Compositional Asymmetry and Transmembrane Movement of Phosphatidylcholine in Vesicular Stomatitis Virus Membranes†

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ABSTRACT: A purified phosphatidylcholine exchange protein has been utilized to follow the transfer of [^{3}H]choline-labeled phosphatidylcholine or sphingomyelin from the bilayer of vesicular stomatitis virus to single lamellar lipid vesicles. Only [^{3}H]choline-labeled phosphatidylcholine was exchangeable. Little if any of the [^{3}H]choline-labeled sphingomyelin was transferred by exchange protein. Results for trypsinized virions containing no glycoprotein spikes revealed a rapidly exchangeable pool comprising 69% of the membrane PC (k_{ves} = 3.4–4.9 h^{-1}). Thirty-one percent of the phosphatidylcholine was in a more slowly exchangeable pool. The rapidly exchanging pool was assumed to be in the outer monolayer of the membrane bilayer and the slowly exchanging pool in the inner monolayer of the bilayer. The slow rate thus identified with the transposition of [^{3}H]choline-labeled phosphatidylcholine across the virus membrane leads to a half-time of 7–11 h for this process. In contrast to spikeless virions, exchange and distribution studies on the intact virions gave a much slower transfer of [^{3}H]choline-labeled phosphatidylcholine to vesicles (k_{ves} = 0.16 h^{-1}) with 58% of the virion phosphatidylcholine in the more rapidly exchangeable pool and 42% in the more slowly exchangeable pool. Complicating the study with intact virions, however, was vesicle adhesion which saturated after a 12-h incubation at about 16 vesicles per virion. This amount of phosphatidylcholine is equivalent to about 50% of the total virion lipid. Furthermore, the intact virions are known to possess negatively charged sialic acid residues in the surface glycoprotein which may decrease the apparent dissociation constant (k_D) for the exchange protein–virion complex. Consequently, results derived from intact virions are less satisfactory in describing the distribution of phosphatidylcholine in vesicular stomatitis virus.

The distribution and the rates of movement of phospholipids between the inner and outer monolayers of the lipid bilayer of a biological membrane are expected to affect membrane integrity and function. In simple bilayer systems compositional asymmetry of phospholipids decays very slowly unless the structure of the bilayer is perturbed by, for example, the presence of different lateral packing in the two monolayers (De Kruijff & Wirtz, 1977) or by the accumulation of phospholipid degradation products in the bilayer (Shaw & Thompson, 1978). The situation in biological membranes is more complex. In an effort to better understand biological systems we have examined compositional asymmetry and transmembrane movement of phospholipids and cholesterol in the membrane of vesicular stomatitis virus, Indiana serotype. Both the phospholipids and cholesterol of the virion membrane are asymmetrically distributed across the bilayer. Cholesterol has previously been shown to undergo transposition across the membrane bilayer with a half-time of 4 to 6 h (Moore et al., 1978; Patzer et al., 1978a,b).

In the present study we have utilized phosphatidylcholine exchange between virions and vesicles catalyzed by an exchange protein to examine the asymmetry and transbilayer movement of phosphatidylcholine in both the intact virion and virions trypsinized to remove the glycoprotein spikes. At the same time the integrity of virions and degree of assiciation of vesicles used in the exchange system with the virion membrane have been carefully monitored. The results show an asymmetric arrangement of phosphatidylcholine across the bilayer similar to that reported for the erythrocyte membrane (Verkleij et al., 1973; Bloj & Zilversmit, 1976). The half-time for transbilayer migration of phosphatidylcholine in the virion membrane is on the order of 10 h.

Materials and Methods

Preparation of Labeled Virions. The Indiana serotype of vesicular stomatitis virus (VSV)[1] was grown in confluent monolayers of BHK-21 cells after infection at a multiplicity of 0.1–1.0 plaque forming units per cell (Barenholz et al., 1976). The BHK-21 cells were grown in the presence of 1 μCi of [*methyl*-^{3}H]choline chloride (60–90 Ci per mmol, New England Nuclear) per mL of growth medium containing no serum and reached confluency within 3 days. Virus was adsorbed to the monolayers for 1 h and the cells were then overlayed with medium. Virus was collected after 16–18 h and purified as previously described (Barenholz et al., 1976). Glycoprotein spikes were removed from purified virions by hydrolysis with trypsin to give spikeless VSV (Moore et al., 1978). Extraction and thin-layer chromatography of the virion lipids revealed the presence of tritium label only in phosphatidylcholine (PC) and sphingomyelin (SPM). The specific radioactivity of both PC and SPM ranged from 60 000 to 80 000 dpm per nmol of phospholipid in the various preparations.

Preparation of Lipid Vesicles. Procedures for the purification of egg PC, cholesterol, and beef brain sphingomyelin have been described previously (Shaw et al., 1977; Moore et al., 1978; Schmidt et al., 1977). Also previously reported is the preparation of single lamellar vesicles of PC or PC/

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[1] Abbreviations used: VSV, vesicular stomatitis virus; PC, phosphatidylcholine; SPM, sphingomyelin; TNBS, 2,4,6-trinitrobenzenesulfonate; NaDodSO$_4$, sodium dodecyl sulfate.

0006-2960/79/0418-0538$01.00/0

SPM/cholesterol (50:25:25 mol %) containing as a nonexchangeable marker less than 0.5 mol % [^{14}C]cholesteryl oleate (50–60 μCi/μmol, New England Nuclear) or trioleoyl[2-^{3}H]glycerol (30–60 μCi/μmol, New England Nuclear and Amersham/Searle) (Moore et al., 1978). The PC/SPM/cholesterol lipid suspension in 100 mM NaCl–20 mM Tris, pH 7.5, was sonicated at 30 °C, whereas the PC suspension in the same medium was sonicated at 10 °C. Small vesicles were separated from large vesicles by high-speed centrifugation (Barenholz et al., 1977).

Purification of a Phosphatidylcholine Exchange Protein. The details of isolation and purification of the phosphatidylcholine-specific exchange protein from beef liver have been published elsewhere (Shaw et al., 1977). Before use, the exchange protein was dialyzed against 10 mM Tris-HCl, 0.1 M KCl, pH 7.5, or 10 mM Tris-HCl, 0.15 M NaCl, 1 mM EDTA, 0.02% NaN_3, pH 7.6, in the presence of 0.1 mg of bovine serum albumin per mL of exchange protein. One unit of exchange protein is defined as the initial rate of exchange of 1 nmol of PC per min at 37 °C when assayed as previously described (Shaw et al., 1977).

Incubation of Virions, Vesicles, and Exchange Protein. Detailed incubation conditions for virions (spikeless or intact) with vesicles in the presence or absence of exchange protein are described in the legend of Figure 1. At selected time intervals during incubation at 37 °C an aliquot was taken and overlayed on top of a 4.5-mL continuous 0–40% potassium tartrate gradient containing 0–20% glycerol maintained in an ice bath. This procedure kept to a minimum the protein catalyzed exchange after the 37 °C incubation. Separate studies showed that about 12% exchange occurred at 10 °C relative to that at 37 °C. The gradients were centrifuged at 35 000 rpm for 2 h in an SW 50.1 Beckman rotor or in some cases, at 16 000 rpm overnight at 4 °C. Vesicles separated from the virus as a floating band at the top of the gradient. The virus, which banded within the gradient, was collected, diluted to 5 mL with buffer and then pelleted at 40 000 rpm in an SW 50.1 rotor for 1 h.

Vesicles and pelleted virion lipids were extracted by the procedure of Bligh & Dyer (1959) after addition of unlabeled carrier PC, SPM, and lysolecithin to virions, and SPM and lysolecithin to vesicles. Negligible counts were present in the upper methanol–water layer after extraction. The chloroform layer was spotted on channeled 20 × 20 cm Quantum thin-layer plates and chromatographed using chloroform/methanol/ammonia/water (65:25:3.5:1.5) solvent. Lipid areas on the plates were lightly stained with iodine vapor and marked. Iodine was then removed by heating the plates under vacuum, and the areas containing [^{14}C]cholesteryl oleate, [^{3}H]PC, [^{3}H]SPM, and lyso-PC (negligible counts) were scraped and measured for radioactivity in a Beckman LS-230 scintillation counter.

The percentage transfer of radiolabeled PC or SPM from virions to vesicles was determined from both the loss of PC or SPM from the virions and the increase in PC or SPM in the vesicles after incubation in the presence and absence of exchange protein. The percentage transfer due to exchange protein alone was obtained from the difference in transfer in the presence and absence of exchange protein.

Miscellaneous Methods. Total virion protein was determined by the method of Lowry et al. (1951) and phosphorus content was assayed as described by Bartlett (1959). Iodination of VSV with lactoperoxidase and TNBS labeling were performed as described by Moore et al. (1977) and Patzer et al. (1978a). Polyacrylamide gel electrophoresis of virion preparations in 1% sodium dodecyl sulfate, 1% β-mercaptoethanol, and 10% glycerol was carried out as described by Patzer et al. (1978b). Cholesterol content was assayed using cholesterol oxidase (Moore et al., 1977). Virions were labeled with [^{3}H]leucine and purified as previously described (Barenholz et al., 1976).

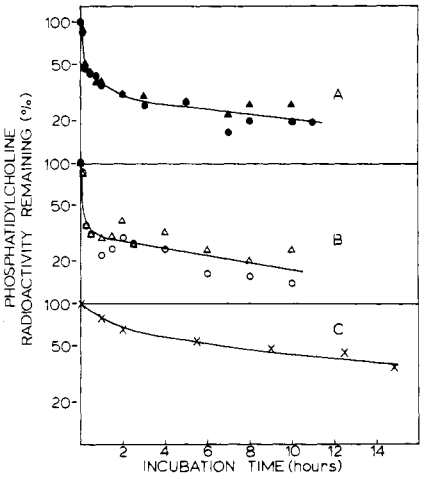

FIGURE 1: Exchange of [*choline*-^{3}H]PC between trypsinized virions and PC/SPM/cholesterol vesicles (A), trypsinized virions and PC vesicles (B), and intact virions and PC vesicles (C). Curves A and B are calculated from data obtained from spikeless virus. Curve C is from experiments on intact virus. Data points were fit to an exponential function with linear regression by the method of least squares. The coefficient of determination varied from 0.55 to 0.99 with the poorest fit occurring at longer time points. The results from three different experimental protocols are shown. (1) T-VSV (1.55 mg, 109 nmol of [*choline*-^{3}H]PC, 6 664 000 dpm/mg of protein in 2.25 mL of 20 mM Tris–0.15 M NaCl, pH 7.5) was incubated with PC vesicles (28.35 μmol of PC and containing [^{14}C]cholesterol oleate, 26 500 dpm in 1.5 mL of 10 mM Tris–0.1 M KCl, pH 7.5), 200 μg of albumin (0.2 mL), 10 mM Tris–0.1 M KCl–0.02% NaN_3, pH 7.5 (1.05 mL) in the presence or absence of exchange protein (7.4 units, 0.12 mg of albumin in 2.0 mL of 10 mM Tris–0.1 M KCl, pH 7.5) in a total volume of 8.0 mL at 37 °C). (2) Trypsinized VSV (1.32 mg, 92.4 nmol of [*choline*-^{3}H]PC, 6 664 000 dpm/mg protein in 1.1 mL of 20 mM Tris–0.15 M NaCl, pH 7.5) was incubated with PC/SPM/cholesterol vesicles (2.52 μmol of PC, 5.58 μmol of total lipid, 139 300 dpm of [^{14}C]cholesteryl oleate/μmol of total vesicle lipid in 0.394 mL of 20 mM Tris–0.1 M NaCl, pH 7.5), in the presence or absence of exchange protein (40.4 units, 0.67 mg of albumin in 6.0 mL of 10 mM Tris–0.15 M NaCl–1 mM EDTA–0.02% NaN_3, pH 7.6) in a total volume of 7.5 mL at 37 °C. After a 5-h incubation another aliquot of PC/SPM/cholesterol vesicles (0.45 μmol of PC, 0.07 mL) and 2.63 mL of exchange protein (17.7 units) or control buffer was added and the incubation continued. (3) Intact-VSV (1.094 mg, 77 nmol of [*choline*-^{3}H]PC, 6 000 000 dpm/mg of protein in 0.45 mL of 20 mM Tris–0.15 M NaCl, pH 7.5) was incubated with PV vesicles (4.37 μmol of PC and containing [^{14}C]cholesteryl oleate, 104 200 dpm/μmol of PC in 0.418 mL of 10 mM Tris–0.1 M KCl, pH 7.5), 0.5 mL of 10 mM Tris–0.1 M KCl–0.02% NaN_3, pH 7.5, in the presence or absence of exchange protein (18.5 units, 0.31 mg of albumin in 4.3 mL of 10 mM Tris–0.1 M KCl, pH 7.5) in a total volume of 5.67 mL at 37 °C. After 12.5-h incubation, 7.7 units of exchange protein, 0.12 mg of albumin, and 0.51 μmol of vesicle PC were added, and the incubation was continued. At different time periods during the incubations, aliquots were taken followed by centrifugation, isolation of virion bands or vesicles, extraction, and thin-layer chromatography of lipids, and finally scintillation counting of silica gel scrapings.

Results

Exchange of Phosphatidylcholine and Sphingomyelin between Virions and Lipid Vesicles. The interpretation of the results presented below is based on the assumption that growth of the virus in the presence of [^{3}H]choline chloride generates PC of the same specific radioactivity in both the inner and outer monolayers of the virion membrane bilayer. The

Table I: Kinetic Parameters for the Exchange of Phosphatidylcholine between Vesicular Stomatitis Virus and Lipid Vesicles[a]

virion	vesicle	rate constants (h^{-1}) k_{ves}	k_o	k_i	$k_o + k_i$	half-time[b] (h)	pool size (% total PC) outer monolayer	inner monolayer
spikeless	PC/SPM/cholesterol	3.4	0.042	0.019	0.061	11	69	31
spikeless	PC	4.9	0.062	0.028	0.090	8	69	31
intact	PC	0.16	0.055	0.040	0.095	7	58	42

[a] Kinetic parameters for the two pool open model shown in Figure 2. Rate constants, half-time for transbilayer equilibration, and pool sizes were determined exactly as described by Bloj & Zilversmit (1976). [b] For PC transbilayer equilibration.

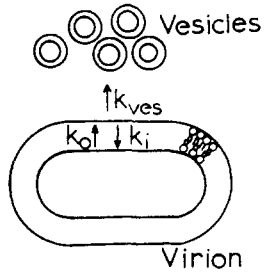

FIGURE 2: Two-pool model. The rate constants are defined in the text.

reasonableness of the assumption is supported by the work of Fong et al. (1976) who found biosynthetically introduced ^{32}P in phosphatidylethanolamine in VSV to have the same specific radioactivity in both faces of the membrane bilayer. In addition, Patzer et al. (1978b) studying the transmembrane movement of cholesterol in VSV obtained identical results with virus labeled biosynthetically by growth on ^{3}H-labeled mevalonate as with ^{14}C-labeled cholesterol introduced into the viral membrane from cholesterol-containing PC vesicles, indicating that cholesterol was also labeled with the same specific activity in both faces of the bilayer.

The rates of transfer of phosphatidyl[^{3}H]choline from both intact and spikeless virions catalyzed by the exchange protein were obtained from measurements of the loss of [^{3}H]PC from virions as well as the gain in [^{3}H]PC by the vesicles. In all experiments these two sets of data give identical results. Experiments using either PC or PC/SPM/cholesterol vesicles also gave very similar results indicating that vesicle composition did not measurably affect the transfer rates. At 37 °C about 70% of the [^{3}H]PC was transferred to vesicles from spikeless virions in about 1 h. A small additional fraction of PC was transferred during the remaining 12 h of incubation. With intact virions exchange was much slower and even after 16 h much less PC was transferred. It is interesting to note that some exchange of [^{3}H]PC from virus to vesicles occurred in the absence of exchange protein. After 10 h of incubation, this exchange, which appeared to be linear with time, was about 10% for spikeless virions and less than 5% for intact virions.

The exchange of [*choline*-^{3}H]SPM was also followed during the course of these experiments. The exchange protein transferred only small percentages of SPM in both spikeless and intact virions. This observation is in agreement with the high specificity of the beef liver protein for PC (Wirtz, 1974; Kamp et al., 1977). The rate of spontaneous exchange of SPM in the absence of exchange protein after a 10-h incubation was found to be about 15% for spikeless virions and 5% for intact virions.

Kinetics of the Exchange of Phosphatidylcholine as Catalyzed by the Exchange Protein. Figure 1 shows semilog plots vs. time of the percent exchange of [*choline*-^{3}H]PC between spikeless virions and PC/SPM/cholesterol vesicles (curve A), spikeless virions and PC vesicles (curve B), and intact virions and PC vesicles (curve C). These data have been corrected for the background exchange noted above.

The data presented in Figure 1 can be fit by the sum of two exponentials. The simplest interpretation of the overall kinetic process of exchange is a two-pool model in which the rapidly exchanging pool is assumed to be the outer monolayer of the membrane bilayer and the slowly exchanging pool the inner monolayer. The slower rate is thus identified with transposition of PC across the bilayer. The two-pool model is shown diagramatically in Figure 2. Since vesicles have been used in excess of virions, back exchange of isotopically labeled PC from vesicles to virions is essentially zero.

Rate constants, pool sizes, and half-times for PC equilibrations in the two-pool system have been determined as described by Bloj & Zilversmit (1976) based on the model shown in Figure 2. Values for the kinetic parameters and pool sizes are given in Table I. Phosphatidylcholine was found to be asymmetrically located (assuming 50% of the phospholipid is allocated to each half of the membrane bilayer) with 69% in the outer monolayer and 31% in the inner monolayer of spikeless VSV. In contrast, the pool sizes for intact VSV were 58% and 42% for outer and inner monolayers, respectively. The half-time for PC transbilayer equilibration ($t_{1/2} = 0.69/(k_o + k_i)$) varied from 7 to 11 h in the three separate experiments. It is interesting that the individual rate constants for transposition of PC between monolayers (k_o and k_i) in spikeless VSV and intact VSV were very similar. The major difference, however, can be seen in k_{ves} (Table I) which represents the rate constant for movement of PC from virion to vesicle (see Figure 2). k_{ves} for spikeless virions is 20–30 times greater than that found for intact virions. The marked variation in k_{ves} can be explained by two factors, the first being a decreased dissociation constant (k_D) of the exchange protein for the membrane of the intact virion. The source of the smaller k_D is probably the strong interaction between negatively charged terminal sialic acid residues in the glycoprotein, and the exchange protein. The phosphatidylcholine-specific exchange protein from beef liver has been reported to be inhibited with a markedly decreased k_D as the concentration of negatively charged lipid (i.e., phosphatidic acid or phosphatidylglycerol) is increased in the bilayer of PC vesicles (van den Besselaar et al., 1975; Wirtz & Moonen, 1977). The second factor which might explain the smaller k_{ves} for the intact VSV data is the presence of vesicles adsorbed to the virion surface. This problem is addressed in the next section.

Adsorption of Lipid Vesicles to Virions during PC Exchange. The adsorption of vesicles to virions was followed during the exchange of phosphatidylcholine by monitoring the amount of [^{14}C]cholesteryl oleate, a nonexchangeable vesicle marker, present in the virion band. Figure 3 shows the percentage of virion-associated lipid derived from adsorbed

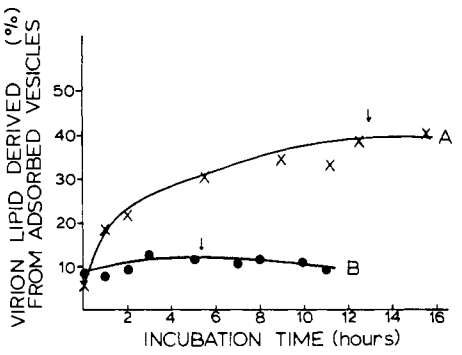

FIGURE 3: Adsorption of PC vesicles to intact virions (A) and PC/SPM/cholesterol vesicles to spikeless virions (B) during incubation in the presence or absence of exchange protein. The incubation conditions and processing of virions are described in the legend of Figure 1 and under Materials and Methods. After thin-layer chromatography of the extracted virion bands, the silica gel areas containing [^{14}C]cholesteryl oleate were scraped and counted. The dpm of [^{14}C]cholesteryl oleate in the virions bands was divided by the dpm of [^{14}C]cholesteryl oleate/nmol of vesicle lipid in the wt original vesicles to determine the nmol of vesicle lipid adsorbed. To calculate the % virion lipid derived from adsorbed vesicles, 20 wt % of the total VSV protein was taken as phospholipid and the ratio 0.72 μmol of cholesterol per μmol of phospholipid was used for determining cholesterol (Patzer et al., 1978a). Consequently, the value of 502 nmol of total lipid (cholesterol and phospholipid) per 1000 μg of protein was used in determining the % virion lipid derived from adsorbed vesicles. Arrows indicate time points when more vesicles and exchange protein were added to the incubation. The precision of the data is indicated by the symbol size.

vesicles as a function of incubation time for both spikeless (curve B) and intact virions (curve A). Lipid adhering to intact virions increased rapidly and reached a plateau at approximately 40%. No additional increase was detected after more vesicles were added at 12.5 h (arrow). In sharp contrast, spikeless virions incubated with PC/SPM/cholesterol vesicles had about 12% adhering lipid during the whole course of the incubation. Addition of fresh vesicles after 5.5 h (arrow) did not change the pattern of adsorption; this result rules out the possibility of breakdown of the [^{14}C]cholesteryl oleate in the vesicles or during incubation since free [^{14}C]cholesterol would be produced and is known to exchange rapidly between vesicles and virions (Patzer et al., 1978b). The utilization of different vesicle types in the experiments shown in curves A and B is not a satisfactory explanation for the differences in degree of absorption since PC vesicles containing cholesterol have been shown to adsorb to virions in a quantitatively similar fashion as PC vesicles (Moore et al., 1978).

The number of vesicles adsorbed per virion can be estimated from a knowledge of the number of virions per mg of protein (4.25×10^{12}) (Dr. E. Dubovi, personal communication) and the known number of lipid molecules per vesicle (3000). For spikeless VSV the average number of vesicles per virion equals 3, whereas the number of vesicles per intact virion equals 7 after 2 h and reaches a maximum value of 16 after 12 h. For intact virions, the association with vesicles has been found by electron microscopy to occur in the outer spike region without direct bilayer–bilayer contact (Moore et al., 1978). Also consistent with the presence of vesicles physically associated with intact virions is the observation by proton NMR of a virion–vesicle complex extensively purified for removal of free vesicles, that revealed broad fatty acyl methylmethylene and PC *N*-methyl resonance signals typical of model phosphatidylcholine liposomal membranes (J. M. Shaw, N. F. Moore, E. J. Patzer, and M. C. Correa-Freire, unpublished results).

The adsorption of vesicles to the virion could explain the low value of $k_{ves} = 0.16$ determined for transfer of [^{3}H]-phosphatidylcholine to vesicles. Thus the greater number of vesicles associated with the intact VSV could prevent access of exchange protein to the virion bilayer and thereby diminish transfer of PC. It is interesting to note, however, that the rate constants for transposition and $t_{1/2}$ for equilibration are almost identical for intact VSV and spikeless VSV. As would be expected, the adsorption of vesicles to virions did not alter these kinetic parameters.

Integrity of Virions during Incubation with Exchange Protein. A number of control experiments were carried out which show that during exchange experiments the viral membrane was impermeable to the 24 000-dalton exchange protein. First, the control experiments performed in the absence of exchange protein illustrate that the [^{3}H]choline-labeled PC and SPM in the virion membranes are not rapidly depleted during the time period of catalyzed exchange by the protein (at 2 h $\leq$ 5%). Second, incubations of spikeless VSV plus vesicles, spikeless VSV plus exchange protein, and spikeless VSV plus exchange protein and vesicles have been performed using similar assay conditions as described in Figure 1. Virions were isolated by gradient centrifugation then labeled by lactoperoxidase catalyzed ^{125}I iodination. $NaDodSO_4$–polyacrylamide gels of the proteins from the ^{125}I-labeled virions showed no significant differences in the small percentage of labeling of the M protein. Third, incubation of intact VSV, intact VSV plus PC vesicles, or intact VSV plus vesicles and exchange protein was carried out for 17 h at 37 °C. The virions were isolated and labeled with TNBS (Patzer et al., 1978a) and the proteins separated on $NaDodSO_4$–polyacrylamide gels. Again no significant differences in the ratio of the labeling pattern of the internal M protein to external glycoprotein among the controls were observed. Fourth, [^{3}H]leucine-labeled spikeless VSV was incubated under a variety of conditions followed by fractionation on a tartrate–glycerol gradient. Ten 0.5-mL fractions were collected and the percentage of dpm of the total on the gradient above the virion band was determined. Spikeless VSV preparations incubated at 4 °C overnight gave 3.4% of the [^{3}H]leucine dpm, whereas at 37 °C for 12 h they gave 5.8%. Spikeless VSV incubated with both PC and PC/SPM/cholesterol vesicles at 37 °C for 12 h gave 12.8% of the dpm above the virion band, whereas incubation with vesicle plus exchange protein at 37 °C for 12 h showed 11.4%. Therefore, approximately 6.3% or 8.7% of the dpms above the 37, or 4 °C control spikeless virions, respectively, must represent small percentages of virion breakdown.

Discussion

In this study of VSV membranes, the pool sizes of exchangeable PC and the inferred compositional asymmetry of the phospholipid are dependent upon the presence or absence of the glycoprotein spikes of the G protein. The more asymmetric composition was found with the spikeless virion (69% of the PC in the outer monolayer). In contrast, the intact virion showed the outer monolayer of the membrane bilayer to contain 58% of the total PC. The data obtained with the intact virus are less certain, however, because of the markedly reduced exchange rate (k_{ves}). This reduction is quite probably due to the presence of sialic acid residues on the glycoprotein resulting in either a decreased k_D, the dissociation constant for the virus–exchange protein complex, or increased adsorption of vesicles to the virus. Adsorption would be expected to not only decrease the apparent specific radioactivity of the [^{3}H]choline-labeled PC of the virus, but also to decrease for steric reasons the availability for exchange of virus PC (Patzer et al., 1978b; Moore et al., 1978). It is also possible that the

Table II: Half-Times for Transbilayer Migration of PC and PC Analogues in Biological Membranes

	membrane system	method	$t_{1/2}$	molecule studied	temp (°C)
1.	influenza A virus[a]	exchange protein	>10 days	PC	37
	LM cell plasma membrane[b]		3.7 days	PC	37
2.	vesicular stomatitis virus[c]	exchange protein	7–11 h		37
	human erythrocytes[d]	spin-label reduction	+ ascorbate, 7 h	spin-labeled PC	37
			− ascorbate, not detected	spin-labeled PC	37
	rat erythrocytes[e]	phospholipase digestion	4.5 h	PC	37
	right-side out ghosts[f]	exchange protein	2.3 h	PC	37
	inside out ghosts[g]	exchange protein	5.3 h	PC	37
	inner mitochondrial membrane[h]	spin-label reduction	+ ascorbate, 4 h	spin-labeled PC	22
			− ascorbate, 25 h	spin-labeled PC	22
3.	vesicles from *E. electricus*[i]	spin-label reduction	+ ascorbate, 4–7 min	spin-labeled PC	15
	Acholeplasma laidawii[j]	spin-label reduction	+ ascorbate, >1 min	spin-labeled PC	0
	Bacillus megaterium[k]	TNBS labeling	3 min	PE	37
	rat liver microsomes[l]	exchange protein	complete exchange, <1 h	PE, PC, PI, PS	30
	rat liver microsomes[m]	exchange protein	complete exchange, <1 h	PC	25, 37

[a] Rothman et al., 1976. [b] Sandra & Pagano, 1978. [c] This report. [d] Rousselet et al., 1976a. [e] Renooij et al., 1976. [f] Bloj & Zilversmit, 1976. [g] Bloj & Zilversmit, 1976. [h] Rousselet et al., 1976b. [i] McNamee & McConnell, 1973. [j] Grant & McConnell, 1973. [k] Rothman & Kennedy, 1977. [l] Zilversmit & Hughes, 1977. [m] van den Besselaar et al., 1978.

glycoprotein spikes mask a fraction of the membrane PC and thus make it less available for exchange.

These results are in qualitative agreement with data obtained in an earlier study using phospholipase C digestion. By this method, however, 94% of the total PC was found to be localized in the outer monolayer of the VSV membrane. This is a larger fraction of PC than was found in the present study using the phosphatidylcholine exchange protein. The reasons for the discrepancy are not known, although two possible factors are evident. On the one hand, the results obtained with the exchange protein could be an underestimate because of the problems of vesicle adhesion to virus and virus–exchange protein interaction discussed above. However, vesicle adhesion was minimal in the spikeless preparations. On the other hand, the results obtained with phospholipase C could be an overestimate of the externally localized PC resulting from reorganization of lipid components caused by the presence of hydrolysis products in the viral membrane bilayer. Recently it has been shown that transbilayer migration of lipid components is considerably enhanced by the presence of lipid degradation products in simple bilayer systems (Shaw & Thompson, 1978). Additional support for this interpretation is found in the recent report by Sundler et al. (1978) in which it was shown that restriction of phospholipase C activity to the outer monolayer lipids of single-lamellar vesicles occurs only when the mole fraction of substrate lipids is less than 0.2. In the VSV membrane the mole fraction of substrate lipids for this enzyme is greater than 0.7 (Patzer et al., 1978a). The discrepancy in the results obtained with VSV using the exchange protein and phospholipase C are in contrast to the good agreement in the results obtained by these two methods when applied to influenza virus by Rothman & co-workers (1976). Presumably in this study they did not encounter the problem of vesicle adhesion to influenza virus during exchange, since the matter was not discussed in their article. In this regard it is perhaps significant that influenza virus contains a membrane-bound neuroninidase and sialic acid is not present in the glycoprotein and glycolipid of the virus membrane (Compans & Choppin, 1975). It should be noted that even trypsin treatment to remove the glycoprotein spikes of VSV does not remove all the sialic acid from the virus, since the VSV membrane is known to contain a small amount of ganglioside when grown in BHK-21 cells (Stoffel et al., 1975; Klenk & Choppin, 1971).

The half-times for transbilayer movement of phosphatidylcholine and phosphatidylcholine analogues in biological membranes have been determined in a limited number of systems. The results are summarized in Table II. It is clear that, depending upon the biological membrane system studied, the values range from several days or more down to minutes. Generally in simple bilayer systems the values of measured half-times are of the order of days. The half-time of 7–11 h we have found in our study of VSV is considerably shorter than the value of 10 days or greater reported by Rothman & co-workers (1976) for influenza virus. Our value is, however, similar to certain of the results in group 2 of Table II obtained for erythrocyte and mitochondrial inner membranes. The half-time for PC transposition is also similar to the value obtained for cholesterol migration in VSV in an earlier study (Patzer et al., 1978b).

The transposition rate of phosphatidylcholine in the VSV membrane determined in this study is in fact a self-transposition rate for this single chemical species. This rate is rapid compared with the duration of time over which the transmembrane compositional asymmetry of this phospholipid persists. Since the virus is biosynthetically incompetent, maintainence of the compositional asymmetry must depend upon interactions with other membrane components. These interactions could result in either an equilibrium or a kinetically limited distribution of components characterized by compositional asymmetry. The fact that the self-transposition rates for both PC and cholesterol in VSV are very rapid does not by itself establish that the observed transbilayer compositions of all components are in equilibrium. The compositional asymmetry could be a kinetically limited configuration. It is obvious that, for decay of the transbilayer compositional asymmetry, mixing across the bilayer of different chemical species must occur; however, the components with the slower self-transposition rates would dominate the overall rate for the mixing of all chemical species in the bilayer. Thus, for example, since the rates for transbilayer movement of the protein components of plasma membranes are immeasurably slow (Rothman & Lenard, 1977), the transmembrane compositional asymmetry of these protein components could impose compositional asymmetries on other components which exhibit rapid self-transposition from one bilayer face to the other. It is apparent that considerably more information must be obtained before the time-average compositional structure of the simple vesicular stomatitis virus membrane can be fully understood.

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