THE PHOSPHOLIPID COMPOSITION OF BACTERIOPHAGE φ6

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Summary. The enveloped bacteriophage φ6 appears to be similar in several ways to the enveloped mammalian viruses. ^{32}P-labelled stocks of φ6 and its host cell were produced and then subjected to phospholipid extraction and analyzation by thin layer chromatography. The results indicate that φ6 and its host cell contain three major phospholipids: cardiolipin, phosphatidylethanolamine, and phosphatidylglycerol. φ6 contains a higher relative amount of phosphatidylglycerol (57%) than does its host cell (29%), suggesting that φ6 does not acquire its membrane directly from an unaltered cellular membrane.

Bacteriophage φ6 differs from all other known bacterial viruses in that it appears to contain a lipid envelope. Vidaver et al. (1) have shown that φ6 is an RNA phage composed of about 25% lipid, which is present in a rather loose-fitting envelope which surrounds a polyhedral proteinacious core. Because of the existence and nature of its envelope, φ6 appears to be a model system for studying various aspects of the biology of the enveloped mammalian viruses. In this paper, I report the phospholipid composition of the φ6 envelope and compare this with the phospholipid composition of the host cell, Pseudomonas phaseolicola HB10Y.

MATERIALS AND METHODS

Routine Growth of φ6. Original stocks of bacteriophage φ6 and its host cell, Pseudomonas phaseolicola HB10Y, were kindly provided by Dr. Anne Vidaver. φ6 was plaque purified and subsequent lysates were produced in NBY medium (1) at 25°C.

To facilitate ^{32}P-labelling of φ6 and host cell material, a tris-buffered minimal medium, designated TG9, was developed. TG9 contains, per liter of distilled water, 12.1 g tris base, 3g KCl, 6g NaCl, 0.2g $MgSO_4$-$7H_2O$, 0.05g KH_2PO_4,

5g glucose, and 20 mg each of arginine, proline, alanine, histidine, leucine, methionine, threonine, tryptophane, and cystine. TG9 is adjusted to pH 7.1 by addition of HCl. The generation time of Pseudomonas phaseolicola HB10Y in TG9 is about 200 minutes, while φ6 produces a burst of about 100 plaque forming units (PFU) per infected cell about 120 minutes after infection. Stocks of φ6 at 2 x 10^{10} PFU/ml are routinely prepared in TG9 and stored at 0°C.

Preparation of Purified ^{32}P-labelled φ6.

To prepare ^{32}P-labelled φ6 and host cells, 60 ml of TG9 medium was inoculated with Pseudomonas phaseolicola HB10Y to 5 x 10^6 cells/ml and aerated overnight at 25° in the presence of 100 μCi $^{32}PO_4$ (New England Nuclear Corp., Boston, Mass.). When the culture reached 1 x 10^8 cells/ml, 10 ml was removed and maintained as an uninfected control. To the remainder of the culture, φ6 (prepared in TG9) was added at a multiplicity of infection (MOI) of 5. Following lysis of the infected culture (3 hours after infection), cellular debris was removed by low speed centrifugation. NaCl (to 0.5 M) and polyethylene glycol 6000 (to 10% W/V) were added to the clarified lysate, which was then stored at 4°C for 4 hours (2). Virus was then pelleted by centrifugation at 16,000 g for 10 min, and resuspended in 1 ml of NBY medium overnight at 4°C. Resuspended virus was then layered onto a 15% - 35% sucrose gradient (in TG9 medium) and centrifuged at 35,000 rpm for 120 minutes in an SW41 rotor. Fractions collected from the gradient were counted by liquid scintillation spectrometry. The peak of radioactivity was also visible as a distinct light scattering source and was titered at greater than 10^{12} PFU/ml.

^{32}P-labelled uninfected cells were washed 3 times to remove non-incorporated label and then stored in TG9 at 0°C.

Extraction and analysis of phospholipids from φ6 and its host cell.

Preparations of ^{32}P-labelled purified φ6 and cells were dried by evacuation. Chloroform: methanol (2:1) was added to the dried viral and cellular preparations and allowed to sit overnight at 22°C. Non-solubilized material

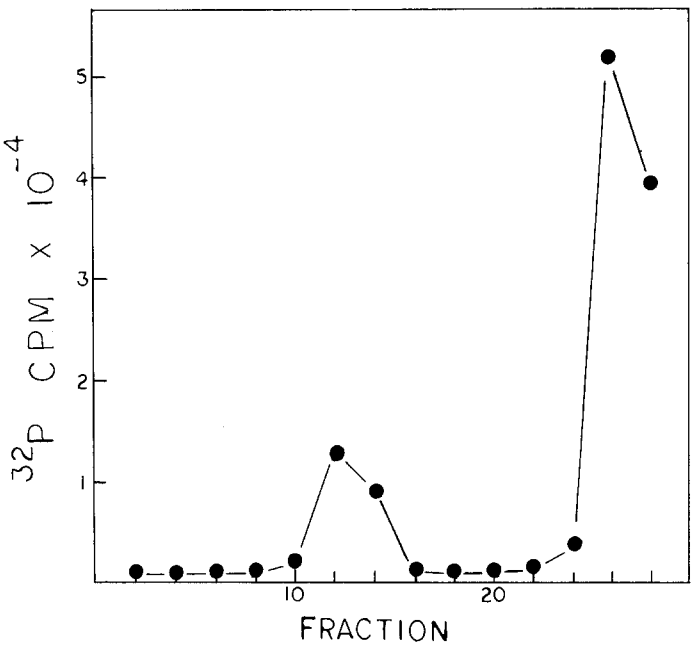

Figure 1: Purification of ^{32}P-labelled φ6 on a 15%-35% sucrose gradient in TG9 medium. Fractions of 0.4 mℓ were collected and assayed for ^{32}P radioactivity and PFU/mℓ. Fraction 12 (peak of radioactivity) contained 2 x 10^{12} PFU, while fractions 8 and 18 contained less than 1 x 10^{9} PFU.

was removed by filtration through glass wool, and the filtrate was dried by evaporation at 55°C. The dried material was solubilized in chrloroform and subjected to thin layer chromatography (500 μ silica gel) in a chloroform: methanol:water (65:25:4) solvent. The chromatography plate was then exposed to Kodak blue x-ray film for 24 hours. Following development of the film, regions on the chromatography plate containing the various [^{32}P]-phospholipids were scraped and added to methanol to solubilize the phospholipids. This solution was filtered, to remove the silica gel, into scintillation vials, dried, and then counted by liquid scintillation spectrometry.

RESULTS AND DISCUSSION

To determine the phospholipid content of bacteriophage φ6 and its host cell, Pseudomonas phaseolicola HB10Y, ^{32}P-labelled preparations of φ6 and HB10Y

Table I

Phospholipid content of bacteriophage φ6 and its host cell

	Percent by Weight[a]		
	C[b]	PE[b]	PG[b]
Host HB10Y	15	56	29
Bacteriophage φ6	8	35	57

[a]. One mole of Cardiolipin contains two moles of P, while one mole of PE and PG each contain 1 mole of P.

[b]. C = cardiolipin, PE = phosphatidylethanolamine, PG = phosphatidylglycerol.

were produced. Labelled φ6 was purified, following polyethylene glycol concentration, on a 15%-35% sucrose gradient in TG9 medium similar to the technique of Vidaver et al. (1). One such gradient profile is shown in Figure 1.

Phospholipids were extracted from virus and host cell preparations and analyzed by thin layer chromatography. Autoradiography of the thin layer chromatogram showed that there are three main phospholipids in both φ6 and its host cell. These phospholipids were identified as cardiolipin (by chromatography of commercially purchased cardiolipin followed by exposure of the thin layer plate to iodine vapors), phosphatidylethanolamine (by chromatography of ^{32}P-labelled phosphatidylethanolamine purified from Pseudomonas BAL-31(3)), and phosphatidylglycerol (by chromatography of ^{32}P-labelled phosphatidylglycerol purified from Pseudomonas BAL-31 (3)).

To quantitate the phospholipid content of φ6 and its host cell, peak regions were scraped from the chromatography plate and counted by liquid scintilla-

tion spectrometry. These results are presented as an average of four experiments in Table 1. As Table 1 shows, the phospholipid composition of φ6 is significantly different from that of its host cell. Specifically, the φ6 envelope appears to have twice the abundance of phosphatidylglycerol than does the host cell. This increased amount of phosphatidylglycerol in φ6 seems to occur at the expense of both cardiolipin and phosphatidylethanolamine.

Bacteriophage φ6 is of special interest because it is similar in several respects to enveloped mammalian viruses. Perhaps the most important of these similarities is the existence of a loose-fitting lipid-containing envelope exterior to a nucleoprotein core. I have here reported the phospholipid composition of the φ6 envelope and shown that it differs significantly from the phospholipid composition of the host cell. This suggests that φ6 does not obtain its envelope from an unaltered cellular membrane. The relative abundance of phosphatidylglycerol in bacteriophage φ6 could arise by one of several mechanisms in the infected cell, such as (i) increased synthesis of or alteration to phosphatidylglycerol, (ii) increased destruction of cardiolipin and phosphatidylethanolamine, or (iii) preferential diffusion or insertion of phosphatidylglycerol into regions of membrane which later become φ6 envelope material.

It is of interest that the only known bacteriophage other than φ6 which contains lipid as an integral component of its structure, bacteriophage PM2, also contains a greater abundance of phosphatidylglycerol than does the PM2 host cell, Pseudomonas BAL-31(3). The lipid of PM2, however, is located at an internal region of the virion (4) and is probably not acquired by a process similar to the budding processes by which many enveloped mammalian viruses acquire their membranes (5).

The mechanism by which φ6 acquires its envelope is unknown. Although φ6 has a fatty acid composition similar to that of its host cell (1), the difference in phospholipid composition between φ6 and its host cell indicates that φ6 does not acquire its envelope from an unaltered region of host cell membrane.

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