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Characterization and N-terminal sequence of a 5 kDa polypeptide in the photosystem I core complex from spinach

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Photosystem I core complexes were isolated from spinach photosystem I particles after heat treatment in the presence of 50% (v/v) ethylene glycol (heat/EG treatment) The core complex from 58°C/EG-treated particles was composed of polypeptides with apparent molecular masses of 63, 60 and 5 kDA, this complex contained the iron sulfur center F_X but lacked center F_A and F_B The core complex obtained from the 70°C/EG-treated preparation lacked F_X and contained a lesser amount of the 5 kDa polypeptide The N-terminal amino acid sequence of the 5 kDa polypeptide did not correspond to the sequence derived from any possible reading frame in the chloroplast DNA of liverwort or tobacco Twelve of the first 29 N-terminal amino acids were hydrophobic, suggesting that this protein is intrinsic to the photosystem I reaction center

Amino acid sequence, Iron sulfur center, P700, Photosystem I protein, Reaction center, (Spinach)

1. INTRODUCTION

The PS I complex from higher plants has been reported to be composed of polypeptide subunits with molecular masses of 83, 82, 18, 17, 10, 11, 10, and 9 kDa (their apparent molecular masses in SDS-PAGE are 63, 60, 21, 17, 13, 11, 9, and 8 kDa, respectively) [1,2]. The reaction center core complex prepared from the PS I complex is composed of two large 83 and 82 kDa polypeptides encoded by the chloroplast genes *psa*A and *psa*B [1,2]. The reaction center core complex binds the reaction center P700 (Chl *a*) with the electron acceptors A_0 (Chl *a*), A_1 (phylloquinone) and F_X (iron sulfur center) [3,4]. The 9 kDa polypeptide encoded by the chloroplast gene *psa*C binds the electron acceptors F_A and F_B (iron sulfur centers) [5,6].

We have demonstrated that heat/EG treatment of spinach PS I particles leads to the dissociation of small polypeptides from the particles and to the selective destruction of iron sulfur centers F_A, F_B and F_X [7]. In our previous work, the core complex isolated from the 60°C/EG treatment contained F_X but not F_A and F_B and was composed of the two large subunits and a small polypeptide with an apparent molecular mass of 5 kDa [7]. Our core complex corresponds to the PS I core protein of Parrett et al. [8], although the 5 kDa polypeptide was not reported in the latter case.

In this paper, we characterize and determine the N-terminal amino acid sequence of the 5 kDa polypeptide.

2. MATERIALS AND METHODS

PS I particles (PS I-200) were prepared by solubilizing spinach thylakoids with Triton X-100 as previously described [9] For the heat treatment, PS I particles were resuspended in a medium containing 0 1 M sorbitol, 10 mM NaCl and 50 mM Tricine-NaOH (pH 7 8) in the presence of 50% (v/v) ethylene glycol at 1 mg Chl/ml The samples were incubated at various temperatures for 5 min and then rapidly cooled to 4°C Heat/EG-treated PS I particles were centrifuged and resuspended in 0.8% (w/v) Triton X-100 at 800 μg Chl/ml. The PS I core complex was isolated from the resulting suspension by sucrose density gradient ultracentrifugation as described in [7]

EPR spectra and flash-induced absorbance changes were measured as described in [7]

Polypeptide analysis was carried out by Tricine-SDS-PAGE [10] The method for protein blotting described in [11] was modified as follows An unstained gel was equilibrated with blotting buffer A containing 25 mM Tris, 40 mM ϵ-aminocaproic acid (pH 9.5), and 20% (v/v) methanol for 15 min at room temperature. The gel was placed on a PVDF membrane and sandwiched between two sheets of filter paper saturated with blotting buffer A and two sheets of filter paper saturated with buffer B containing 25 mM Tris and 20% (v/v) methanol, underlying this assemblage were two sheets of filter paper saturated with buffer C containing 0.3 M Tris and 20% (v/v) methanol Electroblotting was conducted using a semidry-type electroblotter AE-6670 (Atto) Proteins transferred to the PVDF membrane were stained with 0.1% (w/v) CBB in methanol/acetic acid/water (5 2 5, v/v) for 5 min and destained with 90% (v/v) methanol The stained band was cut out, and sequence analysis was performed using Applied Biosystems Protein Sequenator 477A

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Abbreviations· Chl, chlorophyll; CBB, Coomassie brilliant blue R-250; DCIP, 2,6-dichlorophenol indophenol, heat/EG treatment, heat treatment for 5 min in the presence of 50% (v/v) ethylene glycol, PAGE, polyacrylamide gel electrophoresis, PS I, photosystem I, PVDF, polyvinylidene difluoride, SDS, sodium dodecyl sulfate

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3. RESULTS AND DISCUSSION

Heat/EG treatment causes the selective destruction of iron sulfur centers, F_A, F_B and F_X depending on the treatment temperature [7]. 60°C/EG treatment of thylakoid membranes selectively destroyed F_A and F_B without changing the F_X level. 70°C/EG treatment also destroyed F_X [7]. These heat/EG treatment effects can be seen clearly in the flash-induced oxidation and dark re-reduction kinetics of P700 measured at 698 nm (fig.1A). 58°C/EG treatment of PS I particles eliminated the decay phase with a lifetime of 30 ms, the phase which corresponds to the reduction of $P700^+$ by F_A/F_B [3,8,12]. The 1 ms decay phase that appeared after the treatment seems to reflect the reduction of $P700^+$ by F_X [8,12]. The extent of the 1 ms decay phase decreased as the treatment temperature increased, and this phase was almost lost at 70°C (fig.1Ac). These changes of the P700 kinetics paralleled the destruction of F_A/F_B or F_X

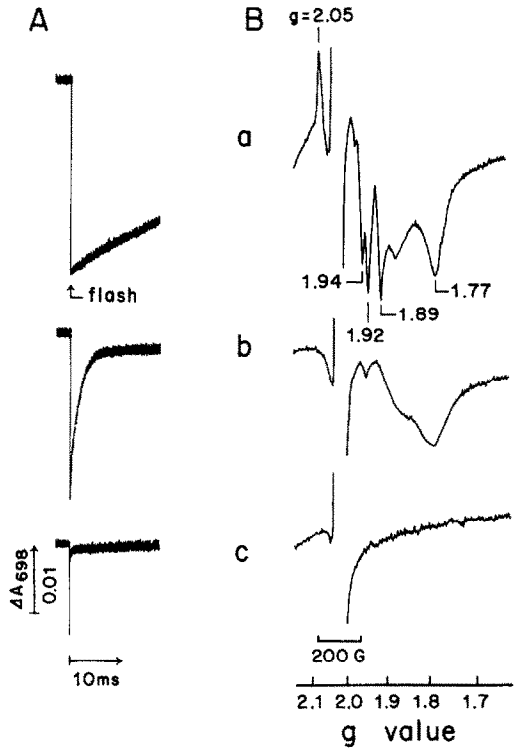

Fig.1. (A) Flash-induced absorption changes at 698 nm in PS I particles. Samples were preincubated with 50% (v/v) ethylene glycol at 25°C (a), 58°C (b) and 70°C (c) for 5 min. Reaction temperature, 15°C. Reaction mixture contained 10 μM DCIP, 1 mM sodium ascorbate, 100 mM sorbitol, 10 mM NaCl; 50 mM Tricine-NaOH (pH 7.8) and PS I particles. (B) EPR spectra of the iron sulfur centers of PS I core complex isolated from heat/EG-treated PS I particles. (a) PS I particles, (b) 58°C/EG core complex, (c) 70°C/EG core complex. EPR experimental conditions: temperature, 8°K; microwave frequency and power, 9.69 GHz and 100 mW, respectively; gain, 1.0×10^5; modulation amplitude, 20 G; Scan width, 3200–4200; time constant, 320 ms. Reaction mixture contained 0.1 M glycine-0.1 M amino methyl propanediol-NaOH (pH 10.0), 50 μM methyl viologen, 50 μM DCIP, 0.7% (w/v) sodium dithionite and PS I core complex (1 mg Chl/ml).

Table 1

P700 content in heat/EG core complex

Samples	Chemical oxidation	Photochemical oxidation
	P700 content % of 58°C/EG core	k_{P700} s^{-1}
58°C/EG Core complex	100 100	8.4
70°C/EG Core complex	78 57	2.3

Extent of chemically determined P700 was measured by the 25 μM ferricyanide minus 0.5 mM ascorbate difference spectrum. Photochemically oxidizable P700 and k_{P700} (first-order rate constant for P700 photooxidation) was measured under continuous light as described in [9]. The reaction mixture contained 1 mM sodium ascorbate, 2 μM DCIP, 0.5 mM methyl viologen, 100 mM sorbitol, 10 mM NaCl, 50 mM Tricine-NaOH (pH 7.8) and the core complex (6 μg Chl/ml). P700 contents in the 58°C/EG core complex measured by chemical and photochemical oxidation were 9.1 and 13.2 mmol/mol Chl, respectively

measured by EPR [7]. However, the 70°C/EG treatment of PS I particles slightly affected the extent of P700 measured under continuous light [7].

The PS I core complex was isolated from PS I particles after 58°C or 70°C/EG treatment by solubilization with Triton X-100. The EPR spectrum of the core complex from 58°C/EG-treated particles (58°C/EG core complex) showed an F_X band but no F_A and F_B signals (fig.1Bb). The band of F_X at $g = 1.77$ was broadened with no shift of the peak position. The 70°C/EG core complex lost all signals of F_A, F_B and F_X

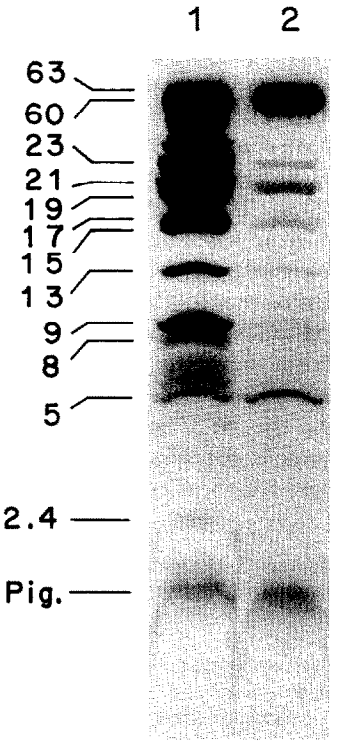

Fig.2. Polypeptide compositions of the PS I core complex. Lane 1: PS I particles; lane 2: 60°C/EG core complex.

Table 2

Content of 5 kDa polypeptide in the PS I core complex

Samples	Ratio of 5 kDa to 60–63 kDa subunits[a]	
	Expt 1[b]	Expt 2[c]
PS I particles	0 53	0 78
60°C/EG core complex	0 67	0 91
70°C/EG core complex	n d[d]	0 39

[a] Molecular masses of large 60–63 kDa and 5 kDa subunits were estimated as 83 kDa [13,14] and 8 4 kDa [17], respectively, based on their DNA coding sequences

[b] Calculated from the absorbance of CBB in SDS-isopropanol extracted from the gel as in [15]

[c] Calculated from the area measured by a densitometer after the gel was stained with CBB

[d] not determined

(fig.1Bc). This core complex still contained P700 assayed by chemical oxidation-reduction (table 1). However, the extent of photooxidizable P700 decreased. This probably can be explained by the reduction of $P700^+$ by A_1^- or A_0^- as suggested by the slower rate constant for P700 photooxidation (table 1).

Fig.2 shows the polypeptide composition of the heat/EG core complex. The treatment removed the low molecular mass polypeptides including the 8 kDa polypeptide (F_A and F_B apoprotein [5,6]) from the core complex obtained after Triton solubilization. The 60°C/EG core complex containing P700 and F_X was composed of the high molecular polypeptides (60 and 63 kDa in the gel used here), the 5 kDa polypeptide, and trace amounts of the 9–23 kDa polypeptides. This core complex seems to be directly comparable to the PS I core protein isolated from urea-treated cyanobacteria by Parrett et al. [8], although they did not report the small molecular mass polypeptide. The 60°C/EG core complex contained the same level of 5 kDa polypeptides as that of PS I particles (table 2), suggesting a close association between this polypeptide and the high molecular mass polypeptides. The content of the 5 kDa polypeptide decreased to 43% in the core complex isolated from 70°C/EG-treated particles lacking F_X (table 2). This suggests that the 5 kDa polypeptide functions to stabilize the PS I reaction center core complex.

The N-terminal amino acid sequence of the 5 kDa polypeptide was determined after extraction of the stained band (fig.3). This sequence did not correspond to the sequence derived from any possible reading frame in the chloroplast DNA of liverwort [13] or tobacco [14], suggesting that this polypeptide is encoded by the nuclear DNA. In the first 29 N-terminal amino acids, twelve are hydrophobic (three phenylalanine, three leucine, two isoleucine, two methionine, one proline and one valine) and only two are charged (aspartic acid and arginine). The sequence indicates the hydrophobic nature of the 5 kDa polypeptide.

To assess possible contamination of the core complex by small polypeptides of non-PS I origin, we also analyzed the soluble stroma proteins prepared from intact spinach chloroplasts isolated by the method described in [16]. However, no protein with molecular mass below 6 kDa was detected (Kamide et al., unpublished data).

Recently, small subunits of PS I with molecular masses below 8 kDa have been studied [17–19]. Franzén et al. [17] have reported the isolation and characterization of cDNA clones encoding three low molecular PS I subunits (P28, P35 and P37) from *Chlamydomonas reinhardtii*. The 5 kDa polypeptide in our work is homologous to P37 which is a mature protein with a 8.4 kDa mass (apparent molecular mass of 3 kDa in SDS-PAGE) [17]. With the exception of four unidentified residues, when the first 25 N-terminal amino acid sequence of the 5 kDa protein is compared with that of P37, the positional identity is 72% (fig.3). They did not speculate the function of the P37 protein [17].

The 5 kDa protein exists in the reaction center core complex containing F_X and dissociates as F_X is destroyed. F_X is postulated to be a [4 Fe-4 S] iron sulfur cluster which bridges the two large-core PS I polypeptides, A and B. The amino acid sequence of the *C. reinhardtii* P37 mature protein as well as that of the 5 kDa protein is rich in hydrophobic amino acids and has two hydrophobic regions (fig.3) capable of forming α-helices. A part between regions I and II of P37 is also hydrophobic (fig.3) and may not be located on the aqueous surface. Almost all parts of this protein are

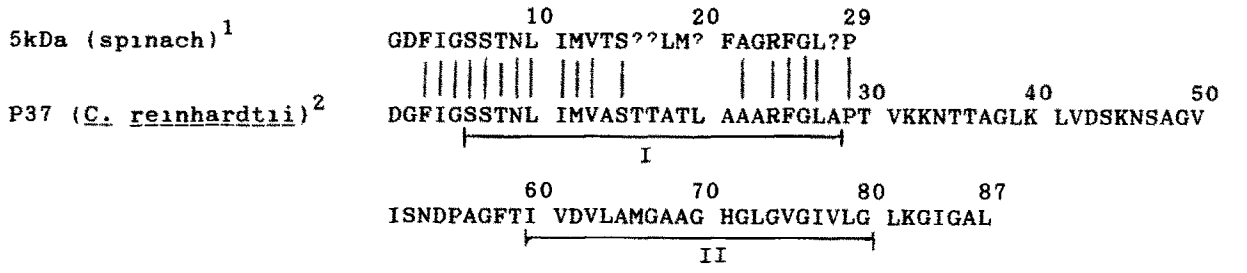

Fig 3 Comparison of the N-terminal amino acid sequence of the 5 kDa polypeptide (this work) and of the P37 mature protein of *C reinhardtii* [17] Amino acids that could not be identified in the sequence of the 5 kDa polypeptide are shown as ? Homologous sequences are shown by vertical lines Regions I and II represent the hydrophobic regions possibly forming intramembranous α-helices [17] [1] This study [2] Franzén et al [17]

presumably buried between the large A and B subunits. This structure as well as observations in the present work suggest that the 5 kDa protein (P37) works to stabilize the PS I reaction center, probably by interacting with both the A and B subunits.

Scheller et al. [18] have demonstrated the presence of polypeptides with apparent molecular masses of 4 and 1.5 kDa in PS I particles from barley, which seem to correspond to the 5 and 2.4 kDa bands in our gel, respectively (fig.2). Møller et al. [19] showed that a 1.5 kDa polypeptide was encoded by a chloroplast gene, designated *psa*I. They speculated that 1.5 kDa and 4 kDa polypeptides may participate in the binding of P700, A_0 and A_1 of PS I in a manner similar to that of the D1 and D2 reaction center polypeptides of PS II because the sequence of *psa*I was partially homologous to the helix E portion of D2. The 2.4 kDa polypeptide, which seems to correspond to the 1.5 kDa *psa*I product, however, was lost in the 60°C/EG core complex which still retained active P700 and F_X signals (table 1 and fig.1Bc) and presumably A_0 and A_1 (phylloquinone [20]). The 70°C/EG core complex, in which the 5 kDa polypeptide was reduced by more than half and no other small polypeptides were retained, still showed P700 activity although F_X was destroyed. Therefore, the small 5 and 2.4 kDa polypeptides do not seem to function in the binding of P700, A_0 and A_1.

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