The Role of the Ganglioside G_{D1a} as a Receptor for Sendai Virus†

Richard M. Epand,*,‡ Shlomo Nir,§ Michelle Parolin,‡ and Thomas D. Flanagan||

Department of Biochemistry, McMaster University, Health Sciences Centre, Hamilton, Ontario, Canada L8N 3Z5, The Seagram Centre for Soil and Water Sciences, Faculty of Agriculture, The Hebrew University of Jerusalem, Rehovot 76100, Israel, and Department of Microbiology, School of Medicine and Biomedical Sciences, State University of New York at Buffalo, Buffalo, New York 14214

Received August 11, 1994; Revised Manuscript Received November 2, 1994⊗

ABSTRACT: The ganglioside G_{D1a}, which serves as a receptor for Sendai virus, also affects lipid polymorphism as determined by ^{31}P nuclear magnetic resonance. The ganglioside promotes the formation of isotropic structures in monomethyldioleoylphosphatidylethanolamine. G_{D1a} also raises the bilayer to hexagonal phase transition temperature of this lipid. The effects of G_{D1a} on the kinetics of viral fusion can be understood on the basis of its role in facilitating the binding of Sendai virus to target membranes as well as its effects on membrane physical properties. Fusion of Sendai virus with liposomes composed of egg phosphatidylethanolamine is particularly sensitive to the presence of ganglioside. In the absence of ganglioside no fusion is observed due to the absence of virus binding to the target membrane. Between 2 and 6 mol % G_{D1a} in egg phosphatidylethanolamine liposomes there is a marked increase in the rate constant of binding of the virus to the liposome but a decrease in the fusion rate constant. The latter effect is found to be common to a number of other amphiphiles that raise the bilayer to hexagonal phase transition temperature. The ganglioside enhances virus binding to liposomes of all the compositions studied, but leakage rates and fusion rate constants are either unaffected or reduced. In the systems studied, the enhanced formation of isotropic structures in liposomes containing the ganglioside does not enhance the kinetics of the actual fusion reaction.

The mechanisms by which receptors affect the rate and extent of viral fusion are not yet very well resolved. Clearly, one function of viral receptors is that of promoting the binding of the virus to the target membrane. However, even this function has to be qualified, since viruses can bind to target membranes devoid of known receptors. Viral receptors also differ greatly in their affinity and specificity for binding to viruses. One of the more specific and higher affinity viral receptors is the CD4 glycoprotein, a receptor for HIV (Lasky et al., 1987). However, even in this case, HIV can also infect cells that do not contain detectable levels of CD4 protein or its mRNA (Cheng-Mayer et al., 1987; Tateno et al., 1989). Conversely there are CD4 expressing cells that cannot be infected by HIV (Maddon et al., 1986; Tersmette et al., 1989). Thus although the presence or absence of viral receptors can often explain viral tropism, these receptors are neither necessary nor sufficient for viral infectivity. We wished to systematically study the role of viral receptors in different membrane environments to assess their effect on virus binding and subsequent fusion to model liposomes.

There have been a number of studies identifying gangliosides as receptors for the fusion of Sendai virus to target membranes (Haywood, 1974a,b; Markwell et al., 1981; Suzuki et al., 1985; Slepushkin et al., 1988). However, an indication that gangliosides alter viral fusion processes by other means, in addition to serving as receptor binding sites, comes from the observation of Tsao and Huang (1985) that the effects of ganglioside on viral-induced vesicle leakage is biphasic. Low concentrations of G_{D1a} promote Sendai-induced membrane lysis but high concentrations of ganglioside inhibit this process. One possible explanation for this biphasic effect is that, in addition to serving as a binding site for Sendai virus, the inclusion of gangliosides to the membrane alters the physical properties of the membrane (Tsao et al., 1987; Ollmann & Galla, 1988). It has recently been observed that G_{D1a} can promote the formation of isotropic phases in phosphatidylethanolamine (Van Gorkom et al., 1991). The formation of such structures has been associated with increased rates of membrane fusion (Ellens et al., 1989). In addition gangliosides are known to form micelles and will therefore affect monolayer curvature in a manner to inhibit the formation of inverted phases. Substances which inhibit inverted phase formation are often found to be antiviral agents and may inhibit membrane fusion (Epand, 1992). We have thus undertaken a study of the effects of G_{D1a} on lipid phase behavior using ^{31}P NMR, on bilayer stability as monitored by vesicle leakage rates, and on the rate of Sendai virus binding to and fusing with liposomes.

It has been deduced (Nir et al., 1988; Stegmann et al., 1989) that the incorporation of 5 mol % of the ganglioside G_{D1a} in liposomes consisting of PC/PE with or without cholesterol resulted in a 3-fold enhancement in the forward rate constant of influenza virus binding. The percents of virions fusing and the fusion rate constants were not affected by the ganglioside. Recently, Alford et al. (1994) concluded

† This work was supported by the Medical Research Council of Canada, Grant MA-7654.

* To whom correspondence should be addressed. Telephone: (905) 525-9140. ext. 22073. Fax: (905) 522-9033. E-mail: epand@fhs.csu.mcmaster.ca.

‡ McMaster University.

§ The Hebrew University of Jerusalem.

|| State University of New York at Buffalo.

⊗ Abstract published in *Advance ACS Abstracts,* December 15, 1994.

0006-2960/95/0434-1084$09.00/0

that when the HA protein of influenza virus was bound to ganglioside it became incapable of participating in fusion. In the case of influenza virus, the same HA protein serves both to bind to the viral receptor as well as to promote membrane fusion. Sendai virus, studied in the present work, is different in that distinct proteins are involved in receptor binding (HN protein) and membrane fusion (F protein). In addition, influenza virus represents an example of acid-induced viral fusion, while Sendai virus fuses with membranes at neutral pH. We will demonstrate that, in the case of Sendai virus, there are different effects of gangliosides on binding and on viral fusion.

EXPERIMENTAL PROCEDURES

Chemicals. All phospholipids were obtained from Avanti Polar Lipids (Alabaster, AL). Gangliosides were purified according to Reed et al. (1987). All lipids showed one spot by TLC at a load of 50 μg. Fluorescent probes were purchased from Molecular Probes (Eugene, OR). All other chemicals and solvents were of reagent grade.

Purification of Sendai Virus. The Cantell strain of Sendai virus was propagated by inoculation of the allantoic sac of 10-day-old embryonated chicken eggs. After 72 h of incubation at 33 °C, the allantoic fluid was harvested and clarified by centrifugation at 3000*g* for 30 min at 4 °C. The virus was pelleted at 60000*g* for 1.5 h at 4 °C. The pellet was dispersed in phosphate buffered saline and centrifuged through a discontinuous gradient of 35%, 40%, 45%, and 60% sucrose at 60000*g* for 2.5 h at 4 °C. The virus was harvested from the band that formed at the 40−45% interface. The virus was pelleted at 60000*g* for 1.5 h and resuspended in HEPES[1] buffered saline, pH 7.4, to a viral protein concentration of 1 mg/mL.

Preparation of Liposomes. Phospholipid and G_{D1a} were dissolved in a solution of chloroform/methanol, 2/1 (v/v). The solvent was evaporated with a stream of dry nitrogen gas, depositing the lipids as a film on the walls of a 13 × 100 mm Pyrex test tube. Samples were placed in a vacuum evaporator equipped with a liquid nitrogen trap for 2−3 h to remove the last traces of solvent. The dried lipid film was suspended by vigourous vortexing at about 45 °C with 5 mM HEPES, 5 mM MES, 5 mM sodium citrate, 150 mM NaCl, and 1 mM EDTA at pH 7.4 (HEPES/MES buffer). These multilammelar vesicle suspensions were used directly for ^{31}P NMR measurements. For studies of liposome leakage and viral fusion, the pH of the buffer was raised to 10.0 for hydration of lipid films not containing ganglioside. This is required in order to be able to extrude these suspensions but is not required for ganglioside-containing vesicles. The lipid suspensions were further processed with five cycles of freezing and thawing, followed by 10 passes through two stacked 0.1 μm polycarbonate filters (Nucleopore) using an extrusion device (Lipex Biomembranes). Vesicles extruded at alkaline pH were reacidified to pH 7.4. Ganglioside-containing vesicles gave similar results in viral fusion studies whether extruded at pH 7.4 or at pH 10. Lipid phosphorus was determined by the method of Ames (1966). Liposome size was determined by quasielastic light scattering and was found to be approximately 115 nm.

^{31}P NMR. Lipid films containing the desired amount of ganglioside were prepared as described above and hydrated with the HEPES/MES buffer at pH 7.4 and a lipid concentration of approximately 150 mg/mL. The samples were freeze−thawed three times and then used directly for the NMR experiment. The spectra were obtained using a Bruker AM-500 spectrometer operating at 202.45 MHz with broad band proton decoupling. The temperature was maintained within ±0.1 °C with a Bruker B-VT 1000 variable temperature unit. Exponential line-broadening of 100 Hz was applied prior to Fourier transformation.

Leakage Assay. All fluorescence measurements were made on an SLM Series 2 fluorimeter. The ANTS/DPX leakage assay was carried out as described by Ellens et al. (1985). Large unilamellar vesicles were prepared by extrusion as described above. Vesicles contained 12.5 mM ANTS, 45 mM DPX, 22.5 mM NaCl, and 10 mM glycine, pH 10.0. LUVs were separated from nonentrapped probes by passage down a Sephadex G-75 column, eluting with 100 mM NaCl, 10 mM glycine, and 0.1 mM EDTA, pH 10. The eluted vesicles were monitored for the ratio of phosphate to ganglioside to ensure that no ganglioside was extracted during the extrusion or gel filtration. The ganglioside was monitored by measuring the sialic acid content of the eluent (Svennerholm, 1957). No loss of ganglioside to the column was observed. Fluorescence intensity was monitored with an excitation wavelength of 360 nm and an emission wavelength of 530 nm. The assay was performed using 50 μM lipid. Leakage was initiated by lowering the pH from 10 to 7.4 with the addition of a small volume of citric acid or by raising the temperature. Leakage was measured by loss of DPX quenching of the ANTS fluorescence due to leakage and dilution. The 100% leakage, F_{100}, was obtained after addition of 40 μL of 10% (v/v) Triton X-100 to the 2 mL sample in the fluorimeter cuvette.

Virus Fusion Assay. Sendai virus was labeled with octadecyl rhodamine (R18) (Molecular Probes) according to the procedure of Hoekstra et al. (1984). Ten microliters of R18 (10 nmol) in ethanol was injected into 1 mL of a suspension of Sendai virus in HEPES/MES buffer. The mixture was allowed to incubate at room temperature for 1 h. Unincorporated R18 was then removed by passing the labeled virus through a Sephadex G-75 gel filtration column eluted with the HEPES/MES buffer and collecting the virus in the void volume. The final viral protein concentration was determined using the BCA assay (Pierce Chemical Co., Rockford, IL). LUVs were diluted into 2 mL of HEPES/MES buffer, pH 7.4, maintained in a thermostated cuvette holder at 37 °C with continual magnetic stirring. Five micrograms of R18-labeled Sendai virus was rapidly injected into the cuvette through a light sealed septum. Fluorescence was recorded using an SLM AMINCO Bowman Series 2 Luminescence Spectrometer interfaced with a 386/20 IBM compatible computer. The instrument used a xenon arc light source with a 560 nm filter between the excitation slit and sample and a 590 nm cutoff filter between the sample and the photomultiplier tube to minimize any contribution of light scattering to the fluorescence signal. The excitation and emission monochromators were set at 565 and 600 nm,

[1] Abbreviations: ANTS, 8-aminonaphthalene-1,3,6-trisulfonic acid; DPX, *para*-xylene-bispyridinium bromide; H_{II}, inverted hexagonal phase; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*'-(2-ethanesulfonic acid); MeDOPE, *N*-methyldioleoylphosphatidylethanolamine; LUV, large unilamellar vesicle; MES, 2-(*N*-morpholino)ethanesulfonic acid); PC, phosphatidylcholine; PE, phosphatidylethanolamine; R18, octadecyl rhodamine B chloride; T_H, L_α to H_{II} phase transition temperature.

respectively. The fluorescence intensity immediately after addition of the labeled virus is taken as F_0. A 40 μL aliquot of 10% Triton X-100 was added in order to measure F_{100}. The percent of R18 dequenching was calculated at time t from

$$\% \text{ R18 dequenching} = 100(F_t - F_0)/(F_{100} - F_0)$$

For kinetic experiments, the fluorescence was recorded over the first 10 min after initiation of fusion. For the final extents of fusion, the cuvettes were wrapped in foil and the fluorescence was measured after 8 h of incubation at 37 °C in a shaking water bath.

Analysis of Viral Fusion Kinetics. The analysis of final extents of fusion of Sendai virus with liposomes was done as previously described (Nir et al., 1986a,b (1990). In the analysis of the kinetics of fusion we have employed three parameters: C, the second-order rate constant of virus adhesion to cells or to liposomes; f, the first-order rate constant of the actual fusion of an adhered virus particle; D, the first-order dissociation rate constant.

Viral Adhesion to Liposomes. Liposome-bound virus was separated from free virus by centrifugation through sucrose. Sendai virus was labeled with R18 as described above. The labeled virus was separated by gel filtration. Five micrograms of the labeled virus was incubated on ice in 2 mL of HEPES/MES buffer, pH 7.4, containing 50 μM phospholipid with or without 6 mol % G_{D1a} in the form of LUVs for 1 h. The solution was then layered on top of 5 mL of 30% sucrose which in turn had been layered, as a discontinuous gradient, over 40% sucrose. These tubes were then centrifuged at 22.5K rpm for 2 h at 4 °C in an SW41 Ti rotor together with virus and lipid controls. The resulting tubes were fractionated into a pellet fraction and fractions corresponding to the lower 30−40% sucrose interface and that of the upper buffer−30% interface. Triton solution was added to each of these fractions to a final concentration of 0.5%. The fluorescence emission intensity was then measured as described above for the viral fusion assay.

RESULTS

Lipid Polymorphism. The ^{31}P NMR powder patterns are characteristic for different lipid phases (Tilcock et al., 1986). In particular, the bilayer powder pattern has a chemical shift anisotropy (width) of about 45 ppm and has a low field shoulder. This is in contrast to the H_{II} phase, which has a high field shoulder and a chemical shift anisotropy of about 20 ppm. In addition, several different kinds of lipid structures, including small vesicles, cubic phases, and others, give rise to narrow isotropic resonances centered at 0 ppm. Structures suggested to be intermediates in membrane fusion are likely to have high curvature and therefore give rise to isotropic resonance lines (Siegel et al., 1989).

We had demonstrated earlier that the ganglioside G_{D1a} induces isotropic ^{31}P NMR resonances in phophatidylethanolamine (PE) but not in phosphatidylcholine (PC) multilammelar vesicles (Van Gorkom et al., 1991, 1994). In the present work we have extended these studies to investigate the effects of G_{D1a} on *N*-methyldioleoylphosphatidylethanolamine (MeDOPE), a lipid which readily forms cubic phases, even as a single component phospholipid (Gruner et al., 1988; Siegel & Banschbach, 1990). As found previously with egg PE, G_{D1a} also markedly facilitates the formation of isotropic phases in MeDOPE (Figure 1). In addition, at 6 mol % G_{D1a} it is evident that the bilayer phase is stable to higher temperatures. Thus at 65 °C there is a substantial amount of bilayer phase still remaining in the sample with 6% ganglioside but not in the sample with pure MeDOPE, and only a small amount is present in the 2% G_{D1a} sample. Thus G_{D1a} has the combined effect of raising the temperature of conversion to nonlamellar phases as well as promoting the formation of isotropic phases.

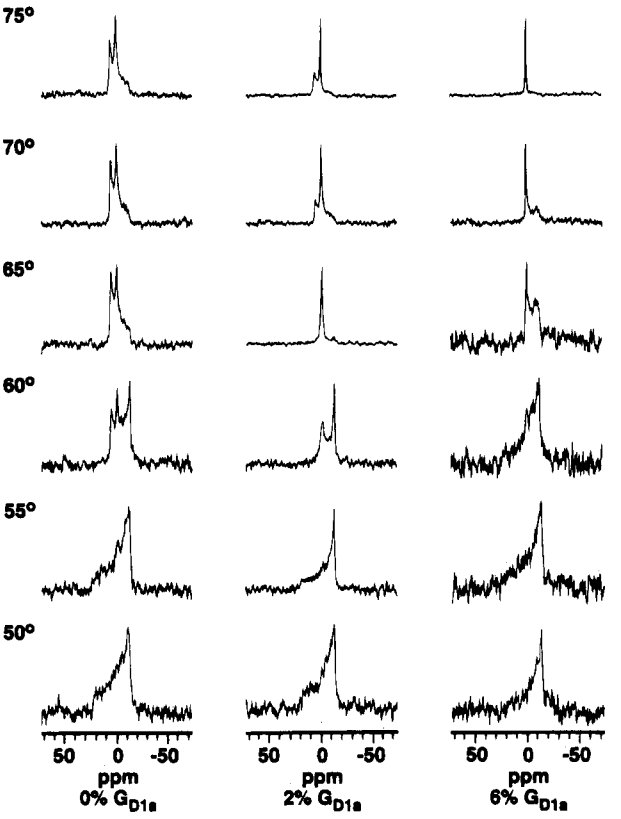

FIGURE 1: ^{31}P NMR of MeDOPE with varying mol % of G_{D1a} as a function of temperature.

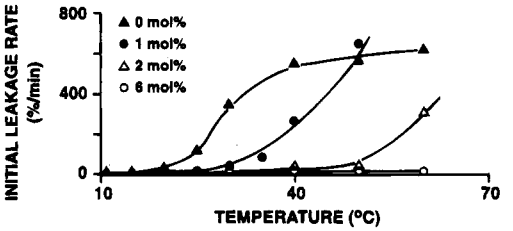

FIGURE 2: ANTS/DPX leakage from LUV of egg PE (▲) or egg PE containing 1 (●), 2 (△) or 6 (○) mol % G_{D1a}. Initial leakage rates are recorded as a function of temperature. Leakage was monitored at pH 7.4 using 50 μM lipid.

Liposome Contents Leakage. A comparison of the leakage rates of vesicles of different compositions is an indication of the stability of these structures. Defects that increase the rate of leakage may also facilitate viral fusion. Egg PC shows no substantial leakage either in the presence or absence of 2 mol % G_{D1a} in the entire temperature range from 10 to 70 °C (not shown). However, both egg PE (Figure 2) and MeDOPE (Figure 3) exhibit substantial rates of leakage at higher temperatures. This leakage is drastically depressed by the presence of ganglioside. Two effects of G_{D1a} on membrane physical properties may affect leakage rates in opposite ways. The promotion of isotropic phases may

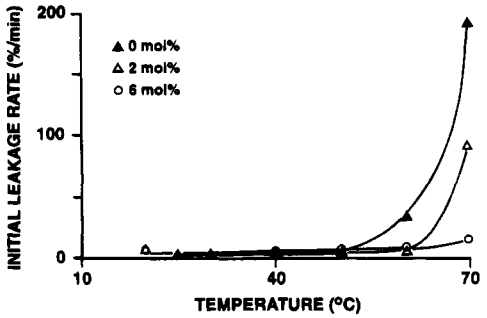

FIGURE 3: ANTS/DPX leakage from LUV of MeDOPE (▲) or MeDOPE containing 2 (△) or 6 (○) mol % G_{D1a}. Initial leakage rates are recorded as a function of temperature. Leakage was monitored at pH 7.4 using 50 μM lipid.

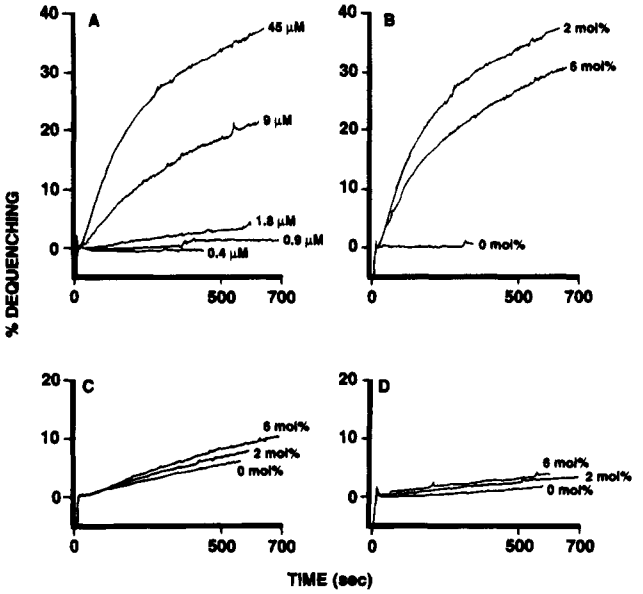

FIGURE 4: Sendai virus fusion with LUV measured by percent dequenching of the fluorescent probe, R18, initially in the viral membrane. The buffer was MES/HEPES, pH 7.4, 37 °C. Sendai virus concentration was 5 μg of viral protein in a final volume of 2.0 mL. (A) Fusion with LUV composed of egg PE with 2 mol % G_{D1a} as a function of lipid concentration. In panels B, C, and D, lipid concentration was 45 μM with varying mol % G_{D1a}. (B) Egg PE; (C) MeDOPE; (D) Egg PC.

facilitate the breakdown of the bilayer permeability barrier, but the decrease in negative curvature strain, indicated by the ability of G_{D1a} to raise the temperature of transition to the H_{II} phase, would have the opposite effect. Apparently the later is the predominant effect.

Sendai Virus Fusion. The kinetics and final extents of fusion of R18-labeled Sendai virus with LUVs of varying phospholipid composition were measured. We have previously determined that trypsinized Sendai virus does not fuse with LUVs composed of egg PE with 5 mol % G_{D1a} and that dithiothreitol-treated Sendai virions exhibited only very low levels of fusion with these liposomes (Cheetham et al., 1994). Thus probe exchange does not occur rapidly in our system, and this lipid dilution assay can be used as a measure of membrane fusion. The dependence of the fusion kinetics on the concentration of LUVs is shown for the case of liposomes composed of egg PE with 2 mol % G_{D1a} (Figure 4A). At the higher lipid concentration of 45 μM a comparison was made of the effect of ganglioside on the fusion of Sendai virus with LUVs composed of the three different phospholipids used in the present study (Figure 4B–D). It can be qualitatively appreciated that the ganglioside has the most dramatic effect on the fusion of Sendai with liposomes composed of egg PE and the least effect with those of MeDOPE. Furthermore, with egg PE, increasing the ganglioside concentration in the liposomes from 2 to 6 mol % actually reduced the rate of viral fusion with the liposomes (Figure 4B). Results from fusion studies of this sort at different virus to liposome ratios as well as measurements of the final extent of fusion after 8 h of incubation were combined and analyzed by the mass action model. A summary of the calculated parameters is given in Table 1.

Table 1: Effect of Lipid Composition on the Kinetics of Sendai Virus Fusion with LUVs[a]

phospholipid	mol% G_{D1a}	% virus capable of fusing	C (M^{-1} s^{-1})	f (s^{-1})
egg PC	0	30 ± 10	ND	ND
	2	35 ± 10	ND	ND
	6	40 ± 10	3×10^7	0.001
MeDOPE	0	40 ± 10	10^7	0.0012
	2	50 ± 10	4×10^7	0.0012
	6	50 ± 10	10^8	0.0012
egg PE	2	60 ± 10	1.8×10^7	0.015
	6	65 ± 10	1.4×10^8	0.005

[a] Percent virus capable of fusing is calculated from a representative series of measurements at different ratios of virus to target membrane. C is the second-order rate constant for the association of virus and LUV and has an estimated uncertainty of 30%, and f is the first-order fusion rate constant for the fusion of bound virus to LUV. It has an uncertainty of 30%. The rate constant for the dissociation of liposome-bound virus, D, is set at 0.02 s^{-1} with an uncertainty of 50%. ND, not determined.

Viral Adhesion to Liposomes. Separation of free virus by centrifugation through sucrose demonstrated that it was mostly in the pellet fraction. The fraction corresponding to the 30–40% sucrose interface contained 9% of the total virus for all samples, including virus in the absence of liposomes, except for egg PE with 6% G_{D1a} which had 11% of the total virus at this location. The amount of virus at the buffer to 30% sucrose interface was 1% for virus alone, 5% for egg PE, 20% for egg PE with 6% G_{D1a}, and 9% for both PC and PC plus 6% G_{D1a}. This upper fraction clearly contains a virus whose density is lowered by binding to liposomes. These results confirm those of the kinetic analysis with regard to the conclusion that the ganglioside most markedly affects the binding of Sendai virus to egg PE and that egg PE in the absence of ganglioside binds less virus than liposomes of other lipid composition.

DISCUSSION

The ganglioside G_{D1a} promoted binding of Sendai virus to liposomes composed of either MeDOPE or egg PE (Table 1). For egg PC or egg PE liposomes interacting with the virus the rate of dequenching in the absence of ganglioside was too slow to be adequately analyzed by the mass action scheme. This result is expected as G_{D1a} serves as a receptor for Sendai virus and may be required for virus binding to liposomes of certain lipid compositions.

The observed rates of Sendai fusion were rapid with LUVs of MeDOPE, either in the presence or in the absence of G_{D1a} (Figure 4C). These results confirm that a receptor is not absolutely required for viral fusion to a target membrane (Klappe et al., 1986; Nir et al., 1986a; Fonteijn et al., 1992; Yeagle, 1993). The results of the mass action analysis clearly

show that addition of ganglioside to liposomes of MeDOPE can further increase the observed rate of fusion by promoting the binding of Sendai without affecting the fusion rate constant (Table 1). Separation of effects on binding and fusion is in accord with the observations that the HN protein is required for virus binding to sialic acid residues (Hsu et al., 1979) while a different protein, the F protein, is required for membrane fusion (Scheid & Choppin, 1974). This is in contrast to the case of influenza virus in which one protein performs both functions. It was suggested that participation of this protein in binding eliminates its participation in membrane fusion (Ellens et al., 1990; Bentz et al., 1990). It should be pointed out that the HN protein of Sendai virus may also have an effect on fusion (Yeagle, 1993).

The rate constant of virus binding to LUVs containing 6 mol % ganglioside increases from egg PC to MeDOPE or egg PE, with the latter two lipids having comparable rates (Table 1). This is the same order as for the stability of the LUVs against leakage, with the least stable liposome having the highest rate of association with the virus. There is also a correlation between the bilayer to hexagonal phase transition temperature (T_H) and the rate constants C and f. Egg PC does not convert to the hexagonal phase and has a lower value for C and f than either MeDOPE or egg PE, which have T_H at about 65 and 35 °C, respectively. The slowest rate of virus association and fusion is with egg PC which has the least tendency to form nonlamellar phases.

At 6% ganglioside, the fusion rate constant, f, exhibits a similar trend to the association rate, but the effects are somewhat larger and the largest change in f is between MeDOPE and egg PE. This is in agreement with the suggestion that lipids with a propensity to form inverted phases promote the rate of membrane fusion (Glaser & Gross, 1994; Zimmerberg et al., 1993). This is not because such inverted phases are fusion intermediates. These inverted phases require bilayer−bilayer contact in order to form and are not observed in LUVs. However, the overall fusion process is facilitated by the presence of lipid with spontaneous monolayer curvatures that also favor the formation of inverted phases. This may be a consequence of the morpholgy of fusion intermediates (Siegel, 1993).

It is interesting that although egg PE shows the highest rates of fusion among the three lipids (Table 1), no fusion is observed with this lipid in the absence of G_{D1a}, even less than in the case of egg PC in the absence of ganglioside (Figure 4). This can be explained by the marked dependency of virus binding to egg PE liposomes on the content of G_{D1a} as shown by the kinetic analysis (Table 1) as well as by direct binding studies using centrifugation. Aggregation of egg PE vesicles may also contribute to the lack of interaction with virus. Although quasielastic light scattering measurements indicated that the size of egg PE liposomes was independent of ganglioside concentration, there may be an undetected small fraction of aggregated liposomes which forms in some cases upon acidification or which forms with increasing time. We also measured the tendency of egg PE liposomes to undergo vesicle−vesicle fusion. We mixed a population of LUV of egg PE containing 5 mol % R18 with a 5-fold excess of unlabeled LUV of egg PE giving a final lipid concentration of 50 μM. The rate of R18 dequenching was only 2% over 500 s. Thus, the inability of Sendai to fuse to liposomes of this lipid composition also is not a consequence of these vesicles fusing with themselves. For egg PE liposomes with or without G_{D1a}, the fusion rate constant increases as the ganglioside content is lowered. Thus for this lipid, addition of ganglioside causes virus binding and fusion rates to change in opposite directions. We suggest that the fusion rate constant is highest for egg PE in the absence of ganglioside but that no fusion is observed under this condition since there is insufficient binding of the virus to liposomes composed of this lipid. This may be a consequence of the fact that PE membranes are the most dehydrated and have interlipid hydrogen bonds that would inhibit the interaction of these LUVs with viral proteins. Although there is a trend for higher fusion rates of the virus with membranes having more curvature strain, the major difference is between MeDOPE and egg PE. For example, at 2 mol % G_{D1a} there is a 10-fold increase in f between these two lipids. However, although the propensity to form H_{II} phases is much greater for MeDOPE than for egg PC, there is little difference in f between these two lipids. This may reflect the fact that, as previously suggested (Lemay et al., 1994), the insertion of the viral fusion protein produces sufficient bilayer destabilization that the system is insensitive to the presence of lipids that promote inverted phases. This apparently is not the case when one uses a very highly strained bilayer such as egg PE, but in such systems it is often difficult to measure fusion because of the lower binding of the virus to the target membrane.

Gangliosides have two effects on lipid polymorphism: they promote the formation of isotropic structures [Figure 1 and van Gorkom et al. (1995)] and they raise T_H. Isotropic structures have been correlated with increased rates of membrane fusion in certain cases (Ellens et al., 1989). However, observing a motionally averaged ^{31}P NMR powder pattern is not an indication of a very specific kind of structure. In the present case the increased amount of isotropic signals observed with the ganglioside does not correlate with increased rates of fusion. This is evident with increasing concentration of G_{D1a} which increases the amount of isotropic signals in MeDOPE and egg PE but does not increase the rate of fusion. Thus either the conditions that give rise to isotropic ^{31}P NMR resonances are not necessarily those that promote the fusion of LUVs, or only specific kinds of structures that exhibit such powder patterns are functionally correlated with increased fusion rates. Gangliosides also raise T_H and this does correlate with the inhibition of membrane fusion in the case of egg PE (Table 1). It appears that compounds that raise T_H, such as G_{D1a}, are most effective in reducing the rate of viral fusion in systems that have a high tendency to convert to inverted phases. It is interesting that a variety of biological fusion events are also sensitive to inhibition by this type of compound (Epand, 1992; Chernomordik et al., 1993). This may be a consequence of an apparent adjustment in the lipid composition of the membranes of certain cells to be in a state close to T_H (Rilfors et al., 1994).

In summary, gangliosides have a primary role to enhance the binding of Sendai virus to target membranes. In addition, with liposomes composed of egg PE, increasing the ganglioside content from 2 to 6 mol % inhibits the fusion rate, presumably as a consequence of the effect of ganglioside on the physical properties of the membrane.

REFERENCES

Alford, D., Ellens, H., & Bentz, J. (1994) *Biochemistry 33*, 1977−1987.

Ames, B. N. (1966) *Methods Enzymol. 8*, 115−118.

Bentz, J., Ellens, H., & Alford, D (1990) *FEBS Lett. 276*, 1−5.

Cheetham, J. J., Nir, S., Johnson, E., Flanagan, T. D., & R. M. Epand (1994) *J. Biol. Chem. 269*, 5467−5472.

Cheng-Mayer, C., Rutka, J. T., Rosenblum, M. L., McHugh, T., Stites, D. P., & Levy, J. A. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 3526−3530.

Chernomordik, L. V., Vogel, S. S., Sokoloff, A., Onaran, H. O., Leikina, E. A., & Zimmerberg, J. (1993) *FEBS Lett. 318*, 71−76.

Ellens, H., Bentz,, J., & Szoka, F. C. (1985) *Biochemistry 24*, 3099−3106.

Ellens, H., Siegel, D. P., Alford, D., Yeagle, P. L., Boni, L. J., Lis, L. J. Quinn, P. J., & Bentz, J. (1989) *Biochemistry 28*, 3692−3703.

Ellens, H., Bentz, J., Mason, D., Zhang, F., & White, J. (1990) *Biochemistry 29*, 8697−9707.

Epand, R. M. (1992) in *Membrane Interactions of HIV: Implications for Pathogenesis and Therapy in AIDS* (Aloia, R. C., Ed.) pp 99−112, Wiley-Liss, New York.

Fonteijn, T. A. A., Engberts, J. B. F. N., Nir, S., & Hoekstra, D. (1992) *Biochim. Biophys. Acta 1110*, 185−192.

Glaser, P. E., & Gross, R. W. (1994) *Biochemistry 33*, 5805−5812.

Gruner, S. M., Tate, M. W., Kirk, G. L., So, P. T. C., Turner, D. C., Keane, D. T., Tilcock, C. P. S., & Cullis, P. R. (1988) *Biochemistry 27*, 2853−2866.

Haywood, A. M. (1974a) *J. Mol. Biol. 83*, 427−436.

Haywood, A. M. (1974b) *J. Mol. Biol. 87*, 625−628.

Hoekstra, D., de Boer, T., Klappe, K., & Wilshut, J. (1984) *Biochemistry 23*, 5675−5681.

Hsu, M.-C., Scheid, A., & Choppin, P. W. (1979) *Virology 95*, 476−491.

Klappe, K., Wilschut, J., Nir, S., & Hoekstra, D. (1986) *Biochemistry 25*, 8252−8260.

Lasky, L. A., Nakamura, G., Smith, D. H., Fennie, C., Shimasaki, C., Patzer, E., Berman, P., Gregory, T., & Capon, D. J. (1987) *Cell 50*, 975−985.

Lemay, C. T. Y., Ceccarelli, D. F. J., & Epand, R. M. (1994) *Biosci. Rep. 14*, 33−42.

Maddon, P. J., Dalgleish, A. G., McDougal, J. S., Clapham, P. R., Weiss, R. A., & Axel, R. (1986) *Cell 47*, 333−348.

Markwell, M. A. K., Svennerholm, L., & Paulson, J. C. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 5406−5410.

Nir, S., Stegmann, T., Hoekstra, D., & Wilschut, J. (1988) in *Molecular Mechanisms of Membrane Fusion* (Ohki, S., Doyle, D., Flanagan, T. D., Hui, S. W., & Mayhew, E., Eds.) pp 451−465, Plenum Press, New York.

Nir, S., Klappe, K., & Hoekstra, D. (1986a) *Biochemistry 25*, 8261−8266.

Nir, S., Klappe, K., & Hoekstra, D. (1986b) *Biochemistry 25*, 2155−2161.

Nir, S., Duzgunes, N., Lima, M. C. P., & Hoekstra, D. (1990) *Cell Biophys. 17*, 181−201.

Ollmann, M., & Galla, H.-J. (1988) *Biochim. Biophys. Acta 941*, 1−10.

Reed, R. A., Mattai, J., & Shipley, G. G. (1987) *Biochemistry 26*, 824−832.

Rilfors, L., Hauksson, J. B., & Lindblom, G. (1994) *Biochemistry 33*, 6110−6120.

Scheid, A., & Choppin, P. W. (1974) *Virology 57*, 475−490.

Siegel, D. P. (1993) *Biophys. J. 65*, 2124−2140.

Siegel, D. P., & Banschbach, J. L. (1990) *Biochemistry 29*, 5975−5981.

Siegel, D. P., Banschbach, J., Alford, D., Ellens, H., Lis, L. J., Quinn, P. J., & Bentz, J. (1989) *Biochemistry 28*, 3703−3709.

Slepushkin, V. A., Starov, A. I., Bukrinskaya, A. G., Imbs, A. B., Martynova, M. A., Kogtev, L. S., Vodovozova, E. L., Timofeeva, N. G., Molotkovsky, J. G., & Bergelson, L. D. (1988) *Eur. J. Biochem. 173*, 599−605.

Stegmann, T., Nir, S., & Wilschut, J. (1989) *Biochemistry 28*, 1698−1704.

Suzuki, Y., Suzuki, T., Matsunaga, M., & Matsumoto, M. (1985) *J. Biochem. (Tokyo) 97*, 1189−1199.

Svennerholm, L. (1957) *Biochim. Biophys. Acta 24*, 604−611.

Tateno, M., Gonzalez-Scarano, F., & Levy, J. A. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 4287−4290.

Tersmette, M., Van Dongen, J. J. M., Clapham, P. R., De Goede, R. E. Y., Wolvers-Tettero, I. L. M., Guerts Van Kessel, A., Huisman, J. G., Weiss, R. A., & Miedema, F. (1989) *Virology 168*, 267−273.

Tilcock, C. P. S., Cullis, P. R., & Gruner, S. M. (1986) *Chem. Phys. Lipids 40*, 47−56.

Tsao, Y.-S., & Huang, L. (1985) *Biochemistry 24*, 1092−1098.

Tsao, Y.-S., Freire, E., & Huang, L. (1987) *Biochim. Biophys. Acta 900*, 79−87.

Van Gorkom, L. C. M., Cheetham, J. J., & Epand, R. M. (1991) *Biophys. J. 59*, 313a.

Van Gorkom, L. C. M., Cheetham, J. J., & Epand, R. M. (1995) *Chem. Phys. Lipids* (in press).

Yeagle, P. L. (1993) in *Viral Fusion Mechanisms* (Bentz, J., Ed.) pp 313−334, CRC Press, Boca Raton, FL.

Zimmerberg, J., Vogel, S. S., & Chernomordik, L. V. (1993) *Annu. Rev. Biophys. Biomol. Struct. 22*, 433−466.