

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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FUNCTIONAL RECONSTITUTION OF PHOTOSYSTEM 2 INTO LIPOSOMES

J. Benešová¹, S.T. Liffers², C. König², E. J. Boekema³, M. Rögner²

¹*Institut of Physical Biology, University of South Bohemia, 373 33 Nové Hradky, Zámek 136, Czech Republic*

²*Faculty for Biology, Department of Plant Biochemistry, Ruhr-University Bochum, D - 44801 Bochum, Germany*

³*Biophysical Chemistry, University of Groningen, NL - 9747 AG Groningen, The Netherlands*

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 Å x 100 Å x 40 Å, this complex also extends about 10 Å out of the membrane in the stromal region and 55 Å in the lumen; the latter is referred to as the oxygen evolving complex, harbouring the water-splitting site. Developing an appropriate 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 water-splitting 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).

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THEORETICAL MODEL OF CU(I)/CU(II) HYDRATION UNDER THE INFLUENCE OF VARIABLE LIGAND FIELD

Jaroslav V. Burda¹, Matěj Pavelka¹, Milan Šimánek¹ and Manoj Shukla²

¹*Department of Chemical Physics and Optics, Faculty of Mathematics and Physics, Charles University, Ke Karlovu 3, 121 16 Prague 2, Czech Republic*

²*Department of Chemistry, Jackson State University, 1325 J. R. Lynch Street, Jackson, Mississippi 39217-0510, USA*

An ab initio second-order perturbation theory (MP2) and DFT calculation were performed on the copper monovalent and divalent cations. Different number of the water molecules was considered for the pure $\text{Cu}(\text{H}_2\text{O})_n^m$ ions where $n = (1 \text{ to } 6)$, $m = 1 \text{ or } 2$. In the next step, copper complexes with four and six ligands were regarded where the number of the ammonium molecules was varied. As a final step complexes of Cu-guanine with 5 water molecules were calculated.

Pure copper hydration clearly shows that while Cu(II) complexes are relatively good stabilized up to coordination with six water molecules, the Cu(I) cation prefers only two-water coordination when electron-correlation effects are included. The other water molecules leave to second hydration sphere. Some interesting theoretical consequences appear when only Hartree-Fock level is taken into account.

Under the ammonium surrounding, the individual stabilities are changed but all qualitative conclusions found for pure water complexes remain valid. Also, some small changes in geometries are apparent as a consequence of different ligand strength of the ammonium molecule.

A slightly stronger ligand - guanine in N7 site also prefers two coordinated Cu(I) complex with the other water

escaped from first hydration sphere of Cu cation. Interesting feature, from the structural point of view, can be regarded as the position where the other water molecules are placed in comparison with the hydration of the plain guanine molecule. On the contrary, hydrated complex Cu(II)-guanine exhibits the largest stabilization among all the studied Cu complexes.

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

Ladislav Bumba^{1,2}, Helena Havelková- Doušová³, Michal Hušák⁴ and František Vácha^{2,4}

¹Faculty of Biological Sciences, University of South Bohemia, Branišovská 31, 37005 C. Budejovice,

²Institute of Plant and Molecular Biology, Czech Academy of Sciences, Branišovská 31, 37005 C. Budejovice,

³Institute of Microbiology, Division of Autotrophic Microorganism, Czech Academy of Sciences, Opatovický mlýn, 37901 Třeboň

⁴Institute of Physical Biology, University of South Bohemia, Zámek 136, 37333 Nové Hradky

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

In this report we present preliminary results on locating 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 *Porphyridium cruentum*.

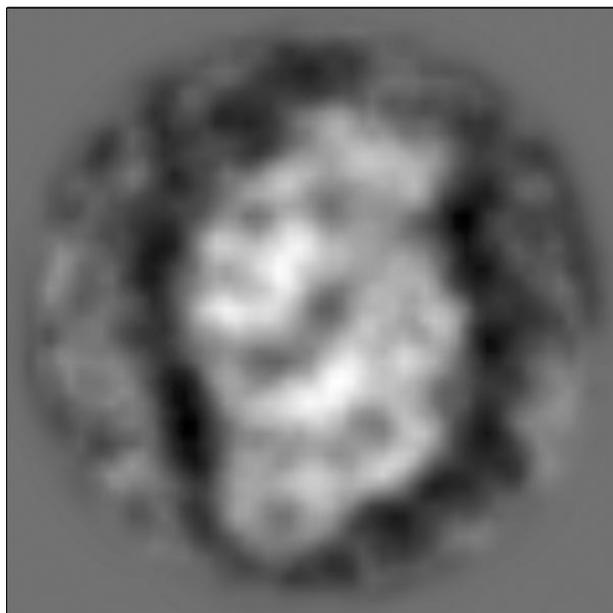


Fig. 1. Averaged projection of negatively-stained preparation of the dimeric PSII complex isolated from *Porphyridium cruentum* in its top-view projection (i.e. perpendicular view to the thylakoid membrane).

The PSII complex was isolated from thylakoid membranes from *P. cruentum*. Sucrose density gradient centrifugation of thylakoid membranes solubilized with β -dodecylmaltoside resulted in the separation of three green bands. On the basis of protein composition, absorption and 77K fluorescence emission spectra, the lowest green band was used for further isolation of the PSII complex. This crude PSII extract were solubilized with β -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 \times 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 luminal 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.