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The 8 kDa polypeptide in photosystem I is a probable candidate of an iron-sulfur center protein coded by the chloroplast gene *frxA*

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The N-terminal sequence of the 8 kDa polypeptide isolated from spinach photosystem I (PS I) particles was determined by a gas-phase sequencer. The sequence showed the characteristic distribution of cysteine residues in the bacterial-type ferredoxins and was highly homologous to that deduced from the chloroplast gene *frxA* of liverwort, *Marchantia polymorpha*. It is strongly suggested that the 8 kDa polypeptide has to be an apoprotein of one of the iron-sulfur center proteins in PS I particles.

Photosystem I particle; Iron-sulfur center; Protein; *frxA* gene; (Liverwort)

1. INTRODUCTION

Photosystem I (PS I) exists as a complex in chloroplast thylakoid membranes and promotes the second energy conserving reaction. Low temperature EPR spectroscopy has revealed that there are at least three iron-sulfur centers, A, B and X [1], serving as electron carriers on the accepter side in PS I. This complex consists of 7 subunits in higher plants, that is, two large subunits (59 and 63 kDa polypeptides) and 5 small subunits (8, 10, 14, 16 and 19 kDa) [2,3]. Extensive efforts have been focused on the subunit(s) carrying the iron-sulfur center(s) and many experimental data have been accumulated.

Lagoutte et al. [4] have recently reported that the 8 kDa polypeptide might be an apoprotein of one of the iron-sulfur center proteins judged from the incorporation experiment of ^{35}S in vivo and by carboxymethylation of cysteine residues with iodo-[^{14}C]acetate. A similar conclusion was obtained by Sakurai and San Pietro [3], showing that the iron-sulfur centers were bound to 59 and/or 63 kDa polypeptide(s) as well as an 8 kDa polypeptide by analyzing zero-valence sulfur atoms covalently bound to these polypeptides, which were converted from the acid-labile sulfides with SDS-treatment [5].

On the other hand, Ohyama et al. [6] have recently determined the whole DNA sequence of chloroplast genome and suggested the existence of a bacterial-type ferredoxin with a molecular mass of 8 kDa.

In the present study, the 8 kDa polypeptide was isolated from spinach PS I particles, and its N-terminal sequence was determined and compared with the sequence deduced from the DNA sequence of the chloroplast gene, *frxA* (Ohyama et al., unpublished).

2. MATERIALS AND METHODS

PS I particles were prepared from spinach chloroplasts by the method of Sakurai et al. [3] with slight modifications. Chromatography on a DEAE-Toyopearl 650 M column (Toyo Soda Co.,

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Tokyo, Japan) was used to purify PS I particles. PS I particles were eluted from this column with a linear gradient system of NaCl concentration from 0 to 200 mM. The PS I particles thus obtained were mixed with an equal volume of *n*-butanol using a vortex mixer for 1 min ×4 with 5-min intervals. After centrifugation at 30000×*g* for 10 min, the aqueous phase was collected and lyophilized. The extract containing 19, 14 and 8 kDa polypeptides was carboxymethylated by the method of Crestfield et al. [7] and applied to a DEAE-Toyopearl 650 M column. The column was developed with a linear gradient system of NaCl concentration from 0 to 700 mM in 50 mM Tris-HCl buffer, pH 7.5, containing 7 M urea. The 8 kDa polypeptide eluted was further purified on a reverse-phase column (Cosmosil 5PhT, 4.6×150 mm, Nakarai Chemical Co., Kyoto, Japan).

Amino acid analysis was performed as described in [8]. Amino acid sequence was determined by a gas-phase protein sequencer (Applied Biosystems, model 470 A) equipped with an on-line connected HPLC, model 120 A.

3. RESULTS AND DISCUSSION

The 8 kDa polypeptide was extracted from the PS I particles with the *n*-butanol treatment and purified by two-step column chromatography. As shown in fig.1 (lane 3), the apparent molecular mass of the 8 kDa polypeptide became slightly larger after carboxymethylation.

The N-terminal sequence of the 8 kDa polypeptide was determined up to the 29th step as shown in fig.2. The typical distribution of 4 cysteine residues in the bacterial-type ferredoxins (Cys-X-X-Cys-X-X-Cys-X-X-X-Cys-Pro) was found in the N-terminal region and further the sequence was highly homologous to that deduced from the DNA sequence of the *frxA* gene in chloroplasts of liverwort, *Marchantia polymorpha* [6]. The first methionine residue translated in the *frxA* gene of liverwort chloroplasts was probably processed to deletion. The 2nd and 4th alanine residues in the liverwort *frxA* gene were replaced by serine residues in the spinach 8 kDa polypeptide. But other residues in the N-terminal sequence were entirely identical up to the 29th step. The amino acid composition of the 8 kDa polypeptide also showed good agreement with that deduced from *frxA:*

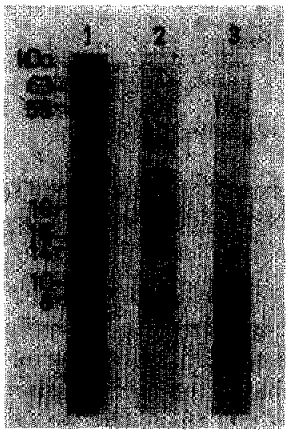

Fig.1. Purification of the 8 kDa polypeptide from PS I particles. SDS-polyacrylamide gel electrophoresis was performed by the method of Schägger et al. [12]. Lanes: 1, spinach PS I particles; 2, polypeptides extracted from PS I particles with the *n*-butanol treatment; 3, carboxymthylated 8 kDa polypeptide purified from the polypeptide mixture of lane 2.

CmC, 8.4(9); Asx, 5.8(7); Thr, 6.1(7); Ser, 5.2(5); Glx, 7.3(6); Pro, 4.2(4); Gly, 5.9(5); Ala, 5.4(6); Val, 5.1(6); Met, 2.3(3); Ile, 3.8(4); Leu, 4.5(4); Tyr, 3.1(3); Phe, 1.7(1); Lys, 4.0(3); His, 1.7(1); Arg, 4.5(6). The values in parentheses are those deduced from the DNA sequence (Ohyama et al., unpublished). Therefore, the 8 kDa polypeptide is probably coded by the *frxA* gene. The arrangement of cysteine residues in the sequence can chelate [4Fe-4S] cluster(s), indicating that the 8 kDa polypeptide must be an iron-sulfur protein functioning as an electron carrier in PS I particles. Furthermore, the 8 kDa polypeptide can form two [4Fe-4S] clusters because the DNA sequence of the *frxA* gene has a very similar sequence to that of the bacterial [8Fe-8S] ferredoxin.

The present experiment shows for the first time that an unidentified product of a gene among other chloroplast genes was identified as a protein functioning in PS I. Since one of the three iron-sulfur centers in PS I particles [1], center X, is considered to be associated with the large subunits (59 and/or 63 kDa polypeptide(s) [9] and to have [2Fe-2S] clusters [10], the 8 kDa polypeptide is probably an apoprotein of center(s) A and/or B).

Malkin et al. [11] isolated an iron-sulfur protein with a molecular mass of 8 kDa from spinach chloroplast membrane and showed the physical

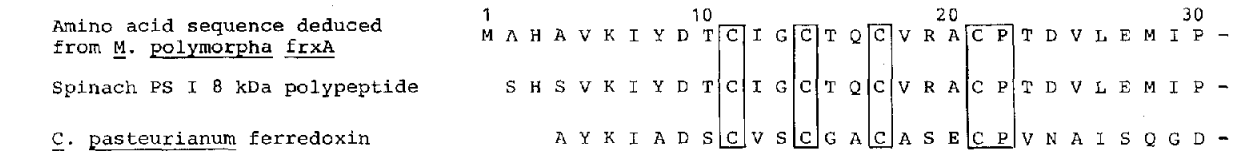

Fig.2. The N-terminal sequence of the 8 kDa polypeptide compared with the sequence deduced from the *M. polymorpha frxA* gene (Ohyama et al. unpublished) and that of *C. pasteurianum* ferredoxin [13]. The typical cysteine and proline residues are framed.

and chemical properties to be similar to those of bacterial-type ferredoxins. However no information is available at the moment about the protein chemical data and therefore, we are uncertain if this protein is identical or not with our 8 kDa polypeptide presented here.

It is noteworthy that the bacterial-type ferredoxin in chloroplasts exists as the membrane-bound form, but those of anaerobic bacteria such as *Clostridium* and *Chlorobium* as soluble forms [13]. Yet there is no clear indication to show such property differences only from the sequences. The soluble ferredoxins of clostridial-type functioned originally only in diverse non-photosynthetic metabolic systems such as carbon and nitrogen metabolic systems, and at an early evolutionary time they diverged to become electron carriers in photosystems of photosynthetic bacteria such as *Chlorobium*. Later the ferredoxins of this type became the bound form on thylakoid membranes in green plants (and probably in cyanobacteria) functioning in photosystem I as electron carriers. Further soluble ferredoxins of [2Fe-2S] type developed to mediate the electron flow from the membrane-bound bacterial-type ferredoxins in plants.

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ADDENDUM

We have recently become aware of a report by T. Hiyama who also obtained a similar result to ours (Abstract in Solar Energy Conversion: Photochemical Reaction Centers and Oxygen Evolving Complexes of Plant Photosynthesis, Japan/US Binational Seminar at Okazaki, Japan, 17–21 March 1987, pp. 64–65).

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