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ARTICLE *in* BIOCHIMICA ET BIOPHYSICA ACTA · NOVEMBER 1993

Impact Factor: 4.66 · DOI: 10.1016/0005-2736(93)90239-V · Source: PubMed

Biochimica et Biophysica Acta, 1152 (1993) 128–134

BBAMEM 76104

Structural requirements for the inhibition of membrane fusion by carbobenzoxy-D-Phe-Phe-Gly

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(Received 26 March 1993)

Key words: Virus replication inhibitory peptide; Lipid polymorphism; Membrane intrinsic curvature; Anti-viral agent; Membrane fusion

The peptide ZfFG is known to inhibit non-bilayer phase formation as well as vesicle-vesicle and viral fusion. In order to ascertain some of the properties or structural features of this peptide which were important for the inhibition of membrane fusion, the blocking group was transferred from the amino to the carboxyl end to make fFGOBz. The fFGOBz lowered the bilayer to hexagonal phase transition temperature of dielaidoylphosphatidylethanolamine and it promoted the formation of isotropic phases in monomethyldioleoylphosphatidylethanolamine. The promotion of non-bilayer phases by fFGOBz appeared to be enhanced by a charged terminal amino group as higher pH or formylation of the amino group both decreased the effectiveness of this peptide to induce formation of the hexagonal phase. With the monomethyldioleoylphosphatidylethanolamine, the fFGOBz also promoted vesicle leakage and fusion as measured by lipid intermixing. The fFGOBz did not inhibit the formation of lipid structures of high curvature, resulting from sonication of phosphatidylcholine, as did ZfFG. Thus, the effects of fFGOBz on membranes are in sharp contrast to those of ZfFG and more closely resemble the behaviour of larger fusion peptides corresponding to the amino-terminal segment of viral fusion proteins. Our results demonstrate that having the carbobenzoxy group on the amino-terminus of fFG is important for giving the peptide derivative the property of inhibiting membrane fusion.

Introduction

The peptide carbobenzoxy-D-Phe-L-PheGly (ZfFG) was found to be a potent anti-measles agent [1]. It was suggested that this peptide might compete with the viral fusion protein for binding to some site on the target membrane [1]. An alternative explanation is that this peptide affects the physical properties of the target membrane so as to make membrane fusion, an essential step in enveloped virus infection, less likely [2]. This latter hypothesis is supported by the fact that ZfFG inhibits membrane fusion [3,4] including viral fusion [4,5]. Thus, despite the fact that this peptide has a superficial resemblance to the 'fusion peptide' of the Sendai viral fusion protein, it inhibits rather than promotes fusion.

There are several differences between ZfFG and the 'fusion peptide' of the viral fusion protein which could account for the opposite effects on membrane fusion. One of the differences is that the viral fusion protein is extended on the C-terminus with other hydrophobic amino acids and has no free carboxyl group in this region. At the same time, the terminal amino group of the 'fusion peptide' of the fusion protein is unblocked and can be protonated. Thus, ZfFG would carry at least a partial negative charge while the 'fusion peptide' of the viral fusion protein would be positively charged in this region.

In order to examine the role of the free amino group on the small peptide in its effects on membranes, we synthesized the peptide derivative D-Phe-L-

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Abbreviations: *N*-methyl-DOPE, *N*-methyl-1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; DEPE, 1,2-dielaidoyl-*sn*-glycero-3-phosphoethanolamine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; egg PC, egg phosphatidylcholine; LUV, large unilamellar vesicle; DPX, *p*-xylylene bis(pyridinium bromide); ANTS, aminonaphthalene-3,6,8-trisulfonic acid; NBD-PE, 1-acyl-2-(2-((7-nitro-2,1,3-benzoxadiazol-4-yl)amino)dodecanoyl)-*sn*-glycero-3-phosphoethanolamine; *N*-Rh-PE, *N*-(lissamine Rhodamine B sulfonyl)-1,2-dioleoyl-*sn*-glycerol-3-phosphoethanolamine; RET, resonance energy transfer fluorescence assay of lipid intermixing; H_{II}, inverted hexagonal phase; T_H, lamellar to H_{II} phase transition temperature.

PheGly benzyl ester (fFGOBz) with the blocking group on the carboxyl-terminus, rather than the amino-terminus. Both ZfFG and fFGOBz have similar hydrophobicities and chemical groups but have opposite charges and opposite ends blocked. This modification alone was sufficient to convert the peptide from an inhibitor of non-bilayer phase formation and membrane fusion and leakage to a promoter of these properties.

Experimental procedures

Materials

N-Methyl-1,2-dioleoyl-*sn*-glycero-3-phosphatidylethanolamine (*N*-methyl-DOPE), dielaidoylphosphatidylethanolamine (DEPE) and dipalmitoylphosphatidylcholine (DPPC) were purchased from Avanti Polar Lipids, Pelham, AL and used without further purification. The purity of these preparations was indicated by the temperature and cooperativity of the thermal transitions. ZfFG was purchased from the Peptide Institute, Osaka. The 1-aminonaphthalene-3,6,8-trisulfonic acid (ANTS) and *p*-xylene bis-(pyridinium bromide) (DPX) were purchased from Molecular Probes, Eugene, OR. Fluorescent-labelled phospholipids and egg phosphatidylcholine (egg PC) were purchased from Avanti.

Synthesis of fFGOBz

The benzyl ester of fFG was made by heating a mixture of 100 mg of this peptide with 57 mg *p*-toluene sulfonic acid and 2.5 ml benzyl alcohol in 2.5 ml anhydrous toluene to 80–90°C for 24 h. At the end of this period most of the solvents were removed under vacuum and 3.5 ml of diethyl ether was added. The mixture was cooled to 4°C and an oil separated on the bottom of the flask. The oil was washed with diethyl ether and dried under vacuum, forming a pale yellow powder. This product was purified by HPLC on a Whatman preparative ODS-3 partisil column using a gradient from methanol/0.1% aqueous trifluoroacetic acid (1:1, v/v) to pure methanol.

The identity and purity of the compound was confirmed by mass spectroscopy, ^{1}H-NMR and TLC. The benzyl ester melted at 75–77°C and had an R_F in TLC of 0.82 using silica gel plates developed with chloroform/methanol/water (65:25:4, v/v).

Synthesis of formyl-fFGOBz

1 ml of a 0.3 M solution of 1,1-carbonyldiimidazole in dichloromethane was added dropwise, with stirring and cooling on ice, to 0.5 ml of a 2 M solution of formic acid in dichloromethane. The mixture was maintained for 3 h in an ice bath with stirring. After this period 1 ml of a 0.07 M solution of fFGOBz in dichloromethane was added dropwise with stirring and cooling on ice. The stirring and cooling was continued for 2 h after which the mixture was warmed to room temperature and left to stand overnight. Following this, two drops of water were added to destroy the excess carbonyldiimidazole. 5 ml of dichloromethane and a small quantity of methanol were added to the opaque mixture until it was transparent. The solution was dried over anhydrous sodium sulfate. The solvent was evaporated and the residue was purified by chromatography on a silica gel column using sequential elution with chloroform followed by chloroform/methanol (5:1, v/v). Final purification was done by preparative TLC on silica gel GF using 6% acetic acid in chloroform as the mobile phase. The R_F of the product was 0.66 which was clearly separated from the starting material whose R_F was 0.21. The product was eluted from the TLC plate and its identity verified by mass spectroscopy and ^{1}H-NMR.

Differential scanning calorimetry (DSC)

Lipid films were made from DEPE dissolved in chloroform/methanol (2:1, v/v) to which varying quantities of peptide dissolved in methanol were added. After solvent evaporation with nitrogen, final traces of solvent were removed in a vacuum chamber for 90 min. The lipid films were suspended in buffer by vortexing at 45°C for 30 s. The final lipid concentration was 5 mg/ml. The buffer used for pH 7.40 was 20 mM Pipes, 1 mM EDTA, 150 mM NaCl with 0.002% NaN_3 and for pH 5.0 was 10 mM citric acid, 150 mM NaCl. The lipid suspensions were degassed under vacuum before being loaded into an MC-2 high sensitivity scanning calorimeter (Microcal Co., Amherst, MA). A heating scan rate of 39 K/h was employed. The bilayer to hexagonal phase transition was fitted to a single van 't Hoff component and the transition temperature reported as that for the fitted curve.

^{31}P-NMR

^{31}P nuclear magnetic resonance (NMR) spectra were obtained with a JEOL FX270 Fourier transform spectrometer in a broad band probe at 25–45°C. A fully phase cycled (32 pulse) Hahn echo sequence was used with a 40 ms echo. The echo sequence eliminates baseline artifacts, removing the need for first order phase corrections [6]. Data were collected prior to the refocussing of the echo, and the FID was transformed from the top of the echo. The ^{1}H decoupler was gated on during the acquisition and off the remainder of the time to prevent sample heating. Exponential line broadening of 50 Hz was used on all spectra. Multilamellar liposomes containing the appropriate phospholipid to peptide mole ratio were prepared for the ^{31}P-NMR experiments as described previously [7], except that chloroform/methanol (2:1) was used to mix the peptide and phospholipid. As reported else-

where, no difference was noted in the behavior observed in the ^{31}P-NMR powder patterns between multilamellar liposomes and large unilamellar vesicles used in the fusion and leakage experiments [8].

Leakage assay

All fluorescence measurements were made on an SLM 8000D fluorimeter. The ANTS/DPX leakage assays were carried out as described by Ellens et al. [9]. Large unilamellar vesicles were prepared for these experiments as described previously [9]. Vesicles contained 12.5 mM ANTS, 45 mM DPX and 22.5 mM NaCl. Fluorescence intensity was monitored with an excitation wavelength of 380 nm and an emission wavelength of 510 nm. All assays were carried out in a total volume of 1 ml. The final lipid concentration was 0.4 μmol/ml. Leakage was initiated by lowering the pH from 9.5 to 4.5 with 25 μl of 2 M sodium acetate/acetic acid buffer. Leakage was measured by dequenching of fluorescence due to leakage and dilution (and dissociation) of the ANTS-DPX complex.

Lipid-intermixing (RET) assay

Processes such as membrane fusion which lead to the intermixing of lipid from different vesicles can be monitored by an assay based on fluorescence resonance energy transfer (RET) between labelled lipid molecules [10]. Lipid films of *N*-methyl-DOPE either with (labelled) or without (unlabelled) 1 mol% *N*-NBD-PE and 1 mol% *N*-Rh-PE were prepared. These films were hydrated with 10 mM glycine, 100 mM NaCl, 0.1 mM EDTA (pH 9.5). The lipid suspension was freeze-thawed five times and then extruded 10 times through a 0.1 μm polycarbonate Nucleopore filter (Lipex Biomembranes, Vancouver, BC). To 2.0 ml of 10 mM glycine buffer (pH 9.5), 10 nmol of labelled LUVs and 50 nmol of unlabelled LUVs were added. Small aliquots of a 5 mg/ml solution of the peptide in methanol was then added. Controls were done to test the effect of methanol whose final concentration was 1%. The cuvette was then acidified to pH 7 or pH 5 with 1 M acetic acid and the fluorescence emission intensity at 530 nm was measured as a function of time ($\lambda_{ex} = 450$ nm) using a Perkin Elmer MPF-44 spectrofluorimeter.

Membrane partitioning assay

N-Methyl-DOPE, deposited as a dried lipid film from chloroform/methanol, was hydrated with either 1.0 ml of buffer or of peptide solution. The peptides were dissolved with difficulty after briefly sonicating, vortexing and warming into either the pH 7.4 Pipes buffer or the pH 5.0 citrate buffer used for DSC. The peptide concentration was determined by absorbance at 257.4 nm after centrifugation and removal of undissolved material and prior to its addition to the lipid films. After the lipid films were hydrated with peptide solution or with buffer, the suspension was vortexed and incubated at room temperature for 30 min. The mixture was then centrifuged at $100\,000 \times g$ for 60 min at 25°C in a TLA-100.2 rotor of a Beckman TL-100 centrifuge. A small amount of peptide will be entrapped within the lipid, rather than bound to it, but since the volume of buffer is orders of magnitude greater than that of the lipid, no correction was made for entrapped material. Even at the highest lipid concentration of 26 mM, the entrapped volume would be less than 1%. Controls were done to ensure that the concentration of peptide in solution in the absence of lipids did not change by this procedure. The peptide concentration in the supernate was determined from its absorbance at 257.4 nm using a molar extinction coefficient of 591 estimated from its chromaphore content [11]. The absorbance of each supernate containing peptide was corrected for the absorbance obtained with a blank supernate from a lipid suspension in buffer without peptide.

Sonication

Sonication was performed with a Branson W350 probe sonicator with an ice bath. First, multilamellar liposomes containing the peptide in the phospholipid bilayer were prepared in the following manner. Egg PC and peptide were solubilized in chloroform/methanol (2:1) at room temperature in the indicated mole ratios. The solvent was removed by evaporation under a stream of nitrogen gas followed by evaporation under high vacuum overnight. The material was then hydrated in D_2O with 50 mM NaCl, sealed under nitrogen gas and vortexed vigorously. The membranes were then sonicated with 5 min sonications followed by a 1 min rest period in which the light scattering of the sample was determined as effective absorbance at 400 nm.

Results

The T_H of DEPE was measured by DSC in samples containing increasing mol fractions of peptide. The lipid chosen for the experiment was DEPE because it exhibits a transition between L_α and H_{II} phases which is highly cooperative and rapidly reversible. It has been used previously as a model system for measuring the effects of peptides and amphiphiles on T_H. For these experiments, low mol fractions of peptide are used in order that the L_α-H_{II} phase transition can be readily observed by DSC and is not too greatly broadened by addition of the peptide. In agreement with previous studies [2], ZfFG raises T_H at pH 7.4 (Fig. 1). The fFGOBz has a weak but opposite effect on T_H, being a H_{II} phase promoter (Fig. 1). At pH 5, ZfFG has a similar effect in raising T_H as at pH 7.4 (Fig. 2) but

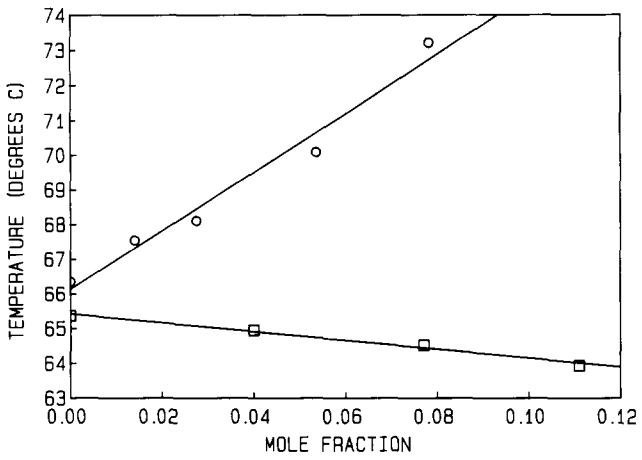

Fig. 1. Effect of ZfFG (○) and fFGOBz (□) on T_H of DEPE at pH 7.4. T_H determined from DSC scans plotted against mol fraction of peptide.

fFGOBz is more potent at lowering T_H at the more acidic pH. Blocking the terminal amino group of fFGOBz by formylation greatly diminishes the ability of the peptide to promote the H_{II} phase. The results of these studies are summarized in Table I.

Monomethyl-DOPE forms cubic phases in addition to the H_{II} phase [12,13]. Precursors to cubic phase formation are probably responsible for narrow line resonances observed with ^{31}P-NMR [14]. The relative intensity of these isotropic resonances have been associated with increased rates of membrane fusion [8]. Thus, for the ^{31}P-NMR experiments, monomethyl-DOPE was chosen as the model lipid since it readily forms isotropic phases. Peptide concentrations and temperatures were chosen so as to monitor the interconversion between bilayer and isotropic phases. It has been shown that ZfFG decreases the relative intensity of the isotropic resonance observed at different temperatures through broadening of the resonance [4,5].

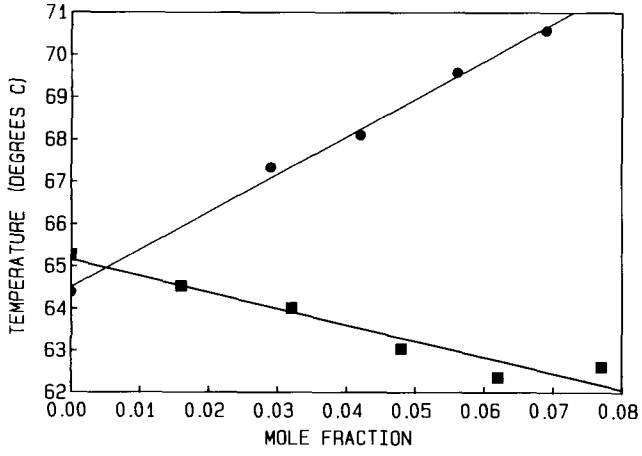

Fig. 2. Effect of ZfFG (●) and (■) fFGOBz on T_H of DEPE at pH 5.0.

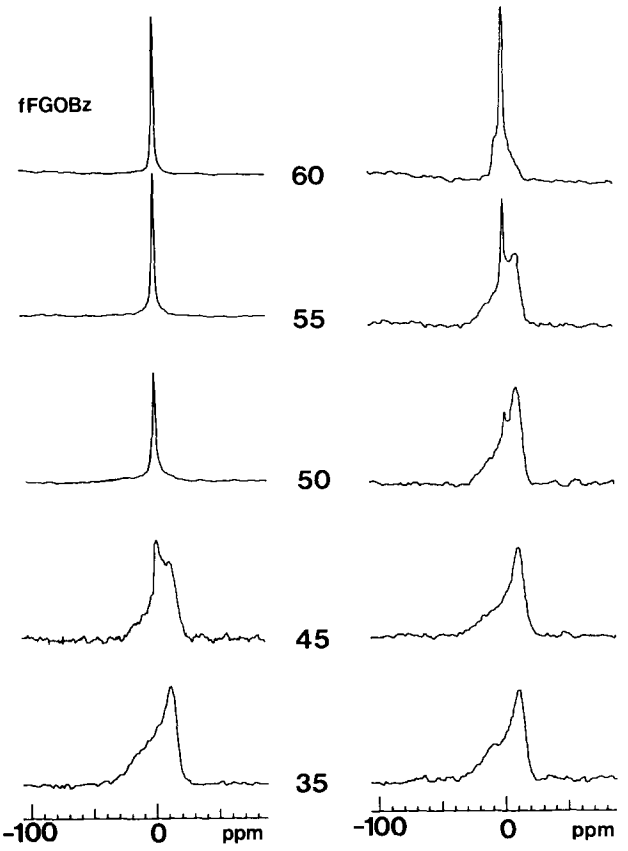

Fig. 3. ^{31}P-NMR of 25 mg of monomethyl-DOPE in the presence (left) and absence (right) of fFGOBz at a 1:4 peptide to lipid mole ratio, pH 7.5, in 100 mM NaCl, 10 mM histidine, 0.1 mM EDTA, at various temperatures.

Curiously, fFGOBz has the opposite effect of increasing the relative intensity of the isotropic signal at 45°C or above (Fig. 3), as was observed with the fusion peptide of measles virus in this same lipid [7].

In agreement with previous findings, ZfFG inhibits ANTS/DPX content leakage from LUV of monomethyl-DOPE (Fig. 4). For these experiments the same lipid was used as for the ^{31}P-NMR studies. Sufficient peptide was used to measure leakage at a moderate rate and the dependence of leakage rates on peptide concentration was studied. The behaviour of fFGOBz is more complex. At low to moderate levels, this

TABLE I

Peptide effects on T_H of DEPE

Peptide	pH	Slope [a]
ZfFG	7.4	84±8
fFGOBz	7.4	−13±1
formyl-fFGOBz	7.4	−8±4
ZfFG	5.0	89±4
fFGOBz	5.0	−39±5
formyl-fFGOBz	5.0	−12±4

[a] Shift of T_H per mol fraction of peptide additive.

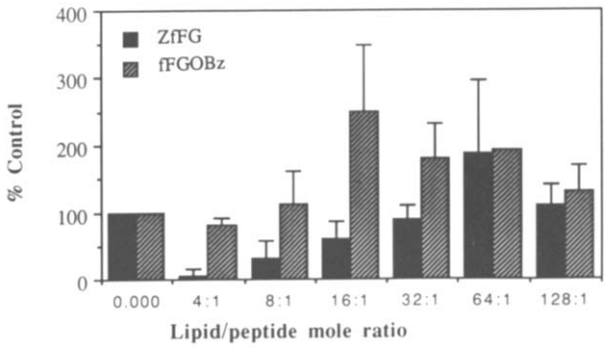

Fig. 4. Effect of peptides at various peptide/lipid ratios on the initial rate of leakage of aqueous contents from LUV of monomethyl-DOPE, pH 4.5, temperature 37°C. Results expressed as % control, with control leakage being measured after the addition of either peptide vehicle (buffer or methanol). ■, ZfFG; ▨, fFGOBz. Average of three independent experiments.

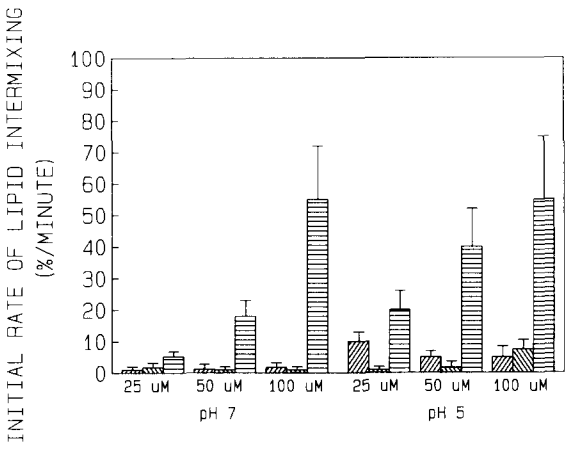

Fig. 5. RET assay for initial rate of fusion of vesicles of monomethyl-DOPE at 37°, pH 7 (left) and pH 5 (right). ▨, methanol control; ▧, ZfFG; ▤, fFGOBz. Lipid concentration = 30 μM. The average and S.E. of three independent experiments are shown. Variation of replicates within one experiment with one batch of vesicles is several fold smaller and always shows similar relative changes in lipid mixing after addition of the various peptides.

peptide causes a stimulation of leakage, though at the lowest and highest levels, there is very little effect. The lack of an effect at low phospholipid to peptide mol ratios (high peptide concentrations) may be due to aggregation of this hydrophobic peptide when injected at high concentrations into an aqueous environment. This problem has been observed in measurements with similar hydrophobic peptides (unpublished results). However, in general the effects of fFGOBz are opposite to those of ZfFG with respect to contents leakage.

Fusion measurements by mixing of aqueous contents are complicated by membrane leakage which will reduce the amount of fusion observed. Since the two peptides have opposite effects on leakage, it is difficult to compare their effects on fusion by this method. Membrane fusion can also be studied by lipid dilution using the RET assay. This method is insensitive to membrane leakage. Using the same lipid, *N*-methyl-DOPE, as for the leakage studies there are again generally opposite effects of the two peptides (Fig. 5). In all cases there is a dramatic, although quantitatively somewhat variable stimulation of lipid mixing by fFGOBz. The inhibitory effects of ZfFG are less consistent. The inhibitory effects are more readily observed at pH 5 where the rate of leakage in the absence of peptide is higher. However, at the highest peptide concentration of 100 μM at pH 5, the ZfFG has little effect. The fFGOBz promotes fusion with the rates generally being higher at pH 5 than at pH 7, except at 100 μM fFGOBz (Fig. 5).

As expected, partitioning of ZfFG into the membrane increased as the pH was reduced. With 6.6 mM *N*-methyl-DOPE and a lipid/peptide molar ratio of 30, at pH 7.4, 45% of the ZfFG was bound to the membrane while at pH 5, 65% was membrane bound. These values are in general agreement with those previously reported [5]. For the fFGOBz, a more extensive study was done (Table II). The concentration of free peptide in solution, C_f, was calculated from the absorbance of the supernate, as described in Experimental procedures. From this, the amount of peptide bound per lipid, X_b, and the partition coefficient, K_p, defined as X_b/C_f were also calculated. At pH 5, K_p is about 230 M^{-1}, except at the lowest lipid concentration. The lower value of K_p at 3.3 mM lipid may be a consequence of electrostatic repulsion preventing accumulation of the cationic peptide at the surface of a zwitterionic lipid [15,16]. Because of the limited amount of data and the lack of knowledge about the pK of the membrane-bound form of the peptide, no attempt was made to apply the Gouy-Chapman theory to account for this effect. One would anticipate that fFGOBz would have a pK between 7 and 8 in water and somewhat lower in a membrane environment. Hence, the state of ionization of this peptide will change between pH 7.4 and 5.0, becoming more cationic at

TABLE II

Binding of fFGOBz to N-methyl-DOPE membranes

Lipid (mM)	Lipid/total peptide	C_f (μM)	% Peptide bound	X_b (mmol/mol)	K_p (M^{-1})
pH 5: 10 mM citrate, 150 mM NaCl					
3.3	21	122	23	11	92
6.6	42	64	60	14	225
13	83	39	76	9.2	237
26	164	22	86	5.3	240
pH 7.4: 20 mM Pipes, 1 mM EDTA, 150 mM NaCl, 0.002% NaN_3					
3.3	19	49	54	18	358
6.6	39	37	65	10.6	287
13	76	29	73	6.0	207
26	153	17	84	3.5	204

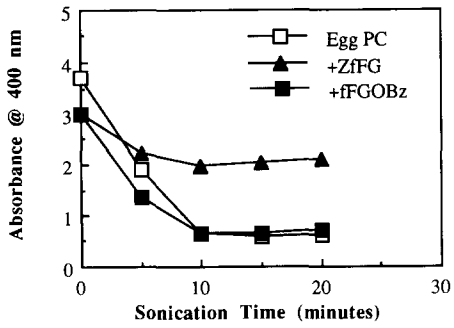

Fig. 6. Time dependence of the clarification of the turbidity of multilamellar suspensions of egg PC in ^2H_2O with 50 mM NaCl at 4°C. □, no peptide; ▲, ZfFG (lipid/peptide = 4); ■, fFGOBz (lipid/peptide = 4). Representative experiment shown. Results confirmed in three separate experiments.

lower pH. At pH 7.4, a similar value for the partition coefficient was obtained but the value of K_p increased somewhat at low lipid concentrations. This suggests an effect of peptide aggregation at low lipid/peptide molar ratios or to a systematic error in the experimental procedure. For the purpose of the present study, the partitioning experiments demonstrate that ZfFG and fFGOBz have similar partition coefficients in lipid and the 3-fold increase in the effect of fFGOBz on T_H of DEPE with a change of pH from 7.4 to 5.0 (Table I) is not a consequence of a markedly greater partitioning of this peptide into lipid at the lower pH, since when different, the partitioning is actually lower at pH 5.0 (Table II).

It has been observed that ZfFG inhibits dispersion of phosphatidylcholine by sonication [17]. Phosphatidylcholine is used for these experiments because it readily forms small unilamellar vesicles upon sonication, unlike DEPE or *N*-methyl-DOPE at neutral pH. This effect is confirmed using egg PC (Fig. 6) instead of DPPC. These studies were done in ^2H_2O to be directly comparable with previous published results, however, we have observed that similar inhibition of sonication by ZfFG also occurs in H_2O. This effect is not observed with fFGOBz which appears to slightly increase the rate of dispersion of egg PC by sonication (Fig. 6). Thus, the ability of ZfFG to inhibit the conversion of multilamellar vesicles of PC to structures with greater curvature is not exhibited by fFGOBz.

Discussion

It is known that ZfFG has antiviral activity [1]. This peptide was designed to mimic the sequence of the putative 'fusion peptide' of the amino-terminus of the Sendai virus fusion protein. There is evidence that the mechanism of this antiviral activity is through the inhibition of membrane fusion [3–5]. One of the differences between ZfFG and the viral 'fusion peptide' is that the terminal amino group of the inhibitory peptide, but not that of the viral protein, is blocked. The activity of ZfFG in inhibiting membrane fusion is associated with effects on model membranes leading to higher T_H [2], less intense isotropic ^{31}P signals than without peptide [5] and stabilization of membrane structures with low curvature [4]. All of these effects on model membranes are consistent with ZfFG inhibiting the formation of highly curved, non-lamellar structures. Decreasing the intrinsic radius of curvature of a lipid monolayer or formation of non-bilayer intermediates have been associated with increased rates of membrane fusion. ZfFG opposes such structural alterations of the membrane and inhibits fusion. The importance of the amino-terminal blocking group in determining the effects of ZfFG on membranes is not known.

In the present study we compare the properties of ZfFG with those of fFGOBz, a peptide of similar structure and hydrophobicity, but one in which the carboxyl-terminus, rather than the amino-terminus, is blocked. The resulting fFGOBz is cationic in contrast to the anionic ZfFG. fFGOBz has effects on membranes which are opposite to those of ZfFG. The fFGOBz promotes the formation of inverted phases and stimulates fusion. The peptide ZfFG has been shown to prevent increased curvature in bilayers composed of lipid exhibiting a large radius of curvature [17]. This provides a mechanism by which ZfFG can even inhibit the rearrangement of membranes with little tendency to form non-bilayer phases. This inhibition is not observed with fFGOBz. Thus, fFGOBz and ZfFG have opposite effects on several membrane properties related to membrane curvature.

Our results suggest a role for a free protonated amino group in the promotion of non-bilayer phases by fFGOBz. This is indicated by the pH dependence of the peptide induced shifts of T_H. The only peptide to promote a statistically significant change in T_H between pH 7.4 and pH 5.0 is the fFGOBz (Table I). This peptide is a 3-fold stronger promoter of H_{II} phases at low pH where the terminal amino group is more protonated. This occurs despite the fact that fFGOBz becomes less hydrophobic at low pH. Generally, more hydrophobic compounds are better promoters of the H_{II} phase. In addition to the pH effect, blocking the terminal amino group of fFGOBz by formylation greatly lowers the ability of the peptide to promote H_{II} phases (Table I). The formyl-fFGOBz is not charged and it has less effect than fFGOBz in lowering T_H (Table I). Interestingly, the uncharged peptide derivative ZfFGOMe, which unlike ZfFG, is not a bilayer stabilizer, has about 100-fold lower anti-measles activity than ZfFG [1]. These results clearly demonstrate the importance of the location of the blocking group in fFG.

A number of *N*-blocked carbobenzoxy peptides have antiviral activity [1,18,19]. With these antiviral peptides, a free carboxyl-terminus appears to be important

for their activity [20]. However, the factors which determine the antiviral potency of peptides are more varied and complex. In fact several carbobenzoxy peptides with free terminal carboxyl groups do not show antiviral activity. Furthermore, the position of the blocking groups is also not the sole factor determining the nature of the interaction of peptides with membranes. Other factors such as the nature of the amino acids, the conformation, hydrophobicity and length of the peptide also influence lipid phase preference. For example, although ZfFGOBz is a weak hexagonal phase promoter, a different doubly blocked peptide, *Z*-Tyr-Leu-NH_2 is a bilayer stabilizer which raises T_H by $+56 \pm 6$ degrees/mol fraction peptide [21]. In addition, other carbobenzoxy peptides with free terminal carboxyl groups are hexagonal phase promoters such as *Z*-D-Phe-L-Leu-Gly-D-Phe-D-Leu-D-Leu-Gly which lowers T_H by -16 ± 2 degrees/mol fraction peptide [22] and *Z*-Ala-Val-Gly which lowers T_H by -10 ± 1 degrees/mol fraction peptide (Epand, Anantharamaiah and Segrest, unpublished results). One thus cannot generalize about the effects of peptides on membranes simply on the basis of their charge and the nature of the blocking groups. However, for several peptide derivatives, such as ZfFG, which exhibit antiviral activity, the presence of an amino-terminal blocking group appears to be important for both the antiviral activity as well as the effects of these peptides on membrane properties. In contrast to these peptides, the promotion of inverted phases and of membrane disruption by fFGOBz may be a common property of amino-terminal segments of several viral fusion proteins [7,23].

The pair of peptides, ZfFG and fFGOBz, provide an interesting contrast. The former stabilizes bilayer membranes and inhibits leakage rates (Fig. 4) and rates of lipid intermixing (Fig. 5) while the opposite is the case with fFGOBz. Thus, fFGOBz appears to be a better model for some of the properties of viral fusion proteins, despite its small size. The results suggest that the ability of the terminal α-NH_2 group to be protonated contributes to the fusogenic properties of these peptides.

Acknowledgements

We are grateful to Ms. Joyce Young for performing the leakage experiments. This work was supported by grant MA-7654 of the Medical Research Council of Canada and grant AI26800 from the National Institutes of Health.

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