

# Evidence for a trimeric organization of the photosystem I complex from the thermophilic cyanobacterium *Synechococcus* sp.

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A photosystem I (PS I) reaction center complex was isolated and purified from the cyanobacterium *Synechococcus* sp. The complex has a molecular mass of about 600 kDa and contains 120 Chl *a* molecules per photoactive Chl *a*<sub>1</sub> (P-700). Electron micrographs show that the PS I complex has the shape of a disk with a diameter of about 19 nm and a thickness of 6 nm. Computer analysis reveals that the complex is composed of three similar units.

Photosystem I; Electron microscopy; Structure analysis; (*Synechococcus*)

## 1. INTRODUCTION

PS I reaction center complexes have been isolated from cyanobacteria by a number of groups (see e.g. [1–4]). Most of these PS I preparations consist of at least two Chl-binding polypeptides of 60–70 kDa, as well as a number of low-molecular-mass subunits. Information on the structure and shape of PS I is rarely available. From freeze-etching electron microscopical studies it was concluded that the native PS I core complex has a diameter of 8 nm [3]. Further studies have

been hampered by the hydrophobic character of these proteins. Triton X-100-solubilized PS I complexes were described as ellipsoid particles of 18 × 8 nm [5].

Here, we report on the isolation and purification of PS I reaction center complexes from the cyanobacterium *Synechococcus* sp. The high homogeneity of the isolated complexes was used for a refined structural analysis.

## 2. MATERIALS AND METHODS

Cells and membranes of the thermophilic cyanobacterium *Synechococcus* sp. were grown and prepared as described in [6,7]. The main part of the PS II system was removed from the membranes with the zwitterionic detergent SB 12 (0.3–0.4%) [7]. The pellet containing the PS I system was extracted with 0.8–1.1% SB 12. The extract was layered on a 10–40% (w/w) sucrose gradient in citric buffer, pH 6.5 (0.025 M Na citrate, 0.01 M MgCl<sub>2</sub>, 0.02 M CaCl<sub>2</sub>, 0.5 M mannitol), and centrifuged in a Beckman SW 27 rotor

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**Abbreviations:** APC, allophycocyanin;  $\beta$ -DM,  $\beta$ -dodecyl-D-maltoside; Car, carotenoid; Chl, chlorophyll; HPLC, high-performance liquid chromatography; OGP, *n*-octyl- $\beta$ -D-glucopyranoside; PS, photosystem; SB, sulfobetain; SDS-PAGE, SDS-polyacrylamide gel electrophoresis

for 16 h at 23000 rpm (4°C). The resulting green band, called SG-1, was solubilized with 0.03%  $\beta$ -DM and supplied to an anion-exchange column (Mono Q HR5/5, Pharmacia) connected to a Knauer HPLC apparatus. The column was equilibrated with the described citric buffer (pH 5.3), containing 20% glycerol (instead of mannitol) and 0.03%  $\beta$ -DM. For elution a pH gradient (pH 5.3–3.0) with the citric buffer was applied.

Reaction center concentrations of the isolated PS I complex were determined by measuring the flash-induced absorbance changes at 703 nm [8], using a molecular extinction coefficient of  $64000 \text{ M}^{-1} \cdot \text{cm}^{-1}$ . Analysis of the protein components (by SDS-PAGE) and estimation of the molecular mass (by HPLC gel filtration) were carried out as outlined in [6].

Specimens for electron microscopy were prepared by the droplet method, using 1% uranyl acetate as a negative stain. For electron microscopy the purified PS I samples were diluted in buffer plus 1.2% OGP or 0.03%  $\beta$ -DM. Electron microscopy was carried out on a Philips EM 300 at 70000 magnification. Selected micrographs were digitized as described in [9]. The scanning step used was  $32 \mu\text{m}$ , corresponding to a pixel (image element) size of 0.47 nm on the specimen level. Image analysis was carried out within the framework of the IMAGIC software system [10] on a Vax 11/780 computer. Alignment procedures and multivariate statistical analysis were carried out as in [9,11].

### 3. RESULTS AND DISCUSSION

Fig.1A shows the elution profile of the SG-1 fraction (solubilized with 0.03%  $\beta$ -DM) on a Pharmacia Mono Q HR5/5 column. A yellow carotenoid fraction and a small APC fraction appear at pH 5.3 of the starting buffer. Upon application of a pH gradient, first a double peak of PS II around pH 4.7 (presumably monomeric and dimeric forms [6]) and then a single peak at pH 4.15, called SG-1/pK 4.15, are eluted. The fractions were identified by their absorption spectra

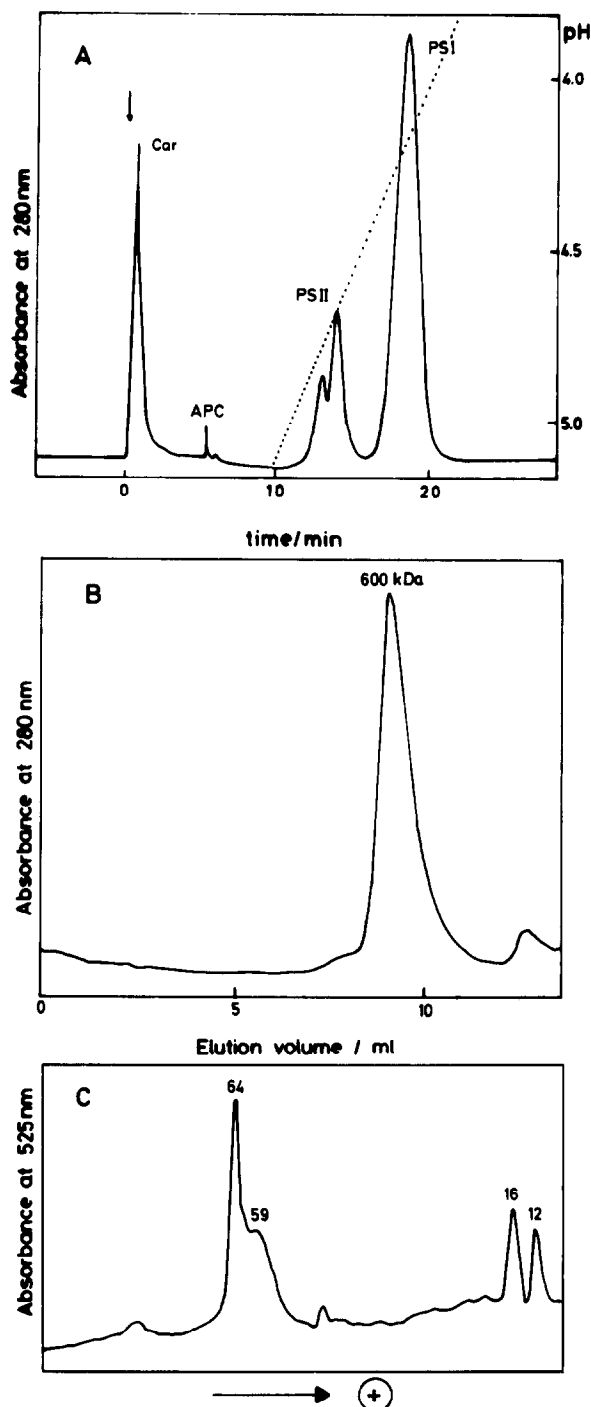


Fig.1. (A) Elution profile on a Pharmacia Mono Q HR5/5 column of the SG-1 material. In the range 0–10 min the pH was kept constant at pH 5.3. The gradient pH 5.3–3 was set up after 10 min. Flow rate

1 ml/min. (B) Elution profile on a TSK 4000 SW gel-filtration column of the isolated SG-1/pK 4.15 complex. (C) Densitometer scan of an SDS-PAGE pattern of the SG-1/pK 4.15 complex.

(not shown). PS II was characterized by a maximum at 673 nm. The SG-1/pK 4.15 fraction was characterized by an absorbance maximum at 679 nm and a pronounced shoulder near 710 nm, thereby being identified as PS I.

The purity of the PS I fraction was determined by HPLC gel filtration (fig.1B), resulting in a homogeneous fraction with a molecular mass of  $600 \pm 50$  kDa. Fig.1C shows the polypeptide composition of the PS I (SG-1/pK 4.15) fraction. Peaks around 64, 59, 16 and 12 kDa are observed which are in accordance with earlier data [1-4]. Activity measurements of the PS I fraction showed that one Chl  $a_1$  (P-700) is oxidized per ~120 chlorophylls in accordance with other intact PS I preparations [1-4].

For electron microscopy we used PS I particles solubilized in  $\beta$ -DM and OGP, respectively. For the change of detergent from  $\beta$ -DM to OGP, the  $\beta$ -DM solution was diluted below the CMC to 0.003%. After 2-3 days at 4°C, the protein aggregated and was carefully centrifuged. The pellet was re-solubilized with 1.2% OGP. In fig.2 one

observes with both detergents two typical projections: an almost circular top view and an elongated side view. Especially with the detergent  $\beta$ -DM, the particles tend to aggregate in the fashion of rolls. A similar behavior has also been observed with purified PS II complexes [6].

For a more refined analysis, a total of 840 top view projections of OGP-solubilized particles was selected from 19 digitized micrographs. The projections were brought into register by computer alignment procedures and were further analyzed by multivariate statistical analysis. Fig.3 shows the sum of all 840 projections. Three large monomers, which need not necessarily be identical, form a trimer. Each monomer shows two regions of higher density, possibly indicating two subunits. It should be pointed out, however, that fig.3 represents a mixture of top and bottom views of the photosystem, masking the exact shape of the subunits.

According to figs 2 and 3, the PS I trimer has a diameter of about 19 nm and a thickness of 6.0 nm. The dimensions of the monomer are

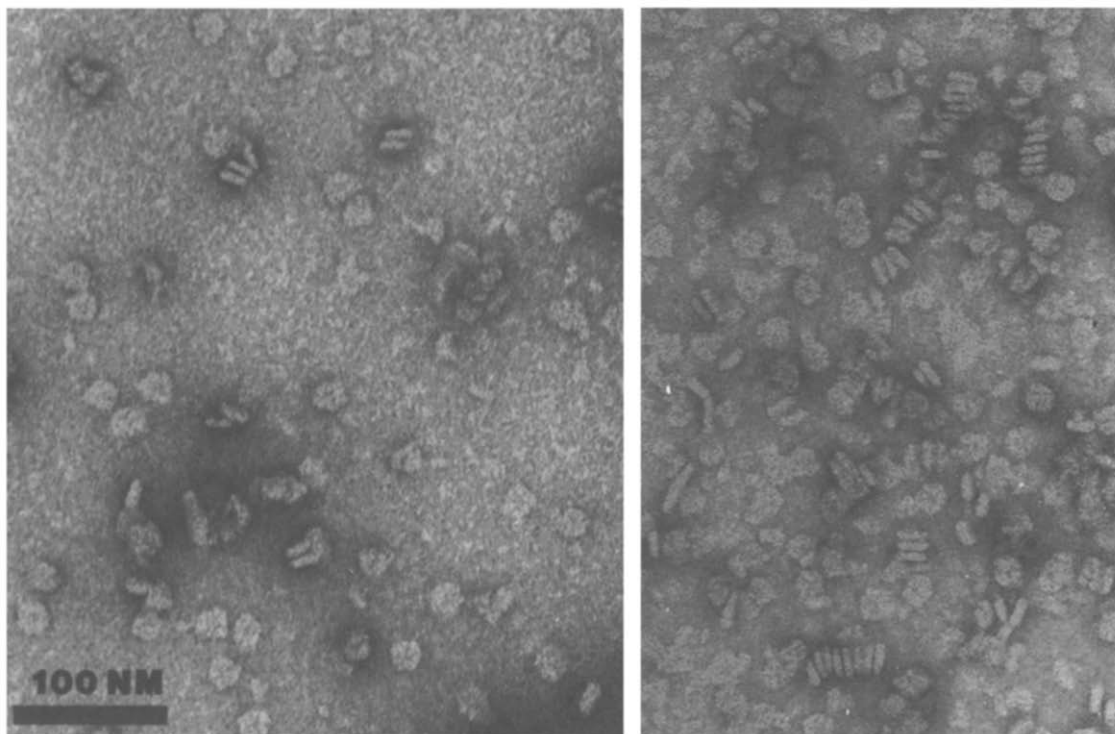


Fig.2. Electron micrographs of SG-1/pK 4.15 complexes prepared in 1.2% OGP (left) and 0.03%  $\beta$ -DM (right) and negatively stained with 1% uranyl acetate.

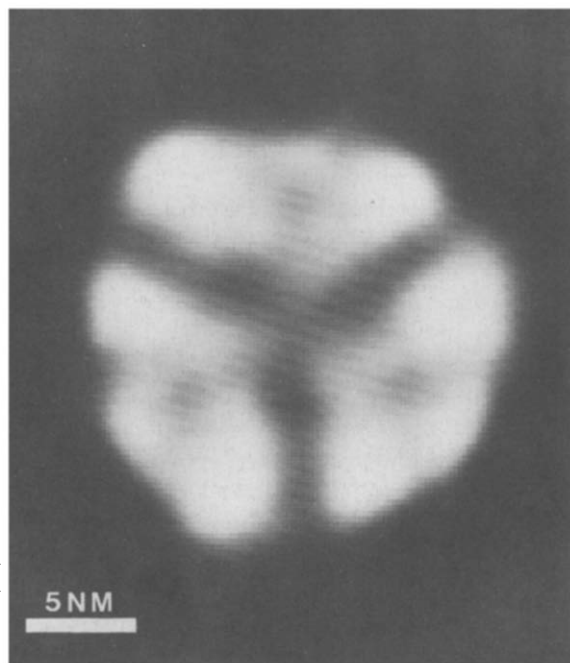


Fig.3. Average images of 840 top view projections of SG-1/pK 4.15 complexes in OGP.

roughly  $77 \times 6.0 \text{ nm}^3$ . Estimated from these dimensions and assuming a specific volume of  $\sim 2 \times 10^{-3} \text{ nm}^3 \cdot \text{Da}^{-1}$ , the molecular mass of one monomer is roughly 230 kDa. This value is in fair agreement with the gel-filtration measurement (fig.1B,  $600 \pm 50 \text{ kDa}$  for the trimer). The molecular mass of each monomer is sufficient to contain the 64 and 59 kDa proteins, 2–4 low-molecular-mass proteins and the pigments.

Our results give, for the first time, evidence for a trimeric organization of the PS I complex. Very likely, the trimer represents the *in vivo* organization of PS I, because we found only one PS I-containing fraction using anion-exchange chromatography (fig.1A) and also only one fraction by the gel-filtration chromatography (fig.1B).

The number of photoactive Chl  $a_1$  (P-700) centers in one trimer is an open question and could be either 1, 2 or 3. If chlorophyll is equally distributed within the trimer, one monomer would contain about 40, 80 or 120 chlorophyll molecules, respectively, because one Chl  $a_1$  can be oxidized per 120 chlorophylls (see above). An argument in favor of the possibility that the Chl  $a_1$  is localized

in only one monomer is that then the number of chlorophylls (40) as well as the mass of this Chl  $a_1$  reaction center are similar to those of the Chl  $a_{11}$  reaction center [6]. This is in line with recent reports of Vierling and Alberty [12] and of Lundell et al. [4] suggesting 4 or 6  $\sim 70 \text{ kDa}$  polypeptides per photoactive PS I reaction center.

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