

Characterization of the phospholipid and fatty acid composition of Sendai virus

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Abstract The lipid composition of Sendai virus, propagated in chicken eggs, was analyzed by high performance liquid chromatography (HPLC), thin-layer chromatography (TLC), and gas-liquid chromatography (GLC). Phosphatidylcholine was found to be the dominant phospholipid (37.3%) with phosphatidylethanolamine (26.8%) and phosphatidylserine (12.0%) also present in significant amounts. Analysis of the fatty acid methyl esters revealed that the dominant fatty acids in total phospholipid were: C16:0 (17.6%), C18:0 (15.4%), C18:1 (n-9) (22.0%), and C24:0 (6.0%). Cardiolipin, phosphatidylserine, and sphingomyelin contained higher levels of saturated fatty acids relative to phosphatidylinositol, phosphatidylethanolamine, and phosphatidylcholine. — Barnes, J. A., D. J. Pehowich, and T. M. Allen. Characterization of the phospholipid and fatty acid composition of Sendai virus. *J. Lipid Res.* 1987. 28: 130-137.

Supplementary key words cardiolipin • phosphatidylcholine • phosphatidylethanolamine • phosphatidylinositol • sphingomyelin • high performance liquid chromatography • two-dimensional thin-layer chromatography

The envelope of paramyxoviruses such as Sendai virus is composed of a lipid bilayer (1) and two integral membrane glycoproteins (HN and F) that project from the viral surface (2, 3). The hemagglutinating and neuraminidase (HN) protein is responsible for attachment of the virus to neuraminic acid-containing receptors on host cells, hemagglutination of erythrocytes, and neuraminidase activity (4, 5), whereas the fusion (F) protein is responsible for virus penetration of host cell membrane, virus-induced hemolysis, and cell fusion (5-7). Viral lipid composition may reflect that of the host plasma membrane from which the virus is derived during a budding process (8, 9). Nevertheless, the ratios of specific viral lipids can differ from those in the host (10) and this is usually attributed to the influence of viral protein (11, 12).

The viral lipid matrix is an essential constituent for viral biological activity since treatment of virions with lipid solvents, detergents, or lipase inactivates their infectivity and hemolytic activities (6, 7, 13). Furthermore, the infectivity of paramyxoviruses is reported to be dependent on the lipid composition of the host (8, 14) and especially on the relative concentration of phosphatidylethanolamine

(PE) and the molar ratio of cholesterol to phospholipid (PL) in the plasma membrane (14). Some viral envelope proteins display host specificity for particular fatty acyl chains during viral assembly. The basis for this specificity is unknown but it is thought to be necessary for correct assembly of nascent virions (15, 16). The preferential binding of certain lipid classes to the boundary layer of an intrinsic protein is one means by which lipid phase separation can be induced and maintained in the membrane (12).

Polyunsaturated fatty acids are known to increase membrane fluidity (17); nevertheless, it is not known how the lipid composition or state of the host cell membrane augments the action of the F protein. Despite the overall biological significance of viral lipids, the lipid composition has been studied in only a few viruses: for example, Sendai and Newcastle disease virus (18, 19), rabies virus (20, 21), Uukuniemi virus (22), and influenza virus (15). No previous study has reported the fatty acid composition of the individual phospholipids of Sendai virus. In this study we report the analysis of Sendai virus phospholipids and their respective fatty acids using advanced analytical techniques.

MATERIALS AND METHODS

Propagation and purification of virus

Sendai (Parainfluenza 1) virus (Cantel strain) was propagated in 10- to 11-day-old embryonated chicken eggs

Abbreviations: PL, phospholipid; HPLC, high performance liquid chromatography; TLC, thin-layer chromatography; GLC, gas-liquid chromatography; CL, cardiolipin; PI, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine; LPE, lysophosphatidylethanolamine; PC, phosphatidylcholine; SM, sphingomyelin; F, fusion protein; HN, hemagglutinin-neuraminidase protein; HBS, (HEPES-buffered saline), 140 mM NaCl buffered with 10 mM 4-(2-hydroxyethyl)-1-1 piperazine ethane sulfonic acid, pH 7.4; HAU, hemagglutinating unit; RSVE, reconstituted viral envelope protein.

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that were inoculated intra-allantoically with 10^6 egg infective doses (EID₅₀) of Sendai virus (7). The eggs were then incubated at 37°C for 72 hr and the allantoic fluid was harvested and the debris was pelleted at 750 *g*. The supernatant was then centrifuged at 17,300 *g* and the viral pellet was dispersed in phosphate-buffered saline (PBS, 0.15 M NaCl, 0.02 M P_i, pH 7.4). The virus was separated as an interfacial band on a sucrose density step gradient (15% and 50% sucrose w/w) at 75,000 *g* for 1 hr at 4°C. The purified virus was finally dialyzed exhaustively against PBS at 4°C and stored at -70°C. The virus was washed with HEPES-buffered saline (HBS) by repeated centrifugation at 100,000 *g* before it was used. Allantoic fluid was also harvested from 13- to 14-day-old embryonated chick eggs which had not been infected with virus.

Purification and characterization of viral HN and F glycoproteins

Sendai virus was washed with HBS and solubilized in the same buffer containing 2% (w/v) *n*-octylglucoside. The detergent mixture (final protein concentration 1.5 to 2.0 mg/ml) was incubated for 1 hr at room temperature. Viral remnants and other nonsolubilized material were removed by centrifugation at 64,000 *g* for 1 hr at 4°C in a Beckman ultracentrifuge using a fixed-angle Ti60 rotor. The supernatant (5.0 ml) was dialyzed in a Spectrophor dialysis tubing against one liter of HBS buffer for 24 hr at 0°C with four buffer changes. In a separate experiment, [¹⁴C]octylglucoside was shown to be completely removed from the dialyzate within 24 hr. The titers of the virus and glycoprotein preparations were determined by hemagglutination assay (23). The lipid composition of reconstituted Sendai virus envelopes, containing HN and F proteins, was determined as a basis for further studies of the role of viral lipids in fusion. The HN and F preparations were found to mimic the hemagglutinating and fusogenic activities of whole virus (17, 19, 24).

Purified Sendai virus and HN and F proteins were negatively stained by a modified procedure of Kruse, Wisniewski, and Popjak (19) and examined with a Philips 300 electron microscope. The protein compositions of the isolated virus and the envelope glycoproteins were analyzed by sodium dodecylsulfate-polyacrylamide gel electrophoresis by a modified Laemmli method (25). Protein concentration was assayed by the method of Peterson (26).

Extraction, separation, and quantitation of phospholipids

Isolated Sendai virus (50 ml, 256,000 HAU/ml) in HBS was lyophilized. The lyophilized material (3.7 g) was extracted for 30 min at room temperature (23°C) with 20 ml of chloroform-methanol 2:1 (v/v) containing 0.01% butylated hydroxytoluene (BHT) as an antioxidant. The residue was further extracted with 20 ml of chloroform-methanol-NH₄OH 35:5:2 (v/v) containing 0.01% BHT

(27). The pooled organic extract was dried under vacuum in order to remove ammonia and methanol, dissolved in 0.2 ml of chloroform, and stored at -20°C.

The concentrated lipid extract was applied to a silicic acid column (15 × 0.9 cm) and the neutral lipids were eluted with chloroform and the phospholipids with methanol. No phospholipids were present in the neutral lipid fractions. The phospholipids were separated on silica gel 60 F254 TLC plates, 20 × 20 cm (Merck Darmstadt, West Germany). One-dimensional chromatograms were developed with chloroform-methanol-water 14:6:1 (v/v) (28). The phospholipids were also separated by two-dimensional TLC first with chloroform-methanol-NH₄OH-H₂O 90:54:5.5:5.5 (v/v) and secondly with chloroform-methanol-NH₄OH-H₂O 90:40:12:2 (v/v) (29). Separated phospholipids were detected by exposure of the chromatograms to iodine vapor. Identification of the spots was confirmed by commercially obtained phospholipids that had been tested by analytical TLC. In addition, ninhydrin spray was used to localize phosphatidylserine, phosphatidylethanolamine, and their lyso derivatives; and α -naphthol spray was used to detect glycolipids. Total cholesterol was determined by the method of Watson (30).

The major portion of the phospholipid fraction from the silicic acid column was dried under nitrogen and dissolved in 0.5 ml of chloroform-diethyl ether 1:2 (v/v) and 40 μ l was injected into the HPLC column (Varian model 5000 solvent delivery system with a UV detector and Merck Chromosphere Si-60 μ m column). The mobile phase (31) was a mixture of acetonitrile-methanol-H₂SO₄ 100:4:0.04 (v/v) at a flow rate of 1 ml/min. Individual phospholipid classes were identified by comparing retention times with those of standards. After separation, each phospholipid class was collected with a Pharmacia Frac 100 fraction collector. Phospholipid phosphorus was determined by the methods of Raheja et al. (32) and Menzel and Corwin (33).

Fatty acid analysis

Phospholipid fatty acids from the purified whole virus and from HN and F glycoprotein preparations were converted into their methyl esters with BF₃-methanol (34). A 14% solution (2.0 ml) of BF₃ in methanol was added to the fraction in the HPLC mobile phase containing each phospholipid class, flushed with N₂, and the mixture was then heated at 110°C for 2 hr. After cooling, 1 ml of distilled water was added and the methylated fatty acids were extracted twice with 2 ml of hexane-diethyl ether 95:5, and purified on silicic acid columns. After drying under a stream of N₂, the fatty acid residue was dissolved in 150 μ l of hexane. Fatty acid methyl esters were separated by GLC (Varian Aerograph 6000 Gas Chromatograph, Varian Canada, Georgetown, Ontario, Canada) and quantified by using flame ionization detectors. Chromatography was performed utilizing a 25-meter SGE 50 QCZ/BP20 capil-

lary column. Helium was used as the carrier gas at a flow rate of 30 ml/min. The injection and detection temperatures were set at 250°C and the column temperature was programmed from 150 to 210°C. Chromatography was completed within 50 min. Fatty acid methyl esters were identified by comparison of retention times with known standards (bacterial fatty acid standards and MaxEPA oil) and by the method of equivalent chain length (35). Peak areas, weight percentage fatty acid composition, and μg values based on the amount of internal standard were computed by a chromatography data system (CDS III, Varian Canada). Hydroxy fatty acid methyl esters were first identified on a nonpolar SE-30 (Varian) column using bacterial standards before being run on a polar column using a mixture of bacterial standards and MaxEPA oil.

RESULTS

A complete separation of viral phospholipids was achieved by one-dimensional TLC (Fig. 1) and two-dimensional TLC (Fig. 2) with the solvent systems described in Materials and Methods. The separation of Sendai virus phospholipids by HPLC is shown in Fig. 3 and the phospholipid contents of whole Sendai virus and the isolated envelope glycoproteins are given in Table 1. A comparison of the individual phospholipids reveals a predominance of phosphatidylcholine (PC), and phosphatidylethanolamine (PE) with relatively lower amounts of phosphatidylserine (PS), cardiolipin (CL), sphingomyelin (SM), and phosphatidylinositol (PI). PC was found to be the dominant phospholipid (37.3%). However, the amounts of PC and PE are at variance with the data of Blough and Lawson (18) who found a comparatively low PC content (8.0%). The cholesterol content was 6.7% of virus dry weight and the molar ratio of cholesterol to phospholipid was found to be 1.0, assuming a mean molecular weight of 775 for phospholipid. These values are in good agreement with the values (7.2% cholesterol, and molar ratio of cholesterol to phospholipid of 0.9) reported by Blough and Lawson (18).

Electron micrographs of negatively stained purified Sendai virus revealed the characteristic heterogeneity of the virions with glycoprotein spikes on their outer surface (not shown). No foreign tissues were observed. The Coomassie blue-stained proteins in the whole virus or in glycoprotein extracts showed only the five characteristic protein bands of the whole virus (7, 19) or the HN and F bands of the isolated glycoproteins, respectively. An assessment of plasma membrane contamination of the purified virus by the 5'-nucleotidase assay (36) indicated that contamination was less than 0.2% with respect to the enzyme activity in chicken fibroblast cells. Several preparations of whole virus contained 30–123 HAU/ μg viral

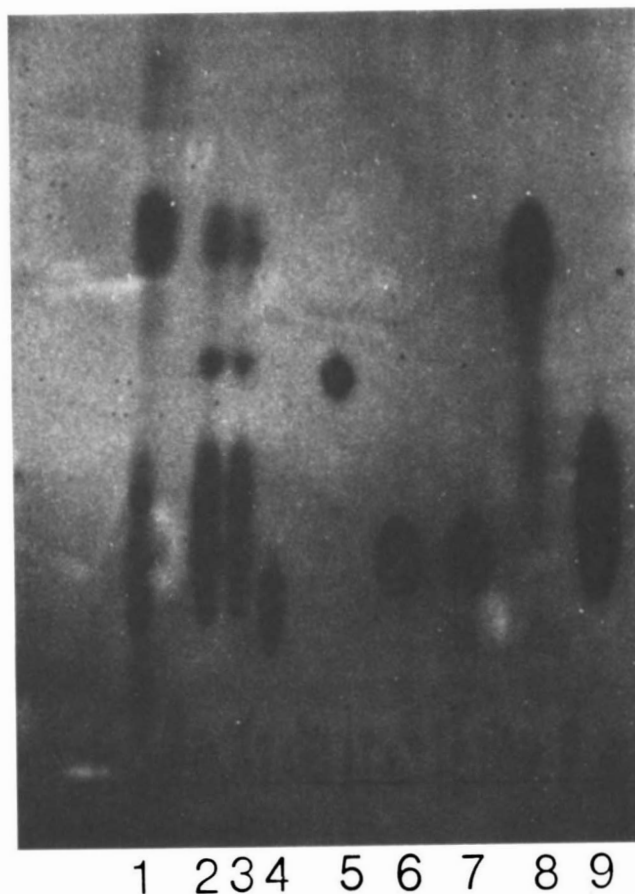


Fig. 1. One-dimensional thin-layer chromatogram of Sendai virus extracted lipids and standard lipids. Lane 1; Sendai virus lipids; lanes 2, 3, mixed lipids; lanes 4, SM; 5, CL; 6, PS; 7, PI; 8, PE; and 9, PC.

protein and the glycoprotein preparations contained 80–330 HAU/ μg HN and F protein. The phospholipid to protein ratio for whole Sendai virus was 0.170 (mg/mg). This corresponds to 0.44 based on a viral HN and F content of 39% of the total viral protein on a weight basis (37). The phospholipid to protein ratio of the detergent-extracted HN and F preparations was 0.45 (mg/mg). These results indicate that there was no lipid depletion in the HN and F glycoprotein preparation compared to HN and F in the viral envelope.

The fatty acid composition of the total phospholipids in whole Sendai virus and of the detergent-extracted HN and F glycoproteins and the fatty acid composition of individual phospholipids are presented in Table 2. The assay was repeated three times with good agreement between assays and the results of the final assay are reported. The relative amounts of individual fatty acids in the whole virus were similar to those in the extracted HN and F proteins. However, the fatty acid compositions of individual phospholipids were markedly different from one another. The major fatty acids were palmitic acid,

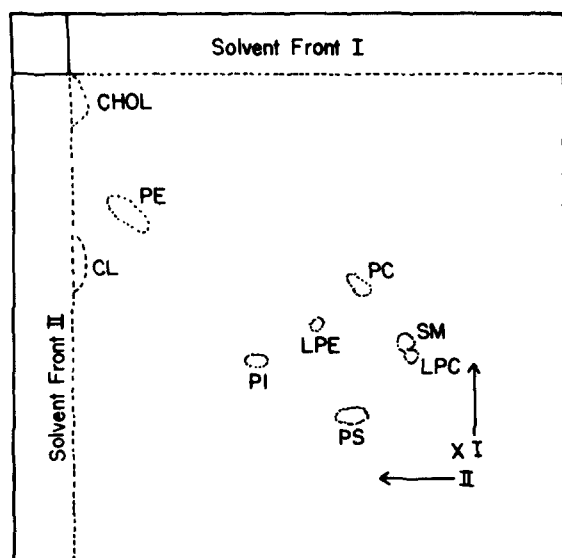


Fig. 2. Two-dimensional thin-layer chromatogram tracing of lipids extracted from Sendai virus. Abbreviations: CHOL, cholesterol; CL, cardiolipin; LPE, lysophosphatidylethanolamine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyelin; x, origin where sample was spotted. Arrows indicate direction of development.

C16:0; stearic acid, C18:0; oleic acid, C18:1(n-9) and the longer chain arachidonic acid, C20:4(n-6) and lignoceric acid, C24:0. In addition, hydroxy fatty acids constituted approximately 10% of the total phospholipid fatty acids. However, it should be noted that this relatively high percentage may be due, in some measure, to contamination with bacterial lipopolysaccharides. These hydroxy fatty acids were not previously reported to be present in Sendai virus lipids.

Cardiolipin contained relatively high amounts of hydroxy fatty acids 2-OH C16:0 (3.5%), I-C17:0 (3.3%). In addition to exhibiting an average percentage (16.1%) of n-6 fatty acids, CL showed a relatively high ratio (1.7) of saturated to unsaturated fatty acids and a moderate unsaturation index (UI) value (see Table 2, footnote). The fatty acid composition of Sendai virus CL contrasts markedly with that found in CL derived from mammalian sources which has a high content of unsaturated fatty acids (38).

In contrast with other phospholipids, PI contained the largest percentage (24.0%) of C20:3 (n-6). PI also contained the largest percentage of n-6 fatty acids, the highest ratio of (n-6)/(n-3) fatty acids, the largest UI value, and the smallest percentage of total monounsaturated fatty acids. There was a relatively high content of mono-unsaturated fatty acids (21.3%) in PS with C18:1 (n-9) being dominant. Both the ratio of saturated to unsaturated fatty acids and the unsaturation index show that CL, PS, and SM contain higher levels of saturated fatty acids as compared to PE, PC, and PI, which contained higher levels of unsaturated fatty acids.

The fatty acid composition of phospholipids of non-infected egg allantoic fluid is shown in Table 3. The amount of individual fatty acids in allantoic fluid was similar to that in the whole virus. However, the content of arachidonic acid, C20:4 (n-6), was reduced by 50% in the allantoic fluid compared with that in the whole virus. There was also a noticeable absence in the allantoic fluid of arachidonic acid and hydroxy fatty acids such as 2-OH C14:0 and 2-OH C16:0, which were present in the virus.

DISCUSSION

The lipid composition of the envelope of paramyxoviruses is approximately 20% of the dry weight (10). The phospholipid and fatty acid components are major factors that influence the physical properties and function of viral membranes. The motional state (fluidity) of the lipid phase

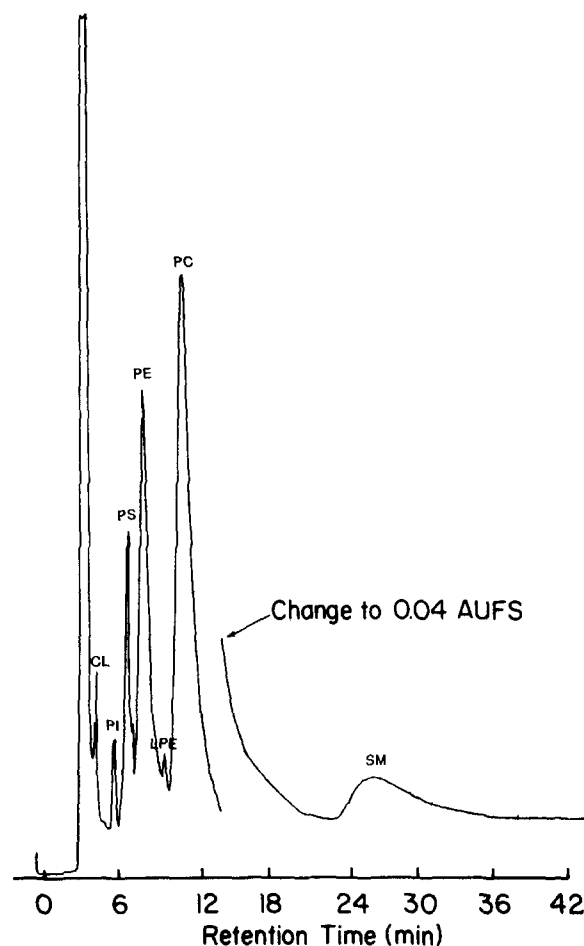


Fig. 3. Chromatogram of phospholipid extract from whole Sendai virus. The mobile phase was a mixture of acetonitrile-methanol- H_2SO_4 , 100:4:0.04 (v/v) at a flow rate of 1 ml/min. Chart speed 20 cm/hr. Sensitivity, 0.16 AUFS changed to 0.04 AUFS before the elution of SM. Phospholipid retention times were: CL, 3.9 min; PI, 5.7 min; PS, 6.6 min; PE, 7.8 min; LPE, 9.6 min; PC, 10.8 min; and SM, 26.1 min.

TABLE 1. Phospholipid composition of whole Sendai virus and the extracted HN and F envelope glycoproteins

Phospholipid	Blough and Lawson Sendai Virus ^a	Whole Sendai Virus ^b	HN + F Protein
	% of total phospholipid		
CL		8.9 ± 0.53	9.0
PI	6.4 ± 0.4	5.5 ± 0.48	6.0
PS	15.0 ± 1.0	12.0 ± 0.84	13.2
PE	37.0 ± 0.5	26.8 ± 1.39	29.1
LPE	2.0 ± 0.0	1.6 ± 0.46	
PC	8.0 ± 0.0	37.3 ± 2.80	37.4
SM	11.8 ± 0.8	8.8 ± 1.10	4.9

CL, cardiolipin; PI, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine; LPE, lysophosphatidylethanolamine; PC, phosphatidylcholine; SM, sphingomyelin; HN + F, Sendai virus hemagglutinin neuraminidase (HN) and fusion (F) proteins.

^aBlough and Lawson, 1968 (18).

^bThis work, based on phosphorus assays with 98–100% recovery of total phosphorus; values represent the mean of six analyses for Sendai virus.

can determine the ability of protein to undergo conformational changes necessary for optimal catalytic activity of enzyme substrate complexes (39). Lipid fluidity is dependent on the nature of the phospholipid head group and its conformation and the nature of the phospholipid acyl chains (40).

Viral lipid composition is dependent on environmental factors (18), the biosynthetic properties of the host cell (9), and the configuration of structural proteins (18). Tiffany and Blough (41) observed that different myxoviruses propagated in homologous membranes under the same environmental conditions had variant phospholipid and acyl chain profiles. Consequently, they postulated that the structural proteins of the viral envelope play a major role in determining the type and orientation of lipids in the virion. Although the production of infective viral particles depends to some extent on the host plasma membrane, the lipids acquired by enveloped viruses do not precisely reflect those of the host plasma membrane (8, 41).

At present it is not known how the lipid composition of the virus, or the lipid composition or state of the host cell membrane, augments the action of the virus fusion protein. Although viral lipids are needed for fusion or viral entry into cell, they have not been shown to exert a modulating role on the infective process (42). When Sendai virus was added to phospholipid liposomes that were in a fully fluid state, only phagocytosis was observed; but when PE and SM were included with these liposomes, fusion occurred (43). Consequently, it appears that specific phospholipids, in addition to a fluid membrane, are necessary for fusion. Sendai virus-induced hemolysis was found to be enhanced by the interaction of neutral glycolipids terminating in galactose and certain phospholipids, primarily PC and SM (44). These results suggest a direct

role for these phospholipids in hemolysis and possibly fusion.

The analytical data presented in this study (Table 1) show higher levels of PC (37.3%) as compared to PE (26.8%). This result is at variance with the results of Blough and Lawson (18) who reported egg-grown Sendai virus content of PE and PC of 37% and 12%, respectively. These workers did not specify the strain of Sendai virus that they analyzed. Nevertheless, Klenk and Choppin (8) reported a PC and PE content of 44% and 17%, respectively, for Simian virus (SV5), another paramyxovirus grown in hamster kidney cells. Possible differences in viral strain could account for discrepancies of Sendai virus PC and PE content. Previous reports showed that viruses contain larger amounts of saturated fatty acids compared to whole cells (8, 9); however, this is not apparent in our study when the unsaturation index from whole virus is examined or from the data of Kruse et al. (19).

The CL content (9.0%) of Sendai virus is similar to that amount (2–10%) found in mammalian tissues (38). It is noticeable that CL from Sendai virus contains a high content (63.1%) of saturated fatty acids. At present no conclusive functional role has been assigned to CL derived from mammalian or prokaryotic sources. Sendai virus CL also contained relatively large amounts of hydroxy fatty acids which are known to be biosynthetic intermediates. The hydroxy fatty acids could be good candidates for maintaining the fluidity of the viral membrane since they could contribute to uneven packing in the lipid bilayer. In all phospholipid classes the long-chain polyunsaturated fatty acids that do not favor close packing of membrane lipids (40) could also contribute to increased membrane fluidity and hence fusion of the viral envelope with the host cell membrane.

The site of maturation of Sendai virus is the plasma membrane lining the allantoic cavity, and the mature virions, which proliferate by budding at the plasma membrane, are shed together with cellular debris directly into the surrounding allantoic fluid (15). Virus multiplication proceeds through several infection cycles and possibly through different cell types in the developing chick embryo. In addition, the highly dynamic nature of the plasma membranes results in complex interactions with their environment (10) and selective uptake of fatty acids from host plasma membrane (18). In view of these considerations and the intrinsic difficulty of finding an ideal control for the lipids of viruses grown in ovo or in selected cell types (10, 15, 18), the phospholipid fatty acid composition of the non-infected allantoic fluid was determined for comparison with the total phospholipid fatty acid composition of the virus. However, it should be noted that it is unlikely that the lipid composition of the egg plasma membranes and that of the allantoic fluid will remain unaltered during the course of virus propagation. A compar-

TABLE 2. Fatty acid composition of Sendai virus phospholipids

Fatty Acid	Total PL	NH + F PL	CL	PI	PS	PE	PC	SM
% of total fatty acids								
C11:0	Tr	Tr	Tr	0.88	Tr	0.50	0.50	ND
C14:0	Tr	0.50	0.78	1.29	0.71	0.83	1.37	1.94
C15:0	Tr	Tr	0.52	1.77	0.96	0.89	1.34	2.20
3-OH C14:0	0.56	Tr	1.39	Tr	Tr	Tr	Tr	Tr
2-OH C14:0	3.90	2.40	ND	Tr	Tr	Tr	Tr	Tr
C16:0	17.60	21.70	25.37	21.50	18.84	20.47	27.04	28.26
C16:1 (n-9)	Tr	1.75	1.30	0.89	0.98	0.59	4.17	1.44
C16:1 (n-7)	1.04	1.75	Tr	0.54	Tr	Tr	0.58	Tr
Cis(9,10) C17:0 Δ^a	Tr	Tr	0.58	0.50	Tr	Tr	Tr	Tr
C17:0	Tr	Tr	Tr	Tr	0.59	0.51	0.62	0.95
I- C17:0	0.54	Tr	3.34	1.30	Tr	ND	Tr	Tr
2-OH C16:0	4.70	2.80	3.53	ND	Tr	ND	Tr	Tr
C18:0	15.40	15.70	15.46	8.67	24.68	21.89	12.91	13.88
C18:1 (n-9)	22.00	21.50	5.75	2.73	19.44	21.69	18.86	5.98
C18:1 (n-7)	0.80	1.50	0.70	Tr	Tr	Tr	Tr	1.05
C18:2 (n-6)	7.70	7.20	1.74	0.68	4.06	5.54	5.27	1.56
C18:3 (n-3)	Tr	Tr	1.03	Tr	Tr	ND	Tr	Tr
C20:0	0.51	ND	Tr	1.21	1.77	0.69	Tr	0.67
C20:1 (n-11)	0.90	Tr	Tr	ND	Tr	Tr	Tr	Tr
C20:1 (n-9)	Tr	Tr	0.89	0.97	2.73	0.63	1.47	2.06
C20:2 (n-6)	Tr	Tr	0.88	ND	Tr	Tr	Tr	ND
C20:3 (n-6)	Tr	Tr	Tr	24.0	Tr	Tr	Tr	0.50
C20:4 (n-6)	9.10	5.70	6.34	4.57	2.25	6.20	6.15	1.08
C20:4 (n-3)	0.82	ND	1.00	Tr	Tr	Tr	Tr	Tr
C20:5 (n-6)	Tr	ND	1.50	Tr	Tr	ND	Tr	Tr
C20:5 (n-3)	Tr	Tr	Tr	Tr	Tr	Tr	Tr	0.50
C22:0	1.60	2.90	Tr	2.83	Tr	Tr	Tr	1.11
C22:1 (n-9)	Tr	Tr	ND	0.50	0.66	Tr	Tr	Tr
C22:4 (n-6)	1.50	0.90	1.30	2.07	Tr	0.50	0.63	0.61
C22:4 (n-3)	0.40	0.77	Tr	Tr	Tr	0.70	0.73	Tr
C22:5 (n-6)	1.00	0.70	0.50	Tr	Tr	0.82	1.45	Tr
C22:6 (n-3)	0.50	4.40	Tr	1.27	1.14	1.25	3.88	Tr
C24:0	6.00	Tr	9.32	11.04	11.04	7.26	4.64	17.39
C24:1 (n-9)	Tr	Tr	1.83	Tr	Tr	0.58	Tr	1.50
C24:4 (n-6)	3.65	Tr	3.63	5.39	3.78	3.52	2.75	9.73
Σ Saturated ^b	49.71	48.00	63.08	53.36	61.55	55.15	51.22	70.72
Σ Monoenoic ^c	25.54	26.80	9.40	4.68	21.26	23.63	24.23	10.16
Σ n-6	23.94	15.54	16.12	37.45	10.97	17.02	16.70	13.83
Σ n-3	2.69	5.54	3.26	2.68	2.28	2.62	5.48	2.17
Ratio saturated/unsaturated	1.09	0.92	1.71	1.14	1.60	1.23	1.05	2.42
Ratio n-6/n-3	8.90	2.80	4.94	13.97	4.80	6.50	3.04	6.37
UI ^d	119.71	106.81	85.77	145.61	72.06	96.02	114.46	75.75

Table summary is based on the total fatty acid profiles, only individual fatty acids greater than 0.5% are reported. ND, not detected; Tr, fatty acid present but less than 0.5% of the total. The following fatty acids were present in trace amounts in total PL (HN and F) and in all PL classes: 2-OH C10:0, C12:0, C13:0, 3-OH C12:0, 2-OH C12:0, C14:1, A-C15:0, I-C15:0, I-C16:0, A-C17:0, C18:03 (n-6), C19:0, C18:4 (n-3), C20:3 (n-9), C22:5 (n-3), and C24:2 (n-6). A, hydroxy substituent in end position; I, hydroxy substituent in interior position.

^a Δ , Methylene hexadecanoic acid.

^bSum of saturated fatty acids.

^cSum of monounsaturated fatty acids.

^dUnsaturation index = $\Sigma[(x)(y)]$ where (x) is the percentage of each unsaturated fatty acid and (y) is the number of double bonds.

ison of the total phospholipid fatty acid composition in the whole virus with that in the allantoic fluid (Table 3) shows differences in content of palmitic acid, stearic acid, oleic acid, arachidonic acid, as well as others.

A number of recent reports on whole and reconstituted viral systems have addressed the question of the role of lipid in fusion and lysis of natural and artificial membranes. Several workers have reported a requirement for

cholesterol for the fusogenic activity of whole virus or reconstituted viral envelope proteins (RSVE) (24, 45, 46). The phospholipid requirement for viral activity is controversial. Ozawa and Asano (45) reported that HN and F proteins reconstituted from Triton X-100 (TX-100) into liposomes of PC or PE and cholesterol were inactive, although Citovsky and Loyer (24) reported fusion between Sendai virus or RSVE from TX-100 and liposomes

TABLE 3. Fatty acid composition of phospholipids of allantoic fluid from embryonated eggs

Fatty Acid	Allantoic Fluid	Whole Virus
% of total fatty acids		
C14:0	0.85	Tr
2-OH C14:0	ND	3.90
C16:0	27.01	17.60
C16:1 (n-7)	1.02	1.04
C17:0	1.36	0.20
2-OH C16:0	ND	4.70
C18:0	18.43	15.40
C18:1 (n-9)	27.54	22.00
C18:1 (n-7)	0.53	0.80
C18:2 (n-6)	13.78	7.70
C20:0	Tr	0.51
C20:3 (n-6)	0.52	Tr
C20:4 (n-6)	4.60	9.10
C22:0	Tr	1.60
C22:5 (n-6)	Tr	1.00
C22:6 (n-3)	1.69	0.50
C24:0	Tr	6.00
C24:1 (n-9)	0.59	Tr
C24:4 (n-6)	ND	3.65

The following fatty acids were present in trace amounts (i.e., less than 0.5% of the total) in the whole virus and the allantoic fluid: 2-OH C10:0, 2-OH C12:0, C13:0, C15:0, C18:3 (n-3), C20:1 (n-9), C20:5 (n-3), and C22:5 (n-3). Tr indicates the presence of the respective fatty acids in trace amounts.

containing only PC and cholesterol. Haywood and Boyer (47), on the other hand, reported a requirement for negatively charged lipid for fusion between Sendai virus and liposomes.

Several reports have demonstrated that HN and F proteoliposomes demonstrate fusogenic activity when incorporated into preparations of total viral lipids (24, 48, 49) or lipid mixtures similar to that of whole virus (45). Inoue, Nojima, and Inoue (50) reconstituted TX-100 extracts of HN and F proteins into liposomes of a variety of lipid compositions and reported that maximum hemolysis and fusion with erythrocytes occurred when the proteins were incorporated into total viral lipids, although activity at lower levels could still be observed when liposomes were composed of only PC and cholesterol. Lipid-depleted protein aggregates also demonstrated hemolysis and fusion activity, but at a low level. Activity of lipid-depleted protein aggregates has also been reported by Kruse et al. (19).

Hemolysis and fusion have been demonstrated to be separate events. RSVE prepared from TX-100 glycoprotein extracts were fusogenic and hemolytic (48) whereas RSVE prepared from *n*-octylglycoside extracts were fusogenic but not hemolytic. Non-lytic fusion occurred between RSVE and liposomes composed of PC and cholesterol, but addition of sialoglycolipid and sialoglycoproteins to the liposome resulted in lytic fusion (24).

Current evidence therefore suggests that, although bilayer lipids may not be essential for the functioning of HN and F proteins, increased biological activities of these glyco-

proteins were observed when they were incorporated into bilayers of total viral lipids. Interaction of total viral lipids with HN and F proteins could, therefore, aid in the induction of conformational changes which are essential for optimal activity of the proteins (51). The analytical data on the phospholipids and fatty acids reported in this study will undoubtedly prove useful in future studies to evaluate the role of lipids in Sendai virus-mediated fusion. ■

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