Session III - Friday, September 25, morning

L15

STRUCTURE ANALYSIS FOR HUMAN PROLIDASE MUTATIONS GIVES INSIGHT INTO THE PROLIDASE DEFICIENCY DISEASE MECHANISMS

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Prolidase is a multifunctional enzyme whose biological relevance, its structure and mechanism of catalysis are still only partially understood [1]. In humans prolidase is the only metalloenzyme that cleaves the iminodipeptides containing a proline or hydroxyproline residue at the C-terminal end. Prolidase deficiency (PD) is a rare recessive disorder characterized by diminished prolidase activity and manifested by variety of clinical symptoms [2].

Several mutations responsible for loss of prolidase activity were identified [3], but the structural basis of the enzyme inactivation mechanism remains unknown. The aim of this study is to determine the influence of single amino acids substitutions or deletions on prolidase structure. These mutant structures will help in understanding the mechanism of enzyme inactivation. In this study several prolidase mutants were studied at BESSY-MX beamlines [4] by single crystal X-ray crystallography.

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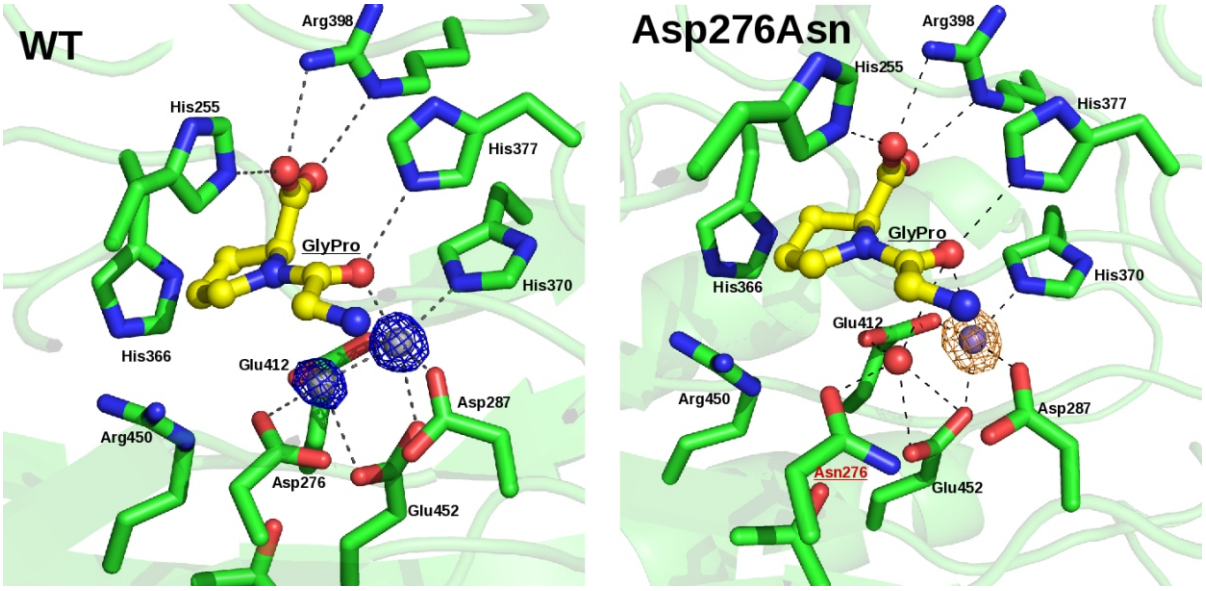

Figure 1: In order to identify ions in the active site crystals were soaked with $MnCl_2$ and GlyPro solution and diffraction images were collected above and below Mn absorption edge. For wild-type (WT) 2Fo-Fc maps contoured at 2.5 shows position of two sodium ions. For mutants anomalous difference maps (AnoDe) contoured at 10 shows positions of manganese ions. Active site mutation is highlighted red. Anomalous difference maps were calculated with the use of *ANODE* [5].

L16

HYPERACTIVITY OF RELAPSED ALL-SPECIFIC cN-II MUTANT IS CAUSED BY AFFECTED ALLOSTERIC REGULATION

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The recent studies demonstrated that 20 % of relapsed acute lymphoblastic leukemia (ALL) are associated with mutations in NT5C2 encoding cytosolic purine 5'-nucleotidase (cN-II). The identified point mutations lead to a biosynthesis of hyperactive enzyme that inactivates drugs used for chemotherapy.

The aim of this project is detailed biochemical and structural characterization of representative cN-II mutants to elucidate determinants of their hyperactivity and to design efficient strategy for the pharmacological intervention. In this study, we scrutinized the properties of the most common mutant R367Q.

Analysis of enzyme kinetics showed the R367Q mutant is constitutively active and does not require, contrasting to the wild-type cN-II, allosteric activator such as ATP for efficient catalysis. Nevertheless, the mutant is fully capable to bind the effectors of cN-II despite rather minor effect on its catalytic properties. As the R367Q aminoacid substitution is located close to oligomeric interface of the cN-II tetramer, we assessed the protein thermostability as well as formation of tetrameric assembly. Biophysical studies revealed that the mutant forms stable tetramers similarly to the wild-type.

X-ray crystallography showed that overall structure of the R367Q mutant is similar that of wild-type. However, superposition of the crystal structures elucidated changes in the arrangement of oligomeric interface altering pattern of intersubunit interactions in the cN-II protein. These observations were supported by analysis using hydrogen/deuterium exchange followed by mass spectrometry. Using this approach the oligomeric interface was identified as major region possessing perturbed exchange rates. It indicates that mutant is locked in active conformation due to changes in dynamic motions of the oligomeric interface.

Using integration of structural and biochemical techniques, our s work identifies oligomeric interface as an important compoment of allosteric regulatotion of cN-II that may become an interesting target for therapy of ALL.

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L17

ACTIVATION AND INHIBITION OF SmCB1, AN ANTIPARASITIC DRUG TARGET

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Digestive protease cathepsin B1 (SmCB1) of the human blood fluke *Schistosoma mansoni* is a potential drug target for the treatment of schistosomiasis, a parasitic disease that afflicts over 200 million people worldwide. SmCB1 is biosynthesized in the form of inactive zymogen in which the propeptide operates as an intra-molecular inhibitor by blocking the active site. We investigated the proteolytic processing through which the propeptide is removed and identified a dual activation pathway for SmCB1 under the complex control of sulfated polysaccharides. The activation mechanism was explained using crystal structures of three molecular forms of SmCB1 along the activation pathway.

Further, we determined crystal structures of the mature SmCB1 complexed with two peptidomimetic vinyl sulfone inhibitors to describe their binding mode. These structural data and biochemical profiling provided insight into the specificity of SmCB1 inhibition. We demonstrated that the severity of phenotypes induced in the parasite by vinyl sulfone inhibitors correlated with inhibition of SmCB1 activity, thus confirming SmCB1 as a valuable drug target. Our data provide a footing for the rational design of anti-schistosomal protease inhibitors targeting SmCB1.

L18

DIMERIZATION-INDUCED ALLOSTERIC CHANGES OF THE OXYANION-HOLE LOOP ACTIVATE THE PSEUDORABIES VIRUS ASSEMBLIN pUL26N, A HERPESVIRUS SERINE PROTEASE

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Herpesviruses encode a characteristic serine protease with a unique fold and an active site that comprises the unusual triad Ser-His-His. The protease is essential for viral replication and as such constitutes a promising drug target [1]. In solution, a dynamic equilibrium exists between an inactive monomeric and an active dimeric form of the enzyme, which is believed to play a key regulatory role in the orchestration of proteolysis and capsid assembly [2]. Previously available crystal structures of herpesvirus proteases correspond either to the dimeric state or to complexes with peptide mimetics that alter the dimerization interface. In contrast, the structure of the native monomeric state has remained elusive. We present the three-dimensional structures of native monomeric, active dimeric, and diisopropyl fluorophosphate-inhibited dimeric protease derived from pseudorabies virus [3], an alphaherpesvirus of swine [4]. These structures, solved by X-ray crystallography to respective resolutions of 2.05, 2.10 and 2.03 Å, allow a direct comparison of the main conformational states of the protease. In the dimeric form, a functional oxyanion hole is formed by a loop of 10 amino-acid residues encompassing two consecutive arginine residues (Arg136 and Arg137); both are strictly conserved throughout the herpesviruses. In the monomeric form, the top of the loop is shifted by approximately 11 Å, resulting in a complete disruption of the oxyanion hole and loss of activity. The allosteric changes revealed by these structures shed light onto the dimerization-induced activation of this class of proteases. Small-angle X-ray scattering experiments confirmed a concentration-dependent monomer/dimer equilibrium of the protease in solution.

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L19

STRUCTURAL STUDY OF THE COMPLEX OF HUMAN GLUTAMATE CARBOXYPEPTIDASE II AND HYDROXAMATE-BASED INHIBITORS

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Small molecule ligands targeting human glutamate carboxypeptidase II (GCPII) are used in diverse diagnostic and therapeutic applications ranging from prostate cancer imaging to the therapy of neurological disorders. Such inhibitors typically consist of a glutamate moiety linked to a zinc-binding group to ensure high specificity and affinity for GCPII, respectively. At present, there are no structural data describing interactions between GCPII and inhibitors harboring a hydroxamate function, the prominent zinc-binding function used in the field. Here we report X-ray structures of several complexes between GCPII and hydroxamate-based inhibitors. Our structures reveal unexpected positioning of hydroxamates in the internal GCPII pocket that differs markedly from binding modes of matching prototypical GCPII inhibitors featuring different zinc-binding groups. The data can be exploited for the structure-assisted design of novel GCPII-specific inhibitors.

L20

CRYO-TEM: A THIRD MEMBER JOINS THE HIGH RESOLUTION BAND

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X-Ray Diffraction (XRD) and Nuclear Magnetic Resonance (NMR) are forming the most famous duo of methods used for structural analysis of biological matter to understand how molecules interact with their target at atomic resolution and to guide the drug discovery. The two techniques complement each other.

Until now, the Cryo-Transmission Electron Microscopy (Cryo-TEM) has been used to resolve tertiary structures of large proteins and quaternary structures of protein complexes at nanometer resolution. Since crystallization or high concentration of the sample are not mandatory prerequisites to obtain a good structure, Cryo-TEM is applicable to most proteins and provides direct observation of the mechanical dynamics and conformation of flexible structures in their natural setting. Because the particles are analyzed separately, the native structure is not distorted by non-natural circumstances. Hence, computational sorting of diverse conformations of proteins or protein complexes in a given preparation enable the visualization of various intermediate states and can provide kinetic information about a specific interaction between individual molecules. As XRD and NMR techniques report an average result based on the summed signal of a large population of individual molecules, such event might be overlooked.

Recently, Cryo-TEM moved to near atomic resolution. This became possible through the development of new powerful microscopes like the FEI Titan Krios, but also through new imaging techniques (beam induced movement correction), contrast enhancement (phase plates), optical aberration correction techniques, and new detectors (direct electron detection cameras). In this presentation, we will review the technical breakthroughs and recent achievements resulting from this shift of paradigm. We will show how Cryo-TEM is consolidating its position as the third member of a dynamic trio (XRD / NMR / TEM).

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ADVANCES IN PROTEIN CRYSTALLOGRAPHY

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This presentation will give an overview of innovative tools that significantly contribute to the success of protein structure determination by X-ray crystallography.

We will discuss how to get more hits using fewer conditions, the latest developments in crystal screens, and how to choose your screens wisely based on your unique project - from membrane proteins, soluble proteins, nucleic acids, complexes and everything else in-between.

A much wider area of crystallization space is now accessible through the use of unique crystal growth screens such as MIDAS, which contains the largest collection of novel precipitants, and the Morpheus® range of screens, which were developed by Fabrice Gorrec at the MRC in Cambridge which use reagents to yield crystals where none might be observed in traditional screens.

In addition we will be available to answer any questions on protein expression, plates, and cryocrystallography, plus imaging systems and incubators.