Structural characterization of virus assembly intermediates

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I. Assembly of PRD1 bacteriophage spike complex

PRD1 is a broad host range bacteriophage with an internal membrane. The icosahedral capsid architecture and DNA replication mode is related to that of the adenovirus [1]. Additionally, both PRD1 and the adenovirus contain a spike complex located on the five-fold vertices. PRD1 vertex complex is composed of proteins P2, P5 and P31. We have obtained low resolution structures for these proteins and their in vitro complexes using solution X-ray scattering and simulated annealing (program DAMMIN)[2]. The P5 trimer is a 28 nm long, rod-like molecule (Fig. 1A). Thus, the P5 trimer constitutes the main spike shaft and its length is similar to the adenovirus spike fiber. The C-terminal domain is elongated but more compact than the N-terminal domain of P5 (Fig. 1B). The P31 pentamer has a compact globular shape (Fig. 1D) and corresponds to the penton base of the adenovirus. Two P5 trimers interact with one to two molecules of P31 to form a long oligomer in which the trimers are interacting via their N-terminal domains (Fig. 1E). Similar heterologous interaction can mediate the incorporation of the P5 spike shaft. This arrangement provides basis for the symmetry mismatch between the pentameric vertex base and the trimeric spike shaft. The P2 protein, which serves as the receptor binding protein, is a stable elongated monomer (Fig. 1C) and interacts with the C-terminal domain of P5 (Fig. 1F). This complex is only transient and produces another symmetry mismatch between the P5 trimer and P2 monomer. It is proposed that the the weak P2-P5 interaction together with the metastable N-terminal domain of P5 are involved in DNA release during infection. P2 binding to the host cell receptor may trigger dissociation of the P5:P31 interaction. The disassembly of P5:P31in turn creates a hole at the five-fold vertex and leads to the release of the membrane enclosed DNA (Fig. 1G).

II. Solution structure and dsDNA packaging of bacteriophages PRD1 and PM2

Bacteriophages PRD1 and PM2 are representatives of dsDNA viruses with an internal lipid bilayer. The internal lipid vesicle serves as a vehicle for DNA packaging and delivery. Although PRD1 virus and coat protein structures are known in detail [1] the PM2 structure has not been characterized. We have obtained SAXS from solutions of PRD1 virus, PRD1 empty capsid (sus1 mutant) and PRD1 icosahedral protein shell (protein P3). The SAXS pattern corresponding to the PM2 virion has also been obtained. Average shell radii of 300±7 Å and 278±5 Å were determined for PRD1 and PM2, respectively. The packaged dsDNA exhibits a diffraction peak at 0.249 $Å^{-1}$ which corresponds to hexagonal packing with 29Å spacing. The quasi-crystalline order extends over domains with an apparent size of 110Å. Comparison of radial density distributions for PRD1 and PM2 revealed distinct difference for the density assigned to DNA. PRD1 DNA is concentrated at the periphery of the internal vesicle in a close contact with the membrane bilayer. In contrast, DNA density within PM2 is uniformly distributed throughout the capsid. Simulations of the packaging process are employed to obtain models of the packaged DNA. The models are then refined using SAXS, electron microscopic and spectroscopic results.

III. Assembly of the bacteriophage ϕ6 nucleocapsid

Bacteriophage ϕ6 serves as a model system for dsRNA viruses that assemble in several steps. In the first step, the procapsid of ϕ6 (PC) is formed by copolymerization of four protein species P1, P2 , P4 and P7 [3]. Three segments of ssRNA are packaged into the PC by protein P4 (ATPase) and replicated inside by the polymerase P2. A nucleocapsid is formed by polymerization of protein P8 onto the surface of PC. We have obtained SAXS from monodisperse solutions of individual proteins. Protein P1 (85 kDa), which constitutes most of the PC mass, is an elongated monomer (Rg=45±2 Å). Protein P4 (35 kDa) forms a ring-like hexamer with Rg=47±2Å. Proteins P1 and P4 associate into a large, asymmetric, complex (Rg=104±5Å, apparent mass 370 kDa) which serves as the precursor for nucleation of procapsid assembly. Protein P7

(17.3 kDa) forms a stable, rod-like, dimer (Rg=35±2Å) and interacts with P1. We have attempted time-resolved study of PC assembly. Although time-dependent increase of scattering intensity (I_0) was observed low concentration and solubility of P1 hindered more detailed study. We have, however, succeeded to reversibly assemble and disassemble protein P8 onto and from the PC cores. P8 trimer assembly onto the PC is much faster than its calcium-triggered self-assembly [4] suggesting significant role of the PC template. SAXS-based low resolution structures of the subunits and electron densities from electron cryomicroscopy [3] will be used to obtain the subunit arrangement within the procapsid and nucleocapsid assemblies.

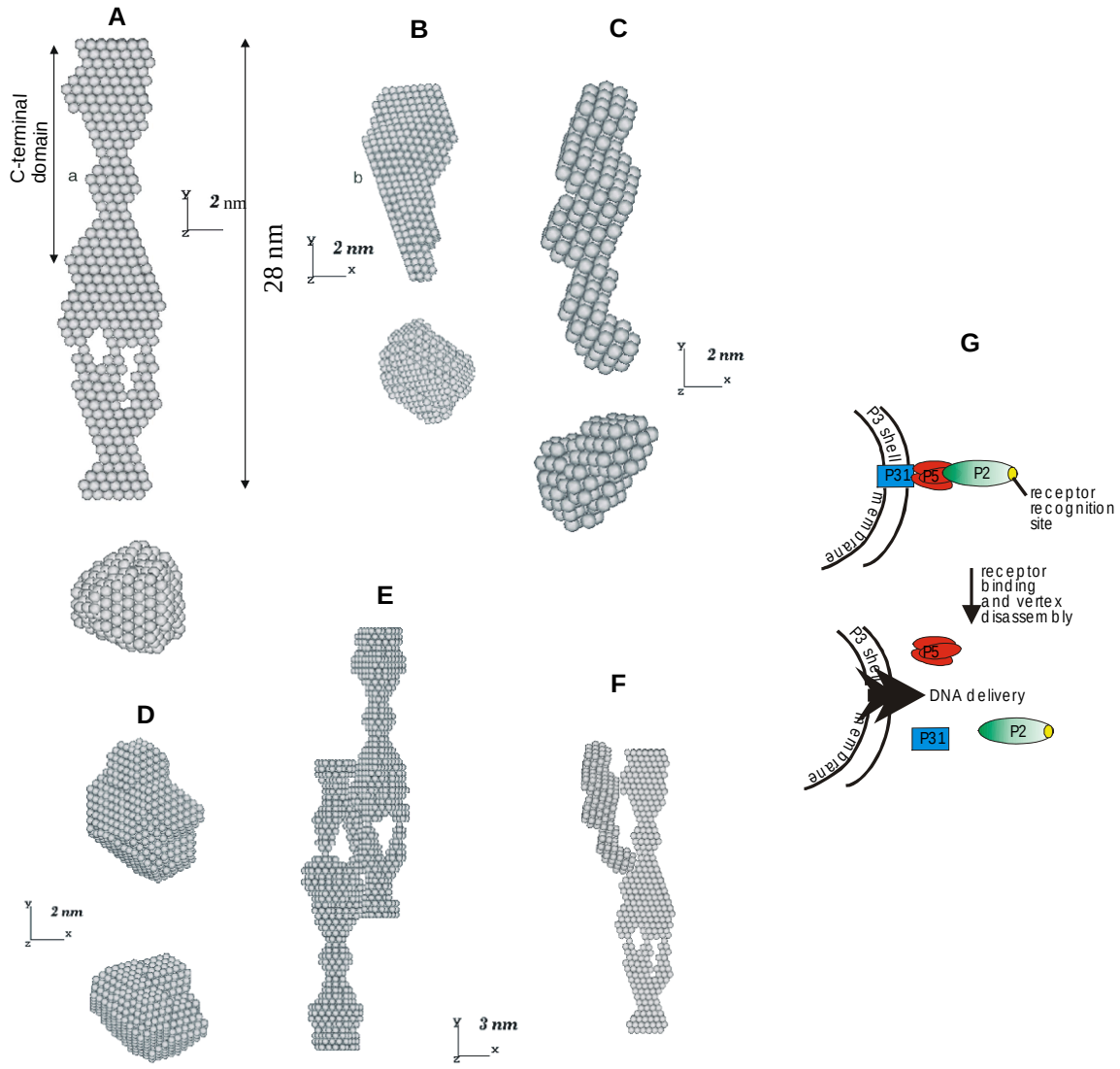

Figure 1: Low resolution structures of PRD1 spike proteins and their in vitro complexes. Two perpendicular views are shown in each panel. (A) P5 trimer; (B) the C-terminal domain of the P5 trimer; (C) P2 monomer; (D) P31 pentamer; (E) $P5_6$:P31 complex; (F).P2:$P5_3$ association model (note that the P2 interacts only with the C-terminal domain); (G) vertex complex disassembly during DNA delivery to the host cell.

References

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