Volume 302, number 1, 15–17 FEBS 10977 May 1992

Assembly and processing of subunit II (PsaD) precursor in the isolated photosystem-I complex

Yuval Cohen and Rachel Nechushtai

Department of Botany, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

Received 9 March 1992

The precursor of photosystem I (PSI) subunit II (pre-subunit II) synthesized in vitro, was found to bind to the holo-PSI complex, both within the thylakoids and outside, after detergent extraction of PSI from the membranes. Chloroplast stromal fraction added to the purified PSI complexes, containing the labeled pre-subunit II, induced the processing of the precursor to the mature form. This implies that processing can occur within the isolated complex, after the integration of the precursor. The results presented suggest that certain aspects of biogenesis of membranal protein complexes can be studied in detergent-extracted purified complexes.

Biogenesis; Photosystem I; Assembly; Processing; PsaD

1. INTRODUCTION

The photosystem I complex (PSI) present in thylakoids of higher plants, algae and cyanobacteria mediates the electron transfer from plastocyanin to ferredoxin. In green algae and higher plants, the complex consists of a light-harvesting antenna (LHCI), and a core component (CCI) in which the charge separation takes places [1,2]. At present CCI is believed to contain 12 polypeptide subunits, about 60 chlorophyll *a* molecules, 1–5 β-carotenes and three [4Fe-4S] clusters. The complex is believed to be organized within the thylakoid membrane with some of its subunits facing the stromal side (PsaC, PsaD, PsaE), and at least one (PsaF) is exposed to the luminal side of the membrane [3,4].

In spinach, subunit II (PsaD) of CCI has been shown to be synthesized in the cell cytoplasm as a precursor of 23.2 kDa [5,6]. Its amino acid sequence did not indicate the presence of any trans-membranal regions in the protein. Hence, the protein is assumed to be a peripheral, rather than an integral membrane protein. Analysis of its transit sequence [7], proteolytic digestion [8] as well as additional biochemical data [9] suggest that subunit II is located on the stromal side of the thylakoid membrane.

PsaD has been shown to be crosslinked to the soluble ferredoxin, and therefore suggested to have a role in docking ferredoxin to the PSI complex [10,11]. In a recent work, Li and his co-workers reconstituted PsaD to depleted CCI core complexes. They have very elegantly shown that by binding of subunit II (PsaD), the binding of subunit VII (PsaC) has been stabilized and improved [12]. Subunit VII contains the two terminal electron acceptors of CCI, and in turn donates the electrons to the soluble ferredoxin [4].

Subunit II (PsaD) has also been shown to play a role in the biogenesis and assembly of the entire PSI complex. Upon exposure of etiolated plants to light, subunit II was the first nuclear polypeptide to accumulate in the leaves [13,14]. In *Chlamydomonas*, in the absence of PsaD, the accumulation of PSI was prevented [15]. Furthermore, *Synechocystis* sp. 6803 mutant, which lacked this subunit, exhibited much lower photosynthetic activity than that of the wild-type [16]. These findings imply the importance of PsaD for the formation of PSI as well as for the function of its reducing site.

In a recent study it was shown that the precursor of subunit II is imported into the chloroplast and inserted into the thylakoid membranes. There, it was processed to the mature form [17]. Both the precursor and the mature forms were confined to the PSI complex. The present study further characterizes this integration, and attempts to find whether the insertion of the protein occurs directly into the PSI complex, without involvement of any additional component.

2. MATERIALS AND METHODS

2.1. *Plant material*

Spinach plants were grown hydroponically for 3 weeks in light (150 $\mu E/m^2/s$/dark cycles of 14 and 10 h, respectively [17].

Abbreviations: PSI, photosystem I; chl, chlorophyll; PAGE, polyacrylamide gel electrophoresis.

Correspondence address: R. Nechushtai, Department of Botany, The Hebrew University of Jerusalem, Jerusalem 91904, Israel. Fax: (972) (2) 666 804.

2.2. *Isolation of the PSI complex*

Plants were harvested and thylakoids were isolated as described [17]. The PSI complex was extracted from the thylakoids with 1% dodecyl-β-D-maltoside and separated by sucrose density gradients (16 h centrifugation in 35,000 rpm in sw41 rotor) [18].

2.3. *In vitro transcription/translation*

The gene coding for the precursor of subunit II (PsaD), which was cloned in a Bluescript vector, was transcribed in vitro. The resulting mRNA was then translated in a wheat germ extract in the presence of [^{35}S]methionine [17].

2.4. *Integration and processing assays*

Assembly of the labeled precursor into the PSI complex and its processing were assayed at 25°C for 30 min in the presence of 10 mM MgATP and 10 mM methionine as described [17]. The protein samples were analyzed on SDS-PAGE [19].

3. RESULTS AND DISCUSSION

In a previous study the precursor of photosystem I, subunit II (PsaD), was shown to integrate exclusively into the pigmented PSI complex [17]. Here we examined the processing of the assembled precursor to its mature form within the isolated complex. Fig. 1 indicates that the precursor that integrated into the PSI complex could be processed by the stromal peptidase to form the mature subunit II. Thylakoids were incubated with the translation products, i.e. the labeled precursor of subunit II [17]. Following the insertion reaction and NaBr wash, the PSI complex was extracted from the thylakoids with 1% dodecyl-β-D-maltoside, and purified on a 5–30% sucrose gradient. The PSI-containing fractions were collected, loaded on a DEAE–cellulose column, and washed with 25 mM MES–NaOH, pH 6.5, and 0.03% dodecyl-β-D-maltoside. The pure complex was eluted with 25 mM $MgSO_4$ (in the same buffer), and concentrated to about 0.5 mg chl/ml in Amicon Centricons (Fig. 1, lane 2). Exposure of this complex to the stromal fraction (containing about 30 μg protein) for 30 min in 25°C resulted in the processing of pre-subunit II to form the mature form (Fig. 1, lane 3).

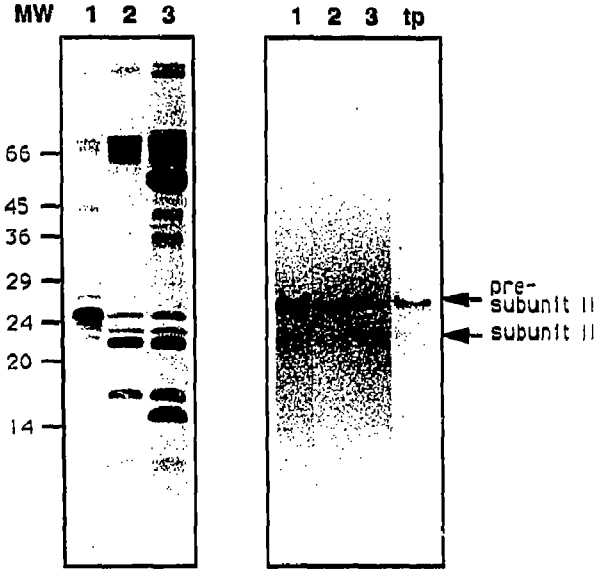

Fig. 1. The precursor of subunit II inserted into thylakoids can be correctly processed following the isolation of the PSI complex. The translation products (the labeled precursor of PSI subunit II) were incubated with isolated thylakoids as described in section 2 and in [17]. The thylakoids were then treated for 20 min with 2 M NaBr to remove unspecific association of the precursor, and a sample (equivalent to 5 μg chl) was analyzed by SDS-PAGE (lane 1). The most abundant proteins in this sample were the LHCII apoproteins (in the range of 25–26 kDa). The remaining thylakoids recovered from the salt-wash were dissociated with 1% dodecyl-β-D-maltoside and loaded on a sucrose density gradient. The PSI-containing fraction (equivalent to 10 μg chl) (lane 2) was incubated for 30 min at 25°C with the chloroplast stromal fraction (30 μg protein) (lane 3). Samples were analyzed by SDS-PAGE. Left panel, stained gel; right panel, fluorography. Note that under the electrophoretic conditions used, the translation products (tp) migrated with an apparent molecular weight of about 26 kDa.

Fig. 2. Insertion and processing of pre-subunit II to its mature form in the isolated PSI complex. Isolated PSI complexes were obtained by detergent extraction and sucrose density gradient, as described in section 2. The isolated complexes were incubated with the in vitro synthesized precursor of subunit II and re-purified on a second 5–20% sucrose gradient. The re-purified PSI complex (equivalent to 10 μg chl) was incubated with the stromal fraction purified from the chloroplasts (30 μg protein) for 30 min at 25°C. Samples were then analyzed by SDS-PAGE. The gel was stained (upper panel), fluorographed and exposed to X-ray film (lower panel). Lane 1, control (the re-purified PSI complex); lane 2, the complex after exposure to the stromal fraction.

In order to further characterize the specificity of the integration we isolated the PSI complex and then examined whether the precursor of subunit II was capable of integrating into this isolated complex. Extraction of the

PSI complex from the thylakoids and purification were followed by incubation of the translation products with the purified complex for 30 min at 25°C. In order to separate the PSI complex from the translation products which failed to associate with it, centrifugation was repeated on a 5–20% sucrose gradient (5 h at 45,000 rpm in sw50 rotor). Fractions of about 0.5 ml were collected, and aliquots were analyzed on SDS-PAGE. Most of the labeled pre-subunit II was detected in the re-purified PSI complex (Fig. 2, lane 1). Some labeled proteins probably derived from non-specific labeling of the wheat germ proteins, and some free precursor was also found in the lower or uppermost fractions of the gradient, respectively. The PSI complex could be re-purified on a DEAE–cellulose column, instead of the second sucrose gradient. Also, in this procedure the labeled pre-subunit II associated with the PSI-containing fractions eluted from the column (data not shown).

Incubation of the re-purified PSI complex with the stromal fraction, as described above, yielded processing of the precursor to its mature form (Fig. 2, lane 2).

These results imply that pre-subunit II does integrate and assemble into the isolated PSI complex. They agree well with results obtained with the reconstitution system in which the PsaD and PsaC proteins were reconstituted into the CCI-core proteins [12]. However, it should be noted that our results were obtained with holo-PSI, which contained *all* the different subunits of the complex. The fact that pre-subunit II could assemble into the holo-PSI suggests that an exchange occurs between the newly integrated subunit and an already existing one. It should be noted that in our system it is the precursor form that assembled into the complex, while Li and co-workers used a cyanobacterial subunit II, which lacks a transit sequence.

The results also indicate that integration of the precursor of subunit II into the PSI complex can occur in isolated complex outside the thylakoid membrane. Information for the assembly of subunit II lies within the PSI proteins independently of the surrounding membrane. The fact that pre-subunit II can properly integrate into the PSI complex implies that no other protein (beside PSI components) is involved in the integration process. All considered, we conclude that the binding site for subunit II is presumably composed of domains in the two high molecular weight subunits, I_a and I_b, together with subunit IV (PsaE) and subunit VII (PsaC) [4,9,12]. The results also prove our previous observation that the precursor form of subunit II is capable of integrating into the PSI complex [17].

The observations that processing of pre-subunit II can occur after integration into the isolated complex imply that the precursor assembles into the complex in a way that its N-terminal transit peptide is accessible to a correct cleavage by the stromal peptidase.

The ability of the precursor of subunit II to bind to the complex after its extraction from the membrane with detergents and to undergo processing in isolated complexes, suggests that the native conformation of both the entire complex as well as the newly integrated pre- subunit II is kept. Hence, such in vitro systems may be useful for studies of integration and maturation of different polypeptide subunits in other membranal complexes as well.

Acknowledgements: This work was supported by BSF grants (No. 87-00049 and 88-00360), and by a research grant from Charles H. Revson Foundation (No. 828/88). We wish to thank Prof. J. Lorch for helpful discussions and suggestions.

REFERENCES

[1] Mullet, J.E., Burke, J.J. and Arntzen, C.J. (1980) Plant Physiol. 65, 814–822.
[2] Thornber, J.P. (1986) in: Encyclopedia of Plant Physiology, vol. 19 (New Series) (Staehelin, L.A. and Arntzen, C.J. eds.) pp. 98–142, Springer-Verlag, Berlin.
[3] Almog, O., Shoham, G. and Nechushtai, N. (1992) in: The Photosystems: Structure, Function and Molecular Biology (Barber, J. ed.) pp. 443–470, Elsevier, Amsterdam.
[4] Golbeck, H.J. and Bryant, A.D. (1991) in: Current Topics in Bioenergetics, vol. 16 (Lee, C.P. ed.) pp. 83–177, Academic Press, New York.
[5] Lagoutte, B. (1988) FEBS Lett. 232, 275–280.
[6] Munch, S., Ljungberg, U., Steppuhn, J. Schneiderbauer, A., Nechushtai, R., Beyreuther, K. and Herrmann, R.G. (1988) Curr. Genet. 14, 511–518.
[7] von Heijne, G., Steppuhn, J. and Herrmann, R.G. (1989) Eur. J. Biochem. 180, 535–545.
[8] Ortiz, W., Lam, E., Chollar, S., Munt, D. and Malkin, R. (1985) Plant Physiol. 77, 389–397.
[9] Oh-oka, H., Takahashi, Y. and Matsubara, H. (1989) Plant Cell Physiol. 30, 869–875.
[10] Zanetti, G. and Merati, G. (1987) Eur. J. Biochem. 169, 143–146.
[11] Zilber, A. and Malkin, R. (1988) Plant Physiol. 88, 810–814.
[12] Li, N., Zhao, J., Warren, P.V., Warden, J.T., Bryant, D.A. and Golbeck, J.H. (1991) Biochemistry 30, 7863–7872.
[13] Nechushtai, R. and Nelson, N. (1985) Plant Mol. Biol. 4, 377–384.
[14] Herrmann, R.G., Westhoff, P., Alt, J., Tittgen, J. and Nelson, N. (1985) in: Molecular Form and Function of the Plant Genome (van Vloten-Doting, L., Groot, G.S.P. and Hall, T.C. eds.) pp. 233–256, Plenum, Amsterdam.
[15] Nechushtai, R. and Nelson, N. (1981) J. Biol. Chem. 256, 11624–11628.
[16] Chitnis, P.R., Reilly, P.A. and Nelson, N. (1989) J. Biol. Chem. 264, 18381–18385.
[17] Cohen, Y., Steppuhn, J., Herrmann, R., Yalovsky, S. and Nechushtai, R. (1992) EMBO J. 11, 79–85.
[18] Nechushtai, R., Peterson, C.C. and Thornber, J.P. (1987) in: Progress in Photosynthesis Research, vol. II (Biggins, J. ed.), pp. 1.41–1.44, Nijhoff, Dordrecht.
[19] Schagger, H. and von Jago, G. (1987) Anal. Biochem. 166, 368–379.