Membrane Binding of Peptides Containing Both Basic and Aromatic Residues. Experimental Studies with Peptides Corresponding to the Scaffolding Region of Caveolin and the Effector Region of MARCKS†

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ABSTRACT: We have studied the binding of peptides containing both basic and aromatic residues to phospholipid vesicles. The peptides caveolin(92−101) and MARCKS(151−175) both contain five aromatic residues, but have 3 and 13 positive charges, respectively. Our results show the aromatic residues insert into the bilayer and anchor the peptides weakly to vesicles formed from the zwitterionic lipid phosphatidylcholine (PC). Incorporation of a monovalent acidic lipid (e.g., phosphatidylserine, PS) into the vesicles enhances the binding of both peptides via nonspecific electrostatic interactions. As predicted from application of the Poisson−Boltzmann equation to atomic models of the peptide and membranes, the enhancement is larger (e.g., 10^4- vs 10-fold for 17% PS) for the more basic MARCKS(151−175). Replacing the five Phe with five Ala residues in MARCKS(151−175) decreases the binding to 10:1 PC/PS vesicles only slightly (6-fold). This result is also consistent with the predictions of our theoretical model: the loss of the attractive hydrophobic energy is partially compensated by a decrease in the repulsive Born/desolvation energy as the peptide moves away from the membrane surface. Incorporating multivalent phosphatidylinositol 4,5-bisphosphate (PIP_2) into PC vesicles produces dramatically different effects on the membrane binding of the two peptides: 1% PIP_2 enhances caveolin(92−101) binding only 3-fold, but increases MARCKS(151−175) binding 10^4-fold. The strong interaction between the effector region of MARCKS and PIP_2 has interesting implications for the cellular function of MARCKS.

Many peripheral membrane-bound proteins contain a cluster of basic residues that interact electrostatically with acidic phospholipids. The cluster of basic residues often acts in conjunction with an N-terminal myristate, e.g., in Src (*1*), or a C-terminal prenyl group, e.g., in K-Ras (*2*−*4*), to anchor the protein strongly to the plasma membrane. The basic clusters in Src and K-Ras do not contain aromatic residues and the interaction of these basic clusters with membranes proceeds via electrostatic interactions alone. Our previous experimental/theoretical work focused on simple basic peptides (e.g., tri-, penta-, and heptalysine), small basic toxins (e.g., charybdotoxin), and peptides corresponding to the N-terminal region of Src, which bind outside the envelope of the polar headgroup region (*5*−*9*). A theoretical treatment that applies the Poisson−Boltzmann equation to atomic models of the peptide and the membrane describes their binding well. This approach, which is summarized in a recent minireview (*7*), correctly predicts how the binding of these peptides depends on the three most important variables that affect electrostatic interactions: the number of basic residues in the peptide, the concentration of salt in the aqueous solution, and the mole fraction of acidic lipid in the bilayer. The binding is independent of the chemical nature of both the monovalent acidic lipid (e.g., PS and PG) and the basic residue (e.g., Arg and Lys), as predicted for a simple electrostatic interaction. Finally, the theory also accounts for the observation that these peptides bind outside the envelope of the polar headgroup, because it predicts the short-range Born/desolvation repulsion is stronger than the long-range Coulomb attraction as the peptide approaches within a distance equal to the diameter of a water molecule.

Src and K-Ras are particularly simple examples of proteins that use electrostatics to bind to membranes. Many peripheral proteins that use electrostatics also have aromatic residues within the cluster of basic residues. For example, the myristoylated, alanine rich C kinase substrate (MARCKS)[1] has five Phe residues within its membrane-binding cluster of 13 basic residues, which is termed the effector domain (*10*−*13*). The membrane-bound scaffolding protein AKAP79 (*14*) contains a cluster of basic residues that is similar in

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sequence to the effector domain of MARCKS. Many different proteins have C2 domains [e.g., protein kinase C (PKC), synaptotagmin, secretory phospholipase A2] that interact with membranes (*15*−*20*). These C2 domains contain both basic and aromatic residues. Several important blood proteins (coagulation factors V and VIII) have membrane-binding regions containing spatially juxtaposed basic and aromatic residues (*21*, *22*). This report focuses on understanding the physical basis for how aromatic and basic residues facilitate membrane binding. We report experimental studies with two peptides and summarize the results of theoretical calculations, which will be described in detail elsewhere (Murray, Arbuzova, McLaughlin, Honig, manuscript in preparation).

In brief, we extend our earlier theoretical approach by adding to the Coulomb and Born/desolvation energies an empirically determined hydrophobic energy due to the insertion of the aromatic residues into the lipid headgroup region. We follow the pioneering work of Wimley and White (*23*) and use their experimentally derived interfacial hydrophobicity scale, which describes the partitioning of amino acid residues into the interfacial region of fluid (liquid-crystalline) PC membranes. We assume the hydrophobic energy is proportional to the area of aromatic residues that are removed from bulk water and inserted into the headgroup region. The inclusion of the aromatic contribution allows us to predict the balance of the Coulomb, Born, and hydrophobic interactions that contribute to the membrane binding properties of peptides containing both basic and aromatic residues.

We performed experimental studies on the membrane binding of peptides corresponding to a portion of the scaffolding region of caveolin and the effector region of MARCKS. We chose these peptides for three reasons. First, MARCKS(151−175) and caveolin(92−101) exist in a nonhelical, extended conformation both when in aqueous solution and when bound to a PC/PS membrane (*24*, *25*; D. Cafiso, personal communication). This simplifies the theoretical analysis greatly, because a peptide that changes from a random coil to an alpha helix upon inserting into a membrane (and many peptides do) derives a substantial fraction of its binding energy from the conformational change, as discussed in detail elsewhere (*26*−*28*). Second, although both peptides have five hydrophobic residues, one Trp, two Phe, two Tyr for caveolin(92−101) and five Phe for MARCKS(151−175)−they contain different numbers of basic residues. Caveolin(92−101) has three, whereas MARCKS(151−175) has 13 positive charges. The different electrostatic contributions provide a good test of our theoretical predictions. Third, we are interested in biological implications of the membrane binding for both caveolin-1 and MARCKS proteins.

[1] Abbreviations: MARCKS, myristoylated alanine-rich C kinase substrate; caveolin(92−101), peptide corresponding to residues 92−101 of human caveolin-1; MARCKS(151−175), peptide corresponding to residues 151−175 of bovine MARCKS; PC, phosphatidylcholine; PG, phosphatidylglycerol; PS, phosphatidylserine; PIP_2, phosphatidylinositol 4,5-bisphosphate; DMSO, dimethyl sulfoxide; PLC, phosphoinositide-specific phospholipase C; K, molar partition coefficient (see eq 1); LUV, large unilamellar vesicle; PKC, protein kinase C; AKAP, A kinase anchoring protein; NEM, *N*-ethylmaleimide; MLV, multilamellar vesicle.

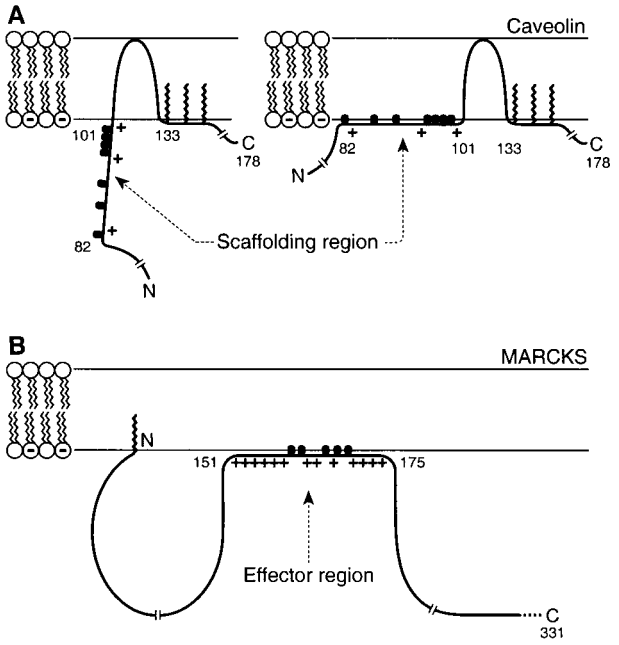

FIGURE 1: (A) Cartoon of caveolin interacting with the plasma membrane. Basic residues are shown as plus signs and aromatic residues as ovals in the scaffolding region (residues 82−101). See text for details. (B) Cartoon of MARCKS interacting with the plasma membrane. The N-terminal myristate inserts into the bilayer and the 13 basic residues (plus signs) in the effector domain (residues 151−175) interact electrostatically with acidic lipids (minus signs). Phe residues in the effector domain, shown as ovals, penetrate the interface. See text for details.

Caveolin-1 is the major protein in caveolae, small (50−100 nm) flask-shaped invaginations of the plasma membrane found in many different cell types (*29*−*33*); caveolae probably play a role in signal transduction (*30*, *33*). There is good evidence that both the N- and C-terminal regions of caveolin-1 are located on the cytoplasmic side of the membrane as illustrated in the cartoon in Figure 1A (*34*−*36*). Residues 102−132 of human caveolin-1 are hydrophobic and should partition into the membrane, although there is no direct evidence they form the membrane-spanning loop shown in Figure 1A and several reviews (e.g., refs *31* and *32*). The proximal C-terminal region contains multiple Cys residues that are palmitoylated (*35*); the palmitates should partition strongly into the bilayer (*37*, *38*). The proximal N-terminal region (residues 82−101) has been proposed to act as a scaffolding region that binds other molecules (*33*). The putative scaffolding region contains three basic residues (Arg, Lys, plus signs) and seven aromatic residues (Trp, Phe, Tyr, filled ovals). This region is usually portrayed protruding into the cytoplasm (*31*, *32*), as shown on the left in Figure 1A, although there is no direct evidence for this orientation. We propose that the scaffolding region binds strongly to the polar headgroup region of the bilayer, as shown on the right in Figure 1A. We show here that a peptide corresponding to part of this region does bind strongly to phospholipid bilayers through nonspecific electrostatic and hydrophobic interactions.

MARCKS is a major substrate of protein kinase C (PKC) in many different cell types. MARCKS binds to calmodulin, membranes, and actin and may play a role in secretion, membrane trafficking, and cell motility (reviewed in refs *10*

and *11*). Although it is essential for brain development (*39*), the biological function of MARCKS remains obscure.

Figure 1B illustrates the mechanism by which MARCKS binds to the plasma membrane (*12*, *13*). The protein is shown with its N-terminal myristate chain embedded into the lipid bilayer and the basic effector region (Arg, Lys; plus-signs) interacting with acidic lipids (minus signs) in the inner leaflet of the plasma membrane; five phenylalanines (filled ovals) penetrate the polar headgroup region. EPR measurements with spin-labeled peptides on vesicles (*24*) and surface pressure measurements on monolayers (see below) show the Phe residues penetrate the polar headgroup region. The N-terminal (residues 2−150) and C-terminal (residues 176−331) regions are highly acidic (net charges of −14 and −25, respectively) and should be repelled from the negatively charged membrane. The highly conserved effector region (residues 151−175) has 13 basic and no acidic residues.

MARCKS is bound to the plasma membrane of a quiescent cell, but it is not distributed uniformly in the plasma membrane of many cell types: in macrophages, for example, MARCKS colocalizes with actin filaments and PKC-α in nascent phagosomes (*40*); in fibroblasts it is concentrated with actin and other proteins in ruffles (*41*). The interactions (probably protein−protein, possibly with actin) that produce these nonuniform distributions of MARCKS in the plasma membrane are not well understood. The results we report here show that although peptides corresponding to the effector region of MARCKS bind weakly to PC vesicles (molar partition coefficient $K < 10^2$ M^{-1}), and moderately to PC/PS vesicles containing < 10% PS ($K < 10^4$ M^{-1}), they bind strongly to PC/PIP_2 vesicles containing 1% PIP_2 ($K = 10^6$ M^{-1}). This result demonstrates there are strong, presumably electrostatic, interactions between this basic effector region of MARCKS and the polyvalent acidic lipid PIP_2 (or, more likely, several PIP_2). The obvious biological implication is that PIP_2 should be concentrated in regions of the cell membrane where MARCKS is concentrated, for example in ruffles.

MATERIALS AND METHODS

Materials. 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine (PC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylserine (PS), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylglycerol (PG), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylethanolamine (PE), and bovine liver phosphatidylinositol were obtained from Avanti Polar Lipids (Alabaster, AL). The ammonium salt of PIP_2 was obtained from Boehringer Mannheim (Mannheim, Germany) or was purified from Sigma (St. Louis, MO) bovine brain extract as describe elsewhere (*42*) and the radiolabeled 1,2-di[1-^{14}C]-oleoyl-L-3-phosphatidylcholine ([^{14}C]DOPC) was purchased from Dupont NEN (Boston, MA).

Peptides and Peptide Labeling. Peptides corresponding to residues 92−101 or 83−101 of human caveolin-1 with or without cysteine added at the N-terminus (acetyl-FTVTKYWFYR-amide, peptide I; FTVTKYWFYR-amide, peptide II; CFTVTKYWFYR-amide, peptide III; acetyl-CGIWKASFTTFTVTKYWFYR-amide, peptide IV) were obtained from both Chiron Technologies (Victoria, Australia) and CASM, SUNY at Stony Brook (Stony Brook, NY); they were >95% pure as determined by high-pressure liquid chromatography and mass spectrometry. For simplicity we refer to these peptides as caveolin(92−101) and caveolin(83−101) and specify which peptide was used. The peptide corresponding to the basic effector region of bovine MARCKS, residues 151−175 (acetyl-KKKKKRFSFKKSFKLSGFSFKKNKK-amide, peptide V), was obtained from CASM, SUNY at Stony Brook (Stony Brook, NY); and the peptide with a cysteine added at the N-terminus (acetyl-CKKKKKRFSFKKSFKLSGFSFKKNKK-amide, peptide VI) was a generous gift from D. Cafiso and was synthesized as described previously (*43*). We refer to these peptides as MARCKS-(151−175) and specify which peptide was used. The peptide with the first lysine replaced by cysteine and the five Phe residues replaced by five Ala, referred to as Phe-Ala MARCKS(151−175) or peptide VII, (CKKKKRASAKKSAKLSGASAKKNKK-amide) was from Chiron Technologies (Victoria, Australia). Note that when unlabeled peptide VII was used, 2 mM DTT (Sigma, St. Louis, MO) was always added to peptide stock and buffer. Acrylodan-CKKKKRFSFKKSFLKSGFSFKKNKK-amide, peptide VIII, was obtained from Boehringer Mannheim (Roche, Mannheim, Germany).

We labeled caveolin(92−101) used for the membrane-binding measurements by reacting Cys on peptide III with *N*-ethylmaleimide (NEM, Sigma, St. Louis, MO) and [^{3}H]-NEM (Dupont NEN, Boston, MA) in DMSO in the presence of DTT for 1 h. MARCKS(151−175) was labeled with [^{3}H]-NEM as follows. We mixed peptide VI (dissolved in 50 mM phosphate buffer, pH 7) with [^{3}H]NEM (dissolved in DMF) and incubated the mixture for 1 h at room temperature. Then we added a 10-fold excess of cold NEM (dissolved in DMF), and incubated for 1 h more. At the last step we added 5-fold excess of DTT over peptide and incubated for another hour. We also labeled peptides III and VI with cold NEM only using the same procedure and checked with MALDI-TOF mass spectroscopy that we obtained a product with the correct mass. Phe-Ala MARCKS(151−175) was labeled with acrylodan using a procedure described previously (*43*, *44*). All labeled peptides were purified using a reverse-phase C8 HPLC column and nonradioactive peptides were characterized using MALDI-TOF mass spectrometry.

Binding Measurements Using Sucrose Loaded Vesicles. The preparation of sucrose-loaded large (100 nm diameter) unilamellar vesicles (LUVs) and the details of the binding measurements have been described previously (*45*). In brief, the vesicles were formed in a solution containing 176 mM sucrose, 1 mM Mops, pH 7. The outside buffer was exchanged to 100 mM KCl, 1 mM Mops, pH 7. The peptide was mixed with sucrose-loaded LUVs, the mixture was centrifuged, and the concentration of peptide in both the supernatant and pellet, which contained the vesicles, was measured. We used both radioactivity measurements with [^{3}H]NEM-peptides and fluorescamine assay with unlabeled peptides to determine the binding of the peptides to sucrose-loaded LUVs.

We describe the binding of the peptides to lipid vesicles by defining a molar partition coefficient, K, that does not require any assumptions about the adsorption mechanism (*6*, *8*, *37*, *46*). *K* is the proportionality constant between the moles of peptide bound per mole of lipid and the molar concentration of peptide in the bulk aqueous phase, [P], $K = [P]_m/([L][P])$.[2] For our conditions ($[P]_{tot} \ll [L]$):

$$[P]_m/[P]_{tot} = K[L]/(1 + K[L]) \quad (1)$$

where $[P]_m/[P]_{tot}$ is the fraction of peptide bound to the vesicle membrane and [L] is the concentration of lipid accessible to the peptide. [L] equals half the total lipid concentration because the membranes are not permeable to the peptides, which are added to the solution bathing the LUVs.

We obtained essentially identical binding constants using the fluorescamine and radioactivity assays. Specifically, results from both radioactivity assay ([^{3}H]NEM-labeled peptide III, open symbols) and fluorescamine assay (unlabeled peptide II, filled symbols) are shown in Figure 2A for the caveolin peptide. Results from both radioactivity assay ([^{3}H]NEM-labeled peptide VI, open symbols) and fluorescamine assay (peptide V, filled symbols) are shown in Figure 3A for the MARCKS peptide. More critically, the value of the molar partition coefficient was independent of the concentration of peptide under our conditions. Specifically, we obtained comparable results using 50 nM, 100 nM, and 5 μM caveolin(92−101). Using 10:1 PC/PS vesicles, 10 nM, 15 nM, 1, and 2 μM MARCKS(151−175) also gave comparable results. [For membranes with a higher fraction of PS, or for PC/PIP_2 membranes, the binding of MARCKS(151−175) is so strong that reliable data could only be obtained using low concentrations of the radioactively labeled peptide; 2 nM in the case of PC/PIP_2.] The observation that K is independent of peptide concentration indicates that the peptide adsorbs as a monomer under our conditions and that the bound peptides do not change significantly the charge density of the bilayer.

We initially attempted to measure the binding of a peptide corresponding to the full length of the scaffolding region, caveolin(83−101), to LUVs. This peptide, however, is not soluble in aqueous solutions (e.g., 100 mM KCl, 1 mM Mops, pH 7.0). When we added caveolin(83−101) dissolved in DMSO to our buffer solution (final peptide concentration 1−10 μM), it appeared soluble by visual inspection, but the peptide was found in the pellet fraction after centrifugation at 100000g for 30 min. This result indicates that the peptide aggregates in the buffer solution, a conclusion confirmed by light scattering experiments (not shown). Increasing the concentration of DMSO or Triton X-100 in the solution to reasonable levels (e.g., <10% DMSO) failed to solubilize the peptide. Presumably caveolin(83−101) exists mainly as a micelle or in some other aggregated form in solution, as observed previously (*48*). There are numerous previous reports that caveolin(82−101) scaffolding domain peptide inhibits the function of a number of important molecules, such as G proteins and Src. Our results support the conclusion of Mumby and co-workers (*48*) that these reports should be regarded with caution: the results may be due more to the caveolin(82−101) peptide's propensity to form micelles in solution than to its ability to bind specifically to G proteins, Src, etc.

To obtain a minimal estimate for the membrane binding capacity of the scaffolding region of caveolin, we synthesized a peptide corresponding to a portion of this region, residues 92−101, added cysteine to the N-terminus and labeled the peptide with [^{3}H]NEM. This peptide, caveolin(92−101), has one fewer Trp and Phe residues than caveolin(83−101), but also has a charge of +3 because of the unblocked N-terminus, and is sufficiently soluble to be used for binding measurements.

Fluorescence Experiments. Equilibrium binding measurements using acrylodan-labeled peptides and data analysis were performed as described earlier (*9*, *43*). Acrylodan is a polarity sensitive probe (*49*). The maximum of the acrylodan emission spectra is blue shifted from 520 nm to about 460 nm and the intensity increases when peptide is bound to a membrane and acrylodan penetrates into the bilayer. We used the increase of the emission intensity at 460 nm after addition of different concentrations of lipid to calculate the amount of peptide bound. We used the ratio of the binding affinities of acrylodan-labeled MARCKS(151−175) and Phe-Ala MARCKS(151−175) peptides to estimate how Phe residues contribute to the peptide-membrane binding. Absolute binding affinities of the fluorescently labeled peptides are higher than those of unlabeled peptides. The probe is hydrophobic and acrylodan-labeled MARCKS peptides bind to membranes 20−50-fold more strongly than the unlabeled peptides. Similarly, acrylodan-labeled heptalysine binds to 5:1 PC/PS vesicles 100-fold more strongly than unlabeled heptalysine (*9*).

Monolayer Experiments. We used a fixed-area circular trough (*50*) to study the penetration of peptides into phospholipid monolayers. In a typical experiment, we formed a monolayer from either PC or a 2:1 PC/PS mixture dissolved in chloroform. A measured volume of the lipid/chloroform solution was deposited on the surface of the subphase (100 mM KCl, 1mM Mops, pH 7.0, 0%, 2%, or 5% DMSO) to produce a monolayer with a surface pressure, π, between 25 and 35 mN/m. We added a small volume of a peptide in water/DMSO to the 15 mL aqueous subphase and recorded changes in π. We obtained identical surface pressure results on PC monolayers with caveolin(92−101) having either a blocked or unblocked N-terminus, peptides I or II. The final concentration of the peptides in the subphase was 1 μM for caveolin(92−101) (peptides I and II), and 0.5 μM for MARCKS(151−175) (peptide V) or Phe-Ala MARCKS(151−175) (peptide VII).

Theoretical Calculations of the Interaction of the MARCKS and Caveolin Peptides with Membranes. We constructed atomic models of the peptide/membrane systems examined experimentally in this paper. We assumed the aromatic Phe, Trp, and Tyr residues insert into the polar headgroup region. Models corresponding to MARCKS(151−175) and caveolin(92−101) were built in extended form using the Insight/Biopolymer molecular modeling package (Insight-II, Biosym Technologies). We varied the side-chain rotamers for the aromatic residues among standard configurations to approximate a peptide conformation in which these side chains can penetrate into the membrane interface simultaneously; we assumed the side chains of all other residues are parallel with the membrane surface. To reduce atomic overlaps and to relax torsional and dihedral constraints, the peptide models were energy minimized using the Insight/Discover molecular modeling package (Insight-II, Biosym Technologies). The minimization consisted of 100 iterations with a conjugate

[2] Note that two different definitions of the molar partition coefficient are used in the literature. We follow Tamm (*46*) and use a standard state of 1 M; our molar partition coefficient, K, has units of M^{-1} (see eq 1). White and Wimley (*28*) define the molar partition coefficient differently. The different definitions of partition coefficients and the conversion between them are discussed elsewhere in the literature (*28*, *37*, *47*).

gradient method in gas phase using the constant value force field (CVFF) and neglecting electrostatic interactions. The minimization did not alter the extended structure of the peptides significantly. Phospholipid bilayer models, corresponding to membranes containing PC, 15:1 PC/PS, 8:1 PC/PS, 5:1 PC/PS, 2:1 PC/PS, and PS, were built as described elsewhere (*51*).

For the electrostatic calculations, we removed the aromatic side chains of the peptides to allow close approach to the membrane surface. The charges and radii used for amino acids were taken from a CHARMM22 parameter set (*52*); those used for the lipids are from (*51*). We solved the nonlinear Poisson−Boltzmann equation (*53*) for the electrostatic potential due to the peptide and the membrane when they are far apart and when they are associated. These potentials were used to calculate the change in the electrostatic free energy of the peptide/membrane system in a particular configuration. The full electrostatic free energy of interaction was calculated, with both the peptide (P) and membrane (M) fully charged, as the difference of the electrostatic free energy when the peptide and membrane are associated (W[P,M]) and when they are far apart (W[P], W[M]):

$$\Delta W_{EL} = W[P,M] - W[P] - W[M]$$

The desolvation penalty was calculated as the sum of (1) the electrostatic interaction free energy with the peptide discharged and (2) the electrostatic interaction free energy with the membrane discharged:

$$\Delta W_{DESOLV} = \{W[P(Q=0),M] - W[P(Q=0)] - W[M]\} + \{W[P,M(Q=0)] - W[P] - W[M(Q=0)]\}$$

where Q is the charge. The electrostatic (Coulomb) attraction was calculated as the difference between the total electrostatic free energy of interaction and the desolvation penalty:

$$\Delta W_{COUL} = \Delta W_{EL} - \Delta W_{DESOLV}$$

Previous work has shown that it is sufficient to consider a single orientation of the peptide with respect to the bilayer in order to calculate how the electrostatic interaction changes as a function of mole percent acidic lipid in the bilayer, ionic strength of the solution and number of basic residues in the peptide (*5*−*8*). In our calculations, we considered only the orientation in which each peptide lies flat at the membrane surface, and calculated the interaction free energy as a function of distance R between the van der Waals surfaces of the peptide backbone and the membrane.

We assumed that only the side chains of the aromatic residues contribute to the nonpolar component of the free energy of interaction. The aromatic contribution was calculated as the product of a maximal value and the fraction of surface area of the aromatic side chains buried in the membrane interface at each distance R between the peptide backbone and membrane surface. The maximum expected nonpolar contribution was obtained from the interfacial hydrophobicity scale of Wimley and White (*23*). When the peptide backbone just touches the membrane surface (R = 0), we assume the aromatic side chains are completely buried and the aromatic contribution is equal to the maximal value. As R increases, the aromatic contribution decreases in proportion to the fractional change in buried surface area. For MARCKS(151−175), the maximum expected nonpolar contribution from the five Phe residues is −6.5 kcal/mol; this decreases to −3.2 kcal/mol at R = 2 Å and to 0 kcal/mol at $R \approx 5$ Å.

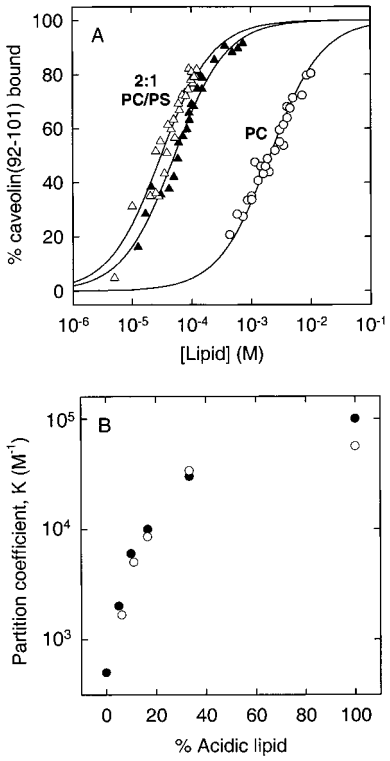

FIGURE 2: Binding of caveolin(92−101) to phospholipid vesicles. (A) The percent peptide bound is plotted as a function of the concentration of lipid accessible to the peptides (half the total lipid concentration) for vesicles formed from the zwitterionic lipid PC (open circles), or a 2:1 mixture of PC with a monovalent acidic lipid: 2:1 PC/PS (open triangles) or 2:1 PC/PG (filled triangles). Each point represents the average of two measurements. The curves are the least squares best fit of eq 1 to the experimental data with the molar partition coefficients $K = 5 \times 10^2$ M^{-1} for PC, $K = 2 \times 10^4$ M^{-1} for 2:1 PC/PG, and $K = 3 \times 10^4$ M^{-1} for 2:1 PC/PS. The aqueous solutions for these experiments, and for the results shown in all other figures, contain 100 mM KCl, 1 mM Mops, pH 7.0. (B) The filled circles illustrate the effect of the acidic lipid PS on the molar partition coefficient, K, which was determined from the experiments shown in panel A and other similar experiments (not shown). The open circles illustrate the theoretically predicted effect of PS on K assuming PS exerts only nonspecific electrostatic effects. The theoretical calculations used atomic models of the membrane and peptide (see text for details). The agreement of theory and experiment is adequate; there are no adjustable parameters in the theory.

RESULTS

Membrane Binding of Peptides Corresponding to Part of the Caveolin Scaffolding Region.[3] The open circles in Figure 2A illustrate that caveolin(92−101) binds to electrically neutral vesicles formed from the zwitterionic lipid phosphatidylcholine (PC) with a molar partition coefficient of $K = 5 \times 10^2$ M^{-1}. (From eq 1 it is apparent that K is the reciprocal of the accessible lipid concentration that binds 50%

[3] We first attempted to measure membrane binding of a peptide corresponding to the full length of the scaffolding region, caveolin-(83−101). The peptide, however, is not soluble in aqueous solutions. For details see Materials and Methods.

of the peptide. If we assume, incorrectly, that the peptide forms 1:1 complexes with the lipid then K is the apparent association constant of the complex.) The work of Wimley and White (*23*) with model peptides shows that partitioning of the aromatic residues (one Trp, two Phe, and two Tyr) of the peptide into the polar headgroup region of the lipid bilayer provides sufficient energy to account for this binding. If these aromatics penetrate the polar headgroup region, caveolin(92−101) should increase the surface pressure of a PC monolayer. It does: at initial PC monolayer surface pressures of $\pi = 35$, 30, or 25 mN/m, adding 10^{-6} M peptide to the subphase increases π by 1, 4, or 10%, respectively (data not shown).[4] As expected, addition of 10^{-6} M caveolin-(92−101) to the subphase produces a larger increase in π if the monolayer contains acidic lipids (data not shown). As discussed in the next paragraph, more of the basic peptide is expected to bind to a unit area of monolayer if it contains acidic lipids.

Figure 2A illustrates that incorporating 33% monovalent acidic lipid, either phosphatidylserine (PS; open triangles) or phosphatidylglycerol (PG; filled triangles), into the vesicles increases the binding of caveolin(92−101) about 100-fold. PS and PG produce similar increases in binding, which suggests the effect of these monovalent acidic lipids is due to a nonspecific electrostatic attraction of the positively charged peptide to the negatively charged surface of the vesicle. PS and PG produce a negative electrostatic potential in the aqueous diffuse double layer (thickness, or Debye length, 1 nm for 100 mM monovalent salt) adjacent to the membrane surface (*58*). The experimentally measured ζ-potentials (electrostatic potential 0.2 nm from the surface) of PC and 2:1 PC/PS or PC/PG vesicles in a 100 mM monovalent salt solution are about 0 and −40 mV, respectively (*58*). If we assume caveolin(92−101) is a trivalent point charge that does not perturb this potential (obviously unrealistic assumptions), the Boltzmann relation predicts the acidic lipids increase the concentration of the caveolin peptide (charge = +3) in the aqueous phase at the surface of the membrane 100-fold [$\exp(-ze\zeta/kT) = 10^{-z\zeta/60} = 100$], a prediction that agrees with the observed ∼100-fold increase in binding (Figure 2A). A more realistic treatment of the electrostatic interaction, using atomic models for the 2:1 PC/PS membrane and the peptide and taking into account the perturbation of the potential adjacent to the membrane by the peptide (described briefly in Materials and Methods), produces a similar conclusion: incorporating 33% acidic lipid should increase the peptide binding about 100-fold due to nonspecific electrostatic effects [see open circles in Figure 2B and (Murray, Arbuzova, McLaughlin, Honig, manuscript in preparation)]. As expected, vesicles with a smaller fraction of acidic phospholipid (PS or PG) bind the peptide less strongly, and vesicles with a larger fraction of acidic phospholipid bind the peptide more strongly (filled circles, Figure 2B). Electrostatic theory describes adequately all the effects of these monovalent acidic lipids (open circles, Figure 2B).

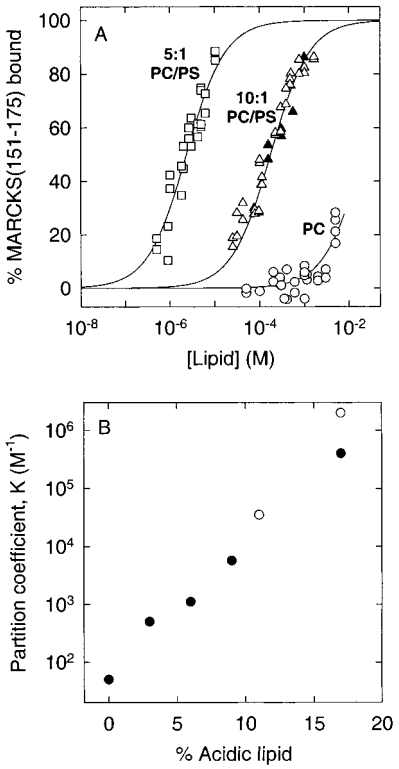

FIGURE 3: Binding of MARCKS(151−175) to phospholipid vesicles. (A) MARCKS(151−175) binds weakly to PC vesicles with a molar partition coefficient $K = 50$ M^{-1} (open circles), but more strongly to 10:1 PC/PS (or PG), $K = 6 \times 10^3$ M^{-1} (open and filled triangles) or to 5:1 PC/PS vesicles, $K = 4 \times 10^5$ M^{-1} (open squares). The solid lines are the least-squares best fits of eq 1. (B) Plot of the molar partition coefficient deduced from data in panel A (and similar data) as a function of the % acidic lipid in the vesicle (filled circles). The open circles show the predicted effect of PS on K from the atomic model calculations. The predictions agree qualitatively with the experimental results.

Membrane Binding of Peptides Corresponding to the MARCKS Effector Region. Figure 3A (open circles) shows that MARCKS(151−175) binds weakly to PC vesicles. The binding can barely be measured at the highest lipid concentrations we can use in our experiments; the molar partition coefficient estimated from the fit of eq 1 to the data is $K = 50$ M^{-1}. Although this binding estimate is uncertain because only 20% of the peptide is bound at the highest lipid concentrations, a similar estimate for K was obtained using an independent technique. We measured the effect of MARCKS(151−175) on the ζ-potential of PC vesicles. Measurements performed with concentrations of MARCKS-(151−175) = 0, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, and 10^{-4} M in a 100 mM KCl, 1 mM Mops, pH 7 solution, produced ζ-potentials of 0, 0, 0, 0, 0, and +10 ± 1 mV ($n = 30$), respectively. The observation that 10^{-4} M MARCKS(151−175) produces a zeta potential of +10 mV when it binds to PC vesicles may be compared with the observation that incorporation of 5% monovalent positive (negative) lipid also produces a zeta potential of +10 (−10) mV (*58*). This suggests that one 13-valent MARCKS peptide is bound per 250 lipids or that the molar partition coefficient of the peptide is 40 M^{-1} (*58*). Thus, two independent measurements suggest

[4] The available evidence suggests that a monolayer surface pressure, π, of 30−35 mN/m corresponds to the pressure in a bilayer membrane (*50*, *54*−*57*). Both the magnitude of the surface pressure change, and the observation that the binding increases as π is lowered, are consistent with the aromatic residues inserting at least partially into the polar headgroup region of the monolayer. As discussed quantitatively elsewhere, the binding increases as π decreases because the aromatic residues must do work, πA, to insert a cross-sectional area A into the monolayer and expand it by this area (*50*).

that the molar partition coefficient for MARCKS(151−175) onto PC vesicles is ∼50 M^{-1}. The work of Wimley and White (*23*) shows that partitioning of the five Phe is sufficient to account for the binding observed.

The other data in Figure 3A show that incorporation of monovalent acidic lipids (PS or PG) into the vesicles greatly increases the binding of MARCKS(151−175).[5] Identical results were obtained using PG and PS, which suggests that there is no chemical specificity of the interaction; the simplest interpretation is that PS and PG exert only electrostatic effects. (We also observed similar, but slightly weaker binding to the vesicles containing phosphatidylinositol, data not shown.) The molar partition coefficients, *K*, deduced from these data (and similar data, not shown, for 3 and 6% PS) are plotted in Figure 3B (filled circles). Note that incorporating 9% PS increases *K* about 10^2-fold and incorporating 17% PS increases *K* about 10^4-fold. The effect of acidic lipids is much larger for MARCKS(151−175) than for caveolin(92−101), as expected based on the difference in their charges (+13 vs +3). The simplest possible theory provides a qualitative explanation for this large effect of acidic lipids on the binding of MARCKS(151−175). The ζ-potentials of 10:1 and 5:1 PC/PS vesicles in a 100 mM monovalent salt are about −20 and −30 mV (*25*, *58*). If we assume MARCKS(151−175) is a point charge with a valence of +13 and the binding of a peptide does not perturb the potential in the vicinity of the membrane, we can use the Boltzmann relation [$\exp(-ze\zeta/kT) = 10^{-z\zeta/60}$]. This predicts that the free concentration of peptide in the aqueous phase at the surface of the membrane, and thus the binding, should increase by factors of about 10^4 and 10^6, more than enough to account for the enhancement observed in Figure 3A, but ∼100-fold larger than observed experimentally. More realistic calculations of the electrostatic potential profiles of a bilayer with an adsorbed MARCKS(151−175) (see Figure 3 in ref *13*) show that the peptide does change the local potential significantly. Our more realistic atomic level theory can describe adequately the effect of acidic lipids on the binding of MARCKS(151−175), predicting that incorporation of 11 or 17% monovalent acidic lipid into PC membrane enhances the binding of MARCKS(151−175) 7×10^2- and 4×10^4-fold (open circles in Figure 3B). The theory can also help us understand how replacing the Phe residues with Ala in MARCKS(151−175) affects its membrane binding.

The Phe residues in MARCKS(151−175) penetrate into the lipid headgroup region of PC/PS monolayers, but a corresponding peptide in which the Phe residues are replaced by Ala, Phe-Ala MARCKS(151−175), does not penetrate the surface. Specifically, adding 0.5 μM MARCKS(151−175) to the subphase increases the surface pressure of 1:1:1 PC/PS/PE monolayers 3, 7, or 12% at 33, 29, or 27 mN/m respectively (data not shown). Under the same conditions, Phe-Ala MARCKS(151−175) does not increase the surface pressure at $\pi > 26$ mN/m. These results are consistent with recent studies by Cafiso and co-workers, who used spin-labeled peptides to show that MARCKS(151−175) penetrates the interface but that Phe-Ala MARCKS(151−175) binds outside the lipid headgroup region (*24*, *59*).

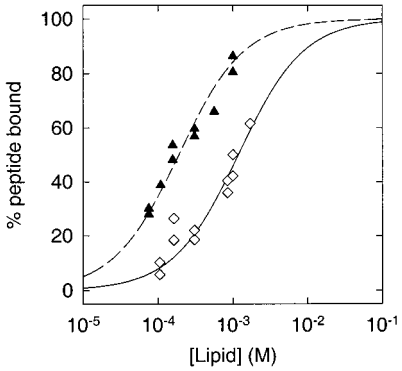

FIGURE 4: Effect of removing aromatic residues. Replacing the five Phe in MARCKS(151−175) by five Ala reduces binding to 10:1 PC/PG vesicles 6-fold. The molar partition coefficient for the binding of MARCKS(151−175) is $K = 5 \times 10^3$ M^{-1} (filled triangles, peptide V, the dashed line is the least-squares best fit of eq 1) and for the binding of Phe-Ala MARCKS(151−175) is $K = 8 \times 10^2$ M^{-1} (open symbols, peptide VII, the solid line is the least-squares best fit of eq 1).

Figure 4 shows that replacing the five Phe of MARCKS-(151−175) with five Ala reduces its binding to 10:1 PC/PG vesicles only 6-fold. Similar results were obtained with acrylodan-labeled MARCKS peptides VIII and VII: acrylodan-labeled MARCKS(151−175) and acrylodan-labeled Phe-Ala MARCKS(151−175) bind to 10:1 PC/PS vesicles with molar partition coefficients of 2×10^5 and 2×10^4 M^{-1} and to 15:1 PC/PS vesicles with the molar partition coefficients 2×10^4 and 3×10^3 M^{-1} (data not shown). Note that although the hydrophobic acrylodan label enhances the binding of the peptides, changing the five Phe to Ala residues has a similar 6−10-fold effect on the peptide binding to PC/PS membranes. Victor et al. (*59*) reported a similar result using spin-labeled peptides. We previously reported that changing two Phe to two Ala reduces the binding to 10:1 PC/PG vesicles 2-fold (*25*). Thus, there is good agreement between all the different experimental techniques: replacement of each Phe residue by Ala decreases the membrane partitioning by only 0.2 kcal/mol. The results of Wimley and White (*23*) suggest a Phe residue could contribute 1.3 kcal/mol more hydrophobic membrane binding energy than an Ala residue. Thus, one might naively expect that replacing the five Phe with Ala residues should decrease the binding about $5 \times 1.3 = 6.5$ kcal/mol or about a factor of 10^4 rather than the factor of ∼10 we actually observe.

As we discuss elsewhere in detail (Murray, Arbuzova, McLaughlin, Honig, manuscript in preparation), the effect of replacing Phe by Ala can be understood in terms of the three major energies that contribute to the binding of MARCKS(151−175), which are shown in Figure 5. First, there is a long range Coulomb attraction of the positively charged peptide toward the negatively charged surface of the membrane (triangles); second, there is a short-range Born/desolvation repulsion of the peptide from the low dielectric interface (squares); and third, there is an effective nonpolar attraction due to the insertion of the aromatics residues into the membrane (circles). At equilibrium MARCKS(151−175)

[5] Kim et al. (*25*) previously demonstrated that the fraction of bound MARCKS(151−175) increases steeply as the mole percent of PG in a PC/PG vesicle increases from 5 to 10%; they used a fluorescamine assay to measure the peptide concentration, which required peptide concentrations > 500 nM. We measured peptide binding to vesicles containing a higher, more physiological fraction of acidic lipid, by using radioactively labeled MARCKS(151−175), which requires only 2 nM of peptide. When the binding is strong, low concentrations of peptide are required to ensure that the bound peptide does not change the charge density of the membrane.

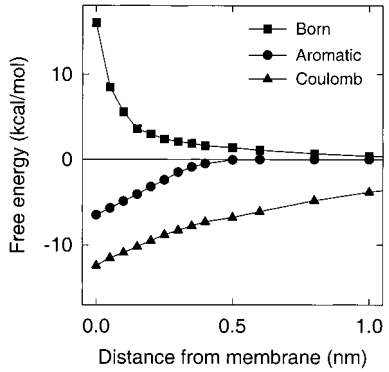

FIGURE 5: Theoretical description of the binding of MARCKS-(151−175) to 5:1 PC/PS membranes. The triangles represent the contribution of the long-range attractive Coulomb forces to the binding energy. The circles represent the contribution of the shorter range hydrophobic forces due to membrane insertion of the five aromatic Phe residues. The squares represent the contribution of the short-range Born/desolvation repulsion forces. The overall binding energy may be calculated from the sum of these three components. The *x*-axis represents the distance between the van der Waals surface of the peptide backbone and the membrane. For Phe-Ala MARCKS(151−175), which lacks the hydrophobic contribution, the minimum in the free energy is shifted to the right; hence the decrease in the Born/desolvation repulsive energy partially compensates for the loss of the aromatic binding term.

is located a distance from the membrane where the sum of the three energies has its minimum. Replacing the five Phe aromatic residues by five Ala residues removes the favorable hydrophobic interactions. At equilibrium Phe-Ala MARCKS-(151−175) is located further away from the surface, at the distance where the sum of the Born/desolvation repulsion and Coulomb attraction has its minimum. The attractive hydrophobic energy that is lost upon replacement of the Phe is partially compensated for by a decrease in the repulsive Born/desolvation energy. (There is also a concomitant, but smaller decrease in the attractive Coulomb energy.)

Effect of PIP_2 on the Membrane Binding of Caveolin(92−101). Figure 6A shows that incorporating 2% of the trivalent (*60*) lipid PIP_2 into PC vesicles increases the binding of caveolin(92−101) 6-fold (incorporating 1% PIP_2 increases the binding 3-fold, data not shown); these data can be compared to those shown in Figure 2B, where incorporating an electrostatically equivalent 6% of the monovalent lipid PS into PC increases the binding of caveolin(92−101) to the same extent. Incorporating 2% PIP_2 into the mixture used to form 2:1 or 5:1 PC/PS vesicles does not increase the membrane binding of caveolin(92−101) significantly (data not shown). Thus caveolin(91−101) interacts with PIP_2, but appears to do so primarily via nonspecific electrostatic interactions with the diffuse double layer potential produced by this acidic lipid.

Effect of PIP_2 on the Membrane Binding of MARCKS-(151−175). Figure 6B illustrates that MARCKS(151−175) binds surprisingly strongly to 99:1 PC/PIP_2 vesicles, $K \cong 10^6\ M^{-1}$. If we assume, probably incorrectly, that the peptide forms a 1:1 complex with PIP_2, the apparent dissociation constant $K_d = 10$ nM or the apparent association constant $K_a = 10^8\ M^{-1}$. In contrast to caveolin(92−101), MARCKS-(151−175) binds much more strongly to 99:1 PC/PIP_2 vesicles than to the electrostatically equivalent 97:3 PC/PS vesicles (Figure 3B). (We observed a similarly large difference between the binding to 98:2 PC/PIP_2 and electrostatically equivalent 94:6 PC/PS vesicles, data not shown.) Clearly the strong binding to PC/PIP_2 vesicles apparent in Figure 6B is not due to a simple electrostatic attraction of the peptide based on the average potential at the surface of the vesicle (magnitude −8 mV from direct ζ-potential measurements), but to a more specific interaction with the multivalent phosphoinositide lipid and/or a redistribution of PIP_2 in the plane of the membrane. A more complete study describing this strong binding of MARCKS effector region peptide to PIP_2 will appear elsewhere (Wang, Arbuzova, Murray, McLaughlin, manuscript in preparation).

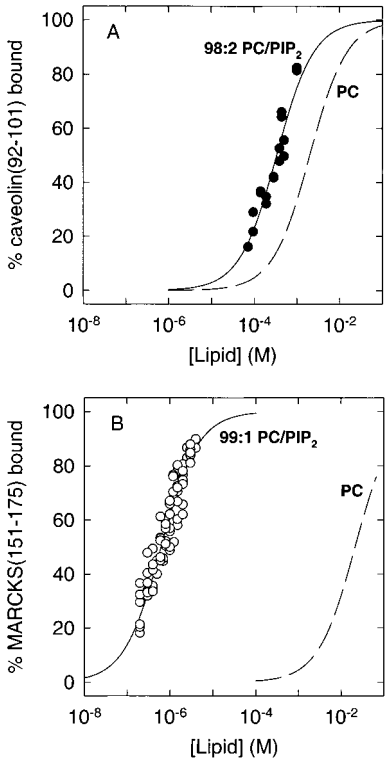

FIGURE 6: Binding of peptides to vesicles containing PIP_2. (A) Binding of caveolin(92−101) ([^{3}H]NEM-labeled peptide III) to 98:2 PC/PIP_2 vesicles. Each filled circle is an average result of two measurements and the solid line is the best fit of eq 1 to the experimental data with the molar partition coefficient, $K = 3 \times 10^3\ M^{-1}$. The dashed line is the best fit to the data obtained with PC vesicles shown in Figure 2A, $K = 5 \times 10^2\ M^{-1}$. (B) Binding of MARCKS(151−175) (2 nM [^{3}H]NEM-labeled peptide VI) to 99:1 PC/PIP_2 vesicles. The solid line is the best fit of eq 1 to the experimental data (open circles) with the molar partition coefficient, $K = 1 \times 10^6\ M^{-1}$. The dashed line is the best fit to the data obtained with PC vesicles shown in Figure 3A, $K = 50\ M^{-1}$. Note that incorporating 1% PIP_2 increases 10^4-fold the binding of the MARCKS(151−175) peptide to the vesicle.

DISCUSSION

We address three topics. First, we discuss briefly the comparison of our experimental results and theoretical predictions, a topic considered in more detail elsewhere (Murray, Arbuzova, McLaughlin, Honig, manuscript in preparation). Second, we summarize the available evidence that MARCKS(151−175) binds strongly to PIP_2. Third, we consider two biological corollaries of the results we obtained with peptides and phospholipid vesicles.

Theoretical Predictions. The theoretical treatment describes adequately two of our main experimental results: (i) addition of negatively charged lipids enhances the binding

of positively charged peptides to membranes (Figures 2 and 3); (ii) replacing Phe residues, which penetrate the membrane interface, with Ala residues, which do not, decreases only slightly the membrane binding of MARCKS(151−175) to PC/PS vesicles (Figure 4).

Figures 2 and 3 show that caveolin(92−101) and MARCKS(151−175) peptides bind to PC vesicles with molar partition coefficients of 500 and 50 M^{-1}, respectively. We observed that incorporating negatively charged lipids into the PC vesicle increases the binding of both peptides; as expected intuitively, the increase is much larger for the more highly charged MARCKS(151−175). A comparison of Figures 2B and 3B shows that incorporating 17% acidic lipid enhances the binding of caveolin(92−101) and MARCKS-(151−175) about 10- and 10^4-fold, respectively. Our theory, which uses atomic models of the peptides and membrane and the nonlinear Poisson−Boltzmann equation, describes adequately the effect of acidic lipid on the binding (Figures 2B and 3B, open circles). Our theory predicts the potential in the immediate vicinity of a highly basic, membrane-bound peptide is positive (*9*, *13*), which helps us understand how MARCKS(151−175) interacts strongly with the multivalent acidic lipid PIP_2.

The experimental results shown in Figure 4 and reported previously (*25*, *59*) suggest that each substitution of Phe by Ala in MARCKS(151−175) decreases the binding free energy to PC/PS membranes by only 0.2 kcal/mol. This is significantly less than the 1.3 kcal/mol decrease in binding free energy to electrically neutral PC membranes observed when Wimley and White (*23*) replaced Phe by Ala in model peptides (with primary sequences WLFLL and WLALL). Consideration of the Born/desolvation repulsion of the charged MARCKS(151−175) from the membrane can account qualitatively for this difference (see Figure 5). The loss of hydrophobic energy when Phe are replaced by Ala in MARCKS(151−175) is partially compensated for by a gain of the Coulomb/Born energy because the peptide shifts away from the membrane to it's new minimum free energy position, where the combination of long-range Coulomb attraction and Born/desolvation repulsion is optimal.

Effect of PIP_2 on the Binding of Peptides to Bilayers. Figure 6 shows that incorporation of 1−2% PIP_2 into PC vesicles produces a very large (10^4-fold) effect on the membrane binding of MARCKS(151−175) but only a small (<10-fold) effect on the binding of caveolin(92−101). The latter result is easy to describe: the effect can be attributed to simple electrostatics because incorporating 6% monovalent PS has same effect as 2% trivalent PIP_2. MARCKS(151−175), valence $z = +13$, clearly interacts much more strongly with PIP_2 than does caveolin(92−101), $z = +3$. We defer a detailed discussion of how MARCKS(151−175) interacts with PIP_2 to a forthcoming manuscript (Wang, Arbuzova, Murray, McLaughlin, manuscript in preparation), but note here that we have observed this strong interaction using three independent techniques: ζ-potential measurements on MLVs, fluorescence measurements with labeled peptide on LUVs, and radioactivity measurements with labeled peptide on sucrose-loaded LUVs (e.g., Figure 6). To extrapolate the result to biological membranes, we would like to know if binding of MARCKS(151−175) to a membrane containing physiological concentrations of PS (e.g., 15−30%) decreases the free concentration or activity of PIP_2 in the membrane. Previously we showed that both MARCKS(151−175) and intact MARCKS protein decrease PLC-induced hydrolysis of PIP_2 on PC/PS/PIP_2 vesicles (*61*). Thus, we predict that the effector region of MARCKS interacts strongly with PIP_2 when the protein binds to the cytoplasmic surface of a plasma membrane, which contains about 1% PIP_2, and a significantly higher (15−30% for a typical mammalian cell membrane) mole fraction of PS.

Biological Implications: Caveolin. Measuring the adsorption of a peptide that corresponds to residues 92−101 of caveolin to PC/PS vesicles produced a minimal estimate of the membrane binding energy of the putative scaffolding region of caveolin. Caveolin(92−101) binds to 2:1 PC/PS vesicles with $K = 3 \times 10^4$ M^{-1} (Figure 2). Peptides corresponding to the full length scaffolding region of caveolin should bind more strongly because they contain additional aromatic residues, but such peptides were not soluble in aqueous solutions (see Materials and Methods). If the caveolin(92−101) results can be extrapolated to the intact protein, a simple calculation using the Boltzmann relation (not shown, see ref *1* for approach) predicts that the scaffolding regions in >99% of the caveolin molecules interact with the bilayer component of the plasma membrane, as shown on the right side of Figure 1A. A more direct test of this prediction requires experiments with caveolin reconstituted into phospholipid vesicles, but we have been unable to reconstitute caveolin-1 with correct orientation into vesicles. Nevertheless, our suggestion that the scaffolding region binds to the membrane through a combination of electrostatic and hydrophobic interactions is consistent with recent studies using deletion mutants of caveolin-1 (*62*).

Biological Implications: MARCKS. MARCKS is not uniformly distributed in the plasma membrane of many cell types: in fibroblasts, it is concentrated in ruffles (*41*); in macrophages, it is concentrated in the nascent phagosomes (*40*). The interactions (presumably protein−protein) that are responsible for these nonuniform lateral distributions are not well understood. There is good evidence that MARCKS binds to the plasma membrane by hydrophobic insertion of its N-terminal myristate into the bilayer and electrostatic interactions of the 13 basic residues in the effector domain with acidic lipids, as shown in Figure 1B. Given our observations that the effector region binds strongly to PIP_2 (e.g., Figure 6), and the high concentration of MARCKS in many cell types (typically 10 μM, comparable to the concentration of PIP_2), we speculate that PIP_2 should be sequestered with MARCKS in these regions of the plasma membrane. This speculation is supported by recent reports showing that PIP_2 is sequestered at high concentrations in membrane ruffles (*63*, *64*).

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REFERENCES

1. Buser, C. A., Sigal, C. T., Resh, M. D., and McLaughlin, S. (1994) *Biochemistry 33*, 13093−13101.
2. Hancock, J. F., Magee, A. I., Childs, J. E., and Marshall, C. J. (1989) *Cell 57*, 1167−1177.

3. Ghomashchi, F., Zhang, X., Liu, L., and Gelb, M. H. (1995) *Biochemistry 34*, 11910−11918.
4. Leventis, R., and Silvius, J. R. (1998) *Biochemistry 37*, 7640−7648.
5. Ben-Tal, N., Honig, B., Peitzsch, R. M., Denisov, G., and McLaughlin, S. (1996) *Biophys. J. 71*, 561−575.
6. Ben-Tal, N., Honig, B., Miller, C., and McLaughlin, S. (1997) *Biophys. J. 73*, 1717−1727.
7. Murray, D., Ben-Tal, N., Honig, B., and McLaughlin, S. (1997) *Structure 5*, 985−989.
8. Murray, D., Hermida-Matsumoto, L., Buser, C. A., Tsang, J., Sigal, C., Ben-Tal, N., Honig, B., Resh, M. D., and McLaughlin, S. (1998) *Biochemistry 37*, 2145−2159.
9. Murray, D., Arbuzova, A., Hangyas-Mihalyne, G., Gambhir, A., Ben-Tal, N., Honig, B., and McLaughlin, S. (1999) *Biophys. J. 77*, 3176−3188.
10. Aderem, A. (1992) *Cell 71*, 713−716.
11. Blackshear, P. J. (1993) *J. Biol. Chem. 268*, 1501−1504.
12. McLaughlin, S., and Aderem, A. (1995) *Trends Biochem. Sci. 20*, 272−276.
13. Arbuzova, A., Murray, D., and McLaughlin, S. (1998) *Biochim. Biophys. Acta 1376*, 369−379.
14. Dell'Acqua, M. L., Faux, M. C., Thorburn, J., Thorburn, A., and Scott, J. D. (1998) *EMBO J. 17*, 2246−2260.
15. Pappa, H., Murray-Rust, J., Dekker, L. V., Parker, P. J., and McDonald, N. Q. (1998) *Structure 6*, 885−894.
16. Sutton, R. B., and Sprang, S. R. (1998) *Structure 6*, 1395−1405.
17. Medkova, M., and Cho, W. (1998) *J. Biol. Chem. 273*, 17544−17552.
18. Chapman, E. R., and Davis, A. F. (1998) *J. Biol. Chem. 273*, 13995−14001.
19. Ball, A., Nielsen, R., Gelb, M. H., and Robinson, B. H. (1999) *Proc. Natl. Acad. Sci. U.S.A. 96*, 6637−6642.
20. Gelb, M. H., Cho, W., and Wilton, D. C. (1999) *Curr. Opin. Struct. Biol. 9*, 428−432.
21. Pratt, K. P., Shen, B. W., Takeshima, K., Davie, E. W., Fujikawa, K., and Stoddard, B. L. (1999) *Nature 402*, 439−442.
22. Macedo-Ribeiro, S., Bode, W., Huber, R., Quinn-Allen, M. A., Kim, S. W., Ortel, T. L., Bourenkov, G. P., Bartunik, H. D., Stubbs, M. T., Kane, W. H., and Fuentes-Prior, P. (1999) *Nature 402*, 434−439.
23. Wimley, W. C., and White, S. H. (1996) *Nat. Struct. Biol. 3*, 842−848.
24. Qin, Z., and Cafiso, D. S. (1996) *Biochemistry 35*, 2917−2925.
25. Kim, J., Blackshear, P. J., Johnson, J. D., and McLaughlin, S. (1994) *Biophys. J. 67*, 227−237.
26. Ladokhin, A. S., and White, S. H. (1999) *J. Mol. Biol. 285*, 1363−1369.
27. Wieprecht, T., Apostolov, O., Beyermann, M., and Seelig, J. (1999) *J. Mol. Biol. 294*, 785−794.
28. White, S. H., and Wimley, W. C. (1999) *Annu. Rev. Biophys. Biomol. Structure 28*, 319−365.
29. Rothberg, K. G., Heuser, J. E., Donzell, W. C., Ying, Y.-S., Glenney, J. R., and Anderson, R. G. W. (1992) *Cell 68*, 673−682.
30. Anderson, R. G. W. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 10909−10913.
31. Simons, K., and Ikonen, E. (1997) *Nature 387*, 569−572.
32. Parton, R. G. (1996) *Curr. Opin. Cell Biol. 8*, 542−548.
33. Okamoto, T., Schlegel, A., Scherer, P. E., and Lisanti, M. P. (1998) *J. Biol. Chem. 273*, 5410−5422.
34. Dupree, P., Parton, R. G., Raposo, G., Kurzchalia, T. V., and Simons, K. (1993) *EMBO J. 12*, 1597−1605.
35. Dietzen, D. J., Hastings, W. R., and Lublin, D. M. (1995) *J. Biol. Chem. 270*, 6838−6842.
36. Monier, S., Parton, R. G., Vogel, F., Behlke, J., Henske, A., and Kurzchalia, T. V. (1995) *Mol. Biol. Cell 6*, 911−927.
37. Peitzsch, R. M., and McLaughlin, S. (1993) *Biochemistry 32*, 10436−10443.
38. Silvius, J. R., and l'Heureux, F. (1994) *Biochemistry 33*, 3014−3022.
39. Stumpo, D. J., Bock, C. B., Tuttle, J. S., and Blackshear, P. J. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 944−948.
40. Rosen, A., Keenan, K. F., Thelen, M., Nairn, A. C., and Aderem, A. (1990) *J. Exp. Med. 172*, 1211−1215.
41. Myat, M. M., Anderson, S., Allen, L. H., and Aderem, A. (1997) *Curr. Biol. 7*, 611−614.
42. Morris, A. J., Rudge, S. A., Mahlum, C. E., and Jenco, J. M. (1995) *Mol. Pharm. 48*, 532−539.
43. Arbuzova, A., Wang, J., Murray, D., Jacob, J., Cafiso, D. S., and McLaughlin, S. (1997) *J. Biol. Chem. 272*, 27167−27177.
44. McIlroy, B. K., Walters, J. D., and Johnson, J. D. (1991) *Anal. Biochem. 195*, 148−152.
45. Buser, C. A., and McLaughlin, S. (1998) *Methods Mol. Biol. 84*, 267−281.
46. Tamm, L. K. (1991) *Biochim. Biophys. Acta 1071*, 123−148.
47. Ben-Shaul, A., Ben-Tal, N., and Honig, B. (1996) *Biophys. J. 71*, 130−137.
48. Huang, C., Hepler, J. R., Chen, L. T., Gilman, A. G., Anderson, R. G. W., and Mumby, S. M. (1997) *Mol. Biol. Cell 8*, 2365−2378.
49. Prendergast, F. G., Meyer, M., Carlson, G. L., Iida, S., and Potter, J. D. (1983) *J. Biol. Chem. 258*, 7541−7544.
50. Boguslavsky, V., Rebecchi, M., Morris, A. J., Jhon, D. Y., Rhee, S. G., and McLaughlin, S. (1994) *Biochemistry 33*, 3032−3037.
51. Peitzsch, R. M., Eisenberg, M., Sharp, K. A., and McLaughlin, S. (1995) *Biophys. J. 68*, 729−738.
52. Brooks, B. R., Bruccoleri, R. E., Olafson, B. D., States, D. J., Swaminathan, S., and Karplus, M. (1983) *J. Comput. Chem. 4*, 187−217.
53. Honig, B. H., and Nicholls, A. (1995) *Science 268*, 1144−1149.
54. Demel, R. A., Geurts van Kessel, W. S., Zwaal, R. F., Roelofsen, B., and van Deenen, L. L. (1975) *Biochim. Biophys. Acta 406*, 97−107.
55. Blume, A. (1979) *Biochim. Biophys. Acta 557*, 32−44.
56. Seelig, A. (1987) *Biochim. Biophys. Acta 899*, 196−204.
57. Seelig, A. (1992) *Biochemistry 31*, 2897−2904.
58. McLaughlin, S. (1989) *Annu. Rev. Biophys. Biophys. Chem. 18*, 113−136.
59. Victor, K., Jacob, J., and Cafiso, D. S. (1999) *Biochemistry 38*, 12527−12536.
60. Toner, M., Vaio, G., McLaughlin, A., and McLaughlin, S. (1988) *Biochemistry 27*, 7435−7443.
61. Glaser, M., Wanaski, S., Buser, C. A., Boguslavsky, V., Rashidzada, W., Morris, A., Rebecchi, M., Scarlata, S. F., Runnels, L. W., Prestwich, G. D., Chen, J., Aderem, A., Ahn, J., and McLaughlin, S. (1996) *J. Biol. Chem. 271*, 26187−26193.
62. Schlegel, A., Schwab, R. B., Scherer, P. E., and Lisanti, M. P. (1999) *J. Biol. Chem. 274*, 22660−22667.
63. Honda, A., Nogami, M., Yokozeki, T., Yamazaki, M., Nakamura, H., Watanabe, H., Kawamoto, K., Nakayama, K., Morris, A. J., Frohman, M. A., and Kanaho, Y. (1999) *Cell 99*, 521−532.
64. Tall, E., Bitter, I., Pentyala, S. N., Spector, I., and Rebecchi, M. J. (2000) *Curr. Biol.* (in press).

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