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Thermodynamic characterization of the association of small basic peptides with membranes containing acidic lipids

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We measured the binding of the peptide acetyl-Trp-Lys_7-amide to membranes formed from mixtures of the zwitterionic lipid 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (PC) and the acidic lipid 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol (PG). Direct filtration and equilibrium dialysis measurements demonstrate that binding increases sigmoidally with the mole fraction of PG as predicted from a simple Gouy-Chapman/mass action theoretical model. We used these measurements to calibrate two binding assays, one based on the increase in Trp fluorescence that occurs when the peptide binds to the membrane, the other on the quenching of Trp fluorescence that occurs when the peptide binds to membranes containing fluorescent lipids. Both fluorescence assays demonstrate that binding does not depend strongly on temperature, which suggests the enthalpy change, ΔH, is small. Calorimetric measurements demonstrate this directly for the analogous basic peptide Lys_5: $\Delta H \simeq +1$ kcal/mol for the binding of Lys_5 to sonicated phospholipid vesicles and $\Delta H \simeq 0$ kcal/mol for its binding to large unilamellar vesicles. Thus, the decrease in the free energy that occurs when these peptides bind to the membrane is due to a positive change in the entropy of the system. Fluorescence measurements demonstrate the binding of the Trp-containing peptide to 4:1 PC/PG membranes is independent of pressure up to 2 kbar, which suggests that binding occurs without a significant change in volume.

Introduction

Acidic phospholipids located on the cytoplasmic surface of plasma membranes [1,2] may associate with basic residues in several different types of proteins [3]. Intrinsic or membrane-spanning proteins, for example, often have clusters of basic residues on their cytoplasmic domains [4]. These clusters are important in determining the transmembrane orientation of proteins [5] and Hartmann et al. [6] suggested the basic residues may associate with acidic lipids. The MARCKS protein [7–11] and neuromodulin [12–14] are not intrinsic proteins, but their acyl chains attach them to the plasma membrane. Both proteins have a cluster of basic residues that binds calmodulin and contains the serines that are phosphorylated by protein kinase C. Houbre et al. [14] suggested the basic residues in these phosphorylation domains could also bind to acidic lipids in the plasma membrane, which would facilitate the phosphorylation by membrane-bound PKC. Peptides that mimic the phosphorylation domains of these proteins do bind strongly to membranes that contain acidic lipids, and the binding decreases when the peptides are phosphorylated by protein kinase C (Kim, Blackshear, Johnson and McLaughlin, unpublished observation). Finally, several membrane-active cytoplasmic proteins contain clusters of basic residues that could bind to acidic lipids. For example, the basic residues in the pseudosubstrate region of protein kinase C [15,16] may bind transiently to acidic phospholipids [17], which act in concert with diacylglycerol to activate this enzyme. Activation probably involves removal of the pseudosub-

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Abbreviations: dansyl PE, *N*-(5-dimethylaminonaphthalene-1-sulfonyl)-L-α-phosphatidylethanolamine; f_v, observed fluorescence intensity from peptide in the presence of vesicles; f_w, fluorescence from peptide in buffer solution without vesicles; F_m/F_w, ratio of Trp molar fluorescence intensities from bound and free peptide; k, intrinsic microscopic association constant between a basic Lys residue and an acidic PG lipid; K_p, partition constant defined in Eqn. 4; LUVs, large unilamellar vesicles; PC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; PG, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol; α_m, fraction of peptide bound to the membrane; δ, thickness of the surface phase; ϵ_r, dielectric constant of water; ζ, zeta potential.

strate region in the regulatory domain from the substrate binding region in the catalytic domain [18]. Several lines of evidence suggest the large cluster of basic residues in the tail region of Myosin I attaches the protein to acidic lipids in the plasma membrane, allowing it to serve as a membrane-associated 'motor molecule' [19–24].

These examples illustrate the importance of understanding how basic residues in proteins bind to acidic lipids in membranes. Studies with well defined model systems consisting of phospholipid vesicles and simple basic peptides have helped characterize the binding [25–30]. Studies with Lys_n, ($n = 2$–5) showed each Lys added to dilysine increased 10-fold the affinity of the peptide for membranes formed from acidic lipids [28]. This result is consistent with a simple Gouy-Chapman/mass action model in which each added lysine binds independently to an acidic lipid such as phosphatidylserine, PS, with a standard Gibbs free energy $\Delta G^0 \simeq 1$ kcal/mol. NMR measurements on vesicles [27] and surface potential measurements on monolayers [28] suggest that basic peptides such as Lys_5 bind outside the envelope of the polar headgroups. The binding appears to be electrostatic in nature because the basic peptides do not bind to membranes formed from zwitterionic lipids such as phosphatidylcholine, there is little selectivity between monovalent acidic lipids such as phosphatidylserine and phosphatidylglycerol for these peptides, and only a 2-fold selectivity of Arg over Lys residues for these lipids [28,29].

The thermodynamic parameters that characterize the association of basic residues and acidic lipids had not yet been determined. In this study we measured the dependence of binding on temperature, which allowed us to infer the enthalpy change from a van 't Hoff plot. We confirmed the conclusions drawn from the van 't Hoff plot [31] by measuring directly the enthalpy change on binding using calorimetry. Finally, we measured the dependence of binding on pressure, which allowed us to infer if a volume change occurs when the peptide binds to a phospholipid vesicle.

We first measured the binding of the peptide acetyl-Trp-Lys_7-amine to large unilamellar vesicles (LUVs) by making equilibrium dialysis and filtration measurements at 25°C and atmospheric pressure, then used these direct measurements to calibrate our two fluorescence assays. One assay exploits the increase in fluorescence that occurs when a peptide with Trp residues binds to a membrane; this approach has been used by several investigators to study the association of peptides with membranes [25,32–34]. The other assay exploits the decrease in fluorescence that occurs when a peptide containing Trp residues binds to a membrane containing the fluorescent lipid dansyl-PE, which quenches the Trp fluorescence; this technique has been used to study lipid–protein interactions [35]. We used both of these fluorescence approaches to study the dependence of binding on temperature and pressure.

High pressure fluorescence measurements provide information about the change in volume that occurs on binding. The dissociation of oppositely charged ions results in a decrease in volume due to the electrostriction of water around a free charge; Le Chatelier's law demonstrates that the application of pressure will thus disrupt salt bridges [36,37]. The change in volume will be less for 'loose' or solvent-separated ion pairs, which have one or more water molecules in the space between the ions, than for 'tight' or contact ion-pairs, which lack the intermediate solvation. This interpretation has been used to explain the difference between the volumes of dissociation for the $RbNO_3$ (-5 ml/mol) and the $TlNO_3$ (-12 ml/mol) ion-pairs [37]. Thus, if most of the lysines in the peptide form 'tight' ion pairs with anionic lipid headgroups, pressure should dissociate the complex with a volume change of order -20 to -50 ml/mol. In this case a pressure of 1 kbar will produce a > 2-fold reduction in the association constant, a change that can be detected readily from fluorescence measurements. Alternately, if the association between a lysine side chain and an acidic phospholipid does not involve the loss of electrostricted water, pressure should not perturb the equilibrium significantly. Information about thermodynamic properties can thus provide some clues about the nature of of the peptide-lipid complexes.

Materials and Methods

The lipids 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (PC) and 1-palmitoyl-2-oleoyl-*sn*-phosphoglycerol (PG) were obtained from Avanti Polar Lipids (Alabaster, AL). *N*-(5-Dimethylaminonaphthalene-1-sulfonyl)-L-α-phosphatidylethanolamine from egg PE (dansyl PE) was from Molecular Probes (Eugene, OR). Acetyl-Trp-Lys_7-amide was obtained from Multiple Peptide Systems (San Diego, CA); peptide purity was $> 99\%$ as determined from reverse phase HPLC. Lys_5 was obtained from Research Plus (Bayone, NJ); purity was $> 95\%$. 4-Morpholinepropanesulfonic acid (Mops) was from Pharmacia (Piscataway, NJ). We studied a fluorescent peptide with seven rather than five Lys residues [28–30] because this peptide binds more strongly to vesicles, allowing us to use lower concentrations of vesicles and minimize scattering in the fluorescence experiments.

Aqueous solutions for the fluorescence and electrophoretic mobility experiments were prepared with 18 MΩ water (Super-Q, Milipore, Bedford MA) that was subsequently bidistilled in an all-quartz still. They were buffered to pH 7.0 with 1 mM Mops. The large unilamellar vesicles (LUVs) for the fluorescence and

direct binding measurements were prepared by extrusion through 0.1 μm polycarbonate filters [38]. Direct binding measurements were performed by determining the concentration of free peptide by means of a fluorescamine assay following dialysis or ultrafiltration [17,28–30]. The preparation of multilamellar vesicles for the electrophoretic mobility measurements and the techniques for making these measurements are described in detail elsewhere [39,40].

Fluorescence was measured as a function of temperature with a Spex Fluorocomp (Edison, NJ) and as a function of pressure with a ISS Greg-PC spectrofluorometer (ISS, Champaign, IL). Hydrostatic presure was applied using a cell based on the design of Paladini and Weber [41]. The fluorescence intensity from the Trp residue increases in a manner similar to other systems when the peptide binds to phospholipid vesicles [25,33]. The observed fluorescence intensity from the peptide in the presence of vesicles, f_v, is

$$f_v = CF_m\alpha_m + CF_w(1-\alpha_m) \quad (1)$$

where α_m is the molar fraction of peptide bound to the surface of the membrane, F_m and F_w are the Trp molar fluorescence intensities from the bound and free peptide, and C is a proportionality factor. The fluorescence from the peptide in buffer without vesicles, f_w, is

$$f_w = CF_w \quad (2)$$

By rearranging Eqns. 1 and 2 we obtain:

$$\alpha_m = ((f_v - f_w)/f_w)/(F_m/F_w - 1) \quad (3)$$

We determined F_m/F_w for vesicles with different mole fractions of the anionic lipid PG as follows: for each vesicle sample we measured the fractional increase in fluorescence intensity, $(f_v - f_w)/f_w$, and determined the fraction of bound peptide, α_m, directly from filtration measurements. The value of F_m/F_w was independent of the fraction of acidic lipid (5–20%) in the membrane; its value appeared to depend slightly on the total lipid concentration, however, increasing from 1.7 for samples containing 0.3 mM lipid to 2.1 for samples containing 1 mM lipid. Once the ratio F_m/F_w is known, fluorescence measurements can be used to calculate the fraction of bound peptide, α_m, as a function of temperature and pressure. This is the classical approach discussed by Bashford and Smith [42] for measuring the binding constant of ligands that change their spectroscopic properties upon binding, except that we calibrated the system by measuring directly the amount of bound peptide rather than by estimating the molar fluorescence intensity of the bound peptide from a saturation plot.

The fluorescence from the Trp residue of the peptide is quenched when the peptide binds to vesicles containing 10% dansyl PE, a fluorescent lipid that bears a charge of -1. We used Eqn. 3 and the procedure described above to calibrate this approach to measuring the fraction of bound peptide. Our fluorescence quenching measurements were less satisfactory than fluorescence enhancement measurements for two reasons. First, the F_m/F_w ratio for quenching decreased as the mol fraction of acidic lipid in the membrane increased. Second, the error from scattering was large in the quenching experiments due to the lower signal/noise ratio. Moreover, scattering is a bigger problem in the high pressure cell than in a normal cuvette.

We measured the fluorescence intensity of the peptide in buffer and in the presence of vesicles made from PC/PG or PC/PG/dansyl PE over the temperature range 16–34°C at atmospheric pressure. In agreement with the results obtained by Dufourcq et al. [25] using the tripeptide Lys-Trp-Lys, we observed a blue shift in the peak of the emission spectrum when our peptide bound to vesicles. Specifically, the peak shifted from 350 to 343 nm (28.57 to 29.15 kk) in the presence of 4:1 PC/PG LUVs at 20°C. In the experiments to determine F_m/F_w and the temperature experiments, samples were excited at 280 nm and the fluorescence intensity was measured at 345 nm. We corrected for the background due to scattering (maximum 5% for the quenching experiments). For the pressure experiments, samples were excited at 280 nm and the reported intensities refer either to the integrated emission peak scanned from 300 to 420 nm or to a 2 min average of the intensity at 350 nm; both gave identical results. To obtain an adequate signal in the pressure experiments, slits were opened to 16 nm, resulting in a low but significant contribution of scattered light. All data were background corrected (maximum 7%).

We define a partition constant:

$$K_p = [P]_m/[P]_w \quad (4)$$

where $[P]_w$ is the free concentration of peptide in the aqueous phase and $[P]_m$ is the concentration of the peptide in the surface phase. Following Guggenheim's approach, we define the surface phase as a slab of finite thickness δ adjacent to the membrane: $\delta = 1$ nm. The partition coefficient calculated in this manner agrees within a factor of two with the value calculated by dividing the mol peptide/g lipid by the mol peptide/g water [33].

Heats of reaction were measured with a Microcal MC-2 high-sensitivity titration calorimeter (Microcal, Northampton, MA) as described elsewhere [43]. In all experiments, unilamellar vesicles were formed from a 1:3 PC/PG mixture (13–14 mM), then placed in a calorimeter cell of volume 1.27 ml. Both sonicated (35 nm diameter) and extruded (50 nm pore size) vesicles

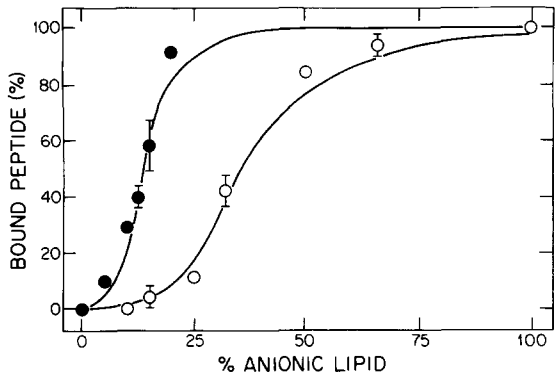

Fig. 1. Binding of acetyl-Trp-Lys$_7$-amide (filled circles) and Lys$_5$ (open circles) to large unilamellar vesicles (LUVs) formed from mixtures of the zwitterionic lipid PC and the monovalent negatively charged lipid PG, as measured by a filtration assay. The aqueous solutions contained 0.1 M KCl buffered to pH 7.0 with 1 mM Mops, $T = 25$°C; 0.8 mM lipid (PC+PG); and either 2 μM peptide (filled circles) or 3 μM peptide (open circles, data from Ref. 30). The curves represent the predictions of a simple Gouy-Chapman/mass action model described in detail elsewhere [30] that assumes a Lys residue on the peptide binds to a PG lipid with standard Gibbs free energy change of 1 kcal/mol.

were used. 10 μl aliquots of 3 mM pentalysine in the same buffer solution (100 mM NaCl, 30 mM Tris (pH 7.4)) were added at 5 min intervals with continuous stirring. About 10 additions were carried out at a temperature of approx. 27°C; the final lipid/peptide ratio was about 50:1. As a control the same pentalysine solution was titrated into the buffer solution without vesicles. The resulting small dilution enthalpy, 9 μcal, was subtracted from the lipid titration.

Results

Fig. 1 illustrates how the peptides acetyl-Trp-Lys$_7$-amide (filled circles) and Lys$_5$ (open circles) bind to PC/PG vesicles. The peptides do not bind significantly to electrically neutral PC LUVs. These peptides, however, do bind to PC/PG vesicles and the binding increases in a sigmoidal manner with the percentage of anionic lipid in the vesicles. The apparent cooperativity of the binding with respect to the anionic lipid arises for two reasons: electrostatics and reduction of dimensionality. First, addition of acidic lipid to the membrane produces a negative electrostatic potential in the aqueous diffuse double layer immediately adjacent to the surface; this potential can be described adequately by the Gouy-Chapman theory of the diffuse double layer [44]. For example, the surface or ζ potential of a PC/PG membrane containing 20% PG is about -30 mV in a 0.1 M monovalent salt solution. This potential attracts the positively charged peptide to the aqueous phase adjacent to the membrane, increasing its concentration. If we assume the peptide is a point charge with an effective valence z_{eff}, we can use the Boltzmann equation to describe this accumulation. Second, when the first residue on a peptide binds to an acidic lipid in the membrane, the other residues are translocated to the surface phase where they encounter a much higher effective concentration of acidic lipids. We treat the surface phase as a region of finite thickness ($\delta = 1$ nm), ignore the size of the peptide and lipids, and describe their interaction with a simple mass action formalism. This Gouy-Chapman/mass action model is described in detail elsewhere [30].

Although it is apparent from Fig. 1 that a peptide with seven Lys residues binds more strongly than a peptide with five Lys residues, we can describe both sets of data with the same Gouy-Chapman/mass action model using the same value for the intrinsic microscopic association constant between a basic Lys residue and an acidic PG lipid, $k \simeq 3$ M^{-1}. A value of $k = 3$ M^{-1} also describes the binding of pentalysine with blocked termini to PC/PG vesicles [29]. Thus, a Lys residue in any of these peptides binds to a PG lipid with a standard Gibbs free energy change $\Delta G^0 = RT \ln k \simeq 1$ kcal/mol. (Two monovalent point charges located 0.4 nm apart in a medium of dielectric 78 have a coulombic energy of 1 kcal/mol.) In our Gouy-Chapman/mass action model we assume the effective valences (the valences used in the Boltzmann relation) of the Lys$_5$ and fluorescent peptides are $z_{eff} = 2.5$ and 3.0, respectively; the effective valence of many small basic peptides is about half their actual value in 0.1 M salt solutions [17,29,30,45–47]. We also took into account the tendency of the hydrophobic Trp residue to partition into the membrane *.

Although the Gouy-Chapman/mass action model provides a surprisingly accurate description of how the binding of a small basic peptide depends on the mole fraction of acidic lipid (Fig. 1), the ionic strength of the solution [30], and the number of basic residues in the peptide [28], it is highly oversimplified. It ignores the discrete nature of the charges on both the membrane and peptide, as well as the structure and size of the peptide. In view of these limitations, we also describe the binding in terms of a partition coefficient defined by Eqn. 4: $K_p = 5 \cdot 10^4$ for membranes containing 20%

* In the calculation of the theoretical curve illustrated in Fig. 1 for the Trp-containing peptide we assumed that each of the 7 Lys can combine with a PG with an intrinsic microscopic association constant of $k = 3$ M^{-1}, that K$^+$ ions combine with PG with an association constant of 1 M^{-1}, and that Trp combines with both PG and PC with an association constant of 2 M^{-1}. An equally good fit is obtained if we ignore the hydrophobic interaction of Trp with the membrane and assume that each Lys combines with a binding constant of 5 M^{-1}. The first interpretation is probably more correct because the Lys residues in Lys$_5$ bind to PG with a $k = 3$ M^{-1} (Fig. 1), and other work suggests Trp residues in simple peptides enhances the binding of the peptide to membranes [25,33].

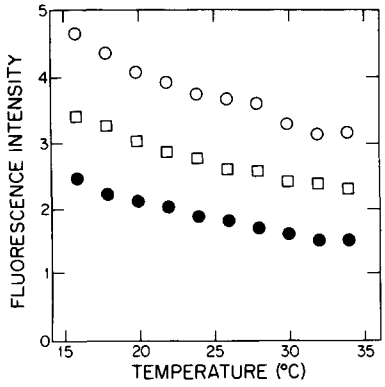

Fig. 2. The fluorescence of the Trp residue in the acetyl-Trp-Lys$_7$-amide peptide plotted as a function of temperature. The aqueous solutions contained 0.1 M KCl buffered to pH 7.0 with 1 mM Mops and 2 μM peptide. They also contained no vesicles (squares), 4:1 PC/PG vesicles (0.3 mM total lipid concentration, open circles) or 8:1:1 PC/PG/danysl-PE (0.3 mM total lipid concentration, filled circles).

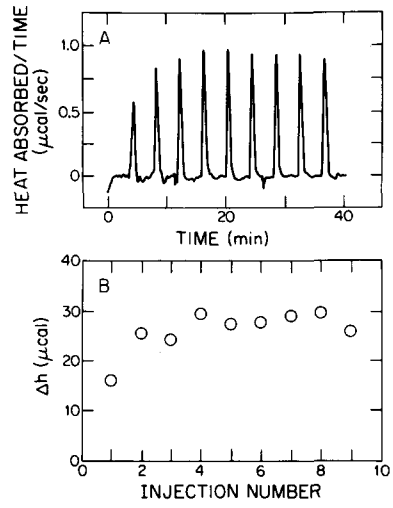

Fig. 4. (A) Calorimeter traces when a Lys$_5$ solution is injected into a solution containing 1:3 PC/PG sonificated vesicles. Upward deviations from baseline indicate absorption of heat. (B) Heat absorbed/injection, Δh (μcal), is evaluated from the area of the calorimeter traces in Fig. 4A. See text for details.

anionic lipid from the data in Fig. 1 for the Trp-containing peptide. This partition constant corresponds to a unitary free energy change of $\Delta G_u^0 = RT \ln K_p \simeq 6$ kcal mol^{-1}.

We used two fluorescence techniques to study how the binding of the Trp-containing peptide to bilayer membranes depends on temperature. The squares in Fig. 2 indicate the fluorescence of the peptide in a solution with no vesicles. The fluorescence increases (open circles) when 4:1 PC/PG vesicles are added to the solution; the fractional increase in fluoresence, $(f_v - f_w)/f_w$ is proportional to the fraction of bound peptide, α_m (Eqn. 3). Direct filtration measurements at 25°C demonstrate that about 50% of the peptide is bound to the vesicles under these conditions (0.3 mM total lipid). The fluorescence decreases (filled circles) when 8:1:1 PC/PG/dansyl-PE vesicles are added to the solution; in this case the fractional decrease in fluorescence should be a measure of the fraction of bound peptide.

Fig. 3 illustrates the dependence of the fraction of bound peptide on temperature, as calculated from the data in Fig. 2 using Eqn. 3. (We assume that F_m/F_w does not depend on temperature.) Both techniques indicate the fraction of bound peptide does not depend significantly on temperature, which suggests that little enthalpy change occurs on binding.

We also used an independent technique to investigate the dependence of the binding on temperature. We measured electrophoretic mobility (zeta potential) of PG and 1:1 PC/PG vesicles at 10, 25 and 40°C in 0.1 M KCl, 1 mM Mops (pH 7.0), in the absence and presence of peptide (10 μM Lys$_5$ or acetyl-Trp-Lys$_7$-

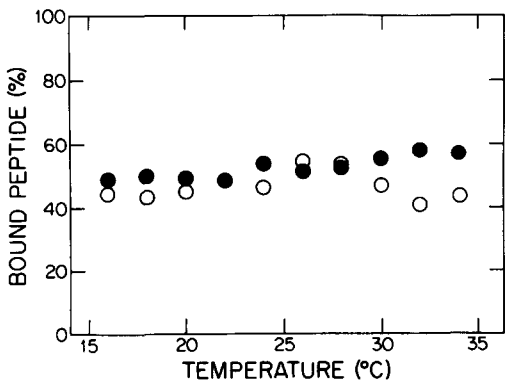

Fig. 3. Binding of Acetyl-Trp-Lys$_7$-amide to large unilamellar vesicles as a function of the temperature. The value of α_m, the fraction of bound peptide, was calculated from the data shown in Fig. 2 using Eqn. 3: open circles represent fluorescence enhancement data, filled circles represent fluorescence quenching data.

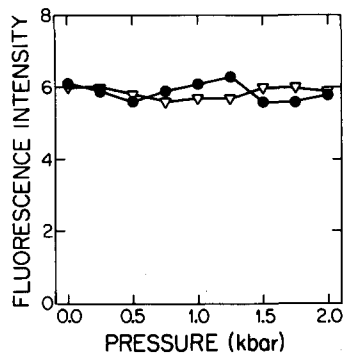

Fig. 5. The fluorescence intensity of the Trp residue in the acetyl-Trp-Lys$_7$-amide peptide as a function of the pressure. The aqueous solutions contained 0.1 M KCl buffered to pH 7.0 with 1 mM Mops, $T = 22$°C; 2 μM acetyl-Trp-Lys$_7$-amide peptide; and 0.3 mM lipid (4:1 PC/PG). About 50% of the peptide was bound to the vesicles under these conditions (see Fig. 3). Symbols indicate results from two separate runs with different samples of vesicles.

TABLE I

Heat absorbed per injection (Δh) and change in enthalpy (ΔH) when pentalysine binds to 1:3 PC/PG unilamellar vesicles

Data on the first line were taken from the experiment illustrated in Fig. 4. Other data from similar experiments. See Materials and Methods and Results sections for details.

Vesicle size (nm)	Δh (μcal)	ΔH (kcal/mol)
35	29±1	1.3±0.05
35	23±2	1.1±0.1
50	12±1	−0.1±0.05
50	12±1	−0.1±0.05

amide). The decrease in the magnitude of the electrophoretic mobility of the negatively charged vesicle produced by the positively charged peptide is a measure of the binding of the peptide to the membrane [28]. Although the mobility depends slightly on the temperature in the absence of peptide (e.g., Ref. 39, see Fig. 2), the ratio of the mobilities in the presence and absence of the peptides is independent of temperature, supporting our inference from fluorescence measurements that binding does not depend significantly on temperature over this range.

These observations suggest the binding is driven by an entropy change, a suggestion that is confirmed by direct calorimetry measurements. Fig. 4A illustrates a typical calorimeter trace obtained when a 3 mM Lys_5 solution was titrated (nine injections of 10 μl each added at 5 min intervals) into a solution containing 14 mM 1:3 PC/PG sonicated vesicles ($d = 35$ nm); essentially all the peptide should bind to the vesicles under these conditions. As shown in Fig. 4B and Table I, the average heat absorbed is 29 μcal per injection. We corrected this number for the enthalpy of dilution before calculating the enthalpy of the binding reaction. The corrected value then yields an enthalpy change of +1.3 kcal/mol for the binding reaction (Table I); this endothermic change indicates that the association is driven by entropy. Table I also illustrates that the reaction enthalpy depends on the size of the vesicles used in the experiments: as the vesicle size increases, the reaction enthalpy changes sign and an exothermic reaction is observed. A similar size-dependence was observed for the binding of Ca^{2+} and La^{3+} ions to phospholipid vesicles (Lehrmann and Seelig, unpublished results). Thus, the fluorescence, electrophoretic mobility, and direct calorimetry measurements all indicate that entropy drives the association of these basic peptides with acidic lipids in membranes.

We also used the fluoresence enhancement technique to study the effect of pressure on the binding of the fluorescent basic peptide to membranes. The pressure experiments were conducted under conditions identical to those illustrated by the open circles in Figs. 2 and 3, where about 50% of the peptide was bound to 4:1 PC/PG LUVs. The fluorescence measurements illustrated in Fig. 5 indicate the binding does not depend significantly on the pressure, at least up to a pressure of 2 kbar. The volume change, ΔV, in the system is related to K_p by $d(-RT \ln K_p)/dP = \Delta V$ [49]; the spread of the data in Fig. 5 suggests the volume change is small, of order 1 ml/mol or less.

Discussion

We draw two main conclusions from our data: the binding of small hydrophilic basic peptides to membranes containing acidic lipids is accompanied by little change in either the enthalpy or the volume of the system.

All the available evidence suggests electrostatic interactions dominate the binding of small basic peptides such as Lys_5 to acidic lipids in membranes: the peptides do not bind to vesicles formed from zwitterionic lipids such as PC (Fig. 1), they exhibit little selectivity between different monovalent acidic lipids [28,29], and they do not penetrate the polar headgroup region [27,28]. We were somewhat surprised to discover that the electrostatic association of these small basic peptides with membranes occurred with little change in enthalpy: entropy-driven binding reactions are characteristic of interactions driven by a classical hydrophobic effect [50]. It should be noted, however, that there are many exceptions to this rule. For example, some small amphipathic molecules adsorb hydrophobically to membranes by means of 'nonclassical' enthalpy-driven processes [51–53]. Furthermore, the binding of inorganic alkaline earth cations such as calcium to acidic lipids in membranes does not depend on temperature [39] and occurs with little change in enthalpy (Lehrmann and Seelig, unpublished data).

We have not attempted to describe theoretically the entropy-driven binding of basic peptides to membranes because many complicating factors affect the reaction. For example, when a peptide binds to a membrane it moves from a three dimensional solution to a two dimensional surface and it must pay an entropy price; one can overestimate this entropy price by treating the peptide as a rigid rotator and calculating the loss of one translational and two rotational degrees of freedom [54]. Other factors must contribute to the entropy-driven binding and overwhelm this term. For example, the binding of one pentavalent Lys_5 peptide to a membrane releases up to five monovalent counterions that were previously confined to the diffuse double layer. Another factor is 'residual entropy', which arises because only some of the basic residues on the peptide will be bound to an acidic lipid at any time. G. Weber (personal communication) calculates that this

residual entropy should be important when the number of binding sites is large (e.g., 50), but not when the number is small (e.g., 5). Finally, the binding of the peptide could cause a decrease in the structure of water adjacent to the peptide and/or membrane for three reasons. First, when two ions of opposite sign approach each other in an aqueous solution, the entropy of the solvent increases; the magnitude of this 'long-range electrostatic entropy effect' is equal to the product of the Coulomb energy and $d\ln\epsilon_r/dT$ where ϵ_r is the dielectric constant of water and T is the temperature [55–57]. Second, when two simple inorganic ions such as Mg^{2+} and SO_4^{2-} form an ion pair, the number of free particles in the system increases because of the release of water molecules from the overlapping hydration shells [56]. Our pressure measurements suggest that this effect is not important for the binding of basic peptides to acidic lipids because the association appears to be of the 'loose' type (rather than the tight or contact ion or inner sphere type), with little change in the electrostriction of water molecules adjacent to the charges on the phosphate moiety of PG or the amino groups on the peptides *. Third, water molecules may be released from other portions of the peptide or the membrane if the association due to multiple electrostatic interactions produces a concomitant decrease in the solvent-accessible surface area. For example, Parsegian and coworkers report that about 60 water molecules are released from the oxygenated form of hemoglobin when it changes its conformation and decreases its accessible surface area upon release of oxygen [58]. In summary, we have shown that entropy drives the association of basic peptides and acidic lipids, but we cannot untangle the relative contributions of the above factors to this entropy-driven reaction.

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* On the other hand, the association of positively charged Lys residues in the peptides with negatively charged phosphate moieties in PG must be more intimate than the electrostatic accumulation of potassium ions in the aqueous diffuse double layer. (Although these K^+ counterions may be considered to be thermodynamically associated with the surface, their average distance from the surface, the Debye length, is about 1 nm for a 0.1 M KCl solution.) The K^+ counterions move in the opposite direction to the vesicle when it is placed in an electric field (i.e., the vesicle has a negative electrophoretic mobility), but the basic peptides associated with the vesicle move with it (i.e., they can reverse the electrophoretic mobility of the vesicle).

References

1 Op den Kamp, J.A.F. (1979) Annu. Rev. Biochem. 48, 47–71.
2 Bishop, W.R. and Bell, R.M. (1988) Annu. Rev. Cell Biol. 4, 579–610.
3 Cafiso, D. (1991) Curr. Opin. Struct. Biol. 1, 185–190.
4 Von Heijne, G. (1986) EMBO J. 5, 3021–3027.
5 Nilsson, I. and Von Heijne, G. (1990) Cell 62, 1135–1141.
6 Hartmann, E., Rapoport, T.A. and Lodish, H.F. (1989) Proc. Natl. Acad. Sci. USA 86, 5786–5790.
7 Graff, J.M., Stumpo, D.J. and Blackshear, P.J. (1989) J. Biol. Chem. 264, 11912–11919.
8 Graff, J.M., Young, T.N., Johnson, J.D. and Blackshear, P.J. (1989) J. Biol. Chem. 264, 21818–21823.
9 Graff, J.M., Rajan, R.R., Randall, R.R., Nairn, A.C. and Blackshear, P.J. (1991) J. Biol. Chem. 266, 14390–14398.
10 McIlroy, B.K., Walters, J.D., Blackshear, P.J. and Johnson, J.D. (1991) J. Biol. Chem. 266, 4959–4964.
11 Hartwig, J.H., Thelen, M., Rosen, A., Janmey, P.A., Nairn, A.C. and Aderem, A. (1992) Nature, 356, 618–622.
12 Skene, J.H.P. (1989) Annu. Rev. Neurosci. 12, 127–156.
13 Apel, E.D., Byford, M.F., Au, D., Walsh, K.A. and Storm, D.R. (1990) Biochemistry 29, 2330–2335.
14 Houbre, D., Duportail, G., Deloulme, J.-C. and Baudier, J. (1991) J. Biol. Chem. 266, 7121–7131.
15 House, C. and Kemp, B.E. (1987) Science 238, 1726–1728.
16 Soderling, T.R. (1990) J. Biol. Chem. 265, 1823–1826.
17 Mosior, M. and McLaughlin, S. (1991) Biophys. J. 60, 149–159.
18 Bell, R.M. and Burns, D.J. (1991) J. Biol. Chem. 266, 4661–4664.
19 Adams, R.J. and Pollard, T.D. (1989) Nature 340, 565–568.
20 Hayden, S.M., Wolenski, J.S. and Mooseker, M.S. (1990) J. Cell Biol. 111, 443–451.
21 Pollard, T.D., Doberstein, S.K. and Zot, H.G. (1991) Annu. Rev. Physiol. 53, 653–681.
22 Swanljung-Collins, H. and Collins, J.H. (1992) J. Biol. Chem. 267, 3445–3454.
23 Doberstein, S.K. and Pollard, T.D. (1992) J. Cell Biol. 117, 1241–1249.
24 Zot, H.G., Doberstein, S.K. and Pollard, T.D. (1992) J. Cell Biol. 116, 367–376.
25 Dufourcq, J., Faucon, J.F., Maget-Dana, R., Pileni, M.P. and Hélène, C. (1981) Biochim. Biophys. Acta 649, 67–75.
26 De Kruijff, B., Rietveld, A., Telders, N. and Vaandrager, B. (1985) Biochim. Biophys. Acta 820, 295–304.
27 Roux, M., Neumann, J.-M., Bloom, M. and Devaux, P.F. (1988) Eur. Biophys. J. 16, 267–273.
28 Kim, J., Mosior, M., Chung, L.A., Wu, H. and McLaughlin, S. (1991) Biophys. J. 60, 135–148.
29 Mosior, M. and McLaughlin, S. (1992) Biochemistry 31, 1767–1773.
30 Mosior, M. and McLaughlin, S. (1992) Biochim. Biophys. Acta 1105, 185–187.
31 Van Holde, K.E. (1985) Physical Biochemistry, 2nd Edition, pp. 56–57, Prentice-Hall, Englewood Cliffs.
32 Surewicz, W.K. and Epand, R.M. (1984) Biochemistry 23, 6072–6077.
33 Jain, M.K., Rogers, J., Simpson, L. and Gierasch, L.M. (1985) Biochim. Biophys. Acta 816, 153–162.
34 Jacobs, R.E. and White, S.H. (1986) Biochemistry 25, 2605–2612.

35 Bazzi, M.D. and Nelsestuen, G.L. (1987) Biochemistry 26, 115–122.
36 Hamann, S. (1980) Rev. Phys. Chem. Japan 50, 147–168.
37 Isaacs, N.S. (1981) Liquid Phase High Pressure Chemistry, Wiley-Intersience, New York.
38 Hope, M.J., Bally, M.B., Webb, G. and Cullis, P.R. (1985) Biochim. Biophys. Acta 812, 55–65.
39 McLaughlin, S., Mulrine, N., Gresalfi, T., Vaio, G. and McLaughlin, A. (1981) J. Gen. Physiol. 77, 445–473.
40 Cafiso, D., McLaughlin, A., McLaughlin, S. and Winiski, A. (1989) Methods Enzymol. 171, 342–364.
41 Paladini, A.A. and Weber, G. (1981) Rev. Sci. Instrum. 52, 419–427.
42 Bashford, C.L. and Smith, J.C. (1979) Biophys. J. 25, 81–85.
43 Wiseman, T., Williston, S., Brandts, J.F. and Lin, L-N. (1989) Anal. Biochem. 179, 131–137
44 McLaughlin, S. (1989) Annu. Rev. Biophys. Biophys. Chem. 18, 113–136.
45 Kuchinka, E. and Seelig, J. (1989) Biochemistry 28, 4216–4221.
46 Schwarz, G. and Beschiaschvili, G. (1989) Biochim. Biophys. Acta 979, 82–90.
47 Stankowski, S. and Schwarz, G. (1990) Biochim. Biophys. Acta 1025, 164–172.
48 Stankowski, S. (1991) Biophys. J. 60, 341–351.
49 Weber, G. and Drickamer, H.G., (1983) Q. Rev. Biophys. 16, 89–112.
50 Tanford, C. (1980) The Hydrophobic Effect: Formation of Micelles and Biological Membranes, Krieger, Malabar.
51 Huang, C.-h. and Charlton, J.P. (1972) Biochemistry 11, 735–740.
52 Seelig, J. and Ganz, P. (1991) Biochemistry 30, 9354–9359.
53 Bäuerle, H-D. and Seelig, J. (1991) Biochemistry 30, 7203–7211.
54 Finkelstein, A.V. and Janin, J. (1989) Protein Eng. 3, 1–3.
55 Israelachvili, J. (1992) Intermolecular and Surface Forces, 2nd Edn., pp. 63–64, Academic Press, San Diego.
56 Marcus, Y. (1985) Ion Solvation, pp. 218–230, Wiley-Interscience, Chichester.
57 Gurney, R.W. (1962) Ionic Processes in Solution, pp. 1–33, Dover, New York.
58 Colombo, M.F., Rau, D.C. and Parsegian, V.A. (1992) Science 256, 655–659.