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Size, shape and mass of the oxygen-evolving photosystem II complex from the thermophilic cyanobacterium *Synechococcus* sp.

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Two different, highly active O_2-evolving photosystem II complexes were purified from the cyanobacterium *Synechococcus* sp. in the presence of the non-ionic detergent β-dodecyl-D-maltoside. Both complexes are homogeneous and have molecular masses of approx. 300 and 500 kDa, respectively. By electron microscopy it was found that both complexes have the shape of an elliptical disk, with a thickness of about 6.5 nm and top view dimensions of 10.5 × 15.5 nm for the 300 kDa particle and 18.5 × 15 nm for the 500 kDa particle. It is concluded that the particles represent monomeric and dimeric forms of photosystem II.

Photosystem II; Electron microscopy; Structure; *(Synechococcus)*

1. INTRODUCTION

Recently, considerable progress was made in the isolation and purification of O_2-evolving PS II complexes from higher plants as well as from cyanobacteria [1–3]. Such preparations are valuable for functional investigations, determination of the protein composition and structural analysis. O_2-evolving complexes from cyanobacteria [4] have been found to be ideal subjects for flash-induced functional analysis of the PS II system (review [5]). The polypeptide composition of PS II from cyanobacteria has been well established in [6] and is very similar to higher plants [2,3] with the exception that the 17 and 23 kDa proteins are absent in cyanobacteria [7]. Less is known about the architecture, i.e., about the size, shape and structural organization of these complexes. When the mass of all components is summed up, a molecular mass of about 300 kDa is expected (see e.g. [6]). A value of about 300–400 kDa for non-oxygen evolving complexes was found by HPLC gel filtration [8], but a higher value of ~500 kDa was found for the O_2-evolving complex from *Synechococcus* [6]. From freeze-etch electron microscopy a diameter of about 8 nm was inferred for the PS II core complex [9,10].

In this contribution we report on a quantitative purification of highly active and homogeneous O_2-evolving PS II reaction center complexes from the cyanobacterium *Synechococcus* sp. and present

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Abbreviations: APC, allophycocyanin; β-DM, β-dodecyl-D-maltoside; Chl, chlorophyll; HPLÇ, high performance liquid chromatography; PC, phycocyanin; PS, photosystem; SDS-PAGE, SDS-polyacrylamide gel electrophoresis

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an estimation of the mass, size and shape of these systems.

2. MATERIALS AND METHODS

Cells of the thermophilic cyanobacterium *Synechococcus* sp. were grown in two 30 l glass columns at 57°C (details in [4,11]). Preparation of the membranes and extraction of the oxygen-evolving PS II complexes was done in principle according to the methods reported by Schatz and Witt [4]. For further purification, 2–4 ml of the extract was layered on a 24 ml of 10–40% (w/w) sucrose gradient in an MCM (20 mM Mes-NaOH, 10 mM $MgCl_2$, 20 mM $CaCl_2$) buffer, pH 6.5. The gradient was run in a Beckman SW 27 rotor for 16 h at 23000 rpm (4°C). Most of the Chl was found in a sharp green band in the lower part of the gradient (called the SG-1 fraction), clearly separated from PC and APC. The SG-1 fraction was dialyzed against MMCM (MCM plus 0.5 M mannitol), pH 6.5, solubilized with 0.045% β-DM (Calbiochem) and layered on a second sucrose gradient (20–40% sucrose in MMCM, pH 6.5, and 0.03–0.05% β-DM). Centrifugation was performed in a Beckman VTi 50 rotor at 50000 rpm for 16 h at 4°C. The resulting upper and lower green bands are called SG-2a and SG-2b, respectively. They are clearly separated from a carotenoid-containing band at the top of the gradient.

SDS-PAGE was carried out on 10–15% gradient polyacrylamide gels. The samples were incubated in 2.5% SDS for 10 min at 56°C, and protein bands were stained with Coomassie brilliant blue. Flash-induced oxygen evolution was measured with a zirconium-dioxide electrode as described in [12]. Reaction center concentrations were measured via flash-induced absorbance changes at 320 nm, indicating the reduction of the primary quinone acceptor Q_A [13]. A $\Delta\epsilon$ value of 12000 $M^{-1} \cdot cm^{-1}$ was used [14]. HPLC was performed on a Waters apparatus consisting of an injector U6K, pump 510, controller and detector M481. Size exclusion chromatography was done with a TSK 4000 SW column (Beckman), using a gel filtration calibration kit (Pharmacia). The flow medium consisted of MMCM, pH 6.5, supplemented with 0.03% β-DM (flow rate 0.5 ml/min). Electron microscopy was carried out on a Philips EM 300 at 70000 magnification. Specimens were prepared by the droplet method, using uranyl acetate as a negative stain.

Micrographs were digitized with a Datacopy model 610F electronic digitizing camera [15]. Projections were brought into register by computer alignment procedures based on correlation methods [16]. Image analysis was carried out within the framework of the IMAGIC software system [17] on a VAX 11/780 computer.

3. RESULTS AND DISCUSSION

Fig.1 shows the HPLC profiles of the SG-2a and SG-2b complexes, the only green complexes observed in the second sucrose gradient. Both appear to consist of very homogeneous fractions having molecular masses of approx. 300 and 500 kDa, respectively. Table 1 shows that both SG-2a and

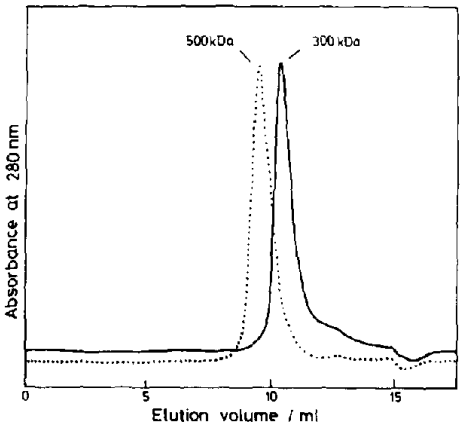

Fig.1. Elution profiles on the TSK 400 SW gel-filtration column of the isolated SG-2a (solid line) and SG-2b complex (dashed line).

Table 1

Average O_2 yield and amount of photo-reducible Q_A per chlorophyll in single turnover flashes in different PS II preparations

Preparation	1/4 O_2/Chl	Q_A/Chl
SB 12 extract	1/(70 ± 20)	1/(60 ± 20)
SG-1	1/(60 ± 10)	1/(50 ± 10)
SG-2a	1/(80 ± 30)	1/(45 ± 5)
SG-2b	1/(80 ± 30)	1/(45 ± 5)

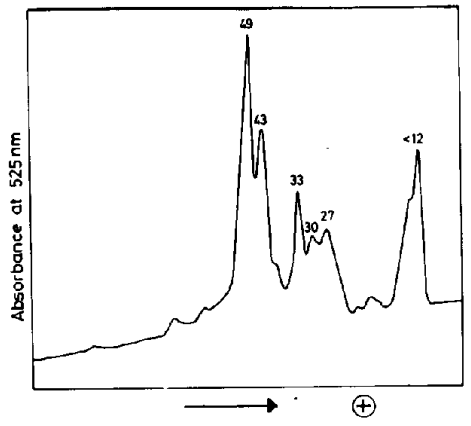

Fig.2. Densitometer scan of an SDS-PAGE pattern of the SG-2a preparation.

SG-2b are able to reduce 1 Q_A/45 Chl and both show considerable water-oxidizing activity. However, after separation of the inactive particles by an ion-exchange column, O_2 capacities of up to 1/4 O_2/50 Chl were obtained (this corresponds to more than 3000 μmol O_2/mg Chl per h).

Fig.2 shows the polypeptide composition of the SG-2a complex. The main bands correspond to proteins with apparent molecular masses of 49, 43, 33, 30 and 27 kDa. The band in the front at <12 kDa contains the apoprotein of cyt-*b*-559. This polypeptide composition is similar to that reported by Ohno et al. [6] and is indicative of a purified PS II reaction center complex (see also [2,3]). The same proteins were also found in the SG-2b complex but in some cases contamination by 19 and 17 kDa APC proteins was observed (not shown).

Fig.3 shows electron microscopic images of the SG-2a and SG-2b particles. Mainly two projections, a top and a side view, are visible. The particles frequently aggregate in such a way that the side view is seen as a repeating unit, especially in the case of the 500 kDa SG-2b fractions.

The dimensions are given in table 2. While the thickness (about 6.5 nm) as well as the length (about 15.5 nm) are similar for the 300 and 500 kDa fractions, the width is 10.5 nm for the 300 kDa complex but 18.5 nm for the 500 kDa one. 106 top view projections of the 300 kDa and 16 top views of the 500 kDa complex were aligned and summed up (see section 2). Unfortunately, top

Table 2

Dimensions of O_2-evolving 300 kDa and 500 kDa PS II complexes, measured from enlarged photographs

	300 kDa	500 kDa
Length	15.8 ± 0.8 nm	15.1 ± 1.5 nm
Width	10.5 ± 0.8 nm	18.5 ± 1.0 nm
Thickness	6.4 ± 0.7 nm	6.6 ± 0.6 nm

view projections of the 500 kDa complex are relatively rare. The result is shown in fig.4. The 500 kDa complex gives the impression of a dimeric structure.

In view of the molecular mass of the components of the PS II complex [6], we conclude that the 300 kDa SG-2a complex represents the monomeric form of the PS II system and the 500 kDa SG-2b complex a dimeric form. Mass and size of the dimer are not exactly twice the value of the monomer; this is understandable because the relative contribution of the boundary layer of detergents and possible fractions of lipids is expected to be smaller for larger particles. Very likely, the smallest dimension of the elliptical disks (6.5 nm) spans the membrane with a hydrophilic surface at the top and bottom of the disk. This attribute is supported by the stain accumulation at these surfaces visible between the aggregated units in fig.3. The existence of isolated, dimeric PS II particles has not been reported before. In principle, dimers could be caused by artificial aggregation of monomers during the preparation procedure. However, it is more likely, that the dimers represent the normal, functional PS II in cyanobacteria because twice as many active PS II reaction centers as phycobilisomes are present [18]; this might indicate that one phycobilisome is attached to one PS II dimer.

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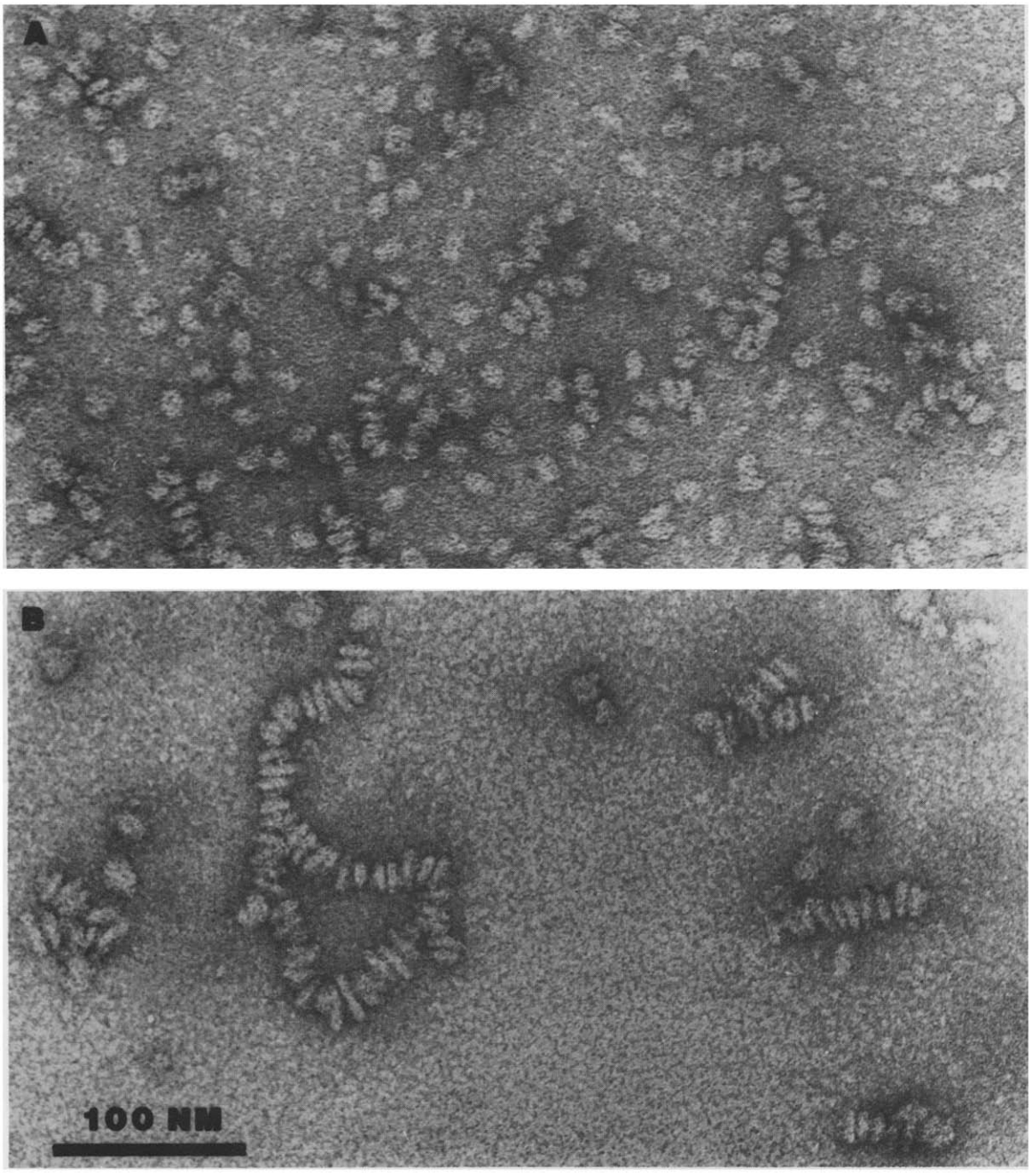

Fig.3. Electron micrographs of SG-2a (A) and SG-2b (B) PS II complexes, prepared in 0.15% β-DM and negatively stained with 1% uranyl acetate.

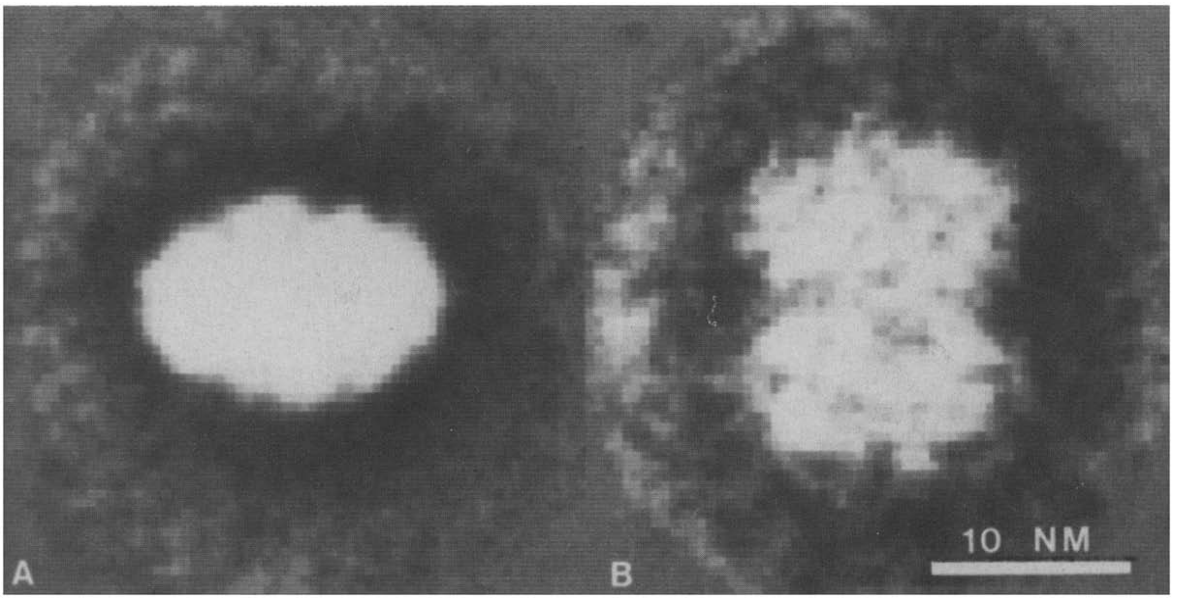

Fig.4. Averaged images of 106 top view projections of the SG-2a complex (A) and of 16 top views of the SG-2b complex (B).

REFERENCES

[1] Murphy, D.J. (1986) Biochim. Biophys. Acta 864, 33–94.
[2] Ghanotakis, D.F. and Yocum, C.F. (1986) FEBS Lett. 197, 244–248.
[3] Satoh, K. (1985) Photochem. Photobiol. 42, 845–853.
[4] Schatz, G.H. and Witt, H.T. (1984) Photobiochem. Photobiophys. 7, 1–4 and 77–89.
[5] Witt, H.T., Schlodder, E., Brettel, K. and Saygin, Ö. (1986) Ber. Bunsenges. Phys. Chem. 90, 1015–1024.
[6] Ohno, T., Satoh, K. and Katoh, S. (1986) Biochim. Biophys. Acta 852, 1–8.
[7] Stewart, A.C., Ljungberg, U., Åkerlund, H.-E. and Andersson, B. (1985) Biochim. Biophys. Acta 808, 353–362.
[8] DeVitry, C., Diner, B.A. and Lemoine, Y. (1987) in: Progress in Photosynth. Res. (Biggins, J. ed.) vol.II, pp.105–108, Martinus Nijhoff Publishers, Dordrecht, The Netherlands.
[9] Staehelin, L.A. (1986) in: Photosynthesis III, Photosynthetic Membranes and Light-Harvesting Systems (Staehelin, L.A. and Arntzen, C.J. eds) pp.1–84, Springer, Berlin.
[10] Zilinskas, B.A. and Greenwald, L.S. (1986) Photosynth. Res. 10, 7–35.
[11] Jüttner, F. (1982) Process Biochem. 17, 2–7.
[12] Meyer, B. (1987) Doctoral Thesis, TU Berlin.
[13] Stiehl, H.H. and Witt, H.T. (1968) Z. Naturforsch. 24b, 1588–1598.
[14] Van Gorkom, H.J. (1974) Biochim. Biophys. Acta 347, 439–442.
[15] Boekema, E.J., Berden, J.A. and Van Heel, M.G. (1986) Biochim. Biophys. Acta 851, 353–360.
[16] Van Heel, M.G. and Stöffler-Meilicke, M. (1985) EMBO J. 4, 2389–2395.
[17] Van Heel, M.G. and Keegstra, W. (1981) Ultramicroscopy 7, 113–130.
[18] Manodori, A. and Melis, A. (1985) FEBS Lett. 181, 79–82.