Student Symposium

Tuesday, June 11

Session I

SL1

AVIAN ORTHOREOVIRUS PROTEIN - NS FORMS PSEUDOCAPSIDS

Barbora Kascakova [1], Zdenek Franta [1], Tatyana Prudnikova [1, 2], Ivana Kuta Smatanova [1, 2], Michal Kuty [1, 2], Roman Tuma [1, 3]

[1]University of South Bohemia, Faculty of Science, Branisovska 1760, CZ-37005 Ceske Budejovice, Czech Republic;
[2]Academy of Sciences of the Czech Republic, Center of Nanobiology and Structural Biology, Institute of Microbiology, Zamek 136, 373 33 Nove Hrady, Czech Republic;
[3]University of Leeds, School of Chemistry, The Astbury Centre for Structural Molecular Biology, Leeds LS2 9JT, UK
barbora.karaffova@gmail.com

The fusogenic avian Orthoreoviruses of Reoviridae family are important pathogens of birds that can cause considerable economic losses in the poultry industry. Avian reoviruses have been associated with a variety of disease conditions in poultry [1]. The avian reovirions are non-enveloped icosahedral particles of 85 nm external diameter with 10 dsRNA genomic segments (23.5 kb) encased within two concentric protein shells, forming the outer capsid and the core [2]. The RNA replication and morphogenesis of reoviruses occurs exclusively within cytoplasmic inclusion bodies, also known as viral factories, or 'viroplasms'. The viroplasms are formed by non-structural protein NS in association with non-structural protein NS [3]. The NS acts as RNA chaperone and destabilizes helical regions of RNAs. The structure is not known. The NS protein was constructed in order to study the process of the viroplasm formation in details. The NS is a non-structural protein approximately 41 kDa large and is composed of 367 amino acids. The homology modelling by Phyre2 prediction server estimated a high - helical structure [4]. The SAXS experiments revealed the elongated pear- shaped structure. The NS protein is homodimer as a biological unit with high probable further hexamerisation. It forms likely octamers in the presence of bound ssRNA in solution by hydrophobic interactions. The NS rapidly bind ssRNA in a sequence-independent manner and then form large nucleoprotein complex [5].

Here we report the results of the expression, purification and further crystallization experiments of the NS protein. Subsequent monitoring of purified samples revealed that protein is quite stable and in presence of ssRNAs forms octamers. In other case, NS forms pseudocapsids that were observed by EM negative staining.

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SL2

EXPERIMENTAL AND COMPUTATIONAL INVESTIGATIONS OF THE HUMAN SYSTEM PAPS-SYNTHASE

A. Shaposhnikova, P. Grinkevich, M. Kuty, I. Kuta Smatanova

Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic, Faculty of Science, University of South Bohemia in Ceske Budejovice, Ceske Budejovice, Czech Republic

PAPS is a derivative of adenosine monophosphate that is phosphorylated at the 3' position and has a sulfate group attached to the 5' phosphate. It is the most common coenzyme in sulfotransferase reactions. PAPS is synthesized from ATP and inorganic sulfate by PAPS synthetase (PAPSS). Proper function of PAPSS is essential for normal physiology in human. Mutations in the PAPSS genes could cause severe disease states. In humans there are three isoforms of PAPSS: PAPSS1, PAPSS2a, and PAPSS2b which are predominantly expressed in brain, cartilage and liver respectively. PAPSS1 and PAPSS2a/b are about 73% similar in amino acid sequences. The kinetics of PAPSS formation between these isoforms are different.

We cloned PAPSS2 using pMAL-c2 and pTrc vectors. Also, we optimized expression by varying the temperature (4, 15, 20, 37 °C), time (3, 14, 20 hours and 2, 5, 7 days) and the concentration of the inducer (with 0.5 mM or 1 mM of IPTG and without IPTG). After expression, we purified protein using amylase and nickel columns. Protein was crystallized using robot also we did optimization for crystallization and tested the crystals on the diffractometer. In the future, we plan to solve structure PAPS-synthase 2 and understand the mechanism of its reaction.

SL3

STRUCTURAL AND FUNCTIONAL STUDIES OF HAD PHOSPHATASE Tt82 FROM *THERMOCOCCUS THIOREDUCENS*

Petra Havlickova[1], Vitezslav Brinsa[2], Jiri Brynda[2,3], Petr Pachl[2], Tatyana Prudnikova[1,4], Jeroen R. Mesters[5], Barbora Kascakova[1], Michal Kuty[1,4], Marc L. Pusey[6], Joseph D. Ng[6], Pavlina Rezacova[2,3] and Ivana Kuta Smatanova[1,4]

[1]*Institute of Chemistry, Faculty of Science, University of South Bohemia, Branisovska 1760, Ceske Budejovice, Czech Republic*

[2]*Institute of Organic Chemistry and Biochemistry, ASCR, Flemingovo namesti 2, Prague, Czech Republic*

[3]*Institute of Molecular Genetics, ASCR, Videnska 1083, Prague, Czech Republic*

[4]*Institute of Microbiology, Center for Nanobiology and Structural Biology, ASCR, Zamek 136, Nove Hrady, Czech Republic*

[5]*Institute of Biochemistry, University of Lubeck, Ratzeburger Allee 160, Lubeck, Germany*

[6]*Biological Sciences, University of Alabama in Huntsville, 301 Sparkman Dr., Huntsville, AL, USA*

The HAD (haloacid dehalogenase) superfamily is one of the largest known group of enzymes and the majority of them catalyze the hydrolysis of phosphoric acid monoesters into a phosphate ion and an alcohol. Phosphatases in general are enzymes classified into the number of superfamilies. Due to their diverse substrate specificity, the understanding of the complete substrate profiling and function is very limited [1]. To gain insight into their biological functions and possible biotechnological applications various biochemical and computational methods can be used. Despite the fact that sequence similarity between HAD phosphatases is generally very low, the members possess some characteristic features, such as Rossmann-like fold, HAD signature motifs or the requirement for Mg^{2+} ion as an obligatory cofactor. This study was focused on new hypothetical HAD phosphatase from *Thermococcus thioreducens*. The protein crystallized in space group $P2_12_12$ with unit-cell parameters a = 66.3, b = 117.0, c = 33.8 Å, and the crystals contained one molecule in the asymmetric unit. The protein structure was determined by X-ray crystallography and refined to 1.75 Å resolution. The structure revealed a putative active site, common to all HAD members. Computational docking into the crystal structure was used to propose substrates for the enzyme. Activity of this thermophilic enzyme towards selected substrates was confirmed at temperature 333 K.

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SL4

PRELIMINARY CRYSTAL STRUCTURE ANALYSIS OF SERPIN 4 FROM TICK

A. Mazur[1,2], B. Kascakova[1], T. Prudnikova[1,2], J. Chmelar[1] and I. Kuta Smatanova[1,2]

[1]Faculty of Science, University of South Bohemia in Ceske Budejovice, Branisovska 31, 37005 Ceske Budejovice, Czech Republic

[2]Center of Nanobiology and Structural Biology, Institute of Microbiology, Academy of Sciences of the Czech Republic, Zamek 136, 37333 Nove Hrady, Czech Republic

mazur@nh.cas.cz

Serpins (serine protease inhibitors) belong to the group of protease inhibitors superfamily. They irreversible inhibit proteases by undergoing to a huge conformational change to break the proteases active site. The changes in serpin structure caused by proteolysis of the reactive-center loop (RCL), which in the native state (also called the S state) has an extended conformation that protrudes from the serpin domain. During the cleavage, the amino-terminal part of the RCL inserts into the central -sheet to form an additional -strand. This structural rearrangement is crucial for protease inhibition and results in the so-called R state of serpin, which is more stable compared with the S state. In the final serpin – protease complex the protease remains covalently linked to the serpin.

Several crystals have been grown in commercial conditions Index (Hampton Research, USA), JBScreen, (Jena Bioscience GmbH, Germany). The vapor diffusion sitting drop method was performed with ratio protein to precipitant 1:1 and 2:1 and stored at temperature 293 K. Optimization of successful crystallization conditions was performed by variation of salt, PEG and protein concentration. Number of diffraction data sets was collected at resolution ranging from 2.0 to 3.0 Å. The image processing was performed by XDS [1] software package and preliminary results shown that serpin crystals belong to the trigonal space group $P3_121$ with unit cell parameters $a = 78.38$ Å , $b = 78.38$ Å $c = 99.57$ Å, = = 90.0°, = 120.0°. The structure with identity above 50 % was found. This particular structure was used as a model for molecular replacement for processed data sets. MolPrep and Phaser as well as automated BALBES pipeline were used to obtain initial models, followed by structure refinement with REFMAC5 from the CCP4 software package. Model building using Coot and refinement in Refmac5 is under way.

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SL5

STRUCTURAL INSIGHT INTO LECTINS FROM *PHOTORHABDUS ASYMBIOTICA*

F. Melicher[1], J. Houser[1,2], G. Jančaříková[2], D. Pokorný[2], M. Wimmerová[1,2,3]

[1]Central European Institute of Technology, Masaryk University, Kamenice 753/5, Brno 625 00, Czech Republic

[2]National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Kamenice 753/5 Brno 625 00, Czech Republic

[3]Department of Biochemistry, Faculty of Science, Masaryk University, Kamenice 753/5, Brno 625 00, Czech Republic

melicherfilip@mail.muni.cz

Lectins are ubiquitous proteins and glycoproteins with the ability to specifically, non-covalently and reversibly bind to the mono-, oligo- and polysaccharides [1]. These sugar-binding proteins can be found in most organisms, ranging from viruses and bacteria to plants and animals. They play an important role in many processes occurring in nature such as cell-cell interaction or recognition of the host by a pathogen [2]. Lectins represent a heterogeneous group of proteins that vary in size, oligomeric state, structure as well as in exhibit specificity. Due to their importance, lectins are studied structurally and functionally to completely understand their role and mechanism of action [3].

Our research focuses on the lectins from gram-negative entomopathogenic bacteria *Photorhabdus asymbiotica*, which live in symbiosis with *Heterorhabditis* nematodes. This symbiotic complex can be found in soil, where it searches for the insect prey [4]. Even though the *Photorhabdus* genus is mainly insect pathogen there are also clinical cases describing a human infection caused by this bacterium [5] *P. asymbiotica* produces the well-characterized lectin PHL [6] which has a seven-bladed -propeller fold and contains two types of binding sites for different ligands. In addition, bioinformatic analysis of the *P. asymbiotica* genome revealed a presence of two additional genes for homologous proteins with the PHL lectin.

Several sugar-binding proteins with unknown function and dual behaviour makes *P. asymbiotica* a compelling organism and further studies of biomolecules produced by this bacterium may reveal their importance in the pathogenic or a symbiotic stage of life.

Selected recombinant lectins were produced in the *E. coli* expression system and purified by affinity chromatography on a mannose-agarose resin. High-throughput crystallization screening was performed to find crystallization conditions using the sitting drop vapour diffusion method. Data collection from obtained crystals was performed on the synchrotron BESSYII. The structure of the PHL2 lectin was solved by molecular replacement using the PHL structure as a model.

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SL6

ESTIMATION OF DIFFRACTION LIMIT USING PAIRED REFINEMENT

M. Malý[1,2], P. Kolenko[1,2], T. Skálová[2], J. Stránský[2], L. Švecová[1,2], J. Dohnálek[2]

[1]FNSPE, Czech Technical University in Prague, Břehová 7, Prague 2, CR
[2]Institute of Biotechnology of the Czech Academy of Sciences, v. v. i., BIOCEV, Průmyslová 595, Vestec near Prague, CR
malymar9@fjfi.cvut.cz

The high resolution diffraction limit is one of the most important parameters of any solved macro-molecular structure. Thus, it should be estimated carefully in order to avoid both covering too noisy data and discarding data containing useful signal. However, the conservative criteria for data quality indicators (*e.g.* $I/\sigma(I)$, R_{pim} and $CC_{1/2}$) are not sufficient to determine the proper high resolution diffraction limit as both instrumentation and software have changed in the last decades. This problem can be overcome with the usage of the *paired refinement* protocol that links crystallographic model and data quality [1]. This approach will be demonstrated on an exemplary dataset.

Diffraction data from a crystal of a heme-dependent globin domain were collected at the Helmholtz-Zentrum Berlin (BESSY II) on beamline 14.1 using a Dectris PILATUS 6M detector. The data were processed in XDS [2]. The initial high resolution cut-off was set to 1.85 Å according to a conservative criterion for $I/\sigma(I)$. Molecular replacement was successful and the structure model was refined in REFMAC5 [3] using isotropic *B*-factors. *R*-values of the obtained structure model were: R_{work} = 0.1924, R_{free} = 0.2413. The structure containing 11,467 atoms has been deposited in the Protein Data Bank under the code 5OHE [4].

Recently, the paired refinement protocol was performed on the original diffraction data to analyse the impact of the data from high resolution. At first, the structure model was refined using data up to resolution 1.80 Å. Both

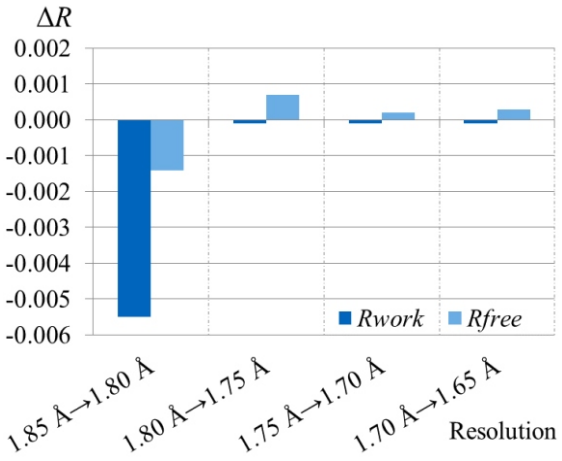

Figure 1. *R*-value analysis of paired refinement results. For each incremental step of resolution from X›Y, the *R*-values were calculated at resolution X.

R-values decreased moderately (Figure 1). Thus, even weak data from the shell 1.85–1.80 Å caused an improvement of the structure model (the quality indicators of data in this shell have following values: $I/\sigma(I)$ = 1.4, $CC_{1/2}$ = 0.820. Refinement against data up to resolution 1.75 Å, 1.70 Å, and 1.65 Å did not cause further improvement as the R_{free}-value increased slightly in these cases.

According to these results, the optimal high resolution diffraction limit was determined as 1.80Å resolution. It does not look like a large difference in comparison with the previous choice (1.85 Å). However, the resolution shell 1.85–1.80 Å contains 9,929 unique reflections (8 % of whole dataset) which is not a negligible number, given the number of refined parameters (45,388).

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Session II

SL7

STUDY OF THE REAL STRUCTURE OF THE LASER-CLADDED STEEL

Karel Trojan[1], Václav Ocelík[2], Jiří Čapek[1] and Nikolaj Ganev[1]

[1]Department of Solid State Engineering, Faculty of Nuclear Sciences and Physical Engineering, CTU in Prague, Trojanova 13, 120 00 Prague 2, Czech Republic

[2]Department of Applied Physics, Zernike Institute for Advanced Materials, Faculty of Science and Engineering, University of Groningen, Nijenborgh 4, 9747 AG, Groningen, The Netherlands

AISI H13 hot working tool steel is one of the most common die material used in metal and casting industries. Dies suffer damage due to wear and thermo-dynamic stresses during their lifetime [1]. Therefore, various methods have been developed for their repair, which is cheaper than manufacturing new ones. A great benefit of laser cladding in this field is a high productivity with minimal influence due to a low heat input on surrounding material by thermal stresses [2]. Therefore, the aim of the contribution is to describe the effects of laser processing on the microstructure of laser cladded H13 tool steel using orientation imaging microscopy (OIM) based on electron backscatter diffraction (EBSD) and other techniques.

Laser cladding was carried using an IPG 3 kW Yt:YAG fibre laser. The laser power density of 114 J/mm^2 was applied to form a volume consisting of five overlapping layers, see Fig. 1. The martensitic structure was observed on the cross-section of the clad using electron backscattering diffraction, see inverse pole figures (IPF) of ferritic phase in Fig. 2. The original austenite grains with a characteristic size of 20–50 μm, which were formed during the transition of the melt into a solid phase and whose were subsequently transformed into martensitic or bainitic laths, are clearly seen in the figure. It has to be noted that the EBSD technique is not able directly distinguish ferrite and martensite.

Further, the clads were subjected to X-ray diffraction measurement, tensile testing, wear resistance and hardness measurement for comprehensive utilization evaluation of laser cladding.

Measurements were supported by the project TH02010664 of the Technology Agency of the Czech Republic and by University of Groningen. This work was supported by the Grant Agency of the Czech Technical University in Prague, grant No. SGS19/190/OHK4/3T/14.

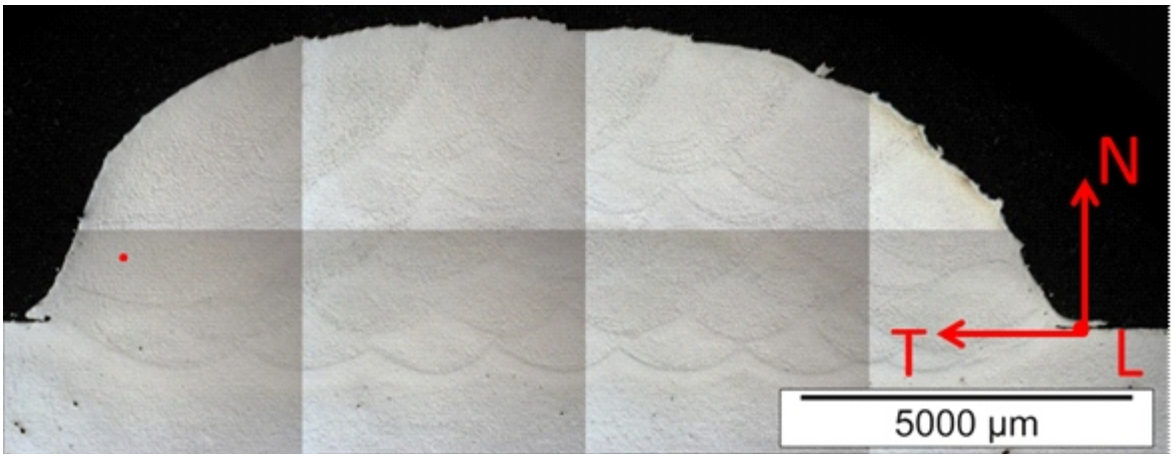

Figure 1: Metallographic cross-section of the clad AISI H13 tool steel with marked directions and area which was observed using OIM.