AKTION ÖSTERREICH - TSCHECHISCHE REPUBLIK

Wissenschafts - und Erziehungskooperation

AKTION ČESKÁ REPUBLIKA - RAKOUSKO

spolupráce ve vědě a vzdělávání

PROJEKT ABSCHLUSSBERICHT / ZÁVĚREČNÁ ZPRÁVA PROJEKTU

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Projektbezeichnung / Název projektu (auch Tschechisch/také česky):

NMR investigations of recombinant PsbH protein in Escherichia Coli

Výzkum rekombinantního PsbH proteinu v Escherichia Coli pomocí NMR

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Projektdauer von / Trvání projektu od: **1. 12.2004** *bis / do*: **1. 12.2005**

Förderungsbetrag / Schválená podpora:

EUR insgesamt / EUR celkem: 5300,- EUR

Kč insgesamt / CZK celkem: 58000,- CZK

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The object of our study, supported by AKTION, is the PsbH protein, one of the proteins of photosystem II (PSII). This study is a part of the project, which shall contribute to understanding of PSII assembly and its proper function, mainly protein-lipid interaction. Within the project the interaction of the PsbH protein of photosystem II with lipids was analysed by combination of CD and NMR spectroscopy and computer modeling. On the molecular biology side the PsbH protein of cyanobacterium Synechocystis sp. PCC 6803 was expressed as a fusion protein with glutathione-S transferase (GST) in *E. coli* growing on the mineral medium that should be later be enriched about 15N isotope. Due to low expression rates we isolated the plasmid and transferred it into new competent cells BL21. The expression of recombinant protein was then induced by IPTG. The fusion protein was isolated using affinity chromatography. After enzymatic cleavage of the fusion protein the PsbH protein was isolated using DEAE chromatography and the concentration of the captured protein was performed by Centricon ultrafiltration units. We obtained PsbH protein in presence of detergent octyl glucoside (OG).

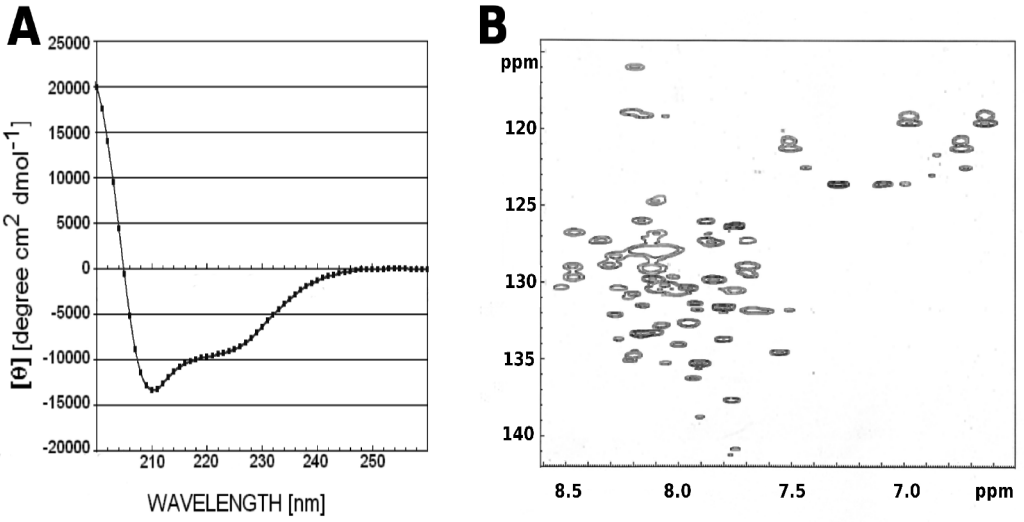

Figure 1: *Circular dichroism spectra of PsbH protein in OG. B (left): ^{1}H-^{15}N-HSQC spectrum of PsbH protein in OG (right)*

Stable isolated protein was then prepared on about 15N isotope enriched media for measurements by NMR in Linz. We measured our test samples on the *Bruker DRX* 500 MHz NMR spectrometer equipped with a 5 mm *TXI* cryoprobe to enhance sensitivity and resolution. The time of measurement was rapidly increased and we had to record data at least 10 hours for a 2D 1H/15N correlated spectrum. In the resulting spectrum we were able to identify each NH group signal from 15N, which shows a poperly folded protein. We isolated and purified the ^{15}N labelled protein to a concentration of 1.1 kg m^{-3} in phosphate buffer (K_2HPO_4 20mM, KCl 100mM, pH 6.8) with presence of OG. ^{1}H-^{15}N HSQC spectra were recorded The protein sample was mixed with D_2O (H_2O/D_2O 1 : 20) to perform the NMR experiments. We recorded spectra of the PsbH protein in OG and then divided sample into four parts and added the liposomes of different lipid classes (sulphoquinovosysldiacylglycerol – SQDG, monogalactosyldiacyl glycerol – MGDG, digalactosysldiacylglycerol – DGDG, phosphatidylglycerol – PG and extract from the thylakoid membrane) to achieve lipid concentration 1% w/w. The standard *Bruker* pulse sequence for these experiments was the *hsqc-etf3-gpsi2* pulse program. For titration experiments we used liposomes prepared by reversed phase evaporation. Non-labelled

PsbH protein was prepared for circular dichroism (CD) spectrometry. CD spectra of the sample were recorded on a *Jasco*-J715 spectropolarimeter, controlled by PC-based *ISA OMA* software at 20 °C in 1-mm quartz cuvettes, after 15 min of temperature equilibration. Five scans were accumulated for each spectrum with a response time of 2 s, a bandwidth of 2 nm, and a scan speed of 10 nm min^{-1} from 190 to 250 nm. Background spectra without protein were subtracted. The protein was diluted to final concentration of 20 μM using a phosphate buffer (K_2HPO_4 20mM, KCl 100mM, pH 6.8) with presence of a non-ionic detergent β-D-octylglucopyranoside (OG) (2%v/w). The cuvette position was next to the photomultiplier to decline light scattering.

A model of the the PsbH protein of cyanobacterium *Synechocystis* sp. PCC 6803 was generated. The three-dimensional model constituted by all non-hydrogen atoms. The resulting structure was minimized to convergence of the energy gradient to less than 0.05 $kcal \cdot mol^{-1} \cdot Ĺ^{-1}$. A preequilibrated POPC bilayer of 128 lipids was used as a model of the biological membrane and the protein was inserted into the cavity. The production runs, without any restraints, were 20 ns long. The simulation was run with GROMACS using the ffG43a2 forcefield with a 2-fs time step. SETTLE (for water) and LINCS were used to constrain covalent bond lengths. Long range electrostatic interactions were computed with the Particle-Mesh Ewald method. The temperature was kept at 300 K by separately coupling the protein, lipids, and solvent to an external temperature bath (τ=0.1 ps). The pressure was kept constant at 1 bar.

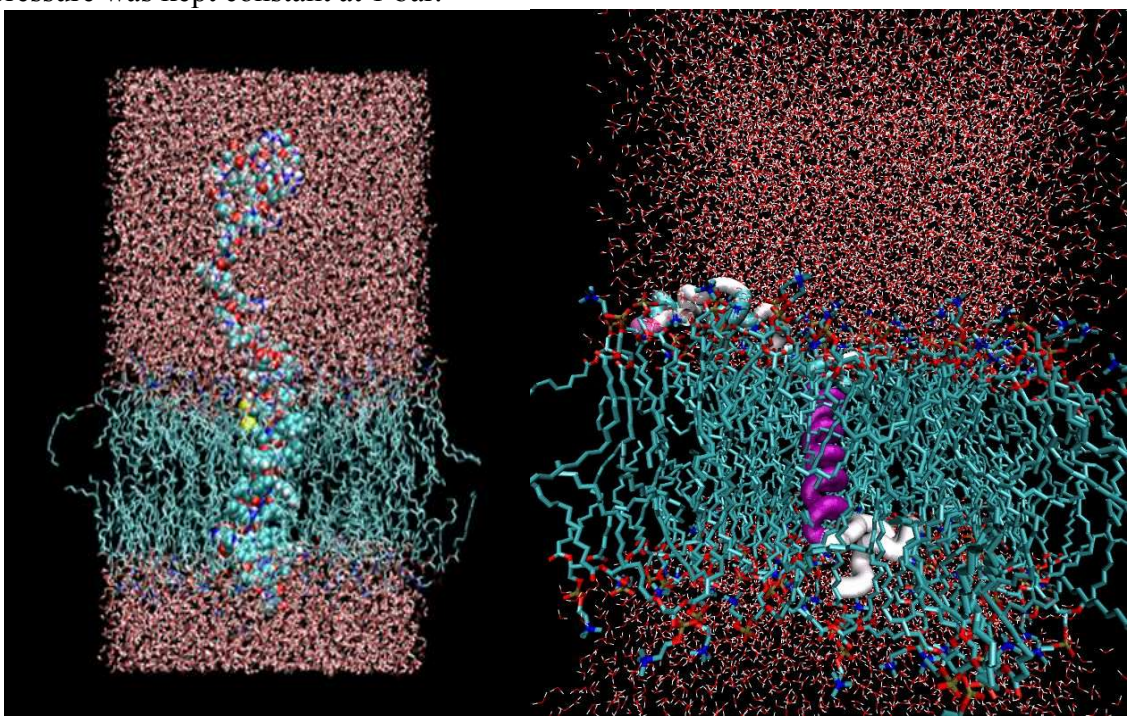

Figure 2: Structure of the PsbH protein *in the lipid bilayer at the beginning of the simulation (left) and at its end (right). Both N- and C- termini submerge in the interface region and the helix is tilted in order to match the hydrophobic regions. The course of the simulation illustrates the fact that the simulation environment is capable to incorporate the membrane protein in conformity with accepted hypotheses on protein-lipid interactions.*

The project addressed scientifically two main questions, whether (a) the structure of the PsbH protein is stable in the lipid bilayer in absence of other proteins of the Photosystem II complex and (b) whether there are lipid molecules which are forming interactions with the PsbH protein which seem to be persistent on a long time scale, in another words whether there is a population of lipids which, when bound to the protein.

The answer gained from our results was positive in both cases. We thus may conclude that there is a fair basis for the hypothesis that the PsbH protein organizes a lipid domain in the thylakoid membrane which may be one of the bases for specific incorporation of other proteins in the particular position in the complex. CD and NMR spectroscopy indicated interactions of PsbH to all bilayer-forming lipids, including those extracted directly from cyanobacterial thylakoid membranes, interact with PsbH non-specifically. The molecular dynamic calculation of PsbH-bilayer forming lipid interactions indicates that both salt-bridge formation and hydrogen bond formation in the lipid-water interface are feasible and stabilize fraction of at least 10 lipid molecules in the neighbourhood of the protein. This may lead to formation of lipid domains which were hypothesized to be involved in assembly of membrane protein complexes.

This AKTION project builds upon the past achievements (supported also by AKTION) of the applicant and the partner. Providing the financial means in a framework of the current project strengthened significantly the former collaboration among the research groups based in the Johannes Kepler University in Linz and the University of South Bohemia in Nové Hrady. It was a collaboration of scientists only, not envolving students directly. Dr. Wolfgang Schoefberger, who started at the beginning as a Post-Doc in the czech laboratory got a contract from February 2005 on the Institution of the Austrian partner. This is cetainly a plus for the austrian partner, but also of advantage for the czech side, as he in Linz continues in the work he started in the czech laboratory and closely collaborates with his former laboratory. On the Czech side, funding was provided for travel expenses of scientists and laboratory consumables. The part of the work done on the Czech side was in the field of molecular biology of proteins and of molecular dynamics calculations. Two scientists from the Austrian partner (Ruth Goessler, Gerhard Zuckerstaetter) were extensively trained in protein expression and purification with an emphasis on expression in labeled medium for NMR purposes. Additionaly, Gerhard Zuckerstaetter and Wolfgang Schoefberger got an introduction into molecular dynamics simulations. On the austrian side the partner was provided with travel expenses and laboratory expenses. His part of the work was the measurement of protein NMR and interpretation of the spectra. Additionaly, MALDI-TOF and circular dichroism was used in the Linz laboratory. Four scientists from the czech side got trained in the field of protein NMR (Rudiger Ettrich, Ivana Kuta Smatanova, Jaroslava Ristvejova, David Kaftan) and will introduce the techniques of spectra interpretation and gaining three-dimensional structures from the data at the Institute in Nove Hrady. Thus this project opened new opportunities for joint research plans, based the individual knowledge on both sides on the same level, and will be continued. The results obtained during this project were recently published in a scientific journal, another regular paper is prepared for submission (see attachments) within the next month and our results were presented on two conferences, a national one (Fourth meeting of Czech and Slovak Structural Biologists) and on the international FEBS conference “The Protein World” in Budapest Hungary.

RNDr. Rudiger H. Ettrich, Ph.D.
řešitel

Nové Hrady, 30.1.2006

Publications:

Regular scientific papers in impacted journals:

Dalibor Stys, Žofie Sovová, Zbyněk Halbhuber, Wolfgang Schoefberger, Josef Komenda, Jaroslava Ristvejova, Norbert Müller and Rüdiger Ettrich: Molecular basis of a lipid-PsbH protein interaction: a structural and computational study. *Manuscript prepared for publication in Protein Science*

Štys D., Schoefberger W., Halbhuber Z., Ristvejova J., Müller M. and Ettrich R.: Secondary structure estimation of recombinant *psbH* encoding a photosynthetic membrane protein of cyanobacterium Synechocystis sp. PCC 6803. *Photosynthetica* (2005) 43 (3): 421-424

Conference abstracts:

Schoefberger W., Halbhuber Z., Ristvejova J., Müller M., Ettrich R. and Štys D.: Structure determination of the psbH membrane protein in different lipid/detergent environments *FEBS Journal* (2005) 272 (s1), G2-114P.

Schoefberger W., Halbhuber Z., Ristvejova J., Müller M., Ettrich R. and Štys D.: Structural measurements of membrane psbH protein in different lipid/detergent environments *Materials Structure*(2005) 12 (1), 39.

Copies of the publications are attached to the final report.