

#14

Reprinted from *J. Mol. Biol.* (1978) 120, 375-379

**Three-dimensional Crystals of the Lipid-enveloped Semliki
Forest Virus**

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(Received 8 November 1977)

Electron micrographs of thin sections of purified, pelleted Semliki Forest virus show highly ordered arrays of virus particles. Examination of these micrographs indicates that the membraned animal virus forms three-dimensional crystals. The unit cell size of the glutaraldehyde-fixed crystals is $a = 735 \text{ \AA}$. The probable space group is $F23$, with the icosahedral virus positioned to share its 23 cubic subgroups with that of the crystal lattice.

1. Introduction

Semliki Forest virus (SFV) is a simple membrane-containing virus. The spherical nucleocapsid, 400 \AA in diameter, consists of a single species of polypeptide and a molecule of single-stranded RNA of 4×10^6 molecular weight. The nucleocapsid is surrounded by a lipid bilayer on the outside of which two (or three) species of glycoproteins form surface projections. The glycoprotein(s) penetrate the bilayer to make contact with the nucleoprotein (Harrison *et al.*, 1971; Garoff & Simons, 1974; Strauss & Strauss, 1976; Kääriäinen & Renkonen, 1977).

The nucleocapsid is icosahedral although there is disagreement on its detailed structure (Osterrieth, 1968; Horzinek & Mussgay, 1969). The observation that the membrane glycoproteins are clustered to form a $T = 4$ icosahedral surface lattice suggests that the icosahedral symmetry extends to the nucleocapsid as well (von Bonsdorff & Harrison, 1975).

It is therefore of interest to note that in infected cell cultures alpha- (and flavi-) viruses have been seen to form "crystalline" aggregates in the extracellular space (e.g., Higashi, 1973; Whitfield *et al.*, 1973). A similar regular arrangement has been observed in thin sections of purified, pelleted SFV (von Bonsdorff, 1973). In this paper we have analyzed such pictures further to show that, under the pelleting conditions, SFV forms true three-dimensional crystals.

2. Materials

Freshly purified SFV (0.5 to 2 mg) was pelleted by centrifugation in the SW 50.1 rotor of a Beckman ultracentrifuge at +4°C for 90 min (Kääriäinen *et al.*, 1969). The pellets were fixed *in situ* with 2% gluteraldehyde and then by 1% OsO₄ and processed for electron microscopy as described previously (von Bonsdorff, 1973).

3. Results

The virus particles, in the thin sections, were in ordered arrays. The largest areas showing a uniform arrangement were up to 5 μm \times 5 μm . The whole section contained such crystals, closely packed but randomly oriented so that they were cut in different planes.

The individual virus particle measured 520 Å, in contrast to the diameter values of 650 to 700 Å obtained with negative staining or from small-angle X-ray scattering experiments (Harrison & Kääriäinen, unpublished observations). This difference has been noticed earlier in individual virus particles and may be caused by the gluteraldehyde fixation or dehydration (von Bonsdorff, 1973). In the particle a dense 300 Å core (nucleocapsid) is surrounded by a 50 Å thick (lipid) layer and outside this the diffusely staining surface proteins are evident (e.g., Fig. 2(b)).

By selecting favorable sectioning planes more detailed data on the three-dimensional arrangement of the virus particles could be established.

Cubic close-packing

Cubic close-packing of SFV particles is demonstrated in an electron micrograph of a thin section, cut obliquely through a crystal, displaying successive hexagonal arrays ((111) planes) of that crystal (Fig. 1(b)).

We may consider the crystal to be formed from close-packed hexagonal layers of virus particles. If the first layer of particles are all at position A (Fig. 1(c)), virus on the second layer will pack between the tops of virus on the first layer at position B (or C). The third layer could then use position A again or position C. The resulting AB, AB (hexagonal close-packing) or ABC, ABC, (cubic close-packing) can repeat to form three-dimensional lattices.

We note a shift of one-third of a lattice spacing between successive hexagonal arrays in Figure 1(b). Referring to Figure 1(c) we see that this is the expected result for arrays centered on A, then B, then C (i.e. cubic close-packing). Thus, the hexagonal layers are not randomly shifted relative to each other as would be expected from a mere close-packing of rotationally disordered spheres. But, instead, a regular cubic close-packing of virus particles is seen indicating that each particle makes repeated specific contacts with its neighbors forming a crystal.

The region between two clear hexagonal arrays (in Fig. 1(b)) can be understood as the image of the tops of virions in one layer and the bottoms of virions in the next layer. Figure 1(d) shows four successive layers of cubically close-packed particles, schematically illustrating both the one-third spacing shifts between successive layers and the details of the image in the cross-over region (see also circled area in Fig. 1(b)). From this analysis we conclude that SFV forms cubically close-packed three-dimensional crystals; the hexagonal arrays we observe being the planes perpendicular to the body-diagonal of a cube (111 planes).

Confirmation of this interpretation is provided by square arrays of virus observed

SW 50.1 rotor
(69). The pellets
were processed for electron

the largest areas
in the section con-
sidered were cut in

diameter values
by X-ray scattering
the difference has
been attributed to
the glutaralde-
hyde cross-linking
of the 300 Å core
outside this the

three-dimensional

electron micrograph of
the hexagonal arrays

hexagonal layers of
viruses (Fig. 1(c)), virus on
the layer at position B
is shifted relative to
the layer at position C. The resulting
interlocking (Fig. 1(d)) can repeat to

successive hexagonal
layers. The expected result
is that, as the hexagonal
layers are expected from a
cubic lattice, a regular cubic
lattice makes repeated

can be understood
the arrangement of
virions in the next
layer. The hexagonal
close-packed particles,
in successive layers
are shifted relative to
the area in Fig. 1(b)).
The hexagonal close-packed three-
dimensional lattice planes perpendicular

of virus observed

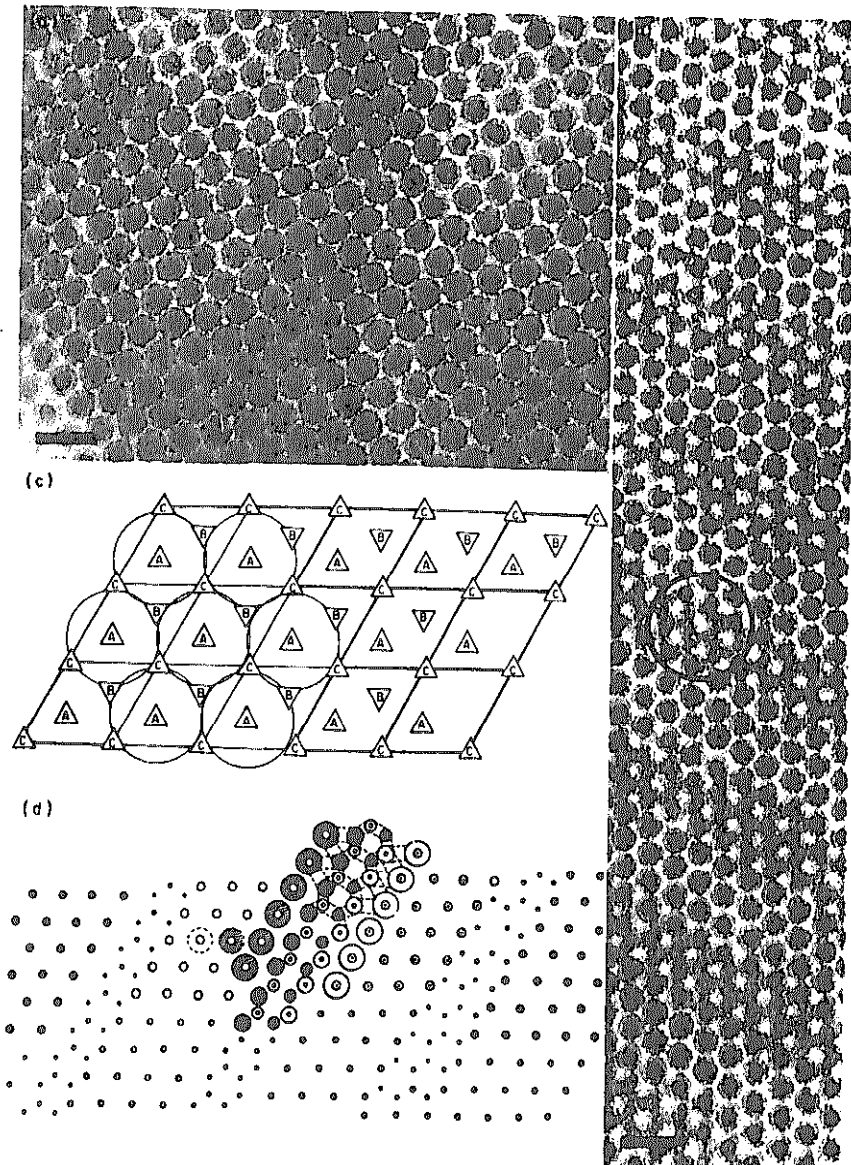


FIG. 1. Thin sections of pelleted Semliki Forest virus as seen in the plane perpendicular to the body diagonal (111 plane). Bar in micrographs: 1000 Å.

(a) Area of hexagonal close-packing of the virus particles (111 plane).

(b) Thin section going through 7 successive layers of hexagonally close-packed viruses. The vertical margins of the picture are cut to demonstrate the continuous one third shift in the position of the successive layers. In the encircled area one can clearly see the details of this shift as the successive layers are "superimposed" (i.e. the tops and bottoms of the particles are seen in the same area).

(c) Schematic representation of the location of consecutive hexagonal layers in cubic close-packing. The lettered triangles represent the centers of particles in successive layers.

(d) Scheme demonstrating the location of virus particles in 4 consecutive layers of hexagonally (111 plane) arranged particles. In the middle the shift between 2 layers is illustrated for full-sized particles. Note the similarity to the encircled area in (b).

in some sections (Fig. 2(a)). The interparticle distance is the same in the hexagonal arrays as expected for views of cubic close-packed crystals down the 2-fold crystal axes (100). Successive square arrays in a cubic close-packed structure are shifted by half a repeat distance and Figure 2(b) is a fortuitous observation of such shifts. These shifts can be understood with reference to Figure 2(c) which shows, schematically, the origin of the square layers in a cubically close-packed structure and how successive square arrays appear shifted by half a repeat distance.

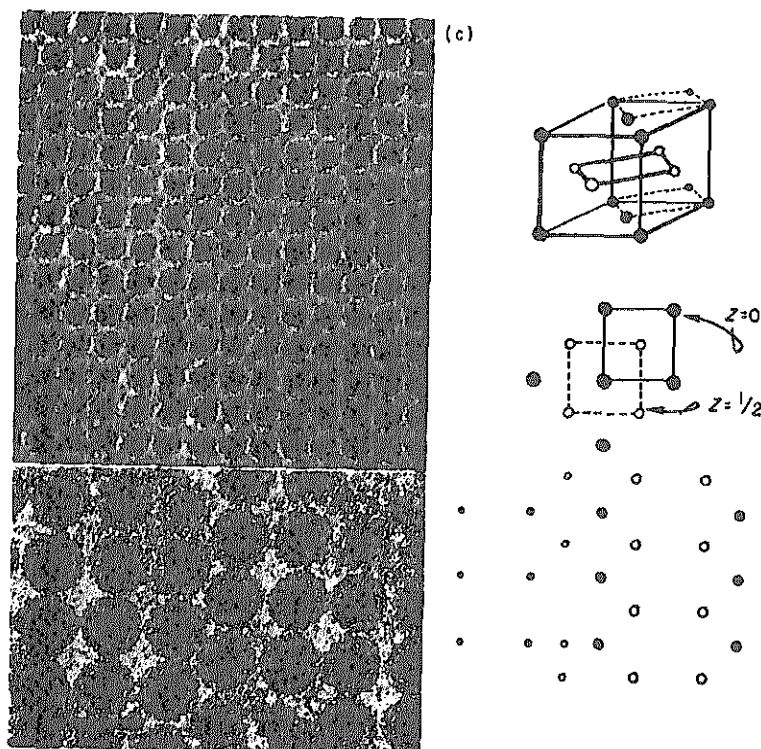


FIG. 2. Thin section of pelleted SFV down the 2-fold (100) axis.

- (a) Area of particles cut through the middle.
 (b) An oblique section through 2 successive layers of cubically close-packed particles. Note that the location of the succeeding plane is shifted by half a repeat distance.
 (c) Schematic representation of a cubically close-packed structure to show the 100 planes (above) and the shift by half a repeat unit in successive layers (solid and open circles).

The optical and calculated diffraction patterns of electron micrographs of the embedded and sectioned crystals of SFV (111 and 100 planes) indicate crystalline order to 100 Å resolution. Two-dimensional Fourier filtered images (not shown) show little detail for two reasons: 100 Å resolution, and the fact that thin sectioning or post-staining procedures reveal different levels within the virus particles across an array, so that the image does not correspond to the projection of repeated structures, but rather (in one array) to views of various thin sections through the virus.

At present, we conclude the lipid membrane containing five microns on a side. The close-packed (face-centered) virus suggests that the space with the virus particles on the crystallization of SFV lipid-membraned particles making the repeated speci

Note added in proof. Sem by conventional crystalli dimension being about 0.1

The skilful technical ass supported by a grant from Science Foundation progr of Health program in allor Biology Organization trav discussions with various r Laboratory of Molecular

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4. Conclusion

At present, we conclude that we have observed adventitious crystallization of the lipid membrane containing Semliki Forest virus into three-dimensional crystals up to five microns on a side. The location of virus particles at the lattice points of a cubically close-packed (face-centered cubic) lattice and the icosahedral (532) surface of the virus suggests that the space group is $F23$ ($a = 735 \text{ \AA}$ for fixed, embedded crystals) with the virus particles on the crystallographic 23 symmetry positions.

The crystallization of SFV demonstrates that the preparations contain homogeneous lipid-membraned particles, which possess a regular surface structure capable of making the repeated specific contacts necessary for crystallization.

Note added in proof. Semliki Forest virus has recently been found to form crystals by conventional crystallization methods. The crystals are still small, their largest dimension being about 0.1 mm. (R. Leberman & K. Simons, personal communication).

The skilful technical assistance of Mr Anssi Mörntinen is acknowledged. The work was supported by a grant from the Sigrid Juselius foundation.

Some of the work was supported by grants to one of us (D. C. W.) from the National Science Foundation program in Human Cell Biology (GB-43573X), the National Institutes of Health program in allergy and infectious diseases (AI-13654), and a European Molecular Biology Organization travel fellowship. One of us (D. C. W.) also gratefully acknowledges discussions with various members of the structure groups of the Medical Research Council's Laboratory of Molecular Biology, Cambridge, during a sabbatical visit there.

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