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

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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

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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.

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

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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].