

ferritin and has been re-discovered as a useful agent to promote crystallization or to increase diffraction quality in a number of cases [Trakhanov 1998]. However, even with a mechanistic explanation of this effect, no rational prediction regarding the probability of success – except statistical evidence – is available!

The specific morphology of thaumatin and cytochrome crystals may depend on factors such as the source of material used during crystal growth and chemicals in the crystallizing buffer in the mother liquor, or on the mother liquor itself. For a single crystal form the angles between the faces are constant, but this is not true if the crystals belong to the different crystal forms such as tetragonal bipyramids and hexagonal prisms as in thaumatin. Their appearance depends on the use of metal salt cations, such as cupric chloride, and partially on the buffer and the precipitating agent used. We assume these metal ions influence evaporation in the protein drop even if they are absent from that drop. As this effect was tested on two different proteins only, we cannot speculate about how universally applicable this will be. However, the influence of Cu^{2+} ions on cytochrome crystal growth appears to be specific, because no other successful combination of ion salts with cytochrome was found among these four salts singly or in pairs. A similar effect was observed even in thaumatin crystallization when conditions with cupric chloride produced thaumatin crystals with a different morphology. The combination of four

particular salts that promote crystallization can be quite reproducible also with other chemicals or even other volumes of the same drop in the remaining drop chambers.

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Acknowledgements

This work is supported by grants MSM6007665808 and LC06010 of the Ministry of Education of the Czech Republic and Institutional research concept AVOZ60870520 of Academy of Sciences of the Czech Republic to I.K.S.

CRYSTALLIZATION AND STRUCTURE - FUNCTIONAL STUDY OF PHOTOSYSTEM II FROM HIGHER PLANTS

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Keywords

Photosystem II, reaction center, counter-diffusion method, vapor diffusion, membrane protein, absorption spectra, precipitation, exciton interaction, transition monopoles, transition dipole moments.

Abstract

Oxygen-evolving complex of photosystem II was crystallized using both counter-diffusion method and common vapor diffusion techniques. Absorption spectra of Photosystem II (PS II) reaction centres (RC) upon reduction of primary acceptor pheophytin of the D1 protein subunit were studied and compared with spectra calculated on the basis of Exciton Limit (Matrix Method).

Introduction

Photosynthesis realized by photosystem II (PSII) uses light energy to couple the formation of molecular oxygen to the fixation of carbon dioxide. This process simultaneously generates an aerobic atmosphere and produces a readily usable carbon source, both of which act to sustain almost all life on this planet. PS II is located in the thylakoid membrane of higher plants, algae and cyanobacteria. Its function to capture sunlight is realized by two antenna proteins CP47 and CP43. They transfer the excitation energy from the antenna system to the photochemical reaction center (RC) with primary electron donor P680, which is formed by chlorophyll *a* (Chl *a*) molecules is excited to P680*, followed by release of an electron that travels along the electron transfer chain [1, 2]. This large multisubunits



protein-pigment complex oxidizes water with releasing of oxygen molecule by a cluster of four manganese atoms. The Mn₄-Ca cluster forming the catalytic site for water oxidation is located near the luminal side of PSII RC mostly surrounded by amino acid side chains from D1. One molecule of oxygen is accumulated in four successive charge separation steps and four positive charge equivalents, utilizing two molecules of water.

PS II consists of four membrane-internal subunits (D1, D2, CP43, and CP47), several smaller internal membrane (including PsbE and PsbF, constituting *cyt b-559*) and three external subunits (PsbQ, PsbP, PsbO in green algae and higher plants) [3]. PSII RC binds more than 50 co-factors and ion species. Cofactors contact to the heterodimeric D1/D2 system at the heart of PSII that include Chl *a*, Pheo *a*, plastoquinone, β -carotene and one non-haem Fe²⁺.

The aim of our experimental part was based on using advanced counter-diffusion and standard vapor-diffusion methods, to observe capability of individual precipitants to influence the crystals growth.

Our theoretical work was based on study of temperature dependence of the optical absorption changes upon reduction of primary acceptor pheophytin *a* (Pheo *a*) of the D1 protein subunit (D1-Pheo *a*) to find out whether pheophytin should be considered as a part of a multimer model.

Materials and methods

Experimental part

Crystallization of macromolecular complex such as PS II is influenced by many parameters. The most important is the protein itself, its purity, homogeneity and some other properties, but mainly its disposition to form crystals. This tendency to produce for diffraction measurement suitable crystals may be optimized combination of using different crystallization techniques and other physicochemical parameters (precipitants, additives, pH, etc.) influencing crystallization [4, 5].

Nowadays counter-diffusion method becomes very popular like substitution of common vapor diffusion techniques: sitting and hanging drops, microbatch under oil, etc. Capillaries of different length and diameters performed in the Granada Crystallization Boxes (GCB-Domino, Triana Sci&Tech, Granada, Spain) are a good choice for counter-diffusion technique. The capillaries (Fig.1) filled by protein {1} are placed into the different precipitant solutions {2} through the layer of 0.1% agarose {3} to fix them. Gradient of supersaturation appears along the length of the capillary due to diffusion of solutions against each other. Optimal condition for producing nice single crystals suitable for X-ray diffraction measurement can arise in some parts of capillary.

The sample with dimeric core complex of PS II (OEC PSII) was obtained from *Pisum sativum* (green pea), purified and isolated to concentration 2-3 mg/ml of chlorophyll *a* (~10-15 mg/ml of protein) and supplemented with 1mM MnCl₂ to crystallization test. 17.5% acrylamid gel was used to analyze protein composition of isolated trials. Fresh sample of OEC PSII was crystallized using counter-diffusion method as well as common vapor diffusion techniques.

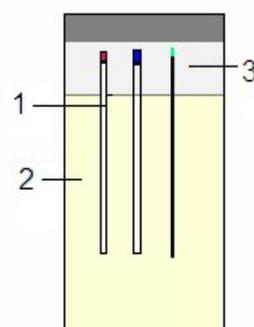


Figure 1. The Granada Crystallization Box with capillaries of different diameters (1 – capillary, 2 – precipitant, 3 – agarose).

Computational part

Exciton interaction between chlorophyll molecules is a general phenomenon in photosynthetic reaction centers. Strong interaction between closely located molecules of pigments leads to a situation where these molecules lose their individual identity and their spectral properties change significantly. The optical properties of chlorophyll-pheophytin complex are determined by the strong interactions between the pigments, which are nearly at Van der Waals' distance to each other.

Exciton interactions between pigments of PSII RC from higher plants upon oxidation or reduction of primary acceptor pheophytin have been studied by using experimental and molecular modeling techniques [7, 8]. For characterization of the spectral properties (absorption and CD spectra) of multimer protein-pigment system, Exciton Limit (Matrix Method) [9] was applied on crystal structure of PSII RC (accession code in protein databank 1S5L [10]). This method consists of two parts, in the first part, one finds the eigenvalues and eigenvectors of the matrix, and the off-diagonal elements are interaction energies between the molecules (pigments) and the diagonal terms represent individual transition energies of pigments. Interaction energies were calculated by two ways, the point dipole and also point monopole method. The point-dipole method [11] evaluates interaction energy between the transition dipole moments of pigments. The point-monopole method [12] describes the interaction energy between transition monopoles distributed on the pigments. This method is an improvement over the point dipoles method [14]. In order to obtain realistic light-adapted absorption spectra of PSII RC we modified site energies (individual transition energies of pigments) by so-called electrochromic shifts as interaction energies between atomic partial charges of D1-Pheo and differential dipole moments of the excited and ground states of PSII RC core pigments [13]. In the second part of calculation, the eigenvectors of this matrix were applied along with the molecular electric transition dipoles to calculate absorption intensity for each of the matrix eigenvalues. Each line of the line spectrum was replaced by a smooth Gaussians function to give the appearance of actual experimental spectra.

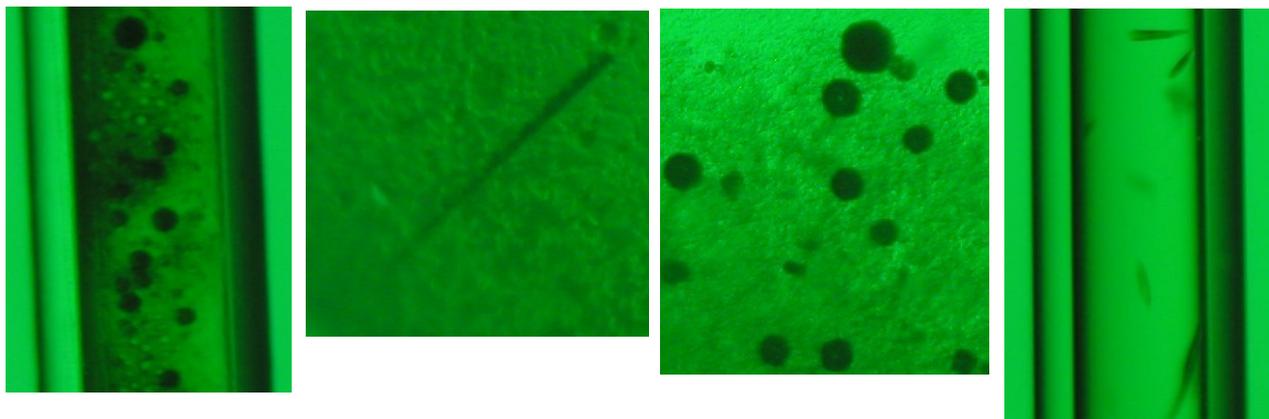


Figure 2. Crystals of higher plants photosystem II.

Results and discussion

Experimental part

Results describing our experimental work when several detergents for membrane protein (-DM, C₁₂E₈), buffers with different pH (MES, HEPES, Tris, KH₂PO₄), cryoprotectants (PEG with several molecular weight, glycerol, MPD) were used, were published by Kuta Smatanova et al. [6]. We also investigated the influence of several salt additives from Hampton Research screening test (Ba, Mg, Ca, Mn, Cd, Cu, Co, Cs, Zn, Y, Ni, Sr) (Hampton Research, CA, USA) to find suitable conditions to produce single crystals of diffraction quality. Crystals of hexagonal shape and needles obtained from different conditions will be measured at the source of synchrotron radiation in DESY, Hamburg (Germany) or ESRF, Grenoble (France).

Computational part

Analogous to our previous experiments we have obtained the temperature dependence of the light-induced difference spectrum under primary acceptor reduction (figure 3). Almost identical differences in intensities of 298 K and 77 K-calculated and 277 K and 77 K-experimental difference absorption spectra clearly supported earlier assumptions [7]. If the molecule of D1-Pheo *a* is a part of the multimer interaction, its reduction would lead to a change in the exciton interaction and consequently to a change in the optical absorption spectrum. Since the process of exciton interaction is not dependent on temperature and the D1-Pheo *a* reduction does not cause any change in the low temperature CD spectrum, we suppose that the D1-Pheo *a* molecule is not coupled in the multimer.

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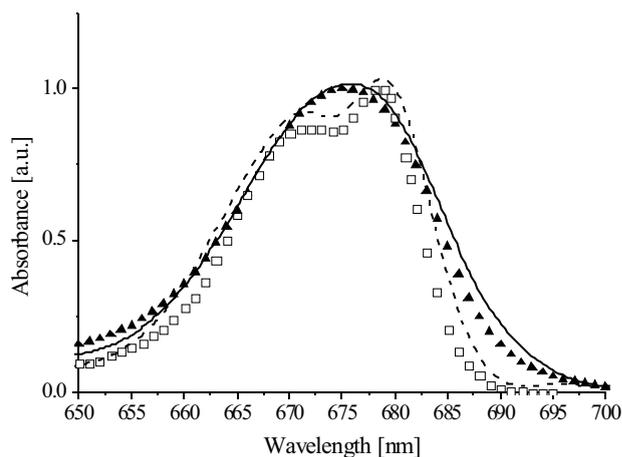


Figure 3. Comparison of calculated absorption spectra of PSII RC pigments calculated at 298K (solid line) and 77K (dashed line) with experimental absorption spectra measured at 273K (triangles) and 77K (squares) [8]. All spectra were normalized to their maxima using arbitrary units (a.u.).

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Acknowledgements

This work is supported by grants NSM6007665808 and LC06010 of the Ministry of Education of Czech Republic and Institutional research concept AVOZ60870520 of Academy of Science of Czech Republic.