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Structural comparison of oxygen-evolving photosystem II core complexes from *Synechococcus elongatus* and *Spinacia oleracea*

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Introduction

Light-induced photosynthetic water cleavage under release of molecular oxygen and protons takes place on the lumenal side of thylakoid membranes within PS II from prochlophytes, cyanobacteria, algae and higher plants. This multisubunit complex consists of 17 to 30 different subunits in dependence of the organism. The minimum complex catalysing water splitting at a manganese containing active centre and plastoquinone reduction is designated as PS II core complex (PS II CC) (Debus, 1992). 3D-single crystals of oxygen evolving PS II CC were obtained from spinach and thermophilic cyanobacteria *Synechococcus elongatus* and *Synechococcus vulcanus*. These were shown to diffract synchrotron X-ray radiation to resolutions of 7, 4 and 3.8 Å, respectively (Adir et al., 1992; Shen and Kamiya (2000); Zouni et al. 2001). 2D-crystals of a reaction centre core complex from spinach were analysed by electron crystallography (Rhee et al., 1997). A first structural model at 3.8 Å resolution has been presented revealing the spatial organisation of the various cofactors of the photochemical reaction centre, its core antenna and the tetrameric manganese cluster (Zouni et al., 2001). Structural comparisons of PS II CC from cyanobacteria, green algae and plants were reported using electron microscopy and single particle analyses (Nield et al., 2000), STEM (Hasler et al., 1997) especially with respect to their forms and sizes. Here we report on the molecular composition of PS II CC from *Synechococcus elongatus* and spinach with special emphasis on the low molecular weight subunits. In addition we addressed the question under which experimental conditions PS II CC of both sources occur as monomers or dimers using gel permeation HPLC, static/dynamic light scattering (SLS/DLS) and analytical ultracentrifugation. The results will be discussed in the light of recent structural models for PS II CC.

Materials and Methods

ß-dodecylmaltoside (ß-DM) solubilised oxygen evolving PS II CC from either *Synechococcus elongatus* or spinach thylakoids were isolated and purified by procedures described in (Zouni et al. 1998, Irrgang et al. 1998). Gel permeation chromatography was carried out either using a TSK G 4000 SW (Beckman) or a Superose 6 FPLC column (Pharmacia) connected to a HPLC at flow rates of 0.8 or 0.5 ml/min. Proteins were detected at 280 nm. Phosphorylated proteins were screened on PVDF membranes with monoclonal/polyclonal antibodies directed against either P-Thr, P-Ser or P-Tyr (Zymed). $Cytb_{559}$ and $cytc_{550}$ were determined by absorbance difference spectroscopy (reduced-$K_3[Fe(CN)_6]$ oxidised form) (Shen et al. 1992,

Cramer and Whitmarsh, 1977). Samples were reduced either by hydroquinone ($cytb_{559}$ HP-form) or $Na_2S_2O_4$ (cytc550, $Cytb_{559}$ LP-form). An extinction coefficient of 15000 M^{-1} cm^{-1} was used for quantifying $cytb_{559}$ (Cramer and Whitmarsh, 1977). SLS/DLS measurements were carried out at 20 ^oC +/-0.2 ^oC using an attenuated frequency-doubled cw Nd-YAG laser (λ=532nm) in combination with an ALV/SP-81 spectrogoniometer equipped with an ALV/5000E digital autocorrelator. Matrix assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF-MS) was performed in a linear mode using a pulsed UV laser (N_2-laser, λ =337 nm, 3ns pulse width, Type RETOF-MS, Bruker-Franzen).

Results and Discussion

The protein compositions of the PS II CC from both organisms were analysed by silver-stained SDS/urea/polyacrylamide gels, western blotting in combination with specific antibodies, MALDI-TOF-MS and absorbance difference spectroscopy. The results obtained are summarised in Tab.1. 17 subunits were identified with certainty for the PS II CC of *Synechococcus elongatus* whereas 15 were detected for that of spinach. The cyanobacterial

protein	**method**			
	SDS/Urea/ PAGE	poly/monoclonal Abs western blotting	MALDI-TOF -MS	difference spectroscopy [red. - ox.]
	A/B	**A/B**	**A/B**	**A/B**
Psb A	+/+	+/+	-	-/-
Psb B	+/+	+/+	-	-/-
Psb C	+/+	n.d.	-	-/-
Psb D	+/+	+/+	-	-/-
Psb E	+/+	+/+	+/+	+/+
Psb F	+/+	n.d.	+/+	
Psb H	-/-	-/+	+/+	-/-
Psb I	-/+	n.d.	+/+	-/-
Psb J	-/-	n.d.	+/+	-/-
Psb K	-/-	n.d.	+/+	-/-
Psb L	-/-	n.d.	+/+	-/-
Psb M	-/-	n.d.	+/(+)	-/-
Psb N	-/-	n.d.	+/(+)	-/-
Psb O	+/+	+/+	+/+	-/-
Psb P	-/-	-/-	-/-	-/-
Psb Q	-/-	-/-	-/-	-/-
Psb R	-/-	-/-	-/-	-/-
Psb S	-/-	-/-	-/-	-/-
Psb T	-/-	n.d.	-/-	-/-
Psb U	+/-	n.d.	+/-	-/-
Psb V	+/-	n.d.	+/-	+/-
Psb W	-/+	-/+	-/+	-/-
Psb X	-/-	n.d.	+/+	-/-
Psb YA1	-/-	n.d.	-/-	-/-
Psb YA2	-/-	n.d.	-/-	-/-
Psb Z	-/-	n.d.	-/(+)	-/-

Tab.1 Identification of different protein subunits of PS II CC from *Synechococcus elongatus naegeli (A) and spinach (B) + = identified, - = not identified , n. d. determined*

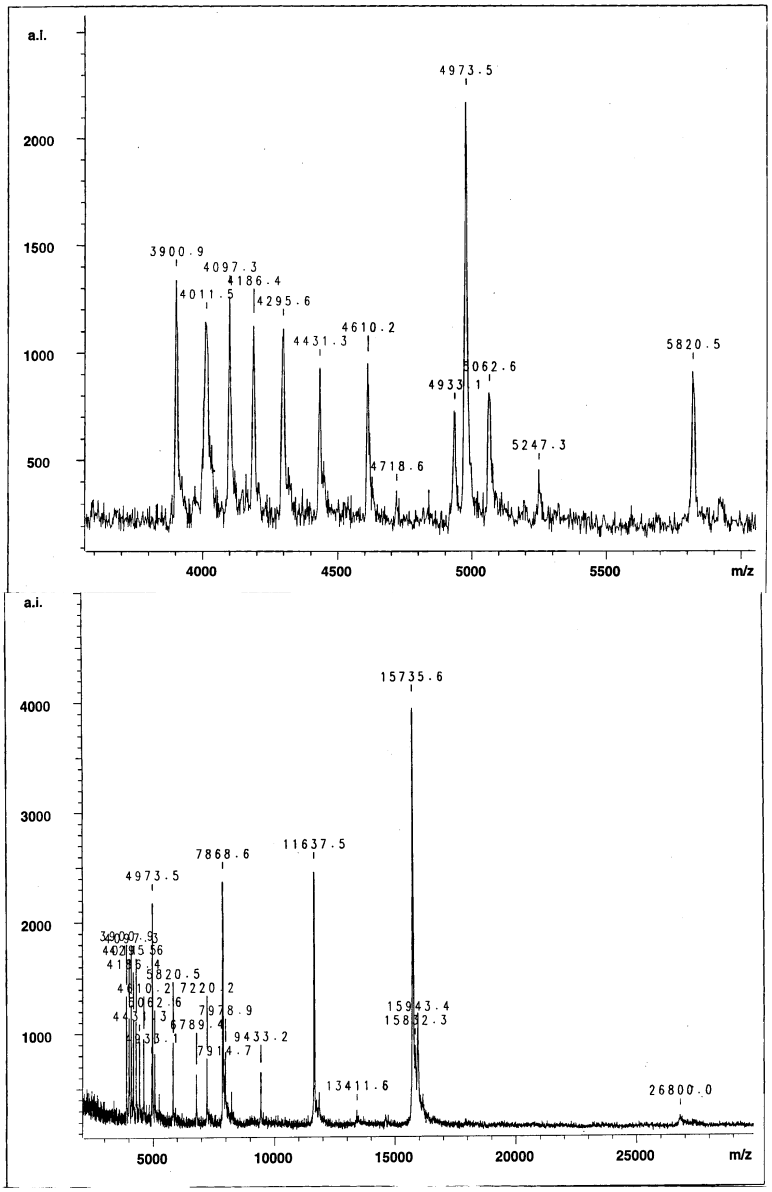

Fig. 1a MALDI-TOF-MS of low molecular mass polypeptides (upper panel) and **1b** of proteins up to 26800 amu [M+H$^+$]$^+$of PS II CC from *Synechococcus elongatus* (lower panel) in solution as well in 3D-single crystals.

complex consists of 14 integral membrane proteins with 32 α-membrane spanning helices and three extrinsic subunits, namely PsbO, PsbU and V. One copy of both cytb559 (HP) and cytc550 (PsbV) per P680 were determined by absorbance difference spectroscopy. These data do not agree with those recently reported (Zouni et al., 2001). The origin of this discrepancy has not yet been clarified. The spinach complex contains at least one more integral polypeptide (Psb W) which is not present in cyanobacterial PS II CC. Another subunit, which was until now unidentified in spinach, shows a mass of 6576 amu, and could be ascribed to the so-called PsbZ protein, formerly designated as Ycf 9 (Ruf et al 2000, Swiatek et al. 2001). Two subunits (PsbM and N) can only tentatively be ascribed to the spinach complex. Assuming that both polypeptides are stably associated with the complex, 34 α-helices should be present in spinach PS II CC excluding the spectroscopically determined second cytb559 (Irrgang et al, 1998). In contrast to the plant PS II CC, no phosphorylated polypeptide (P-Thr, P-Ser) could be detected in the cyanobacterial complex (data not shown). The apparent molecular mass of both PS II CC were analysed by gel permeation chromatography, SLS/DLS and analytical ultracentrifugation. HPLC analyses indicated 450 +/- 20 kDa for both ß-DM solubilised complexes. The diffusion coefficients D_z were obtained from the DLS autocorrelation function at protein concentrations of 0.2-2.0 mg/ml. The D_z for the cyanobacterial complex decreased linearly from the extrapolated value at c=0 mg/ml of 3.8 +/- 0.2 x10^{-7} cm^2s^{-1} to 2.1 +/- 0.2 x10^{-7} cm^2s^{-1}. This corresponds to hydrodynamic radii R_H = 5.02 +/-0.15 nm to R_H = 9.04 +/- 0.15 nm and apparent molecular masses of 4.26 +/-0.1 10^5 g/mol to 25+/- 0.1 10^5 g/mol. At low protein concentrations PS II CC seems to be monomeric whereas at higher concentrations larger aggregates of unknown composition and structure are formed (Fig. 2). Performing the same kind of experiments with the spinach CC revealed that the latter

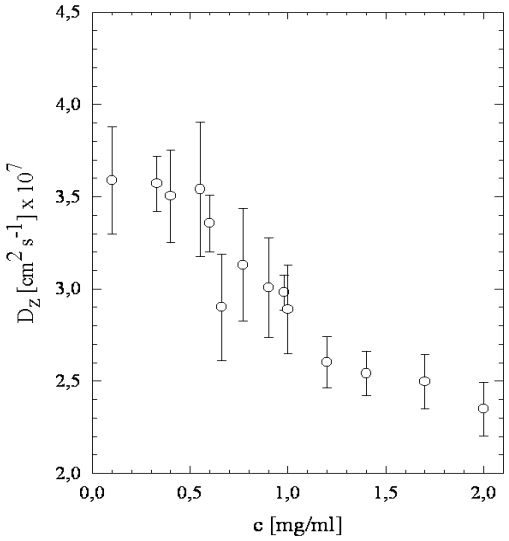

Fig. 2 Dependence of the apparent diffusion coefficient D_z on the protein concentration of PS II CC from *Synechococcus elongatus*

aggregates at even lower concentrations (0.2 mg/ml), very likely due to its higher hydrophobicity (data not shown). In summary these results indicate that both PS II complexes are monomeric at low protein concentrations (spinach < 0.2mg/ml, *Synechococcus elongatus* < 0.5 mg/ml). The same types of samples were recently investigated under comparable conditions in an analytical ultracentrifuge. Preliminary evaluation of these data shows a dimeric form for both PS II CC (MW ~700 +/-20) even at low protein concentrations. These results and the occurrence of dimers in the crystals of cyanobacterial PS II CC (Zouni et al, 2001) suggest that the experiments with HPLC and SLS/DLS should be considered with caveat. The reason is that (1) PS II CC were solubilised with detergent and their peaks in HPLC were calibrated against soluble proteins, and (2) SLS/DLS cannot reliably distinguish between monomers and dimers as the hydrodynamic radii (R_H) would differ only by about 20% for assumed spherical particles, barely above the limit of these methods**.**

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