PHOSPHOLIPIDS IN AN *ESCHERICHIA COLI* BACTERIOPHAGE

Jeffrey A. SANDS and Stephen P. CADDEN
Department of Physics and Molecular Biology Program, Lehigh University, Bethlehem, Pa. 18015, USA

Received 21 July 1975

1. Introduction

Two lipid-containing bacterial viruses, PM2 [1–3] and ϕ6 [4–6] have been studied in recent years as model systems for investigating the structure and assembly of biological membranes, virus envelopes in particular. One difficulty with each of these systems is due to the host bacteria, *Pseudomonas* BAL-31 for PM2 and *Pseudomonas phaseolicola* HB10Y for ϕ6: namely, neither of these species can be manipulated genetically nearly as well as *Escherichia coli.* Recently, Bradley and Rutherford [7] reported the preliminary characterization of a bacteriophage which can infect *E. coli* cells which carry an appropriate drug-resistance plasmid. This bacteriophage, PR4, was presumed to contain lipid based on its buoyant density, its sensitivity to chloroform, and its morphology as observed by electron microscopy. In this report, we show directly that PR4 virions produced from *E. coli* do contain lipid. Specifically, we have determined the phospholipid composition of PR4 in comparison to that of the *E. coli* host cell. We find that more than half the phospholipids of PR4 are due to two species, phosphatidylserine and an unknown phospholipid, which are present in only small amounts in *E. coli* (K12) CR34.

2. Materials and methods

2.1. *Routine growth of cells and virus*

Two media were used in the experiments reported here. NT medium contains 10 g nutrient broth and 12.1 g Tris (2-amino-2-(hydroxy-methyl)-1,3-propanediol) per liter of distilled water. TGN^+ medium contains 12.1 g Tris, 1.5 g KCl, 1.5 g NaCl, 0.2 g $MgSO_4 \cdot 7H_2O$, 1 g NH_4Cl, 5 g glucose, 12 mg KH_2PO_4, 75 mg K_2HPO_4, and 20 mg each of leucine, threonine, thymidine, and nutrient broth per liter of distilled water. Both media are adjusted to pH 7.6 by addition of HCl.

Escherichia coli CR34 was routinely grown in either NT or TGN^+ medium with aeration at 37°C. Bacteriophage PR4 lysates were produced by infecting cell cultures of 10^8 cells/ml with virus at a multiplicity of infection of > 1. Following lysis, cellular debris was removed by low speed centrifugation and the resulting supernatant was stored at 4°C.

2.2. *Production and purification of labeled virus*

^{32}P-labeled PR4 virions were produced as follows. To 100 ml of NT or TGN^+ medium, about 3×10^8 *E. coli* CR34 cells and 300 μCi ^{32}P phosphate were added and the cells were grown with aeration at 37°C for 5 generations to about 10^8 cells/ml. At this time, about 5×10^{10} plaque forming units (PFU) of PR4 were added and aeration was continued. After lysis of the infected cultures, cellular debris was removed by low speed centrifugation.

^{32}P-labeled PR4 virions were purified by several rounds of ultracentrifugation. Virions were pelleted by centrifuging crude lysates at 20 000 rev/min for 2 h in an SW27 rotor (the same rotor as used for all the purification steps which follow). Virions resuspended from the pellets were layered onto 15%–30% linear sucrose gradients (in TGN^+ medium) and centrifuged at 25 000 rev/min for 75 min. Three-ml fractions were collected and assayed for radioactivity. The peak fractions were layered onto 30%–60% linear sucrose gradients (in TGN^+ medium) and centrifuged at 27 000 rev/min for 180 min. Three-ml fractions again were collected and assayed for radio-

activity. Peak fractions of the 30%–60% gradients were diluted 10-fold and centrifuged at 20 000 rev/min for 180 min to pellet the virions.

2.3. *Thin layer chromatographic analysis*

Pellets of purified ^{32}P-labeled PR4 virions and CR34 cells (uninfected aliquots removed from the main cultures just before infection and incubated until after lysis of infected cultures) were resuspended in 20 ml chloroform:methanol (2:1). Lipids were extracted by incubation at 25°C for 24 h followed by 55°C for 4 h. Subsequent steps were as previously described [3,5]. The identities of phospholipid spots were identified by chromatography of commercial standards.

2.4. *Sources of materials*

Stocks of *E. coli* CR34, a derivative of strain K12 containing the drug-resistance plasmid RP1, and bacteriophage PR4 from the laboratory of Drs Bradley and Rutherford [7] were obtained from Dr W. Snipes, Pennsylvania State University, University Park, Pa., USA. ^{32}P-phosphate (carrier free, 10 mCi/ml) was obtained from Schwarz-Mann, Orangeburg, N.Y., USA. Phospholipid standards were purchased from Sigma Chemical Co., St. Louis, Mo., USA.

3. Results

To detect and analyze the phospholipids presumed to be present in bacteriophage PR4, we produced ^{32}P-labeled PR4 stocks by prelabeling *E. coli* CR34 for 5 generations, infecting with PR4 in the continued presence of ^{32}P, harvesting the resulting phage by differential centrifugation, and then purifying the labeled virions by velocity sedimentation through sucrose gradients. Measurement of unincorporated ^{32}P in the medium in control experiments showed that less the half of the 300 μCi originally added to the cell culture had been incorporated by the time of harvesting of virus. Thus, the radioactivity determinations which follow are a valid indication of total phospholipid content of cells and virus.

Centrifugation profiles of PR4 are shown in fig.1. Virions were centrifuged in a 15%–30% linear sucrose gradient. Virus particles from the peak fraction collected from this gradient were then further purified by centrifugation in a 30%–60% linear sucrose gradient. All analyses reported here were conducted on virus samples which had been 'doubly' purified in this manner.

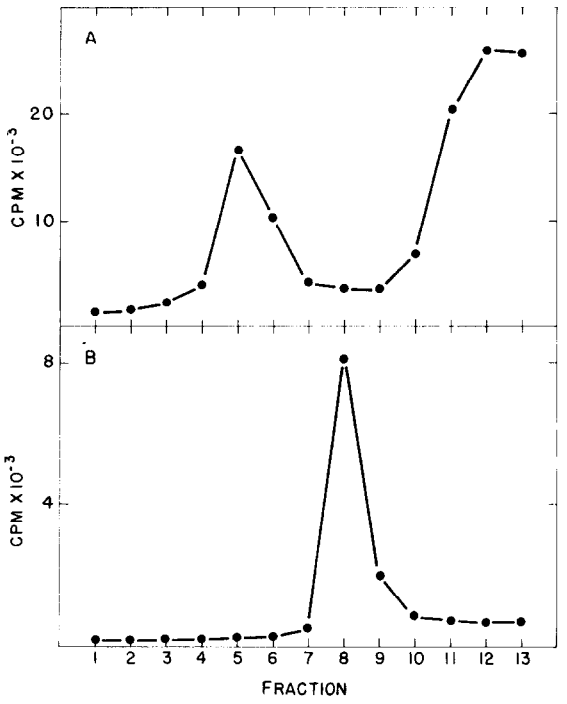

Fig.1. Purification of ^{32}P-labeled PR4 virions. (A) 15%–30% sucrose gradient sedimentation of virions resuspended from a pellet. (B) 30–60% sucrose gradient sedimentation of the peak fraction from the 15%–30% gradient. The peak fraction from the second gradient routinely contained greater than 10^{11} plaque forming units per ml. See Materials and methods for details.

Lipids were extracted from ^{32}P-labeled *E. coli* CR34 cells and doubly purified PR4 virions which had been produced in cells of the same strain. Phospholipid analysis was carried out by thin layer chromatography. To determine the identity of the various phospholipids detected, co-chromatography of phospholipid standards was employed. The relative mobilities of various standard phospholipids are given in table 1 along with the mobilities of the phospholipids extracted from *E. coli* CR34. Phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and cardiolipin (C) account for about 90% of the cellular phospholipid. In addition, small amounts

Table 1

Chromatographic mobilities of phospholipids[a]

Phospholipid	Commercial standards %	*E. coli* CR34
Phosphatidic acid (PA)	124[c]	
Phosphatidylethanolamine (PE)	100	100
Phosphatidylglycerol (PG)	85	87
Cardiolipin (C)	80	82
Phosphatidylserine (PS)	62	61.5
PX[b]		25

[a] Silica gel plates. Chloroform:methanol:water (65:25:4) solvent.
[b] PX: unknown phosphorous-containing lipid.
[c] Mobilities measured relative to PE.

(see below) of phosphatidylserine (PS) and an unknown phospholipid (PX) are present in this cell. This unknown phospholipid might be phosphatidylglycerol-phosphate [8] or CDP-diglyceride [9].

^{32}P-labeled lipids of *E. coli* CR34 and bacteriophage PR4 separated by thin layer chromatography and detected by autoradiography were scraped from the TLC plates and counted by liquid scintillation spectrometry to determine the quantitative phospholipid composition of the virus and host cell. The results are shown in table 2. Bacteriophage PR4 contains PE, PG, C, PS, and PX, as does *E. coli* CR34. Virions produced in either growth medium, NT or TGN$^+$, contain large amounts of PS and especially PX; specifically, these two lipids account for more than 50% of the lipid phosphorous in the virus.

As also shown in table 2, the phospholipid composition of *E. coli* CR34 depends on the growth medium, and this difference is reflected somewhat in the PR4 virions.

Table 2

Phospholipids of bacteriophage PR4 and it *E. coli* host

Organism	Growth media	PE	PG	C	PS	PX
E. coli CR34	NT	57[a]	30	7	3	3
	TGN$^+$	37	43	7	10	3
Bacteriophage PR4	NT	21	20	6	20	33
	TGN$^+$	16	23	7	15	39

[a] All values given as per cent of total. Estimated accuracy is ± 3%.

4. Discussion

The data presented in this report show that bacteriophage PR4, which can infect *E. coli,* does indeed contain lipid. In addition, we have shown that PR4 has a phospholipid composition that is markedly different quantitatively from that of its *E. coli* host cell.

The lipid-containing bacteriophage PM2 and ϕ6 also have phospholipid compositions different from that of their host cells [5,10]. These phages have been shown to alter cellular phospholipid synthesis during infection for the apparent purpose of facilitating virus assembly ([3], unpublished data from this laboratory). It will not be surprising if a somewhat similar process occurs in PR4-infected *E. coli* cells.

The main importance of the existence of bacteriophage PR4 is that it is the only known lipid-containing bacterial virus which can productively infect *E. coli*. Many membrane related mutants of *E. coli* exist which could greatly facilitate an investigation of the molecular membrane assembly process of PR4. As an example, we mention the recent isolation of a mutant of *E. coli* which is temperature sensitive for the final step in the synthesis of phosphatidylethanolamine (PS → PE) [11]. This and other mutants could become hosts for bacteriophage PR4 by receiving the drug resistance plasmid from strain CR34.

Acknowledgements

This work was supported by grant BMS 74-05587 from the U.S. National Science Foundation.

References

[1] Espejo, R. T. and Canejo, E. S. (1968) Virology 34, 738.
[2] Harrison, S. C., Casper, D. L. D., Camerini-Otero, R. D. and Franklin, R. M. (1971) Nat. New Biol. 229, 197.
[3] Snipes, W., Douthwright, J., Sands, J. and Keith, A. (1974) Biochim. Biophys. Acta 363, 340.
[4] Vidaver, A. K., Koski, R. K. and Van Etten, J. L. (1973) J. Virol. 11, 799.
[5] Sands, J. (1973) Biochem. Biophys. Res. Commun. 55, 111.
[6] Sands, J., Cupp, J., Keith, A. and Snipes, W. (1974) Biochim. Biophys. Acta 373, 277.
[7] Bradley, D. E. and Rutherford, E. L. (1975) Can. J. Microbiol. 21, 152.
[8] Randle, C. L., Albro, P. W. and Dittmer, J. C. (1969) Biochim. Biophys. Acta 187, 214.
[9] Raetz, C. R. H. (1975) Proc. Nat. Acad. Sci. USA 72, 2274.
[10] Camerini-Otero, R. D. and Franklin, R. M. (1972) Virology 49, 385.
[11] Hawrot, E. and Kennedy, E. P. (1975) Prod. Nat. Acad. Sci. USA 72, 1112.