Comparative Ultrastructural Studies of Insect Granulosis and Nuclear Polyhedrosis Viruses

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(Accepted 9 December 1975)

SUMMARY

Optical diffraction studies indicated that the periodic lattice structure in electron micrographs of the capsids of two granulosis and two nuclear polyhedrosis viruses were indistinguishable. The capsid is composed of stacked rings spaced 4.5 nm apart.

Comparison of the intracellular forms of Bombyx mori nuclear polyhedrosis virus with negatively stained virus particles leads us to encourage the more general use of the term 'capsid' instead of 'intimate membrane' and the term 'virus membrane' in place of 'developmental membrane'. These terms are consistent with those currently used for most animal and plant viruses.

INTRODUCTION

The genus Baculovirus consists of the nuclear polyhedrosis and the granulosis (or capsule) viruses, and their classification into the one genus relies on the fact that they are both rod shaped and contain double-stranded DNA. However the virus inclusion bodies differ widely in size, shape and numbers of occluded virus particles.

In this investigation we have made a comparative study of the fine structure of four viruses, two from the granulosis group and two from the nuclear polyhedrosis group. We have concentrated particularly on the capsids and nucleocapsids of these viruses and have used the technique of optical diffraction (Klug & Berger, 1964) to analyse electron micrographs of the virions. The results are used as a basis for discussion of the relationships of these two groups of viruses and to clarify inconsistencies in the literature relating to the nomenclature of components of the virus particle structure.

METHODS

Viruses. Table 1 lists the names of the insects from which the four viruses were isolated. Following the convention of Krywienczyk & Bergold (1960a, b) we have used an abbreviated symbol consisting of a prefix denoting the virus 'genus' (P for polyhedrosis and C for 'capsule' or granulosis) followed by the initials of the host organism.

Isolation of virus particles for negative staining. (a) Polyhedra and capsules were isolated by differential centrifugation of homogenates of whole insects. Final purification was carried out by layering on sucrose density gradients of 30 to 70 ° (w/v) for granuloses and 50 to 85 ° (w/v) for polyhedroses. Polyhedra or capsules were washed with distilled water to remove the sucrose, centrifuged and vacuum dried. (b) To release virions from polyhedra
Table 1. Abbreviations of names of viruses studied and their host insect species

<table>
<thead>
<tr>
<th>Symbol*</th>
<th>Common name of host insect</th>
<th>Specific name of host insect</th>
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<tbody>
<tr>
<td>CPo</td>
<td>Potato moth</td>
<td><em>Phthorimaea operculella</em> (Zell.) Gelechiidae (Lepidoptera)</td>
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<tr>
<td>CPr</td>
<td>Cabbage white butterfly</td>
<td><em>Pieris rapae</em> (L.) Pieridae (Lepidoptera)</td>
</tr>
<tr>
<td>PBm</td>
<td>Silkworm</td>
<td><em>Bombyx mori</em> (L.) Bombycidae (Lepidoptera)</td>
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<tr>
<td>PS1</td>
<td>Cluster caterpillar</td>
<td><em>Spodoptera litura</em> (F.) Nectuidae (Lepidoptera)</td>
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* The first letter represents capsule (= granulosis) virus (C) or nuclear polyhedrosis virus (P).

and capsules, the inclusion body protein was dissolved in an aqueous solution of 0.01 M-
Na₂CO₃/0.05 M-NaCl. (c) For negative staining the above suspension was further purified by sedimenting virions at 100,000 g for 15 min and resuspending the pellet in distilled water. The suspension was mixed in equal proportions with either a 2% aqueous solution of potassium phosphotungstate at pH 7.0 or a 2% aqueous solution of sodium silicotungstate. Microdrops of these suspensions were sprayed on to carbon-coated grids with an atomizer.

Infection of insects for studies of the developmental cycle. In vivo studies of the developmental cycle were carried out in *Bombyx mori* only. Fifth instar larvae were infected by allowing them to ingest polyhedra. Three days after infection (p.i.) larvae were fixed by injection with cold 2.5% glutaraldehyde in 0.05 M-cacodylate buffer at pH 7.2 containing 0.15 M-sucrose. After 5 min, pieces of integument, fat body, tracheae, testes and gut were dissected and fixed for 2 to 12 h in the glutaraldehyde solution. Specimens were then washed in cacodylate buffer and post-fixed in 1% osmium tetroxide in veronal acetate buffer at pH 7.2, containing 0.15 M-sucrose. After fixation, specimens were dehydrated with ethanol, transferred to propylene oxide and embedded in Araldite. Sections approx. 50 nm in thickness were mounted on carbon coated grids and stained with uranyl acetate and lead citrate.

Electron microscopy. Negatively stained preparations and sections were examined in a Siemens Elmiskop I electron microscope operated at 80 kV and fitted with a liquid nitrogen cooled anticontamination device. Through-focus series of micrographs were recorded at electron optical magnifications up to 80,000 times.

Optical diffraction. Apparatus similar to that described by Markham (1968) was used for optical diffraction. A 1.0 milliwatt He-Ne gas laser was used as a light source. The lenses and light source for diffraction and reconstruction were mounted on a 3 metre optical bench. Electron microscope negatives were routinely contact printed or copied at 1 to 1 enlargement on to Ilford Projector Slide plates to make a positive transparency suitable for use as the diffraction object in the diffractometer. Diffraction patterns were recorded on Polaroid Type 55 film.

RESULTS

Negative staining of viruses and optical analysis of micrographs

When polyhedra and capsules are treated with the carbonate/salt solution, the occlusion body protein is dispersed and virions are released, initially within the virus membrane. Longer treatments cause nucleocapsids to be released from this membrane. After negative staining these appear as shown in Fig. 1 to 5, and 7. Two distinct types of image are observed: (a) intact nucleocapsids which appear as rods of relatively low electron density (Fig. 1 to 4) and (b) empty capsids, in which the structure of the capsid is more apparent since the nucleic acid core has been lost and which are wider than intact nucleocapsids because they flatten during drying (Fig. 2, 4, 5 and 7). Occasionally, empty capsids were
Comparative structure of insect viruses

Fig. 1 to 4. Negatively stained virions (Vi) and capsids (C). The inset optical diffraction patterns were produced from the indicated areas on each micrograph.

Fig. 1. Spodoptera litura polyhedrosis virus (PSI).
Fig. 2. Phthorimaea opercullela granulosis virus (CPo).
Fig. 3. Pieris rapae granulosis virus (CPr).
Fig. 4. Bombyx mori polyhedrosis virus (PBm).
Fig. 5. *Phthorimaea operculella* granulosis virus (CPo) capsid.

Fig. 6. Optical transform produced from the area of the capsid shown in Fig. 5. The two reciprocal lattices, corresponding to diffraction by the two sides of the flattened tubular capsid are indicated. Dotted lattice is from the side of the capsid that has been better negatively stained.

Fig. 7. Partially disrupted, unfolded capsid of *Phthorimaea operculella* granulosis virus (CPo).

Fig. 8. Optical transform of the encircled area of the capsid in Fig. 7.
Comparative structure of insect viruses

Fig. 9-11. Development of *Bombyx mori* polyhedrosis virus (PBm) in the nuclei of cells of fifth instar *B. mori* larvae.

Fig. 9. Early stage of development showing unenveloped capsids and nucleocapsids. Fat body cell.
Fig. 10. Later stage of development showing 2 nucleocapsids during their envelopment within the virus membrane. Fat body cell.
Fig. 11. Membrane-bound virus particles at the stage of inclusion into the polyhedron. Note triple-layered structure of virus membrane (arrows). Testis cell.
Fig. 12. Section of part of an isolated PBm polyhedron.
Comparative structure of insect viruses

Fig. 13.

1. Naked nucleocapsid
2. Enveloped nucleocapsid
3. Enveloped nucleocapsid with electron dense material condensed onto inside of envelope
4. Inclusion into polyhedron
5. Mature virion with condensed electron dense material on both inside and outside of envelope

observed in a partially disrupted state, with the tubular capsid unfolded into a sheet, and the individual capsomeres clearly visible (Fig. 7).

Micrographs of representatives of each of the four viruses studied are shown in Fig. 1 to 4. The optical diffraction patterns obtained from the marked areas provide convincing evidence that the capsids of all four viruses are similarly constructed. The two reciprocal lattices, corresponding to diffraction by the images of the upper and lower surfaces of the capsid are shown in Fig. 6. The prominent meridional spot which corresponds to diffraction by the rings of subunits from which the capsid is constructed is common to both lattices and may be used to measure the ring spacing by comparison with the known spacings on diffraction patterns of catalase (Wrigley, 1968). For the purpose of these measurements, catalase and virus were deposited on the same grid and negatively stained. Where possible, fields containing both intact nucleocapsids and catalase were recorded on the same micrograph. Such measurements made from all four types of nucleocapsid gave an average value of 4.5 nm (s.d. 0.14 nm) for the ring spacing. Numbers of particles measured were as follows: CPR-7, CPO-10, PSL-15, PBm-8. There was no significant difference between ring spacings of any of the viruses studied. The spacings were the same for the empty capsids also (Fig. 2, 4, 5, 7).

To check the assignment of diffraction spots to reciprocal lattices, an image of a partially disrupted capsid was subjected to optical diffraction. The capsid and the diffraction pattern obtained from the encircled area are shown in Fig. 7 and 8. It is obvious that the 6 spots in this diffraction pattern (3 above and 3 below the large zero order spot) are on the reciprocal lattice marked with dotted lines in Fig. 6.

Cells infected with PBm

The detailed development of several different nuclear polyhedrosis viruses including PBm has been well documented elsewhere (Summers & Arnott, 1969; Harrap, 1972b; Raghow & Grace, 1974). The four stages represented by Fig. 9 to 12 have been included merely to correlate the electron microscopic images of virus components obtained by the negative staining technique with those seen in sections of infected cells.
Capsids or nucleocapsids first appear within patches of virogenic stroma (Fig. 9). Subsequently single particles or groups of particles are enveloped by a triple-layered virus membrane (Fig. 10). This membrane is never observed to be closely adpressed to the nucleocapsids at any stage of development. The minimum spacing between virus membrane and nucleocapsids and also between adjacent nucleocapsids is approx. 12 nm. The volume within the virus membrane that is not occupied by nucleocapsids is filled with material of low electron density. During occlusion of the bundles of virus particles within polyhedra, dense layers condense first on the inner and then on the outer faces of the virus membrane. The triple-layered structure of the membrane may still be discerned in the peripheral regions of developing polyhedra (Fig. 11), but not in deeply occluded virus bundles (Fig. 12). A diagrammatic representation of the morphological changes in the virus membrane during development is shown in Fig. 13. In the section of a CPo capsule shown in Fig. 14 the structure of the membrane surrounding the virion is similar to that described in PBm.

DISCUSSION

The terms ‘intimate membrane’ and ‘developmental membrane’ were coined by insect virologists specifically for the occlusion body viruses. The use of these terms in reference to particular structural components seems to have varied according to the author. There is no doubt that the term intimate membrane has in general been used to define what is homologous to the capsid in all other plant and animal viruses (e.g. see Harrap, 1972b), and we suggest strongly that future use of the meaningless term intimate membrane be discontinued completely and that capsid be used exclusively in its place.

The term ‘developmental membrane’ is also rather meaningless in that there are no data available which relate to its true role in virus development or infection. Unlike the capsid, this component is very likely a true membrane in the sense that it possesses a structure
Comparative structure of insect viruses

typical of the other triple-layered lipoprotein membranes of the host cell. It would be tempting to suggest that this outer membrane is homologous to the virus envelope of other animal viruses. However, the latter term is usually reserved for that part of the host cell plasma membrane retained by the virion when it buds off the cell at final maturation. It is not known whether the so-called developmental membrane is coded for by the virus or is virus modified host cell membrane, so we suggest instead the use of the more general term virus membrane to replace developmental membrane. Our suggested term implies no special role for the membrane and should be more acceptable to the majority of virologists.

A number of previous studies has shown the existence of a regular lattice of subunits on the capsids of nuclear polyhedrosis (Harrap & Juniper, 1966; Teakle, 1969; Khosaka, Himeno & Onodera, 1971) and granulosis (Smith & Hills, 1962; Summers & Paschke, 1970) virions. The structure has been described as either helical (Smith & Hills, 1962) or crossbanded (Harrap & Juniper, 1966; Teakle, 1969; Khosaka et al. 1971).

The technique of optical diffraction is sensitive for the demonstration of focal level and astigmatism in high magnification electron micrographs (Johansen, 1972), and also for the indication of symmetry as we have shown here. In fact it is possible to carry out optical diffraction on published electron micrographs (Bancroft, Hills & Markham, 1967). Thus we have been able to check the symmetry of published micrographs of other granuloses and nuclear polyhedroses as well as other rod-shaped insect viruses. Harrap (1972b) was hampered in his interpretation of the structure of the polyhedrosis virus he studied because he was unable to obtain optical diffraction patterns from his micrographs. We obtained a diffraction pattern from his Fig. 9 that was quite similar to those we have presented here. The pattern consisted of 7 spots, corresponding to the zero order and the 6 other spots closest to it. We also used our diffractometer to examine published micrographs of an intra-nuclear, non-occluded, rod shaped virus from midgut cells of a whirligig beetle (Gouranton, 1972). Optical transforms of Gouranton’s Fig. 5 established that the symmetry of the virus particle was helical and not of the stacked disc type, since diffraction spots were obtained 10° to 15° off the meridian. The basic form of this virus appears similar to that of lettuce necrotic yellows virus in aphid tissues as reported by O’Loughlin & Chambers (1967). Optical transforms of Fig. 6 of O’Loughlin & Chambers (1967) and transforms of negative stain preparations of the same virus (Wolanski, Francki & Chambers, 1967) appear quite similar to those from the virus of the whirligig beetle.

Our results show that for the granulosis and nuclear polyhedrosis capsids the basic structure is one of stacked rings of subunits rather than a single stranded helix. More important, however, is the observation that the lattice structures of the capsids of the granulosis and nuclear polyhedrosis virions studied here are identical. These structural similarities provide additional evidence for the taxonomic similarities of the two groups of insect viruses. Bellett (1969) has already shown that there are considerable serological relationships and probable genetic relationships between the granulosis and the nuclear polyhedrosis viruses. A close relationship between the two groups is now accepted and the International Committee for the Nomenclature of Viruses has included them in a single ‘genus’, Baculovirus (Wildy, 1971).

There seems to be little difference between synthesis of one or a large number of virions per occlusion. Arnott & Smith (1968) in their study of the structure of abnormal capsules in a granulosis virus of the Indian meal moth, reported finding not only a range of shapes and sizes of capsules, but also capsules containing a number of virions. Thus it appears that with minor genetic variation granulosis viruses could develop similar occlusion bodies to those of the nuclear polyhedrosis viruses.
Other similarities between nuclear polyhedrosis and granulosis viruses have also been reported in the literature. For instance, the inclusion body protein of a granulosis (Longworth, Robertson & Payne, 1972) has been shown to possess gross similarities to that of a nuclear polyhedrosis virus (Harrap, 1972a). Polar structures described as nipple and claw arrangements have been seen in virions of a granulosis (Summers & Paschke, 1970; Summers, 1971) and a nuclear polyhedrosis (Kozlov & Alexeenko, 1967; Teakle, 1969).

We conclude that these previous studies combined with our own structural findings indicate that there is very little difference between the two groups of viruses.

We thank Dr T. D. C. Grace of the Division of Entomology, CSIRO, for providing viruses and infected insects.

REFERENCES


*(Received 13 October 1975)*