

Saturday, March 16, Session VII

L25

THE CRYSTAL STRUCTURE OF THE PHI6 MAJOR CAPSID PROTEIN**Boura E.^{1,2}, Nemecek, D.^{3,4}, Plevka P.⁵ and Hurley J. H.²**¹*Institute of Organic Chemistry and Biochemistry AS CR, v.v.i., Prague, CZ*²*The National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, USA*³*Central European Institute of Technology, Department of Structural Biology, Brno, CZ*⁴*National Institutes of Health, National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, MD, USA*⁵*Department of Biological Sciences, Purdue University, 915 W. State Street, West Lafayette, IN 47907*

Double-stranded RNA (dsRNA) viruses share several distinctive features, regardless of whether they infect mammals, fungi, or bacteria. Their genomes comprise multiple linear segments; the virions consist of up to three nested shells; the innermost capsid is icosahedrally symmetric with a nonequivalent packing of its subunits. Our goal was to devise an atomic model of dsRNA virus maturation. In order to achieve that we have determined the crystal structure of its major capsid protein, P1, at 3.6 Å resolution. Self rotation function revealed that the P1 protein crystallized as one pentamer in the asymmetric unit. Thus, the structure

was solved by molecular replacement using a cryo-electron map of a pentamer at 7 Å resolution as a search model and the phases were extended using non-crystallographic symmetry. The P1 subunit is shaped like a kite with sides of ~91 Å by 73 Å. The thickness varies between 14 Å and 38 Å at the edges, reaching 47 Å in the center. The fold is novel and mostly alpha helical. Fit of the crystal structure into high-resolution cryoEM map of the procapsid revealed conformational changes during virus maturation.

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CRYO-EM RECONSTRUCTION OF THE BACTERIOPHAGE 6 PROCAPSID AT NEAR-ATOMIC RESOLUTION SHOWS CONFORMATIONAL CHANGES IN DSRNA VIRUS MATURATION**Nemecek, D.,^{1,2} Boura, E.,^{3,4} Wu, W.,² Cheng, N.,² Qiao, J.,⁵ Mindich, L.⁵ Heyman, B.,² and Steven, A. C.²**¹*Central European Institute of Technology, Department of Structural Biology, Brno, CZ*²*National Institutes of Health, National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, MD, USA*³*Institute of Organic Chemistry and Biochemistry AS CR, v.v.i., Prague, CZ*⁴*The National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, USA*⁵*University of Medicine and Dentistry of New Jersey, Department of Microbiology, NJ, USA*

Bacteriophage 6 is the type member of dsRNA bacterial viruses that share many structural and mechanistic features with other dsRNA viruses regardless if they infect mammals or fungi. The mature virion consists of multiple concentric shells that enclose the segmented dsRNA genome, viral RNA polymerase and other accessory proteins. The genome is replicated inside the innermost icosahedral capsid with a non-equivalent packing of 120 subunits. The inner capsid of 6 is first assembled as the precursor procapsid that undergoes major conformational changes as it matures. We have determined the procapsid structure by cryo-electron microscopy at 4.5 Å resolution and compared the crystal structure of the major capsid protein (P1) with its two conformers in the procapsid shell (P1_A and

P1_B). The P1_A and P1_B subunits exhibit distinct conformations from each other and from the crystal structure. The P1_A's fit snugly together and comprise inverted 5-fold vertices with a central channel wide enough (~ 20 Å) for translocation of ssRNA. The P1_B's bind to the outer rim of the P1_A vertex and connect 12 vertices into the procapsid shell. In maturation, the P1 subunits pivot mostly as rigid bodies and interlock into an almost spherical shell in a stepwise manner that controls packaging of the tripartite genome. These observations have also implications for interaction of P1 subunits with the viral RNA polymerase and for regulation of packaging and replication of the segmented genome.



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COMPLEXES OF HIV-1 RT AND DNA/RNA HYBRID REVEAL STRUCTURE COMPATIBLE WITH RNA DEGRADATION

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Reverse transcriptase (RT) is a multifunctional enzyme used by retroviruses to transcribe their single-stranded RNA genome into double stranded DNA capable of integration into host cell chromosomes. Several crystal structures of human immunodeficiency virus type 1 (HIV-1) RT complexed with different DNA-RNA hybrid substrates were solved. Structures of polymerase and RNaseH active sites are described as well as a number of insights into mechanisms of polymerization and RNA degradation

are presented. Interactions between RT and DNA-RNA hybrids are discussed in comparison with other structures of apo- and DNA-bound forms of RT. Also, presence of non-nucleoside reverse transcriptase inhibitors in some structures may help better understand the mechanisms of inhibition, which can be used to generate potent anti-AIDS agents that specifically target the HIV-1 RT.

Lapkouski M, Tian L, Miller JT, Le Grice SF, Yang W.; *Nat Struct Mol Biol.* 2013 Feb; 20 (2):230-6.

L28

ROLE OF THE BURIED HALIDE-BINDING SITE OF HALOALKANE DEHALOGENASE DBEA

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The crystal structure of haloalkane dehalogenase DbeA from *Bradyrhizobium elkanii* USDA94 revealed the presence of a unique second halide-binding site for chlorides. A double mutant DbeA03 (I44L+Q102H), which should have the second binding site removed, was constructed and biochemically characterized. Molecular modeling was employed to prove successful removal of the second halide-binding site in the mutant and to study its role in the catalysis.

Comparison of calculated binding energies of chloride ions bound at the second halide-binding site in DbeA03 and wild type DbeA suggested the successful removal of the second halide-binding site. The calculated difference in the binding energies between wild type DbeA and DbeA03 was 8.7 ± 2.7 kcal.mol⁻¹. This conclusion was confirmed experimentally by stopped flow fluorescence measurement of chloride binding to both enzymes. Obtained dissociation constant showed an order of magnitude decrease in chloride binding affinity to DbeA03 compared to DbeA wt.

The effect of the second halide-binding site on the catalysis was consequently probed by molecular dynamic simulations at constant pH conditions. The pK_a of the catalytic histidine in wild type DbeA

(pK_a = 7.1 ± 1.4) without chloride anion bound at the second halide-binding site was comparable to pK_a in DbeA03 (pK_a = 7.3 ± 0.5) where the second halide-binding site was removed. In the case of wild type DbeA with the chloride anion present, the pK_a of the catalytic histidine was significantly increased (pK_a = 9.6 ± 0.8) making it a much stronger base. This effect is in agreement with transient kinetic experiments revealing that the rate of hydrolysis was significantly decreased by introduced mutations.

This study showed that the presence of the second halide-binding site in haloalkane dehalogenase DbeA significantly alters its catalytic properties. Thus, engineering of buried halide-binding sites into the protein core represents a novel strategy for the construction of novel catalysts.