The molecular organization of cypovirus polyhedra

Infection of insect larvae with cypoviruses (figure 1a) and baculoviruses leads to polyhedrosis, a fatal disease which causes significant losses in silkworm cocoon harvests [1]. Cypoviruses (Cytoplasmic Polyhedrosis Virus, CPV) are the only known members of the reoviridae family where the viral RNA is protected by polyhedra. The polyhedrin protein (figure 1b) functions as a three-dimensional in vivo crystal, rather than as a single molecule or oligomer. Thousands of viral particles are packed into such persistent micron-sized protein crystals called polyhedra.
The molecular basis leading to the the remarkable stability of these polyhedra and the mechanisms of disassembly in the alkaline mid-gut of insect larvae are unknown.

We developed methods for purification and X-ray analysis of in vivo grown CPV polyhedra that allowed us to determine its structure down to 2 Å resolution [2]. The crystals had dimensions of 5-12 microns and are the smallest yet used for *de novo* protein X-ray structure solution. Crystals were spread onto micro-fabricated mounts and illuminated at 100 K by the X-ray beam at the micro-diffractometer (MD2) station at beamline X06SA of the Swiss Light Source. Data were processed in cubic space group I23 and structure factor phases were obtained from four different isomorphous heavy atom derivatives. The structure of infectious polyhedra was refined to 2 Å with R = 9.3% and R_{free} = 15.4%.

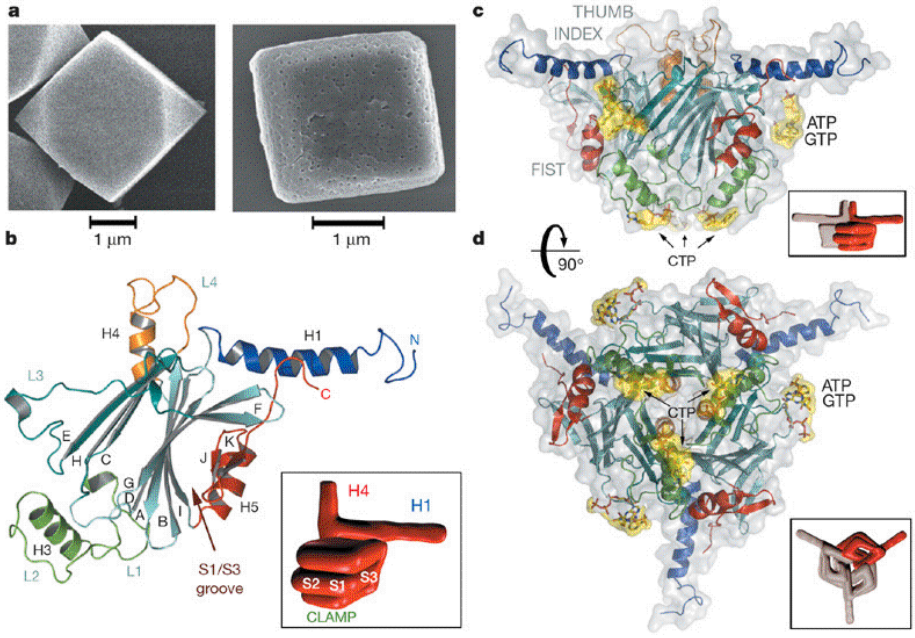

Figure 1. a, Scanning electron micrographs of recombinant (left) and infectious (right) polyhedra. **b,** Polyhedrin has the shape of a left hand (inset): The finger (H1), thumb (H4), fist (S1-S3) and clamp (H3) all contribute to the organization of polyhedra. **c, d,** The polyhedrin trimer in two orthogonal views with bound nucleotides are shown in a yellow surface. The insets represent a trimer models with one of the subunits highlighted in red.

The structure presented in Figure 1 reveals the molecular organization of the polyhedra. The basic building block is a trimer of polyhedrin proteins, which is mainly formed through hydrophobic interactions (Figures 1c, d). The 3-fold symmetry axes of the trimers coincide with the crystallographic three-folds of the cubic lattice so that a single polyhedrin protein constitutes the asymmetric unit of the crystal. A tetrahedral cluster of four trimers forms the next level of organization in polyhedra. Two of these tetrahedral clusters constitute the unit cell of the crystal which is repeated a few hundred times along each axis to form micron-sized cubic polyhedra. The interconnected trimers form an extensive, tightly packed network that shields over 70 % of the polyhedrin surface from solvent. Remarkably, the dense packing is interrupted only by narrow channels between unit cells and by closed cavities at the centre of the tetrahedral clusters. Thus, polyhedra are tightly sealed containers of extraordinary chemical and physical stability that shield embedded virus particles from the external environment. Based on the structure we are also able to propose a mechanism for virus release at high pH values: Crystals dissolve readily at pH 10 - 11 presumably due to deprotonation of a particular group of amino acids that are essential for the stability of the tetrahedral clusters.

Due to their robustness and size CPV polyhedra are easy to manipulate and we could show that they can accommodate a wide range of cargos. The structural characterization of polyhedra provides a sound basis for structure-based engineering of polyhedrin to create variants that, for example, release their cargo at less extreme pH values. Such an approach would certainly pave the road for promising developments of a new generation of nano-containers.

References

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