Available online at www.sciencedirect.com
ScienceDirect
Biochimica et Biophysica Acta 1768 (2007) 2568–2577
Biochimica et Biophysica Acta BBA
www.elsevier.com/locate/bbamem

Transbilayer distribution of phospholipids in bacteriophage membranes

Simonas Laurinavičius [a,b], Dennis H. Bamford [b], Pentti Somerharju [a,*]

[a] *Department of Biochemistry, Institute of Biomedicine, University of Helsinki, Biomedicum Helsinki (Haartmaninkatu 8), Room C205, PO Box 63, 00014 Helsinki, Finland*
[b] *Institute of Biotechnology and Department of Biological and Environmental Sciences, Viikki Biocenter, University of Helsinki, 00014 Helsinki, Finland*

Received 20 March 2007; received in revised form 31 May 2007; accepted 12 June 2007
Available online 19 June 2007

Abstract

We have previously demonstrated that the membranes of several bacteriophages contain more phosphatidylglycerol (PG) and less phosphatidylethanolamine (PE) than the host membrane from where they are derived. Here, we determined the transbilayer distribution of PG and PE in the membranes of bacteriophages PM2, PRD1, Bam35 and phi6 using selective modification of PG and PE in the outer membrane leaflet with sodium periodate or trinitrobenzene sulfonic acid, respectively. In phi6, the transbilayer distributions of PG, PE and cardiolipin could also be analyzed by selective hydrolysis of the lipids in the outer leaflet by phospholipase A_2. We used electrospray ionization mass-spectrometry to determine the transbilayer distribution of phospholipid classes and individual molecular species. In each bacteriophage, PG was enriched in the outer membrane leaflet and PE in the inner one (except for Bam35). Only modest differences in the transbilayer distribution between different molecular species were observed. The effective shape and charge of the phospholipid molecules and lipid–protein interactions are likely to be most important factors driving the asymmetric distribution of phospholipids in the phage membranes. The results of this first systematic study on the phospholipid distribution in bacteriophage membranes will be very helpful when interpreting the accumulating high-resolution data on these organisms.

Keywords: Transbilayer phospholipid distribution; Membrane asymmetry; Bacteriophage; Molecular species; Effective shape; Lipid–protein interaction

1. Introduction

Bacteriophages have proven to be valuable models when studying various aspects of molecular biology such as genome replication and virion architecture and assembly [1,2]. Some phages contain a lipid membrane which plays an important role at certain stages of the viral life-cycle. For instance, bacteriophage PRD1, which infects broad range of Gram-negative bacteria [3], forms a membranous tube which penetrates the host cell envelope and fuses with the host cytoplasmic membrane [4], thus priming the delivery of the viral genome to the host cytoplasm. However, many aspects of phage membrane biology are poorly understood. For example, it is not known (i) how the viral membrane is assembled inside the host, (ii) what determines the lipid composition of the phage membrane and (iii) what role does the membrane lipid composition plays in the assembly of phage particles or in the infection process.

We have previously determined in detail the lipid class and molecular species compositions of three bacteriophages, i.e. PRD1, Bam35, phi6 [5,6] and PM2 ([7,8]; Supplementary information of this study). They all were found to contain more PG and less PE than the respective host membranes (Table 1). The mechanisms underlying such enrichment of PG (or depletion of PE) in the phage membrane have not been established, but there are several possible explanations. For instance, the phage could obtain its lipids from putative PG-rich/PE-poor domains of host membrane [9]. Another possibility is that phage proteins might selectively attract negatively charged PG molecules. Yet, particular physicochemical properties of phospholipids, such as the "effective" molecular shape [10,11] could drive selective incorporation of

* Corresponding author. Tel.: +358 9 191 25410; fax: +358 9 191 25444.
E-mail address: pentti.somerharju@helsinki.fi (P. Somerharju).

0005-2736/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2007.06.009

Table 1
Phospholipid compositions of lipid-containing bacteriophages and the cytoplasmic membrane of their host bacteria

Phage/host	Phospholipid (mol%±SD)[a]			Reference
	PE[b]	PG	CL	
PM2	36.2±4.2	63.8±4.2	n.d.[c]	[7,8][d]
Pseudoalteromonas sp. ER72M2	75.2±1.7	24.8±1.7	n.d.	[7,8][d]
PRD1	51.2±2.2	43.4±2.4	5.4±0.2	[6]
Salmonella enterica DS88	78.8±3.0	16.2±4.2	5.0±1.2	[6]
Bam35	29.9±7.0	53.6±5.2	16.5±2.0	[6]
Bacillus thuringiensis HER1410	57.9±2.5	29.7±3.3	12.4±1.6	[6]
Phi6	57.4±1.6	38.2±3.1	4.4±2.4	[5]
Pseudomonas syringae HB10Y	80.8±3.4	16.1±2.9	3.1±0.8	[5]

[a] Compositions are expressed as mol% of the total phospholipids excluding trace amounts of phosphatidic acid and acyl-phosphatidylglycerol that are found in PM2 and its host (see supplementary material) and in *Salmonella enterica*.
[b] PE—phosphatidylethanolamine, PG—phosphatidylglycerol, CL—cardiolipin.
[c] Not detected.
[d] Also this study (see supplementary material).

host lipids to the highly curved phage membrane [5,6]. Elucidation of the phospholipid transbilayer distributions could help to discriminate between the various models for PG enrichment in bacteriophage membranes. This information is also needed to determine the phospholipid compositions of individual membrane leaflets, which are necessary when interpreting the diffraction data aiming to establish the complete structures of bacteriophages. For instance, in a recent X-ray study on bacteriophage PRD1 it was found that the outer leaflet contains more electrons than the inner one, which possibly indicates asymmetric transbilayer distribution of phospholipids therein [12]. The lipid head groups, however, could not be positively identified due to their mobility/disorder; thus more direct information on the phospholipid compositions of the inner and outer leaflets in PRD1 membrane is needed to confirm this.

Bacteriophage PM2 infects the Gram-negative *Pseudoalteromonas espejiana* and is structurally somewhat similar to PRD1 despite the differences in genome organization [13,14]. The transbilayer distribution of phospholipids in the membrane of PM2 has been addressed previously [8,15], but the results were not conclusive.

Bacteriophage Bam35, infecting the Gram-positive bacterium *Bacillus thuringiensis*, is structurally very similar to PRD1 [16], although there is very little sequence similarity [17]. There are major differences between these phages in the lipid compositions [6] and bilayer curvature profiles, which affects the organization of membrane proteins [16]. The transbilayer distribution of phospholipids in the Bam35 membrane has not been studied previously.

No such data exist also for bacteriophage phi6 infecting Gram-negative *Pseudomonas syringae.* This phage contains an external lipid envelope [18] and thus differs from the other phages discussed above, which have an internal membrane. In this respect, phi6 is also unique among bacteriophages, but similar to enveloped animal viruses, such as *Coronaviridae*, *Retroviridae*, *Orthomyxoviridae* (www.ncbi.nlm.nih.gov/ICTVdb/).

Here, we carried out a detailed study on the transbilayer distribution of phospholipids in the membranes of bacteriophages PM2, PRD1, Bam35 and phi6 using chemical reagents that have been commonly employed to determine the transbilayer distribution of phospholipids [19]. In case of phi6, we also could use phospholipase A_2 digestion to assess the transbilayer distribution of PG, PE as well as cardiolipin. The progress of the reactions was monitored with electrospray ionization mass spectrometry (ESI-MS), which allows sensitive and facile determination of the transbilayer distribution of phospholipid classes and individual molecular species as well.

We find that both the PG and PE classes distribute asymmetrically over the membranes of all bacteriophages studied. PG is always enriched in the outer membrane leaflet, while PE is enriched in the inner one in all but one phage. Modest differences in the transbilayer distribution were also observed for some PE molecular species in PRD1 and some PG species in Bam35 and other phages.

2. Materials and methods

2.1. Chemicals

Tetra-14:0 and tetra-18:1 cardiolipin (CL) and di-14:1, di-20:1 and di-22:1 phosphatidylcholine (PC) were purchased from Avanti Polar Lipids. Di-14:1, di-20:1 and di-22:1 phosphatidylethanolamines (PE), di-14:1, di-20:1 and di-22:1 phosphatidylglycerols (PG) were synthesized from the corresponding PC species using phospholipase D-mediated transphosphatidylation [20]. Trinitrobenzene sulfonic acid (TNBS), glycylglycine, phospholipase D from *Streptomyces* species, bee venom phospholipase A_2, Tris–base and Tris–HCl were obtained from Sigma. Sodium periodate was from Aldrich and sodium thiosulfate from Serva. Lysozyme was obtained from Boehringer Mannheim and sucrose from BDH. Ammonia, acetic acid, sulfuric acid, citric acid, sodium chloride, ammonium sulfate and hydrochloric acid were from Riedel-de Häen. Other chemicals were from Merck. All chemicals were of analytical grade. Solvents (Merck) were either of analytical (lipid extraction) or HPLC (mass-spectrometry) grade.

2.2. Growth and purification bacteriophages

PM2 was propagated in *Pseudoalteromonas* sp. strain ER72M2 grown at 28 °C in SB medium and purified as described previously [21]. The final purification was accomplished by equilibrium centrifugation in CsCl gradients [22]. The phage was collected by centrifugation and resuspended in PM2 buffer (100 mM NaCl, 5 mM $CaCl_2$, 100 mM MOPS, pH 7.2) or in 200 mM Na-borate buffer (pH 8.5).

Agar stocks of wild-type PRD1 were prepared on *Salmonella enterica sv.* typhimurium DS88 and those of the *sus1* mutant (deficient in genome packaging, [23]) on a *S. enterica* suppressor strain PSA. Wild-type PRD1 and the *sus1* mutant were propagated in DS88 grown in LB medium at 37 °C and purified by rate-zonal and equilibrium centrifugations [24], collected by centrifugation and suspended in 200 mM Na-phosphate buffer (pH 7.4 or 8.5).

A clear plaque derivative of phage Bam35 was propagated in *B. thuringiensis* HER1410 grown in LB medium at 37 °C, purified by rate zonal centrifugation, ammonium sulfate precipitation and equilibrium centrifugation in CsCl gradients [17]. Two light scattering bands representing mature and DNA-less Bam35 particles were aspirated into separate tubes, collected by centrifugation and suspended in 200 mM K-phosphate buffer (pH 8.5).

Phi6 phage was propagated in *P. syringae* HB10Y grown in LB medium at 28 °C and was purified by rate zonal and equilibrium centrifugations essentially as described previously [24]. The phage was concentrated either by differential centrifugation or by filtration through Amicon Ultra (Millipore, cut off 100 kDa) concentrator filters and suspended in either 200 mM

Na-phosphate (pH 8.5) or 200 mM Tris buffer (pH 7.4 or 8.0) and kept on ice. The transbilayer distribution of phospholipids was always determined within 6 h after purification.

2.3. Determination of the transbilayer distribution of phospholipids

Phosphatidylglycerol (PG) in the outer leaflet was selectively oxidized with sodium meta-periodate (5 mg/ml in H_20 or in 200 mM Na-borate buffer, pH 8.0) [25]. The reactions were performed at room temperature (RT; 23–24 °C) or on ice. The reaction was stopped by mixing 200 μl aliquot of the reaction mixture with 50 μl 60% sodium thiosulfate and incubating for at least 5 min on ice and then kept at −20 °C until extracted. Phosphatidylethanolamine (PE) in the outer leaflet was selectively derivatized with 5 mM (final concentration) trinitrobenzene sulfonic acid (TNBS) in the same buffer in which the virus was suspended [26] at RT, unless stated otherwise. Aliquots of 200 μl were taken from the reaction mixture at time intervals, mixed with 250 μl of ice-cold quenching buffer (0.5 M glycylglycine, 0.1 M citric acid, pH 5.0) and kept on ice or at −20 °C until extracted. Bee venom phospholipase A_2 (PLA_2) was used to selectively digest the outer leaflet phospholipids of bacteriophage phi6 membrane. Approximately 1–5 ng PLA_2 in 0.1 M Tris–HCl, 2 mM $CaCl_2$ buffer (pH 7.4) was added to virus corresponding to 100 nmol of lipid and the mixture was incubated at RT. Samples were taken at time intervals and mixed with equal volume of quenching-solution (5 mM EDTA, 50 mM HCl), incubated on ice for at least 5 min and kept at −20 °C until extracted.

To determine the extent of phospholipid modification under conditions where both membrane leaflets are accessible to the reagents, during the reaction the viral particles were disrupted by several pulses of sonication (tip sonicator, 1–2 min pulses of sonication followed by 3–5 min pause). During these experiments the samples were kept on ice.

2.4. Lipid analysis

Lipids were extracted according to Folch et al. [27]. To avoid nonspecific association of negatively charged lipids with glass surface when analyzing distribution of PG, silanized Kimax tubes were used and 0.1 M HCl was included at a single-phase state. After addition of water and mixing, the upper phase was removed, and the lower phase was washed twice with the theoretical upper phase. The final lower phase was spiked with di-14:1-, di-20:1-, and di-22:1-PE and PG internal standards and the solvent was evaporated under a nitrogen stream. Lipids were dissolved in 100–200 μl of chloroform/methanol/25%NH_3 (25:50:3; v/v) and analyzed immediately.

The ESI-MS system consisted of an Ultimate pump (LC Packings), a Famos autosampler (LC Packings) and a Quattro Micro mass-spectrometer (Micromass, Manchester, UK). Autosampler injected 10 μl of each sample into the continuous flow of chloroform/methanol/25%NH_3 (25:50:3; v/v) which was lead to the mass-spectrometer operated in the negative mode with essentially the same settings as previously [28]. When necessary, scans of neutral loss of 141 *m/z* in positive ion mode were performed to selectively detect and quantify PE molecular species.

2.5. Data analysis

The MS spectra were converted to peak lists, which was copied to Microsoft Excel, and the phospholipid molecular species were quantified using the LIMSA software tool developed in the laboratory [29].

3. Results

3.1. Transbilayer distribution of phospholipid classes in different bacteriophages

The transbilayer distribution of PG determined by incubating freshly isolated phage particles in the presence of sodium periodate. Periodate preferentially oxidizes the non-acylated glycerol moiety of the PG molecules located in the outer leaflet [25,30]. However, since also the molecules in the inner leaflet are slowly oxidized due to penetration of periodate through the membrane [19,25], it was necessary to determine the time-course of PG oxidation to reliably distinguish the rapidly (outer) and slowly (inner) reacting PG pools. Extrapolation of a linear fit to the data representing the slower phase of oxidation to time zero gives the fraction of PG in the outer leaflet [31]. The transbilayer distribution of PE was determined analogously using trinitrobenzene sulfonic acid (TNBS), which reacts with the amino group of PE, as the modifying reagent [8,32,33]. Since TNBS can also penetrate the bilayer [19], the fraction of PE labeling in the outer leaflet was determined from the time course plot as described above for PG. Incomplete reaction of PE with TNBS, possibly due steric crowding, has been reported for PE monolayers [34]. We therefore also carried out the modification while sonicating the samples. A virtually complete reaction was observed under such conditions, thus indicating that all PE molecules are accessible to the reagent. The same was found true for PG as well (see below).

3.2. PRD1

Upon incubation of bacteriophage PRD1 with sodium periodate at RT, 56±3% of PG was rapidly oxidized and was thus assigned to the outer leaflet (Fig. 1A, open circles). The size of this rapidly reacting pool did not depend on the pH (pH 7.4 vs. 8.5) or the concentration of sodium periodate (not shown). When the membrane was disrupted by sonication during the reaction, only a single and rapid phase of oxidation was observed, virtually all of PG (>97%) being oxidized in less than 30 min (Fig. 1A, diamonds).

We also studied the PG transbilayer distribution in the *sus1* mutant of PRD1, which lacks the packaging ATPase P9 and is thus devoid of DNA. Oxidation of PG in *sus1* particles at RT was very rapid and extensive (Fig. 1B, open circles), indicating that also the inner leaflet was readily accessible to the probe, possibly via the open portal vertexes [35]. When the reaction was carried out on ice, two pools could be readily distinguished and 52±3% PG was assigned to the rapidly reacting, outer leaflet pool (Fig. 1B, full circles). Notably, the slower phase was not due to depletion of periodate, because nearly all PG was oxidized during an overnight (720 min) incubation. Accordingly, the transbilayer distribution of PG in *sus1* particles is very similar to that in the *wt* phage, thus indicating that DNA does not influence the transbilayer distribution of PG in the intact phage.

When PRD1 particles were incubated with TNBS at RT, 36±4% of PE was found in the rapidly reacting pool and was thus assigned to the outer leaflet (Fig. 1C, open circles). This value is consistent with that obtained for bacteriophage PR4, a close relative of PRD1, in which less than half of PE was found in the outer leaflet [36]. When PRD1 particles were sonicated during the reaction, rapid and complete (>97%) modification of PE was observed (Fig. 1C, diamonds). In DNA-less PRD1 particles (*sus1* mutant) the transbilayer distribution of PE was

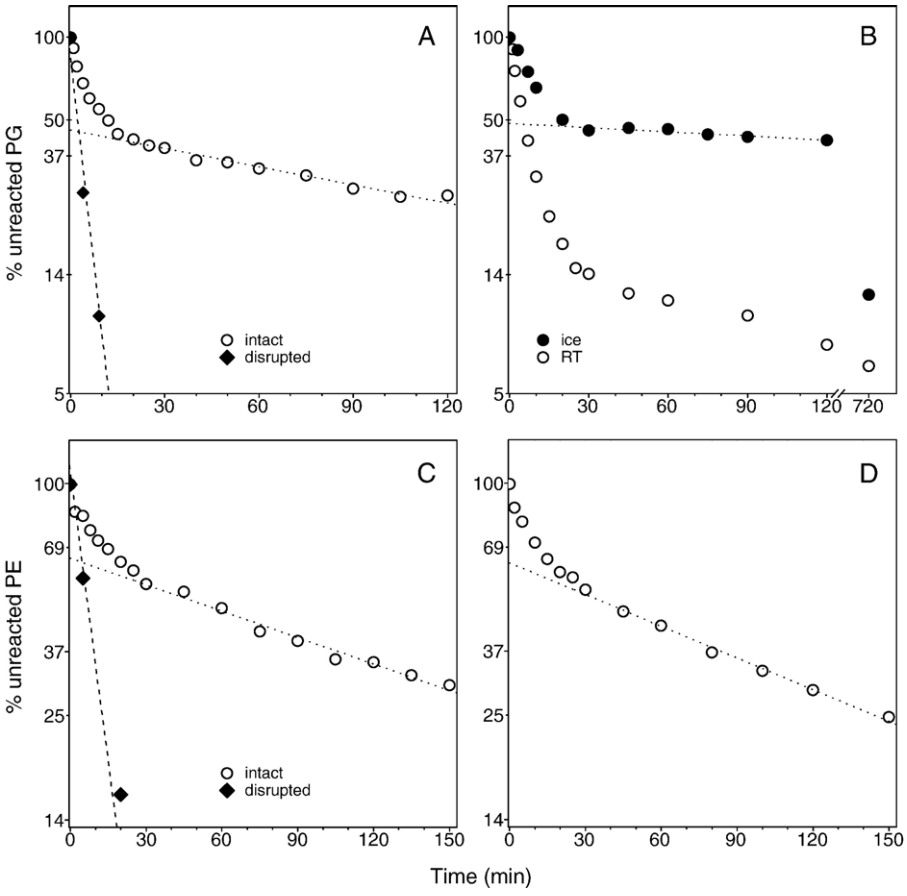

Fig. 1. Time-course plots for modification of PG and PE in bacteriophage PRD1 and the *sus1* mutant. Freshly prepared PRD1 or *sus1* particles were incubated with sodium periodate or with trinitrobenzene sulfonic acid (TNBS) at RT or on ice. Aliquots of the reaction mixture were removed, mixed with a quenching solution and the lipids were extracted and analyzed as described in Materials and methods. (A) Oxidation of phosphatidylglycerol (PG) with sodium periodate in intact phage (open circles; $n=2$) at RT or in phage disrupted by sonication (diamonds; $n=1$). The last data point of PG oxidation in sonicated particles is below the *X*-axis ($Y=2.9\%$). (B) Oxidation of PG with sodium periodate in the *sus1* particles at RT (open circles, $n=3$) or on ice (solid circles). The latter plot represents a single experiment, but essentially the same PG distribution in *sus1* particles was found in another, similar experiment. Note the break in the *X*-axis of panel B. (C) Modification of phosphatidylethanolamine (PE) by TNBS in intact (open circles; $n=2$) or sonication-disrupted (diamonds; $n=1$) PRD1 particles at RT. The last data point for PE modification in sonicated particles is below the *X*-axis ($Y=2.7\%$) (D) Modification of PE by TNBS in the *sus1* mutant ($n=3$) at RT. The dotted lines are the linear fits to the data points of the slower phase of reaction, and its intercept with the *Y*-axis indicates the fraction of the lipid in the inner membrane leaflet. The dashed line is a linear fit to the data for disrupted PRD1 particles. Note that the *Y*-axis has a logarithmic scale in each panel.

identical to that in *wt* particles (Fig. 1D), thus indicating that the DNA has no influence on PE distribution either.

3.3. PM2

In the case of bacteriophage PM2, it was difficult to reliably distinguish the fast and slow phases of oxidation at RT (not shown), probably because of a rapid penetration of periodate through the membrane. However, when the reaction was carried out on ice, the two phases could be readily distinguished (Fig. 2A). Under these conditions 59±2% of PG reacted rapidly, thus indicating that a corresponding fraction of this lipid is in the outer leaflet of the PM2 membrane. When PM2 particles were incubated with TNBS at RT to assess the transbilayer distribution of PE, it was again difficult to reliably distinguish the slower phase of the reaction (Fig. 2B, open circles). However, when the reaction was carried out on ice, two phases could be distinguished and 40±2% PE was assigned to the rapidly reacting pool and thus to the outer leaflet (Fig. 2B, full circles).

These data on PG and PE transbilayer distribution differ significantly from those obtained in two earlier studies for PM2 [8,15]. The reasons for this are not clear, but we note that one of those earlier studies employed sulfanilic acid diazonium salt, a reagent rarely used to assess phospholipid distribution, and reaction kinetics were also not presented [15]. In the other previous study [8], the transbilayer distribution of PE was not determined for intact particles, but for membrane vesicles formed in the host cells infected with a temperature sensitive mutant of PM2. Distribution of PG was not studied.

3.4. Bam35

In case of the bacteriophage Bam35, 57±2% of PG and 54±3% of PE were found in the rapidly reacting pool and thus probably in the outer leaflet of the membrane (Fig. 3).

Similar results were obtained with DNA-less Bam35 particles (data not shown).

3.5. Phi6

In phi6 particles 52±2% of PG was assigned to the rapidly oxidized pool (Fig. 4A), while 35±3% of PE reacted rapidly with TNBS (Fig. 4C), suggesting that the corresponding fractions of these lipids reside in the outer leaflet. Since the lipid envelope is the outermost layer in phi6, we could also use phospholipase A_2 (PLA_2) to assess the fraction of phospholipids in the outer leaflet. PLA_2 hydrolyzed 53±3% of PG (Fig. 4B) and 34±3% of PE (Fig. 4D), in a close agreement with the data obtained with the chemical reagents (Fig. 4A and B). Very similar results were obtained with phi6 particles, from which the membrane protein spikes had been removed by either treating with butylated hydroxytoluene [24] or with proteinase K digestion (not shown), thus indicating that surface proteins do not limit the hydrolysis of phospholipids by PLA_2. Cardiolipin, which constitutes approximately 5% of total phospholipids of phi6, was also digested by PLA_2. Half (50±2%) of CL was accessible to the enzyme (not shown), indicating that the corresponding fraction is in the outer leaflet of the phi6 membrane.

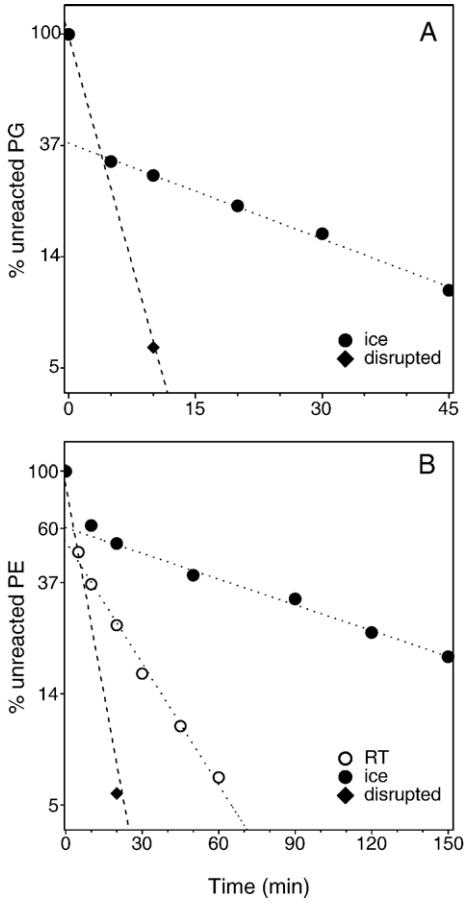

Fig. 2. Time-course plots for modification of PG and PE in bacteriophage PM2. The experiments were carried out as described in the legend of Fig. 1 and in Materials and methods. (A) Oxidation of PG in intact particles by sodium periodate on ice (solid circles). To obtain similar reaction times as at RT, higher concentration (20 mg/ml) of sodium periodate was used. A similar distribution of PG was found also in two other similar experiments. (B) Modification of PE by TNBS at RT (open circles; $n=2$) or on ice (solid circles; $n=2$). Virtually all of PG (>98%) and PE (>99%) was rapidly modified in PM2 particles sonicated during the reaction (Panels A and B, diamonds). The last data points for modification of sonicated particles are below the *X*-axes in both panels. See the legend of Fig. 1 for other details.

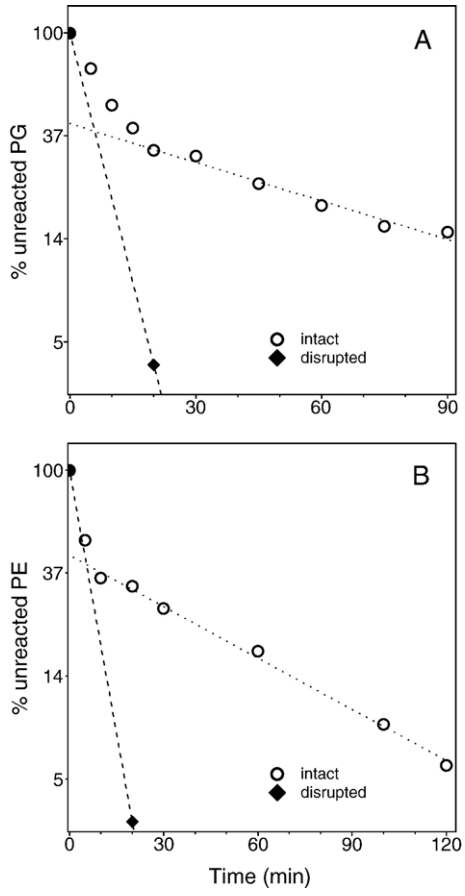

Fig. 3. Time-course plots for modification of PG by periodate and PE by TNBS in bacteriophage Bam35 at RT. (A) PG. (B) PE. Data points represent averages of three independent experiments. Dotted lines represent linear fits to the data points of the slow reaction phase. More than 96% PG and 98% PE was rapidly modified in sonicated Bam35 particles during 20 min (Panels A and B, diamonds). See the legend of Fig. 1 for other details.

The transbilayer distribution of phospholipid classes found for the different bacteriophages are summarized in Fig. 5. The phospholipid compositions of the inner and outer membrane leaflets, calculated from the asymmetry data and the overall lipid compositions ([5,6]; and Supplementary material), are given in Table 2.

3.6. Transbilayer distribution of individual molecular species

Since ESI-MS allows direct quantification of the individual PG and PE molecular species [5,6], their transbilayer distributions could also be assessed (cf. Fig. 6). In most cases, only minor or no differences between the different species within a lipid class were observed (Supplementary Fig. 2). In few cases, however, significant differences were detected. In PRD1, the 32:2 and 33:2 PE species (Fig. 6, lower panel) as well as the

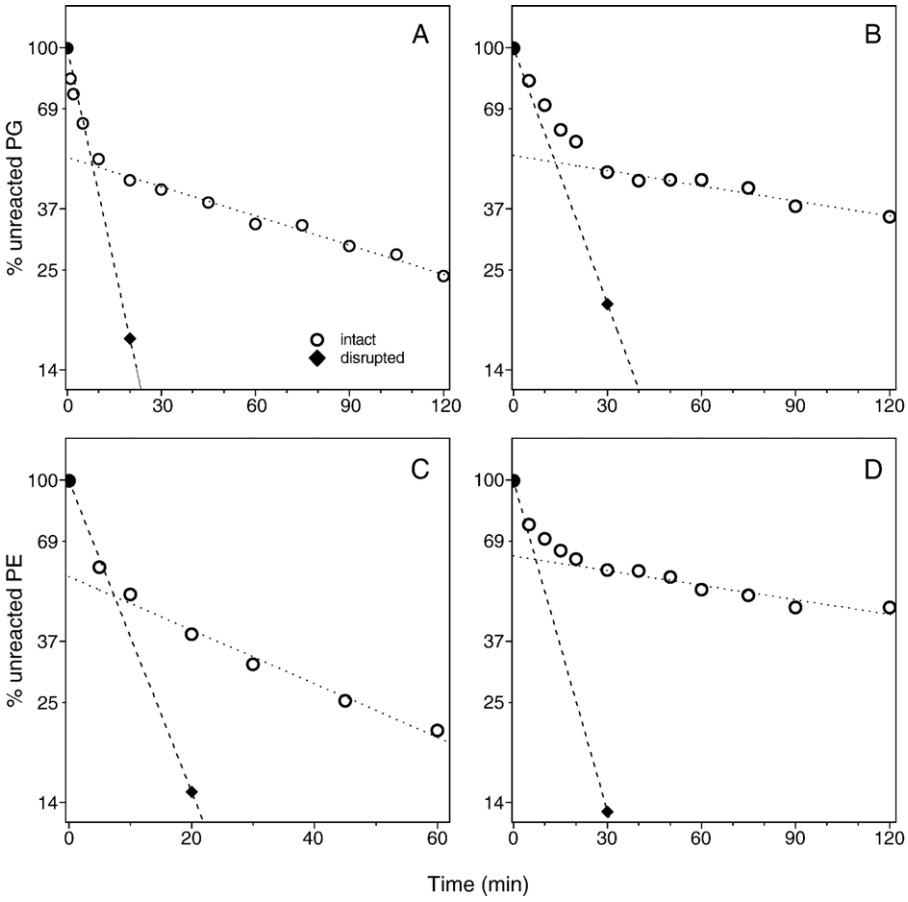

Fig. 4. Time-course plots for modification of PG and PE in bacteriophage phi6. PG and PE in phi6 were modified by sodium periodate and TNBS, respectively, or by enzymatic hydrolysis using phospholipase A_2 as described in Materials and methods. (A) Oxidation of PG by sodium periodate on ice ($n=3$). (B) Hydrolysis of PG by PLA_2 at RT ($n=2$). (C) Modification of PE by TNBS at RT ($n=3$). (D) Hydrolysis of PE by PLA_2 at RT ($n=2$). Upon disruption of phage particles by sonication, almost all of PG and PE was modified rapidly (A–D, diamonds). See the legend of Fig. 1 for other details.

30:1, 31:1, 32:2 and 33:2 PG species (Supplement, Fig. 2) were more abundant in the inner leaflet than PG or PE as a class, respectively. On the other hand, the 32:0, 35:1, 35:2 and 36:2 PE species were slightly more abundant in the outer leaflet relative to the PE class (Fig. 6 and Supplementary Fig. 2).

In Bam35, unsaturated PG species tended to be more abundant in the inner leaflet than saturated PGs with same total acyl chain length (29:0 vs. 29:1, 30:0 vs. 30:1, etc.). The shortest saturated PG species, i.e., 27:0 was more abundant in the inner leaflet than PG on the average (Fig. 6 and Supplementary Fig. 2). Minor acyl-chain saturation-dependent differences in transbilayer distribution of PE species might also exist in case of PM2 and PRD1, as well in that of PG species in phi6 (Supplementary Fig. 2).

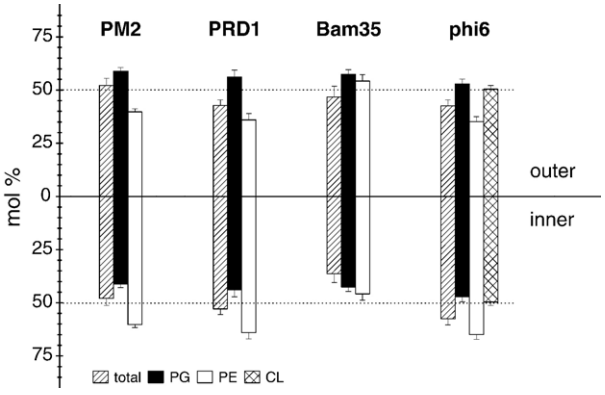

Fig. 5. Summary of the transbilayer distributions of phospholipids in the bacteriophages studied. Bars represent the distribution of total phospholipids (total, striped bars), phosphatidylglycerol (PG, black bars), phosphatidylethanolamine (PE, white bars) and cardiolipin (CL, cross-striped bars) between the inner and outer leaflets. The molar percent of total phospholipids is calculated from the asymmetry data and phospholipid compositions determined previously [5,6], and includes cardiolipin only in case of phi6; thus they do not sum up to 100% in case of PRD1 and Bam35. PM2 contains trace amounts of acyl-PG which is not included in the calculation. The distributions of the individual phospholipids are expressed as the mol% within the class. Error bars represent standard deviations ($n=3$–6 for PG and PE, and 2 for CL of phi6).

4. Discussion

4.1. Transbilayer phospholipid distribution

The data obtained here shows that PG is enriched in the outer membrane leaflet in all four bacteriophages studied, while PE is enriched in the inner leaflet of all except Bam35, which, unlike the others, infects a Gram-positive host (Fig. 5). The transbilayer distribution of cardiolipin could only be

Table 2
Phospholipid compositions[a] of the inner and the outer membrane leaflets of lipid-containing bacteriophages

Phage	Inner leaflet			Outer leaflet		
	PE	PG	CL[b]	PE	PG	CL
PM2	45.2±5.6	54.8±4.3	n.d.[c]	27.9±3.7	72.1±5.1	n.d.
PRD1	60.2±4.5	34.8±3.2	5.0±0.4	40.2±4.8	53.8±4.3	6.0±0.5
Bam35	30.8±7.4	50.8±5.5	18.4±2.1	29.5±7.1	55.5±5.7	15.0±1.7
phi6	64.9±3.3	31.4±3.3	3.8±2.1	47.4±4.0	47.4±4.4	5.2±2.8

[a] Compositions expressed as mol% of the total phospholipids in a given leaflet; ±SD. Standard deviations appear large, because they are derived from standard deviations of phospholipid compositions and asymmetry data.
[b] The proportions of CL in the leaflets of PRD1 and Bam35 were calculated assuming that it is equally distributed between the leaflets.
[c] Not detected.

determined for phi6, since the external localization of its membrane allowed us to use phospholipase A_2. Accordingly, the transbilayer distribution of total phospholipids could be accurately determined only for phi6 as well as for PM2, which lacks cardiolipin and contains only trace amounts of other lipids ([37,38], Supplement).

In the membrane of PM2, there was slightly more phospholipid in the outer than the inner leaflet, while on case of phi6 and PRD1 the opposite was true. However, in each case the fraction of phospholipid in the outer leaflet was significantly less than that calculated based on the relative surface areas of the outer and inner leaflets (calculated assuming that the thickness of the bilayer is 5 nm, and that the viral bilayer vesicle is approximately spherical, and its diameter is equal to the average of vertex-to-vertex and facet-to-facet distances of the icosahedral particle), i.e., 0.52 vs. 0.62 for PM2; 0.47 vs. 0.61 for PRD1; 0.55 vs. 0.61 for Bam35 and 0.42 vs. 0.57 for phi6 (we assume that CL is symmetrically distributed over the membranes of PRD1 and Bam35). This area discrepancy can be accounted only to a minor degree by the enrichment of PG, which occupies a larger surface area than PE (59 and 49 $Å^2$, respectively [39,40]) to the outer leaflet. The rest of the area "lacking" in the outer leaflet is likely to be occupied by protein (see below).

The transbilayer distributions of the individual PG and PE molecular species were generally similar to that of the respective phospholipid class, albeit modest deviations were observed in some cases (Fig. 6 and Supplementary Fig. 2). This implies that the polar head group is a more significant factor than the acyl chains in determining the transbilayer distribution of phospholipids. The putative tendency of the more unsaturated molecular species to be more abundant in the inner membrane leaflet than the less unsaturated ones with same total acyl chain length, might indicate that some lateral segregation of the species takes place (see below).

4.2. Phospholipid composition of the membrane leaflets

The compositions of the outer and inner leaflets of the bacteriophage membranes can be calculated using the transbilayer distribution data presented here and the total lipid compositions determined previously [5,6]. Except for Bam35, the phospholipid compositions of the inner and outer leaflets were significantly different (Table 2). PG was the most abundant lipid in the outer leaflet of all phages, except in phi6, which contained an approximately equal amount of PE. No such regularity was observed for the inner leaflet, in which either PG (PM2 and Bam35) or PE (PRD1 and phi6) was the most abundant lipid.

The mole fraction of PG was quite high (≥50 mol%) in the outer leaflet in all phages as well as in the inner leaflet of PM2 and Bam35 (Table 2). Since PG molecules can only avoid being next to each other when their concentration is ≤33 mol%, such high concentrations the negatively-charged PG seems energetically unfavorable due to mutual electrostatic repulsion [41,42]. However, it is very likely that many PG molecules interact with positively charged amino acid residues of viral proteins (see below), thus neutralizing their negative charge. PG molecules

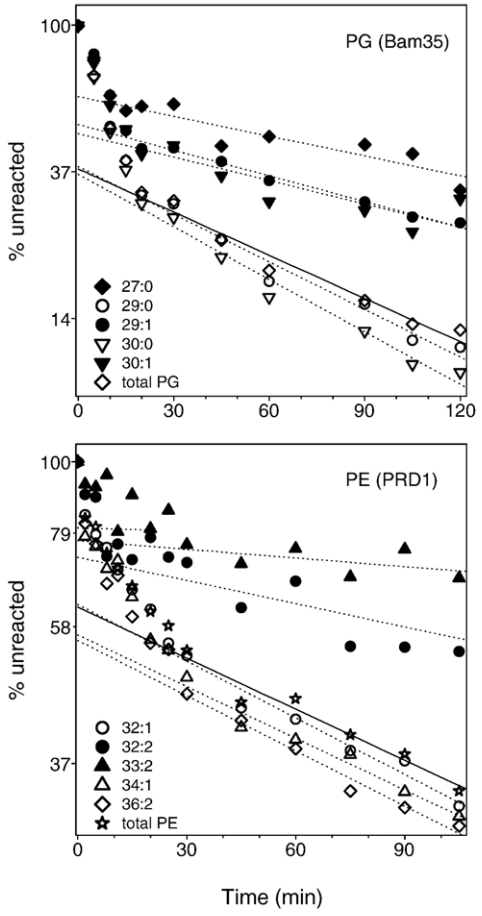

Fig. 6. Examples of time-course plots for modification of individual molecular species. The experiments were carried out as described in the legend of Fig. 1 and data analysis was performed with the LIMSA software as described in Materials and methods. Upper panel: oxidation of PG molecular species by periodate in Bam35. The data points are means of three independent experiments. Lower panel: modification of PE molecular species by TNBS in PRD1. The data points are means of two independent experiments. Data are shown only for select species for clarity. The dotted lines represent linear fits to the slower phase of the reaction for individual molecular species, while the solid lines are fits for the respective phospholipid classes. See Supplementary Fig. 2 for other details.

could also be partially neutralized by sodium or other cations as indicated by a recent molecular simulation study [43], which would be consistent with that the highest PG content was observed for PM2, which maturates in a marine bacterium growing in a high-salt medium [21].

In a recent X-ray study on PRD1 it was proposed, based on modeling of the electron densities of the inner and outer leaflets, that all PG and CL molecules reside in the outer membrane leaflet and most of PE molecules in the inner one [12]. While these results qualitatively agree with ours, we find that the asymmetry of PG is less extreme, i.e., only 55% of this lipid appears to be present in the outer leaflet (our data do not allow us to draw conclusions regarding CL distribution in PRD1). One possible reason for this discrepancy is that membrane proteins, which are abundant in PRD1 [14,44], could not be included when modeling the electron density data, but the membrane was assumed to consist entirely of lipids [12]. Our analysis indicates that the number of lipid molecules in the inner leaflet of PRD1 membrane is similar to that of outer one (Fig. 5) despite the larger surface area of the latter. Thus it is very likely that a significant fraction of the outer leaflet is occupied by protein (see above). Another reason for the discrepancy could be that counter-ions, solvating water molecules and other small molecules possibly present, which all contribute to the electron density, could not be resolved in the X-ray study.

The observed asymmetric transbilayer distribution of PG and PE in the phage membranes could be achieved by several different mechanisms, which are not mutually exclusive. These will be now discussed.

4.3. "Inheritance" of the host membrane lipid asymmetry

The lipid transbilayer asymmetry in several animal viruses is similar to the host membrane they obtain their lipids from [45,46]. Accordingly, it is possible that also bacteriophages "inherit" their membrane lipid asymmetry from their hosts, at least partially. Unfortunately, the phospholipid transbilayer distributions in the host membranes are not known and, therefore, it is not possible to estimate the contribution of this phenomenon to the asymmetry of phage membranes.

4.4. Matching lipid shape and membrane leaflet curvature

The observation that in all bacteriophages (except Bam35) PG is enriched in the outer leaflet and PE in the inner one strongly suggests that the distribution of phospholipids may relate to their effective geometrical shape and the different curvatures of the outer and inner membrane leaflets. The cross-sectional area of the PE polar head group is smaller than the combined cross-sectional area of the acyl chains and thus the "effective" shape of an unsaturated PE molecule can be considered similar to that of an inverted cone [41,47,48]. Such shape is more compatible with the negative curvature of the inner leaflet of the phage membranes (leaving less area for the head group) and could thus contribute to the enrichment of PE to this leaflet (Fig. 5). In contrast, the effective cross-sectional area of the head group of PG is as large (or even larger in some cases) as that of the acyl chains [10], due to higher degree of hydration and the negative charge of the phosphoglycerol head group [11]. Thus, the effective shape of PG is that of a cylinder or a modest cone depending on the acyl chains [41]. Accordingly, the effective shape of PG is more compatible with the positively curved outer leaflet (allowing more space for the head group) and is thus likely to contribute to its enrichment in this leaflet.

In Bam35, unlike in other phages, there is somewhat more PE in the outer leaflet than in the inner one (Fig. 5). While this seems inconsistent with the shape concept discussed above, it is notable that in Bam35 all PE species are either saturated or mono-unsaturated [6]. The effective shape of such species is closer to that of a cylinder rather than an inverted cone [41] and thus they should have no significant preference for the negatively curved inner leaflet.

PM2, PRD1 and Bam35 are icosahedral particles and thus their membrane contain highly curved areas coinciding with the vertexes [12]. The shape concept thus implicates that partial lateral segregation of molecular species might occur based on (slight) differences in their effective molecular shape. In the outer leaflet, the high positive curvature at the vertexes should attract PG molecules containing saturated acyl chains due to their more pronounced cone-shape. Respectively, PE molecules with short/unsaturated chains are likely to segregate/enrich to the vertexes in the inner leaflet due their pronounced inverted cone shape.

4.5. Selective protein–lipid interactions

It is known that peripheral membrane proteins often bind negatively charged lipids via positively charged amino acid residues located at the membrane-interacting domains [49]. Recent structural studies provide evidence that capsid proteins of PM2, PRD1 and Bam35 interact intimately with membrane lipids [12,16,50,51]. For example, some of the N-terminal, alpha-helixes of the major coat protein P3 interact with in the outer leaflet of PRD1 membrane [51]. Similar protein–membrane interactions occur in PM2 and Bam35 as well [16,50]. Chemical cross-linking studies with the bacteriophage PR4, a close relative to PRD1 [52,53], indicate that PG specifically interact with the major coat protein and could thus be responsible for enrichment of PG [36]. Thus, preferential association of the viral proteins on the outside of the membrane with negatively charged PG (and CL) molecules could contribute to the enrichment of PG in the outer leaflet. Also positively charged amino acid residues of integral membrane proteins could contribute to the enrichment of PG to the outer leaflet, but this remains uncertain since the topologies of most integral membrane proteins in bacteriophages are not known.

4.6. Effect of membrane–DNA interactions

Enrichment of PG in the outer leaflet was most evident for PM2, PRD1 and Bam35, in which the membrane is in a close contact with DNA [12,16,50,51]. Because both DNA and PG are negatively charged, their mutual repulsion could contribute to the enrichment of PG in the outer leaflet. However, the

transbilayer distribution of PG in DNA-less PRD1 and Bam35 particles were essentially the same as in intact phage particles, thus essentially dismissing the contribution of DNA–membrane interactions to phospholipid asymmetry.

4.7. The biological significance of viral membrane asymmetry

Our data indicate that the phospholipid asymmetry in the phage membranes is intrinsic, i.e., is generated by matching the lipid shape to the monolayer curvature as well as via selective lipid–protein interactions. Therefore, the transbilayer asymmetry might be important for (i) allowing the membrane to adopt optimal curvature, (ii) stabilizing the conformation of the integral membrane proteins, (iii) optimizing the interaction of peripheral capsid protein with the membrane and/or (iv) promoting the interaction of the phage with the host during infection.

4.8. Enrichment of PG in the membranes of bacteriophages

We have previously proposed that the enrichment of PG in the bacteriophage membranes relative to the host membrane could occur if PG and PE enrich to the outer and inner leaflets, respectively and, secondly, the area covered by phospholipids in the outer leaflet should be significantly higher than in the inner one [5]. While the present data are consistent with the former requirement (only partially in case of Bam35), the area covered by phospholipid molecules is similar in inner and outer leaflets in each case. Thus such mechanism cannot alone account for the PG enrichment in the bacteriophage membranes, and additional factors have to be considered.

Theoretically, it is possible that selective synthesis of PG and/or degradation of PE are affected during phage assembly, and could contribute to the enrichment of PG in the viral membrane. However, previous studies have shown that infection by PM2, PRD1 or phi6 does not induce neither synthesis nor degradation of phospholipids in the host [5,54,55]. Another possibility is that the host membrane contains PG-rich domains, and the phages derive their lipids mainly from such domains. However, lateral segregation of phospholipids in bacterial membranes is still uncertain, albeit some evidence has been obtained [9]. Alternatively, it is possible that PG-rich microdomains in the host cytoplasmic membrane are induced by insertion of integral viral membrane proteins that usually contain positive amino acid residues close to their transmembrane helixes. However, since neither the numbers of integral membrane proteins and their topologies in the membrane nor the numbers of positively charged amino acid residues interacting with lipids are known, it is not possible to estimate the relevance of this mechanism at present.

4.9. Concluding remarks

This is the first comprehensive and detailed study on the transbilayer distribution of phospholipid classes and molecular species in bacteriophage membranes. It provides strong evidence that, in all phages studied, the phospholipids are distributed asymmetrically over the membrane, PG being more abundant in the outer leaflet and PE in the inner one. Such asymmetric distribution of phospholipids is probably due to two major factors: (1) matching the effective shape of the phospholipid molecule to the different curvatures of the inner and outer membrane leaflets and (2) preferential association of acidic phospholipids with viral proteins. Finally, the present data should be very helpful when interpreting the structural data obtained for these bacteriophages in the past and the future.

Acknowledgments

We are grateful to Petri Papponen for purification of PM2, and other members of Bamford and Somerharju labs for skillful help. S.L. is a fellow of Helsinki Biomedical Graduate School. This investigation was supported by the Center of Excellence Program (2006–2011) of the Academy of Finland grants (No. 1213992, 1213467) to D.H.B. and a grant from Academy of Finland (No. 111936) to P.S.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbamem.2007.06.009.

References

[1] L. Mindich, Packaging, replication and recombination of the segmented genome of bacteriophage Phi6 and its relatives, Virus Res. 101 (2004) 83–92.
[2] M.M. Poranen, R. Tuma, Self-assembly of double-stranded RNA bacteriophages, Virus Res. 101 (2004) 93–100.
[3] R.H. Olsen, J.S. Siak, R.H. Gray, Characteristics of PRD1, a plasmid-dependent broad host range DNA bacteriophage, J. Virol. 14 (1974) 689–699.
[4] A.M. Grahn, R. Daugelavičius, D.H. Bamford, Sequential model of phage PRD1 DNA delivery: active involvement of the viral membrane, Mol. Microbiol. 46 (2002) 1199–1209.
[5] S. Laurinavičius, R. Käkelä, D.H. Bamford, P. Somerharju, The origin of phospholipids of the enveloped bacteriophage phi6, Virology 326 (2004) 182–190.
[6] S. Laurinavičius, R. Käkelä, P. Somerharju, D.H. Bamford, Phospholipid molecular species profiles of tectiviruses infecting Gram-negative and Gram-positive hosts, Virology 322 (2004) 328–336.
[7] S.N. Braunstein, R.M. Franklin, Structure and synthesis of a lipid-containing bacteriophage: V. Phospholipids of the host BAL-31 and of the bacteriophage PM2, Virology 43 (1971) 685–695.
[8] G.J. Brewer, R.M. Goto, Accessibility of phosphatidylethanolamine in bacteriophage PM2 and in its gram-negative host, J. Virol. 48 (1983) 774–778.
[9] S. Vanounou, A.H. Parola, I. Fishov, Phosphatidylethanolamine and phosphatidylglycerol are segregated into different domains in bacterial membrane. A study with pyrene-labelled phospholipids, Mol. Microbiol. 49 (2003) 1067–1079.
[10] B. de Kruijff, Lipid polymorphism and biomembrane function, Curr. Opin. Chem. Biol. 1 (1997) 564–569.
[11] J.N. Israelachvili, Theoretical considerations on the asymmetric distribution of charged phospholipid molecules on the inner and outer layers of curved bilayer membranes, Biochim. Biophys. Acta 323 (1973) 659–663.
[12] J.J. Cockburn, N.G. Abrescia, J.M. Grimes, G.C. Sutton, J.M. Diprose, J.M. Benevides, G.J. Thomas Jr., J.K. Bamford, D.H. Bamford, D.I. Stuart, Membrane structure and interactions with protein and DNA in bacteriophage PRD1, Nature 432 (2004) 122–125.
[13] D.H. Bamford, J.K.H. Bamford, Lipid-Containing Bacteriophage PM2,

the Type Organism of Corticoviridae, in: R. Calendar (Ed.), The Bacteriophages, Oxford University Press, New York, 2006, pp. 171–174.
[14] A.M. Grahn, S.J. Butcher, J.K.H. Bamford, D.H. Bamford, PRD1: Dissecting the Genome, Structure, and Entry, in: R. Calendar (Ed.), The Bacteriophages, Oxford University Press, New York, 2006, pp. 161–170.
[15] R. Schäfer, R. Hinnen, R.M. Franklin, Structure and synthesis of a lipid-containing bacteriophage. Properties of the structural proteins and distribution of the phospholipid, Eur. J. Biochem. 50 (1974) 15–27.
[16] P.A. Laurinmäki, J.T. Huiskonen, D.H. Bamford, S.J. Butcher, Membrane proteins modulate the bilayer curvature in the bacterial virus Bam35, Structure 13 (2005) 1819–1828.
[17] J.J. Ravantti, A. Gaidelytė, D.H. Bamford, J.K. Bamford, Comparative analysis of bacterial viruses Bam35, infecting a gram-positive host, and PRD1, infecting gram-negative hosts, demonstrates a viral lineage, Virology 313 (2003) 401–414.
[18] J.M. Kenney, J. Hantula, S.D. Fuller, L. Mindich, P.M. Ojala, D.H. Bamford, Bacteriophage phi 6 envelope elucidated by chemical cross-linking, immunodetection, and cryoelectron microscopy, Virology 190 (1992) 635–644.
[19] A.L. Hubbard, Z.A. Cohn, Specific labels for cell surfaces, in: A.H. Maddy (Ed.), Biochemical Analysis of Membranes, Chapman and Hall, London, 1976, pp. 427–501.
[20] R. Käkelä, P. Somerharju, J. Tyynelä, Analysis of phospholipid molecular species in brains from patients with infantile and juvenile neuronal-ceroid lipofuscinosis using liquid chromatography-electrospray ionization mass spectrometry, J. Neurochem. 84 (2003) 1051–1065.
[21] H.M. Kivelä, R.H. Männistö, N. Kalkkinen, D.H. Bamford, Purification and protein composition of PM2, the first lipid-containing bacterial virus to be isolated, Virology 262 (1999) 364–374.
[22] H.M. Kivelä, N. Kalkkinen, D.H. Bamford, Bacteriophage PM2 has a protein capsid surrounding a spherical proteinaceous lipid core, J. Virol. 76 (2002) 8169–8178.
[23] L. Mindich, D. Bamford, T. McGraw, G. Mackenzie, Assembly of bacteriophage PRD1: particle formation with wild-type and mutant viruses, J. Virol. 44 (1982) 1021–1030.
[24] D.H. Bamford, P.M. Ojala, M. Frilander, L. Walin, J.K.H. Bamford, Isolation, purification, and function of assembly intermediates and subviral particles of bacteriophages PRD1 and phi6, Methods Mol. Genet. 6 (1995) 455–474.
[25] R.P. Huijbregts, A.I. de Kroon, B. de Kruijff, On the accessibility of phosphatidylglycerol to periodate in *Escherichia coli*, Mol. Membr. Biol. 14 (1997) 35–38.
[26] T. Pomorski, R. Lombardi, H. Riezman, P.F. Devaux, G. van Meer, J.C. Holthuis, Drs2p-related P-type ATPases Dnf1p and Dnf2p are required for phospholipid translocation across the yeast plasma membrane and serve a role in endocytosis, Mol. Biol. Cell 14 (2003) 1240–1254.
[27] J.M. Folch, M. Lees, G.H. Sloane-Stanley, A simple method for the isolation and purification of total lipids from animal tissue, J. Biol. Chem. 226 (1957) 497–509.
[28] M. Hermansson, A. Uphoff, R. Käkelä, P. Somerharju, Automated quantitative analysis of complex lipidomes by liquid chromatography/mass spectrometry, Anal. Chem. 77 (2005) 2166–2175.
[29] P. Haimi, A. Uphoff, M. Hermansson, P. Somerharju, Software tools for analysis of mass spectrometric lipidome data, Anal. Chem. 78 (2006) 8324–8331.
[30] J. de Bony, A. Lopez, M. Gilleron, M. Welby, G. Laneelle, B. Rousseau, J.P. Beaucourt, J.F. Tocanne, Transverse and lateral distribution of phospholipids and glycolipids in the membrane of the bacterium *Micrococcus luteus*, Biochemistry 28 (1989) 3728–3737.
[31] S.S. Zumdahl, Chemistry, 4th ed., Houghton Mifflin Company, Boston, 1997.
[32] D.G. Bishop, J.A. Op den Kamp, L.L. van Deenen, The distribution of lipids in the protoplast membranes of *Bacillus subtilis*. A study with phospholipase C and trinitrobenzenesulphonic acid, Eur. J. Biochem. 80 (1977) 381–391.
[33] J.E. Rothman, E.P. Kennedy, Asymmetrical distribution of phospholipids in the membrane of *Bacillus megaterium*, J. Mol. Biol. 110 (1977) 603–618.
[34] D.G. Bishop, E.M. Bevers, G. van Meer, J.A. Op den Kamp, L.L. van Deenen, A monolayer study of the reaction of trinitrobenzene sulphonic acid with amino phospholipids, Biochim. Biophys. Acta 551 (1979) 122–128.
[35] N.J. Strömsten, D.H. Bamford, J.K. Bamford, The unique vertex of bacterial virus PRD1 is connected to the viral internal membrane, J. Virol. 77 (2003) 6314–6321.
[36] T.N. Davis, J.E. Cronan Jr., An alkyl imidate labeling study of the organization of phospholipids and proteins in the lipid-containing bacteriophage PR4, J. Biol. Chem. 260 (1985) 663–671.
[37] R.D. Camerini-Otero, R.M. Franklin, Structure and synthesis of a lipid-containing bacteriophage: XII. The fatty acids and lipid content of bacteriophage PM2, Virology 49 (1972) 385–393.
[38] N. Tsukagoshi, M.N. Kania, R.M. Franklin, Identification of acyl phosphatidylglycerol as a minor phospholipid of Pseudomonas BAL-31, Biochim. Biophys. Acta 450 (1976) 131–136.
[39] P. Garidel, A. Blume, 1,2-Dimyristoyl-*sn*-glycero-3-phosphoglycerol (DMPG) monolayers: influence of temperature, pH, ionic strength and binding of alkaline earth cations, Chem. Phys. Lipids 138 (2005) 50–59.
[40] T.J. McIntosh, S.A. Simon, Area per molecule and distribution of water in fully hydrated dilauroylphosphatidylethanolamine bilayers, Biochemistry 25 (1986) 4948–4952.
[41] J.N. Israelachvili, S. Marcelja, R.G. Horn, Physical principles of membrane organization, Q. Rev. Biophys. 13 (1980) 121–200.
[42] P. Somerharju, J.A. Virtanen, K.H. Cheng, Lateral organisation of membrane lipids. The superlattice view, Biochimi. Biophys. Acta 1440 (1999) 32–48.
[43] W. Zhao, T. Rog, A.A. Gurtovenko, I. Vattulainen, M. Karttunen, Atomic-scale structure and electrostatics of anionic palmitoyloleoylphosphatidylglycerol lipid bilayers with na+ counterions, Biophys. J. 92 (2007) 1114–1124.
[44] T.N. Davis, E.D. Muller, J.E. Cronan Jr., The virion of the lipid-containing bacteriophage PR4, Virology 120 (1982) 287–306.
[45] E.J. Patzer, N.F. Moore, Y. Barenholz, J.M. Shaw, R.R. Wagner, Lipid organization of the membrane of vesicular stomatitis virus, J. Biol. Chem. 253 (1978) 4544–4550.
[46] G. van Meer, K. Simons, J.A. Op den Kamp, L.M. van Deenen, Phospholipid asymmetry in Semliki Forest virus grown on baby hamster kidney (BHK-21) cells, Biochemistry 20 (1981) 1974–1981.
[47] P.R. Cullis, B. de Kruijff, Lipid polymorphism and the functional roles of lipids in biological membranes, Biochim. Biophys. Acta 559 (1979) 399–420.
[48] P.R. Cullis, M.J. Hope, C.P. Tilcock, Lipid polymorphism and the roles of lipids in membranes, Chem. Phys. Lipids 40 (1986) 127–144.
[49] A. Arbuzova, L. Wang, J. Wang, G. Hangyas-Mihalyne, D. Murray, B. Honig, S. McLaughlin, Membrane binding of peptides containing both basic and aromatic residues. Experimental studies with peptides corresponding to the scaffolding region of caveolin and the effector region of MARCKS, Biochemistry 39 (2000) 10330–10339.
[50] J.T. Huiskonen, H.M. Kivelä, D.H. Bamford, S.J. Butcher, The PM2 virion has a novel organization with an internal membrane and pentameric receptor binding spikes, Nat. Struct. Mol. Biol. 11 (2004) 850–856.
[51] N.G. Abrescia, J.J. Cockburn, J.M. Grimes, G.C. Sutton, J.M. Diprose, S.J. Butcher, S.D. Fuller, C. San Martin, R.M. Burnett, D.I. Stuart, D.H. Bamford, J.K. Bamford, Insights into assembly from structural analysis of bacteriophage PRD1, Nature 432 (2004) 68–74.
[52] D.H. Bamford, L. Rouhiainen, K. Takkinen, H. Soderlund, Comparison of the lipid-containing bacteriophages PRD1, PR3, PR4, PR5 and L17, J. Gen. Virol. 57 (1981) 365–373.
[53] A.M. Saren, J.J. Ravantti, S.D. Benson, R.M. Burnett, L. Paulin, D.H. Bamford, J.K. Bamford, A snapshot of viral evolution from genome analysis of the tectiviridae family, J. Mol. Biol. 350 (2005) 427–440.
[54] D.L. Diedrich, E.H. Cota-Robles, Phospholipid metabolism in Pseudomonas BAL-31 infected with lipid-containing bacteriophage PM2, J. Virol. 19 (1976) 446–456.
[55] M.M. Poranen, J.J. Ravantti, A.M. Grahn, R. Gupta, P. Auvinen, D.H. Bamford, Global changes in cellular gene expression during bacteriophage PRD1 infection, J. Virol. 80 (2006) 8081–8088.