NANOSECOND MOLECULAR DYNAMICS OF HIV PROTEASE- INHIBITOR COMPLEXES:INSIGHTS INTO THE DIFFERENTIAL BINDING POTENCY OF DIASTEREOISOMERES

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The inhibitory potency of four nanomolar diastereomeric inhibitors of HIV-1 protease [1] was studied by molecular dynamics simulations and MM-GBSA/PBSA analysis. As a starting point we used the crystal structures of protease-inhibitor complexes [2, 3]. Having added hydrogens, we surrounded the complexes with a box of explicit water molecules and added counterions to neutralize the box. Using AMBER 7 program package [4], we minimized, heated and equilibrated the system after which we ran 2-nanosecond-long production dynamics. Periodic boundary conditions were used and long-range electrostatics was treated by particle mesh Ewald (PME) technique.

An analysis of the molecular dynamical trajectories was performed and their quality assessed. The protease-inhibitor binding energies were calculated with MM-GBSA/PBSA approach. The effect of the length of the simulation, method to calculate solvation energy, and other factors upon the results was determined.

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1. J. Konvalinka, J. Litera, J. Weber, J. Vondrášek, M. Hradílek, M. Souček, I. Pichová, P. Majer, P. Štrop, J. Sedláček, A. M. Heuser, H. Kottler and H. G. Kraeusslich, *Eur. J. Biochem.* **250**, (1997), 559-566.
2. J. Weber, J. R. Mesters, M. Lepšík, J. Prejdová, M. Švec, J. Šponarová, P. Mlčochová, K. Střížovský, T. Uhlíková, M. Souček, L. Machala, M. Staňková, J. Vondrášek, T. Klimkait, H. G. Kraeusslich, R. Hilgenfeld & J. Konvalinka, *J. Mol. Biol*, **324** (2002), 739-54, pdb code 1IZH.
3. Mesters et al, unpublished data
4. D. A. Case, D. A. Pearlman, J. W. Caldwell, T. E. Cheatham III, J. Wang, W. S. Ross, C. L. Simmerling, T. A. Darden, K. M. Merz, R. V. Stanton, A. L. Cheng, J. J. Vincent, M. Crowley, V. Tsui, H. Gohlke, R. J. Radmer, Y. Duan, J. Pitera, I. Massova, G. L. Seibel, U. C. Singh, P. K. Weiner & P. A. Kollman (2002), AMBER 7, University of California, San Francisco.

FULL CHARACTERIZATION OF NATURAL KILLER CELL MEMBRANE MICRODOMAINS

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Natural killer (NK) cells are cytotoxic effector lymphocytes, which do not express antigen-specific cell surface receptors. NK receptors that mediate signals leading to the initiation or supression of natural cytotoxicity processes are poorly characterized. Moreover, these receptors may associate with other co-stimulating molecules and adaptor proteins, which transduce the signal from the receptor to the cell. Such complexes may be observed as biochemically distinct parts of plasmatic membranes and are commonly referred to micro domains or glycosphingolipid enriched microdomains (GEMs). They are enriched in GPI-anchored and acetylated proteins and in cholesterol and glycosphingolipids. It is believed that they may aid in signal transduction as well as in trafficking through the secretory and endocytic pathways and in cell to cell interaction.

In this study we focused on membrane microdomains from rat NK leukaemia cell line (RNK-16). For detailed characterization we used a shotgun strategy based on microcapillary HPLC - tandem mass spectrometry. Aditionally, we applied techniques of native electrophoresis for detailed mapping of protein complexes present in the GEMs. We have identified a large number of proteins (e.g. gp-42, CD2, LAT, CD161, CD44 or g-proteins in GEM and tubuline in non-GEM fractions).

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HIGH AFFINITY LIGANDS FOR HUMAN LYMPHOCYTE RECEPTOR CD69

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CD69 is one of very important activating receptors expressed on the surface of human lymphocytes. This molecule exists as a homodimer, each of its subunits being terminated with the globular domain related to the C-type lectin family. Although the crystal structure of this domain has been recently solved [1], its potential ligands and the function of the whole receptor remain unclear. In our labo-

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.

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1. S. Natarajan et al., *Biochemistry*, **39** (2000) 14779-14786.
2. K. Bezouška et al. *BBRC*, 208 (1995) 68-74.
3. J. W. Dennis et al. *Science*, 236 (1987) 582-585.
4. E. Gorelik et al. *Cancer and Metastasis Reviews*, **20** (2001) 245-277.

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

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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.

1. X.-P. Xu, S. C. F. Au-Yeung, *J. Phys. Chem. B*, **104** (2002) 5641-5650.
2. Http://www.bmrb.wisc.edu.
3. L. Trantírek, R. Štefl, J. E. Masse, J. Feigon, V. Sklenár, *J. Biomol. NMR*, **23** (2002) 1-12.

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

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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