

STRUCTURAL ANALYSIS OF INFECTIOUS BURSAL DISEASE VIRUS CAPSID.

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Structural polymorphism of macromolecules and macromolecular assemblies is an intrinsic feature required for the control of all fundamental processes in biology. The structure of viral capsids provides a paradigm in the analysis of non-equivalent interactions among identical subunits. Structural polymorphism, together with the extensive use of symmetry, is an example of nature's efficient use of limited coding capacity. The knowledge of the virus structure at the highest resolution must be achieved to understand its properties, e.g. viral morphogenesis and antigenicity. We have used cryo-electron microscopy (cryo-EM), combined with three-dimensional reconstruction techniques, to determine the structure of a dsRNA virus, the infectious bursal disease virus (IBDV), classified in the family *Birnaviridae*, and IBDV-related assemblies, at 7-12 Å resolution. This study addresses the mechanisms that control conformational flexibility of the major structural protein in the IBDV icosahedral capsid which allow to acquire quasi-equivalent conformational states, and their functional implications.

The IBDV single shelled T=13 viral particle is composed of two major proteins, VP2 and VP3. The molecular basis of the conformational flexibility of the major capsid protein precursor, pVP2 (512 residues), is an amphipatic α -helix formed by the sequence GFKDIIRAIR (C-terminal domain residues 443-453). pVP2 undergoes a variety of defined C-terminal processing events to render the mature VP2 (441 residues). However, a VP2 variant containing this α -helix is able to assemble into the T=13 capsid only when expressed as a chimeric protein with an N-terminal His tag (variant HT-VP2-466). An amphiphilic α -helix, which acts as a conformational switch, is thus responsible for the inherent structural polymorphism of VP2. On the other hand, the His tag mimics the VP3 C-terminal region closely and acts as a molecular triggering factor. Assembly control of the complex IBDV T=13 capsid thus requires the interaction of two separate polypeptide elements that can be disengaged in our system. Using cryo-EM difference imaging, both polypeptide elements were detected on the capsid inner surface. We propose that electrostatic interactions between these two morphogenic elements are transmitted to VP2 to acquire the competent conformations for capsid assembly. This analysis also implies that VP3, whose function is successfully restored by the His tag, acts as a canonical scaffolding protein rather than as a permanent component of the final capsid. To our knowledge, the IBDV and the virus-like HT-VP2-466 capsids are the largest true T=13 quasi-equivalent shells, built of a single protein with no additional components.

The availability of three-dimensional maps at subnanometric resolution (7-9 Å) allows the direct identification of secondary structure elements [1]. In addition, X-ray derived maps at atomic resolution can be combined with cryo-EM derived maps with high reliability. These approaches are being used with VP2 assemblies (VP2 alone forms exclusively T=1 capsids, i.e., all-pentamer capsids [2]), and T=13 virion and chimeric capsids [3] together with the recently determined atomic structure of VP2 [4], with the final goal of envisage the structural intermediates at quasi-atomic resolution.

References

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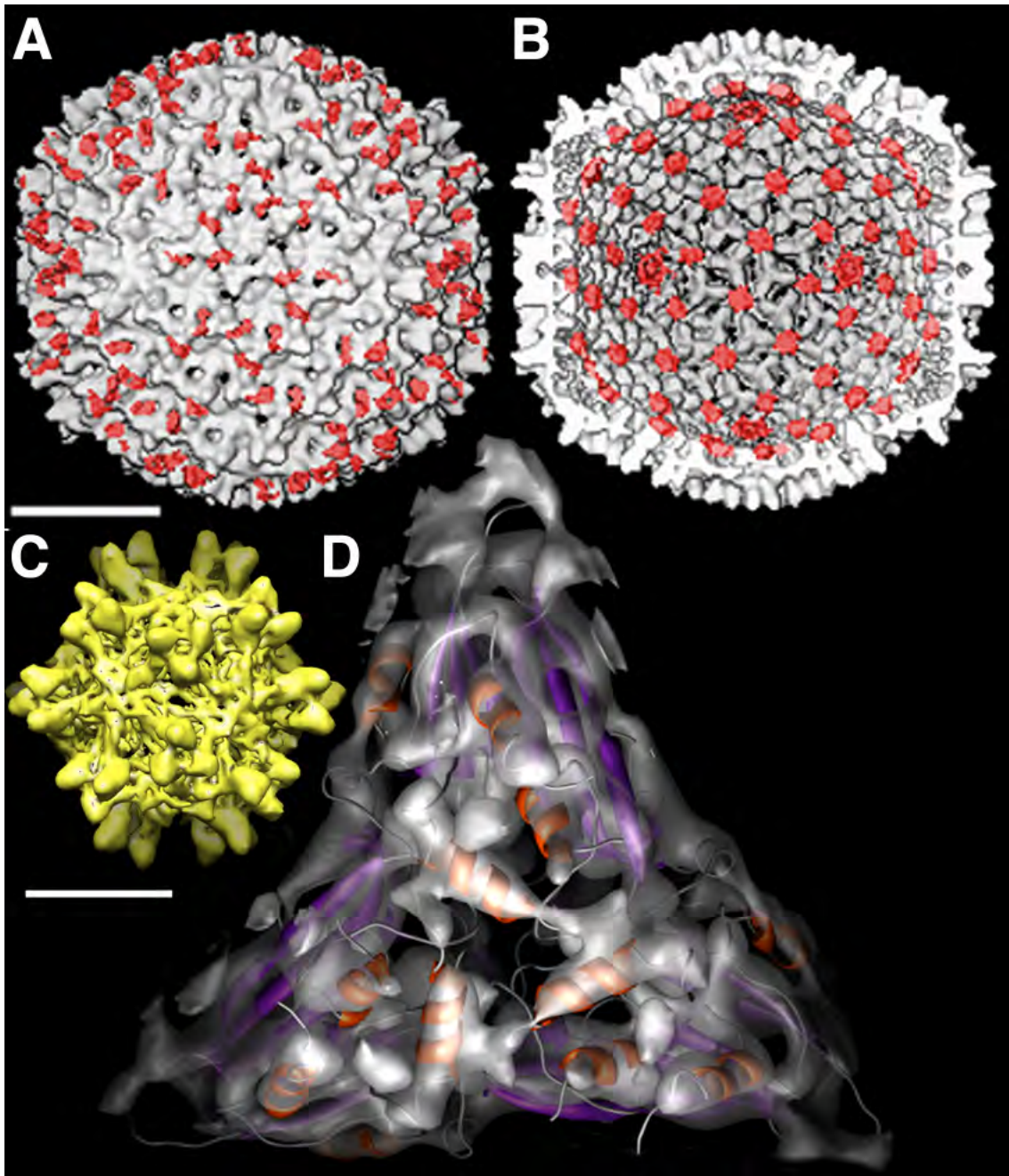


Figure 1. (A) Difference map calculated by subtracting HT-VP2-466 from IBDV capsid. The resulting difference map is represented in red on the outer surface of an IBDV capsid. (B) Difference map calculated by subtracting IBDV from HT-VP2-466 capsid. The resulting difference map, showed as 132 bigger blobs, is shown in red on the inner surface of a HT-VP2-466 capsid. Bar, 200Å. (C) Three-dimensional structure of the VP2-456 T=1 capsid at 7.5 Å resolution. Bar, 100Å. Maps in A, B and C are viewed down a 2-fold axis. (D) T=1 capsid inner surface viewed down a 3-fold axis, showing the fit of three VP2 monomers.