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# Crystallization of Viruslike Particles Assembled from Flock House Virus Coat Protein Expressed in a Baculovirus System

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**Flock house virus coat protein expressed in a baculovirus system spontaneously assembles into viruslike particles, which undergo an autocatalytic postassembly cleavage equivalent to that of the native virus. Mutations of the asparagine at the Asn/Ala cleavage site result in assembly of provirion-like particles that are cleavage defective. Crystals of the mutant provirions have been grown, and they diffract X rays beyond 3.3-Å (0.33-nm) resolution. The crystals are monoclinic space group $P2_1$ ($a$ = 464.8 Å [46.48 nm]; $b$ = 333.9 Å [33.39 nm]; $c$ = 325.2 Å [32.52 nm]; β = 91.9°) with two provirion-like particles per unit cell. Thus, it should be possible to determine the high-resolution structure of the provirion, which will be compared with the crystal structure of the mature authentic virion. This collation should provide mechanistic detail for understanding the cleavage event. Moreover, this demonstrates that the baculovirus expression system displays sufficient fidelity to permit crystallographic analysis of the assembly process of biological macromolecules.**

Icosahedral viral capsids formed from a single gene product are among the simplest biological assemblies, yet the details of their morphogenesis are poorly understood. Frequently, the maturation of a virus includes a postassembly cleavage of the capsid protein that confers stability and infectivity on the virus particle. We have chosen members of the insect nodaviruses to investigate the biochemical and biophysical aspects of viral morphogenesis and the postassembly processing of the capsid protein. Members of the *Nodaviridae* family are the simplest animal viruses that support this paradigm (5, 6).

The nodavirus capsid architecture consists of 180 identical capsid proteins assembled into a T=3 lattice (1). Each icosahedral asymmetric unit contains three chemically iden-

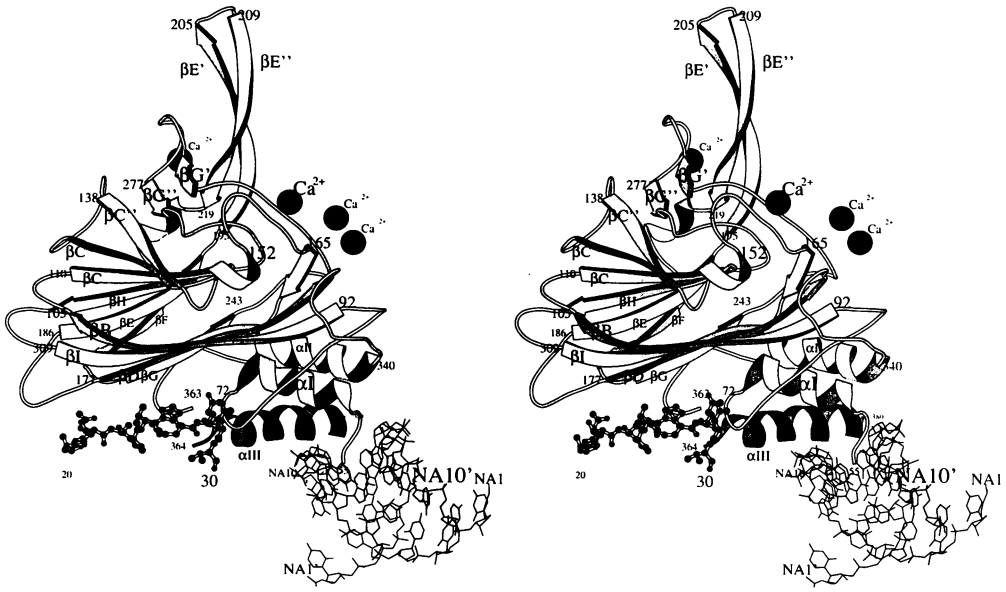


FIG. 1. Stereoribbon diagram showing internal location of the gamma protein (black helix) in the C subunit of the coat protein of FHV (reproduced from reference 3 with permission). The view is from the side with the capsid surface above and the cavity surface below. The separation of Asn-363 on the C terminus of the beta chain and Ala-364 on the N terminus of the gamma chain suggests a shift in chain position following maturation cleavage of N-363/A-364 in the alpha chain precursor.

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FIG. 2. Crystals of provirion-like particles assembled from mutant FHV capsid protein expressed in a baculovirus system. Crystallization conditions are described in the text. The largest crystal is 0.3 mm across the body diagonal.

tical but structurally unique polypeptide chains. Subunits designated A form pentameric units, and subunits designated B and C form hexamers about the icosahedral threefold axes. After particle assembly, the coat proteins are cleaved, yielding a particle resistant to disassembly by 1% sodium dodecyl sulfate (SDS) (5). The cleavage occurs at the conserved site Asn-363/Ala-364 to produce peptides beta and gamma ($\alpha \rightarrow \beta + \gamma$), which contain 363 and 44 amino acids, respectively (8). This maturation cleavage is first order and autocatalytic (5) and is necessary for virus infectivity (15).

The 3.0-Å (0.3-nm) resolution structure of flock house nodavirus (FHV) has recently been solved (Fig. 1) (3). The cleavage site is visible in the A and C subunits and occurs in the virus interior; thus, the gamma peptide is inside the capsid. The first 17 residues of the gamma peptide in the C subunit form an $\alpha$ helix (the last 27 residues lack icosahedral symmetry and are not visible). This helix and the icosahedral twofold related gamma helix form a clamp that binds duplex RNA (Fig. 1, bottom right). The nucleoprotein interaction contributes to the formation of subunit contacts that occur only at the icosahedral twofold joints. The gamma peptide of the A subunit also forms an $\alpha$ helix that lies near the icosahedral fivefold axes. These five gamma helices circle the fivefold icosahedral axes reminiscent of the five VP4 chains at the base of the pentamer center in picornaviruses (7).

The B subunit differs from A and C in that neither its gamma chain nor its cleavage site is visible, indicating disorder. Biphasic cleavage kinetics in FHV suggest that one of the three chains in the asymmetric subunits is cleaved more slowly and less completely than the other two (see Fig. 5 in reference 5). The observed disorder of the B subunit might be explained if it consisted of two populations, one cleaved and the other not, and if maturation cleavage were accompanied by a shift in the position of its gamma chain.

The mechanism of cleavage was not apparent from the analysis of the structure, although a conserved aspartic acid (Asp-75) (9) resides near the cleavage site. The cleavage mechanism and the relationship between cleavage and particle stability should be established if the structure of the uncleaved provirion can be determined and compared with that of the native virus. Since this cleavage is required for

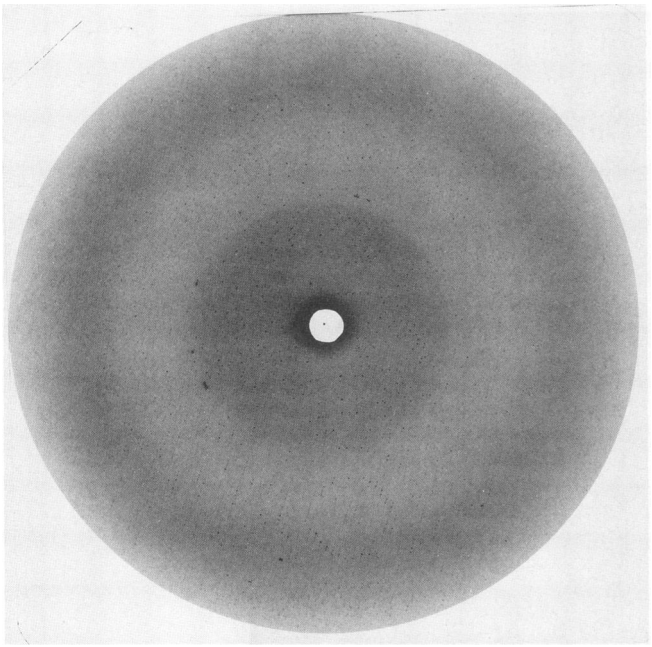

FIG. 3. A 0.5° oscillation diffraction pattern obtained from a crystal shown in Fig. 1, taken at the F1 line at the Cornell High Energy Synchrotron Source. The wavelength was 0.910 Å (0.091 nm), and the crystal-to-film distance was 220 mm. The resolution at the edge is 3.3 Å (0.33 nm). A total of 10,727 reflections with $I/\sigma(I) \geq 2.0$ were accepted for processing on this film.

infectivity, an assembly-competent foreign expression system is needed to produce mutants.

A recombinant baculovirus was created by inserting a cDNA clone of the FHV capsid protein in place of the polyhedron gene. The FHV wild-type capsid protein expressed in this system spontaneously assembles into virus-like particles that package the mRNA of the capsid protein and mature in a fashion similar to that of authentic virus (14). A construct in which the cleavage site was mutated (N363T) resulted in expressed protein that assembled into viruslike particles but was incapable of cleaving into the mature virion (14).

In this paper, we report the crystallization of these provirion-like particles, which were produced from the spontaneous assembly of mutant FHV capsid protein expressed in a baculovirus system. The crystals diffract X rays beyond 3.3-Å (0.33-nm) resolution. This demonstrates the fidelity of the baculovirus expression system in studying the assembly process of macromolecules at atomic resolution.

Viruslike particle production and purification are described by Schneemann et al. (14). However, we included an additional purification step by precipitating the virus in 8.0% (wt/vol) polyethylene glycol 8000–0.2 M NaCl for 6 h at 4°C. The concentration of the viruslike particles was estimated by assuming the same extinction coefficient as that of the native virus; $A_{260}$ (1 mg/ml; 1-cm path) = 4.15.

We often obtained a yield of 1 to 2 mg of viruslike particles per $80 \times 10^6$ cells. The $A_{260}/A_{280}$ ratio was similar to that of native virus, suggesting that the viruslike particles package similar quantities of RNA. Electrophoretic analysis of protein of mutant viruslike particles on SDS-polyacrylamide gels indicated that the sample was pure and that no cleavage occurred (not shown).

Provirion-like particles were crystallized by hanging-drop vapor diffusion (12). The reservoir consisted of 5.0% (wt/vol) polyethylene glycol 8000 in 10 mM sodium phosphate–20 mM $CaCl_2$, pH 6.8. The drop was produced by mixing equal volumes (4 to 5 μl) of reservoir buffer with provirion-like particle solution at 12 mg/ml in 10 mM Bis-Tris, pH 6.0, on a plastic coverslip. The coverslip was inverted over 1 ml of reservoir solution, sealed, and allowed to equilibrate at room temperature. This differs from the conditions that generated crystals of the authentic mature virus, which were grown by sitting a drop against a reservoir of 2.8% (wt/vol) polyethylene glycol 8000 buffered at pH 6.0 by Bis-Tris (4).

Rhombohedrally shaped crystals grew in 4 weeks (Fig. 2). This morphology was similar to that of native FHV, which crystallized in the trigonal space group R3 (4). The provirion-like particle crystals diffracted X rays from a synchrotron radiation source beyond 3.3-Å (0.33-nm) resolution (Fig. 3). Autoindexing of the diffraction pattern (10) suggested that the crystals were not trigonal, but monoclinic, with the following unit cell parameters: $a$ = 464.8 Å (46.48 nm), $b$ = 333.9 Å (33.39 nm), $c$ = 325.2 Å (32.52 nm), and β = 91.9°. The volume-per-molecular-weight ($V_m$) calculation (11) implied two provirion-like particles per unit cell with a value of 2.74 $Å^3$ (0.00274 $nm^3$)/Da. This compares with 2.70 $Å^3$ (0.00270 $nm^3$)/Da for the trigonal native FHV crystals, indicating equivalent solvent content in crystals.

Diffraction intensities were measured with the program developed by Rossmann (13). An analysis of reflection systematic absences showed that the lattice is primitive. Examination of particle packing suggested that the space group is $P2_1$ with the 300-Å (30-nm) particles at (1/4, $y$, 1/4); however, *0k0* reflections were not present on the diffraction patterns analyzed.

These provirion-like particle crystals are not the same space group as native FHV, which was propagated in cultured *Drosophila melanogaster* cells. This difference may result from surface changes caused by the uncleaved capsid protein that would alter the crystal packing. The provirions, however, bind to the cellular receptor with the same affinity as mature virus (15); thus, the difference in space groups may be a consequence of the particles being produced in two different cell lines with different purification procedures. Because these crystals are not the same space group as native FHV, a complete data set must be collected. The structure will be solved by molecular replacement with the native FHV structure as a phasing model. The phases will be refined by real-space averaging of electron density over the 60-fold noncrystallographic symmetry in the $P2_1$ space group.

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