Lipids of Transmissible Gastroenteritis Virus and their Relation to those of Two Different Host Cells

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SUMMARY

The lipids of two cell types (primary pig kidney and secondary adult pig thyroid) and those of transmissible gastroenteritis virus (TGEV) grown in these cells were studied using 14C-palmitic acid. Differences were demonstrated between the incorporation of isotopically labelled lipid precursors in the two cell types and it was found that the phospholipid and glycolipid profiles of purified TGEV closely resembled those of the host cell in which it was grown.

Transmissible gastroenteritis virus (TGEV) is a porcine coronavirus (Tajima, 1970) bounded by a lipid-containing envelope. Work is in progress at this Institute to investigate the antigenic nature of the structural components of TGEV. Since glycolipids may be antigenic and may vary between cell types (Hakomori & Jeanloz, 1970) it was of interest to determine whether TGEV incorporates host glycolipid which might alter virus antigenicity. This report describes the incorporation of radioactively labelled palmitic acid and orthophosphate into the lipids of the virus grown in two different types of host cells.

Primary pig kidney cells (PK/1) derived from newborn piglets (Luther, 1972) and secondary adult pig thyroid cells (APT/2; Rutter & Luther, 1973) were used. Total cell lipids were labelled with U-14C-palmitic acid (The Radiochemical Centre, Amersham, Bucks., sp. act. 256 mCi/mmol), using a procedure similar to that of Robbins & Macpherson (1970). A solution of benzene containing 50 #Ci palmitic acid was evaporated to dryness under a stream of nitrogen. The residue was resuspended by sonication in 2 ml of either lamb serum for use with PK/1 cells or calf serum for APT/2 cells. The mixtures were then added to serum-free medium ELG (Garwes & Pocock, 1975) or medium 199/gal each containing 20 mM-HEPES (Pocock & Garwes, 1975) to give a final serum concentration of 2%, and passed through a 450 nm filter.

Phospholipids were labelled by adding 32P-orthophosphate (The Radiochemical Centre, Amersham, Bucks.) to ELG, in which the orthophosphate concentration was lowered to 10 #M from 0.9 mM, while maintaining the sodium ion concentration at 124 mM by the addition of extra NaCl (ELG-low P). The 32P was added to give a final concentration of 2.5 #Ci/ml of medium. Monolayer cultures of PK/1 and APT/2 cells in 4 oz flat glass bottles were infected with the FS772/70 strain of TGEV (Garwes & Pocock, 1975) at an input multiplicity of 1 to 5 p.f.u./cell and were incubated together with uninfected controls for 36 h (PK/1) or 18 h (APT/2) at 37 °C in medium containing either 14C-palmitic acid or 32P-orthophosphate.

After incubation the medium was removed from the uninfected monolayer cultures, which were washed twice with saline. A small volume of distilled water was added and the bottles were frozen at -20 °C. The monolayers were then thawed, scraped off the glass and sedimented at 1500 g for 10 min at 4 °C. The pelleted material was washed briefly in methanol and dried under nitrogen.

Infected cultures were frozen at -20 °C after incubation and the virus was purified as
Table 1. Extraction of lipids from PK/I and APT/2 cells labelled with $^{14}$C palmitic acid and TGEV grown in those cells: two-dimensional chromatography

<table>
<thead>
<tr>
<th>Spot no.</th>
<th>Probable identity</th>
<th>PK/I</th>
<th>APT/2</th>
<th>PK/I</th>
<th>APT/2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Phosphatidyl ethanolamine</td>
<td>9.3</td>
<td>7.8</td>
<td>8.9</td>
<td>4.1</td>
</tr>
<tr>
<td>2</td>
<td>Phosphatidyl choline</td>
<td>21.2</td>
<td>54.6</td>
<td>28.5</td>
<td>52.1</td>
</tr>
<tr>
<td>3a</td>
<td>Sphingomyelin</td>
<td>35.7</td>
<td>14.8</td>
<td>31.7</td>
<td>16.1</td>
</tr>
<tr>
<td>3b</td>
<td>Lysolecithin</td>
<td>1.8</td>
<td>N.D.*</td>
<td>1.6</td>
<td>N.D.</td>
</tr>
<tr>
<td>4</td>
<td>Phosphatidyl serine</td>
<td>2.7</td>
<td>1.8</td>
<td>2.5</td>
<td>1.6</td>
</tr>
<tr>
<td>5</td>
<td>Phosphatidyl inositide</td>
<td>10.3</td>
<td>15.7</td>
<td>13.8</td>
<td>16.4</td>
</tr>
<tr>
<td>6</td>
<td>Monohexose ceramide</td>
<td>1.5</td>
<td>0.5</td>
<td>1.8</td>
<td>N.D.</td>
</tr>
<tr>
<td>7</td>
<td>Dihexose ceramide</td>
<td>N.D.</td>
<td>0.3</td>
<td>0.5</td>
<td>N.D.</td>
</tr>
<tr>
<td>8</td>
<td>Trihexose ceramide</td>
<td>4.3</td>
<td>1.4</td>
<td>2.7</td>
<td>0.4</td>
</tr>
<tr>
<td>9</td>
<td>Tetrahexose ceramide</td>
<td>3.9</td>
<td>0.7</td>
<td>2.5</td>
<td>0.4</td>
</tr>
<tr>
<td>10</td>
<td>Aminoglycolipid</td>
<td>9.3</td>
<td>2.4</td>
<td>5.5</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td>Unknown spots, breakdown product etc.</td>
<td>501330</td>
<td>220652</td>
<td>106150</td>
<td>56420</td>
</tr>
</tbody>
</table>

* N.D. = no spot detected on autoradiogram.

described by Garwes & Pocock (1975). The final preparation, containing no detectable host protein or nucleic acid, was dried under nitrogen.

Lipids were extracted from the cell and virus preparations with chloroform/methanol (2:1 by vol.) at 4 °C for 18 h followed by a fresh extraction at room temperature for 5 min. The extracts were pooled, reduced to small volume under nitrogen and spotted on to one corner of a 250 μm thick, 20 x 20 cm silica gel G chromatography plate (type 5737, E. Merck, Darmstadt, Germany) previously activated at 110 °C for 30 min. The plate was then run in the two-dimensional system of Gray (1967). Radioactive lipids were located by autoradiography using Kodak AP54 ‘no-screen’ X-ray film, their identity being tentatively established by comparison with published data (Gray, 1967; Robbins & Macpherson, 1971) and by chromatography of known standards. Quantification was carried out by scraping the spots from the plate into scintillation vials, using the exposed film as a template. A toluene-Triton X-100 based scintillation fluid was added and the level of radioactivity determined in a scintillation counter.

One-dimensional chromatography was also carried out. The samples were prepared as described above, and chromatographed on pre-run, activated silica gel G plates using the following solvent system: petroleum ether (boiling range 40 to 60 °C); diethyl ether; glacial acetic acid, in the proportions 85:15:2 by vol. (Gloster & Fletcher, 1966). Autoradiography was carried out as before; the labelled spots were identified by comparison with known standards and quantified by scintillation.

The results obtained from typical experiments are shown in the tables. Table 1 summarizes a typical lipid analysis from one experiment, using two-dimensional chromatography. The identity of spots 1 to 5 as phospholipids was established in experiments in which the lipids were labelled with $^{32}$P-orthophosphate. Some variation was found between this and other similar experiments, but these data are representative of the results obtained. Both types of cells, and TGEV grown in these cell contain similar classes of glycolipids; however in PK/I cells and virus, grown in PK/I cells, the glycolipids appeared as incompletely resolved
double spots (treated as single spots for quantification) whereas analysis of APT/2 cellular and virus material gave single spots. The glycolipids in both cell types were mainly composed of monohexose ceramides, more being found in the APT/2 material than in the PK/I sample. The next most abundant glycolipid was found to be a tetrahexose ceramide, although this was present in the APT/2 material in only small amounts. The spot corresponding to the position of aminoglycolipid was also more pronounced in the PK/I material than in that from the APT/2. The dihexose ceramide made a small contribution to the total glycolipid pattern, while the trihexose ceramide was detected at very low concentration in the PK/I virus material, although in other experiments it has been found in trace amounts in both cell types and in the virus grown in them.

Both cell types were found to contain a large amount of $^{14}$C-palmitic acid incorporated into phospholipid. Sphingomyelin and lyssolecithin were not fully separated by the solvent systems used and were treated as one component for quantification. However, it was noted that, while in the autoradiogram of the PK/I lipids, the radioactivity associated with these two lipids appeared to be similar when the blackness of the film was taken as a guide, the APT/2 material showed a considerable drop in the proportion of lyssolecithin to sphingomyelin. These two phospholipids and phosphatidyl choline were the most abundant phospholipids in the PK/I material, with phosphatidyl ethanolamine and phosphatidyl inositide present in lesser amounts. Phosphatidyl serine formed a small proportion of the total. Phosphatidyl choline was the major phospholipid in the APT/2 extract, the sphingomyelin/lyssolecithin component contributing a smaller proportion. The proportions of phosphatidyl ethanolamine and phosphatidyl inositide were lower than in the PK/I material, and there was no detectable phosphatidyl serine. The pattern of incorporation of $^{14}$C-palmitic acid into the phospholipids and glycolipids of TGEV resembled that of the host cell in which it was grown.

In Table 2, which summarizes the results from one-dimensional chromatography, an obvious difference between the cell and virus was the apparent absence of cholesteryl and fatty acid esters in the virus autoradiogram. There was an appreciable drop in the proportion of triglyceride present, but an increase in diglyceride. While the amount of cholesterol and other sterols remained constant, there was a significant rise in the proportion of phospho-
lipids and glycolipids. A disproportionate amount of labelled free fatty acid was found in the APT/2 grown TGEV sample, but with this exception, where differences occurred between the two cell types, the virus showed a corresponding change.

This work on the lipids of TGEV and its host cells has shown that the PK/I and APT/2 cells have recognizably different profiles, as judged by the incorporation of $^{14}$C-palmitic acid. No attempt has been made to obtain detailed chemical information about the lipids found because of the small quantities of virus material available, but it has been shown that while TGEV grown in two cell types has some ability to influence the incorporation of the neutral lipids into its envelope, it has little, if any, control over the incorporation of the phospholipids and glycolipids.

All the analyses of host lipid composition were determined using extracts from whole cell material. Similar work has been carried out on Sindbis virus by Hirschberg & Robbins (1974), Rous sarcoma virus by Quigley, Rifkin & Reich (1971), and parainfluenza virus SV5 by Klenk & Choppin (1969) using the plasma membranes isolated from the host cell as a source of host lipid. TGEV, in contrast to these viruses, matures by budding into cytoplasmic vesicles (Okaniwa et al. 1966). The composition of the membrane bounding these vesicles has not been established so a study of the lipids of the whole cell might be more meaningful than a possibly misleading study of the plasma membrane.

The normal host for TGEV is the intestinal epithelial cell. The glycolipid composition of intestinal cells has been shown to differ from parenchymatous tissue such as the kidney (Hakomori & Jeanloz, 1970). It is likely, therefore, that, because the labelling of the glycolipids of TGEV with $^{14}$C-palmitic acid reflects that of its host cells, the virus will have a different glycolipid composition when grown in intestinal cells from that grown in PK/I cells. These findings may be relevant in studies on the antigenic differences between TGEV grown in different host cells, both in vitro and ultimately in vivo.

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REFERENCES


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