

CRYSTALLIZATION OF PHOTOSYSTEM II CORE COMPLEX FROM *PISUM SATIVUM*

KUTA SMATANOVA I.^a, VACHA F.^b, GAVIRA J.A.^c, GARCIA-RUIZ J.M.^c

^a*Institute of Physical Biology USB CB & Institute of Landscape Ecology AS CR, Zamek 136, 373 33 Nove Hradky, Czech Republic,* ^b*Institute of Physical Biology USB CB, Zamek 136, 373 33 Nove Hradky, & Institute of Plant Molecular Biology AS CR, Branisovska 31, 370 05 Ceske Budejovice, Czech Republic,* ^c*Laboratorio de Estudios Cristalograficos, IACT CSIC-UGRA, Facultad de Ciencias, Avd. Fuentenuva s/n, 18002 Granada, Spain*

KEYWORDS: membrane protein; photosystem II; crystallization

Membrane proteins are responsible for enzymatic reactions, which play an important role in all fundamental processes of life. The fact that protein crystals are quite unstable, highly temperature- and light-sensitive along with complicated composition of membrane proteins are responsible for difficult crystal growing and solving their structure.

Monomeric photosystem II (PSII) core complex consisting of reaction center proteins D1 and D2, the chlorophyll-containing inner-antenna subunits CP43 and CP47, α and β subunits of cytochrome b_{559} , several low molecular weight subunits, pigments and cofactors and three extrinsic proteins of oxygen-evolving complex [1] was isolated from *Pisum sativum*, purified and concentrated to 4-6 mg/ml chlorophyll *a*. Freshly isolated and frozen samples of monomeric PSII core complex were crystallized using the counter-diffusion technique implemented in single capillaries [2] and traditional vapor diffusion method in sitting drops. In both cases the protein solution was gelled with tetramethyl orthosilicate (TMOS) or agarose at different concentration. Gel free experiments were prepared in parallel. Different types of precipitants, inorganic salts, different pH values and variant protein:precipitant concentration ratios were tested experimentally. As a general observation, only fresh purified and non-frozen protein was suitable for crystallization trials. Both, the use of gels and crystallization in capillaries, were proved as useful crystallization method.

Plate crystals of monomeric PSII core complex were grown in sitting drops from precipitant solution containing PEG4000, NaCl, Bis-Tris pH 7.00 at 283K. Needle-shaped crystals of protein were grown in sitting drops and in capillaries from the same precipitant solution in the presence of TMOS or agarose at RT. Green crystals were tested at synchrotron DESY (Hamburg) at 100K. Crystallization experiments on PSII membrane protein complexes are still in the progress.

Acknowledgements: This work is supported by the Ministry of Education of the Czech Republic (grant LN00A141), by the Grant Agency of the Czech Republic (grant 206/03/D061) and by the joint C.S.I.C. and AS CR project 01CZ0001, in the frame of the co-operation agreement 2003CZ0013.

[1] Zouni, A., Witt, H.-T., Kern, J., Fromme, P., Krauss, N., Saenger, W. and Orth, P.: *Nature*, 2001, **409**, 739-743.

[2] Garcia-Ruiz, J.M., Gonzales-Ramirez, L.A., Gavira, J.A. and Otalora, F.: *Acta Cryst*, 2002, **D58**, 1638-1642.