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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.

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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.

for full paper see page

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].

Four haloalkane dehalogenase genes of different origin were used to construct a hybrid gene: *dhlA* cloned from *Xanthobacter autotrophicus* GJ10 [4], linB from *Sphingomonas paucimobilis* UT26 [5], *dhaA* from *Rhodococcus rhodochrous* NCIMB13064 [6] and *dhmA* from *Mycobacterium avium* N85 [7]. The technique called Degenerate Oligonucleotide Gene Shuffling was used for *in vitro* recombination of four different genes [8]. Altogether twelve hybrid genes were constructed using one pair of degenerate oligonucleotides.

For preliminary characterization, hybrid proteins were expressed in *Escherichia coli* BL21(DE3) and tested in the resting cells assay for activity towards six halogenated aliphatic compounds. Four out of twelve hybrid proteins keep good expression and ten proteins showed obvious catalytic activity. Comparison of relative activities determined for the hybrid enzymes with the activities of wild type enzymes suggests that constructs do not possess novel substrate specificities.

All hybrid genes were cloned to pET-32(a) vector to support high level of expression an stability of hybrid proteins. His-taggged tail was introduced to C-terminus of hybrid proteins. All hybrid haloalkane dehalogenases were successfuy expressed in fusion with thioredoxin in host cells *E. coli* HB101. Optimization of purification conditions on Ni-NTA agarose and cleavage of hybrid protein-thioredoxin complexes is under progress.

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TOWARDS TRUE SSTABILIZATION ENERGIES OF H-BONDED AND STACKED DNA BASE PAIRS

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The study of binding energies of Adenine...Thymine and Guanine...Cytosine base pairs in vacuo was aimed to get an information on relative order of different structure motives on the energetical scale with special focuse on differences between H-bonded and stacked structures. We also aimed to find a general way to reliable interaction energies of the weakly bonded complexes.

Structures taken from previous MD/quench studies were fully optimized at RI-MP2 [1,2] level with TZVPP [5s3p2d1f]/[3s2p1d] basis set. To approach complete basis set (CBS) limit, convergency of both HF energy and MP2 correlation energy was studied employing augmented correlation-consistent basis sets aug-cc-pV(D,T,Q)Z. Is was found that molecular interaction energies close to the CBS limit may be obtained by 2-point extrapolation [3] using aug-cc-pVDZ and aug-cc-pVTZ basis sets.

To account for higher order correlation effects convergency of CCSD(T) MP2 correction term (E_{corr}^{MP2} - E_{corr}CCSD(T)) was investigated. For the formamide… .formamidine complex (Fig. 1) which is a model for adenine..thymine interaction MP2 and CCSD(T) correlation interaction energies were evaluated with various basis sets up to aug-cc-pVTZ (Fig. 2). It was shown that unlike the correlation energy itself the CCSD(T) MP2 difference is almost basis set independent. Rather accurate values were obtained with relatively small 6-31G*(0.25) and cc-pVDZ(0.25,0.15) basis sets. Because the latter one performs well also for stacked complexes [4] it can be recommended for evaluation of the term of extended complexes possessing both H-bonded and stacked structures.

Interaction energies of the DNA base pairs obtained by combination of the CBS extrapolations of MP2 interaction energies and the CCSD(T) MP2 correction are in good agreement with experiment.

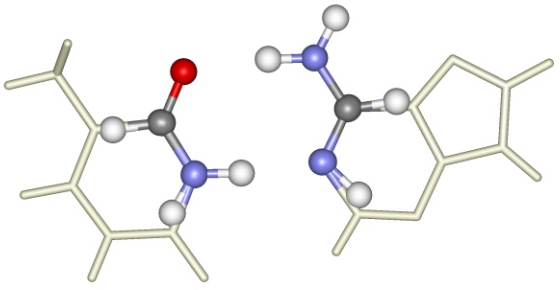

Figure 1