The architecture and function of the polymerase of a dsRNA virus. J.M. Grimes1, S. Butcher2, E. Makeyev2, D. Bamford2 and D.I. Stuart1, 1Division of Structural Biology, Wellcome Trust Centre for Human Genetics, Oxford, OX3 7BN, UK. 2 Dept. Biosciences, P.O.Box 56 (Viikinkaari 5), 00014 University of Helsinki, Finland.

Keywords: dsRNA virus, bacteriophage, polymerase.

Double-stranded RNA (dsRNA) viruses usually replicate and transcribe their genome within an icosahedral complex, using a polymerase which can act on both single-stranded and double-stranded RNA templates.

We have determined the structure of the polymerase of the dsRNA virus bacteriophage phi-6, at 2.0Å using X-ray crystallography. Phases were determined from a single crystal of seleno-methionated protein, using the MAD method. The protein crystallised in space group P32, with 2 molecules in the asymmetric unit. There are 50 selenium atoms in the asymmetric unit, whose positions were solved using Shake’n’Bake. These atoms provided sufficient phasing power to give an electron density map, which allowed unambiguous interpretation of the entire structure.

Although, from the amino acid sequence, the protein has no detectable similarity to other polymerases, the structure has the generic polymerase fold, with characteristic fingers, palm and thumb domains, and catalytic aspartates in the expected positions. Moreover the structure most closely resembles the polymerase from HCV. This similarity is sufficient to argue for a close evolutionary link between dsRNA viruses and flaviviruses.

Complexes of the enzyme with template fragments and NTPs have been analysed, which suggest a role for the C-terminus, in conjunction with the template, in providing a mechanism for priming the reaction. This mechanism may be common to dsRNA viruses and some ssRNA viruses such as HCV.


Keywords: plasma glycoprotein, membrane adhesion

The human plasma-protein β2-glycoprotein I (β2GPI) is a key antigen in the autoimmune disease Antiphospholipid Syndrome (APS), and it is implicated in blood coagulation and clearance of apoptotic bodies from the circulation. The protein is a member of the Regulators of Complement Activation (RCA) and consists of 5 Short-Consensus Repeat (SCR) domains. Its fifth domain is an aberrant SCR domain with a 6-residue insertion and a 19-residue C-terminal extension. The fifth domain has been indicated to be directly involved in binding to anionic phospholipid layers. Adhesion to membranes is very likely an essential aspect of this protein, that is common to the observed effects in APS, blood coagulation and apoptosis.

The crystal structure of this heavily glycosylated membrane-adhesion protein has first been solved to 2.7 Å resolution1. The five SCR domains form an elongated chain of 200 Å long yielding an overall fish-hook shape appearance of the molecule. In the crystal the molecules form a 3-dimensional ‘chicken-wire’ with huge solvent channels yielding a solvent content of 86% v/v. The first four domains display regular SCR folds, whereas the 6-residue insertion and 19-residue extension form a new face on the fifth and aberrant SCR domain. Based on the crystal structure a model for membrane adhesion is proposed, which includes a large positive charged area on the new face of the fifth SCR domain for electrostatic interactions with anionic lipid headgroups and a hydrophobic ‘membrane-anchoring’ loop, containing Trp-316, that supplies specificity for lipid layers. The observed spatial arrangement of the domains in the crystal structure suggests that the non-glycosylated N-terminal domains are projected away from the membrane surface into solution providing potential protein-adhesion sites.

Most recently, we solved the structure of an isoform of β2GPI, that is accumulated in man during severe oxidative stress (e.g. cardiac arrest, drowning). Biophysical characterization and crystal-structure determination revealed an unexpected and surprising feature of this isoform. The method of inactivation of the membrane-adhesion function both yields new insights into mechanism of membrane-adhesion and the potential roles of β2GPI in human plasma.