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Purification and crystallization of Photosystem I complex from a phycobilisome-less mutant of the cyanobacterium Synechococcus PCC 7002

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Abstract

An active photosystem (PS I) complex was isolated from a phycobilisome-less mutant of the mesophilic cyanobacterium Synechococcus PCC 7002 by a mild procedure. Purification of PS I was achieved using a sucrose density gradient and an isoelectric focussing subsequent to the extraction of PS I from thylakoids with dodecyl-β-maltoside. Electron microscopy and gel filtration HPLC suggested that the isolated complex represents a trimeric form of PS I. The trimeric form was resistant to pH or detergent exchange. A ‘molecular weight’ of 690 kDa to 760 kDa has been determined for the complex by gel filtration HPLC in several detergents or mixtures of detergents.

The PSI complex contains the polypeptides of the psaA, psaB, psaC, psaD, psaE, psaL gene products and two small polypeptides as determined by SDS-PAGE and N-terminal sequencing; its antenna size is 77 ± 2 Chl a/P700. The full set of Fe–S clusters (F_A, F_B and F_X) was observed by EPR-spectroscopy. A preliminary characterization of crystals obtained from this preparation was carried out using SDS–PAGE, optical and EPR spectroscopy.

Abbreviations: BA – benzamidine; CAS – 6-amino-n-caproic acid; C₆-G – octyl-β-D-glucopyranoside; C₁₂-M – lauryl-β-D-maltoside; C₁₀-M – decyl-β-D-maltoside; C₉-TG – octyl-β-D-thioglucoside; Chl a – chlorophyll a; EPR – electron paramagnetic resonance; F_A, F_B, F_X – iron–sulfur centers; HPLC – high performance liquid chromatography; kDa – kilodalton(s); LDAO – lauryldimethylamine oxide; MES – 2-(N-morpholino)ethanesulfonic acid; PS I – Photosystem I; PS II – Photosystem II; P₇₅₀ – primary electron donor; SB12 – sulfobetain 12; SDS–PAGE – sodium dodecyl sulfate polyacrylamide gel electrophoresis; Tris – tris(hydroxymethyl)-aminomethane

Introduction

Photosystem I (PS I) of plants and eukaryotic algae is a membrane protein complex which catalyses the light-driven electron transport from reduced plastocyanin to oxidized ferredoxin. The cyanobacterial PS I is similar in structure, function and molecular aspects to the PS I of the chloroplasts. PS I consists of at least 10 polypeptides. Two of them are membrane-spanning proteins with a molecular weight of 83 kDa (psaA and psaB gene products). In SDS–
PAGE, these two polypeptides migrate as a diffuse band in the 50–70 kDa region (Fish et al. 1985). The sequences of these gene products have been reported for several plants and for *Synechococcus* PCC 7002 which was formerly called *Agnemenellum quadruplicatum* (for a review see Golbeck and Bryant 1991). Both polypeptides bind a large number (60–90) of light-harvesting Chl a molecules and several carotenoids. Additionally, the primary electron donor P$_{700}$, the primary electron acceptor A$_0$, the intermediate electron acceptor A$_1$ and the iron–sulfur center F$_{X}$ are bound. P$_{700}$ seems to be a Chl a dimer (Ikegami and Itoh 1988, Rutherford and Setif 1990), A$_0$ was shown to be a Chl a monomer (Wasielewski et al. 1987). Two quinone molecules seem to be present (Takahashi et al. 1985, Schoeder and Lockau 1986). F$_{X}$ is proposed to be a 4Fe–4S cluster (Golbeck 1987, Petrouleas et al. 1990). It has been suggested that this e$^{-}$-acceptor binds as a bridging cluster to the two large polypeptides, using two cysteines of each polypeptide to ligand the 4Fe–4S cluster (Golbeck 1987).

PSI contains six to eight small polypeptides in the molecular weight region of 4–20 kDa. One of these, the psaC gene product, binds the two terminal electron acceptors F$_{A}$ and F$_{B}$ (Oh-oka et al. 1987) both of which are 4Fe–4S clusters. The other polypeptides are members of three topologically diverse groups. PsaD, psaE and psaH (only in plants) gene products are located on the stromal side, the psaF gene product on the luminal side. The psaI, psaJ, psaK and psaL gene products are very hydrophobic and predicted to form transmembrane α-helices.

PSI complexes from thermophilic and mesophilic cyanobacteria have been purified and crystallized (Ford et al. 1987, Witt et al. 1987, Reily and Nelson 1988, Shoham et al. 1989). Recently, diffraction to 4 Å resolution was reported for crystals of the trimeric form of PSI from a thermophilic *Synechococcus* sp. (Witt et al. 1988) and to 5.5 Å for crystals of a monomeric PSI from the thermophilic cyanobacterium *Mastigocladus laminosus* (Almog et al. 1991). Both the trimeric and monomeric complexes have been crystallized, but which form is present in the native cyanobacterial membrane is an open question (Ford and Holzenburg 1988, Hladík and Sofrová 1991).

The quaternary structure of solubilized PSI has been investigated using electron microscopy (Boekema et al. 1987, Ford and Holzenburg 1988), non-denaturing electrophoresis (Takahashi et al. 1982, Ford 1987) and HPLC (Boekema et al. 1987, Witt et al. 1987, Rögner et al. 1990a,b). In the case of the thermophilic cyanobacteria, electron microscopy and subsequent image processing gave evidence for a trimeric organisation of the PSI complex (Ford and Holzenburg 1988, Boekema et al. 1989). The same method provided evidence for the monomeric nature of PSI particles purified from mesophilic (Williams et al. 1983) and from thermophilic cyanobacteria (Ford and Holzenburg 1988, Rögner et al. 1990a).

The trimeric PSI from a thermophilic *Synechococcus* sp. has a molecular weight of 670 ± 35 kDa (Rögner et al. 1990a). In contrast, a possible trimeric form of PSI from *Synechocystis* PCC 6803 has a molecular weight of 750 ± 50 kDa (Rögner et al. 1990b). This difference might be due to a different number of small polypeptides present in both complexes. The same difference exists in the molecular weights between the monomeric forms from both organisms. The monomeric PSI from the thermophilic *Synechococcus* sp. has a molecular weight of 235–260 kDa and from the mesophilic *Synechocystis* PCC 6803 of 300 ± 20 kDa.

Here we present the isolation and crystallization of PSI from phycobilisome free mutant (Δ apc cpc) of the mesophilic cyanobacterium *Synechococcus* PCC 7002. Optical and EPR-spectroscopy were used for the characterization of crystals obtained from this preparation.

**Materials and methods**

**Protein purification**

The *Synechococcus* PCC 7002 Δ apc cpc strain was a kind gift of Dr D. Bryant. The Δ apc cpc mutant is a spontaneous mutant which was isolated from the Δ apcAB mutant. While the Δ apcAB mutant (in which the allophycocyanin