Structural and Interaction studies in and between the extrinsic proteins of photosystem II in higher plants (AT-CZ 69p26)

Final report

This project was a continuation of the previously granted project aimed at structural analysis of proteins PsbP and PsbQ from oxygen-evolving complex of higher plants photosystem II (PSII). The aim of the project was focused on the structural analysis of further proteins from oxygen-evolving complex of higher plants photosystem II named PsbO and PsbR and interaction studies between selected proteins of oxygen-evolving complex, including PsbP and PsbQ, which have been structurally studied within our collaboration during last years.

During the process of photosynthesis oxygen is released to the atmosphere, thus giving the basis for aerobic life. One of the major aims of plant physiology studies is the understanding of mechanism and kinetics of water oxidation. The higher plant OEC consists of an inorganic Mn_4Ca cluster and extrinsic proteins named PsbO, PsbP, PsbQ [Ghanotakis *et al.*, 1984; Ghanotakis&Yocum, 1985; Miyao&Murata 1985; Seidler, 1996]. Additionally, a new protein, PsbR, has been discovered in plant PSII, however the functional role and properties of PsbR, also known as the 10-kDa PSII polypeptide, have remained poorly characterized [Stockhaus *et al.*, 1990; Ljungberg *et al.*, 1986; Suorsa *et al.*, 2006]. Our research is focused on structural and interaction studies of mentioned proteins PsbP, PsbQ, PsbO and PsbR.

1. Optimization of the purification protocol for isolation of recombinant PsbO protein

First aim of this project was optimization of the purification protocol for recombinant protein PsbO to analyze folding and mis-folding of PsbO protein by using of CD spectroscopy in different buffers.

PsbOlabeled with ^{15}N isotope was subjected to measurement by solution NMR spectroscopy. At the initial phase, 2D experiment ^{1}H, ^{15}N HSQC was recorded since it serves as a "fingerprint" spectrum and gives an initial idea about the fold and dynamic properties of a protein. First screening revealed that although the protein could be sufficiently concentrated to make a good NMR sample, the spectrum indicated an unfolded protein. Given that it was already proposed in the literature to be a natively unfolded protein (IDP), another methods were used to investigate its structural properties (CD, fluorescence) [Lydakis-Simantiris *et al.*, 1999]. Since we got ambiguous data from these three spectroscopic methods, we decided to improve the expression procedure. A broad-range optimization was carried with respect to optimal buffer, pH, temperature and redox conditions. A set of HSQC spectra were recorded for various samples including PsbO stabilized with sorbitol or mannitol (replacement for glycerol which interferes with NMR measurement), PsbO mixed with detergent (LDAO) and equilibrated at different pH values (3.6, 6.0, 8.0). Since none of those conditions helped to improve the fold of PsbO a refolding step was implemented into the preparation. PsbOisolated from inclusion bodies and refolded with the help of glutathione was so far the best sample suitable for characterization by solution NMR spectroscopy. ^{1}H, ^{15}N HSQC of this new preparation of ^{15}N PsbO showed very nice separation of cross peaks as opposed to the previous cases. The peak pattern corresponds to a protein with high yield of β-sheets, which is agreement with the literature[2]. Following relaxation (T1) data and heteronuclear NOEs confirmed that the protein is in folded form and contains high amount of flexible parts. Moreover, a special version of ^{1}H, ^{15}N HSQC, which is designed for larger proteins (> 30 kDa), was carried out as well and yielded even better resolved spectra confirming the assumption of having a natively folded PsbO (33 kDa). In addition such refolded PsbO responds to the changes in pH from 8.0 to 6.0 and vice versa. As was reported previously, such behaviour is unique to PsbO compared to other extrinsic proteins of oxygen evolving

centre (OEC)[Shutova *et al.*, 1997; Shutova *et al.*, 2005]. The induced conformational change is associated with the ability to bind Ca^{2+} and Mn^{2+} ions as well as with the water and H^+ transport in the OEC [Shutova *et al.*, 2005]. Our results from NMR spectroscopy were corroborated by the results from CD and fluorescence spectroscopy. Next step of the research is to prepare doubly labelled (^{15}N, ^{13}C) PsbO that will be used for 3D NMR experiments designed to elucidate the resonance assignment (publication in draft).

2. Preparation of the recombinant PsbR protein

PsbR likes transmembrane protein causes problems with isolation and optimization of the purification protocol and thus preparation of PsbR protein for structural analysis was the second aim of this project. From preliminary results it was evidence that we have to choose carefully the construct for overexpression of PsbR protein. To this time we prepared construct of GST-PsbR and TRX-PsbR proteins and optimized protocol for isolation of soluble protein with anchor. The experiments of digestion and the analysis of folding-misfoldingof the PsbR without anchor will be next steps to get protein suitable for NMR and X-ray analysis.

3. Interaction studies within and between extrinsic proteins

Assessing the interactions within and between of the extrinsic proteins was the third aim of this project. We got almost complete chemical shift assignment of PsbP (publication under revisions) and we are in the process of determination the full PsbP structure in solution. For the PsbQ, assignment [Horničakova *et al.*, 2011] and a 3D solution structure were also carried out in group (submitted to Protein Science). Thus we are able to monitor changes to the unstructured regions at the atomic level resolution, along with detailed studies on structures and the associated changes in dynamics via NMR relaxation (T_1, T_2 and $T_{1\rho}$) and NOE experiments as well as interactions by mutual titration in NMR. Additional experiments included titrations of PsbO into PsbP in the absence and presence of Ca^{2+}. The most recent data revealed new conformation of PsbP upon addition of PsbO, however the details of those interactions require further time-demanding experiments to specify the exact atoms involved.

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This AKTION project strengthened significantly the collaboration among the research groups based in the Johannes Kepler University in Linz and the University of South Bohemia in České Budějovice. As one main focus of the project were preparation of recombinant proteins suitable for NMR studies several research stays of the austrian experts in the Czech republic were necessary to discuss optimization protocol in minimal medium, analysis of folding and misfolding of proteins by biophysical methods and delivery of prepared protein sample for measurements in Linz (Petr Rathner, Adriana Walnerova - April, June, November) and preparation of manuscripts (Norbert Müller, Adriana Walnerova, Petr Rathner -November) These stays were fully covered on the Czech side from the funded grant. Scientists from the Czech partner (Michal Kamenický, Jaroslava Kohoutová and Jiří Heller) were using the newly availbale NMR instrumentation and circular dichroisms in Linz to analyse samples of recombinant proteins. First PsbO recombinant protein was analysed by CD and NMR (July, September) and it was shown that protocol has to be optimize to produce protein with right folding. After optimization of protocol by refolding steps was sample again analyse and first

NMR spectra of PsbO protein recorded (November). All results of this grant, manuscripts and following experiments were discussed during the final meeting in February 2015 in Nove Hrady.

Project results have been partially published and reported in:

Conferences:

Kamenicky M., Heller J., Walnerova A., Rathner P., Smatanova I., Ettrich R., Müller N., Kohoutova J.: Recombinant preparation and initial NMR spectroscopic investigation of extrinsic PsbO from the oxygen-evolving complex of higher plants, in: FEBS Advanced Course: Ligand-binding Theory and Practice, 29. 6. – 6. 7. 2014, NovéHrady, Czech Republic.

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Adriana Walnerova, Petr Rathner, Kousik Chandra, ReinhardWimmer, Norbert Müller: Novel results on Psb proteins of Photosystem II by solution NMR spectroscopy, in: North-Austrian/Bavarian Bio-NMR Workshop, 23. 10. 2014, Munich, Germany

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Petr Rathner, Kousik Chandra, JaroslavaKohoutova, Michaela Hornicakova, Rüdiger Ettrich, Reinhard Wimmer and Norbert Müller: Solution and crystallographic structures are different for the extrinsic photosystem II protein PsbQ of higher plants, XVII. Annual Linz Winter Workshop, 30. 1. – 2. 2. 2015, Linz, Austria

Publications:

1. Adriana Walnerová, Kousik Chandra, Petr Rathner, Michaela Horničáková, Judith Schlagnitweit, JaroslavaKohoutová, RüdigerEttrich, Norbert Müller: **Resonance assignment of PsbP –an extrinsic protein from photosystem II of *Spinacia oleracea,*** *Biomolecular NMR Assignments* (BNMR)-accepted after revision.

2. Petr Rathner, Adriana Walnerová, Michaela Horničáková, Christian Wohlschlager, Kousik Chandra, Jaroslava Kohoutová, Rüdiger Ettrich, Reinhard Wimmer and Norbert Müller: **Solution Structure of PsbQ from Photosystem II of Higher Plants** (submited to *Protein Science*)

In preparation:
Purification and structural analysis of recombinant PsbO protein.
Expression and purification of recombinant transmembrane protein PsbR.

V Českých Budějovicích, dne 25.3.2015

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