MS29.P01

Acta Cryst. (2011) A**67,** C409

Trapping novel allosteric regulatory pockets on herpesvirus protease

Aida Baharuddin, Gregory M. Lee, Tina Shahian, Jonathan E. Gable, Charles S. Craik, *Department of Pharmaceutical Chemistry, University of California, San Francisco, CA, (USA).* E-mail: Aida.Baharuddin@ucsf.edu

We have identified a small molecule (DD2) that inhibits an essential dimeric protease of the human Kaposi's sarcoma-associated herpesvirus (KSHV Pr). Proteolytic activity of KSHV Pr is disrupted by dissociation of dimeric state. Circular dichroism, chemical shift perturbation mapping and hydrogen exchange experiments indicate that a significant loss of structure at the C-terminal helices occurs concurrently with the dimer to monomer transition [1]. Complementary methods including size exclusion chromatography and 2D NMR spectroscopy show that the monomeric protease is enriched in the presence of DD2, leading to loss of enzymatic activity. $^{1}H - ^{15}N$ HSQC titration studies mapped the inhibitor-binding site to the dimer interface and mutagenesis studies targeting this region confirmed this binding mode [2]. As currently there are no specific treatments for herpesviral infections and with increased incidents of drug resistance, there is a need for the identification of new human herpesvirus therapeutics. The crystal structure of a monomeric form of KSHV Pr in complex with the small molecule inhibitor bound at the dimer interface was solved at 2.0 Å resolution. The final R_{factor}/R_{free} is 20.5%/24.3%, respectively. The complex was crystallized in the I222 space group. Two monomers of the truncated KSHV Pr were observed in the asymmetric unit with one DD2 molecule bound to monomer A, and two DD2 molecules bound to monomer B. The crystal structure is consistent with a mechanism where inhibitor binding prevents dimerization through the conformational selection of an inactive monomeric intermediate. This represents the first crystallographic structure of an inhibited monomeric Human Herpesvirus Protease to date.

[1] A.M. Nomura, A.B. Marnett, Shimba N,V. Dotsch, C.S. Craik *Nature Structural & Molecular Biology* **2005,** *12*, 1019-1020. [2] T. Shahian, G.M. Lee, A. Lazic, A.A. Leggy, P. Velusamy, C.M. Roels, R.K. Guy, C.S. Craik *Nature Chemical Biology* **2009**, *5,* 640-646.

Keywords: KSHV pr, dimer disruptor, herpesvirus

MS29.P02

Acta Cryst. (2011) A**67,** C409

Structural analysis of bacteriophage-encoded peptidoglycan hydrolase domain KMV36C

K. Van Hecke,[a] L. Vanpraet,[a] Y. Briers,[b] M. Walmagh,[b] D.D. Rodríguez,[c] I. Usón,[c] R. Lavigne,[b] L. Van Meervelt.[a] [a]*Biomolecular Architecture and* [b]*Laboratory of Gene Technology, KULeuven, Heverlee, (Belgium).* [c]*Instituto de Biología Molecular de Barcelona, Barcelona, (Spain).* E-mail: kristof.vanhecke@chem.kuleuven.be

Pseudomonas phage φKMV is a member of the *Podoviridae*, which have a short, non-contractile tail. During infection, these phages must degrade the peptidoglycan layer and establish a protein channel, in order to eject the viral DNA into the host cell.

In phage phiKMV, genome and proteome analysis revealed three gene products (gp35, 36, 37) as the predicted functional virion proteins [1,2]. The muramidase domain (KMV36C), responsible for degrading the peptidoglycan, is contained in the C-terminus of gp36. KMV36C was shown to be a mesophylic protein (Tm=50.2°C) with highly thermoresistant properties (k=0.595 h-1 at 95 °C). This thermoresistance is influenced by aggregation and a two-state endothermic unfolding transition of this protein [3]. To ascertain the structural background into the reasons for this remarkable thermoresistance, aggregation phenomena and the manner in which the ejection channels are formed, we here analyze the crystal structure of gp36C.

KMV36C was crystallized by the hanging-drop vapor-diffusion method. A complete data set to 1.6 Å resolution was collected at beamline PXI of the SLS synchrotron in Villigen (Switserland). The crystals belong to the cubic space group *P*432 with cell parameters $a = b = c = 102.52$ Å.

Despite several molecular replacement (MR) trials, the structure could only be solved with the program ARCIMBOLDO, a general method for *ab initio* phasing up to 2 Å data, based on a combination of location of model fragments like small α-helices with PHASER and density modification with SHELXE, supported by a grid of computers running CONDOR [4].

[1] R. Lavigne, M.V. Burkal'tseva, J. Robben, N.N. Sykilinda, L.P. Kurochkina, B. Grymonprez, B. Jonckx, V.N. Krylov, V.V. Mesyanzhinov, G. Volckaert. *Virology* **2003**, *312*, 49-59. [2] R. Lavigne, J.-P. Noben, K. Hertveldt, P.-J. Ceyssens, Y. Briers, D. Dumont, B. Roucourt, V.N. Krylov, V.V. Mesyanzhinov, J. Robben, G. Volckaert. *Microbiology* **2006**, *152*, 529-534. [3] Y. Briers, R. Lavigne, P. Plessers, K. Hertveldt, I. Hanssens, Y. Engelborghs, G. Volckaert. *Cell. Mol. Life Sci.* **2006**, *63*, 1899-1905. [4] D.D. Rodríguez, C. Grosse, S. Himmel, C. Gonzalez, I.M. de Ilarduya, S. Becker, G.M. Sheldrick, I. Usón, I. *Nature Methods* **2009**, *6*, 651-U39.

Keywords: bacteriophage, lysozyme, ab initio phasing

MS29.P03

Acta Cryst. (2011) A**67,** C409-C410

Structure of the membrane-piercing phage tail spike

Petr G. Leiman,[a] Christopher Browning,[a] Mikhail Shneider,[b] [a]*Ecole Polytechnique Federale de Lausanne (EPFL), (Switzerland).* [b]*Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry (RAS), Moscow, (Russia).* E-mail: petr.leiman@epfl.ch

The needle or spike protruding from the center of the baseplate is a conserved feature found in all known contractile tail phages. It has been proposed that this needle punctures the outer membrane of the cell during phage infection and tail contraction [1]. The structure of one of these spikes, the phage T4 gp5-gp27 complex, was reported earlier [1]. However, the structure of the membrane-interacting tip of the T4 needle is unknown because it is formed by a yet unidentified protein, which decorates the gp5 beta-helix [2].

We report here the crystal structures of three spike proteins from two contractile tail phages and from the R-type pyocin. These are gene product V (gpV) from bacteriophage P2, gp138 from phage φ92, and the product of gene *pa0616* from Pseudomonas aeruginosa PAO1. Using cryoEM, we show that gp138 and PA0616 form the central baseplate spike of φ92 and the R-type pyocin, respectively, and that there is no other protein at the tip of the needle.

GpV, gp138 and PA0616 are long trimers and consist of two domains, the N-terminal OB-fold domain and the C-terminal triple-stranded beta-helical domain. The OB-fold and the beginning of the beta-helix of all the three structures superimpose onto the corresponding N-terminal part of T4 gp5 very well despite exhibiting about 10% sequence identity on average. This strongly suggests that all the four proteins have a common ancestor.

The beta-helical domains of gpV, gp138 and PA0616 are different to that of gp5 in that they taper strongly towards the C terminus and end in a very sharp tip (only 10 Å in diameter), making the proteins look like a sharpened pencil. However, the topologies of the polypeptide

chain forming the beta-helices are very different. Nevertheless, gpV, gp138 and PA0616 are water soluble and SDS-resistant proteins and show no obvious membrane affinity. They are unlikely to unfold upon interaction with the membrane during phage attachment.

GpV, gp138 and PA0616 contain a conserved cluster of histidines at the tip of the beta-helical domain. These histidines bind a Fe atom in the octahedral configuration. In addition to Fe, gpV also contains Ca and Cl near the spike's apex.

The peculiar topology and thermodynamic stability of gpV, gp138 and PA0616 suggests that these proteins are used as rigid and sharp needles to breach the outer membrane of the host cell using the energy of the contractile sheath. These spikes appear to create an opening in the host cell membrane into which the tail tube in inserted for subsequent DNA release into the host cell.

[1] S. Kanamaru, P.G. Leiman, V.A. Kostyuchenko, P.R. Chipman, V.V. Mesyanzhinov, F. Arisaka, M.G. Rossmann. *Nature* **2002**. *415(6871)*, 553-557. [2] V.A Kostyuchenko, P.G. Leiman, P.R. Chipman, S. Kanamaru, M.J. van Raaij, F. Arisaka, V.V. Mesyanzhinov, M.G. Rossmann. *Nat Struct Biol*, **2003**. *10(9)*, 688-693.

Keywords: bacteriophage, beta-helix, membrane-piercing protein

MS29.P04

Acta Cryst. (2011) A**67,** C410

Structure of influenza B nucleoprotein and its functional characterization

Pang-Chui Shaw,[a] Andy Ka-Leung Ng,[a] Mandy Ka-Han Lam,[a] Jia-Huai Wang,[b] *[a]Centre for Protein Science and Crystallography, School of Life Sciences, The Chinese University of Hong Kong, Hong Kong, (China). [b]Dana-Farber Cancer Institute, Harvard Medical School, Boston, (USA).* E-mail: pcshaw@cuhk.edu.hk

The Influenza viruses are classified into three types: A, B and C. While influenza A virus is widely recognized as the most devastating one, influenza B virus also causes severe damages, in particular substantial mortality among patients younger than 18 years old. Influenza B virus is prevalent in Hong Kong. It accounts for 32% of the 554 laboratory-confirmed cases from February 27, 2011 to April 2, 2011. Among the influenza viral proteins, nucleoprotein (NP) is the major component of the ribonucleoprotein complex, which is crucial for the transcription and replication of the viral genome. We have recently determined the crystal structure of influenza B NP to a resolution of 3.2 Å. Two NP molecules, namely chains A and B, are found in an asymmetric unit. Homologous to the structure of influenza A NP [1,2], influenza B NP is composed of the head and body domains and a tail loop. Influenza B NP forms a tetramer in the crystal structure with two A chains and two B chains, in contrast to the trimer observed in influenza A NP. The homo-tetramer formation is the result of tail loop insertion from one NP molecule to its neighboring NP. Another major role of NP is to bind the genomic RNA of the virus. The putative RNA-binding regions are exposed in the influenza B NP tetramer. Residues involved in oligomerization and in RNA binding have been studied biochemically by static light scattering and surface plasmon resonance. The functional significance of these residues towards the ribonucleoprotein activities of the virus has also been investigated. The structure-function relationship of influenza B NP has enriched the current knowledge on influenza NP and provides valuable information for the design of anti-viral agents.

This work was supported by a grant from the General Research Fund (Project no. 473810) from the Research Grants Council of Hong Kong SAR.

[1] A.K.L. Ng, H. Zhang, K. Tan, Z. Li, J. Liu, P.K.S. Chan, S.M. Li, W.Y. Chan, S.W.N. Au, A. Joachimiak, T. Walz, J.H. Wang and P.C. Shaw *The FASEB Journal* **2008**, *22*, 3638-3647. [2] A.K.L. Ng, J.H. Wang and P.C. Shaw *Science in China Series C: Life Sciences* **2009,** *52*, 439-449.

Keywords: influenza virus, nucleoprotein, structure

MS29.P05

Acta Cryst. (2011) A**67,** C410

Packing disorder: structure of soluble domains of Hepatitis A Virus 2B protein

Laia Vives-Adrián,[a] Damià Garriga,[b] Mònica Buxaderas,[a] Pedro Pereira,[c] Sandra Ribeiro,[c] Núria Verdaguer,[a] *[a]Institut de Biologia Molecular de Barcelona, CSIC, Parc Científic de Barcelona (Spain), [b]Centro Nacional de Biotecnología, CSIC, (Spain). [c]IBMC - Protein Crystallography Group, Porto (Portugal).* E-mail: lvacri@ibmb.csic.es

Hepatitis A Virus (HAV) is a common cause of acute hepatitis worldwide, transmitted by fecal-oral route. HAV is classified within *Picornaviridae* family but it has some distinct biological characteristics like its slow viral replication and not inducing cellular lysis. Picornavirus genome encodes for a large polyprotein, which is processed by viral proteinases into a variety of precursor and mature proteins. The polyprotein is organized in structural proteins, the P1 region, and non-structural proteins, P2 and P3 regions. The P2 region includes proteins 2A, 2B and 2C involved in the virus life cycle. The role of 2A in proper capsid assembly and the implication of 2B in viral RNA synthesis and in the release of the virus particles from the cell, make them an interesting focus of study. The C-terminal domain of 2B is predicted to be organized as a transmembrane helix, which would allow its presence in cellular membranes.

We have obtained small tetragonal crystals from the P2 N-terminus (including 2A and the soluble part of 2B) and determined the X-ray structure up to 2.7Å resolution. Only the structure corresponding to the 2B region (145 amino acids) could be determined because the 2A polypeptide appeared disordered in the crystals. The asymmetric unit (a.u.) contains two 2B molecules, organized in two domains: the first one shows a pseudo β-barrel organization and the second is an α-helix bundle. The two molecules in the a.u. are connected through their respective β-hairpins, included in the β-domain. This connection is extended by the crystal contacts, forming a fiber-like crystal packing along C-axis. Thus, the crystal network consists of parallel fibers of 2B separated by big spaces, where the disordered 71 residues of 2A protein seem to be allocated.

Keywords: hepatitis A, 2B protein, packing

MS29.P06

Acta Cryst. (2011) A**67,** C410-C411

Fab'-induced folding of intrinsically disordered HIV-1 Tat

Jennifer Serrière, Richard Haser, Patrice Gouet, Christophe Guillon, *Laboratoire de Biocristallographie et Biologie Structurale des Cibles Thérapeutiques, BMSSI-UMR5086, Lyon, (France).* E-mail: jennifer.serriere@ibcp.fr

The human immunodeficiency virus type 1 (HIV-1) is the agent responsible for acquired immunodeficiency syndrome (AIDS). Besides the canonical *gag*/*pol*/*env* retroviral genes, HIV-1 codes for additional accessory and regulatory proteins that act at different stages of the viral replication cycle. Among regulatory proteins, the transcriptional activator protein Tat contributes to the transactivation of viral genes