force field doesn't contain force constants needed to describe the modified parts of the phosphonate analogs [9]. The completion was made on the base of ab initio calculations [3].

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1. E. Uhlmann and A. Peyman, *Chem. Rev.*, **90** (1990) 544-584.
2 D. Rejman, J. Snasel, R. Liboska, Z. Tocik, O. Paces, S. Kralikova, M. Rinnova, P. Kois, I. Rosenberg, *Nucleosides Nucleotides & Nucleic Acids*, **20** (2001) 819-823.
3. I. Barvík Jr., J. Štěpánek, J. Bok, *J. Biomol. Struct. Dyn.*, **19** (2002) 863-875.
4. I. Barvík Jr., J. Štěpánek, J. Bok, *Comp. Phys. Comm.*, **147** (2002) 158-161.
5. J. Hanuš, I. Barvík Jr., J. Štěpánek, P.-Y. Turpin, J. Bok, I. Rosenberg, M. Petrová, *Nucl. Acid Res.*, **29** (2001) 5182-5194.
6. I. Barvík Jr., J. Štěpánek, J. Bok, *Czech. J. Phys.*, **48** (1998) 409-415.
7. E. De Clercq, A. Holý and I. Rosenberg, *Antimicrob. Agents Chemother.*, **33** (1989) 185-191.
8. N. Atsumi, Y. Ueno, M. Kanazaki, S. Shuto and A. Matsuda, *Bioorg. & Med. Chem*, **10** (2002) 2933-2939.
9. W. D. Cornell et al., *J. Am. Chem. Soc.*, **117** (1995) 5179-5197.

FUNCTIONAL RECONSTITUTION OF PHOTOSYSTEM 2 INTO LIPOSOMES

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Most recent structural data on photosystem 2 (PS2), the first membrane protein complex in the photosynthetic electron transport chain, confirm that this complex exists as a functional dimer in the thylakoid membrane of cyanobacteria [1, 2]. Besides the membrane embedded part of this dimer with dimensions of 190 A x 100 A x 40 A, this complex also extends about 10 Ĺ out of the membrane in the stromal region and 55 A in the lumen; the latter is referred to as the oxygen evolving complex, harbouring the water-splitting site. Developing an approriate method to reconstitute dimeric PS2 into liposomes should finally help to answer the fundamental question concerning its structure-based function: Is a dimeric structure a prerequisite for optimal watersplitting activity (monomeric complexes, solubilized by detergent, are active, too, although at a lower level) and which is the impact of the lipid-phase-composition on the water-splitting activity? Here we present data on the orientation of reconstituted dimeric PS2 from the cyanobacterium *Thermosynechococcus elongatus* and also give indications for its activity within the liposomes and monomer-dimer distribution (by EM analysis).

1. A.Zouni, H.T. Witt, J. Kern, P. Fromme, N. Krauss, W. Saenger, & P. Orth, *Nature* **409** (2001) 739 - 743.
2. N. Kamiya & J.R. Shen, *Proc. Natl. Acad. Sci USA,* **100** (2003) 98-103.

ELECTRON MICROSCOPY AND SINGLE PARTICLE ANALYSIS OF PHOTOSYSTEM II FROM RED ALGA *PORPHYRIDIUM CRUENTUM*

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Photosystem II (PSII) is a multisubunit pigment-protein complex embedded in the thylakoid membranes of higher plants, algae and cyanobacteria [1-3]. It performs series of photochemical reactions resulting in the reduction of plastoquinone, the oxidation of water, and the formation of a transmembrane pH gradient. The essential components of the PSII complex are intrinsic membrane proteins that are almost identical between cyanobacteria and higher plants: they include the D1 and D2 reaction center proteins, chlorophyll *a*-binding proteins CP47 and CP43, and subunits of cytochrome *b-559* (cyt *b-559*) and several low-molecular weight proteins with unknown functions [3, 4].

In addition, there are extrinsic proteins associated with PSII, which play important roles in maintaining the function and stability of the oxygen-evolving complex [5]. As both cyanobacteria and higher plants contain 33-kDa extrinsic subunit they differ in composition of the other lumenal subunits. While higher plants and green algae contain the 23 and 16 kDa extrinsic subunits, in cyanobacteria, these proteins are replaced by another two proteins coded by *psbU* and *psbV* gene (cyt c_{550} and 12-kDa subunit) [6]. Red algal PSII complex has a 20 kDa protein in addition to the three cyanobacterial proteins [7]. Recently, structure of the PSII complex isolated from two cyanobacterial strains has been presented [8,9]. These models give the idea of the

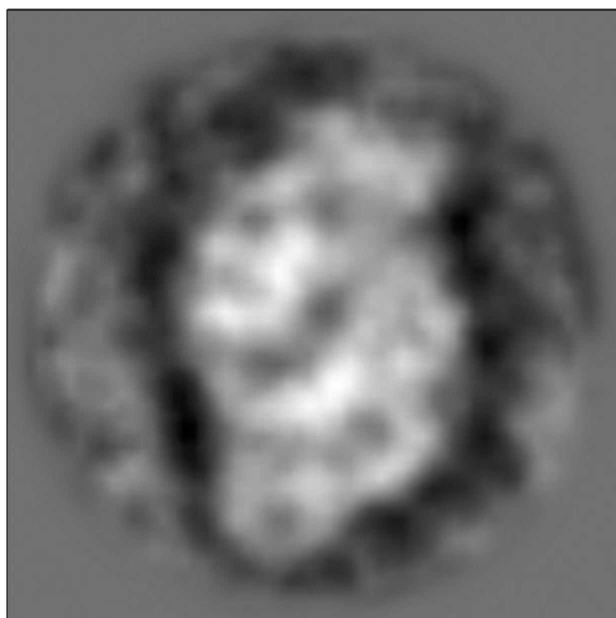

Fig. 1. Averaged projection of negatively-stained preparation of the dimeric PSII complex isolated from Porhyridium cruentum in its top-view projection (i.e. perpendicular view to the thylakoid membrane).

arrangement of the intrinsic and extrinsic subunits. However, a location of the 20 kDa extrinsic subunit within the red algal PSII is still unknown.

I this report we present preliminary results on locating the the 20 kDa extrinsic protein using a transmission electron microscopy and single particle image analysis of negatively-stained preparations of Photosystem II isolated from a red alga *Porhyridium cruentum*.

The PSII complex was isolated from thylakoid membranes from *P. cruentum*. Sucrose density gradient centrifugation of thylakoid membranes solubilized with -dodecylmaltoside resulted in the separation of three green bands. On the basis of protein composition, absorption and 77K fluorescence emmision spectra, the lowest green band was used for further isolation of the PSII complex. This crude PSII extract were solubilized with ß-dodecylmaltoside and loaded on DEAE-Sepharose CL-6B column according to [7]. The purified PSII complexes were eluted with 200 mM NaCl and analyzed.

Electron microscopy was performed with a Philips TEM 420 at magnification of 60,000 . The PSII complexes were applied on the glow discharged carbon-coated copper grids and negatively stained with 2% uranyl acetate. The image analysis was carried out with SPIDER software according to [10]. A total number of 253 single particle top-view projections were extracted from 14 negatively-stained electron microscopy images. The averaged top-view projection of PSII complex indicated a trapezoid particle with a dimension of 21 13 nm (Fig 1). Although no symmetry has been imposed during the image analysis clearly two-fold rotational symmetry around the center of the complex is visible indicating the dimeric nature of the PSII complex. This result is consistent with similar PSII preparations from both cyanobacteria and higher plants suggesting the PSII complex is structurally very similar in organism performing oxygenic photosynthesis [8, 9, 11 - 13].

In order to investigate the organization of the extrinsic subunits (and mainly the location of 20 kDa protein) the PSII particles will be exposed to various salt treatments which remove the lumenal subunits. Thus, a difference map of averaged projections of PSII complexes with and without the extrinsic subunits would enable to determine the location of the 20 kDa subunit.

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1. O. Hansson and T. Wydrzynski, *Photosynth. Res.* **23** (1990) 131-162.
2. D. F. Ghanotakis and C. F. Yocum, *Photosynth. Res.* **7** (1985): 97-114.
3. J. Barber, J. Nield, EP Morris, D. Zheleva and B. Hankamer *Physiol. Plant.* **100** (1997) 817-827.
4. B. Hankamer, E. P. Morris, J. Nield, A. Carne and J. Barber *FEBS Lett.* **504** (2001) 142-51.
5. A. Seidler, *Biochim. Biophys. Acta* **1277** (1996) 35-60.
6. J. R. Shen, M and Inoue Y Ikeuchi, *FEBS Lett.* **301** (1992) 145-149.
7. I. Enami, H. Murayama, H. Ohta, M. Kamo, K. Nakazato and J. R. Shen, *Biochim. Biophys. Acta* **1232** (1995) 208-216.
8. A. Zouni, H. T. Witt, J. Kern, P. Fromme, N. Krauss, W. Saenger and P. Orth, *Nature* **409** (2001) 739-743.
9. N. Kamiya and J. R. Shen , *Proc. Natl. Acad. Sci. USA*, **100** (2003) 98-103.
10. van Heel & Frank, *Ultramicroscopy*, **6** (1981) 187-194.
11. E. J. Boekema, B. Hankamer, D. Bald, J. Kruip, J. Nield, A. F. Boonstra, J. Barber and M. Rogner, *Proc. Natl. Acad. Sci. USA 92* (1995) 175-179.
12. J. Nield, O. Kruse, J. Ruprecht, P. da Fonseca, C. Büchel and J. Barber, *J. Biol. Chem.* **275** (2000) 27940-27946.
13. B. Hankamer, E. P. Morris, J. Nield, C. Gerl and J. Barber, *J. Struct. Biol.* **135** (2001) 262-269.