MS110.O03

The crystal structure of the C-terminal domain of the Ebola virus nucleoprotein

Z. Derewenda[1], P. Dziubanska[1], U. Derewenda[1], J. Ellena[2], K. Haley[3], D. Engel[3]

[1]University of Virginia, Department of Molecular Physiology and Biological Physics, Charlottesville, VA, USA, [2]University of Virginia, Department of Chemistry, Charlottesville, VA, USA, [3]University of Virginia, Department of Microbiology, Immunology and Cancer Biology, Charlottesville, VA, USA

Ebola virus (EBOV) and Marburg virus are members of the family Filoviridae. Both are highly pathogenic and cause hemorrhagic fever, lethal in 90% of infected people. There is fear that the viruses can be used as bioterrorism agents. There are no approved vaccines, and intense effort is underway to discover drugs targeting these viruses. The EBOV genome encodes seven proteins, two of which have no known structures: RNA polymerase (L) and nucleoprotein (NP). NP is essential for packaging viral genomic RNA into the nucleocapsid. Other viruses also contain nucleoproteins, but only the Ebola and Marburg NP proteins contain two distinct domains. The C-terminal domain (Ct; ~100 residues) has no homologues; it acts as a hub for protein-protein interactions important for the assembly of the nucleocapsid and for the interaction with the VP40 matrix protein, embedded in the viral membrane. We obtained three distinct crystal forms of the Ct domain of NP from EBOV, and solved the structures using anomalous scattering from Se, and Molecular Replacement. High-quality NMR data were also collected. The models were refined at 1.6-2.0 Å resolution to R factors ~20%. The protein has a novel fold, with topology distantly related to the β-grasp fold. In spite of its small size, the Ct domain shows high melting temperature of ~60ºC. Our efforts focus on the identification of how the C-terminal domain of NP binds to its partners. As part of an effort towards anti-filovirus drug discovery, proteins NP, VP24, VP35 and VP40 are being targeted for small molecule inhibition using a yeast-based phenotypic assay. Each protein, when expressed in budding yeast, produces a slow-growth phenotype. Chemical suppressors of the slow-growth phenotype will be identified and used in viral growth assays to confirm their antiviral activity. The structure of NP will be used to complement small molecule screening methods.

Keywords: Ebola virus, new structure, new fold