Fine Structure of the Iridescent Virus Type I Capsid

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SUMMARY

The structure of the iridescent virus type I was examined using negative staining technique. After storage for 1 to 2 months at 4°C or treatment with chloroform, the icosahedral capsid breaks up into its structural elements – pentagons and triangles, each consisting of 31 and 55 subunits respectively. When such a preparation is centrifuged through a linear sucrose gradient (10 to 40%), 5 zones are revealed. An electron microscopic analysis showed the following distribution of the material (from top to bottom of the tube): (1) free triangles; (2) associated triangles, ‘core’; (3) partially disrupted virions; (4) free intact virus particles; (5) virus aggregates.

A model for iridescent virus type I capsid is proposed, consisting of 12 pentagons (372 subunits) and 20 triangles (1100 subunits). The total number of subunits is 1472.

INTRODUCTION

Iridescent virus type I (Tipula iridescent virus) is one of the first viruses for which the regularity of capsid structure has been shown. The icosahedral shape of the virus was established by electron microscopy, using the two-directional shadowing method (Williams & Smith, 1958). Further investigations showed that the iridescent virus type I shell is an icosadeltahedron and is composed of 812 subunits (Smith & Hills, 1960; Steinhaus & Leutenegger, 1963).

However, these data about the number of subunits and their arrangement were rather indirect because of the poor resolution of individual subunits on the negatively stained virus particles (Day & Mercer, 1964; Mercer & Day, 1965; Almeida, Waterson & Plowright, 1967). It was shown that crude extracts from insects infected with iridescent virus type I or other iridescent viruses consisted of partially disrupted virions, in which the triangular arrangements of subunits could be distinguished (Almeida et al. 1967; Smith & Hills, 1959).

In crude preparations of the iridescent virus type II (Sericesthis iridescent virus) stored for a long period, Wrigley (1969) found a great number of triangles, pentagons and linear fragments, which, according to the opinion of the author, are structural groups of the iridescent virus type II shell; the total number of subunits was claimed to be 1592 (although the models with 1472 or 1292 subunits were not excluded. For the iridescent virus type I capsid Wrigley (1970) proposed a 1472-subunit structure (alternative 1592-subunit and 1292-subunit models were also considered).

In this paper more direct data are given, showing that the iridescent virus type I capsid consisted of 1472 morphological subunits. A corresponding model is presented and the possibility of a separation of the virus structural components is shown.
METHODS

The virus was extracted from larvae of the greater bee moth *Galleria mellonella*, purified by two cycles of differential centrifugation at 4000 g for 20 min and at 30000 g for 30 min and additionally purified by centrifugation through a linear sucrose gradient (10 to 40%). For partial disruption, the virus was stored at 4°C in distilled water or in 0.01 M-borate buffer (pH 7.0) for 1 to 2 months, or shaken with an equal volume of chloroform for 1 h at 4°C. The separation of virus components was carried out by centrifugation of the partially disrupted virus preparation through the same sucrose gradient (10 to 40%) at 40000 g for 40 min.

Samples for electron microscopy were prepared by negative staining with 2% (w/v) potassium phosphotungstate (pH 6.5) or with 1% (w/v) uranyl acetate (pH 4.5) and examined in a JEM-7A electron microscope at 80 kV.

RESULTS AND DISCUSSION

Morphology of intact particles

Numerous polyhedral particles of 140 to 160 nm in diam. and differently oriented were observed in freshly extracted purified preparations negatively stained with potassium phosphotungstate (Fig. 1a). The presence of particles in twofold, threefold and fivefold orientation confirms the previously established icosahedral shape of the iridescent virus type I (Williams & Smith, 1958; Smith & Hills, 1960; Steinhaus & Leutenegger, 1963). The preparation was practically free of any contamination with cellular and virus fragments, though some particles appeared to be penetrated by the stain ("empty" capsids). Morphological subunits could sometimes be seen on the surface of some virus particles; however, their arrangement was indistinguishable, possibly because of superposition of many capsomers (Mercer & Day, 1965).

Partially disrupted virus

Considerably more information about the iridescent virus type I capsid was obtained when partially disrupted virus was examined by electron microscopy. In the preparation stored for 2 months in distilled water or in 0.01 M-borate buffer one can see, besides intact particles, a great number of capsid fragments (Fig. 1b). The virus treated with an equal volume of chloroform (Fig. 1c) looked similar. Individual subunits and their regular assemblages were sometimes visible on the surface of disrupted particles. These groups of subunits, looking like triangles and pentagons, were loosely packed in such a way that the spherical shape of the virus capsid remained. Similar pentagons and triangles were observed on the supporting film in a free state; in some of them, individual capsomers could be discerned, having the shape of globules of 7 nm in diam. (Fig. 2a). It is interesting that some capsomers appeared to be composed of smaller subunits but we have not defined their number per capsomer nor their structure.

An analysis of the triangles ('trisymmetrons', t, according to Wrigley's 1969 classification) shows that each of them consists of 55 hexagonally packed subunits, forming equilateral triangles with an edge-length of 70 nm (10 subunits per edge). The triangles often form groups; in a group, triangles contact each other not corner to corner but are dislocated by three subunits. Such a dislocation was observed in most cases; if the individual capsomers were indistinguishable, the determination of the 'overlap' (a): triangle edge-length (b) ratio (Wrigley, 1970) \( a/b = 0.33 \pm 0.01 \) allowed us to draw the conclusion that two neighbouring triangles were shifted by three subunits relative to each other.
Iridescent virus type I capsid structure

Fig. 1. (a) Iridescent virus type I purified by ultracentrifugation through a sucrose gradient. Negative staining with potassium phosphotungstate. (b) Virus preparation stored for 2 months and stained with uranyl acetate. Partially disrupted virions and capsid fragments are seen. (c) Virus particles disrupted by shaking with chloroform: uranyl acetate staining.

Pentagons as elements of partially disrupted virions and in a free state were observed only rarely. This is probably connected with the fact that after disruption of the capsid a number of triangles remain bound to pentagons masking their structure. Individual capsomers (especially apical ones) were very rarely visible in the pentagons; the possible
Fig. 2. Structural elements of iridescent virus type I capsid partially disrupted by storage. Negative staining with potassium phosphotungstate. (a) Triangles in which individual capsomers are visible; (b) pentagon with five neighbouring triangles; (c) 'evolvent' of approximately one half of the virus capsid.
Iridescent virus type I capsid structure

Fig. 3. Three-dimensional model of iridescent virus type I capsid viewed along twofold axis (a), fivefold axis (b) and threefold axis (c); (d) group of a triangle and three pentagons corresponding to coordinates $t = 55$, $p = 31$ and $d = 0$ of Goldberg's diagram (Goldberg, 1937). The same group is shaded on the model (c).

explanation of the fact is that, unlike the flat triangles, the pentagons are three-dimensional and their capsomers overlap one another. Nevertheless, in some cases it was possible to discern 4 subunits forming the edge of a pentagon; an estimation of the ratio between the edge-length of pentagons and the diameter of the subunit — 28 nm: 7 nm — also gives 4. The pentamer group with 4 subunits forming its edge may consist, in toto, of 31 subunits; therefore, the 'pentasymmetron', $p$ (Wrigley, 1969), of the iridescent virus type I equals 31.

We have not found any linear fragments ('disymmetrons', $d$; Wrigley, 1969) in the preparations of partially disrupted virus. Thin filaments of 2 to 3 nm in diameter observed in some preparations are probably the products of capsomer decay or fragments of virus nucleoprotein. Thus, the capsid of the iridescent virus type I is composed of pentasymmetrons and trisymmetrons connected with each other in a certain way.

We also observed groups of structural elements located on the supporting film as a plane 'evolvent' of a part of the virus capsid (Fig. 2b). The presence of wedge-shaped slits between
elements is clear from stereometrical considerations. In Fig. 2(c) the 'evolvent' of almost one half of the capsid is shown; one can clearly see the pentagons and triangles forming the capsid.

A model of iridescent virus type I capsid

The above mentioned data allowed us to build a model of the iridescent virus type I capsid, which is composed of 12 pentasymmetrons (p), each consisting of 31 subunits, 20 trisymmetrons (t) [55 subunits each] and no disymmetrons (d); hence, the total number of subunits (N) is:

\[ N = 12p + 20t + 0d = (12 \times 31) + (20 \times 50) = 1472. \]

Fig. 3 shows the described model in projections corresponding to twofold (Fig. 3a), fivefold (Fig. 3b) and threefold (Fig. 3c) axes of symmetry. Fig. 3(d) represents a fragment of Goldberg’s diagram, describing all possible arrangements of morphological subunits on the surface of an icosahedron (Goldberg, 1937). Comparing this fragment, composed of a triangle and three pentagons (diagram coordinates p = 31, t = 55 and d = 0), with the group marked on the surface of the model (Fig. 3e) one can see that they are identical. Thus, it is evident that the variant of the arrangement of subunits theoretically predicted by Goldberg (1937) is actually realized in the shape of the icosahedral capsid of the iridescent virus type I.

Sucrose gradient centrifugation

When the virus, which has been partially disrupted by storage or by treatment with chloroform, has been centrifuged through a linear sucrose gradient (10 to 40%), 5 zones were obtained. The upper zone consisted of single and dimerized triangles, pentagons and small multi-edged groups of capsomers. As well as these there was a considerable number of individual capsomers – probably the result of a spontaneous disruption of the capsid elements on the supporting film. Groups of triangles and pentagons looking like 'evolvents' of the virus capsid were seen in the second wide zone. Dense spherical particles with a diam. of 90 nm (probably 'core') were also found in this zone. In the third zone partially disrupted virus particles were seen. The largest part of the centrifuged material was concentrated in the fourth dense zone which consisted of intact single virions. The fifth zone contained virus aggregates and virions bound to cell membranes. Approximately the same distribution of the components was observed when the crude virus preparations were centrifuged under the same conditions.

The possibility of separating the virus into its structural components opens the way for a further more detailed investigation of the structure of the iridescent viruses.

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REFERENCES


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