L15

SOLVATION OF NUCLEIC ACID BACKBONE: A DFT STUDY

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A negatively charged phosphate group of nucleic acid backbone interconnecting two (2-deoxy)ribose units represents one of the most important solvation sites in nucleic acids. An impressive amount of work has been done on characterizing the structure of the solvation shell of canonical DNA as well as of other backbone patterns found in RNA. Surprisingly narrow regions of water occurrence in the direct contact (H-bond) with phosphate group have been observed in crystals. The presence of physiological monovalent and divalent cations in the phosphate first solvation shell was also confirmed [1].

The X-ray identification of 3rd period alkali metal ions (Na^+, Mg^{2+}) is not a straightforward task since these ions and the water molecule possess the same number of electrons. In many cases, the methods of molecular spectroscopy can be used for metal ion recognition [2-4]. We investigated the possibility of characterizing the specific interactions of metal ions with nucleic acids by NMR spectroscopy. Ab-initio computational methods were applied to selected nucleic acid structural patterns including explicit solvent molecules. We outline several options for monitoring the presence of metal ions in contact with nucleic acids.

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L16

THE INFLUENCE OF BACKBONE AND SOLVENT DYNAMICS ON ^{31}P CHEMICAL SHIFT TENSORS IN DICKERSON DODECAMER: A COMBINED MD/DFT STUDY

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^{31}P chemical shift tensors ($_{ii}$) can aid nucleic acid structure determination [1]. Due to the lack of experimental data, theoretical calculations are a valuable method of choice to obtain $_{ii}$. Previous results of such calculations on static models of phosphate groups proved to provide useful, yet rather limited information, as they do not account for dynamical effects [2,3]. Internal conformational motion as well as the continuous breaking and forming of hydrogen bonds between solvent molecules and phosphate oxygens influence ^{31}P chemical shift tensors considerably. Therefore, we have performed classical molecular dynamics (MD) simulation of $[d(CGCGAATTCGCG)]_2$ and used the snapshots from the MD trajectory for chemical shift tensor calculations. Small cluster models consisting of dimethyl phosphate and water molecules within the first solvation shell have been employed. Calculations were carried out at the density functional level (DFT) of theory, applying gradient-corrected BP86 functional and IGLO-III basis set. Changes in chemical shift tensors introduced 1) by extending the explicit solvent beyond the first solvation shell and 2) by adding implicit solvent or partial point charges to the small cluster models have been analysed. In order to assess the direct effect of hydrogen-bonding, the results obtained are also compared to chemical shift tensors calculated for dimethyl phosphate without any coordinated water molecules.

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Lectures - Friday, March 13, Session V

L17

DNA CONFORMATIONS AND THEIR SEQUENCE PREFERENCES

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We analyzed phosphodiester backbone conformations of almost eight thousand DNA dinucleotides from about 500 carefully selected crystal structures containing naked and complexed DNA extracted from the Nucleic Acid Database (NDB, [1]). The analysis identified all the known major conformers of the B, A, and Z types thereby confirming the validity of the procedure but we also observed various intermediate BI/BII and B/A conformers. We investigated how the conformers build larger, often deformed or unusual DNA structures as bent or kinked DNA, tetraplexes, and junctions. To summarize, the BI-form ("canonical" B-form) is by far the most populated in both naked and complexed DNA, BII is a distinct B-form but both BI and BII are bridged in protein complexes by a series of intermediate conformers. We confirmed that DNA in protein complexes acquires quite frequently A-like conformations and even pure A-form. Proteins in general broaden the DNA conformation space and induce existence of conformers not or rarely observed conformers as intermediate BI/BII or mixed B/A.

The wrapping of DNA around histone proteins in a nucleosome-core particle is achieved by a fairly regular alteration of BI and BII conformers, BII is occasionally substituted by deformed BI or B/A conformers. Even in the highly deformed histone-wrapped DNA two or more BII conformers follow each other in sequence only very rarely.

For naked DNA, we examined possible sequence preferences of various dinucleotide conformers by chi-square statistical tests. In the closely studied B-like duplexes, the conformers were grouped into five broad categories, BI, BII, A-to-B, B-to-A, and "non-classified". We observed important sequence preferences between these conformers. Here we highlight just a few: Homogenous RR and YY steps (except GG) are over-represented in BI; TG and its Watson–Crick counterpart, CA, prefer BII; the CG and GC steps show a high propensity for mixed B/A conformations. The GC step shows mixed conformational preferences and many GC steps are structurally highly unusual; of all dinucleotides, this step has conformationally the most complicated behavior.

This study concludes our exploration of the conformational space of DNA [2] and RNA [3, 4] dinucleotides.

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L18

MOLECULAR DYNAMICS STUDY: INTRINSIC DYNAMICS OF RNA THREE-WAY JUNCTION

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Ribosome is a large biomolecular machine which resembles a brick-box composed of **molecular building blocks -** RNA motifs having different shapes, flexibilities, capabilities to interact with ribosomal surrounding elements, e.g K-turns possessing elbow dynamics [1], RNA helix showing anisotropic bending [2] or segment of specific rigid shape [3]. Molecular dynamics (MD) simulation is a suitable method for characterizing properties and topology of individual RNA segments.

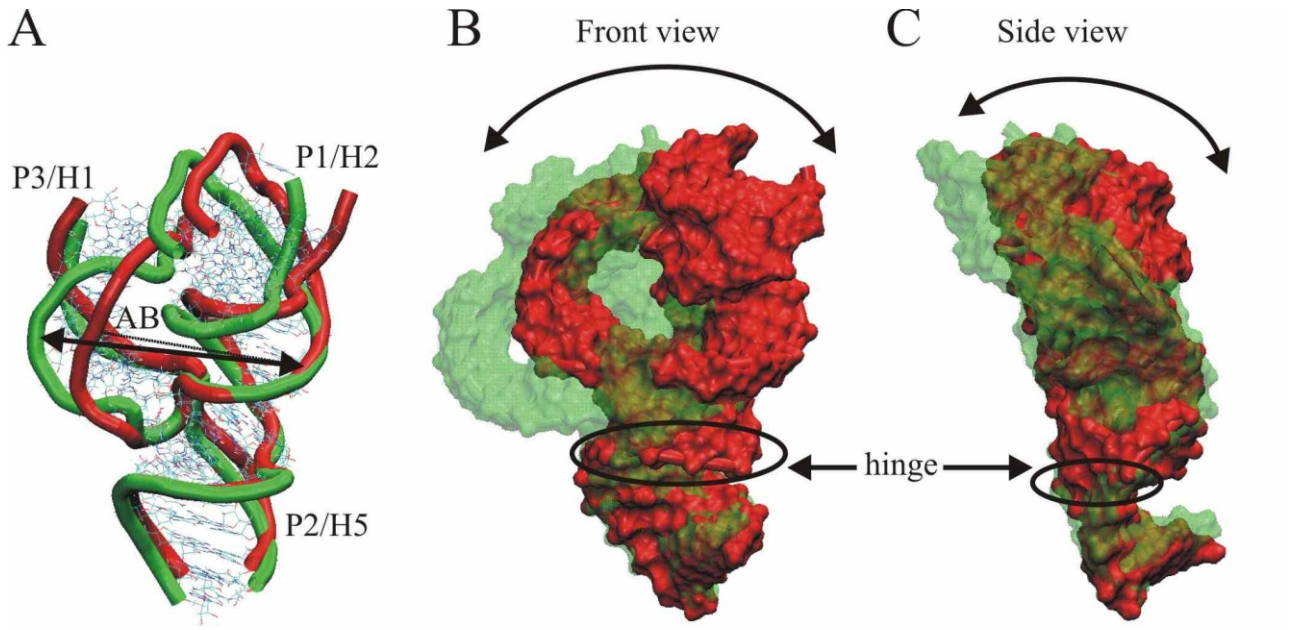

Figure 1. Overlay of 5S 3WJ structures in its extremal substates of two characteristic intrinsic dynamics (A) breathing- like motion, (B, C) hinge- like motion.

This research is focused on dynamics of one of the important ribosomal building block - RNA three-way junction (3WJ) family C. [4] The structure is composed of three helices P1, P2 and P3 diverging from one point, while P1 and P2 helices are coaxially stacked. [4] There are also tertiary interactions between stems P1 and P3, which are characteristic especially for the family C. [4] The study involves three junctions - Peptidyl Transferase Center 3WJ (helices 90-92), GTP-ase associated center 3WJ (helices 40-42) and 3WJ from the 5S rRNA. Crystallographic structures from archea Haloarcula Marismortui and bacteria *Escherichia coli* were used.

This extensive molecular dynamics study including simulations of total length more than 0.6 s showed two dominant structural motions which are very similar for all three 3WJs. The first dynamics includes hinge-like fluctuations between the coaxially stacked stems P1/P3 (forming a compact RNA part of the structure) and P2, classified as a hinge-like motion (see Figure 1 B-C). The second one is internal dynamics of stems P1 and P3 called as a breathing-like motion (see Figure 1 A).

All three studied junctions are associated with extended regions of negative electrostatic potential which are in many cases major binders of monovalent cations with 100% occupancy and very slow exchange of ions.

To sum up, 3WJs belong to RNA building blocks including a characteristic intrinsic dynamics of hinge- and breathing- like motion.

L19

DOUBLE-HELICAL LADDER STRUCTURAL TRANSITION IN THE B-DNA IS INDUCED BY A LOSS OF DISPERSION ENERGY

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The role of the dispersion energy and electrostatic energy on the geometry and stability of the B-DNA helix was investigated. Both molecular dynamics simulations with empirical force field and hybrid quantum mechanical/ molecular mechanics molecular dynamics simulations, where the dispersion or electrostatics term is suppressed/increased, have shown that the lack of the dispersion term leads to an increase of the vertical separation of the bases as well as to a loss of helicity, thus resulting in a ladder-like structure. A decrease of the electrostatic term produces a separation of the DNA strands.

L20

STRUCTURE-BASED DRUG DESIGN OF SELECTIVE 5'-NUCLEOTIDASES INHIBITORS

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The monophosphate 5'-nucleotidases, including 5'(3')-deoxyribonucleotidase, belong to a family of enzymes that catalyze the dephosphorylation of nucleoside monophosphates. The ribonucleotides and deoxyribonucleotides could be synthesized *de novo* from low-molecular-weight precursors or by salvage from nucleosides or nucleobases coming 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 synthesis of DNA and RNA. The phosphorylation by cellular nucleoside kinases is opposed by 5'-nucleotidases that dephosphorylate ribo- and deoxyribonucleoside monophosphates [2,3,4]. 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 main goal of this project is the search for potent and selective inhibitors of mammalian 5'-nucleotidases based on nucleoside phosphonic acids and their derivatives and comparison of sensitivity of 5'-nucleotidases isolated from various sources toward individual inhibitors.

We have prepared 2 types of human 5'-nucleotidase: cytosolic and mitochondrial by recombinant expression in *E. coli*. The inhibitory properties of a series of nucleoside phosphonic acids derivatives are tested and for the most promising compounds the enzyme-inhibitor structure will be determined to serve as a lead for structure-based drug design efforts.

In general, compounds of strong and selective inhibitory potency are of high medicinal interest as antimetabolites for anticancer and antiviral therapy.

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L21

SIMULATING A-TRACT DNA: THE INFLUENCE OF SUBSTATES AND THE MEASURE OF BENDING

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A-tracts, or stretches of duplex DNA comprising at least four consecutive adenines or thymines without a TA step, exhibit special features in the context of the B-DNA conformational family. A-tracts embedded in a DNA sequence composed of G or C result in a structure bent by roughly 20 deg into the minor groove of the A-tract; moreover, both experiments and simulations suggest that they are exceptionally stiff and adopt a particular structure. Since their discovery more than 25 years ago [1], a large number of crystallographic [2], NMR [3], and other [4] experimental studies have been devoted to A-tracts, trying to decipher their structural features and in particular the origin of bending. Existing computational studies using atomistic molecular dynamics (MD) either include now obsolete force fields and trajectories too short by contemporary standards [5], or use restraints on the A-tract geometry

[6]. With the emergence of the Amber parmbsc0 force field which fixes a fundamental deficiency of the previous parm94/99 versions (namely the irreversible flips in alpha/gamma backbone torsions resulting in the unwinding of the double helical structure in long simulations), the question arises as to how this new force field is able to reproduce A-tract bending and other features. We present the analysis of all-atom MD simulations of DNA oligomers containig the A-tract A_4T_4 sequence, and those containing the non-A-tract T_4A_4 , either as one copy or as two copies in phase. Each MD trajectory has been prolonged to 150 ns. In the course of the analysis, we confronted the problem of properly measuring the global bending of a DNA tract. We present a critical analysis of the existing methods and propose a new one, based on mathematically rigorous averaging of base-fixed coordinate frames. The study represents a stringent test of the reliability of the current Amber force field, and the proposed bending measurement method may be applied to other nucleic acids systems as well, for instance to ribosomal RNA motifs where the overall geometry plays an important functional role.

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Lectures - Friday, March 13, Session VI

L22

CONDUCTIVITY OF NATURAL AND MODIFIED DNA MEASURED BY SCANNING TUNNELING MICROSCOPY. THE EFFECT OF SEQUENCE, CHARGE AND STACKING

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The conductivity of DNA covalently bonded to a gold surface was studied by means of the STM technique. Various single- and double-stranded 32-nucleotide-long DNA sequences were measured under ambient conditions so as to provide a better understanding of the complex process of charge-carrier transport in natural as well as chemically modified DNA molecules. The investigations focused on the role of several features of DNA structure, namely the role of the negative charge at the backbone phosphate group and the related complex effects of counterions, and of the stacking interactions between the bases in Watson-Crick and other types of base pairs. The measurements have indicated that the best conductor is DNA in its biologically most relevant double-stranded form with Watson-Crick base pairs and charged phosphates equilibrated with counterions and water. All the studied modifications, including DNA with non-Watson-Crick base pairs, the abasic form, and especially the form with phosphate charges eliminated by chemical modifications, lower the conductivity of natural DNA.

L23

THERMODYNAMIC ANALYSIS OF THE ENANTIOSELECTIVITY OF HALOALKANE DEHALOGENASE DBJA AND ITS VARIANTS DBJA AND DBJA +H139A TOWARDS BROMINATED ESTERS AND -SUBSTITUTED BROMOALKANES

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Haloalkane dehalogenase DbjA from *Bradyrhizobium japonicum* USDA110 [1] shows excellent enantioselectivity towards brominated esters (*E*-value > 200 and high enantioselectivity towards -substituted bromoalkanes (*E*-value 145). Substrate mapping revealed that brominated esters are kinetically resolved by DbjA as well as two other haloalkane dehalogenases DhaA *Rhodococcus rhodochrous* NCIMB13064 and LinB from *Sphingobium japonicum* UT26, while -substituted bromoalkanes only by DbjA.

Structural analysis and comparison of the primary sequence of DbjA with protein sequences of other family members revealed the presence of an inserted fragment unique to DbjA, which is located on the protein surface. Construction and characterization of DbjA variant carrying deletion of the extra amino acid sequence showed that the fragment is involved in enantioselectivity of DbjA with -substituted bromoalkanes, but not with the brominated esters. Following detailed comparison of crystal structures of DbjA and DbjA enzymes indicate importance of amino acid residue H139 to chiral recognition of -substituted bromoalkanes, resulting in the construction of DbjA +H139A variant. Its kinetic characterization revealed modulation of enantioselectivity towards -substituted bromoalkanes and no changes in enantioselectivity with brominated esters.

In this work, the thermodynamic analysis has been used to address the origin of enantiomeric discrimination by determining differential activation enthalpy and entropy for the reaction of DbjA, DbjA and DbjA +H139A enzyme variants with selected brominated esters and -substituted bromoalkanes. Differential activation enthalpy was found to be a major determinant of chiral recognition for brominated esters by all three enzyme variants. Enzyme enantioselectivity towards brominated esters could be explained by different binding interactions of individual enantiomers with the residues of the active site in the Michaelis complex and/or the transition state of dehalogenation reaction. On the other hand, entropy was found to play equally important role as enthalpy for enantiomeric discrimination of 2-bromopentane by DbjA and DbjA +H139A. Interestingly, DbjA showed reversed dependence of enantioselectivity on temperature for 2-bromopentane when entropy dominated over enthalpy contribution towards enzyme enantioselectivity. Importance of entropy for kinetic resolution of -substituted bromoalkanes by all studied enzymes can be related to different flexibility of both enantiomers in the enzyme active site and different solvation/desolvation of the enzyme active site upon binding.

In summary, obtained results imply that DbjA possesses two chemically distinct bases of enantioselectivity towards brominated esters and -substituted bromoalkanes and that enzyme variants with different thermodynamic contributions towards enantioselectivity can be constructed by protein engineering.

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L24

STRUCTURAL STUDIES OF SELECTED HUMAN RYANODINE RECEPTOR 2 DOMAINS

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Human ryanodine receptor 2 (RyR2) is one of the three isoforms of the ryanodine receptor, the ion channel that mediates Ca^{2+} release from intracellular calcium stores. RyR2 is responsible for releasing Ca^{2+} ions necessary for contraction in cardiac myocytes. It is a large homotetramer, composed of four ~5000 amino acid residues. Because the whole molecule is very large, we identified, cloned, expressed and purified individual domains of RyR2 and performed first crystallization experiments.

The first domain prediction, obtained by program Pfam, suggested the existence of 14 domains in the RyR monomer. The prediction was further adjusted by comparison with RyR2 secondary structure prediction and with the 3D structure of the IP3-binding domain of the inositol-3-phosphate receptor (PDB ID 1N4K). The cDNA sequence involving residues 1 - 759 (Tunwell *et al.*, *Biochem J.* 318:477-487, 1996) was obtained from Prof. F.A. Lai (University of Cardiff). A fragment from the central part of the RyR was synthesized by GeneScript, USA. Three N-terminal domains and two central domains were cloned and expressed with His-Tag fusion at C-terminus. The N-terminal domains were also expressed with Nus or Trx fusion partners to increase solubility and obtain correct folding of expressed proteins. All constructs were expressed in *E. coli,* BL21 (DE3) at optimized temperature, IPTG concentration and expression time. His-Tag purification was used as the main purification step. The monomeric state of each purified fragment was assessed by gel filtration. The tested N-terminal domains differed markedly in their expression level, solubility and monomeric state. Crystallization experiments with one of the fragments, performed with the crystallization kit Structure Screen 1 (Molecular Dimensions), yielded crystals with dimensions ~0.2 x 0.1 x 0.05 in 1.5 M Lithium sulfate, 2% PEG 8000. Crystallization of other fragments and testing their diffracting power is under way.

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L25

DIRECTION OF IMMUNE SYSTEM IN T1D PATHOGENESIS – ANALYSIS BY USING GENE MICROARRAY AND PATHWAY ANALYSIS (GENEGO, METACORE)

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Introduction

Diabetes mellitus is a disorder of metabolism that manifests itself as type I or type II. The Type I (T1D, the object of this study) is caused by destruction of the insulin-producing beta-islet cells of the pancreas. It results in development of well known disorder of carbohydrate metabolism which leads to life-lasting necessity of insulin injection administration. It seriously impact not only the patient itself but it has also strong socioeconomic consequences. Nowadays that situation becomes worse in the Czech Republic due to increase in T1D occurrence among infants and children.

Aim

The aim of our study was to monitor *in vitro* changes in gene expression (mRNA transcription) in peripheral blood mononuclear cells (PBMCs) after stimulation with diabetogenic autoantigens within one family related to different manifestation of the T1D with respect to early detection of developing destructive insulinitis.

Method

PBMCs were divided into equal halves and one half was stimulated using derived syntetic peptides such as glutamic acid decarboxylase 65 (GAD65), IA2 (islet antigen-2, tyrosinephosphatase) and proinzulin. After 72hours incubation totalRNA was isolated from both groups - stimulated and intact which represents basal gene expression.

After RNA isolation, amplification and fluorescent labeling, the samples were competitively hybridize on the high density whole-genome microarray HOA (Phalanx Biotech). The microarray contains over 30 000 genomic probes. Hybridized slides were scanned and analyzed using GeneSpring GX (Agilent) and Pathway analasis software MetaCore (GeneGo). The microarray part was done as a service in microarray facility of Central European Biosystems s.r.o..

Results and conclusion

We identified more than 100 genes which belong mainly to regulatory factors and transcription factors. In accordance with previously published results we found out the similar expression pattern between basal expression and expression in stimulated samples depending on the diabetes development. As a promising results seems especially a decline in the expression of some cytokinines and chemokines in prediabetic phase. Important increase in gene expression after specific stimulation was observed for following genes in this study group ($p < 0.05$): IFN-gamma, IL-1,-2,-6,-13,-22,-31, GATA-3, JUNB, IL-6R, STAT-6, TGF-beta. Moreover probably the most important difference was observed for IL-23R which was importantly downregulated in this group (12 fold difference) and also in T1D patients group (23 fold). In T1D patients we also observed an important activation of IL-2,- IL-33, JUNB genes after specific stimulation but strong downregulation of IL-4. STAT-6 and GATA-3 genes were also slightly downregulated in this group.

The study confirms that there can be find differential expression pattern and markers which preveal risk of diabetes.

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L26

ROLE OF 14-3-3 PROTEINS IN THE REGULATION OF G-PROTEIN SIGNALING

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In response to specific agonist signals, G protein-coupled receptors (GPCR) act as guanine nucleotide exchange factors and promote the exchange of GDP for GTP on the G subunit of heterotrimeric G proteins. This process is followed by a dissociation of GTP-bound G from the G subunit. Both G and G are thus activated and can propagate downstream signaling via effectors and second messengers [1]. The deactivation of G protein-mediated signaling occurs via the inherent GTPase activity of the G_{α} subunit, which causes the hydrolysis of GTP to GDP and subsequent assembly of G protein heterotrimer.

Regulator of G protein signaling (RGS) proteins share a highly conserved 125-amino-acid large domain that was first identified by its ability to negatively regulate GPCR signaling [2]. To date, more that 25 proteins containing RGS or RGS Homology domains have been identified. Some RGS proteins consist of little more that the RGS domain (e.g. RGS1, RGS2, RGS4) while others posses long N-terminal or C-terminal extensions (e.g. RGS3, RGS7, RGS9) that usually contain additional protein-protein interaction motifs and domains [3]. RGS proteins function as GTPase-activating proteins (GAPs) for the G subunit of heterotrimeric G proteins. They bind specifically to the GTP-bound forms of G and significantly stimulate GTP hydrolysis by stabilizing the transition state.

The activity of RGS proteins is tightly regulated through various mechanisms including posttranslational modifications and interactions with other proteins. Several RGS proteins, e.g. RGS3, RGS4 and RGS7, have been found to interact with 14-3-3 proteins. 14-3-3 proteins are a family of acidic regulatory proteins that function as molecular scaffolds by modulating the structure of their binding partners. Results published by several groups suggested that the 14-3-3 protein binding to selected RGS proteins decreases their inhibitory effect on G protein signaling presumably by blocking the interaction between RGS and G_{α} subunit [4,5].

To elucidate the mechanism of 14-3-3 protein-dependent regulation of RGS function, we performed biophysical characterization of interactions between RGS3 and 14-3-3 protein. Our results show that 14-3-3 interacts with both phosphorylated motif located within the N-terminal part of RGS3 and C-terminally located RGS domain. Association of RGS3 with 14-3-3 induces a significant structural change within the RGS domain, which can explain the observed 14-3-3 protein-dependent inhibition of RGS3 function.

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