FYZIOLOGICKÝ ÚSTAV AV ČR, V.V.I.

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Oponentský posudek

na doktorskou disertační práci Mgr. Jaroslavy Kohoutové nazvanou: „Structural analysis of extrinsic proteins from the oxygen-evolving complex of photosystem II from higher plants."

Ve své doktorské disertační práci se Mgr. Jaroslava Kohoutová zabývá problematikou přípravy a studia strukturní analýzy proteinů PsbQ a PsbP z fotosystému II a jejich možnými interakcemi. Tyto proteiny jsou částí tzv. kyslík-vyvíjícího se komplexu, který se účastní fotosyntetického procesu. Strukturní znalost vnějších proteinů, která by vedla k predikci uspořádání těchto proteinů na lumenálním místě vyšších rostlin PSII, je stále ještě relativně malá a může významně přispět k pochopení funkce těchto důležitých bílkovin.

Ke studiu těchto proteinů Mgr. Jaroslava Kohoutová použila různé biochemické techniky jako crosslinking a řadu biofyzikálních technik jako je např. NMR spektroskopie, Ramanova spektroskopie včetně tzv. drop coating deposition metody (DCDR), modelování, rentgenová krystalografie, povrchová plasmonová resonance (SPR), mikroskopie atomové síly (AFM). První část práce obsahuje teoretický úvod, který charakterizuje danou problematiku, principy použitých metod, hlavní cíle práce a stručný přehled získaných výsledků s jejich diskusí. Druhá část práce se skládá ze tří již publikovaných prací a dvou rukopisů v recenzním řízení na diskutovaná témata, na kterých je Mgr. Jaroslava Kohoutová buď prvním autorem nebo spoluautorem.

Práce Mgr. Jaroslavy Kohoutové na problematice vnějších proteinů vázajících kyslík přinesla řadu nových výsledků. Byl připraven rekombinantní PsbQ protein expresí v *E. coli,* byl vytvořen homologní model kompletní struktury PsbQ proteinu. Pomocí FTIR a Ramanovy spektroskopie byla určena sekundární struktura proteinu v roztoku. Práce ukázala, že odstranění prvních 12 aminokyselin vede k trvalým strukturním změnám tohoto proteinu. Dalším studovanou bílkovinou byl protein PsbP, který byl také připraven expresí v *E.* coli jako fúzní protein s His-tagovou kotvou. Byla vyřešena krystalová struktura tohoto proteinu (ze *Spinacia oleracea)* s rozlišením 2.06 Å. Následně byla konfrontována krystalová struktura tohoto proteinu s daty z DCDR a Ramanovy spektroskopie. Byla naměřena Ramanova spektra proteinu v roztoku, dále pomocí DCDR a konečně ze vzorku proteinového krystalu. Vzájemné porovnání spekter ukázalo na rozdíly v krystalové struktuře s ohledem na strukturu v roztoku. Tyto informace následně umožnily domodelovat nevyřešené (neuspořádané) části krystalové struktury PsbP. V další části práce byla studována interakce mezi PsbP a PsbQ. Ukázalo se, že dvojstupňová crosslinková reakce není specifická a tudíž není možno určit typ konjugátu a interakční oblast. Proto byl navržen experiment za použití afinitní chromatografie a ukázalo se, vazba těchto dvou proteinů je závislá na obsahu soli. Pro interakce byla

stanovena disociační konstanta K_d pomocí povrchové plasmovové rezonance (SPR) mikroskopie atomové síly (AFM).

Mgr. Jaroslava Kohoutová je autorkou 3 publikací (první autorkou 2 publikací) otištěných v mezinárodních časopisech s IF, další její dvě práce, na kterých je první autorkou, jsou v recenzním řízení. Doktorská disertační práce je psána anglicky, formální úroveň je dobrá, výskyt překlepů je minimální. Zde mám pouze jednu připomínku týkající se obráceného umístění poslední strany s posterem.

K problematice diskutované v doktorské disertační práci mám pouze tři drobné dotazy:

1. Pro interakční studie vnějších proteinů PsbP a PsbQ jste používala chemický crosslinking a afinitní chromatografii. Byly zkoušeny i jiné techniky, jako je např. nativní elektroforéza, esej na bázi změny anizotropie fluorescence či analytická ultracentrifugace?

2. Jaká je přesnost měření nové techniky Ramanovy spektroskopie – drop coating deposition (DCDR)? Nemůže vysušení vzorku vést k denaturaci studovaného vzorku?

3. Jaká je výhoda měření disociační konstanty metodou AFM oproti měření pomocí povrchové plasmonové rezonance (SPR)?

Závěrem konstatuji:

Předložená doktorská disertační práce Mgr. Jaroslavy Kohoutové představuje cenný přínos ke studiu kyslík-vyvíjícího se komplexu. Práce je psána srozumitelně, pečlivě, výsledky byly publikovány v impaktovaných mezinárodních časopisech. Autorka ve své disertační práci dokázala, že je samostatnou vědeckou pracovnicí, schopnou samostatné výzkumné práce.

Jelikož předložená práce Mgr. Jaroslavy Kohoutové vyhovuje všem požadavkům kladeným na doktorskou disertační práci, plně ji doporučuji k přijetí.

Praha 8. ledna 2010

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Seibersdorf, 8th Feb 2010

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Review of the PhD thesis

Structural analysis of extrinsic proteins from the oxygen-evolving complex of photosystem II from higher plants

submitted by

Mgr. Jaroslava Kohoutová

In her PhD thesis Mgr. Jaroslava Kohoutová worked on one of the most important enzyme complexes, the photosystem II. Green plants, algae and cyanobacteria make use of this amazing enzyme system to harbor the energy from the sunlight and convert it to chemical energy. Thereby, ATP and NADPH are produced under the consumption of water and generate molecular oxygen. The success of the plants' energy production strategy led to the development of the today's earth atmosphere, which contains ~20% breathable oxygen and to the formation of the ozone layer. Together both events paved the way for life outside the oceans. Despite many years of intense investigations crucial details of the function of the photosystem II complex, especially of the oxygen-evolving complex, remain unknown. The core structure of photosystem II of cyanobacteria has been solved to medium resolution in the last decade. Structure and interaction of the extrinsic proteins PsbO, PsbQ, PsbP and PspH, which are necessary for normal photosynthesis, are the focus of this PhD thesis. Important contributions to our knowledge of these extrinsic proteins are described.

The PhD thesis is divided into four main parts: a description of the involved proteins, explanation of the used methods, discussion and interpretation of the results and a collection of the publications that resulted from the work carried out during the PhD. The introduction gives an overview on photosynthesis and a thorough description of the structures of the extrinsic proteins of higher plants and the knowledge of the structural assembly of the oxygen-evolving complex. In the methods section protein purification and the chemistry of the cross-linking experiments is outlined. Crystal structures of the PsbQ extrinsic protein became available during the PhD work. Even though the structure was obtained at high resolution, a crucial part of the structure was not resolved. Combining a number of spectroscopic techniques with molecular modeling, Mgr. Jaroslava Kohoutová was able to build a model of the missing loop. Details of the interaction between PsbQ and PsbP have been elaborated and it has been shown that the interaction is driven by charge interactions.

In the manuscripts, a structural study of the integral membrane protein PsbH, part of the photosystem II complex, the dynamic studies of the PsbQ N-terminal loop, crystallization of the PspP protein are

described. In the submitted paper important methodological advances are achieved that allow *in situ* assessment of the conformational integrity of proteins during crystallization.

Conformational flexibility of protein loops and entire proteins are a very important topic in general and are a central topic in the presented PhD thesis. Further discussion of the topic would be welcome.

- By which experimental techniques can flexibility be investigated on a per-residue basis?
- Protein model creation is a demanding task. Especially difficult is the building of long loops for which no template is available? A discussion of the methodology and the challenges is welcome.

Mgr. Jaroslava Kohoutová has published three publications, two of them as first author. An additional one is submitted and a fifth is in the final stages of preparation. This underlines the outstanding achievement of Mgr. Jaroslava Kohoutová. I can therefore state that the PhD thesis submitted by Mgr. Jaroslava Kohoutová fullfills by far all requirements for being accepted as a basis for being awarded the PhD title, and recommend Mgr. Jaroslava Kohoutová for the admission to the PhD defence.

Best regards

Dr. Thomas Stockner

Report on Ph.D. Thesis of Mrs. Jaroslava Kohoutová

This thesis concerns structural studies of two lumenal extrinsic proteins of Photosystem II PsbQ and PsbP stabilizing the oxygen-evolving complex in higher plants. Crystal structures of recombinant proteins are used to model their possible interactions. This rather complicated approach is necessary as detailed PSII crystal structures are available only for cyanobacteria and even in them the lumenal proteins are not well resolved. Moreover, composition of extrinsic proteins in higher plants and cyanobacteria is largely different so the use of the cyanobacterial structure for higher plant OEC is limited.

The thesis is organized in manuscript format with three papers already published, one submitted and another in preparation.

The Introduction is quite thorough in giving the background of the research – an overview of PSII extrinsic proteins, their roles and different approaches how to study them.

From Material and Methods in both thesis and respective publications it is evident that Mrs. Kohoutová devoted great part of her work to improve and modify protein isolation protocols for individual proteins and their variants.

With regard to PsbQ, the thesis deals with the potentially important N-terminal loop of PsbQ, not resolved in the available crystal structure. The authors used molecular dynamics simulation to model this loop and infrared and Raman spectroscopy of the recombinant protein to indicate relevance of several models. Modeling of the protein lacking the first 12 amino acids (physiologically relevant) explained inability of this protein to bind PSII. Moreover, Mrs. Kohoutová devised protocol for the isolation of isotope-labeled PsbQ for NMR spectroscopy.

Concerning PsbP, the protein from spinach was cloned, expressed in *E. coli,* purified as a His-tagged protein and crystallized. Its structure was determined by X-ray crystallography and compared with the available structure of the tobacco PsbP. Interestingly, both crystals failed to show the structure in two loops of the protein. These loops were modeled, based on the data from Raman spectroscopy, leading to a complete PsbP structure.

The last manuscript, for me the most interesting one, is dealing with PsbP-PsbQ interaction and clearly shows that the two proteins do interact and that the interaction is salt dependent. Unfortunately, this manuscript (to be submitted) is only torso consisting of several parts of results written by different people, with missing Discussion, not providing the overall picture for these nice data. As such, it would be better to include Mrs. Kohoutová data from this manuscript into Chapter 3 and combine them with the crosslinking studies.

Overall, the thesis is well-written and easy to follow (with the exception of the last manuscript). The English is understandable with reasonable number of mistakes.
An important asset of this thesis is that it is compact, well focused to the main topic – structure of two proteins and their mutual interaction. The methodology is carefully chosen to serve its purpose.

In summary, I find this thesis to be a solid piece of work clearly deserving the award of the Ph.D. degree.

Specific questions:

There are numerous reports of PsbP and PsbQ having their cyanobacterial homologues functioning in PSII. Would, in your opinion, solving crystal structure of cyanobacterial PSII containing these subunits help to elucidate their structural and functional roles in plants?

There are multiple genes in plant genomes coding for PsbP. As various functions are suggested for PsbP, do you think that it is possible that different PsbPs could be some-how specialized? How it is in spinach, do you think that it is important to choose the "right" PsbP for your study?

There is one report of PsbP being the Mn-binding protein. What do you think about this report, would you see Mn in your structure?

As a non-specialist, I do not understand how the methods used in the last manuscript (together with modeling) helped you to suggest the nature of the interaction and to propose amino acid residues suitable for mutagenesis. Are there some other methods that could be used before mutagenesis to make your list of residues shorter?

In the last manuscript you are stating that it is possible to separate PsbO protein from PsbP and PsbQ, but that it is difficult to separate PsbP and PsbQ (as not shown data). As this is an important finding, could you present the data – how difficult it is?

Could you compare previous models of PsbP-PsbQ arrangement (e.g. Fig. 10b) with your interaction model (Fig. xx3, last manuscript)?

Martin Tichý

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