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Trapping novel allosteric regulatory pockets on herpesvirus protease

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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.

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Structural analysis of bacteriophage-encoded peptidoglycan hydrolase domain KMV36C

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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].

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Structure of the membrane-piercing phage tail spike

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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