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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Projektnummer/číslo projektu:

60p21

Projektbezeichnung / Název projektu :

Study of photosynthetic PsbI protein's interaction with the thylakoid membrane

Studium interakce fotosyntetického proteinu PsbI s thylakoidní membránou

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PROJEKT ABSCHLUSSBERICHT / ZÁVĚREČNÁ ZPRÁVA PROJEKTU

Oxygenic photosynthesis is one of the essential processes on Earth as it results in energy production with water and sunlight as inputs. It takes place on the thylakoid membranes of phototrophic organisms where the four major multicomponent protein-pigment engines are embedded. The first of these units is photosystem II (PSII), where a water molecule is split into electrons and oxygen. The major attention is paid to examine the bigger and functionally most relevant subunits of PSII, but there is still a little known about single-helical small subunits. One of these is PsbI, that is suspected [1] to be one of the first PSII subunit being inserted into the membrane and to its alter local composition. The photosynthetic membrane composes mainly of galactolipids, contrary to animal membranes, that are made of mainly from phopholipids.

The main aim of this project was to reproduce the behaviour of PsbI protein in the thylakoid membrane by the means of molecular dynamic simulations and the experimental verification of predicted membrane properties by NMR. As till start of the project no computational model of the thylakoid membrane was available, we started with its construction. We managed to construct models of pure monogalactosyl diacylglycerol (MGDG), digalactosyl diacylglycerol (DGDG) and sulfoquinovosyl diacylglycerol (SQDG) that reproduce phase transition temperatures in agreement with experiments available [2]. The models were constructed in the Gromos53A6 force field and the simulations are stable with a time step of 2.5 fs. Later, we mixed these component together with phosphatidyl glycerol (PG; parameters known) to make a model of the thylakoid membrane composed of 72 molecules of MGDG (40 %), 36 molecules of DGDG (20 %), 54 molecules of SQDG (30 %) and 18 molecules of PG (10 %). These proportions were taken from experiments recently published [3]. Each sugar head group was joined to one palmitoyl and one alpha linenoyl chain.

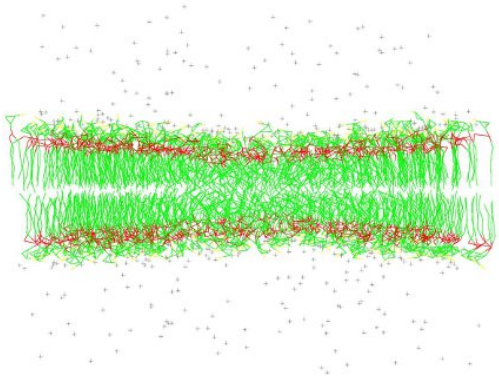

Fig.1 Equilibrated coarse-grained membrane in two different phases to determine the phase transition temperature. The unordered liquid-crystalline phase is in the center of the membrane, the ordered gel is at both sites. DSSQDG with counter-ions (designated by a gray cross) is shown here. Glycerol beads are shown in red, SO3- group is in yellow and the lipid tails are in green.

After insertion of the PsbI protein into the relaxed membrane, we ran the simulation for 300 ns with the time step for 2.5 fs. The most important finding is that the protein (after the system equilibration) moves relatively fast with respect to the lateral movement of the lipids. Furthermore, PsbI interact with nearby lipids via hydrogen bonds, what makes the lipids follow the protein. Interestingly, if we look which type of lipid is within 0.5 nm from the protein, we notice that the amount of DGDG is more than doubled after the equilibration phase than it is on the start of the production run. This may either means that PsbI attracts DGDG molecules or in the case of our simulation, that PsbI requires interaction with any lipids. Having looked on the initial frame of the simulation we discover, that PsbI is close to cluster of SQDG and DGDG, where the DGDGs are slightly further than 0.5 nm, that was chosen as the arbitrary criterion for the close molecules. This may mean, that DGDG were the closes available molecules with respect to the protein a for this reason they bounded to PsbI. To judge with of the previously

mentioned options is the correct one, further simulations with the different initial positions of PsbI are necessary. With respect to the development of coarse-grain glycolipid parameters to sample the conformational behaviour at least on a μs timescale we developed MARTINI force field paramters for the thylakoid membrane in collaboration with the Marrink group in Groningen that enable a time step of at least 20 fs. The parameters were developed by a standard method using the atomistic simulations with the Gromos45a4 force field described above. At the moment we test parameters for all three glycolipids – monogalaktosyl-diacylglycerole, digalactosyldiacylglycerole a sulfoquinovosyldiacylglycerole for conditions, in which experimentally phase transitions take place.

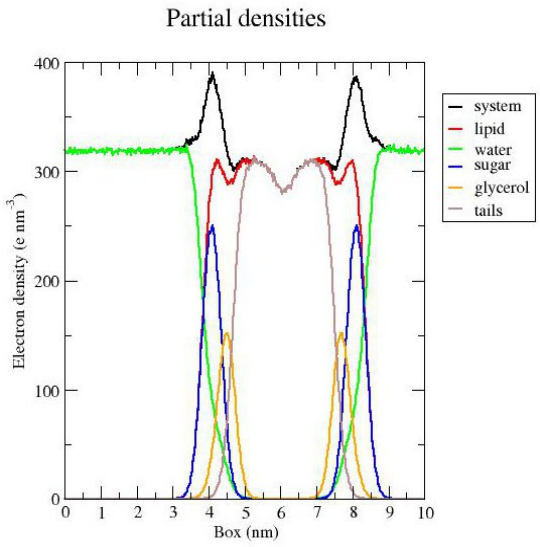

Fig.2 Electron density profile of the DSMGDG fully hydrated bilayer in the liquid-crystalline phase. The final modeled coarse-grained membrane has a correct thickness of 4.0 nm.

Embedded PsbI from *Synechocystis* at physiological conditions (i.e. 310 K, atmospheric pressure and a high degree of hydratation in which the membranes are in liquid crystalline phase) shows a behaviour in accordance with our atomistic simulations and all experimental data available to date. For PsbI from *Synechocystis* we generated a homology model based on the crystal structure 3ARC_I as a template that was refined by steepest-descent minimization and a short simulated annealing protocol. The protein has 38 amino acids, with the N-terminal 24 forming a trans-membrane helix and the following 14 folded into a random coil structure., with 7 of these 14 residues charged, hinting on a probable role in binding to the light-harvesting antennas.

The protein was simulated in the MARTINI force field for at least 2 μs in several membranes. These simulations are currently evaluated in detail. First results show the protein to be stable in the time period monitored with the random coil structure being flexible while the trans-membrane helix is constrained and shows a typical kinking in the membrane of approximately 20 degrees. The random coil structure in a very short time starts to interact with the membrane surface and therefore the membrane composition influences its flexibility.

The NMR investigations on the reconstituted membranes were initiated with the assessment of the methodology, which made use of the novel instrumentation available through the new Austro-Czech NMR Research Centre in Linz (REgional Research Infrastructure Upper Austria South Bohemia: RERI-uasb). The delay in opening this centre (official opening in December 2011) also had a minor impact on the NMR part of this project, which will be handled in an extension application. We have assessed the feasibility of approaches to measure the orientation of lipid components and proteins in the reconstituted membranes. It turned out, that determination of order parameters from the ^{2}H NMR spectra of selectively and partially labelled lipid components is feasible in principle, but prohibitively expensive for all but the simplest lipids. Therefore an alternative approach exploiting the residual $^{1}H^{13}C$ dipolar couplings (RDCs) to determine molecular order parameters was chosen. This methodology works even without isotope labelling for membranes made of a single lipid and the major lipid components of mixed ones. Some ^{13}C labelling will be required for minor lipid components and full ^{15}N isotope labelling by recombinant techniques is needed to determine

the order parameter of embedded proteins, i.e. PsbI. These strategies were discussed and refined at the December reunion of scientists from both countries.

The project was originally planned and written as a two years project, though financed only for one year. We fulfilled all steps planned within the first year, however we will apply for a prolongation as we need to gain more experimental NMR data to fine-tune the glycolipid parameters and characterize the thylakoide membrane in *Synechocystis* and finalize the data analysis for PsbI.

References:
[1] Dobáková M., Tichý M., Komenda J. (2007): Role of PsbI Protein in Photosystem II Assembly and Repair in Cyanobacterium *Synechocystic* sp. PCC 6803. Plant Physiol. Vol 145, pp 1681 – 1691
[2] Sen A, Williams W.P., Quinn P.J. (1981): The structure and thermotropic properties of pure 1,2-digalactosylglycerols in aqueous systems. BBA 663: 380-389
[3] Sakurai I., Shen J.R., Leng J., Ohashi S., Kobayashi M., Wada H. (2006) Lipids in Oxygen-Evolving Photosystem II Complexes of Cyanobacteria and Higher Plants. J. Biochem. 140(2): 201-209

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 Nove Hrady and helped to start the regional research infrastructure RERI-uasb. As one main focus in the first year of the project were the theoretical simulations and predictions, including their detailed analysis with respect to experimentally measureable predicted data, several research stays of the Austrian experts in the Czech republic were necessary to evaulate the data and plan the NMR experiments in detail (Norbert Müller, 30.11-2.12, Beate Hager 7.-9.12, Maria Pöschko, 7.-9.12, Michaela Hornicakova 8.12) These stays were fully covered on the Czech side from the funded grant. One scientist from the Czech partner (Sabina Novakova) was extensively trained in using the newly available NMR instrumentation in Linz (14.11. - 18.11.2011). 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. First preliminary results obtained in the initial phase of this project were recently published in a scientific journal, and another regular paper is prepared for submission within the next month and our results were presented on one international conference (1st Visegrad Symposium on Structural Systems Biology, Czech Republic).

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

Nove Hrady, 30.1.2012

Univ.Prof. Mag. Dr. Norbert Müller

Linz, 31.1.2012

Regular scientific paper in an impacted journal:

M Horničáková, J Kohoutová, J Schlagnitweit, C Wohlschlager, R Ettrich, R Fiala, W Schoefberger, N Müller (2011) Backbone assignment and secondary structure of the PsbQprotein from Photosystem II *Biomolecular NMR Assignments* 5: 2. 169-175.

A copy of the full publication is attached to the final report.

Conference abstracts:

Schlagnitweit J, Hornicakova M, Mueller N.: Accerelerating Biomolecular NMR by Multiplex Techniques, Book of Abstracts: 1st Visegrad Symposium on Structural Systems Biology, Nove Hrady, Czech Republic, 2011 p. 17.

Sovova Z, de Vries AH, Ettrich, R, Marrink SJ.: DGDG bilayer as a first step in thylakoid membrane building, Book of Abstracts: 1st Visegrad Symposium on Structural Systems Biology, Nove Hrady, Czech Republic, 2011 p. 39.