying subunit from *Anabaena* (see fig.2 for the polypeptide composition of the photosystem preparations used). Plastoquinone-9 (PQ-9), which elutes with a retention time of 18 min, was detected in the membranes only. The quinone eluting at about 10 min was present in all samples. It co-chromatographed with authentic vitamin K_1 in both HPLC (retention times; 9.8 min in methanol:H_2O (95:5), 5.7 min in absolute methanol on the reverse-phase column used) and TLC in 6 different systems on silica gel, $AgNO_3$-impregnated silica gel (which separates also on the basis of double bonds [18]), and on reverse-phase plates. The UV spectrum of the oxidized quinone closely corresponds to that of the authentic vitamin (fig.3). Upon reduction with $NaBH_4$, the expected absorption maximum at 243 nm developed (not shown). The quinone pre-

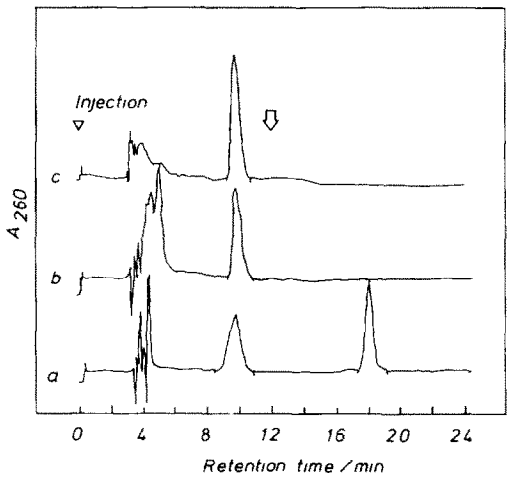

Fig.1. HPLC elution profiles of the quinone analysis of *A. variabilis*. Traces: a, membranes; b, PS I prepared with Triton X-100; c, P700 reaction center derived from PS I by treatment with SDS. At the second arrow, the eluent was switched from 95% methanol to 100% methanol. Phylloquinone eluted after about 10 min, PQ-9 after 18 min. See section 2 for details.

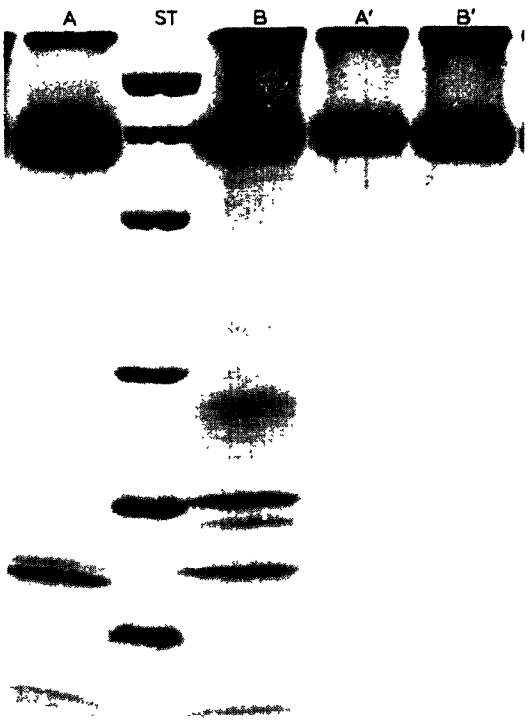

Fig.2. Polypeptide composition of the PS I preparations. After electrophoresis in linear (12–18%) acrylamide gradient gels containing SDS, polypeptides were stained with Coomassie brilliant blue R-250. Samples containing approx. 80 pmol P700 were applied to each track. (A,B) PS I from *Anabaena* and spinach, respectively, prepared with Triton X-100; (A',B') P700 reaction center derived from A and B by treatment with SDS; (ST) molecular mass standards (from top to bottom: 92.5, 66.5, 45, 31, 21.5, 14.3 kDa).

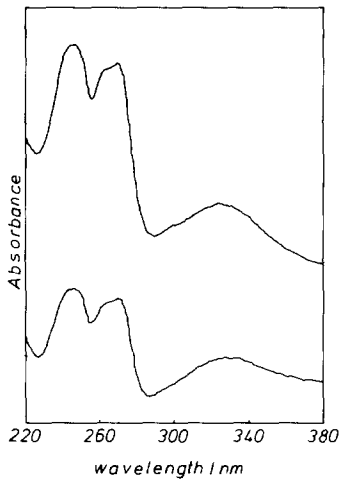

Fig.3. UV absorption spectra of authentic vitamin K_1 (top) and of the quinone extracted from PS I of *Anabaena*, dissolved in methanol:H_2O (95:5). The *Anabaena* quinone was purified by TLC and HPLC as described in section 2.

sent in the photosystem is thus phylloquinone. Other quinones were not detected on the TLC plates employed for the initial purification (see section 2).

3.2. *Quantitative analysis*

The molar ratios of phylloquinone and of PQ-9 to P700 in the various fractions are given in table 1. We will first describe the results obtained with the cyanobacterium. When related to PS I, the PQ-9 content of the parent membranes seems surprisingly low. This is due to the high PS I concentration in the membranes which contain only 200 chl per P700, but 600 chl per cytochrome b_6/f complex [17], i.e. approx. 10 PQ-9 molecules are present per cytochrome *f*. Nearly identical results have been obtained for a unicellular cyanobacterium, *Synechococcus*, by Aoki et al. [19].

About 60% of the phylloquinone was recovered in the multi-subunit PS I prepared with Triton X-100, giving a quinone/P700 ratio of 2.0. PQ-9 was absent, with an estimated detection limit of <0.1/PS I (cf. fig.1). The somewhat higher phylloquinone content of the P700 reaction center may be attributed to a partial inactivation by the SDS treatment employed.

The phylloquinone and PQ-9 contents of spinach thylakoids (table 1) compare well with those given in [20] on a chlorophyll basis. The quinone analysis of the higher plant PS I gave results which are not as clear cut as for the cyanobacterium. Firstly, both the multi-subunit and the single-subunit PS I preparations contained some (residual?) PQ-9 which could not be removed by extensive washing of the preparations with buffer containing Triton X-100. Secondly, in all samples somewhat less than 2 phylloquinone molecules/P700 were determined. This difference from *Anabaena* may, at least in part, reflect an uncertainty in the extinction coefficients of P700, the usually accepted [21] coefficient for spinach being 10% lower than that given for *Anabaena*

Table 1

Phylloquinone and PQ-9 contents of thylakoid membranes, PS I and the P700 reaction center from *Anabaena* and spinach

	Membranes	PS I	P700 reaction center
(a) *Anabaena*			
Phylloquinone/P700	3.5	2.0	2.2
PQ/P700	3.1	<0.1	<0.1
(b) Spinach			
Phylloquinone/P700	1.7	1.8	1.9
PQ/P700	11.0	0.2	0.1

Ratios are given on a molar basis. The values are means of 2–4 determinations (in the case of spinach for each of 2 independent preparations) which deviated from the means by ± 10%

[12], despite the great similarity of the photosystems from higher plants and cyanobacteria [22]. However, again an enrichment in phylloquinone on the P700 reaction center of PS I is observed with a strong decrease in the PQ-9/P700 ratio from 11 (membranes) to 0.2. All of the phylloquinone of the membranes seems to be bound to PS I.

3.3. *Concluding remarks*

The chemical analysis presented here does not prove a function of phylloquinone in PS I. The observation that the naphthoquinone resides on the P700 reaction center of the photosystem, however, does suggest such a function since the electron acceptor A_1, proposed to be a quinone species, also seems to be located there [2]. The $E_{1/2}$ for a one-electron reduction of the closely related 2,3-dimethyl-1,4-naphthoquinone, determined by cyclic voltammetry in acetonitrile, is −600 mV vs the normal hydrogen electrode [23], i.e. at least 100 mV more positive than the assumed redox potential of A_1 (see [3]). The binding site of the quinone would thus have to contribute to the redox potential by providing an aprotic environment and perhaps a negative charge which would destabilize the semiquinone anion [24]. The observation of more than one phylloquinone per reaction center, the true stoichiometry probably being two, is reminiscent of the situation in PS II and in the reaction center of purple bacteria where two benzo- or naphthoquinones have a two-electron gate function (see [25]). However, electron transport in PS I appears to occur by a series of one-electron transfers.

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NOTE ADDED IN PROOF

After submission of this manuscript, a report by Takahashi et al. [26] was brought to our attention. These authors detected about two phylloquinone molecules per P700 in PS I preparations from the cyanobacterium *Synechococcus*. In contrast to our results, they obtained circumstantial evidence that a large fraction of the quinone is associated with polypeptides of 14 and 10 kDa.

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