6th Discussions in Structural Molecular Biology

Academic and University Center, Nové Hrady, March 29 - 310, 2007

Conference Committee

Bohdan Schneider, Ivana Kutá Smatanová, Radomír Kužel, Dalibor Štys, Jindřich Hašek

Lectures - Thursday, March 29

L1

THE THREE-DIMENSIONAL STRUCTURE OF RYEGRASS MOTTLE VIRUS AT 2.9 Å RESOLUTION

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The crystal structure of ryegrass mottle virus (RGMV) has been determined at 2.9 Å resolution. The icosahedral capsid contains 180 copies of the coat protein arranged with T = 3 quasi-symmetry. The coat protein has a jellyroll -sandwich fold similar to the other sobemoviruses. A comparison of the sequences and structures of viruses in the genus shows that the RGMV coat protein has a deletion in one of the loop regions that are conserved among the other sobemoviruses. The full-size loop contains a helix that participates in the stabilization of the N-terminal arms. The structure of RGMV appears to compensate for the deletion by having longer â-strands in the N-terminal arm. The interactions of coat proteins within the icosahedral asymmetric unit of sobemoviruses usually involve calcium ions. We could not identify any density for metal ions in the proximity of the conserved residues normally involved in calcium binding. Nevertheless, calcium ions are necessary for RGMV particle stability at neutral pH, since particles depleted of calcium disassemble at high ionic strength. A likely reason for the absence of the calcium ion is the low pH of the crystallization buffer.

L2

CRYSTALLOGRAPHIC STUDIES OF INTERMOLECULAR INTERACTIONS OF Fc-FRAGMENT OF IMMUNOGLOBULIN

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IgG antibodies are one of the key mediators of immune system response to some viral infections. They are also used in medicine for many different purposes, such as strengthening of immune system, detection of primary tumors and microscopic metastasis using scintigraphy, etc.

Antibodies are composed of three globules (fragments): two Fab-fragments (Fragment-antigen binding), Fc-fragment (Fragment-crystallizable). Fab-fragments are responsible for antigen detection, Fc-fragment (tertiary structure shown in Fig. 1) plays an important role in immune system activation. In our structure solution [1], the Fc-fragment of mouse monoclonal IgG2b against carbonic anhydrase MN CA IX was cleaved from intact antibody with papain.

The Fc-fragment is functional as a dimer. A monomer is formed by two domains, named C 2 and C 3, joined by a

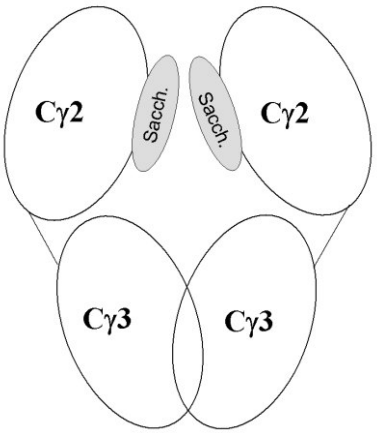

Figure 1. Diagram of tertiary structure of the Fc-fragment of an antibody.

linker composed of four amino acids. Two C 3 domains form a compact non-covalent dimer. The C 2 domains are linked by hydrogen bonds between oligosaccharides covalently attached to asparagines, disulfide bridges between cysteins in the region linking these domains to the Fab fragments were found only in a few structures in the PDB database [2]. In our case [1], the area of disulfide bridges is disordered (flexible) and without a possibility of clear structure interpretation.

The PDB database [2] contains thirty records with structures of Fc-fragment. They can be divided into four groups: Fc-fragments cleaved from antibodies (by papain, pepsin, etc.), intact antibodies, Fc-fragment in complex with a ligand attached via the C2-C 2 interface, Fc-fragment in complex with a ligand attached via the C 2-C 3 interface. An extensive analysis of structure and interaction of oligosaccharide chains was performed and the results summarized.

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L3

PROTEIN AS A FLEXIBLE STRUCTURE: CASE STUDY OF THE HIV-PROTEASE

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In order to find properties of the minimal model of protein flexibility, we introduce a two-variable coarse-grained model describing the rigidity of a three-dimensional structure—protein. We follow local changes of rigidity after removing a certain fraction of contact interactions among amino-acid residues. In this way, mobility of a single residue may be estimated. The model is mapped to a constraint satisfaction problem and solved via the Belief Propagation iterative message–passing algorithm.

For the HIV protease, the resulting flexibility profiles are compared with the experimental temperature factors data, burial profile predictions and Molecular Dynamics simulations. Although we cannot estimate the actual presence or absence of a physical interaction between specific residues, we show that the contact interactions in the HIV protease are distributed in the way that maximizes the overall flexibility of the protein. This confirms the notion of the HIV Protease as a flexible albeit compactly folded structure.

L4

TLS ANALYSIS OF ANTIBODY STRUCTURE SHOWS STABILIZATION BY EPITOPE BINDING

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Specific antibodies interfere with function of human carbonic anhydrase IX (CA IX), and attract attention as tools for anti-cancer interventions. This work presents a comparison between structural elements and thermodynamic parameters of association of an antibody fragment, Fab M75 [1], to a peptide representing its epitope in the proteoglycan-like domain of CA IX. Comparisons of crystal structures of free and liganded Fab fragment reveal major re-adjustments of H1 and H3 CDR loops. In contrast, shapes and positions of H2 and L2 CDR loops remain unaltered and their positively charged residues may thus present a fixed frame for epitope recognition and for the consecutive induced fit. Attainment of the altered H3 CDR loop conformation in the complex structure is accompanied with evident local stabilization, i.e. with decreased mobility, as measured with residual atom displacement parameters (ADPs). Analysis of domain mobility with translation-libration-screw (TLS) parameterization shows

that librations of entire heavy chain variable domain (V_H) become decreased in magnitude and reoriented in the complex. This effect corresponds well with extensive involvement of the heavy chain in the ligand binding. Isothermal titration microcalorimetry (ITC) [2] yields high unfavorable entropy term, attributable, in part, to local stabilization of the H3 CDR loop. Molecular dynamic simulations indicate major roles for the charged residues of epitope.

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L5

INTERACTION OF FORKHEAD TRANSCRIPTION FACTOR FOXO4 WITH DNA

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FoxO4 belongs to the "O" subset of forkhead transcription factors, which participate in various cellular processes. The forkhead DNA binding domain (DBD) consists of three-helix bundle resting on a small antiparallel â-sheet from which two extended loops protrude and create two wing-like structures (Fig. 1). The main DNA recognition site is -helix H3 that makes contacts with the major groove of DNA. Other regions of forkhead domain that can make important interactions with DNA are both wings or N-terminal extension upstream of helix H1 [1, 2].

Within the large family of Fox transcription factors, the proteins FoxO1 (FKHR), FoxO3a (FKHR-L1), FoxO4 (AFX) and FoxO6 constitute the "O" subfamily. Members of this subfamily play an important role in cellular proliferation, survival, and in mediating effects of insulin and growth factors on metabolism [4]. All FoxO proteins function under the control of the phosphoinositide-3-kinase–protein kinase B (PI3K–PKB) pathway. Phosphorylation by PKB creates two binding sites for the 14-3-3 protein and induces phosphorylation of additional sites by casein kinase 1 and dual-specificity tyrosine(Y) regulated kinase 1A. PKB-mediated phosphorylation induces binding of the 14-3-3 protein and the resulting complex is then translocated to the cytosol where the bound 14-3-3 protein prevents re-entry of FoxO into the nucleus likely by masking its nuclear localization sequence [5-7].

The DNA binding potential of FoxO–DBD is controlled by multiple mechanisms. The most important factors seem to be the PKB-induced phosphorylation and the binding of the 14-3-3 protein [6-9]. PKB phosphorylation site and the 14-3-3 protein binding motif are located in the basic region of wing W2 at the C-terminus of FoxO–DBD (Fig. 1). Structures of HNF-3 (FoxA3) and Genesis (FoxD3) complexes revealed that cluster of basic residues in the wing W2 is involved in DNA binding [1,10]. Moreover, the removal of wing W2 abolishes DNA binding of forkhead transcription factor HNF-3 . Sequence alignment between HNF-3 , Genesis and FoxO sequences suggests that analogues basic residues forming the second FoxO PKB phosphorylation site and the 14-3-3 binding motif might participate in DNA binding as well. This similarity could explain the inhibitory effect of both the phosphorylation and the 14-3-3 protein on DNA binding activity of FoxO proteins. However, the regulation of DNA binding among various FoxO proteins seems to differ significantly. It has been shown that phosphorylation of the second PKB/14-3-3 binding motif in the wing W2 suppresses DNA binding of FoxO1 and FoxO6 factors [9]. On the other hand, the PKB-induced phosphorylation of both DAF-16 (*Caenorhabditis elegans* FoxO homologue) and FoxO4 (fragment 11-213) does not by itself affect their binding to the target DNA [6,8]. Binding of the 14-3-3 protein to phosphorylated FoxO4 and DAF-16 has been shown to be necessary for complete inhibition of their binding to the DNA.

To better understand these differences among FoxO–DBD, we investigated the role of N-terminal loop (portion located upstream of first helix H1) and C-terminal region (loop known as wing W2) of forkhead domain of transcription factor FoxO4 in DNA binding. While the deletion of either portion partly reduces the FoxO4–DBD binding to the DNA, the simultaneous deletion of both regions inhibits its DNA binding significantly. Förster resonance energy

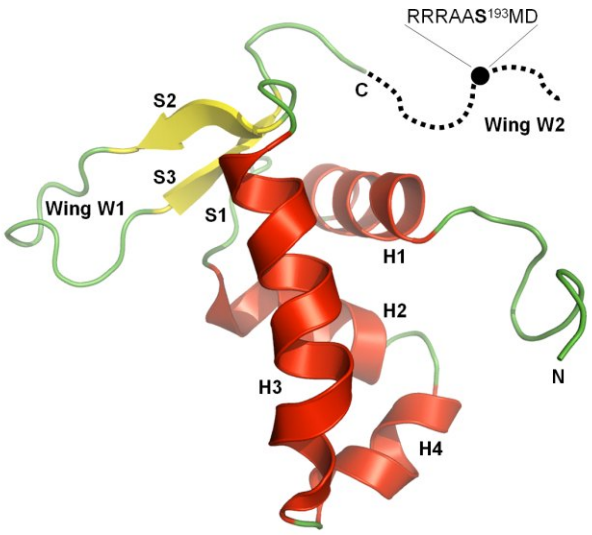

Fig. 1. The ribbon representation of solution structure of FoxO4–DBD sequence Ser^{92}-Gly^{181} [3]. The missing part of wing W2 is schematically shown as dotted line. Black circle represents approximate location of PKB phosphorylation site Ser^{193}.

transfer measurements and molecular dynamics simulations suggest that both studied N- and C-terminal regions of FoxO4–DBD directly interact with DNA. In the presence of N-terminal loop the PKB-induced phosphorylation of wing W2 by itself has negligible effect on DNA binding. On the other hand, in the absence of this loop the phosphorylation of wing W2 significantly inhibits the FoxO4–DBD binding to the DNA. The binding of the 14-3-3 protein efficiently reduces DNA binding potential of phosphorylated FoxO4–DBD regardless of the presence of N-terminal loop. Our results show that both N- and C-terminal regions of forkhead domain are important for the stability of FoxO4–DBD/DNA complex [11].

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L6

STRUCTURAL MECHANICS OF DNA: FROM ATOMIC SCALE TO BIOINFORMATICS

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The storage and retrieval of genetic information and their regulation depend on interaction of DNA with numerous proteins. It is now well established that the protein-DNA interactions may operate through two different mechanisms: so-called direct readout where specific chemical contacts between protein and DNA are made, or indirect readout, in which it is the three-dimensional structure or the mechanical deformability of the molecules, in particular the DNA, that guides the binding.

We set us a goal to investigate sequence-dependent DNA structure and mechanical deformability at a wide range of length scales. The general approach we use consists in performing large-scale, atomistic molecular dynamics (MD) simulations of DNA in explicit solvent, and employing methods from statistical physics to parametrize coarse-grained models on different length scales from the MD data.

We consider a rigid basepair and a rigid base model, where base pairs or bases are treated as interacting rigid bodies in contact with a thermal reservoir. Assuming harmonic (quadratic) interaction energy, the shape parameters and stiffness constants can be inferred from structural fluctuations observed in an unconstrained simulation. Similar models have already been parametrized, using either an ensemble of crystal structures or atomistic MD trajectories, but all of them rely on the assumption that only nearest-neighbour base pairs or bases contribute to the interaction. We studied the full, nonlocal problem and found significant contribution to the interaction energy beyond nearest neighbours. The rigid base model is clearly more complete than the rigid basepair one, and we found that it is also much more physically realistic.

In order to obtain comprehensive sequence-dependent data, we take part in an initiative (the ABC consortium) aimed at performing atomistic MD simulation of a pool of oligomers involving all possible tetrameric sequences. The simulations are close to completion and the first analysis is under way. In our lab we concentrate on obtaining the full set of sequence-dependent shape and stiffness parameters for tetrameric sequences and on constructing comprehensive, nonlocal models based on them.

The knowledge of sequence-dependent parameters for a rigid base model enables one to predict the deformation energy (within harmonic approximation) for a deviation from the equilibrium geometry and thus help quantify the energetic cost of indirect readout by a protein. This opens the possibility to include the shape and mechanical stiffness of a sequence as additional parameters in the analysis of sequence similarity, routinely based only on the sequence viewed as a text, i.e. a sequence of letters. The possibility that very different sequences have similar mechanical properties and thus may be involved in similar

biological functioning would be readily detected in this way.

The length scales from individual base pairs up to ca. a hundred of base pairs of DNA are crucial in protein-DNA interactions. The latter scale, for instance, is the one on which DNA is wrapped in the nucleosome, and on which loops of DNA are involved in the regulation of gene expression. Recent experiments on the formation of DNA minicircles suggest that DNA of ca. 100 base pairs in length can form loops with a rate much higher than predicted by standard theories. It has been proposed that some kind of transient local deviation from the double helical structure (bubbles, or kinks) may provide the explanation. We studied sharply bent DNA in a series of atomistic simulations of 94-bp DNA minicircles and found that kinks, but not bubbles, indeed arise during the simulation. The kinks involve a sharp bending into the minor groove, and their number depends on the supercoiled state of the molecule. The results suggest a microscopic basis for models of DNA looping beyond the harmonic approximation.

L7

TIME-RESOLVED STRUCTURAL DYNAMICS - A CHALLENGE FOR FUTURE

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Knowledge of structure and dynamics of molecular systems is the prerequisite for understanding of function. While static structures of molecules are usually obtained from methods like X-ray crystallography or NMR spectroscopies, information about dynamics is typically obtained from various forms of time-resolved spectroscopies. The most fundamental processes in chemistry and biology usually occur on the ultrafast time scale of femtoseconds to picoseconds. This is the time scale of elementary chemical reactions and of electronic and nuclear motions in molecules, thus this time scale is critical for following the most elementary processes such as bond breaking and formation or electron and energy transfer. Thus, our fundamental understanding of chemical and biological dynamics ultimately relies upon a thorough explanation of the ultrafast processes. Today we combine results from structural and dynamical methods to obtain insight into the function of molecular systems and understanding the molecular mechanisms of dynamic processes. It is a long nourished dream to get *both* structure *and* dynamics from the same experiment, *i.e.* to directly obtain time-resolved structures showing the three-dimensional evolution of a molecular system in the course of a chemical reaction. A number of different techniques are presently pursued towards this goal. Perhaps the most ambitious approach is to extend the present structural methods, X-ray or electron diffraction, to yield 3D structures on the molecular time scale. Besides this 'direct' approach, alternative methods for obtaining dynamical structural information, such as time-resolved X-ray absorption or multidimensional coherent femtosecond spectroscopies, emerged in past few years. The aim of this contribution is to give an overview of the state-of-art methods applied nowadays for obtaining dynamical structures of molecules at very short time scales, to compare their pros and cons, and their potential for future applications.

Lectures - Friday, March 30, morning

L8

ORIENTATIONAL RESTRAINTS IN NMR STRUCTURE

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The lecture will provide a general introduction to the restraints obtained in partially aligned samples of biomacromolecules. Methods of alignment will be mentioned and observed interactions of different physical origins will be briefly discussed. Methods of validation of the obtained data prior to structure calculation will be presented.

L9

IS LOCAL FLEXIBILITY OF M-PMV MATRIX PROTEIN RELATED TO ITS BINDING AFFINITY WITH A MOLECULAR MOTOR?

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Mason-Pfizer monkey virus (M-PMV) belongs to the genus of betaretroviruses in which the matrix protein (MA) plays the essential role in certain stages of their life cycle (e.g. in assembly, transport and budding of new viral particles). Several single or double point mutants are known to cause dramatic changes in the virus life cycle [1]. In particular, the single point mutation R55F in MA redirects the assembly of the viral capsid to the plasma membrane instead of to cytoplasm, which is the place of assembly of the wild type form (WT).

The NMR assignments and three-dimensional structures of WT and R55F have been solved recently by our group [2]. Both structures are composed of four -helices, however the relative orientation of the N-terminal domain (helices 1 and 2) with respect to the C-terminal one (helices 3 and 4) is different. Such reorientation causes different accessibility of Cytoplasm Targetting/Retention Signal (CTRS) sequence for the interaction with a molecular motor dynein . It is expected that the degree of flexibility of the CTRS sequence will affect binding affinity with the Tctex-1 which is one of the light chains of dynein.

Therefore, we carried out a detailed motional analysis of the loop connecting helices 2 and 3, the least structured part of CTRS. Relaxation parameters of amide ^{15}N and carbonyl ^{13}C nuclei have been studied by NMR spectroscopy on uniformly labelled (^{15}N and $^{15}N/^{13}C$) samples. Additionally, MD trajectory analysis have provided a complementary view on the mobility of CTRS sequence which is crucial for understanding the interation of Gag with dynein.

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L10

RNA RECOGNITION BY THE ADAR2 DSRBMS: A 50 KDA PROTEIN-RNA COMPLEX BY NMR

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Adenosine deaminases that act on RNA (ADARs) tune and regulate gene expression. Although ADARs act mostly as nonspecific enzymes, they can recode certain genes in a highly specific manner. This results from preferential binding of the ADARs to certain RNA substrates. To understand how ADARs bind RNA, we investigate the N-terminal region of ADAR2 in complex with a 71 nucleotide RNA encoding the R/G site of the GluR-B (MW ~50 kDa), using nuclear magnetic resonance (NMR) spectroscopy. We chose solution-state NMR technique as a number of groups failed in making crystals of ADAR-RNA complexes. The studied complex represents a challenge for structure determination by NMR because of its size and elongated shape. However, we identified that two structured domains (double-stranded RNA-binding domains, dsRBMs) located in N-terminal region of ADAR2 bind RNA substrate in independent manner, each domain binding a different site on RNA. This allowed us to make two subcomplexes in which all necessary NMR experiments for structural determination could be measured. The full-length complex is being reconstructed using long-range structural information derived from residual dipolar couplings (RDCs) measured on a 50 kDa ADAR-RNA complex. This study demonstrates how NMR can be used for structural determination of large protein-RNA complexes, provided that rationale design of studied constructs along with deuteration and TROSY techniques are used.

L11

CALCULATION OF SCALAR COUPLINGS IN THE BACKBONE OF NUCLEIC ACID

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Global architecture of nucleic acids corresponds to conformation of nucleic acid backbone. Structure of the backbone is usually described by the torsion angles measured along the backbone, . Calculation of scalar coupling constants between the 31P, 13C, and 1H nuclei correlate strongly with the backbone torsion angles.

Distinct patterns of RNA backbone by X-ray crystallography at the dinucleotide level [1] were used as structural models for the theoretical calculation of all relevant scalar couplings. It was shown that the calculated scalar couplings can facilitate their accurate and reliable structural interpretation [2]. In particular: i) proposed computational strategy allows for the determination of the multidimensional character for scalar couplings, i.e. for example the effect of sugar pucker (-torsion) on the scalar couplings correlated only with the neighboring torsion, ii) new correlation of the 2J(P,C) couplings indicative of the trans, gauge+, and gauge- conformations of the - and -torsion was calculated, iii) the stepwise procedure for assignment of different scalar couplings in torsion space for nucleic acid backbone was proposed.

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L12

THEORETICAL NMR STUDY OF WATSON-CRICK/SUGAR EDGE RNA BASE PAIR FAMILY

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Ribonucleic acid, one type of nucleic acids, has large variety of forms and also shows an astonishing variability of base-pairing [1, 2].

Classical Watson-Crick type of base pairing found in DNA represents only 50% of base pairing in RNA statistically[1, 2]. There are many non-canonical base pairs in RNA that have no counterpart in DNA. A role of the non-WC base pairs in RNA is fundamental since they participate in folding and stabilization of RNA tertiary structure. Detection of binding motives in rather complicated and highly variable RNA macromolecules can help in better understanding of chemical processes of nucleic acids.

Each nucleoside possesses three edges [1] shown in Figure 1: WC edge, *Hoogsteen* edge (for purines) or *CH* edge (for pyrimidines), and *Sugar* edge (SE). A given edge of one nucleoside can in principle interact with one of the three edges of a second nucleoside. This interaction can be either *cis* or *trans* with respect to sugar moiety. According to Leontis and Westhof [1], all possible combinations lead to twelve families of distinct geometry patterns.

Quantum chemistry study of nuclear magnetic resonance parameters of all members of cis- and trans- Watson-Crick/Sugar edge base pair family was provided by mean of calculation of indirect spin-spin coupling constants with the CP-DFT method. RNA base pair interact via hydrogen bonds. Inter and intra indirect spin-spin couplings of local binding motif are calculated and are compared to experimental data and obtained structural dependences of spin-spin couplings are described. Forty-two complexes are studied and effects of local structural modification, water mediation and solvent effect are calculated. Each H-bond pattern has its own set of representative J-coupling which can be used for the identification of particular RNA bonding pattern via NMR spectroscopy.

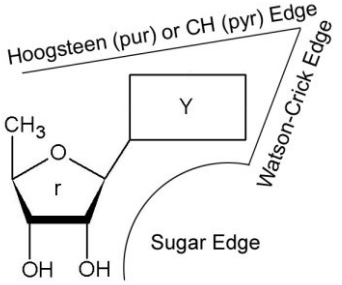

Figure 1. Classification of the interaction edges for RNA nucleoside by Leontis [1]

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L13

COMPUTATIONAL STUDY ON SPECTRAL PROPERTIES OF THE SELECTED PIGMENTS FROM VARIOUS PHOTOSYSTEMS; STRUCTURE – TRANSITION ENERGY RELATIONSHIP

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In this study, the most important kinds of pigments (chlorophylls, bacteriochrophylls, phycobilins, and carotenoids) from various photosystems were explored. For the most stable conformations, electronic transitions were determined at the TDDFT/6-31+G(d) level with B3PW91 functional and compared with measured spectra. The group of carotenoids was also investigated at the TDA/TDDFT level with the functional BLYP. The energies of Q_y transitions are systematically blue shifted by about 50-100 nm in the case of (bacterio)chlorophyll and pheophytin molecules. Nevertheless, the correct relative order of the Q lines among various chlorophyll types was obtained in comparison with experimental results. Much better agreement was obtained for the Soret band where the differences between calculated and measured transitions are at most 35 nm. In the case of phycobillins the first transition line is estimated to be at lower frequencies (around 500 nm) with a very similar blue shift of by about 100 nm from experimental values. The influence of anchoring cysteine side chain(s) was found marginal. Dominant role of linear polyene chain on determined spectral lines in the case of carotenoids was found. Nevertheless, the impact of cycles, epoxy- or keto-groups is clearly visible, too. The high intensity of the first allowed transition matches different character of HOMO and LUMO. On the contrary to the Q_y line of chlorophyll molecules, in the case of carotenoids, calculated transitions are red shifted in average by about 70 nm from the measured spectra.

L14

ELECTRONIC STRUCTURE OF THE NON-HEME IRON CENTER IN PHOTOSYNTHETIC APPARATUS OF PLANTS AND BACTERIA. COMPUTATIONAL DFT STUDY

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The non-heme iron center (Fe-center) is a part of electron-transfer (ET) chain located in Photosystem II (PS II) and bacterial reaction centers (BRC), which is responsible for primary charge separation in photosynthesis. The Fe-center represents a bridge between two quinone molecules: Qa and the terminal electron acceptor, Qb. However, its role in the ET process remains unclear [1]. Although the structure of Fe-center is highly conserved among photosynthetic organisms, experimental studies revealed that, in case of PS II, various ligands can bind reversibly to the Fe-center (the "native" ligand being bicarbonate).

We present a density functional theory investigation for molecular models of the Fe-center. Calculations on the small model system predict that the high-spin (quintuplet) state is the ground state in both PS II and BRC. However, much smaller energy differences of the spin states were found in case of BRC. Disctinct local minima were found for both intermadiate- (triplet) and low-spin (singlet) configurations. These minima differ only in the lengths of Fe-N and Fe-O bonds. This can explain why both low- and high-spin states were observed in PS II. From this point, more accurate x-ray structures would be required.

Spin states energies are only slightly affected when the bicarbonate ligand is removed in PS II. Unlike in the "native" Fe-center, electron affinity of the center without bicarbonate appears sufficient to accept electron from Qa- in our small model. In this way we assume the blocking of the possible electron transfer.

The extended Fe-center+Qa model shows that the reduction of Qa is accompanied by proton transfer from the H-bonded histidine residue, in accord with previous studies on simplified Zn-replaced model [3].

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L15

THEORETICAL DFT STUDY OF SOLVENT EFFECTS ON PLATINUM COMPLEXES INTERACTIONS WITH CYSTEINE AND METHIONINE

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Interactions of two cisplatin hydrated forms cis-$[Pt(NH_3)_2ClH_2O]^+$ and cis-$[Pt(NH_3)_2(OH)H_2O]^+$ with cysteine and methionine in vacuum and with implicit solvent were simulated. In the first step, reaction mechanism involves formation of monodentate intermediates where aqua ligand is replaced with the amino acid. In the next stage, another platinum ligand is replaced by one of the remaining donor atoms of the amino acid creating a chelate structure.

Structures were optimized using DFT method with splitted valence double-zeta basis set extended by polarization and diffuse functions on heavy atoms. Solvent effects were described utilizing SCRF/COSMO solvation model in both optimization and single point calculations. Core electrons of platinum, sulfur and chlorine atoms were described by quasirelativistic pseudopotentials. Energy decomposition together with the NPA population and MO analysis were performed using 6-311++G(2df,2pd) basis set. Reaction energies were determined in the so-called supermolecular approach as well as in the model of isolated molecules.

Bond dissociation energies were calculated with counterpoise correction. In solvent, a modified protocol for basis set superposition corrections was applied to take into account interaction with solvent.

Estimation of pK_a values of all investigated complexes was done. Several implicit solvent methods were used and the best results were obtained for the B3LYP/6-311++G(2df,2pd) method with DPCM solvation scheme and UAHF cavities. Spheres around platinum ligands were constructed following UAHF rules using atomic charges obtained from NPA analysis within the single point CPCM calculations.

Lectures - Friday, March 30, afternoon

L16

COMPUTATIONAL CHEMISTRY AS A HELPFUL TOOL FOR BIOCHEMIST – STRUCTURE-FUNCTIONAL STUDY AND CORRELATION EN ROUTE TO PROTEIN ENGINEERING

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The current computational capacity and methods allow to perform sophisticated simulations and modelling of complicated, yet, in regard to the essentials of living organisms' existence, fundamental processes, namely biomolecular interactions. These approaches embody a great potential regarding experimental time and budget savings.

Lectins are proteins capable of binding saccharide structures with high affinity as well as high specificity. Saccharides display wide variability of conformational alternatives, being it intramolecular isomery or intermolecular linkage, and therefore able to serve as recognition agents, with lectins serving as the receptors. The interconnection of practical methods of molecular biology, structural and functional experimental methods is crucial for understanding the relationship between protein structure and its properties. The more complete this understanding, the easier and more effective the process of designing proteins with precisely defined and desired properties – protein engineering.

The study of affinity changes related to mutations of lectin PA-IIL by combination of the aforementioned methods revealed the crucial role of the amino-acid composition of the binding site for binding preferences[1]. The studied mutants were created in silico as well, and the resulting structures were used for docking experiments. The docking results were correlated with experimental data. The aim of the project is to develop a reliable method of precognition of future, unknown interactions, both from thermodynamic and structural point of view, enabling the protein engineering approach.

1. Adam J., Pokorná, M., Sabin, C., Mitchell, E. P., Imberty, A., and Wimmerová, M., article submitted.

L17

STRUCTURE AND DYNAMICS OF THE OXYGEN EVOLVING COMPLEX OF PHOTOSYSTEM II: ROLE OF THE N-TERMINAL LOOP OF PSBQ

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Infrared and Raman spectroscopy were applied to identify restraints for the structure determination of the 20 amino acid loop between two beta-sheets of the N-terminal region of the PsbQ protein of the oxygen evolving complex of photosystem II from *Spinacia oleracea* by restraint-based homology modeling. One of the initial models has shown a stable fold of the loop in a 20 ns molecular dynamics simulation that is in accordance with spectroscopic data. Cleavage of the first 12 amino acids leads to a permanent drift in the root means square deviation of the protein backbone and induces major structural changes.

The probable binding site of PsbQ to the complex could be formed by the lysyl rich region of the helix bundle and the N-terminal loop region around Asp24 and thus would contain a large positively charged region and a small negatively one. We hypothesize that after binding to PSII the loop loses its high flexibility and bends in the direction of Lys96 with Thr20 and Glu21 interacting with this residue and so burying it under the accessible surface (Fig. 1). Thus Lys96 could probably behave as a molecular hook holding Glu21 by a salt bridge.

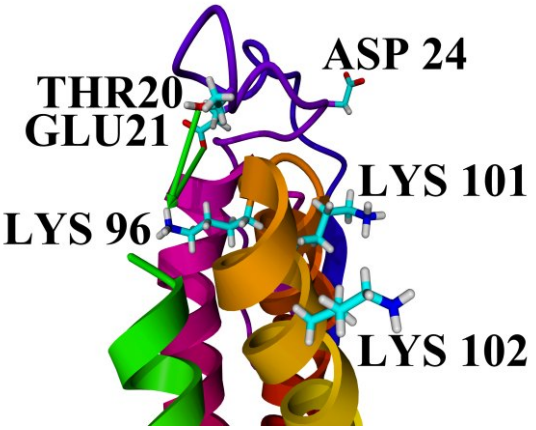

Figure 1. Lys96, one of the four lysyl residues which are probably orientated to the lumenal facing intrinsic proteins of PSII, lies on the opposite side as are the other three lysyl residues and the conserved loop residue Asp24, in a distance from 6–12 Å and 7–13 Å to the loop residues Thr20 and Glu21, respectively.

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L18

NEW APPROACHES TO STRUCTURE AND FUNCTION STUDIES OF RS20L LECTIN FROM *Ralstonia solanacearum*

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Lectins are sugar-binding proteins of non-immune nature that play a role in cell agglutination or glycoconjugates precipitation. These lectins bind to sugar moieties in cell walls or membranes and thereby change the physiology of the membrane, thus cause agglutination, mitosis, or other biochemical changes in the cell.

Ralstonia solanacearum is a plant bacterial pathogen, which causes a wilt disease in several economically impor-

tant agricultural crops, such as potatoes, tomatoes, peppers, eggplant, and banana.[1]

Plant and animal pathogens use protein-carbohydrate interactions in their strategy for host recognition and invasion.

Until our knowledge now, the *R. solanacearum* bacterium has been producing three soluble lectins. RSL (MW 9900), which exhibits sugar specifity to L-fucose [2] and partial sequence homology to mushroom *Aleuria aurantia* lectin AAL [3], RS-IIL (MW 11601) lectin [4] resembles PA-IIL from human pathogen *Pseudomonas aeruginosa* in structure and properties but differs in sugar specifity [5]. The last one is RS20L (MW 19903), which displays L-fucose and D-mannose and D-xylose binding ability.

This presentation describes, structurally and functionally, the RS20L, a 20 kDa lectin, which has no sequence similarity to any known lectin amino acid sequence, but the solution of crystal structure showed high structural similarity to animal galectins. However it doesn't display any sugar specificity to D-galactose.

Further functional studies using surface plasmon resonance (SPR) and isothermal titration calorimetry (ITC) allowed to define binding properties (afinity, kinetics) and thermodynamic parameters.

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L19

COMPUTATIONAL STUDIES ON PA-IIL LECTIN-CARBOHYDRATE INTERACTIONS

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Lectins are proteins of nonimmune origin that non-enzymatically selectively bind to mono or oligosaccharides. Multifarious activity of carbohydrates in biophysiological pathway, such as immune activity, tumor metastasis, cell-cell recognition, bacterial pathogenecity, open an avenue for the lectin-carbohydrate interaction research, which is also a big challenge for theoretical modeling due to the polar flexible saccharide moiety. One of the lectins, PA-IIL that produced by *Pseudomonas aeruginosa*, which play significant role in cystic fibrosis disease, motivated our study on PA-IIL-carbohydrate interactions.

The structure can provide a static view of the macromolecules, but for the full understanding of protein-ligand interactions it is necessary to know all the accessible spatial orientations of the ligand in the receptor binding pocket. It is often seen that the binding energy calculated over the sampled structure by molecular dynamics could give the insight of the interactions between protein and ligand.[1, 2] We used the MM/PBSA approach to calculate the binding free energy of the lectin-carbohydrate. The entropy contribution to the binding free energies was obtained by normal mode analysis. Unfortunately these conventional methods are not precise enough to accurately distinguish the binding order of saccharide series. Thus, to gain the insight of the spatial orientation of the monosaccharide and conversely the ligand induced fit in the receptor binding site, the binding energy and the pocket conform to the specific orientation of the saccharide was correlated. The spatial proximity of carbohydrate inside the binding domain is the key factor for the lectin-carbohydrate binding, which was identified by statistical clustering. One of the interesting finding was ions behavior, revealing their paramount significance for the binding.

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L20

CONFORMATIONAL SPACE OF NUCLEIC ACIDS

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Explosion of information on nucleic acid (NA) structure available in the public archive [1] has enabled detailed analyses of NA, mostly RNA, conformational space [2-5]. The RNA Ontology Consortium, ROC [6], is a collaborative framework that coordinates effort of several research groups to lay firm foundation to description of RNA structure. Consensual description of RNA backbone conformations sponsored by the ROC is before finish [Richardson *et al*. in preparation, 2007]. To complement the analysis of RNA conformations, we investigated conformations of more than eight thousand nucleotides from over four hundred well resolved DNA X-ray structures. Here we compare basic features of conformational behavior of RNA and DNA nucleotides.

For both DNA and RNA, the double helical A and B forms represent a large majority of populations. In the DNA conformational space, gradual changes lead from A forms with C2'-endo sugar puckers to B forms with C3'-endo sugars and several A-to-BI sub-states with mixed sugar puckers were identified. Existence of intermediates with deoxyribose in the O4'-endo region was detected not only in protein/DNA complexes but significantly also in high resolution structures of naked DNA. There are also sub-states of the BI and especially BII conformations, most are induced by interactions with other molecules, mostly proteins. All these conformational sub-states or intermediates keep the basic double helical arrangement. For instance, DNA in complex with histone core particles acquires its circular shape by a combining nucleotides in the BI and BII conformations. DNA bound to TATA-box binding proteins seems to be extremely deformed, opens up its minor groove and bends away from the protein but it keeps its double helical arrangement and only locally changes from B to A type. In summary, DNA undergoes "plastic deformation" and its conformation can be changed from right-handed double helix only under very specific circumstances and into a few structurally defined states, as specific sequence can form G-tetraplexes or a certain sequence can form Z form DNA at high salt.

In contrast, widely diverse RNA conformations seem to form isolate islands in the conformational space. The extra hydrogen bond donor and acceptor, the hydroxyl -O2'H at the ribose ring, stabilizes conformations that lead to bulges, loops, and consequently to RNA molecules globally folded in three dimensional space. When RNA is disrupted from its most stable A form, it "jumps" to conformations incompatible with the rigid right handed helix.

This work has been supported by an NSF grant DBI 0110076 to the NDB and by a grant LC512 from the Ministry of Education of the Czech Republic.

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L21

ELECTRONIC COUPLING IN THE EXCITED ELECTRONIC STATE OF STACKED DNA BASE HOMODIMERS

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The nature of the excited states of DNA bases, in particular the electronic coupling between adjacent bases in their excited state is addressed in this contribution. One of the possibilities how DNA can protect itself from UV damage is a fast transfer of excitation energy which prevents the localization (trapping) of electronic energy and subsequent reaction. The energy transfer becomes faster with increasing electronic coupling between excited states.

The electronic coupling results from both the orbital overlap in the short-range limit and interaction of transition dipole moments (dipole-dipole interaction) in the intermediate-range limit. It is a generally accepted view that the extent of the delocalization depends on the base sequence as well as on the structure of DNA helix which effects their mutual orientation.

In this contribution we report the results of ab initio study of the electronic coupling between two adjacent stacked DNA bases where the orbital overlap is the dominant part. These studies were performed for adjacent cytosine, thymine, and adenine in both A- and B-DNA conformations.

It is shown that the electronic coupling depends considerably on the geometry of two adjacent bases. In addition, the character of the lowest excited states of thymine complex is different compared to that of cytosine complex. We believe that these studies can bring some contribution to the understanding of an observed different photochemical behavior, with respect to cyclobutane formation, in particular.

L22

see page 12 for the paper

Lectures - Saturday, March 31

L23

NEW PRINCIPLE OF THE MOLECULAR PHOTO-FET

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We report a possibility of a reversible formation of charge carrier traps in molecular system consisting of polymer matrix containing photochromic species. One can envisage a construction of photo-FET device in which source-drain current in a semiconductor film is influenced by photo formed dipolar fields associated with charge carrier traps in a photoactive "gate" layer. The results demonstrate that the suitable photochromic reaction can modulate the transport of the charge carriers in a semiconducting film between the source and drain electrodes. The speed of the modulation is limited by the speed of the photochromic transformation – in a suitable system with a free volume of the polymer it can reach microseconds scale. The speed can be improved using the polarity of the exciting state where picoseconds scale can simply be reached. The experimental effects presented here, leading to the decrease of charge carrier mobility and therefore of the photocurrent by factor ca. 3, strongly depends on the sample geometry, thicknesses of the films and the concentration and orientation if photochromic additive in the "gate" polymer layer.The finding of the suitable parameters is in progress. DFT calculations on model dimer $(H_2Pc)_2$ show changes in electronic structure caused the induced dipole which lower the carrier mobility.

L24

CHIPAS, TOOL FOR CHIPONCHIP ANALYSIS

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ChIP-on-chip, also known as genome-wide location analysis, is a technique for isolation and identification of nucleotide sequences occupied by specific DNA binding proteins in cells. These binding sites may indicate functions of various transcriptional regulators and help identify their target genes during animal development and disease progression. The identified binding sites may also be used as a basis for annotating functional elements in genomes. The types of functional elements that one can identify using ChIP-on-chip include promoters, enhancers, repressor and silencing elements, insulators, boundary elements, and sequences that control DNA replication.

The chip-on-chip technology can typically be used for identification of genes that respond to the transcription factor in study. We present here software CHIPAS (released under GPL2) that makes it possible to obtain a set of the best binding sites for given transcription factor together with the identification and location of its consensus binding nucleotide sequences. Application of this software is demonstrated in serching for protein-binding sites in the mouse PAX6 gene.

L25

STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF HUMAN PROTEIN KINASE CKI EPSILON

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Casein kinase I epsilon (CKI) is one of the crucial components of Wnt signaling pathway that is required for normal development and cell proliferation. However, the role of CKI in this signaling remains still unclear. It has been shown that mutations in some genes encoding proteins regulating the Wnt cascade (-catenin, axin, APC) are common in dysregulated development and multiple cancers. Recently, Fuja *et al.* (2004) identified eleven point mutations in gene for CKI in human breast cancer cells. In this project, we would like to address the effect of mutations on casein kinase I epsilon 3D structure and its activity. In par-

allel, we plan to identify proteins, which stably associate with CKI under *in vivo* conditions and potentially characterize the topology of the CKI complex. These investigations will provide a molecular basis for understanding of CKI function and for design of drugs modulating CKI activity.

This work was supported by Grant Agency of the Czech Republic (301/07/0814).

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L26

SUBTILASES AND METAL BINDING – THE WEAK BINDING SITE OF SUBTILISINS REVISITED

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Subtilisins form a family of / hydrolases with broad specificity towards the cleaved peptide sequence [1]. Relationship between their structure and activity was extensively studied by means of X-ray crystallography. Representatives of this family such as subtilisin BPN´/NOVO, Carlsberg or Savinase became subjects of systematic studies of metal ion binding in relation to the enzymatic activity, stability and structure since 1969 when the first subtilisin structure was published [2]. Enzymes from the superfamily of subtilisin-like proteases have their catalytic domain homologous to that of subtilisins with some additional domains and/or secondary structure elements.

In the earlier studies a strong calcium binding site (CaI, formerly named site A) and a weak calcium binding site (CaII, formerly named site B) were identified in subtilisins [3]. The site CaI plays a crucial role in stabilizing the molecular structure but the role of the site CaII is slightly more complicated and depends on concentration of sodium ions in the environment [4]. As the number of structures of subtilisins in the PDB [5] was growing it became obvious that in some cases the contents of site CaII was misinterpreted.

Two novel structures of subtilisin-like proteases were determined by single crystal X-ray diffraction. The extracellular proteases are produced by bacteria *Bacillus* sp. TY145 and *Bacillus halmapalus.* The new subtilases bring experimental evidence for Na^+ instead of Ca^{2+} bound in site NaII (2.7 Å away from site CaII) and for three new Ca^{2+}-binding sites in subtilases. We have attempted to re-classify the typical metal binding sites of subtilisins and subtilisin-like proteases and to reflect on the most likely nature of the ions in site CaII in the publicly available structures.

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L27

LIGAND DOCKING TO BIOCHEMICAL TARGETS. CRYSTALLOGRAPHY AND MODELLING

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X-ray structure analysis is an excellent tool for study of molecular recognition (i.e. specific adhesion between two macromolecules of biological origin) and therefore it plays a key role in elucidation of molecular mechanisms of many biochemical processes. A high interest of crystallographers was also devoted to specific interactions of drugs in active sites of enzymes and their research has already been reflected in many practical results of the rational drug design. Also in the case of drugs, we can usually see high affinity of the ligand to the target protein because it is the aim of the human effort to break down the enzymatic function permanently. The molecules of interest are in these cases usually well ordered in crystal and therefore relatively easily resolved by protein crystallography.

The situation is not so easy with ligands possessing lower affinity to the protein molecule (as for example polymers). One has to cope usually with molecules which are only partly localized on the protein surface with the remaining parts floating freely in the crystallization buffer (for diffraction experiment invisible). In addition, parts of the ligand adhering to protein surface (the only visible fragments in the maps of electron density) have often lower occupancy and also can appear in multiple conformations accompanied by multiple configuration of the surrounding water molecules of buffer. Therefore to localize these low affinity ligands, we need relatively good experimental data and additional work connected with careful localization of water molecule networks forming the hydration shell of the protein. It was believed until recently that protein crystallography cannot describe well the structure of solvent near the protein surface and thus neither localize well the low affinity ligands on the protein surface. These difficulties are probably the reason why crystallographers did not pay attention to these low adhesion molecules in past.

However, many low affinity ligands have already been proved as highly efficient tools practically used in health care. Several tens of studies testing practical usage of these low affinity ligands have been published every year (drug carriers for safe delivery and release of drugs in the target tissue, coating materials protecting the biologically degradable molecules during their transport, control of drug release rate, artificial additives in food, etc.). In spite of a clear importance of the subject, our knowledge of how the low affinity ligands bind to protein surface remains limited until now.

In spite of the fact that polyethyleneglycol (mostly PEG2000) has been intensively used in crystallization experiments for a long time [1], only marginal attention has been paid to soaking of various polymers into the protein crystals and to crystallographic studies of adhesion between proteins and polymers (low affinity materials of non-biological origin). Here we show on several examples [2-10] a number of general problems tackled when one tries to study polymers and the complex molecular systems mentioned above. Namely we focus on the problems connected with determination of all conformation states, conformational freedom of the bound ligand influencing the entropy of the system, the flipping problem of His, Gln, Asn, determination of water sites in the first hydration shell, verification of water molecule networks and localization of the relevant parts of polymer ligands.

It can be said generally that the experimentally derived view of the molecular structure (the 3D-map of electron density) contains information about all conformational states and motions of the molecular system realized during the time of the measurement (usually several minutes). In the case of a good measurement, parts of the molecular system remaining stable during the measurement are usually well resolved in the map of electron density, any single electron can be resolved and the individual atom positions can be localized with an accuracy up to 1 pm (0.01 Å). However, electron density of atoms in areas with higher mobility of atoms is blurred over larger areas. It is usually described by higher temperature factors and by disorder of some functional groups. A better alternative way, although less compact, is to deposit a definite number of models similarly as it is generally used in the NMR structure analysis [10].

The fact that we cannot expect high specificity of these interactions, because proteins did not pass any genetic selection with respect to these compounds in past, is on the other hand a great advantage. Molecular adhesion of such low affinity materials is not critically dependent on a specific protein, and thus we hope that it will be much easier to generalize the observations received on a number of experimentally determined structures of proteins of different origin and to form rules which govern the properties of these molecular complexes.

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STRUCTURAL STUDIES OF THE REPRESSOR CGGR FROM *Bacillus subtilis*

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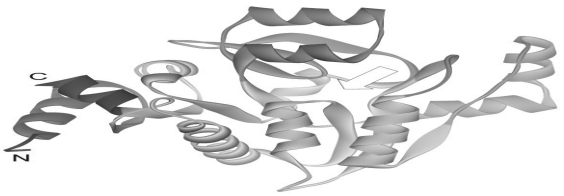
The crucial glycolytic enzyme, glyceraldehyde-3-phosphate dehydrogenase (GapA), is in *Bacillus subtilis* negatively regulated at the transcriptional level by the central glycolytic gene regulator CggR. CggR belongs to a large family SorC/DeoR family of prokaryotic transcriptional regulators with the N-terminal DNA binding domain and larger C-terminal effector binding domain. When no glucose is present in the growth media, CggR interacts with its target sequences in the *gapA* operon and blocks transcription. By contrast, the availablity of glucose induces expression of GapA enzyme. *In vitro* experiments demonstrated that the effector molecule that abolishes CggR DNA binding activity is fructose-1,6-bisphoshate (FBP) [1]. *In vivo* studies suggested that CggR can also synergically to other signals derived from aminoacid anabolism [2].

The structure of CggR C-terminal effector-binding domain (residues 89-340, referred to as C-CggR) was determined by a single-anomalous dispersion (SAD) using the selenomethionyl-substituted protein crystals and was refined using data to 1.65 Å resolution. The C-CggR has an open 3 layer / / sandwich architecture (Figure 1). The central part is formed by a seven-stranded parallel -sheet surrounded on both sides by five -helices and is similar to an NAD-binding Rossmann fold. Three lateral subdomains are added in C-CggR to the central part so that the overall structure resembles a trefoil in its shape. A deep surface cleft located between the lateral subdomains and the central part represents the effector binding site (indicated by an arrow in Figure 1).

With the aim to gain structural information on the effector binding, we determined crystal structures of C-CggR in complex with various ligands. In addition to previously identified effector FBP, we also observed the binding of glyceraldehyde-3-phosphate (G3P), glucose-6-phosphate (G6P), and fructose-6-phosphate (F6P) in our crystal structures. The binding of these ligands in solution was investigated by the isothermal titration calorimetry and their effect on C-CggR oligomeric state was studied through chemical cross-linking.

We conclude that C-CggR is able to bind several different small molecules and the ligand binding is accompanied with changes in C-CggR oligomeric state. Our results strongly suggest that CggR could be modulated by various effectors.



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