CRYSTALLIZATION OF KILLER PROTEIN SPOIISA AND ITS ANTIDOTE SPOIISB FROM *BACILLUS SUBTILIS*

Patrik Florek[1], Katarína Muchová[1], Richard J. Lewis[2] and Imrich Barák[1]*

[1]*Institute of Molecular Biology, Slovak Academy of Sciences, 845 51 Bratislava 45, Slovakia*
[2]*Laboratory of Molecular Biophysics, The Rex Richard Building, University of Oxford, South Parks Road, Oxford OX1 3QU, UK*

The *B. subtilis spoIISA* gene encodes a 248-residue protein containing three predicted transmembrane domains [2] with the last two-thirds of protein being located in the cytoplasm. The *spoIISB* gene codes for a hydrophilic 56-residue protein. None of these proteins shares any sequentional similarity to a protein of known function, providing no clue to their function and evolutionary origin. The *spoIISB* translation start codon overlaps the *spoIISA* translation stop codon what is a strong indication that the two genes constitute an operon [1].

A null mutation in *spoIISB* leads to the strong sporulation defect, whereas disruption of either *spoIISA* or whole *spoIIS* locus has no effect on sporulation. Altogether, this facts indicates that a) *SpoIISA* prevents normal progression of the sporulation process; b) *SpoIISB* neutralizes the action of *SpoIISA*; and c) *spoIIS* locus does not play essential role in sporulation process. The strain carrying the *spoIISB* null allele does not exhibit any obvious defect during exponential growth. This immunity of exponentially growing cells to the absence of *SpoIISB* most likely reflects the existence of a threshold concentration below which *SpoIISA* does not significantly impair cell viability, since the induced expression of additional *spoIISA* gene copy led to rapid drop in optipcal density of exponential phase cell [1].

Since it has structural features of an integral membrane protein, *SpoIISA* could act as a holin and allow some endolysin to gain access to the peptidoglycan [3]. Local solubilization of the cell wall would lead to membrane disruption and consequently to the large plasmolysis zones which were observed by electron microscopy [1]. However, *SpoIISA* does not show any similarity to known holins and is significantly larger than holins identified so far [3]. It is therefore quite possible that the cytoplasmic membrane itself is the target of the toxic action of *SpoIISA*.

In our work we over-expressed cytosolic part of *SpoIISA* His-tag fusion protein together with intact *SpoIISB* protein in *Escherichia coli*. The both proteins were purified using single step metal chelate affinity chromatography, and therefore isolated proteins formed stable complex, which indicates their specific interactions. The gel filtration and electrophoresis experiments showed that the most abundant form of the complex is oligomer consisting of two SpoIISA and two SpoIISB molecules. This observation confirms the results gain using genetic complementation experiments, which predicted that SpoIIA acts as an oligomer [1]. The purified SpoIISA-SpoIISB protein complex was used for crystallization trials.

Work in authors laboratories is supported by grant 2/1004/22 from the Slovak Academy of Sciences and Wellcome Trust Project and Collaborative Research Initiative Grants (056247/Z/98/Z and 066732/Z/01/Z, respectively).

1. E. Adler, I. Barák, and P. Stragier. Bacillus subtilis locus encoding a killer protein and its antidote. *J. Bacteriol.* **183** (2001) 3574-3581.
2. M. G. Claros and G. von Heijne. TopPred II: an improved software for membrane protein structure prediction. *Comput. Applic. Biosci*. **10** (1994) 685-686.
3. I.-N.Wong, D. L. Smith, and R. Young. Holins: the protein clocks of bacteriophage infections. *Annu. Rev. Microbiol.* **54** (2000)799-825.

SHORTCUTS TO MUSHROOMS: NMR AND MS ANALYSES OF FUNGAL PYRANOSE OXIDASE

P. Halada[1], P. Sedmera[1], C. Leitner[2], K. Bezouška[1], J. Volc[1] and D. Haltrich[2]

[1]*Institute of Microbiology, Academy of Sciences of the Czech Republic,Vídeňská 1083, CZ-142 20 Prague 4, Czech Republic*
[2]*Division of Biochemical Engineering, Institute of Food Technology, University of Agricultural Sciences, Muthgasse 18, A-1190 Vienna, Austria*

Pyranose 2-oxidase (P2O, EC 1.1.3.10), a fungal periplasmic homotetrameric flavoprotein (~300 kDa), has received increased attention due to its potential analytical and biotechnological applications [1]. This enzyme catalyzes C-2/C-3 oxidation of numerous sugars to their corresponding dicarbonyl derivatives (aldos-2-uloses or glycosid-3-uloses), accompanied with the reduction of flavin adenine dinucleotide (FAD), an obligatory cofactor. P2O has a great biotechnological potential as a catalyst in the key step of C-2 oxidation of D-glucose and D-galactose in the production of modern low-caloric sweeteners D-fructose and D-tagatose.

Our research on the fungal pyranose oxidase followed three subjects. The first one was the study of the enzyme substrate specificity and characterization of its reaction products by spectral analyses (NMR, FAB mass spectrometry) [2,3]. Further, we applied MALDI mass spectrometry with post-source decay (PSD) analysis to determine sequence segments suitable for designing PCR primers for cloning cDNA corresponding to the P2O gene [4]. Finally, we elucidated the structure of the P2O flavin-binding domain, which is of importance for understanding the enzyme reaction mechanisms and possible optimized application. The combination of PSD-MALDI MS and electrospray ion trap mass spectrometry (ESI IT-MS) on the isolated flavopeptide identified flavopeptide sequence, flavin type and flavin linkage site. The type of the aminoacyl flavin covalent link was determined by NMR spectroscopy resulting in the structure STXW with X = 8 -(N^3-histidyl)-FAD [5].

The work was supported by Program for Scientific-Technical Cooperation AKTION, Austria-Czech Republic (2002-9) and Institutional Research Concept AV0Z5020903.

1. F. Giffhorn, S. Köpper, A. Huwig & S. Freimund, *Enzyme Microb. Technol.* **27** (2000) 734-742.
2. J. Volc, C. Leitner, P. Sedmera, P. Halada & D. Haltrich, *J. Carb. Chem.* **18** (1999) 999-1007.
3. J. Volc, P. Sedmera, P. Halada, P. Dwivedi & M. Costa-Ferreira, *J. Carb. Chem.*, in press (2003).
4. P. Halada, C. Leitner, J. Volc, D. Haltrich & V. Havlíček, *Collect. Czech. Chem. Commun.* **65** (2000) 1669-1676.
5. P. Halada, C. Leitner, P. Sedmera, D. Haltrich & J. Volc, *Anal. Biochem*., in press, (2003).

RECONSTITUTION OF MEMBRANE PROTEIN PSBH INTO NATURAL ALGAL LIPIDS

Zbyněk Halbhuber[1] and Dalibor Štys[1]

Photosynthesis Research Center, Institute of Physical Biology, University of South Bohemia, Zamek 136, 373 33 Nové Hrady, Czech Republic

Study of membrane proteins in their native environment is restricted from the complexity of native membranes, interference with other membrane constituents and other reactions. To understand organization of the biological membranes and the interaction-taking place between proteins, lipids and variable cofactors, artificial membranes are very useful. The PsbH protein is associated with the reaction centre of PSII in higher plants, algae and cyanobacteria. In our study *psbH* gene from cyanobacterium *Synechocystis sp.* PCC 6803 was cloned into a plasmid expression vector, which allowed a synthesis of the PsbH protein as a glutathione-S transferase (GST) fusion protein in *E. coli* BL21(DE3) cells. Although the exact role of the protein PsbH is not clear, it seems to be important for the structure and function of photosystem II. These structural and functional role could be closely associated with lipidic environment surrounding the protein. Moreover the protein could bind some cofactors e.g. pigments or in literature mentioned carbon dioxide [1].

Lipids were extracted from Synechocystis sp. PCC 6803 using method of Bligh and Dyer [2]. Extracted lipids were used to prepare liposomes by reversed phase evaporation. The detergent mediated reconstitution was performed according to Lévy et al. [3]. Interaction of lipids and other bound compounds was monitored by measurement of circual dichroism. Interaction of chlorophyls and protein was detected by low temperature fluorescence.

1. J. Komenda, L. Lupínková & J. Kopecký, *Eur. J. Biochem.*, **269** (2002) 610-619.
2. E. G. Bligh & W. J. Dyer, *Can. J. Biochem. Physiol.*, **37** (1959) 911-917.
3. D. Levy, A. Bluzat, M. Seigneuret & J. L. Rigaud, *BBA.*, **1025** (1990)179-190.

STRUCTURAL BIOLOGY ON THE SODIUM PUMP: A COMBINED APPROACH LEADING TO A FULL CHARACTERIZATION OF THE CATALYTIC DOMAIN

K. Hofbauerová[1,2], V. Kopecký Jr.[1,2,3], R. Ettrich[4], M. Kubala[2,3], J. Teisinger[2], O. Ettrichová[2,4] and E. Amler[2]

[1]Department of Biochemistry, Faculty of Sciences, Charles University, Albertov 2030, CZ-128 40 Prague 2, Czech Republic, email: hofbauer@biomed.cas.cz
[2]Institute of Physiology, Czech Academy of Sciences, Vídeňská 1083, CZ-142 20 Prague, Czech Republic
[3]Institute of Physics, Charles University, Ke Karlovu 5, CZ-121 16 Prague 2, Czech Republic
[4]Laboratory of High Performance Computing, Institute of Physical Biology USB and Institute of Landscape Ecology AS CR, University of South Bohemia, Zámek 136, CZ-373 33 Nové Hrady, Czech Republic

In this paper we summarize our previous work on the catalytic part of Na^+/K^+-ATPase. The nucleotide-binding domain of the subunit of mouse brain of Na^+/K^+-ATPase was expressed and isolated from *Escherichia coli* cells. The secondary structure of the expressed domain was experimentally determined by UV circular dichroism and Raman spectroscopy. By computer modeling was generated a three-dimensional model with and without docked ATP and predicted amino acids involved in the ATP binding site. ATP binding of wild type was followed by Raman difference spectroscopy and point mutants were measured by fluorescence spectroscopy with TNP-ATP. The set of eight amino acids residues was identified to form the complete ATP recognition site.

for full paper see page

APPLICATION OF DEGENERATE OLIGONUCLEOTIDE GENE SHUFFLING FOR CONSTRUCTION OF HYBRID HALOALKANE DEHALOGENASES

Andrea Jesenská[1], Yuji Nagata[2] and Jiří Damborský[1]

[1]National Centre for Biomolecular research, Faculty of Science - Masaryk University, Kotlářská 2, Brno, 611 37, Czech Republic
[2]Department of Environmental Life Sciences, Graduate School of Life Sciences, Tohoku University, Sendai 980-8577, Japan

Haloalkane dehalogenases are bacterial enzymes catalyzing cleavage of the carbon-halogen bond of halogenated aliphatic compounds by a hydrolytic mechanism. Improvement of cataytic properties of these environmentally important enzymes can be reached by application of non-recombinant directed evolution techniqes [1,2] or recombining several homologous genes [3].