ratory [2] we established that calcium ion can be bound tigtly in the molecule and this binding increases the affinity of the protein to N-acetylglucosamine and N-acetylgalactosamine. The positions of binding sites has been suggested by molecular modeling and proved by site-directed mutagenesis.

These data allowed us to find potential high affinity ligands among branched oligosaccharides terminated with N-acetylglucosamine units. We isolated these molecules by deglycosylation of ovomucoid and characterized them by mass spectrometry. From results of our binding studies we can conclude that pentaantenary structure is the ligand with the highest known affinity for CD69 molecule. It has been published [3, 4] that similar structures are expressed on the surface of some tumor cells. This finding indicates that one role of CD69 molecule on the cells of the immune system may be to attract killer lymphocytes to the tumor sites.

Acknowledgement: *This work was supported by Institutional Research Concept No. AV0Z5020903 (for Institut of Microbiology) and by grants MSM 113100001, GACR 203/01/1018 and A7020006.*

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A DFT INVESTIGATION OF STRUCTURE-CHEMICAL SHIFT RELATIONSHIPS FOR 13C AND 15N IN DNA

Jana Přecechtělová, Markéta L. Munzarová and Vladimír Sklenář

National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Kotlárská 2, CZ-611 37 Brno, Czech Republic

Density functional theory has been applied to explore the dependence of ^{13}C and ^{15}N chemical shifts in deoxyribonucleosides on various structural features such as the orientation about the glycosidic bond, the CH2OH group conformation, the sugar pucker, and the hydrogen bonding. Geometry optimizations have been performed with sugar-phosphate backbone dihedral angles frozen to their average experimental values in BI-DNA. Results obtained in NMR parameter calculations have been compared to available experimental data for C1`, C2` and N9.

The effect of the glycosidic torsion angle c has already been studied [1] but we wished to involve the relaxation of the geometry after changing c, which has not been considered in the previous work [1]. C1`, C2` and N1/N9 chemical shifts appeared to be influenced most by the base orientation. The trends uncovered in chemical shifts are significantly different from those reported previously [1] and the absolute chemical shift values are in the case of C2` approximately the same for all deoxyribonucleosides, except for the anti orientation of the base. On the contrary, for C1` and N1/N9 the trends for purine nucleosides differ from those for pyrimidine nucleosides and the absolute N1 chemical shifts in deoxycytidine are found upfield relative to deoxythymidine.

Besides the influence of varying the glycosidic torsion angle, we wanted to assess the effect of the sugar puckering and the hydroxymethyl rotation, both of which were studied on deoxyguanosine. N9 experienced the largest changes, namely 10 or 8 ppm difference between the south and north conformation in both the syn and anti region, respectively. The N9 chemical shift for deoxyguanosine (*S, anti, gg*) differed significantly from the other two CH_2OH-rotamers.

The comparison with the experiment has been carried out using the data from BMRB database [2] (C1`, C2`) and the data for the $[d(G_4T_4G_4)]2$ quadruplex (C1`, N9) [3], on which changes upon the hydrogen bonding have also been studied.

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NATURE OF STACKING INTERACTIONS BETWEEN INTERCALATORS AND DNA BASE PAIRS. AB INITIO QUANTUM-CHEMICAL, DENSITY FUNCTIONAL THEORY AND EMPIRICAL POTENTIAL STUDY.

David Řeha, Martin Kabeláč, Filip Ryjáček, Jiří Šponer and Pavel Hobza

J. Heyrovský Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, and Center for Complex Molecular Systems and Biomolecules, 182 23 Prague 8, Czech Republic

Properties of isolated intercalators (ethidium (E), daunomycin (D), ellipticine (EL) and 4,6'-diaminido-2-phenylidone (DAPI)) and their stacking interactions with adenine…thymine (AT) and guanine…cytosine (GC) nucleic acid base pairs were investigated by means of a nonempirical correlated *ab initio* method [1]. All intercalators exhibit large charge delocalization and neither of them (including dicationic DAPI) exhibit a site with dominant charge. All intercalators have large polarizability and are good electron acceptors while base pairs are good electron donors. MP2/6-31G*(0.25) stabilization energies of complexes intercalator…base pair are large (E…AT : 22.4 kcal/mol; D…GC :17.8 kcal/mol; EL…GC :18.2 kcal/mol; DAPI…GC :21.1 kcal/mol) and are well reproduced by modified AMBER potential (vdW radii of intercalator atoms are enlarged and their vdW energy depths are increased). Standard AMBER potential give less satisfactory results especially for DAPI containing complexes. Because DAPI is the best electron acceptor (among all

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

T. Skálová, H. Petroková, J. Hašek, J. Dohnálek, E. Buchtelová, J. Dušková

Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, Heyrovského nám. 2, 162 06 Praha 6, Czech Republic

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

Helena Synková[1], Renáta Pechová[3], Michal Hušák[2], František Vácha[2], Pavel Šiffel[2]

[1]Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Na Karlovce 1a, CZ-160 00 Praha 6
[2]Institute of Physical Biology, University of South Bohemia, Branišovská 31, 370 05 České Budějovice
[3]Department of Plant Physiology, Faculty of Sciences, Charles University, Viničná 5, CZ-128 44 Praha 2, Czech Republic

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