The Lipid Content of Two Iridescent Viruses

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SUMMARY

Lipids extracted from extensively purified iridescent viruses (types 2 and 6) have been analysed by thin-layer chromatography. The lipid forms 9% by weight of the iridescent virus particle and is equivalent to $59 \times 10^6$ daltons per particle. Negatively stained preparations and thin sections of the virus particles viewed with the electron microscope indicated that a membrane is located internally. There is sufficient lipid in the virus particles to form a continuous lipid bilayer at this internal site.

Iridescent viruses are large icosahedral cytoplasmic DNA-containing viruses, which are not enveloped by a conventional 'unit membrane' (Kelly & Robertson, 1973), and which are claimed to be ether resistant (Day & Mercer, 1964). However, there are reports that some iridescent viruses (types 1 and 2) contain small amounts of lipid (Thomas, 1961; Glitz, Hills & Rivers, 1968), though Bellett (1968) and Kelly & Robertson (1973) suggest that the lipid may be a contaminant. Recent studies by Stoltz (1971) by electron microscopy of iridescent viruses types 1 and 3 suggest that these viruses possess an internal 'membrane', although the chemical nature of this 'membrane' has not been determined. Our results in this paper demonstrate that iridescent viruses types 2 and 6 contain lipid, and that sufficient lipid is present to form an internal membrane structure.

Iridescent viruses types 2 and 6 (Tinsley & Kelly, 1970; Kelly & Robertson, 1973) were grown in late instar Galliera mellonella L. larvae and extensively purified as previously described (Kelly & Tinsley, 1972). Viruses so purified did not contain host antigens or host DNA, were homogenous when viewed under the electron microscope, and no 'enveloped' particles were observed when at least 1000 particles were counted at random (Kelly, 1972). For comparative purposes the lipids of some conventionally enveloped viruses were also extracted - Sendai virus, and two strains of Newcastle disease virus (Ulster, avirulent; Texas, virulent), viruses which acquire their envelope at the plasma membrane, and are similar in size to the iridescent viruses. These paramyxoviruses were grown in ova and were purified as will be described (D. C. Kelly & N. J. Dimmock, unpublished observations). Electron microscopy was performed as described by Kelly & Tinsley (1973).

Lipids were extracted from 1 ml suspensions of viruses in NTC buffer solutions at pH 7.1 (Franklin, Salditt & Silbert, 1969) by the method of Folch, Lees, & Stanley (1957). The total lipid extract was dissolved in chloroform: methanol (1:1, v:v), and 5 µl samples were applied to 0.25 mm silica gel Polygram SIL G thin-layer chromatography plates (CamLab, Cambridge). The phospholipids were separated in a solvent system of chloroform:methanol:water (100:42:6, by vol.) and the non-polar lipids separated in a solvent system of benzene:ethyl ether:ethyl acetate:acetic acid (80:10:10:0.2, by vol.) (Skipski & Barclay, 1969). The lipids were detected by iodination, or the gel was impregnated with 50% (v/v) sulphuric acid and charred. The phospholipid content of the viruses was determined by the method of Dittmer & Wells (1969). The relative amounts of lipid were determined by scanning negative photographs of charred plates in a Joyce Loebli microdensitometer, which was calibrated
Fig. 1. Thin-layer chromatograms of the phospholipids extracted from iridescent viruses and paramyxoviruses. 1, total lipid extract of BHK 21 cells; 2, total lipid extract of late instar Galleria mellonella larvae; 3, iridescent virus type 2; 4, iridescent virus type 6; 5, sphingomyelin; 6, phosphatidyl choline; 7, Newcastle disease virus – strain Ulster; 8, Sendai virus; 9, Newcastle disease virus – strain Texas; 10, total lipid extract of late instar Galleria mellonella larvae. A, non-polar lipid; B, glycolipid; C, phosphatidyl ethanolamine; D, phosphatidyl glycerol/phosphatidyl inositol; E, phosphatidyl choline; F, sphingomyelin; O, origin.
Table 1. The lipid composition of some iridescent viruses and paramyxoviruses

<table>
<thead>
<tr>
<th>Virus</th>
<th>Total lipid</th>
<th>Phospholipid</th>
<th>Non-polar lipid</th>
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<tbody>
<tr>
<td>Iridescent, type 2</td>
<td>9</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Iridescent, type 6</td>
<td>9</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Sendai</td>
<td>30</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Newcastle disease (Ulster)</td>
<td>27</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>Newcastle disease (Texas)</td>
<td>27</td>
<td>13</td>
<td>14</td>
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</tbody>
</table>

with standards photographed under identical conditions. The protein and DNA content of the virus suspensions were determined by the methods of Lowry et al. (1951) and Burton (1956) respectively, using bovine serum albumin and calf thymus DNA as standards.

The amount of lipid obtained from the virus particles is shown in Table 1. The results were taken from thin-layer chromatography plates of the phospholipids and non-polar lipids (Figs. 1, 2). Whereas the 9% lipid content of the iridescent viruses is higher than previous estimates of 2 to 5% (Thomas, 1961; Glitz et al. 1968), the values obtained for the paramyxoviruses are in good agreement with those reported by Blough & Lawson (1968) and Klenk & Choppin (1969). A striking difference between the phospholipids of the iridescent viruses and the paramyxoviruses is that sphingomyelin is present only as a minor component of the iridescent viruses (Fig. 1). Spingomyelin is present in relatively high amounts in the plasma membrane of BHK cells and other plasma membrane preparations (Renkonen et al. 1971) and therefore is found in relatively high quantities in such viruses as the paramyxoviruses that obtain their lipid envelope by budding from the plasma membrane. In contrast, iridescent viruses are completely assembled in the cytoplasm of cells and budding from the plasma membrane does not play a role in their release from cells (Kelly & Tinsley, 1973). It is also apparent that iridescent viruses contain a high proportion of sterol esters when compared with the non-polar lipids of paramyxoviruses (Fig. 2).

Since the viruses are highly purified and free from contaminating material, a lipid content of 9% of the particle weight is not likely to be an artifact of purification, especially since the relative proportions of lipids found in the iridescent virus particles are quite different from those of the total lipid extracts of late instar Galleria mellonella larvae (Figs. 1, 2). The question then arises as to the role of lipid in the architecture of iridescent viruses. Negatively stained iridescent viruses (Fig. 3) and thin sections of iridescent viruses (Fig. 4) show that a ‘membrane’ is sandwiched between two shells, presumably protein, of the virus capsid. From a lipid content of 9% we have calculated that iridescent viruses contain about $59 \times 10^8$ daltons of lipid per particle. To cover the surface of the virus particle (diam. 130 nm; surface area 53,000 nm$^2$) approx. $78 \times 10^8$ daltons of lipid would be required (assuming that a non-polar lipid-phospholipid complex occupies 1 nm$^2$ in a bilayer (Engelman, 1969) and has a mol. wt. of 1400). The area of 35,300 nm$^2$ occupied by a membrane sandwiched between two capsid layers at 106 nm diam. requires $49 \times 10^8$ daltons of lipid at that diam. to form a bilayer. Hence, it appears that the lipid content of the iridescent viruses is consistent with the location of a continuous bilayer within the virus particle. In addition, it should be noted that Harrison et al. (1971) demonstrated by X-ray analysis of structure that an internal lipid bilayer was present in the smaller bacteriophage PM2 (diam. 60 nm), which has a striking morphological resemblance to the iridescent viruses.
Fig. 2. Thin-layer chromatograms of the non-polar lipids extracted from iridescent viruses and paramyxoviruses. 1, total lipid extract of late instar Galleria mellonella larvae; 2, iridescent virus type 2; 3, iridescent virus type 6; 4, cholesterol; 5, cholesterol palmitate; 6, total lipid extract of BHK 21 cells; 7, Newcastle disease virus – strain Texas; 8, Newcastle diseases virus – strain Ulster; 9, Sendai virus; 10, total lipid extract of BHK 21 cells. A, sterol esters; B, triglycerides/free fatty acids; C, sterols; D, unidentified non-polar lipid; E, phospholipid and the origin.
Fig. 3. Particles of iridescent virus type 2 showing the site of the internal membrane (arrowed), stained with 2% (w/v) uranyl acetate.
Fig. 4. Thin section of iridescent virus type 6 particles in the cytoplasm of cultured *Antherea eucalypti* cells 144 h after infection at 21 °C. The outer shell of the virus is well defined and the location of the internal membrane is arrowed.

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