S23-004

The assembly of the peripheral PsaD subunit into the photosystem I complex of different organisms

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Keywords: photosystem I, assembly, specificity of recognition.

Introduction

Photosystem I (PSI) is a multi-subunit photosynthetic complex present in the thylakoid membranes of organisms performing oxygenic photosynthesis. In cyanobacteria, PSI consists of 12 subunits of which nine are integral membrane proteins (PsaA, PsaB, PsaF, PsaI-PsaM and PsaX) and three (PsaC, PsaD, PsaE) are membrane extrinsic subunits [Jordan et al., 2001]. In green algae and higher plants, PSI comprises 13 subunits of which eight are nuclear encoded. As in cyanobacteria, PsaC, PsaD and PsaE are peripheral stromal-facing proteins. The remaining 10 subunits are intrinsic membrane proteins (PsaA-PsaB, PsaF-PsaL and PsaN). PSI catalyzes the oxidation of reduced plastocyanin or cytochrome c_6 and the reduction of ferredoxin or flavodoxin. Although PSI carries out the same function in all oxygenic photosynthetic organisms, PSI of cyanobacteria differs from that of algae and plants in the organization and content of the various subunits [Golbeck 1992]. While in green algae and plants PSI exists as a monomer only, the cyanobacterial PSI appears both in monomeric and trimeric forms. PsaD is a key subunit in the assembly, stability and functionality of PSI. The assembly pathway of PsaD into the thylakoid membranes requires neither ATP nor the presence of a stromal component [Cohen et al., 1992a]. Recently, we have shown that a newly synthesized PsaD is able to assemble into a fully-assembled PSI by replacing the native PsaD subunit.

The present study aims at characterizing the ability of recombinant PsaD of the thermophilic cyanobacteria *Mastigocladus laminosus* to assemble into PSI of the green alga *Chlamydomonas reinhardtii* and into that of pea *vis-a-vis* the ability of recombinant spinach PsaD to assemble into PSI of *M. laminosus* and *C. reinhardtii.*

Materials and Methods

Over-expression and purification of PsaD

The mature *psaD* gene of spinach and the *psaD* gene of *M. laminosus* were cloned into the pET20b and pET21b(+) [Jin et al., 1999] vectors that encode a six histidines tag at the C-terminus of the proteins. Over-expression and purification of the recombinant proteins were performed as described in [Lushy et al., 2000].

Photosynthetic material

Wild type *C. reinhardtii* cells were grown on a TAP medium, at 25^{o}C under dim light (40μE/m^2/sec). Pea seedlings (Pisum sativum L. cv. Alaska) were grown as described in [Cohen et al., 1992a]. *M. laminosus* cells were grown as described in [Lushy et al., 2000].

PSI isolation

PSI of pea and *C. reinhardtii* were isolated as described in [Cohen et al., 1992b]. PSI of *M. laminosus* was isolated as described in [Lushy et al., 2000].

Integration/assembly reaction

Isolated PSI complexes containing 40 μg Chlorophyll were incubated with 5 μg homogeneous recombinant PsaD protein for 10 minutes at 4°C. Following incubation, the reaction mixture was loaded on a 5-30% sucrose gradient and centrifuged using a SW50 rotor for 4.5 hours at 45,000 rpm. Fractions of 0.5ml were collected and analyzed by western blot using anti-his antibodies.

Results and discussion

It was recently shown that recombinant PsaD of spinach and *M. laminosus* were able to assemble into isolated PSI of pea and *M. laminosus* respectively, via an exchange mechanism. To further characterize this assembly, both proteins were tested for their ability to assemble into PSI that was isolated from organisms of different origin.

Spinach PsaD was incubated with PSI isolated from *C. reinhardtii*. The reaction mixture was then separated on a sucrose gradient to differentiate between PSI-assembled PsaD and free PsaD that did not integrate into the complex. Analysis of the different gradient fractions by western blot with antibodies raised against his-tag, showed spinach PsaD to be present within the fractions containing PSI of *C. reinhardti* (Fig. 1A). Incubation of the protein with PSI isolated from *M. laminosus,* indicated that most of it integrated properly into the PSI complex (Fig. 1B).

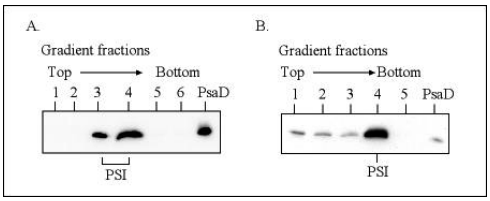

Figure 1. The recombinant spinach PsaD assembled in a stable manner into PSI of *C. reinhardtii*, and *M. **laminosus*.** Isolated spinach PsaD was incubated with purified PSI complex. Following assembly, the reaction mixture was chromatographed on a sucrose gradient and its different fractions were collected and analyzed by Western blot using anti-his antibodies. Panel A: Assembly reaction of spinach PsaD into PSI of *C. reinhardtii*. Panel B: Assembly reaction of spinach PsaD into PSI of *M. laminosus.*

The assembly of the procaryotic recombinant PsaD of *M. laminosus* into eucaryotic PSI isolated from *C. reinhardtii* and pea was carried out similarly. Figure 2A shows that following incubation with *C. reinhardtii* PSI, most of the PsaD of *M. laminosus* co-migrated with the fractions containing PSI of *C. reinhardtii*. Figure 2B shows that PsaD of *M. laminosus* was absent from the fraction containing PSI of pea.

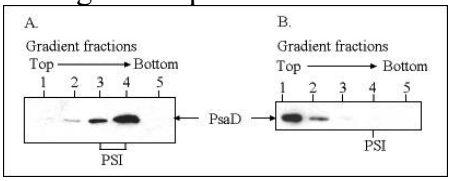

Figure 2. The recombinant *M. laminosus* PsaD assembled into PSI of *C. reinhardtii* but could not assemble in a stable manner into pea PSI. Assembly reaction of *M. laminosus* PsaD into isolated PSI and Western blot analysis were carried out as described in Figure 1. Panel A: Incubation of *M. laminosus* PsaD with PSI of *C.reinhardtii*. Panel B: Incubation of *M. laminosus* PsaD with PSI of pea.

These findings, obtained by *in vitro* assembly studies, indicate that the determinants essential for the recognition and stable assembly of PsaD within the PSI complex remained conserved among cyanobacteria and green algae. However, the evolutionary distance between plants and cyanobacteria probably denied the identification of the cyanobacterial PsaD by the plant PSI, though the plant PsaD could still be recognized by the cyanobacterial PSI. This probably indicates that PsaD bears special identity domains/residues that are being recognized by specific determinants in the PSI complex, with which they interact. The mutual identification of the PSI complex and the individual PsaD subunit is necessary for the interaction between PsaD and PSI in order that a stable assembly of the subunit within the complex can be enabled.

The recent structural model of PSI of the cyanobacteria *S. elongatus* [Jordan, 2001] shows that PsaD interacts with several subunits of the PSI complex. PsaA, PsaB, PsaC, PsaE and PsaL are in contact with PsaD via residues that are scattered throughout the PsaD protein (Fig. 3). In an attempt to find out whether the residues that take part in the interaction of PsaD with its neighbours are conserved, the sequences of PsaD of *Spinacia oleracea* (spinach*)*, *C. reinhardtii, M. laminosus* and *S. elongatus* were compared.

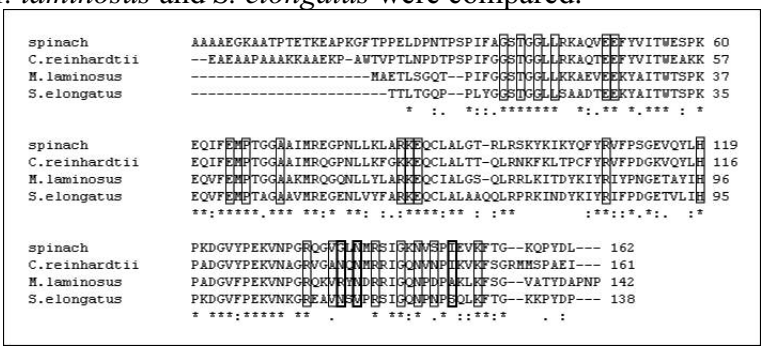

Figure 3. Sequence alignment of mature PsaD of spinach, *C. reinhardtii M. laminosus* and ***S. elongatus***. The 4 sequences were aligned using Clustal W (1.81) software. Shared identity: spinach:*C. reinhardtii*-63%, spinach:*M. laminosus*-59%, spinach:*S. elongatus*-57%,*C. reinhardtii*:*M. laminosus*-54%, *C. reinhardtii*:*S. elongatus*-52%, *M. laminosus*:*S. elongatus*-68%. Amino acids that interact with other PSI subunits are marked in squares [Jordan 2001]. Bold squares indicate those residues that are not conserved.

* identical or conserved residues. : conserved substitutions. . semi-conserved substitutions.

Figure 3 shows that the four sequences share over 50% homology. The vast majority of the residues of PsaD that were found to interact with the different subunits of PSI [Jordan, 2001] are conserved among the four organisms. Yet, three exceptions are located at the C-terminus of PsaD. (Figure 3, bold squares). In *S. elongatus,* these residues were shown to bind PsaC (Asn 113, Val 115) and PsaB (Ser 126). Among these residues, the only one that is not conserved among spinach and *C. reinhardtii* PsaD as compared with *M. laminosus* PsaD, is at position 113 of *S. elongatus.* At this position, spinach PsaD has a Gly while *C. reinhardtii* PsaD possesses an Asn and *M. laminosus* PsaD has an Arg residue. The replacement of Gly by an Arg is much less conserved than the replacement of Asn by an Arg. In spinach PsaD the presence of Gly probably enables a flexibility of the C-terminal loop of PsaD, which is not possible in the presence of Arg. In addition, the interactions that Asn and Arg are able to form with other amino-acids are similar, while this is not the case with Gly. This may explain the inability of *M. laminosus* PsaD to assemble properly into spinach PSI while it can assemble into *C. reinhardtii* PSI. It is of note, that the differences at the C-terminus of PsaD involve amino-acids that are essential to the interaction of PsaD with the core subunit PsaB and with the neighbour of PsaD in the stromal ridge, namely, PsaC. This may stress the importance of these subunits for the correct assembly of PsaD within PSI. Modelling of the structure of PsaD of spinach, *M. laminosus* and *C. reinhardtii,* using the coordinates of the structure of S.

elongatus PSI, while considering the interaction with PsaB and PsaC subunits, should help us better understand the phyla-specific assembly of PsaD.

Acknowledgments

We thank Dr. P.R. Chitnis from Iowa State University for the *E. coli* cells expressing *M. laminosus* PsaD. Special thanks to Dr. Petra Fromme from the Technisch Universitat of Berlin, for valuable data on the structure of PsaD.
This work was given the support of the DA'AT consortium of the Magnet foundation of the Office of the Chief Scientist of the Ministry of Industry and Trade of Israel.

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