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Two new components of 9 and 14 kDa from spinach photosystem I complex

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Two formerly-uncharacterized subunits of 9 kDa and 14 kDa were found in spinach PSI complex. The 9 kDa subunit was released upon removal of antenna chlorophyll complex, whereas the 14 kDa subunit was tightly bound to the core complex. We determined the N-terminal amino acid sequence of the 9 kDa, and an internal sequence of the 14 kDa subunit after protease treatment, since the N-terminus of the latter protein was blocked. These partial sequences suggested that both subunits are new PSI components.

Protein sequence; Photosystem I; Spinach

1. INTRODUCTION

PSI is organized in thylakoid membranes as a large protein complex consisting of many different subunits. Recent analyses of protein and DNA sequences have registered up to 11 species of components in PSI of higher plants, of which genes are named *psa*A to *psa*K [1,2]. Besides these, we have observed and reported the presence of at least two more components of 14 and 9 kDa in spinach PSI complex, although their identities were uncertain [3]. Direct sequencing of a newly detected protein is one of the best ways to determine whether it is new or corresponds to a previously characterized protein. However, protein sequencing of those two components has not yet successfully been done, because the 9 kDa protein co-migrated with other PSI components, and the N-terminus of the 14 kDa protein was blocked.

Here we report partial amino acid sequences of these two subunits, suggesting that both are new PSI components.

2. MATERIALS AND METHODS

Native PSI complex retaining LHCI and PSI core complex depleted of LHCI were isolated from spinach as described in [3,4]. Protein subunits were resolved by SDS-urea-PAGE with a 16–22% acrylamide gel containing 7.5 M urea [5]. For sequencing the 9 kDa subunit, the gel was directly subjected to electroblotting onto an Immobilon membrane (Millipore) according to [6]. For sequencing the proteolytic fragments, substrate proteins in the gel were visualized by 30 min-staining with 0.04% (w/v) Coomassie brilliant blue R-250 (Bio-Rad) followed by 1 h-destaining with 40% (v/v) methanol, and then excised. After equilibration with 0.1% (w/v) SDS, 1 mM EDTA, 30% (w/v) sucrose, and 60 mM Tris-HCl (pH 7), the substrate proteins were electrophoresed into the second SDS-urea-gel together with the protease employed basically according to Cleveland et al. [7]. The second gel was essentially the same as the first gel except for the absence of urea in the stacking gel. After the gel pieces were loaded on the second gel, a protease solution containing 0.1% (w/v) SDS, 1 mM EDTA, 15% (w/v) sucrose, 60 mM Tris-HCl (pH 7), and 100 μg/ml *Staphylococcus aureus* V8 protease (Boehringer Mannheim) was overlaid. Proteolytic digestion was accelerated by turning off the electrophoresis for 30 min at 25°C when the electrophoretic front reached the bottom of the stacking gel. After completion of electrophoresis, proteolytic fragments were transferred to the Immobilon membrane. N-Terminal amino acid sequences of proteins and proteolytic fragments were determined with a protein sequencer (model 477A, Applied Biosystems) as described in [8].

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Abbreviations: LHCI, light-harvesting chlorophyll complex associated with photosystem I; PSI, photosystem I; SDS-PAGE, sodium dodecylsulfate-polyacrylamide gel electrophoresis

3. RESULTS AND DISCUSSION

The 9 kDa band resolved from spinach native PSI complex appeared to consist of a sharp doublet band (Fig. 1, lane of native PSI) superimposed on a diffuse band (Fig. 1, lane of PSI core). In fact, when the doublet band of the native PSI complex was subjected to sequencing, three sets of amino acid signals with comparable intensity were obtained at each cycle. However, when the diffuse band obtained from the PSI core complex was sequenced, a single set of signals corresponding to PSI-C (Fe-S center A/B protein) was obtained. One of the other two sets of signals, which were missing in the core complex, corresponded to PSI-G [9]. After subtracting these two already known sets of signals, the rest revealed the following sequence:

Gly-Val-Ile-Asp-Glu-Tyr-Leu-Glu-Lys-Ser-Lys-Ala-Asn-

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When we applied the same sequence analyses to a pea 9 kDa band of native PSI complex (Fig. 1 of [3]), a similar sequence accompanied by PSI-C and PSI-G sequences was obtained as follows:

Ser-Val-Phe-Asp-Ala-Tyr-Leu-Glu-Lys-Ser-Lys-
Ala-Asn-

These two sequences were homologous to each other but did not correspond to either of the open reading frames of chloroplast DNA, any known nuclear-encoded subunits of PSI or any proteins in Protein Database. The fact that the 9 kDa protein was associated with the native PSI complex in both spinach and pea strongly suggests that this is a new type of PSI component, although it was not retained in the PSI core complex. Since no compatible homologue has yet been found in cyanobacterial PSI, this 9 kDa subunit might be involved in association of LHCI with the core complex in green plants, as already suggested for PSI-H [10].

When we identified PSI-C, PSI-D, PSI-E, PSI-F, PSI-G and PSI-H bands of spinach PSI complex by protein sequencing in our previous papers [3], we observed a band at 14 kDa, of which N-terminus is blocked. Since this protein was tightly bound to the PSI core complex (Fig. 1), it was inferred to be a real PSI component. Recently Obokata et al. [11] reported the presence of a similar blocked protein of about 15 kDa in tobacco PSI complex. They cloned cDNA by use of the partial amino acid sequence of its proteolytic fragment. Since the cloned gene was found to be very similar to tobacco *psa*E encoding the unblocked protein (81% homology on amino acid level), they assigned it as the second *psa*E. Similarly, Münch et al. [12] reported that the spinach genome contains at least two *psa*E genes, although they cloned and characterized only one of them. Our sequence data of spinach 12 kDa protein (14 amino acid residues) completely matched with the gene sequence published by Münch et al. [12], except that about one-third of our protein began with the second residue, Ala. Taking these into account, we first assumed a working hypothesis that our 14 kDa protein might be a product of the second *psa*E gene.

Both the 14 kDa protein and PSI-E were isolated from spinach PSI complex (Fig. 1) and digested with *Staphylococcus aureus* V8 protease, which specifically cleaves the peptide bond on the carboxyl side of Glu residue. Clearly, the proteolytic profiles are different from each other (Fig. 2): many bands (11.5, 10, 5.5, 5 and 4 kDa) were generated from PSI-E, while the 14 kDa protein seemed to be cleaved into two major fragments of 9 and 5 kDa (Fig. 2). The 4 kDa and 5.5 kDa bands found in PSI-E digest corresponded to the

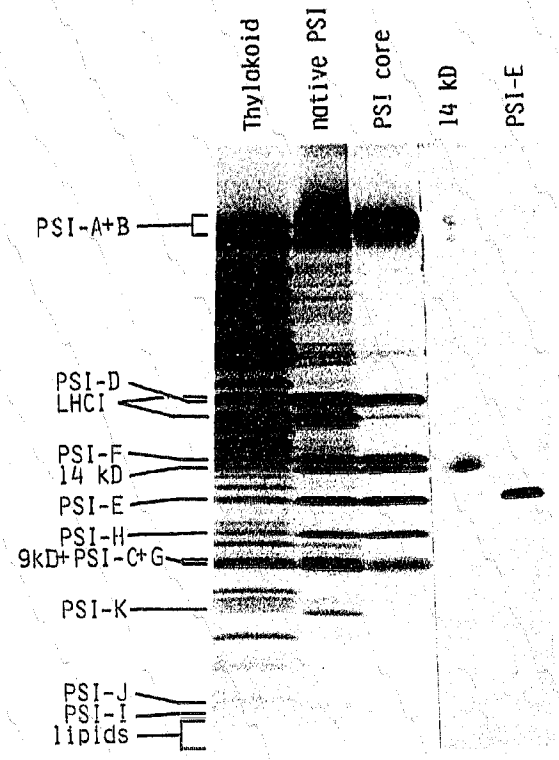

Fig. 1. SDS-PAGE profiles of thylakoid membranes, native PSI complex, PSI core complex and isolated 14 kDa and PSI-E proteins.

Fig. 2. Proteolytic digestion profile of PSI-E and the 14 kDa protein on Immobilon membrane. The proteins were fragmented and resolved in the second gel by co-electrophoresis with *Staphylococcus aureus* V8 protease (V8), electroblotted to Immobilon membrane and then stained with Amido black 10B.

internal sequences of PSI-E as expected. The N-terminus of the 9 kDa fragment obtained from the digest of the 14 kDa protein was blocked, indicating that this fragment originated from the N-terminal part of the 14 kDa protein. On the other hand, N-terminal sequencing of the 5 kDa fragment in the same digest revealed a following sequence:

Gly-Glu-Pro-Ser-Ile-Ala-Pro-Ala-Leu-Thr-Leu-Thr-Gly-Arg-Lys-(Lys)-Gln-Pro-?-Gln-Leu

Probably this fragment was derived from the C-terminal part of the original protein. Since this sequence did not correspond to any part of PSI-E, any other known proteins or any open reading frames of chloroplast DNA, the 14 kDa blocked protein cannot be the product of the second *psa*E gene, but is probably a new PSI component encoded by nuclear genome. Based on these, we assume that the second *psa*E product, if expressed, would have co-migrated with the authentic one as a single band of 12 kDa or that the second one did not accumulate at detectable levels. In either case we may exclude the possibility that the content of PSI-E-related subunits is twice as much as PSI-D or PSI-F, as suggested for tobacco PSI complex [10]: the staining intensity of PSI-E band per its molecular mass was almost identical to those of PSI-D and PSI-F (see below).

It turned out very recently that the above internal sequence from spinach corresponds to the C-terminal part of a barley PSI component of 14 kDa (proposed as *psa*L) (Okkels, J.S. and Scheller, H.V., personal communication). It was furthermore revealed that the barley 14 kDa protein has blocked N-terminus like our spinach 14 kDa protein, while the other nuclear-encoded PSI subunits carry open N-termini. It is thus very likely that both 14 kDa proteins from spinach and barley are of the same origin, consistent with our conclusion that the protein is a new PSI component. Notably, a protein significantly homologous to the plant 14 kDa subunit was already found as a 12 kDa protein in *Synechococcus* PSI complex, although it was at first reported as a possible homologue to the plant PSI-G based on weak sequence homology [13].

Strangely, the 14 kDa protein (PSI-L) shows an unusual stoichiometry. The relative subunit abundance calculated for spinach PSI complex was: PSI-L (14 kda); PSI-D (17.9 kDa); PSI-E (9.7 kDa); PSI-F (17.3 kDa) = 0.6; 1.1; 1.0; 1.0. More or less the same unusual stoichiometry has been observed not only for *Synechococcus vulcanus* [13] but also for *Synechocystis* PCC6803 and *Anabaena* ATCC29413 (Ikeuchi, M., Nyhus, K., Inoue, Y. and Pakrasi, H.B., unpublished results). It seems that the low abundance of PSI-L cannot be an artifact but a characteristic of this component.

In conclusion, the present study together with our previous report [3] have registered 11 subunits (PSI-C, PSI-D, PSI-E, PSI-F, PSI-G, PSI-H, PSI-I, PSI-J, PSI-K, 14 kDa protein (PSI-L) and 9 kDa protein) in PSI complex of higher plants by protein sequencing. There are no more discrete bands in PSI complex except for the 60 kDa PSI-A/B bands. In cyanobacterial PSI complex, PSI-C, PSI-D, PSI-E, PSI-F, PSI-J, PSI-K, PSI-L have been confirmed by protein sequencing [13]. Recently PSI-I was also found in *Anabaena* PSI complex [14], although the other subunits (PSI-G, PSI-H and 9 kDa protein) have not yet been detected. There is one PSI component of 5 kDa, which is found in cyanobacteria [13] but not in higher plants [3]. These differences in subunit composition between cyanobacteria and higher plants may reflect the different antenna system of PSI, namely LHCI in higher plants vs no apparent antenna in cyanobacteria. However, it should be kept in mind that some differences in subunit composition can occur due to a loss from the PSI complex during isolation. More careful study would be needed to settle the consensus about the subunit composition of PSI complex of cyanobacteria and higher plants.

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