L11

CHARGE TRANSPORT THROUGH DNA/DNA DUPLEXES AND DNA/RNA HYBRIDS: COMPLEX MECHANISM STUDY

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The position of oligonucleotides among biological and man-made polymers is unique due to its ability to preserve, transfer, and transmit information. Charge transfer through oligonucleotides may serve as an indicator of changes in many physical and chemical properties or to repair some of their lesions. Oligonucleotides conduct electric charge via various mechanisms and their characterization and understanding is very important and complicated task. In this work, experimental and theoretical approaches (time-resolved and steady state fluorescence spectroscopy, melting point measurements plus Density Functional Theory) were combined to study charge transport processes in short DNA/DNA and RNA/DNA duplexes with virtually equivalent sequences. The optical spectroscopy measurements revealed higher values of charge transport through RNA/DNA hybrids. The experimental results were consistent with the theoretical model - the delocalized nature of HOMO orbitals and polarons, base stacking, electronic coupling and conformational flexibility form the conditions for short distance charge transport processes in RNA/DNA hybrids via a coherent charge transport mechanism. In contrast, more localized polarons and molecular orbitals without significant electronic overlap between adjacent base pairs in DNA/DNA duplexes create less effective conditions for charge transport that can occur via incoherent multistep random transport mechanism.

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L12

PLANT NUCLEASE TBN1 INVOLVED IN APOPTOTIC PROCESSES BLOCKS ITS ACTIVE SITE BY A SURFACE LOOP – SIGN OF REGULATORY FUNCTION?

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Tomato multifunctional nuclease (TBN1; UniProt accession no. Q0KFV0), which belongs to the nuclease type I family, plays an important role in specific apoptotic functions, vascular system development, stress response, and tissue differentiation in plants [1]. Furthermore, TBN1 exhibits anticancerogenic properties [2]. The enzyme posseses endonuclease and exonuclease-like activity on ds and ss RNA and DNA and on structured RNA, with production of 5'-mono- and oligonucleotides [3]. TBN1 consists of 277 aminoacids with a molecular mass of 31.6 kDa (about 37 kDa when glycosylated).

Structures of wild type TBN1 and mutant N211D were solved by our group by the means of X-ray crystallography [4]. Molecules of TBN1 form super-helices generated by crystal symmetry, where contacts are provided by the active site of one molecule and a surface loop of a neighboring molecule. This motif is conserved in all the known crystal structures of the enzyme but the rest of crystal packing differs across different crystallization conditions. Formation of intermolecular contacts in crystals suggests the way of assembly of molecules into oligomers in solution. The interaction of the active site and the surface loop is best

resolved in the currently reported structure, where the active centre at the zinc cluster is occupied by phosphate ion. It correlates with the behavior of TBN1 in phosphate buffer, observed with dynamic light scattering. The phosphate ion binds in the same fashion as the corresponding part of a substrate analog in the structure of Phospholipase C [5] with highly homologous active centre.

Properties of mutants, designed to modify dimerization and activity of TBN1, suggest that deliberate disruption of the loop-active site contacts by mutations limits expression of the active enzyme. Therefore formation of TBN1 oligomers together with phosphate binding are hypothesized to have regulatory roles in apoptotic-like and senescense processes in plant cells.

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L13

PREPARATION, CRYSTALLIZATION AND PRELIMINARY STRUCTURAL ANALYSIS OF AHP2 PROTEIN, THE SIGNAL TRANSMITTER FROM *ARABIDOPSIS THALIANA*

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Histidine-containing phosphotranfer proteins from *Arabidopsis thaliana* (AHP1-5) function as a signal transmitters between sensor histidine kinases and response regulators via multistep phosphorelay (MSP). AHP proteins mediate and potentially integrate various MSP signalling pathways (e.g. cytokinin, ethylene, osmosensing). However, structural information about AHP proteins and its importance in the MSP signalling is scarce.

To get insights into structural determinants of AHP-mediated signalling, the coding sequence of AHP2 protein was cloned and purified to the homogeneity. To enhance its crystallization behaviour, AHP2 was transferred to the buffer optimal for its thermodynamic stability prior to crystallization. The obtained crystals diffracted up to 2.5 Å (BESSY, Berlin) and show significant anisotropic behaviour. AHP2 structure was determined by SIRAS protocol using the anomalous signal of the Lutetium.

According to the preliminary results AHP2 consists of a four-helix bundle with the phophorylated His residue in the center of the second helix, thus representing the conserved core common for all known HPt structures. The final structure refinement is currently being in progress.

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L14

HUMAN CARDIAC RYANODINE RECEPTOR: STRUCTURAL STUDY OF THE N-TERMINAL REGION

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Human ryanodine receptor (hRyR2) is a cardiac calcium ion channel present in sarcoplasmatic membrane [1,2]. It mediates the calcium ions release in response to electrical stimulation during excitation-contraction coupling. Human RyR2 is large homotetramer, composed of four subunits with a molecular weight of 560 kDa [3]. N-terminal (aa. 1-655) and central (aa. 2100-2500) region are believed to be involved in channel gating regulation [4]. Mutations located in these two regions are linked to several heart diseases [5]. To understand hRyR2 gating mechanism and prevent its malfunction, it is of high importance to know the atomic structure of the key regions as well as the entire molecule.

This contribution reports structural study of hRyR2 1-606 region. This region was analysed by x-ray crystallography as well as by small angle x-ray scattering analysis (SAXS). Samples for crystallization and SAXS analysis were prepared through IMAC and size exclusion chromatography. Quality of the samples was tested *via* SDS and native PAGE and stability was analysed with DLS. After a successful crystallization, diffraction quality crystals and diffraction data to 2.5 Å were obtained. Model of the hRyR2 1-606 structure was created using software package CCP4 and the phase problem was solved by molecular replacement using homolog rabbit RyR1 structure (PDB ID: 2XOA). The structure model has revealed conserved three domain structure, similar to rabbit RyR1 (2XOA) [6] and inositol 1,4,5-trisphosphate receptor (3UJ0) [7]. SAXS analysis led to low resolution model of hRyR2 1-606 showing regions missing in x-ray model. Docking of x-ray model into SAXS envelope was done and modelling of missing parts is yet to be done.

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L15

STRUCTURAL STUDY OF LEDGF/P75 BINDING PARTNERS

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Lens epithelium-derived growth factor p75 (LEDGF/p75) is a prominent cellular cofactor for human immunodeficiency virus (HIV) integration. LEDGF/p75 tethers the preintegration complex to the host chromosome and this process is crucial for HIV replication. HIV integrase interacts with the C-terminal part of LEDGF/p75, region designated integrase-binding domain (IBD, amino acids residues 347 - 429). Interaction interface between HIV integrase and LEDGF/p75 became an attractive target for design of small molecule inhibitors blocking this interaction [1].

While the role of LEDGF/p75 in HIV integration is well characterized, very little is known about its physiological function. As a transcriptional co-activator, LEDGF/p75 is implicated not only in HIV replication, but also in human cancer and autoimmunity. The LEDGF/p75 was shown to interact through its IBD with several cellular proteins and recent evidence implies that LEDGF/p75 is a general adaptor protein tethering various factors to chromatin [2].

In this work, we set to prepare two LEDGF/p75 physiological binding partners JPO2 [2] and pogo transposable element (pogZ) [3]. The aim of our study is to obtain structural information on the LEDGF/p75 interaction with its physiological binding partners JPO2 and pogZ, respectively. Such structural information is essential for understanding the LEDGF/p75 biological role and might help in design of inhibitors selectively blocking interaction with HIV integrase while not interfering with the LEDGF/p75 biological function.

The IBD, interaction domain of LEDGF/p75 was cloned and expressed in *E. coli*. The protein was purified with yields sufficient for binding and structural studies. The JPO2 and pogZ were cloned and isolated as a full-length proteins and several alternative constructs encompassing individual domains with IBD binding affinity. Both proteins were biophysically characterized as well as the reconstituted complex of JPO2 and IBD. The JPO2 protein was found to be intrinsically flexible, which prevented us getting any crystals and forced us into an alternative path of structural characterization of the complex of JPO2 and IBD.

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L16

THE POWER IS IN DETAIL - STRUCTURAL ANALYSIS OF AN *ALEURIA AURANTIA* LECTIN MUTANT

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Lectins are able to specifically and reversibly bind carbohydrates and therefore are widely used for detecting, labelling and isolation of glycoproteins and other sugar containing moieties. Changes in glycosylation are connected to various processes including cancer development. Among sugar moieties exhibited on the cell surface, the important role is played by L-fucose (6-deoxy-L-galactose) [1]. Most known fucose-specific lectin is AAL isolated from *Aleuria aurantia*. Despite the long-term investigation of this protein including the solution of its structure [2], more detailed characterization is demanded in order to create varieties of more strict specificity and/or increased affinity.

The preparation of several artificial constructs based on AAL protein was reported [3]. We have crystallized one such mutant that has been proved to have stronger affinity towards 1-6 bound fucose. We were able to collect several datasets of high quality with resolution up to 1.09 Å. The detailed insight into the structure revealed the principal of the affinity change, shading the light upon variability of AAL's binding sites differences. Furthermore, we utilized *in silico* approach to describe the contribution of particular amino acids in each of five AAL's binding sites. The combination of experimental and computational techniques is a powerful tool to decipher a complex interaction, where both hydrogen bonds and dispersion forces take part. This research helps us to improve our knowledge of not yet fully understood background of sugar binding variability among AAL lectin family which includes also proteins from opportunistic pathogens such as *Burkholderia ambifaria* [4] or *Aspergillus fumigatus*.

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