intercalators studied) this difference is explained by the importance of the charge transfer term which is not included in the AMBER potential. The Hartree-Fock and DFT/B3LYP methods not covering the dispersion energy fail completely to describe any energy minimum at the potential energy curve of the E…AT complex and these methods thus cannot be recommended for a study of intercalation process. On the other hand, a modified version of DFT method which covers London dispersion energy yields for all complexes very good stabilization energies well comparable with referenced *ab initio* data. Besides vertical dependence of interaction energy twist dependence of interaction energy was also investigated by both, reference correlated *ab initio* method as well as empirical potentials. It is concluded that despite the charged (E +1, D +1, DAPI +2) or polar (EL) character of intercalators investigated it is the dispersion energy which predominantly contributes to the stability of intercalator…DNA base pair complexes. Any procedure which does not cover dispersion energy is thus not suitable for studying the process of intercalation.

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ANALYSIS OF INTERACTIONS IN COMPLEXES OF HIV-1 PROTEASE AND ITS PEPTIDOMIMETIC INHIBITOR

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HIV-1 protease is a 22 kDa protein of the human immunodeficiency virus. The function of this protein is to cleave polyprotein of immature virus and thus to contribute to formation of active matured virus. Inhibition of the protease is therefore one of possible ways of fighting with disease AIDS, caused by the human immunodeficiency virus.

Our research was focused on interaction analysis of HIV-1 protease and its peptidomimetic inhibitor Boc-Phe- $[CH_2CH_2NH]$-Phe-Glu-Phe-NH_2, denoted as OE. The inhibitor was developed in the laboratory of J. Konvalinka (Institute of Organic Chemistry and Biochemistry, Academy of Sciences CR). Native and mutant (A71V, V82T, I84V) HIV-1 protease were expressed and purified in the laboratories of J. Sedláček (Institute of Molecular Genetics, Academy of Sciences CR) and J. Konvalinka. In our research group, crystallization of complexes of OE with native and mutant protease was performed, X-ray diffraction of crystals on the synchrotron source of radiation was measured and structures of both complexes were determined ([1], [2]).

As a result, we have two structures with *R*-factors 18 % (native protease complex, diffraction limit 2.45 A) and 20.3 % (mutant protease complex, diffraction limit 2.2 A). Both complexes crystallized in space group P61 and inhibitor OE was found in the active site in two approximately C2 symmetrical positions, following thus pseudo-symmetry of the protease. This fact makes interpretation of interactions between the protease and inhibitor more difficult. Therefore, standard structural analysis of contacts between the protease and inhibitor was completed by two energy analyses of interactions in the active site. The inhibitor binding modes to both proteases are similar from the structural point of view and interpretation of small details could be ambiguous. However, energy analysis of both complexes confirms the interpretation of changes caused by mutation of the protease. Mutated residue Thr 182 forms an aromatic hydrogen bond to the inhibitor phenyl group in P1 position. Mutation I84V causes a decrease in van der Waals interaction between residue 84 and the OE inhibitor.

The research was supported by the Grant Agency of the Academy of Sciences of the Czech Republic (projects A4050811/1998 and B4050312/2003) and by the Academy of Sciences of the Czech Republic (project AVOZ4050913).

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MEMBRANE PSEUDO-CRYSTAL STRUCTURES IN PSSU-IPT TOBACCO CHLOROPLASTS

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Our study is focused on native pseudo-crystalline structures, which were observed in chloroplasts of transgenic tobacco overproducing plant hormones cytokinins. The structures were not positively identified until now. We suppose that they are formed by light harvesting protein (LHC) aggregating in a form of 2D crystal, which then constitute membrane stacks. Our hypothesis is supported by fluorescence emission spectra, which showed certain bands corresponding to LHC aggregates and higher emission of chlorophyll *b* in chloroplasts izolated from transgenic plants.

The aim of this experiment was the estimation of relative size of pseudo-crystals compared to chloroplast and the size of basic cell unit, which can be determined from analysis of TEM images from ultrathin sections of leaves and isolated chloroplast suspensions.

Transgenic tobacco containing a supplementary ipt-gene under a control of the promoter for the small subunit of RuBPCO (P*ssu-ipt*) was grown as grafts on non-transgenic

tobacco (*Nicotiana tabacum* L. cv. Petit Havana SR1) rootstock as described by Synková et al. (J. Plant Physiol. 155: 173-182, 1999) or as rooted plants (kanamycin resistant progeny of the transgenic grafts). Samples for TEM were taken from the central part of the young fully developed leaf or isolated chloroplast suspension and after overnight fixation in 3 % glutaraldehyde were embedded in Spurr's resin. Ultrathin sections were stained by uranyl acetate and Reynold's lead citrate and examined in JEM 1010 (Jeol, Japan). Analysis of serial sections by program IMOD 2.42 enabled three dimensional (3D) reconstructions of chloroplasts and pseudo-crystals. The size of basic structural unit was calculated using MRC Cambridge Image Processing System (1994).

3D reconstruction showed that pseudo-crystalline structures occupy up to 20 % of chloroplast volume (at least in that part of chloroplast which was studied).

The average size of basic cell unit was calculated as: a = 11 nm, b = 12 nm, = 100 .This size parameters support our hypothesis, although dehydration preceding embedding in epoxide resin cause a shrinkage of natural structures. Therefore further experiments are needed to prove it.

Acknowledgements

The authors thank to Dr.J. Nebesářová and the staff of her laboratory (IP, Lab.of EM, České Budějovice) for the assistance in electron microscopy. The work was supported by grant of the Grant Agency of the Czech Republic No. 206/01/1061.

THE ROLE OF STRUCTURAL DIFFERENCES OF FLAVANOLIGNAN SILYBIN STEREOIZOMERS IN BINDING TO HEPATOCYTES

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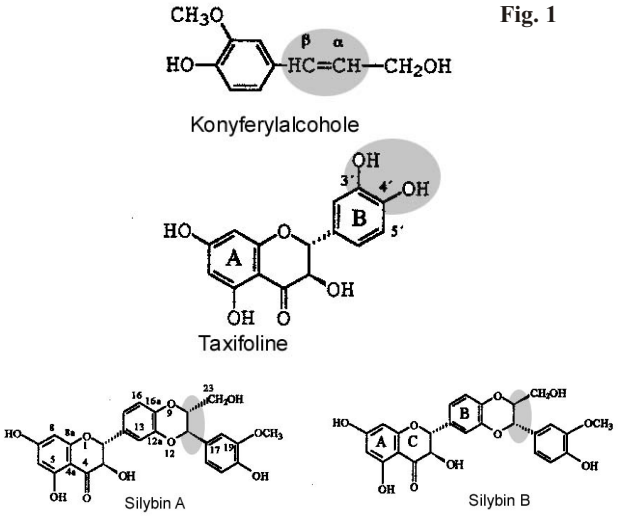
Hepatoprotective effects of Milk Thistle (*Silybum marianum*) have been known since ancient Greece and Roma very well. Flavanolignans (called silymarine) extracted from Milk Thistle seeds were shown to help against hepatotoxic effects of many natural toxins (i.e. alga toxin microcystine, mushroom toxins amanitin and phaloidin, fungal toxins cyclosporines, etc.). The main active substance of silymarin is silybin.

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.

Fig. 1



This work was supported by grants GACR 204/98/P129, MSMT CEZ:J06/98:123100001 and GACR 521/99/D098.

OVEREXPRESSION AND PURIFICATION OF RECOMBINANT MEMBRANE PROTEIN PSBH IN ESCHERICHIA COLI

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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)