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

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Received 25 September 1989

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 psaA and psaB [1, 2]. The reaction center core complex binds the reaction center P700 (Chl a) with the electron acceptors $A_0$ (Chl a), $A_1$ (phyllloquinone) and $F_X$ (iron sulfur center) [3, 4]. The 9 kDa polypeptide encoded by the chloroplast gene psaC 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 Tris-NaOH (pH 7.8) in the presence of 25% (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 2-amino-2-(hydroxymethyl)-1,3-propanediol 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, underlyng 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 semidyne-type electrophotri- 

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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00145793/89/$3 50 © 1989 Federation of European Biochemical Societies

305
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.1A). These changes of the P700 kinetics paralleled the destruction of $F_A$/$F_B$ or $F_X$.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Chemical oxidation</th>
<th>Photochemical oxidation</th>
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<tbody>
<tr>
<td></td>
<td>% of 58°C/EG core</td>
<td>$k_{P700}$ s$^{-1}$</td>
</tr>
<tr>
<td>58°C/EG Core complex</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>70°C/EG Core complex</td>
<td>78</td>
<td>57</td>
</tr>
</tbody>
</table>

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.1](image1.png)

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, $1.0 \times 10^5$; 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).

![Fig.2](image2.png)

Fig.2. Polypeptide compositions of the PS I core complex. Lane 1: PS I particles; lane 2: 60°C/EG 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 FX and dissociates as FX is destroyed. FX 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 \( \alpha \)-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
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 psa1. They speculated that 1.5 kDa polypeptide to the helix E portion of D2. The 2.4 kDa polypeptide, which seems to correspond to the 1.5 kDa psa1 product, may participate in the binding of P700, AO and A1 of PS I in a manner similar to that of the D1 and D2 reaction center polypeptides of PS II because the sequence of psa1 was partially homologous to the helix E portion of D2. The 2.4 kDa polypeptide, which seems to correspond to the 1.5 kDa psa1 product, however, was lost in the 60°C/EG core complex which still retained active P700 and FX signals (table 1 and fig.1Bc) and presumably AO and A1 (phylloquinone [20]). The 70°C/EG core complex, in which the 5 kDa polypeptide was reduced by more than half and no small polypeptides were retained, still showed P700 activity although FX was destroyed. Therefore, the small 5 and 2.4 kDa polypeptides do not seem to function in the binding of P700, A0 and A1.

Acknowledgements: We are grateful for Drs L.G. Franzén and B.L. Møller, who kindly allowed us to read their unpublished papers ([17] and [19]) during the preparation of this manuscript. A part of this work was presented during the VIIIth International Congress on Photosynthesis, Stockholm, August 6–11, 1989. We thank Prof. Y. Fujita for his support and helpful comments, Dr. H. Kagamiyama of Osaka Medical School for analyzing amino acid sequences, Ms M. Iwaki and Mr. M. Kawamoto for their technical assistance; and Mr. B. Kemp for reading the manuscript. This work was supported in part by a Grant-in-Aid for Scientific Research on Priority Areas (63621003, 01621003) from the Ministry of Education, Science and Culture, Japan.

REFERENCES