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Phospholipid Interactions of Synthetic Peptides Representing the N-Terminus of HIV gp41

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ABSTRACT: Peptides representing the N-terminal 23 residues of the surface protein gp41 of LAV_{1a} and LAV_{mal} strains of the human immunodeficiency virus were synthesized and their interactions with phospholipid vesicles studied. The peptides are surface-active and penetrate lipid monolayers composed of negatively charged but not neutral lipids. Similarly, the peptides induce lipid mixing and solute (6-carboxyfluorescein) leakage of negatively charged, but not neutral, vesicles. Circular dichroism and infrared spectroscopy show that at low peptide:lipid ratios (approximately 1:200), the peptides bind to negatively charged vesicles as α-helices. At higher peptide:lipid ratios (1:30), a β conformation is observed for the LAV_{1a} peptide, accompanied by a large increase in light scattering. The LAV_{mal} peptide showed less β-structure and induced less light scattering. With neutral vesicles, only the β conformation and a peptide:lipid ratio-dependent increase in vesicle suspension light scattering were observed for both peptides. We hypothesize that the inserted α-helical form causes vesicle membrane disruption whereas the surface-bound β form induces aggregation.

Enveloped viruses such as influenza and human immunodeficiency virus (HIV) infect their target cells in a process involving cell-specific binding followed by fusion of the viral envelope membrane with the appropriate cellular membranes (White, 1990; Blumenthal, 1984). Influenza viruses bind to cells expressing surface sialic acid residues, are endocytosed,

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and fuse with the endosomal membrane when the pH becomes acidic within the endosome (Stegmann et al., 1987). The HIV viruses bind to cells expressing the CD4 antigen (e.g., helper T-cells) and can subsequently fuse directly with the cellular membrane rather than requiring endocytosis and acidification (Stein et al., 1987). In each virus, one particular viral membrane protein is involved in both binding and fusion. By far the most extensively characterized is the influenza hemagglutinin protein [reviewed by Wiley and Skehel (1987)]. It is composed of two subunits, HA1 and HA2, which are formed by posttranslational proteolytic cleavage of a precursor protein. HA1 contains the cell surface binding site and most of the antigenic determinants. HA2 is bound to the viral membrane at its C-terminus and contains an approximately 20-residue conserved sequence at its N-terminus that is necessary for infective fusion. This N-terminal peptide is highly hydrophobic and has a high tendency to interact with phospholipid membranes (Lear & DeGrado, 1987).

The HIV virus, similar to influenza, expresses a membrane glycoprotein, gp160, on its surface. This protein is proteolytically cleaved into two subunits that appear from mutation studies (Kowalski et al., 1987) to have different functions. One subunit, gp120, contains the binding site for cell-surface CD4 antigens. The other, gp41, contains a C-terminal transmembrane-anchoring sequence and a highly hydrophobic N-terminal sequence with some homology to that of other putative "fusion peptides", including HA2 (Gallaher, 1987). Mutagenesis (McCune et al., 1988) has established that the gp160 cleavage reaction is necessary for fusogenic activity. Mutagenesis of the first 15 N-terminal residues of an analogous protein, gp32 of the simian immunodeficiency virus (closely related to HIV), has been used to probe the role of this sequence in viral fusion. Mutations that increase the overall hydrophobicity of this segment enhance virus-induced cell fusion reactions whereas polar substitutions reduce or abolish the fusogenic activity (Bosch et al., 1989). This further supports the "fusion peptide" assignment and suggests that the isolated gp41 N-terminus, like that of influenza HA2, directly interacts with the hydrocarbon portion of lipid bilayer membranes in a fusion proceses catalyzed by the virus.

Numerous studies [e.g., Doms et al. (1985)] with the water-soluble (bromelain-cleaved) form of influenza hemagglutinin, BHA, have shown that the N-terminal "fusion peptide" of influenza HA2 becomes available for interaction with other proteins or membranes when the pH is lowered to approximately 5. Harter et al. (1989) have recently shown that this peptide is the only portion of the hemagglutinin molecule that is labeled by photoactive, lipid-bound probes when BHA solutions are acidified in the presence of probe-containing liposomes. The lipid membrane-interactive properties of synthetic peptides comprising approximately the first 20 residues of the N-terminal segment of influenza HA2's from different virus strains have been extensively studied (Lear & DeGrado, 1987; Murata et al., 1987; Wharton et al., 1988). Under conditions specified in these studies, all of the peptides bind and perturb phospholipid vesicles, causing leakage of entrapped solutes, fusion of small unilamellar vesicles (SUV's), and erythrocyte lysis. The peptides appear to bind to membranes as α-helices, the N-termini of which are sufficiently hydrophobic to bind to the acyl chain hydrocarbon portion of the phospholipid bilayer (Lear & DeGrado, 1987). The membrane penetration is hypothesized to disrupt the bilayer structure, accounting for the observed membrane disruption. Similar mechanisms have been proposed to account for the membrane-perturbing effects of toxins such as melittin (Dawson et al., 1978, DeGrado, 1988). In this paper, we show that peptides representing the gp41 N-terminal segments of two different strains of HIV are also highly perturbing to negatively charged lipid membranes. Both peptides are highly surface-active (indicating attraction to polar/apolar interfaces) and can "penetrate" negatively charged lipid monolayers, even at surface pressures above the peptides' own maximum values, indicative of strong lipid/peptide interactions. At low peptide to lipid ratios, the peptides bind in an α-helical conformation to negatively charged vesicles and disrupt the membranes sufficiently to cause the release of entrapped small molecules. At higher peptide:lipid ratios, intervesicular lipid mixing is observed. These results support the idea that, similar to influenza HA2, the surface activity of the gp41 N-terminus can play a significant role in the viral infection process.

Materials and Methods

Materials. Protected amino acids were purchased from Bachem and Advanced Chemtech, and 4-methylbenzhydrylamine resin was from Bachem. Lipids were from Avanti Polar Lipids, and 6-carboxyfluorescein (6-CF) was from Molecular Probes Inc. (Eugene, OR).

Peptides. Peptides HIV_{Arg} and HIV_{Ala} representing the N-terminus of gp41 from strains LAV_{1a} and LAV_{mal} (Figure 1) were synthesized by the Merrifield solid phase method (Stewart & Young, 1984) on a 4-methylbenzhydrylamine resin using Boc-protected amino acids and a DuPont RaMPS system. Amino acid symmetric anhydrides were formed by mixing a 6-fold excess of protected amino acid with a 3-fold excess of diisopropylcarbodiimide in dimethylformamide. Couplings of the symmetric anhydrides with resin-bound peptide required between 0.5 and 2 h. Completed peptide was cleaved from the resin by treatment with HF/anisole (9:1) at 0 °C for 1 h. The peptides were purified by reversed-phase HPLC using a preparative Vydac C_{18} column and linear gradients of increasing concentrations of aqueous acetonitrile containing 0.1% trifluoroacetic acid. They were homogeneous by criteria of analytical HPLC, fast atom bombardment mass spectroscopy, and amino acid analysis.

Surface Tension Measurement. Surface pressure was determined by the de Noüy method (Adamson, 1976) using a Fisher Autotensiomat Model 215. For measuring equilibrium spreading pressures, a concentrated solution of the peptide in DMSO was injected beneath the surface of phosphate-buffered saline (PBS: 50 mM phosphate/100 mM NaCl, pH 7.0), and the surface tension was monitored until a constant value was reached. Above 0.5 μM, the final value obtained was independent of peptide concentration. For monolayer insertion experiments, a lipid monolayer was spread from a chloroform solution to attain surface pressures slightly below the peptides' equilibrium, saturating surface pressure. The peptide was added by injecting a concentrated solution beneath the monolayer and the resulting change in surface pressure observed.

Vesicle Preparation. Stock solutions of the desired lipids in chloroform were mixed and dried to a film using a stream of nitrogen, and the residue was further dried under reduced pressure for at least 3 h. Large unilamellar vesicles (LUV's) were prepared by adding the appropriate buffer to the dried film and vortexed to give a 10 mg/mL suspension. This was then freeze–thawed 5 times using liquid nitrogen/lukewarm water cycles and then extruded 10 times through two stacked, 0.1-μm polycarbonate filters (Nucleopore Corp.) in a pressure extruder (Lipex Biomembranes Inc., Vancouver). For small unilamellar vesicles (SUV's), dried lipids were hydrated in buffered solutions to a final concentration of 10 mM, followed by sonication under nitrogen at 20–25 °C until no turbidity

gp41 N-Terminus

(a) Ala-Val-Gly-Ile-Gly-Ala-Leu-Phe-Leu-Gly-Phe-Leu-Gly-Ala-Ala-Gly-Ser-Thr-Met-Gly-Ala-Arg-Ser

(b) Ala-Ile-Gly-Leu-Gly-Ala-Met-Phe-Leu-Gly-Phe-Leu-Gly-Ala-Ala-Gly-Ser-Thr-Met-Gly-Ala-Ala-Ser

FIGURE 1: Amino acid sequences of peptides from the gp41 N-terminus of LAV_{1a} (HIV_{Arg}) (a) and LAV_{mal} (HIV_{Ala}) (b). Synthetic peptides used in this work were amidated at their carboxyl termini.

was evident. For vesicle leakage measurements, 6-CF (100 mM for SUV's, 50 mM for LUV's) was encapsulated in vesicles, and gel filtration (Sephadex G50) was used to remove nonentrapped dye.

Fluorescence Measurements. Fluorescence spectra were measured with a Perkin-Elmer 650-40 fluorescence spectrophotometer equipped with a thermostated cell holder and a magnetic stirring device. Experiments were performed at 25 °C with constant mixing of the medium. Peptides were added to vesicle/buffer mixtures from a concentrated stock solution (approximately 1 mM) in DMSO (dimethyl sulfoxide) such that the final concentration of DMSO did not exceed 1%. DMSO at these concentrations had no measurable effect on vesicle stability.

Infrared Measurements. Infrared (IR) spectra were measured with a Nicolet Series 60SX Fourier transform infrared spectrophotometer. KRS-5 ATR prisms (45°, 50 × 20 × 3 mm) were from Harrick Scientific. Planar multilayers were prepared by dissolving the peptide and lipid (POPG, 1-palmitoyl-2-oleoylphosphatidylglycerol; or POPC, 1-palmitoyl-2-oleoylphosphatidylcholine) together in hexafluoro-2-propanol at molar ratios of peptide to lipid of 1:30 or 1:200 (55 μM peptide concentration). Solvent was evaporated using a stream of nitrogen and the residue dried under reduced pressure overnight. The dried film was resuspended in water (20 min) and then sonicated for 10 min at 25 °C. Multilayers were formed by drying the vesicles onto a KRS-5 plate using a gentle current of nitrogen until traces of liquid water were no longer visible, and spectra were recorded immediately. If it was necessary to store the multilayers for any length of time (more than 20 min), they were placed in a sealed container saturated with water vapor. No change in spectra was observed when the samples were stored for up to 3 days in this manner.

Circular Dichroic (CD) Measurements. Spectra were recorded on a Jobin Yvon Dichrograph V spectropolarimeter in 1-mm cells, at 25 °C. A stock solution of the HIV_{Arg} peptide (Figure 1) in methanol/water (1:1) was added to PBS buffer (0.1 M NaCl/0.05 M sodium phosphate, pH 7.0) with or without vesicles such that the final concentration of methanol was less than 0.5%, and the spectra were averaged over 20 scans.

Peptide-Induced Vesicle Leakage. This was measured in both SUV's and LUV's by the increase of fluorescence intensity of 6-CF caused by its dilution upon leakage from a self-quenching concentration (100 or 50 mM) inside the vesicles. Results are expressed as the percent of the intensity obtained after Triton X-100 addition to solubilize the vesicles.

Peptide-Induced Lipid Mixing. This was measured in SUV's with a fluorescence probe dilution assay, based on resonance energy transfer (Struck et al., 1981). Both probes, *N*-NBD-DOPE [*N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)-dioleoylphosphatidylethanolamine; donor of energy] and *N*-RhB-PE [*N*-(lissamine rhodamine B sulfonyl)diacylphosphatidylethanolamine; acceptor of energy], were added together at 0.3 mol % each to the lipids used to prepare vesicles. Both labeled and unlabeled vesicles were mixed in a 1:1 ratio. The fluorescence intensities were measured at 530 and 590 nm (excitation wavelength 465 nm, 5-nm slit widths). The ratio of the emission intensities increases with dilution of the energy transfer pair upon mixing of the labeled and unlabeled vesicle lipids. Complete lipid mixing of the vesicles would result in membranes containing 0.15 mol % of each fluorescent phospholipid. As observed previously (Lear & DeGrado, 1985), SUV fusion and resulting intervesicular lipid mixing exhibited an initially rapid rate up to a level well below complete mixing, followed by little or no further change with time. Consequently, we here define peptide-induced lipid mixing as the percent of the theoretical maximum increase in the fluorescence intensity ratio (I_{530nm}/I_{590nm}) observed after a time sufficient to indicate little further change was occurring. The theoretical maximum increase value was measured directly on freshly prepared vesicles containing 0.15 mol % of each probe.

Table I: Surface Pressure Measurements

peptide	initial surface	final obsd surface pressure (mN/m)
HIV_{Arg}	clean, air/water interface	25
HIV_{Arg}	POPC, 22 mN/m	24
HIV_{Arg}	POPG, 22 mN/m	33
HIV_{Ala}	clean, air/water interface	23
HIV_{Ala}	POPC, 20 mN/m	25
HIV_{Ala}	POPG, 20 mN/m	30

Light Scattering. The 90° light-scattering measurements were done by using the Perkin-Elmer 650-40 fluorescence spectrophotometer with both emission and excitation wavelengths set at 400 nm, 3-nm slit widths.

RESULTS

Peptides. Peptides (Figure 1) representing the first 23 residues of gp41 from HIV strains LAV_{1a} and LAV_{mal} (Gallaher, 1987) were synthesized as their C-terminal carboxamides. The peptide from LAV_{1a} contains a positively charged Arg residue at position 22 as the sole ionizable side chain, and will be referred to as HIV_{Arg}. The other peptide (HIV_{Ala}) has an alanine residue in the corresponding position along with several other conservative changes. To further explore the effect of charge, this peptide was also synthesized with an acetylated N-terminus (Ac-HIV_{Ala}). However, this peptide showed very low solubility in aqueous solution, so most studies were conducted on the slightly more soluble HIV_{Ala} and HIV_{Arg} peptides.

Monolayer Binding Properties. At submicromolar concentrations, both the HIV_{Ala} and HIV_{Arg} peptides were highly surface-active, lowering the surface tension of the air/water interface by 23 and 25 mN/m, respectively, at saturating peptide concentrations (0.5 μM or greater). For comparison, the equilibrium spreading pressure of melittin is 18 mN/m (Fidelio et al., 1981). The ability of the HIV_{Ala} and HIV_{Arg} peptides to insert into phospholipid monolayers was assessed by injecting the peptides beneath a phospholipid monolayer with an initial surface pressure slightly less than the equilibrium spreading pressure of the peptide, and monitoring the consequent change in the surface pressure while holding the surface area constant. Table I summarizes the results of these

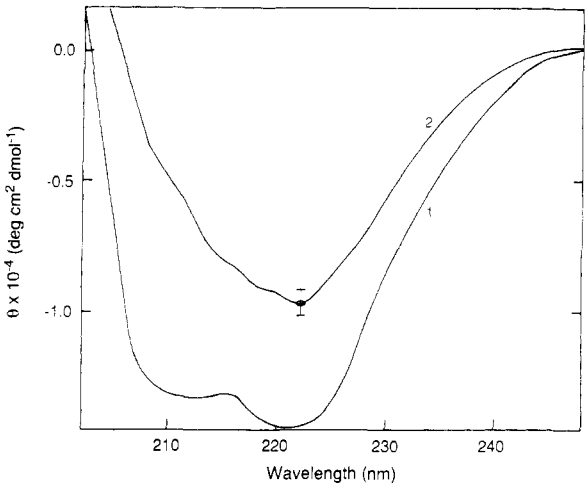

FIGURE 2: CD spectra of HIV_{Arg}/POPG complexes in PBS buffer. The peptide was added from a concentrated stock solution (approximately 0.2 mM in 50% aqueous methanol) to preformed vesicles (POPG concentration 1 mM) to give a final peptide:lipid ratio of 1:30 (2) or 1:200 (1).

experiments. The HIV_{Ala} and HIV_{Arg} peptides were capable of increasing the surface pressure of negatively charged POPG monolayers—but not neutral POPC monolayers—beyond the equilibrium spreading pressure of the peptide. Such interactions have been interpreted as evidence for insertion into a lipid monolayer (Briggs et al., 1985), indicating the HIV_{Ala} and HIV_{Arg} peptides bind and insert into POPG monolayers.

Vesicle Binding and Conformational Studies. In aqueous solution, the HIV_{Arg} peptide displayed a CD spectrum indicative of a random conformation with a mean residue ellipticity (θ_{222}) = −1500 ± 1000 deg cm^2 dmol^{-1}. However, if the peptide was added to a solution containing phospholipid vesicles, the spectra observed (Figure 2) were indicative of the formation of substantial secondary structure (Greenfield & Fasman, 1969). At a peptide concentration of 5 μM and at low peptide:lipid ratios (1:200) with POPG vesicles, a spectrum with minima at 222 and 208 nm was observed, consistent with a predominantly α-helical conformation. At higher peptide:lipid ratios (1:30), a spectrum consistent with a predominantly β-sheet conformation was observed. With POPC vesicles, CD spectra showed a shape consistent with a β conformation, although the intensity at 217 nm (−6000 deg cm^2 dmol^{-1}) was significantly lower than would be expected from reference spectra (Greenfield & Fasman, 1969). Intensity dampening of CD signals is known to accompany light scattering, which would be likely to occur as a result of vesicle aggregation (see within) in these experiments. The change in shape of the spectra, however, indicates that the peptides bind to POPC vesicles, but are apparently unable to insert, as indicated by the monolayer experiments.

CD experiments with the HIV_{Ala} peptide were technically difficult because of its low solubility. Moreover, quantitative interpretation of the CD spectra was not possible because of the increased light scattering from vesicles in the presence of both peptides. We therefore turned to IR spectroscopy as an independent method to determine conformation. Figure 3 illustrates the IR spectra of the peptide/lipid multilayers in the amide I region. The intense band centered at 1735 wave numbers (cm^{-1}) is due to the ester carbonyl absorbance, while the bands between 1600 and 1700 cm^{-1} are due to the peptide amide groups and are conformationally sensitive. Disordered and α-helical structures give bands between 1650 and 1660 cm^{-1} while antiparallel β-sheets generally give a very sharp band near 1630 cm^{-1} and a less intense band near 1690 cm^{-1}

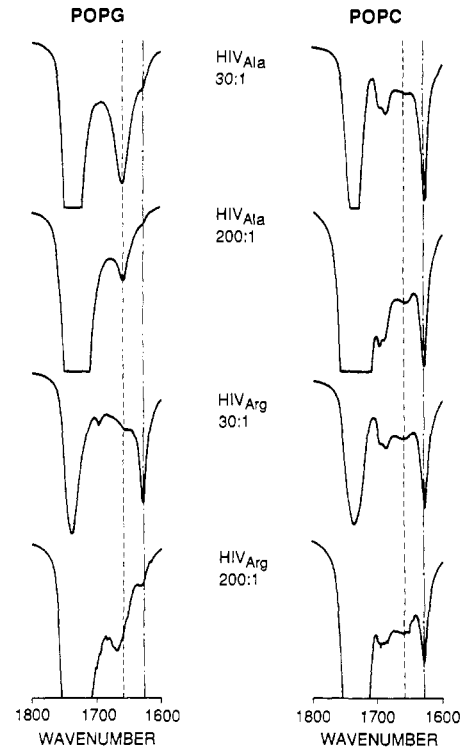

FIGURE 3: IR absorption spectra of HIV_{Ala} and HIV_{Arg} peptides in POPG and POPC multilamellar membranes. Experimental conditions are described under Materials and Methods. The amide I band near 1660 cm^{-1} is assigned to α-helical structure and the band near 1630 cm^{-1} to β conformation.

(Surewicz & Mantsch, 1988). The IR spectra of HIV_{Arg} in POPC monolayers are consistent with an almost exclusively antiparallel β-sheet conformation at both low and high peptide to lipid ratios. The HIV_{Ala} peptide also gave spectra consistent with a predominantly antiparallel β-sheet conformation at both peptide to lipid ratios in POPC multilayers. Together with the CD results, the IR spectra of HIV_{Ala} in POPG multilayers indicate that it adopts a predominantly α-helical conformation at both 1:200 and 1:30 peptide:lipid ratios. HIV_{Arg} in POPG appears α-helical at low peptide:lipid ratios (1:200) but exhibits a significant fraction of antiparallel β-sheet conformation at high peptide:lipid ratios (1:30). It should be noted that it is difficult to differentiate between α-helical and disordered conformations on the basis of IR spectroscopy using the amide I bands (DeGrado & Lear, 1984), but, on the basis of CD measurements, we consider the band near 1660 cm^{-1} to arise from the α-helical conformation.

Vesicle Leakage. The interaction of both of the HIV peptides with negatively charged lipid vesicles leads to membrane disruption as measured by the peptide concentration-dependent leakage of 6-CF entrapped in the vesicles. The HIV_{Ala} and HIV_{Arg} peptides caused rapid leakage of 6-CF from SUV's (data not shown) and LUV's composed of POPG (Figure 4) but not POPC. With POPG vesicles, a rapid burst of leakage was observed at the earliest time point (approximately 10 s), followed by a slower phase. In contrast, very little leakage over the background rate was observed with vesicles composed of POPC. Figure 4 describes the lipid: peptide ratio dependence of the leakage in LUV's (measured after 10 min, sufficient to complete the rapid phase of the kinetics) at a constant (8 μM) lipid concentration. The HIV_{Arg} peptide was somewhat more potent than the HIV_{Ala} peptide, possibly because of electrostatic interaction between the positively charged Arg side chain and the negatively charged vesicles.

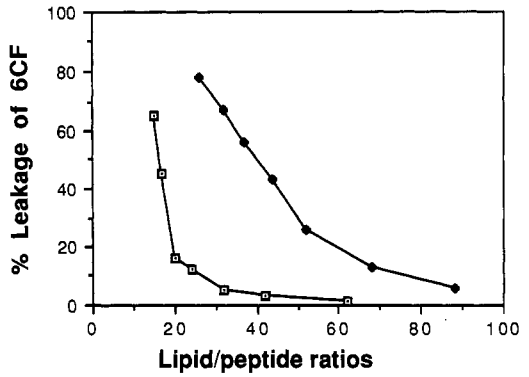

FIGURE 4: Leakage of 6-CF from large unilamellar vesicles of POPG initiated by the addition of peptide to vesicle suspensions at different lipid:peptide ratios. The amount of leakage was monitored 10 min after addition of peptide to vesicles (POPG concentration = 8 μM) suspended in PBS. (♦) HIV_{Arg} peptide; (□) HIV_{Ala} peptide.

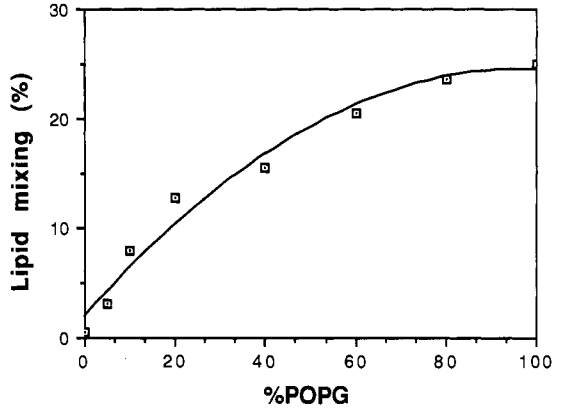

FIGURE 5: Effect of membrane composition on intervesicle lipid mixing induced by 3 μM HIV_{Arg} peptide in PBS, peptide:lipid molar ratio = 1:17.

Lipid Mixing. The ability of the peptides to induce intervesicular lipid mixing of SUV's was assessed by using a technique that measures the dilution of fluorescent lipids when mixing occurs between fluorescently labeled and unlabeled populations of vesicles (Struck et al., 1981). When this method was used, both the HIV_{Ala} and HIV_{Arg} peptides were seen to be capable of catalyzing lipid mixing in vesicles composed of negatively charged (POPG and POPS, 1-palmitoyl-2-oleoylphosphatidylserine) but not neutral (POPC) phospholipids. Figure 5 illustrates the extent of mixing induced by the HIV_{Arg} peptide as a function of the mole percent of POPG in SUV's composed of mixed POPC and POPG phospholipids. As the percent of negatively charged phospholipid is increased, the degree of mixing also increases, suggesting that electrostatic interactions contribute to the process. This was confirmed qualitatively in experiments (data not shown) where nearly a 2-fold decrease in lipid mixing was seen when the salt (NaCl) concentration was varied from 0.01 to 1.0 M. The HIV_{Ala} peptide, although inducing mixing more slowly in the initial stage, also showed a similar decrease in the final extent of lipid mixing at high salt concentrations, suggesting that the α-amino group rather than the arginine guanidino group (present in the HIV_{Arg} but not the HIV_{Ala} peptide) might more likely be responsible for the salt effect.

Vesicle Aggregation. The HIV_{Arg} peptide appeared also to be capable of causing aggregation of negatively charged phospholipid vesicles. Addition of this peptide to POPG SUV's gave rise to a milky suspension indicative of the formation of very large particles. A control with only peptide added gave no detectable change. In comparison, HIV_{Ala} (at an equal degree of lipid mixing) caused significantly less turbidity. For example, when 3 μM peptide was added to vesicles (final POPG concentration, 50 μM, to give about 25% lipid mixing after 10 min), the light scattering at 400 nm increased 10-fold for HIV_{Arg} and 7-fold for HIV_{Ala}. This phenomenon was further studied by using POPC SUV's where, in the absence of appreciable lipid mixing, the turbidity increases were due primarily to vesicle aggregation. Addition of HIV_{Arg} to POPC vesicles (concentration 50 μM) gave a 44% increase in light scattering—the corresponding value for HIV_{Ala} was 67%. This order of aggregational potency for POPC vesicles was also observed using a fluorescence energy transfer method (Gibson & Loew, 1979) employing donor and acceptor probes on separate POPC SUV's. In these experiments, aggregation of vesicles results in an increase of energy transfer (data not shown).

Discussion

The findings of this paper provide general information concerning hydrophobic peptide/phospholipid vesicle interactions. We find that the ability of the peptides to insert into monolayers of a given type of lipid correlates with their ability to disrupt vesicles composed of the same lipid. The increase in monolayer surface pressure is a result of energetically favorable peptide/phospholipid interactions. In a vesicular system exposed to peptide, the energy of these interactions could conceivably lower the activation energy for whatever structural reorganizations are involved in the fusion process.

Also correlated with the peptides' abilities to insert into and disrupt membranes of a given lipid type are their abilities to form helices at low peptide:lipid ratios. Helices allow the peptide amide groups to form internal hydrogen bonds, an effectively necessary energetic condition for their insertion into the apolar membrane interior (Roseman, 1988). At higher peptide:lipid ratios (for HIV_{Arg}), or with neutral vesicles where the monolayer data predict no insertion, the peptides appear from the IR data to be bound predominantly as antiparallel β-sheets. Other workers (Briggs et al., 1986) have similarly found that hydrophobic signal sequences are α-helical when inserted into monolayers but are in β-conformations when surface-bound.

In the present study, we find that vesicle aggregation accompanies the formation of antiparallel β-sheets. This finding suggests that the peptides form large, extended sheets that partially coat the vesicles, and may cause aggregation because of their extreme hydrophobicity. Also consistent with this view is the charge selectivity shown by the peptides; the more positively charged HIV_{Arg} peptide was more efficient at aggregating negatively charged vesicles, while HIV_{Ala} showed specificity for neutral vesicles.

The penetration and membrane disruption effects of both of the peptides showed clear specificity for negatively charged vesicles. This is interesting because the only ionizable group of the HIV_{Ala} peptide is the N-terminal α-amino group. The salt concentration dependence of the peptide lipid interactions is, however, surprisingly modest for a purely aqueous electrostatic interaction. This observation could reflect opposing electrostatic effects (e.g., peptide/vesicle versus vesicle/vesicle interactions). Alternatively, the N-terminus of the peptide helix might be inserted into the bilayer at sufficient depth to interact with the membrane phosphates without experiencing major counterion screening effects from ions located in the Stern layer.

These results are also of interest in connection with the general mechanism of action of gp41 in the membrane fusion events accompanying HIV viral infection. Our finding here, together with previously mentioned work with influenza HA2

peptides, is that the N-terminal peptides can adopt helical structures and interact strongly enough with phospholipids to disrupt bilayer membranes. The exact significance of this in biologically relevant fusion events remains to be established.

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