Session IV-b, Wednesday, September 11

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BIOINFORMATIC ANALYSIS OF PROTEIN/DNA COMPLEXES

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Recognition between proteins and DNA is a key step of essential biological processes. To investigate structural features of the recognition we analyzed the geometry of the protein/DNA interface calculated from more than thousand crystal structures of protein/DNA complexes.

Novelty of the analysis lies in our fine-grained categorization of protein and DNA local conformations involved in the interactions. The conformations of proteins and DNA were categorized into structural classes according to the previously published structural alphabets. Protein structures were sorted into sixteen pentapeptide units called "*peptide blocks*" [1] and DNA into 14 dinucleotide conformers derived from the original dictionary of conformers [2]. We determined what *peptide blocks* and *dinucleotide conformers* formed the protein/DNA interface and which of them were in mutual contact. The resulting matrix showing which *peptide block* contacted which *dinucleotide conformer* was called *interaction matrix*. The *interaction matrices* were constructed and analyzed for various types of protein/DNA complexes such as DNA complexes of polymerases, transcription factors, or structural proteins. The variability of these interaction matrices characterizes differences of recognition patters of different types proteins. The *interaction matrices* were constructed and analyzed for direct polar contacts between protein and DNA atoms and for contacts mediated by water molecules and also separately for the DNA atoms forming the minor groove, major grove, and the phosphate group. Water-mediated contacts are almost as frequent as the direct polar ones and the contacts to the minor groove display distinctly different patterns from the major groove and phosphate atoms.

This work was supported by the Czech-France collaboration Barrande (MEB021032) in years 2009-2010 and is supported by the Czech Science Foundation (P305/12/1801). BS and JC are supported by the institutional grant AV0Z50520701.

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SELECTIVE INHIBITORS OF 5´-NUCLEOTIDASES

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The monophosphate 5`-nucleotidases are ubiquitous enzymes that catalyze the dephosphorylation of nucleoside monophosphates and regulate thus nucleotide and nucleoside pools in cell [1-6]. The ribonucleotides and deoxyribonucleotides could be synthesized *de novo* from low molecular weight precursors or by a salvage pathway from nucleosides or nucleobases originating from catabolism of nucleic acids [1]. In this salvage pathway, ribonucleotides and deoxyribonucleotides are phosphorylated by nucleoside and nucleotide kinases to maintain sufficient pools of dNTP's and NTP's for nucleic acid synthesis. The phosphorylation by cellular nucleoside kinases is opposed by the activity of 5'-nucleotidases that dephosphorylate ribo- and deoxyribonucleoside monophosphates [2-4]. Besides their role in the regulation of physiological dNTP pools, substrate cycles between ribonucleotidases and kinases may affect the therapeutic action of pyrimidine nucleoside analogs used as anticancer and antiviral agents. Such compounds require the nucleoside kinases activity for phosphorylation to their active forms. Results of clinical and in vitro studies propose that an increase in nucleotidase activity can interfere with nucleoside analogue activation resulting in drug resistance [5].

The aim of this project is structure-assisted design of highly selective inhibitors of mammalian 5'-nucleotidases based on nucleoside phosphonic acid derivative scaffold. In general, the compounds with a strong and selective inhibitory potency are of high medicinal interest as antimetabolites for anticancer and antiviral therapy.

Using the structure of human mitochondrial 5'-nucleotidase [6] and our structure of cytosolic 5'-nucleotidase we performed *in silico* screening of a virtual library containing 29 thousand compounds. A set of compounds with highest scores was synthesized and screened for their inhibitory ef-

fect toward two isoforms of human 5'-nucleotidase: cytosolic (cdN) and mitochondrial (mdN).

By this approach we have discover compounds specifically inhibiting the mdN and cdN with inhibitory potency two orders of magnitude better compared to the formerly reported mdN and cdN inhibitors, respectively. Structural details of interactions of newly identified compounds with both nucleotidases were investigated through determination of high-resolution crystal structures.

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This work was supported by the Grant agency of the Czech Republic (research project No. GA 203/09/0820). Diffraction data have been collected on BL14.1 and BL14.2 operated by the Helmholtz-Zentrum Berlin (HZB) at the BESSY II electron storage ring.

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CRYSTALLOGRAPHIC STUDIES OF SELECTED HALOALKANE DEHALOGENASES

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Haloalkane dehalogenases (EC 3.8.1.5) belong to the group of enzymes hydrolyzing carbon-halogen bonds in a wide range of halogenated compounds. Many of those organic compounds are environmental pollutants. The potential use of haloalkane dehalogenases is in bioremediation applications, in application of these enzymes as industrial biocatalysts, as active components of biosensors, and also in synthesis of pharmaceuticals, agrochemicals or food additives.

Several haloalkane dehalogenases have been selected for structural studies perforce to study particular enzyme structures and clarify precise function of those enzymes.

Crystallization procedures and parameters affecting crystal growth will be discussed during the lecture.

This research is supported by the GACR (P207/12/0775), ME CR (CZ.1.05/2.1.00/01.0024) and by the AS CR.

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MODIFICATION OF ACTIVITY, SPECIFICITY AND STABILITY OF HALOALKANE DEHALOGENASES BY ENGINEERING OF BURIED HALIDE-BINDING SITES

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Haloalkane dehalogenases (HLDs; EC 3.8.1.5) are bacterial enzymes cleaving a carbon-halogen bond by a hydrolytic dehalogenation [1]. Crystal structure of the haloalkane dehalogenase DbeA from *Bradyrhizobium elkani* USDA94 was solved to 2.2 Å resolution and revealed the presence of two binding sites for chloride aniont. The *first* chloride anion was found in the active site in between two conserved halide-stabilizing residues Asn38 and Trp104. This binding site is common for all members of the subfamily HLD-II [2]. The *second* chloride anion is placed about 10 Å from the *first* binding site, buried deep in the protein core, where it is coordinated by the side chains of Gln274, Gln102, Gly37 and Thr40. This *second* halide-binding site is unique to DbeA and its closely related enzyme DbjA [3,4] from *Bradyrhizobium japonicum* USDA110 (71% sequence identity) and has not been observed in any other crystal structure of HLD-II enzymes.

To elucidate the effect of the second halide-binding site on the structure and function of DbeA enzyme, two-point variant I44L+Q102H lacking the second halide-binding site was constructed, purified and biochemically characterized. Its comparison with the wild type enzyme revealed that elimination of the second halide-binding site decreased the stability of the enzyme in the presence of chloride salt and decreased its catalytic activity without change of its catalytic mechanism. Moreover, the two-point substitution resulted in a shift of the substrate-specificity class, which is the first time this has been demonstrated for the haloalkane dehalogenase enzyme family. Changes in the catalytic activity of the variant were attributed to deceleration of the rate-limiting hydrolytic step, mediated by lower basicity of the catalytic histidine. Our study demonstrates that engineering of the buried halide-binding sites has significant impact on enzyme′s activity, specificity and stability. Rational design of buried halide-binding sites could represent a novel strategy for engineering of biocatalysts with desired catalytic properties.

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