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Lipogastrins as potent inhibitors of viral fusion

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Abstract

The rate and extent of membrane fusion is markedly sensitive to membrane interfacial properties. Lipopeptides with hydrophilic peptide moieties will insert into membranes, leaving the peptide portion at the membrane-water interface. In this work, we have used a lipopeptide composed of the peptide [Nle^{15}]-gastrin-(2–17)-amide covalently linked to 1,2-diacyl-3-mercaptoglycerol-N^{α}-maleoyl-β-alanine to give DM-gastrin or DP-gastrin having 14 or 16 carbon atom acyl chains, respectively. The fluorescence emission from the two Trp residues of these lipopeptides exhibited little or no blue shift upon addition of liposomes of egg-phosphatidylethanolamine containing 5 mol% G_{D1a}. Iodide quenching of DP-gastrin fluorescence was also independent of lipid. These results indicate that the peptide moiety is exposed to the aqueous environment even though the lipopeptide is firmly anchored to the membrane. Both DM and DP-gastrin markedly raise the bilayer to hexagonal phase transition temperature of dipalmitoleoyl phosphatidylethanolamine. However, DM-E_5 lowers this phase transition temperature. These lipopeptides have effects on the overall fusion of Sendai virus to liposomes in accord with their opposite effects on lipid curvature. The lipogastrins are potent inhibitors of viral fusion, while DM-E_5 slightly promotes this process. Truncated forms of DM-gastrin are also inhibitory to viral fusion, but are less inhibitory than the full lipopeptide. Analysis of the fusion kinetics shows that DP-gastrin causes a reduction in the final extent of fusion and a marked lowering of the fusion rate constant. Binding of Sendai virus to the ganglioside receptor-containing liposomes was not affected. Consideration of the various contributions to the mechanism of inhibition of viral fusion suggests that effects of lipogastrin on membrane intrinsic monolayer curvature is of primary importance. © 1997 Elsevier Science B.V.

Keywords: Sendai virus; Membrane fusion; Anti-viral agent; Membrane surface; Lipopeptide

Abbreviations: LPG, lipophosphoglycan from *Leishmania donovani*; R18, octadecyl rhodamine; LUV, large unilamellar vesicles; NL-HG, [Nle^{15}]-human gastrin (1–17) peptide amide; DM, (2*R*,*S*)-1,2-dimyristoyl-3-mercaptoglycerol-N^{α}-maleoyl-β-Ala-; DP, (2*R*,*S*)-1,2-dipalmitoyl-3-mercaptoglycerol- N^{α}-maleoyl-β-Ala-; DiPoPE, dipalmitoleoyl phosphatidylethanolamine; PE, phosphatidylethanolamine; T_H, bilayer to hexagonal phase transition temperature

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1. Introduction

Lipidation of peptides with a double chain lipid anchor firmly attaches the lipopeptide to the membrane [1]. This can allow the placement of specific binding sites on a membrane surface. In addition, there will be non-specific effects resulting from the lipopeptide altering the physical properties of the membrane. The lipopeptide, *a*-factor, has been found to raise the bilayer to hexagonal phase transition temperature of phosphatidylethanolamine [2]. Many substances having this property also inhibit viral fusion [3]. Membrane fusion is inhibited by making the contacting membrane interfaces more polar and hydrated as well as by expanding the headgroup area of the outer monolayer so as to produce positive curvature strain [4]. Lipid-anchored hydrophilic polymers may be effective agents to inhibit membrane fusion. These substances will be sequestered to the membrane, but they will also make the membrane interface more polar and expanded. In this work we explore the non-specific interactions between a lipopeptide and the fusion of an enveloped virus. We have studied how several lipid-anchored peptides affect the polymorphism of model membranes and their fusion with virions. We have used lipopeptides which have been made by covalently linking a peptide to a lipid anchor containing two acyl chains (Fig. 1). The acyl chains were either C14 or C16, which are sufficiently long to sequester the lipopeptide in the membrane. The peptides were largely hydrophilic and therefore stayed at the membrane interface and/or in the aqueous environment above the membrane [5,6].

A lipid anchored polymer, lipophosphoglycan from *Leishmania donovani* (LPG), has been found to be a potent inhibitor of the fusion of Sendai virus and influenza [7]. We wished to explore other membrane-anchored polymers, to determine if they also have an effect on viral fusion. The lipopolysaccharide, LPG, has a molecular mass of 9500 while lipopeptides used in this work have a molecular mass of about 2700 or less. The lipopeptides represent a class of molecules which are chemically and structurally very different from LPG. In addition these lipopeptides are smaller than LPG and therefore would contribute less steric interference to viral fusion. Furthermore, the general strategy of using lipopeptides as inhibitors of viral fusion provides much flexibility as to the nature of the tethered peptide.

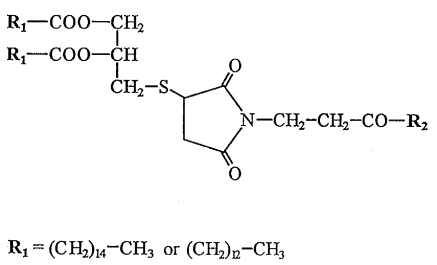

Fig. 1. Structure of the lipid anchor, the ((di-fatty acyl-thioglyceryl)-succinimidyl) propionyl moiety attached to the N-terminus of peptides (R_2).

2. Materials and methods

2.1. Chemicals

All phospholipids were obtained from Avanti Polar Lipids (Alabaster, AL, USA). Gangliosides were purified according to Reed et al. [8]. All lipids showed one spot by TLC at a load of 50 μg. Fluorescent probes were purchased from Molecular Probes (Eugene, OR, USA). Lipogastrins and derivatives were synthesized as described by Romano et al. [5]. All other chemicals and solvents were of reagent grade. The structures of these lipopeptides are given in Table 1.

2.2. Preparation of large unilamellar vesicles

Phospholipid and the ganglioside 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 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 vigorous vortexing with 5 mM HEPES, 5 mM MES, 5 mM sodium citrate, 150 mM NaCl, 1 mM EDTA at pH 7.4 (HEPES/MES buffer). The lipid suspensions were further processed with 5 cycles of freezing and thawing, followed by 10 passes through two stacked 0.1 μm polycarbonate filters (Nucleopore Filtration Products, Pleasanton, CA, USA) using an Extruder (Lipex Biomembranes) at room temperature [9,10].

Lipid phosphorous was determined by the method of Ames [11]. The lipogastrins were added subsequent to liposome formation. The lipogastrins are expected to partition into the liposomes because they are double acyl chain amphiphiles which have limited solubility in water. If the lipid partitioning were incomplete, this would indicate that the membrane bound forn of the lipogastrins are actually more potent than we state. However, this is not likely since Moroder and colleagues have found that lipogastrin rapidly inserts into liposomes [6]. In addition, we have demonstrated that the Trp fluorescence from DP-gastrin changes when LUVs are added but after a few minutes no further changes are observed.

2.3. Quasi-elastic light scattering

Particle sizing was carried out with a laser light scattering instrument from Brookhaven Instrument Corp., equipped with a BI-20sm goniometer, version 2.0 and a BI-900AT Digital Correlator System. Size distribution analysis was calculated using a non-negatively constrained least squares method, using software provided by the instrument manufacturer. Liposome size was found to be approximately 115 nm.

2.4. Virus preparations

The Cantell strain of Sendai virus was propagated in the allantoic sac of 10-day-old embryonated chicken eggs by incubation at 33°C for 72 h. Virus was isolated by discontinuous sucrose gradient centrifugation. The virus was washed and the final preparation was resuspended in HEPES-buffered saline, pH 7.4 at a viral protein concentration of 1 mg/ml. The virus was stored in the frozen state at −80°C.

2.5. Virus fusion assay

Sendai virus was labelled with octadecyl rhodamine (R18) (Molecular Probes, Eugene, OR, USA) according to the procedure of Hoekstra et al. [12]. Ten microliters of R18 (10 nmol) in ethanol were injected into 1 ml of a suspension of Sendai virus in HEPES/MES buffer containing approximately 1 mg of viral protein. The mixture was allowed to incubate at room temperature for 1 h. Unincorporated R18 was then removed by passing the labelled 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, USA). 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. Lipogastrins were added to the liposome suspension and incubated for 10 min. Then 5 μg of R18-labelled Sendai virus was rapidly injected into the cuvette. 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, respectively. The fluorescence intensity immediately after addition of the labelled virus is taken as F_0. A 40 μl aliquot of 10% Triton X-100 was added in order to measure F_{100}. The percentage of R18 dequenching was calculated at time t from:

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

For kinetic experiments, the fluorescence was recorded over the first 10 min after addition of the virus following a 15 min incubation period with LUV's and lipopeptides. For the final extents of fusion, the cuvettes are wrapped in foil and the fluorescence was measured after 20 h of incubation at 37°C in a shaking water bath. The use of a fusion assay based on membrane mixing requires elimination of nonspecific probe transfer. The early studies employing the R18 assay indicated experimentally that probe transfer was minimal at incubation times of up to 1 h. In Nir et al. [13,16] incubation times of Sendai virus with liposomes were 24 h. The liposome concentrations in the latter study varied between 2.5–50 μM of lipid. Shorter incubation times, such as 8 h had little effect on the outcome. A model that assumed partial fusion activity could simulate the final extents quite adequately, whereas a model based on

probe partition fails to explain such results. An example was given later in Larsen et al. [17] for a comparison between the ability of these two models to explain the results of final extents of fluorescence increase. In the current study the model could yield fair predictions for both the final extents and kinetics of fluorescence increase for a 4-fold variation in lipid concentration (results not shown). Hence, although a certain degree of probe exchange cannot be ruled out, it cannot contribute to more than 10% of the total fluorescence increase. The results in Table 4 demonstrate about 2-fold reduction in the percentages of virions capable of fusing when 1.8 mol% of DP-gastrin was included in the liposomes.

2.6. Analysis of data

The analysis of final extents and kinetics of fusion of Sendai virus with liposomes was done as previously described [13]. In the analysis of the kinetics of fusion we have employed three parameters: *C*, the second order rate constant of virus adhesion to liposomes; *f*, the first order rate constant of the actual fusion of an adhered virus particle; *D*, the first order dissociation rate constant.

2.7. Differential scanning calorimetry (DSC)

Lipid films were made from dipalmitoleoyl phosphatidylethanolamine (DiPoPE) dissolved in chloroform/methanol 2/1 (v/v). After solvent evaporation with nitrogen, final traces of solvent were removed in a vacuum chamber for 90 min. The lipid films were suspended in 20 mM PIPES, 1 mM EDTA, 150 mM NaCl with 0.002% NaN_3, pH 7.40 by vortexing for 30 s at room temperature. The final lipid concentration was 10 mg/ml. Lipogastrin solutions were prepared by adding 5 μl of NH_4OH to a weighed amount of peptide, then adding HEPES/MES buffer pH 7.4 to obtain the desired concentration and finally readjusting the pH to 7.4. Appropriate amounts of lipogastrins in buffer solution were added to the lipid and vortexed vigorously. The lipid suspension was degassed under vacuum before being loaded into an MC-2 high sensitivity scanning calorimeter (Microcal Co., Amherst, MA, USA). A heating scan rate of 37°C/h was generally employed. The observed phase transitions were independent of scan rates between 10 and 60°C/h. The bilayer to hexagonal phase transition was fitted using parameters to describe an equilibrium between two phases, using a single van't Hoff enthalpy [14]. The transition temperature is reported as that of the fitted curve.

2.8. Tryptophan fluorescence

Fluorescence emission spectra of tryptophan in lipogastrins, with and without LUV's of egg PE with 5% G_{D1a}, were measured using an SLM Aminco Bowman Series II luminescence spectrometer. The

Table 1
Lipopeptide: peptide sequences

	Peptide moiety
NL-HG[20]	pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Nle-Asp-Phe-NH_2
DM-gastrin (2–17)[5]	Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Nle-Asp-Phe-NH_2
DP-gastrin (2–17)[5]	Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Nle-Asp-Phe-NH_2
DM-gastrin (7–17) [a]	Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Nle-Asp-Phe-NH_2
DM-gastrin (9–17) [a]	Glu-Glu-Ala-Tyr-Gly-Trp-Nle-Asp-Phe-NH_2
DM-gastrin (11–17) [a]	Ala-Tyr-Gly-Trp-Nle-Asp-Phe-NH_2
DM-CCK	Arg-Asp-Tyr-Gly-Trp-Nle-Asp-Phe-NH_2
DM-AAe[21]	Ala-Ala-D-iso-Glu
DM-E_5 [b]	Glu-Glu-Glu-Glu-Glu

DM = (2*R*,*S*)-1,2-dimyristoyl-3-mercaptoglycerol-N^{α}-maleoyl-β-alanyl
DP = (2R,S)-1,2-dimyristoyl-3-mercaptoglycerol-N^{α}-maleoyl-β-alanyl
pGlu = pyroglutamic acid
CCK = cholecystokinin

[a] Lutz et al., Eur. J. Biochem., submitted. [b] Synthesized according to procedures described in [5].

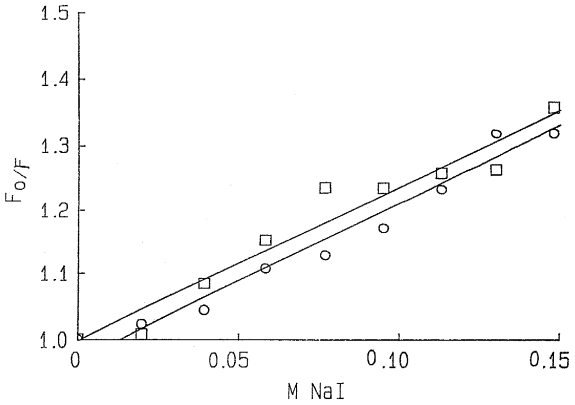

Fig. 2. NaI quenching of: □, DP-gastrin in buffer and ○, in LUV's.

liposomes were suspended in HEPES/MES buffer pH 7.4. An excitation wavelength of 295 nm was used. The contribution of the Raman scattering peak at 238 nm was discounted using LUVs as background.

2.9. Leakage

Aqueous content leakage from liposomes was determined using the ANTS-DPX assay [15]. Films were hydrated with 12.5 mM ANTS, 45 mM DPX, 68 mM NaCl and 10 mM TES at pH 7.4. After making LUV's of approximately 100 nm diameter by extrusion, the liposomes were separated from untrapped dye by passage through Sephadex G-75. Lipid was collected in the void volume and the concentration was determined by phosphate analysis. The fluorescence assay was performed in 2 ml of 10 mM TES, 0.15 M NaCl, 0.1 mM EDTA, pH 7.4, at 37°C. Fluorescence was recorded as a function of time using an excitation wavelength of 360 nm and an emission wavelength of 530 nm. Leakage was initiated by adding 0.9 μM lipopeptide to 50 μM LUV's in the cuvette. The value for 100% leakage was obtained by adding 40 μl of 10% Triton X-100

Table 2
Tryptophan fluorescence at 37°C in HEPES/MES buffer pH 7.4

	Peptide [μM]	LUV's [a] [μM]	Emission maximum (nm)
DP-gastrin	0.9	0	350
	0.9	50	348
	50	1 100	348
DM-gastrin	0.9	0	350
	0.9	50	348
NL-HG (1–17)	150	0	353
	150	1 500	353
DM-gastrin (7–17)	131	0	344
	131	1 500	344
DM-CCK	0.9	0	352
	0.9	50	345

[a] LUV's composed of egg PE-5% G_{D1a}

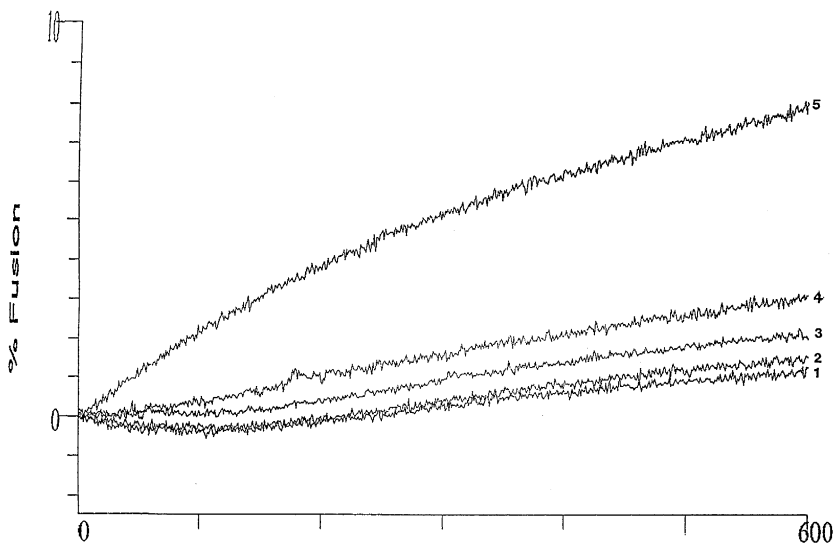

Fig. 3. Inhibition of fusion of Sendai virus with liposomes by DP-gastrin at 37°C. R18 dequenching curves for 50 μM LUV's of egg PE with 5% G_{D1a} containing the following concentrations of DP-gastrin: (1) 2.7 μM, (2) 1.8 μM, (3) 0.9 μM, (4) 0.45 μM, (5) 0 μM.

approximately 10 min after measurements were initiated.

3. Results

3.1. Tryptophan fluorescence

There are two tryptophan residues, at positions 3 and 13, in the lipogastrins and one, at position 5, in the cholecystokinin chain (Table 1). In buffer all lipopeptides show an emission maximum at 350 nm, indicating aqueous exposure of the tryptophan residues. The shorter lipopeptide DM-gastrin (7–17) is an exception, with a slight blue shift in buffer (Table 2). The presence of LUV's also causes a small or a negligible blue shift. This indicates that the tryptophans remain exposed to the aqueous environment. The peptide NL-HG, lacking a hydrophobic tail, retains a maximum emission wavelength of 353 nm both in the presence and absence of LUV's.

Table 3
Bilayer to hexagonal phase transition shifts in DiPoPE

	Slope [a] (°C/mol fraction)
NL-HG	180 ± 1
DM-AAe	133 ± 14
DM-E_5	−101 ± 14
DM-gastrin	500 ± 35
DP-gastrin	1100 ± 106
DM-CCK	1200 ± 190

[a] Slope of a plot of T_H vs. mol fraction of peptide or lipopeptide added.

Trp fluorescence of DP-gastrin was also measured after successive additions of 10 μl of NaI (containing 0.5 M of $Na_2S_2O_3$ to prevent iodide oxidation) in HEPES-MES buffer pH 7.4 (Fig. 2). The quenching of the Trp fluorescence was the same in the presence and absence of vesicles.

3.2. DSC

DM-gastrin, DP-gastrin, and DM-CCK were the most powerful stabilizers of the bilayer phase relative

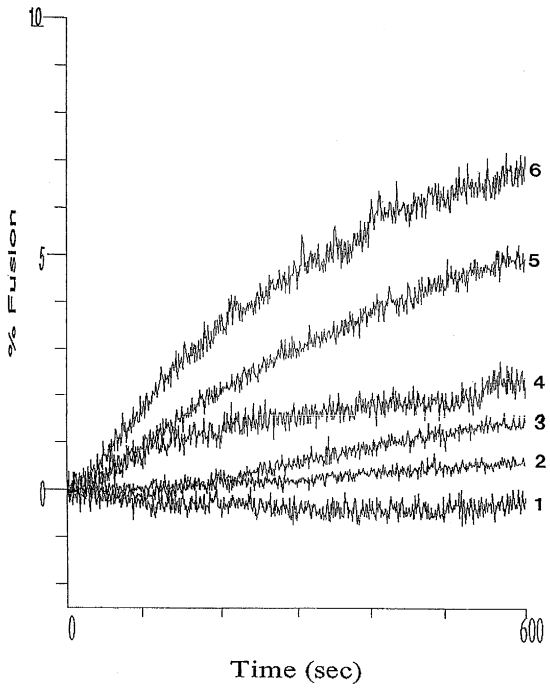

Fig. 4. Temperature dependence of the R18 dequenching of 0.9 μM DP-gastrin added to 12.5 μM LUV's composed of egg PE with 5% G_{D1a}. (1) DP-gastrin at 25°C, (2) LUV's at 25°C, (3) DP-gastrin at 37°C, (4) DP-gastrin at 45°C, (5) LUV's at 37°C, (6) LUV's at 45°C.

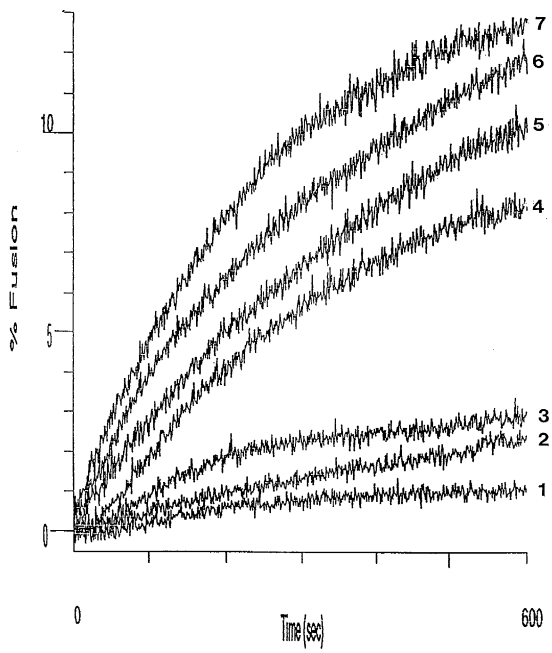

Fig. 5. R18 dequenching curves for 50 μM LUV's composed of egg PE with 5% G_{D1a} with added (1) 2 μM DM-gastrin, (2) 5 μM DM-gastrin (9–17), (3) 5 μM DM-gastrin (7–17), (4) 6 μM DM-gastrin (11–17), (5) LUV's of egg PE with 5% G_{D1a}, (6) 2 μM NL-HG, (7) 12 μM DM-E_5. DM-gastrin (9–17) and DM-gastrin (11–17) required some methanol to remain in solution.

to the inverted hexagonal phase (H_{II}) of DiPoPE. The peptide NL-HG as well as the short lipopeptide DM-AAe, had small positive slopes in comparison, while DM-E_5 had a negative slope, making it a weak hexagonal phase promoter (Table 3).

3.3. Sendai virus fusion

DP-gastrin is a potent inhibitor of Sendai virus fusion to LUV's composed of egg PE with 5% G_{D1a} (Fig. 3). Viral fusion proceeds more rapidly at higher temperatures, but at each temperature tested, 1.8 mol% of DP-gastrin causes potent inhibition (Fig. 4). The curves of viral fusion kinetics are determined by the affinity of the virus for the target liposome, the final extent of fusion as well as the first order rate for fusion of the bound virus to the target liposome. We have analyzed the kinetics of the fusion reaction using several ratios of virus to liposome, as well as measuring the final extents of fusion after incubation at the indicated temperature for 20 h. We find that at all temperatures the DP-gastrin has a marked effect in reducing the final extent of fusion as well as lowering the fusion rate constant, f (Table 4).

We have also studied the effect on virus-liposome fusion of a variety of lipopeptides and free peptide structurally related to DP-gastrin (Fig. 5). DP-gastrin is a 16 amino acid peptide (residues 2–17 of gastrin) covalently linked at its N-terminus to the DP anchor (Fig. 1). The homologous lipid anchor DM-gastrin, has about the same inhibitory potency as DP-gastrin. Attaching partial sequences of gastrin, truncated at the amino terminus and lacking 1, 3 and 5 Glu's in their sequence, to the DM lipid anchor (DM-gastrin (7–17), DM-gastrin (9–17) and DM-gastrin (11–17), respectively), results in peptides with lower potency to inhibit Sendai fusion (Fig. 5). These truncated sequences could not be studied comparatively in great detail due to their insolubility in water, requiring differing amounts of methanol for keeping them in solution. Attaching the oligopeptide Glu-Glu-Glu-Glu-Glu to the DM anchor produces a lipopeptide which actually promotes fusion somewhat (as would be expected from its small bilayer destabilizing effect, observed by DSC). The free gastrin peptide, with no lipid anchor as well as DM-AAe, have only a very small effect on Sendai fusion with LUV's.

We have also tested DM-CCK, a lipopeptide with

Table 4
Effect of 1.8 mol% DP-gastrin on sendai virus fusion with egg PE containing 5 mol% G_{D1a}

(A) Final extents of fusion

Composition of LUV's	Temperature (°C)	% Virions capable of fusing
Control	25	55 (±10)
+DP-gastrin	25	20 (± 5)
Control	37	68 (±10)
+DP-gastrin	37	35 (± 5)
Control	45	72 (±10)
+DP-gastrin	45	38 (± 5)

(B) Kinetic parameters [a]

Composition	Temperature (°C)	C ($M^{-1}s^{-1}$)	f (s^{-1})	D (s^{-1})
Control	25	4×10^{7}	5×10^{-4}	0.005
+DP-gastrin	25	4×10^{7}	2×10^{-4}	0.005
Control	37	6×10^{7}	0.0035	0.01
+DP-gastrin	37	5×10^{7}	0.001	0.01
Control	45	7×10^{7}	0.004	0.015
+DP-gastrin	45	7×10^{7}	0.002	0.015

[a] Kinetic parameters defined according to the scheme which describes the initial events in virus-liposome fusion: virus + liposome $\underset{D}{\overset{C}{\rightleftharpoons}}$ virus-liposome $\xrightarrow{f}$ fusion product. See Nir et al. [13] for a more complete description. The estimated error is 25% for C and f and 50% for D.

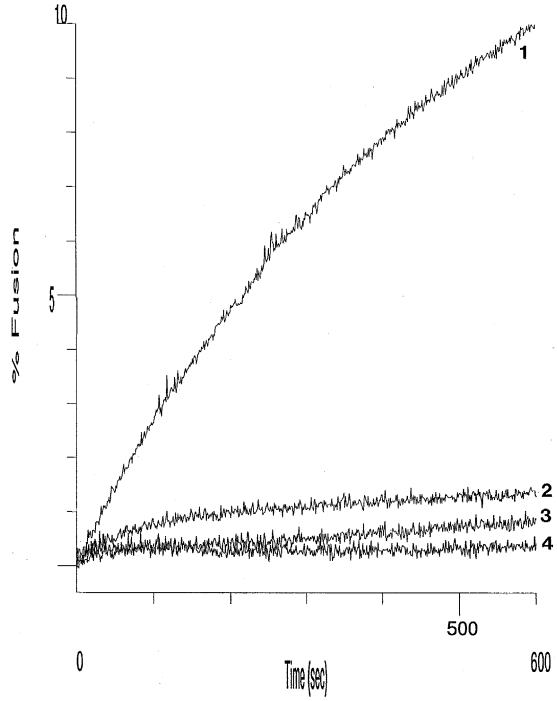

Fig. 6. Comparison of inhibition of Sendai virus fusion when adding 0.9 μM lipopeptides to 12.5 μM LUV's composed of egg PE with 5% G_{D1a} at 37°C. (1) no addition, (2) DM-CCK, (3) DP-gastrin, (4) DM-gastrin.

eight amino acid residues attached to the DM anchor (see Table 1). The sequence is such that the six carboxy-terminal amino acids of DM-CCK correspond to the six carboxy-terminal amino acids of the gastrin lipopeptides used in this study. The DM-CCK proved to be a strong bilayer stabilizer (Table 3) and also an inhibitor of Sendai virus fusion. Its fusion inhibitory power is somewhat lower than that of DP-gastrin or DM-gastrin, however (Fig. 6). For example, at 37°C and after 24 h, 60% of fusion occurred for 12.5 μM LUV's, 44% for 0.9 μM DM-CCK, 38% for 0.9 μM DP-gastrin and 24% for 0.9 μM DM-gastrin, added respectively to 12.5 μM LUV's in cuvette. These trends are maintained for all concentrations of LUV's and their reproducibility in the same batch of virus is within 15%.

To test whether the lipopeptides acted on the virus or on the target membrane, Sendai virus was incubated with 0.9 μM DP-gastrin or DM-gastrin for 10 min at 37°C. Then an aliquot of this mixture was added to the fluorimeter cuvette containing 50 μM LUV's in 2 ml HEPES/MES buffer pH 7.4, so that the final concentration of virus (5 μg) would correspond to that of a standard fusion assay. Fluorescence dequenching was then followed as a function of time. No inhibition of fusion was seen in this case, indicating that lipogastrins had to be incorporated into the target membrane to exert their inhibitory effect on the virus.

3.4. Leakage

No leakage was observed with this vesicle system, with or without added lipogastrins, in the first hour following 15 min of initial thermal equilibration to 37°C. A small amount of leakage was observed after 20 h of incubation either with or without lipogastrin.

4. Discussion

Enveloped viruses must fuse with a cellular membrane for infectivity. A strategy to inhibit the infectivity of these viruses is to block membrane fusion by altering the surface properties of target membranes. We have studied the fusion of Sendai virus to large unilamellar vesicles made of egg phosphatidylethanolamine (PE) with 5 mol%, G_{D1a} with or without the addition of 1.8 mol% of a lipogastrin. The octadecylrhodamine lipid dilution assay [12] was used to monitor the overall kinetics of viral fusion. The DP-gastrin greatly reduced the apparent rate of fusion. The fusion kinetics were analyzed to separate the rate constants of association and dissociation of the virus with the target membrane, as well as the rate constant for membrane fusion. Addition of 1.8 mol% DP-gastrin reduces the fusion rate constant 3.5-fold at 37°C without affecting the reversible binding of the virus to the target liposome (Table 4). There is also a 2-fold decrease in the final extent of fusion, a measure of the fraction of virus that is bound in a manner that can proceed to fusion [16]. DP-gastrin is a negatively charged lipopeptide. It has been observed that the final extents of membrane fusion are reduced upon incorporating in the target membrane anionic amphiphiles that raise the T_H [18], such as DP-gastrin (Table 3). The negatively charged amphiphile, DM-E_5, which lowers T_H, does not reduce the final extent of fusion (not shown). It is less

common for an amphiphile to lower the actual fusion rate constant, *f*. However, certain amphiphiles [3,18], as well as the lipid-anchored polymer LPG [7], also have this property.

We have begun to evaluate the factors contributing to the inhibitory action of DP-gastrin. We have also studied the free gastrin peptide NL-HG, as well as the lipid anchor itself. Neither of these components of DP-gastrin had any viral inhibitory activity (Fig. 5). In addition, forms of lipogastrin truncated from the amino terminus had either lower or no inhibitory activity. The truncated gastrins, the free peptide and the lipid anchor, all had weaker effects compared with DP-gastrin in raising T_H (Table 3). The inhibitory effect of DP-gastrin does not exhibit a high degree of specificity. Although the truncated peptides were weaker, they were also inhibitory. In addition, DM-CCK was also inhibitory (Fig. 6). However, not all lipopeptides exhibit significant inhibitory action. The lipopeptide, *a*-factor, which raises T_H by 217 ± 14 degrees/mol fraction lipopeptide [2] is a much weaker bilayer stabilizer than DP-gastrin. The *a*-factor is not a potent inhibitor of viral fusion (unpublished observations). The peptide anchored to the farnesyl moiety of *a*-factor is much more hydrophobic than is either gastrin or CCK. Despite these correlations, we recognize that other factors also contribute to inhibition of viral fusion and that the relationship between fusion inhibition and the increase in T_H is not quantitative. For example, DM-CCK is more effective in raising T_H but is a slightly weaker inhibitor of Sendai fusion than DM-gastrin.

We have also considered the role of steric interference. Among the lipopeptides used, the greatest steric interference should occur with DP-gastrin. However we have calculated that the maximal protrusion of this lipopeptide from the membrane surface when it is a fully extended chain, is only 50 Å, less than half of the 120 Å that the F-protein protrudes from the membrane (Fig. 7). Alternatively if the peptide covered the surface of the liposome, the cross-sectional surface area of the peptide would be sufficient to cover all of the lipid. However, we believe this arrangement to be unlikely since fluorescence and CD measurements show the DP-gastrin to be devoid of secondary structure and be exposed to an aqueous environment [6]. In addition, the Pro residue and the sequence of five Glu residues would be expected to cause the peptide to protrude more from the membrane surface. It appears, as well, that flexible polymers tethered to the membrane surface do not inhibit viral fusion. Thus, glycophorin, a sialoglycoprotein receptor for Sendai which possesses a 25 kDa extracellular portion, does not appear to show any steric inhibition, even at high mol fractions [19]. Thus lipogastrins provide novel inhibitors of viral fusion whose likely mechanism is to prevent the membrane bilayer from forming highly curved structures required as intermediates in membrane fusion. Lipogastrin inhibits not only Sendai fusion to liposomes but also HIV-induced syncytia formation (unpublished observation).

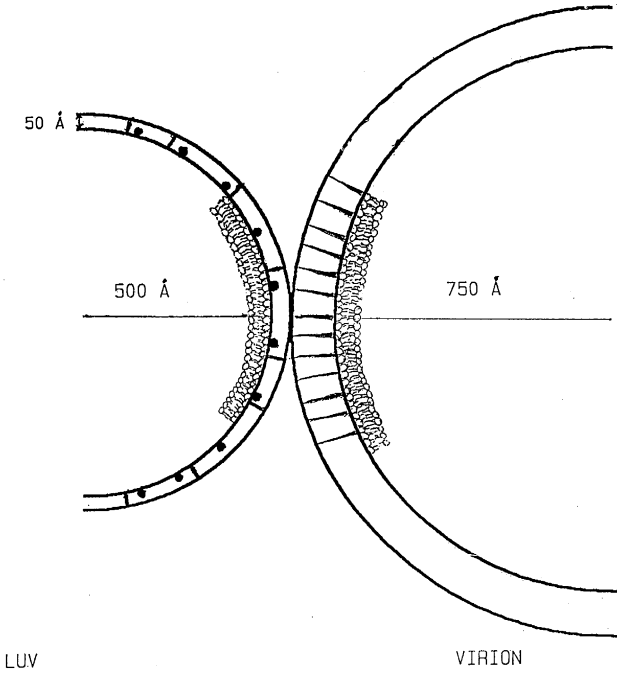

Fig. 7. Scale model of a Sendai virion approaching a LUV containing DP-gastrin. Model of a virion, with protein spikes protruding 120Å, approaching a LUV 1000 Å in diameter represented here with evenly distributed 5% G_{D1a} (●) molecules protruding 25Å, and 1.8% DP-gastrin (■) molecules protruding about 50Å from the surface. The representation illustrates that the relative size and membrane surface density of lipogastrin still allows access of the viral spike proteins to the target membrane. The size of the extended gastrin (2–17) chain was calculated using the Biograf Program, V3.0, Molecular Simulations (Density = 4 dots/Å, van der Waals scale = 0.89 Å).

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