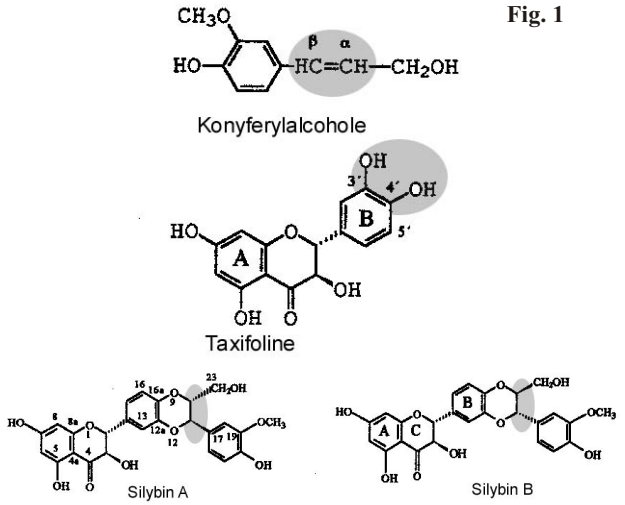

Fig. 1

Recent studies revealed that many transport and metabolic processes in the cell are stereospecific. Silybin occurs in two stereoisomers (A and B) that differ in the bound between konyferyl and taxifolin (Fig. 1). We developed a new method for preparation and purification of these silybin stereoisomers and for their specific labelling by radioactivity (3H, 125I) at positions 6 and 8. Transport of four stereoisomers was studied. The best affinity of transport systems were found for 6-[125I]silybinA, which is taken 100 times better than the other silybin stereoisomers.

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A THEORETICAL STUDY OF THE PHOTOSYNTHETIC REACTION CENTRE PSII

Josef Šeda, Jaroslav V. Burda

Department of Chemical Physics and Optics, Faculty of Mathematics and Physics, Charles University, Ke Karlovu 3, 12000 Prague, Czech Republic.

Mutlireference perturbation theory (CAS-PT2), random phase approximation (RPA), configuration interaction with singles (CIS) (all at various basis sets) and semiempirical ZINDO methods were used for the determination of the excited states energies of free-base porphin and Mg-porphin. On the basis of these results an error estimation of the methods used for larger systems (molecules in PSII reaction center) calculation can be established.

The PSII model in ref. (1) was used for the electron-exication spectra determination. First, individual ZINDO spectra of monomers chlorophyll-a and pheophytine-a were estimated for semiempirically (PM3) fully optimized structures.

As a next step, partial optimizations (the hydrogen atoms only) on all the monomers, selected dimers, trimers, tetramers, and hexamer were done at the same PM3 semiempirical level, keeping the positions of all the "x-ray determined atoms" fixed. Selection of studied dimeric and oligomeric structures is based on the considerations of the multiparticle (multimolecular) excitations. These multiparticle specta exhibits complex non-additive character where all the basic spectra lines (Qx, Qy, Soret lines) are shifted unevenly from their positions in isolated molecules. This uneven character speaks out about the different importance of individual molecules in the various excitations.

Anothe comparison of electron-excitation spectra using ZINDO and RPA was performed for the chlorophyll-a and pheophytine-a molecules, as well. For deeper elucidation, the structures used in comparison were both fully-optimized and "hydrogens-optimized" (taken from RC model).

(1) S. Ruffle, D. Donnelly, T. Blundell, J. Nugent, A 3-Dimensional model of the photosystem-II reaction center of Pisum-Sativum, Photosynthesis research, 34 (1992), 287-300.

OVEREXPRESSION AND PURIFICATION OF RECOMBINANT MEMBRANE PROTEIN PSBH IN ESCHERICHIA COLI

Zbyněk Halbhuber[1], Zdenka Petrmichlová [1,2], Kassimir Alexciev[3], Eva Thulin[4] and Dalibor Štys[1,2,5]

[1]Photosynthesis Research Center, Institute of Physical Biology, University of South Bohemia, Zamek 136, 373 33 Nové Hrady, Czech Republic
[2]Department of Autotrophic Microorganisms, Institute of Microbiology CAS, 37901 Trebon - Opatovický mlýn
[3]CanAg Diagnostics, 414 55 Gothenburg , Sweden
[4]Biophysical Chemistry, Lund University, Box 124, 22100 Lund, Sweden5
[5]Institute of Landscape Ecology, Academy of Science of the Czech Republic, Zamek 136, 373 33 Nové Hrady

In this work we featured an expression system that enables the production of sufficient quantities of membrane PsbH protein (~mg´s quantities) for solid-state NMR as well as other biophysical studies. PsbH is a small membrane protein associated with the photosystem II complex in higher plants, algae and cyanobacteria. Although the exact role of PsbH is not clear, it seems to be important for the structure and function of photosystem II.

In this approach a synthetic psbH gene from cyanobacterium *Synechocystis sp.* PCC 6803 was cloned into a plasmid expression vector, which allowed a direct synthesis of the PsbH protein as a glutathione-S transferase (GST) fusion protein in *E. coli* BL21(DE3) cells. A relatively large GST anchor overcome foreseeable problems with the low solubility of membrane proteins and the toxicity caused by protein incorporation into the membrane of the host organism. As a result, the majority of fusion protein was obtained in a soluble state and could be purified from crude bacterial lysate by affinity chromatography on immobilised glutathione under non-denaturating conditions. The PsbH protein was cleaved from the carrier protein with Factor Xa protease and purified on DEAE- cellulose column with yields of up to 2.1 μg protein/ml of bacterial culture. Details of sample optimization for small membrane proteins as well as the impact constitutive cell protection mechanism against host membrane proteins are discussed.