Interaction of Peptide Fragment 828–848 of the Envelope Glycoprotein of Human Immunodeficiency Virus Type I with Lipid Bilayers

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ABSTRACT: The interaction of the peptide fragment 828–848, called P828, from the carboxy-terminal region of the envelope glycoprotein gp41 of HIV-I with model membranes composed of phosphatidylcholine (PC) and phosphatidylglycerol (PG) was investigated using microelectrophoretic mobility of liposomes, fluorescence polarization of labeled lipids, NMR, and differential scanning calorimetry. The peptide binds to negatively charged lipid surfaces. No interaction between P828 and neutral PC surfaces is observed. The interaction between the peptide and the lipid is exclusively electrostatic with the six positively charged arginines of P828 acting as binding sites for PG. Circular dichroism measurements of P828 indicate that the peptide undergoes a transition from a random coil to an ordered conformation upon binding to negatively charged PG bilayers or SDS micelles, but not in the presence of neutral PC bilayers. The ordered structure has an apparent helical content of 60%. In DOPG/DOPC mixtures containing 20 mol % DOPG, the peptide causes the formation of lipid domains enriched in DOPG, as assessed by measurement of fluorescence energy transfer between labeled PG and PC. The formation of these domains requires energy and therefore reduces the strength of peptide binding to the lipid matrix. Our data support and quantitate the results from antibody binding studies [Haffar, O. K., Dowbenko, D. J., & Berman, P. W. (1988) *J. Cell Biol. 107*, 1677–1687] that the carboxy-terminal segment of the envelope glycoprotein gp41 interacts with microsomal membranes.

The envelope glycoprotein gp160 which coats human immunodeficiency virus (HIV) has two cleavage products, gp120 and gp41, both of which are implicated in the early stages of infectivity leading to AIDS. The gp41 fragment (residues 512–856 of isolate HXB2R) is an integral membrane protein. Haffar et al. (1988) demonstrated, using antipeptide antibodies specific for various segments of gp41, that the extracellular domain of the protein extends to residue 684 and that residues 707–856 constitute the intracellular domain. Studies on viruses where the carboxy-terminal region of the intracellular domain has been modified indicate that this region of the gp41 is implicated in the cytotoxicity of the virus (Fisher et al., 1986; Hirsch et al., 1987; Lee et al., 1989; Gabuzda et al., 1992). In addition, Miller et al. (1991, 1992) recently reported strong cytolytic effects on both prokaryotic and eukaryotic cells of a peptide which represents a segment of the carboxy terminus of the gp41.

In their study using antibodies raised against carboxy-terminal fragments of the gp41, Haffar et al. (1988, 1991) observed that this region of the protein interacts with microsomal lipid bilayers. In order to improve our understanding of this interaction and its potential functional consequences, we investigated the membrane interaction of a peptide fragment corresponding to the region 828–848 (labeled P828) of gp41. This fragment has the sequence RVIEVVQGACRAIRHIPRRIR and contains six positively charged arginine residues. It has been shown that as an α-helix P828 displays an extraordinarily high degree of amphiphilicity (Venable et al., 1989; Eisenberg & Wesson, 1990). In analogy to known amphipathic α-helices, it was predicted that this sequence could interact strongly with a lipid surface or span a lipid bilayer as an aggregate of helices.

We monitored the interaction of P828 with both neutral and negatively charged phospholipid membranes by a variety of techniques including ^{31}P and ^{2}H NMR, microelectrophoretic mobility, fluorescence spectroscopy, and differential scanning calorimetry (DSC).[1] Structural changes in the peptide upon binding to the phospholipid bilayer were observed by circular dichroism (CD) spectropolarimetry. Our results show that the peptide binds at the surface of the membrane and that this interaction is entirely electrostatic. The charge neutralization at the lipid/water interface which results from the binding of the positively charged peptide with negatively charged liposomes was used to quantitate the interaction.

MATERIALS AND METHODS

Synthesis and Purification of the Peptide Fragment. The P828 peptide was synthesized on a MilliGen/Biosearch Model 9600 peptide synthesizer (San Rafael, CA) using tboc chemistry. The crude peptide was purified by HPLC with a 250 mm × 10 mm Synchropak RP-4 column (SynChrom, Lafayette, IN). The identity and purity of the peptide were confirmed by ^{252}Cf mass spectrometry.

Phospholipids. L-α-Dipalmitoylphosphatidylcholine (DPPC), L-α-[^{2}H]dipalmitoylphosphatidylcholine (DPPC-d_{62}), L-α-dimyristoylphosphatidylcholine (DMPC), L-α-dioleoylphosphatidylcholine (DOPC), L-α-dimyristoylphosphatidylglycerol (DMPG), and L-α-dioleoylphospha-

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[1] Abbreviations: P828, H_3N-Arg-Val-Ile-Glu-Val-Val-Gln-Gly-Ala-Cys-Arg-Ala-Ile-Arg-His-Ile-Pro-Arg-Arg-Ile-Arg-COOH; PC, phosphatidylcholine; PG, phosphatidylglycerol; DPPC, L-α-dipalmitoylphosphatidylcholine; DPPC-d_{62}, L-α-[^{2}H]dipalmitoylphosphatidylcholine; DMPC, L-α-dimyristoylphosphatidylcholine; DOPC, L-α-dioleoylphosphatidylcholine; DMPG, L-α-dimyristoylphosphatidylglycerol; DOPG, L-α-dioleoylphosphatidylglycerol; APC, 1-acyl-2-[12-(9-anthryl)-11-*trans*-dodecenoyl]-*sn*-glycero-3-phosphatidylcholine; PPC, 1-acyl-2-[9-(3-perylenoyl)nonanoyl]-*sn*-glycero-3-phosphatidylcholine; APG, 1-acyl-2-[12-(9-anthryl)-11-*trans*-dodecenoyl]-*sn*-glycero-3-phosphatidylglycerol; SDS, sodium dodecyl sulfate; DTT, 1,4-dithio-L-threitol; CD, circular dichroism; DSC, differential scanning calorimetry.

tidylglycerol (DOPG) were purchased from Avanti Polar Lipids Inc., Alabaster, AL. All products were judged to be at least 98% pure by thin-layer chromatography.

The fluorescence labeled phospholipids 1-acyl-2-[12-(9-anthryl)-11-*trans*-dodecenoyl]-*sn*-glycero-3-phosphatidylcholine (APC), 1-acyl-2-[9-(3-perylenoyl)nonanoyl]-*sn*-glycero-3-phosphatidylcholine (PPC), and 1-acyl-2-[12-(9-anthryl)-11-*trans*-dodecenoyl]-*sn*-glycero-3-phosphatidylglycerol (APG) were synthesized in the Shemyakin Institute of Bioorganic Chemistry, Moscow, Russia (Molotkovsky et al., 1984).

Circular Dichroism. Spectra were recorded on a J-600 spectropolarimeter (JASCO, Tokyo, Japan) using a 0.02-cm cylindrical cell. The peptide concentration in all samples was 50 μM. Unilamellar liposomes of DOPG (0.9 mg of DOPG in 2.2 mL of 15 mM NaCl, 1 mM Na_2HPO_4, and 1 mM DTT, pH 7.0) were prepared by ultrasonication. The CD spectra presented are the average of 16 accumulations. The helical content of the peptide conformation was calculated using version 1.02 of the secondary structure estimation program provided by JASCO (Chang et al., 1978).

NMR. Both ^{31}P and ^{2}H NMR spectra were recorded as described previously (Gawrisch et al., 1992). Lipid mixtures were prepared by dissolving both lipids in chloroform. Most of the solvent was then removed in a stream of nitrogen. Remaining chloroform traces were removed in vacuum. For sample preparation, the peptide was dissolved in 0.1 M NaCl/10 mM MOPS buffer solution in H_2O or D_2O adjusted to pH 7.0 or pD 7.4, respectively. Typically, 0.5 mL of solution was added to 50 mg of dry phospholipid. To ensure equilibrium, the dispersion was taken through at least three freeze–thaw cycles with vortexing between cycles. Also before any measurements were carried out, all samples were kept in the liquid-crystalline lipid phase for several hours (room temperature for DOPG/DOPC and 50 °C for DPPC). Unilamellar vesicles of DOPG, DOPC, and their mixtures in 10 mM NaCl/D_2O and 10 mM MOPS buffer, pD 7.4, were prepared by ultrasonication. P828 was added as a stock solution in the same buffer. At the completion of the high-resolution ^{31}P NMR experiments, the vesicles were converted into multilamellar liposomes by lyophilization and hydration with 50 wt % H_2O for the subsequent solid-state NMR investigations.

Microelectrophoresis. The measurements were performed on a Mark II apparatus from Rank Brothers (Bottisham, England) using the cylindrical cell. If not stated otherwise, a 0.1 M NaCl solution, containing 1 mM MOPS buffer adjusted to pH 7.0, was used. Lipids were mixed as mentioned above for NMR experiments. A 1 wt % stock solution of the lipid in buffer was prepared by vortexing. The final lipid concentration in the measurements was always 0.01 wt %. P828 was added to the lipid dispersion as a stock solution in MOPS buffer. The final dispersions were vortexed at high speed. To reduce any effect due to peptide binding to the glass surface, the cell was flushed first with a peptide solution of the appropriate concentration. Measurements were performed going from low to high peptide concentrations. The electric field along the capillary was measured using the inner cell electrodes of the cylindrical cell attached to a high input resistance electrometer. All data points are the average of backward and forward velocities of 10 particles. The ζ-potentials were calculated using the Helmholtz–Smoluchowski equation (Aveyard & Haydon, 1973).

Fluorescence Measurements. A Perkin-Elmer LS 50 fluorescence spectrometer (Beaconsfield, England) interfaced to an IBM PS/2 computer was used to measure fluorescence polarization and energy transfer. A stock solution of the lipid was dispersed in 0.1 M NaCl containing 1 mM MOPS buffer which was adjusted to pH 7.0. The final lipid concentration was 50 μg of lipid/mL. The fluorescence-labeled lipids were added as a stock solution of the labels in ethanol. The peptide was added as a stock solution in MOPS buffer. For homogenization, the dispersion was first vortexed and then ultrasonicated for 1 min. Fluorescence polarization is somewhat sensitive to the radius of curvature of membrane particles. In order to obtain reproducible results, any small unilamellar liposomes formed after ultrasonication were destroyed in a freeze–thaw cycle. For fluorescence polarization measurements, the anthrylvinyl fluorescence was excited at a wavelength of 376 nm with a slit width of 10 nm. Emission was observed at 433 nm and a slit width of 4 nm. Emission intensities were measured with an integration time of 10 s. Polarization values are the average of at least three independent measurements.

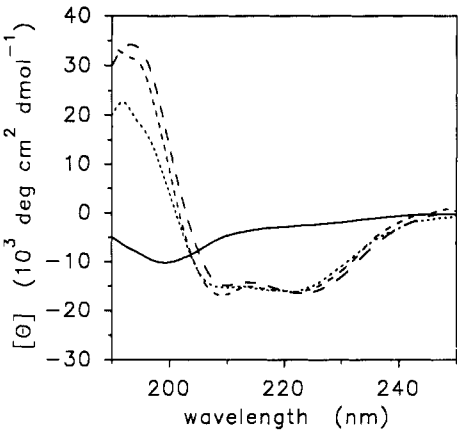

FIGURE 1: CD spectra of 50 μM P828 in different preparations: (—) 0.1 M NaCl, 1 mM Na_2HPO_4, and 1 mM DTT, pH 7.0; (– –) 0.05 wt % SDS, 15 mM NaCl, 1 mM Na_2HPO_4, and 1 mM DTT, pH 7.0; (- - -) 0.05 wt % DOPG unilamellar liposomes, 15 mM NaCl, 1 mM Na_2HPO_4, and 1 mM DTT, pH 7.0; (···) methanol.

For fluorescence energy transfer, the anthrylvinyl fluorescence was excited at 376 nm, and the emission of perylenoyl was measured at 520 nm.

Calorimetry. The experiments were performed on an MC-2 differential scanning calorimeter (MicroCal, Inc., Northampton, MA) with a heating rate of 45 °C/h. One milligram of lipid was dispersed in 2.5 mL of a 10 mM NaCl/10 mM MOPS buffer solution at pH 7.0. The P828 peptide was added as a stock solution in buffer. For homogenization, the samples were ultrasonicated and taken through one freeze–thaw cycle.

EXPERIMENTAL RESULTS

Circular Dichroism. Circular dichroism spectra were obtained on P828 in (i) 0.01–0.3 M NaCl, 1 mM Na_2HPO_4, and 1 mM DTT, pH 7.0; (ii) 0.05 wt % SDS, 15 mM NaCl, 1 mM Na_2HPO_4, and 1 mM DTT, pH 7.0; (iii) 0.05 wt % DOPG unilamellar vesicles, 15 mM NaCl, 1 mM Na_2HPO_4, and 1 mM DTT, pH 7.0; and (iv) methanol.

The peptide dissolves readily in water and was dissolved in methanol at concentrations below 50 μM. The CD spectra (Figure 1) show that the free peptide in aqueous buffer is not structured. It forms a partly ordered structure if dissolved in methanol or in buffer containing 0.05 wt % SDS or negatively charged phospholipid. The spectral fits indicate a helical content of about 60%.

Sodium chloride concentrations in CD measurements were kept low because heavy light scattering at wavelengths around

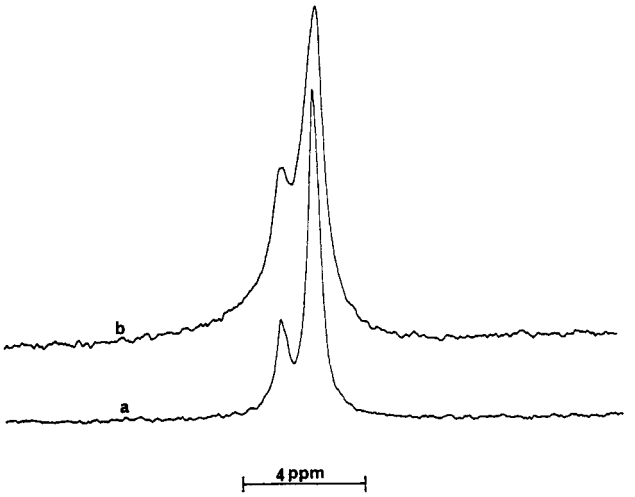

FIGURE 2: ^{31}P NMR spectra of a dispersion of unilamellar liposomes (28 mol % DOPG in a DOPG/DOPC mixture) produced by ultrasonication in 10 mM NaCl/10 mM MOPS, pH 7.0, without (a) and with (b) addition of P828 (DOPG/P828 = 10/1 mol/mol).

200 nm made measurements at higher ionic strength in the presence of micelles or liposomes impossible. No differences between CD spectra recorded at concentrations of 15 mM NaCl and 150 mM NaCl were observed in the range from 250 to 210 nm.

The P828 molecule may form dimers in solution via disulfide bridges between two cysteines. The measured helical content of bound dimerized peptide is significantly lower than that of monomeric peptide (results not shown). Dimer formation was avoided by addition of 1 mM DTT.

NMR. NMR spectroscopy provides information about the phase state of the lipid as well as the nature of the lipid/peptide interaction. The influence of P828 on both neutral and negatively charged membranes was investigated using high-resolution ^{31}P NMR on unilamellar vesicles of DOPC and of DOPG/DOPC mixtures containing 28 mol % DOPG. If the pH of the dispersion is kept constant, the signals of phosphate groups are not shifted by the addition of P828. However, addition of the peptide to the negatively charged vesicles results in an increase in the line widths of the ^{31}P resonances (see Figure 2), presumably because the peptide causes the vesicles to aggregate or fuse.

Both ^{31}P and ^{2}H solid-state NMR experiments were carried out on phospholipid/water dispersions containing up to 1 molecule of P828 per 20 molecules of either DOPC, DPPC, DOPG, or DOPG/DOPC mixtures. The ^{31}P NMR anisotropies of chemical shift are identical to preparations not containing the peptide. The quadrupolar splittings of the methylene groups of the deuterated fatty acids in DPPC-d_{62} do not change after addition of P828.

Calorimetry. The midpoint of the main-phase transition temperature of DMPC (24.3 ± 0.1 °C) and the enthalpy of the transition (4.7 ± 0.3 kcal/mol) do not change in the presence of a 10 μM solution of P828 (lipid/P828 = 50/1 mol/mol). The main-phase transition temperature of DPPC in the presence of P828 was measured on a Perkin-Elmer DSC-2 calorimeter at a water concentration of 50 wt %. At a DPPC/P828 molar ratio of 20/1, the phase transition temperature (41.7 ± 0.2 °C) is identical to the transition temperature of pure DPPC.

Addition of P828 to multilamellar liposomes of DMPG or DMPG/DMPC (1/2 mol/mol) mixtures broadens the main-phase transition significantly. Several distinct peaks are visible. The midpoint of the transition shifts upward by a few degrees. Additional ultrasonication and freeze–thaw cycles change the appearance of the thermograms but do not reduce the width of the transition region.

Fluorescence Spectroscopy. The fluorescence polarization of APC in DOPC liposomes does not change upon addition of 20 μM P828 to the solution. However, addition of the peptide to DOPG liposomes increases the APG fluorescence polarization significantly. In DOPG/DOPC mixtures containing 20 mol % DOPG, the addition of P828 results in a moderate increase of the fluorescence polarization of APC and an almost 2-fold increase of the polarization of APG (Table I). Samples containing 20 mol % DOPG and 1 mol % APG show significant self-quenching of fluorescence after addition of P828.

It was shown previously that between anthrylvinyl and perylenoyl labels in membranes excitation energy is transferred (Molotkovsky et al., 1984). The energy transfer from APC or APG to PPC in DOPG/DOPC multilamellar liposomes (1/4 mol/mol) was investigated in the presence and absence of peptide. The rate of transfer depends critically on the ability of lipids to form domains. After addition of 20 μM P828 to the liposomes, the rate of transfer from APG to PPC decreases (see Figure 3) whereas the energy transfer from APC to PPC remains unchanged within experimental error. The APG concentration in these experiments was kept as low as possible (0.2 mol %) to avoid self-quenching of the APG fluorescence.

Microelectrophoresis. The binding of positively charged P828 to the lipid surface changes the ζ-potential of liposomes. The ζ-potential of pure DOPC liposomes is close to 0 and does not change even upon addition of 100 μM peptide.

In Figure 4, the ζ-potential of DOPG/DOPC membranes is given as a function of the DOPG concentration. Within experimental error, the ζ-potentials measured without addition of peptide are in good agreement with data reported by Winiski et al. (1986). The presence of 20 μM P828 causes the ζ-potentials at DOPG concentrations above 10 mol % to decrease significantly.

In a second series of experiments, we determined the dependence of the ζ-potential on the peptide concentration in solution in the range from 1 to 100 μM P828. These results are presented in Figure 5. An initial estimate of the strength of the interaction between the peptide and the lipid can be obtained by extrapolating the measurements to a lipid concentration where the ζ-potential becomes 0. If we assume that the lipid and peptide charges are equally effective in changing the ζ-potential, then this is the peptide concentration at which the concentrations of positive and negative charges at the lipid surface are identical. The experiments indicate that binding of the peptide to a membrane composed of pure DOPG is stronger than binding to a membrane containing only 20 mol % DOPG. The strength of binding of the peptide to pure DOPG liposomes increases slightly if the ionic strength of the electrolyte solution decreases from 100 to 10 mM (Figure 6). The binding of P828, blocked at the N- and C-terminals, is identical to the binding of the unblocked peptide.

Calculation of Lipid/Peptide Binding Constants. Measurement of peptide binding to negatively charged lipid surfaces as a function of both lipid and peptide concentration permits the quantitation of lipid/peptide association. Analysis of the data is based on the model of lipid/peptide interactions of McLaughlin and colleagues (Kim et al., 1991; Mosior & McLaughlin, 1991, 1992a,b). An essential feature of this model is the ability to relate the experimentally measured ζ-potential to the lipid/peptide binding constant.

Table I: Fluorescence Polarization of APC and APG in Liposomes of Different Composition Formed in 0.1 M NaCl/1 mM MOPS, pH 7.0

	APC		APG	
sample	no P828	20 μM P828	no P828	20 μM P828
100 mol % DOPC	0.054 ± 0.002	0.053 ± 0.002		
100 mol % DOPG			0.084 ± 0.002	0.126 ± 0.002
20 mol % DOPG	0.050 ± 0.002	0.066 ± 0.007	0.073 ± 0.002	0.162 ± 0.016

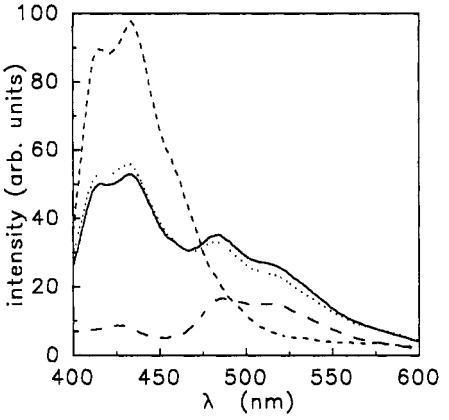

FIGURE 3: Fluorescence emission spectra of APG and PPC in a DOPG/DOPC (20 mol % DOPG) dispersion in 0.1 M NaCl/1 mM MOPS, pH 7.0, λ_{ex} = 376 nm. Fluorescence energy is transferred from APG to PPC. Addition of P828 reduces energy transfer. (- - -) 0.2 mol % APG; (– –) 0.5 mol % PPC; (—) 0.2 mol % APG + 0.5 mol % PPC; (···) 0.2 mol % APG + 0.5 mol % PPC + 20 μM P828.

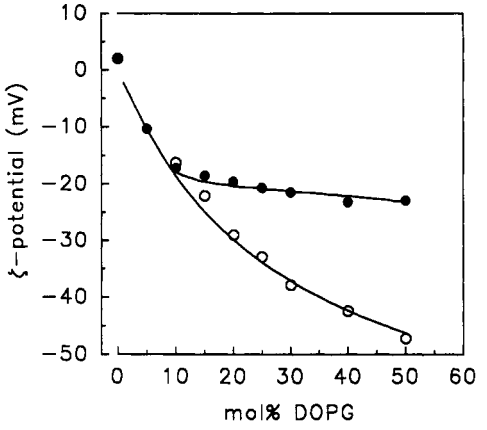

FIGURE 4: ζ-potential of DOPG/DOPC liposomes dispersed in 0.1 M NaCl/1 mM MOPS, pH 7.0, as a function of DOPG concentration. The potentials measured without addition of peptide (○) can be interpreted using an association constant of 1 L/mol for the Na^+–DOPG interaction (Winiski et al., 1986). The potential in the presence of 20 μM P828 (●) was fitted using an arginine–DOPG association constant of 3.5 L/mol. For DOPG concentrations from 0 to 20 mol %, it was assumed that the peptide carries +5 elementary charges and from 20 to 50 mol % that the effective peptide charge changes linearly with concentration from +5 to +4 charges.

The peptide does not bind to neutral lipids. The ζ-potential of the negatively charged liposomes changes as a result of adsorption of positively charged peptide molecules. It is assumed that peptide and lipid charges are equally efficient in changing the potential at the plane of shear. Further, it is assumed, for the purpose of calculating a microscopic association constant for each binding site, that each of the six arginine residues in P828 contributes equally to binding to the negatively charged DOPG. The surface charge density at the membrane surface is equal to the sum of all peptide and lipid charges per unit area. The effect of the negative charge of the glutamic acid residue is to lower the ζ-potential. An alternative approach would have been to consider the formation of an internal salt bridge between an arginine and glutamic

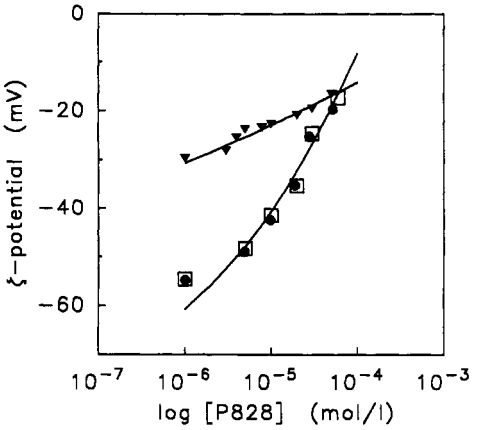

FIGURE 5: Measured ζ-potentials of DOPG and DOPG/DOPC liposomes in 0.1 M NaCl/1 mM MOPS, pH 7.0, as a function of P828 concentration. The data were fitted using the following arginine–lipid association constants (K_R) and the effective number of elementary peptide charges (z): (▼) 20 mol % DOPG in DOPG/DOPC, K_R = 3.5 L/mol, z = +5.0; (●) DOPG liposomes, K_R = 10 L/mol, z = +0.5; (□) DOPG liposomes + P828 with blocked N- and C terminals, K_R = 10 L/mol, z = +0.5.

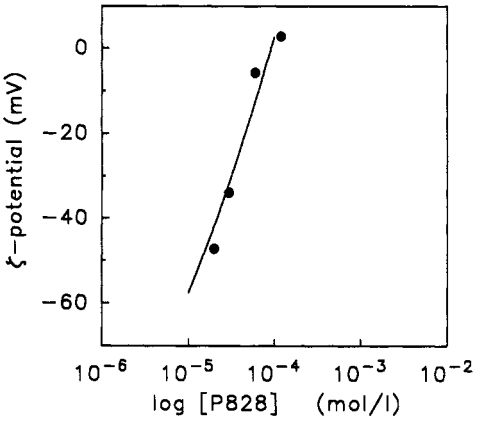

FIGURE 6: Measured ζ-potentials of DOPG liposomes in 0.01 M NaCl/1 mM MOPS, pH 7.0, as a function of P828 concentration (K_R = 15 L/mol, z = 0.5). The strength of binding of P828 to DOPG increases with decreasing electrolyte concentration.

acid, which would reduce the number of binding sites for lipids to 5. Both assumptions lead to similar binding behavior. Also, any additional contribution from the positive charge of the histidine residue in P828 to binding is neglected. Because the corresponding P828 with both positively and negatively charged end groups blocked binds with identical strength to the DOPG bilayers, the positive charge at the N-terminal amino group in P828 is not considered to represent a binding site.

The peptide concentration at the membrane surface [P_0] is calculated from the Boltzmann equation: [P_0] = [P] exp($-ze\zeta/kT$). Here [P] is the peptide concentration in solution, ze is an effective charge per molecule which is equal to or lower than the sum of all charges on the peptide, and ζ is the measured ζ-potential. To calculate the binding constants, the dependence of the ζ-potential on the peptide concentration (Figure 5) is extrapolated to peptide concentrations at which

Table II: Parameters of P828/DOPG Binding[a]

sample	c (mol/L)	K_R (L/mol)	z (elementary charges)	ΔG (kcal/mol)
20 mol % DOPG	0.1	3.5	+5.0	4.5
100 mol % DOPG	0.1	10.0	+0.5	8.3
100 mol % DOPG	0.01	15.0	+0.5	9.7

[a] Abbreviations: c, electrolyte concentration; K_R, arginine–DOPG association constant; z, effective number of elementary charges per P828 molecule; ΔG, free energy of binding of P828 to DOPG in bilayers.

the ζ-potential is zero. At this condition, $[P_0] = [P]$, and binding is described solely by an association constant K_R. At all other peptide concentrations, binding depends on the apparent net charge per peptide, ze, as well as on K_R. Peptide binding as a function of P828 concentration can be fitted well by one K_R and one ze value per curve (see Table II). The free energy of binding (ΔG) is calculated from the equation $\Delta G = nRT \ln K_R$, where n is the number of binding sites per peptide. This ΔG value does not take into account the difference between peptide in solution and at the membrane surface caused by the field of the electric double layer. Typical membrane surfaces have a negative potential and lower the free energy of positively charged peptides. For a ζ-potential of −25 mV and an effective number of peptide charges of 5, this energy difference would be of the order of 3 kcal/mol.

The association constant K_R was higher for pure DOPG membranes than for DOPG/DOPC mixtures. Its value decreased with increasing ionic strength. The effective charge per peptide is low when the peptide interacts with pure DOPG membranes and reaches values close to the sum of all negative and positive peptide charges at concentrations of 20 mol % DOPG in DOPG/DOPC mixtures.

DISCUSSION

Our observations using various physical techniques to study the interaction of P828 with model membranes can be summarized as follows: (i) The peptide interacts with negatively charged lipid bilayers but not with neutral PC bilayers (NMR, microelectrophoretic mobility, calorimetry, fluorescence polarization). (ii) It interacts preferentially with the negatively charged PG in PG/PC mixtures (fluorescence polarization). (iii) P828 binding decreases transfer of fluorescence energy between PG and PC. (iv) The peptide has no structural order if dissolved in an electrolyte solution at neutral pH (CD spectroscopy). (v) It forms an ordered structure upon interaction with negatively charged SDS or DOPG or if dissolved in methanol (CD spectroscopy).

According to the solid-state ^{31}P NMR experiments, all investigated lipid dispersions remain in the lamellar liquid-crystalline phase after addition of P828. The interaction of P828 with DOPG or DOPG/DOPC mixtures does not change the ^{31}P NMR anisotropies of chemical shift, which indicates that conformation and motions of the lipid phosphate groups are not altered by interactions. Addition of the peptide does not change the fatty acid order parameters of deuterated DPPC as measured from the ^2H NMR quadrupolar splittings.

The critical question here is the location of the bound peptide with respect to the polar headgroups of the bilayer. Fluorescence spectroscopy and microelectrophoresis experiments show that P828 associates with the negatively charged PG in the membrane. The results suggest that the interaction is exclusively electrostatic and that its strength decreases with increasing electrolyte concentration (see Table II). If the peptide were penetrating the lipid bilayer, one would anticipate a hydrophobic contribution to the lipid/peptide interaction,

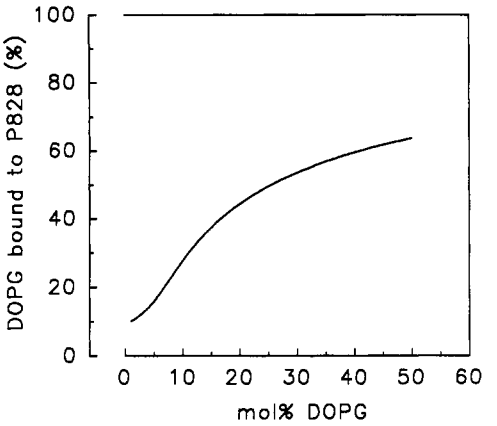

FIGURE 7: Amount of DOPG (in percent) bound to P828 as a function of DOPG concentration in DOPG/DOPC liposomes. Binding of DOPG to P828 increases rapidly in the range from 10 to 20 mol % DOPG. The curve was calculated using the experimental data of Figure 4.

which is not observed. The most plausible conclusion is that the peptide is located at the lipid/water interface and that it does not penetrate the membrane.

Binding of 20 μM P828 to membranes as a function of DOPG concentration is characterized by a high degree of cooperativity (see Figure 4). Below 10 mol % DOPG, the concentration of peptide bound to the membrane surface is negligible. Above 10 mol % DOPG, binding of P828 keeps the ζ-potential at a level which is almost constant. McLaughlin's model was used to calculate the amount of DOPG molecules in the outermost monolayer of liposomes bound to P828 as a function of total DOPG concentration (Figure 7). The calculation confirms the rapid increase of binding in the range from 10 to 20 mol % total DOPG. The high degree of cooperativity of binding of P828 to membranes parallels observations for a variety of positively charged peptides (Mosior & McLaughlin, 1992a,b). Two factors are responsible for the behavior [see Mosior and McLaughlin (1992a)]: (i) A linear increase of the concentration of charged lipid makes the surface potential more negative. This results in an exponential increase of the concentration of positively charged peptide at the membrane. (ii) The formation of the first contact between peptide and lipid reduces the dimensionality of the system. The consecutive binding sites encounter much higher lipid concentrations after the peptide has been attached to the membrane surface.

Peptide Structure. According to circular dichroism measurements, the peptide forms an extended chain conformation or a random coil in water. In the presence of a negatively charged surface or if dissolved in methanol, the peptide exists in a more ordered structure with a helical content of about 60%. In aqueous solution at neutral pH values, the arginine residues each carry a positive charge and therefore are mutually repulsive. The neutralization of the charges on the arginine side chains by a set of negative charges on a surface appears to be necessary for the formation of the ordered peptide structure. A simple decrease in electrostatic repulsion alone by addition of sodium chloride up to concentrations of 300 mM is insufficient to induce a conformational change. The decreased polarity of a lipid/water interface or a methanol solution appears to be a cofactor for the induction of the structural transition.

The nature of the ordered conformational state observed for P828 is important to understanding the molecular basis of lipid/peptide association. If the peptide forms an amphipathic α-helix as suggested previously (Venable et al., 1989;

Eisenberg & Wesson, 1990), the charged side of the peptide would face the bilayer surface. This would require exposure of the hydrophobic side of the peptide to the water which would be energetically unfavorable. If the hydrophobic side interacts with the lipids, we would expect to observe some binding of P828 to neutral lipids which was not the case. One possibility is that P828 forms oligomers at the membrane surface in which the hydrophobic side chains interact with each other. Simple geometrical considerations indicate that the packing density of peptide molecules at the membrane surface necessary to achieve charge neutralization of lipids is high. The existence of oligomers could ease the packing constraints for the peptide molecules. Further structural studies of the peptide in the bound state will help to answer this question.

Formation of Lipid Domains. At low concentrations of DOPG in DOPG/DOPC mixtures, the local DOPG concentration may be insufficient to permit formation of six P828–DOPG contacts. If, however, the DOPG is collected into domains, six contacts could be possible which would result in a stronger macroscopic interaction. The fluorescence data obtained on DOPG/DOPC mixtures suggest that such domain formation does occur. In these domains, the local PG concentration is increased (self-quenching), and the average distance between PG and PC molecules increases (decreased fluorescence energy transfer). However, the separation of DOPG and DOPC into domains would result in a lower entropy for the lipid matrix which is thermodynamically unfavorable. In agreement with the experimental observation, this entropy reduction results in a lower association constant, K_R, for the interaction of P828 with DOPG/DOPC mixtures. The difference in free energy of binding between pure DOPG and DOPG/DOPC mixtures is 3.8 kcal/mol, which would correspond to about 0.6 kcal/mol of DOPG if the peptide in fact makes six contacts with the lipid.

Peptide binding is a sensitive probe to study the thermodynamics of domain formation. The energy necessary to form domains may vary for different lipids. If domain formation is already triggered by factors other than lipid/peptide interactions such as the actual lipid composition or the presence of cations, then the binding of the peptide to the lipid would not require additional rearrangement of the lipid matrix. The strength of the lipid/peptide association would then be much higher. This is one possible explanation for the observation that low concentrations of divalent cations such as Ca^{2+}, which are known to cause domain formation in charged lipid mixtures, are important cofactors for binding of a number of membrane proteins.

Relation to the C-Terminus of gp41. The binding studies of Haffar et al. (1988, 1991) show that the C-terminus of gp41 binds sufficiently tightly to a microsomal membrane to prevent antibody recognition. An important feature of the various isolates of gp41 is conservation of the number of positively charged residues in the carboxy-terminal region. The high concentration of positively charged residues accounts at least in part for the binding of the C-terminus of gp41 to membranes. The binding energies of P828 to mixed DOPG/DOPC bilayers are somewhat low to explain Harffar's result. However, such considerations as domain formation in the membrane, formation of protein oligomers at the membrane surface, and enhancement of binding from residues adjacent to the segment corresponding to P828 could significantly enhance binding. One function of the carboxy terminus of gp41 could be to tether this region to the membrane surface.

There are numerous parallels between P828 and magainins involving their interactions with membranes (Williams et al., 1990). Both peptides have a similar number of positive charges, and both are highly amphipathic as α-helices. Both peptides form structures with increased helical content upon binding to negatively charged membrane surfaces, and both show similar antimicrobial activity (H.-C. Chen, unpublished results). Also, both peptides similarly increase membrane permeability, i.e., negatively charged lipid vesicles in the case of the magainins (Matsuzaki et al., 1991) and negatively charged black lipid membranes for P828 (Chanturia et al., 1993).

Cloyd and Lynn (1991) have indicated that the primary mechanism of HIV cytopathology is a perturbation of the cell membrane which results in an increased membrane permeability. It has also been established that the carboxy-terminal region of the gp41 is involved in cytopathology. Although it is not obvious that the synthetic peptide P828 models functional properties of the protein gp41, a better understanding of the molecular basis for increased membrane permeability from studies on P828 hopefully will contribute to the understanding of the mechanism of cytopathology of gp41.

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