# A Limiting Law for the Electrostatics of the Binding of Polypeptides to Phospholipid Bilayers†

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ABSTRACT: Two cysteine-substituted variants of a peptide derived from the first 25 residues of the presequence for subunit IV of cytochrome *c* oxidase were synthesized and modified with a nitroxide spin label. The equilibrium partitioning of these spin-labeled peptides into negatively charged phospholipid vesicles was studied with electron paramagnetic resonance (EPR) to investigate the binding energetics. It is found that the binding equilibrium constant is an explicit function of a unique variable, the membrane surface potential $\Psi$ in the Gouy−Chapman−Stern theory. Moreover, at low $\Psi(<0.5RT/F)$ the binding equilibrium is described by the linear dependence of the transfer free energy $\Delta G^{el}$ on $\Psi$ with a slope equal to the full formal charge of the peptides. However, the partition constant levels off at higher $\Psi$, suggesting departure from the ideal limiting behavior.

Direct adsorption or insertion of polypeptides into membranes has wide implications in important biological processes such as ligand binding (Sargent & Schwyzer, 1986), protein translocation (Wickner, 1988), cell entry of toxins (Cramer et al., 1990), and protein kinase C function (Newton, 1993). For example, the leader sequences on the precursors of the mitochondrial proteins which are essential for the exclusive protein transport to mitochondria are capable of inserting into phospholipid bilayers (von Heijne, 1986; Roise, 1992), suggesting that insertion into the bilayer is perhaps a step prior to the recognition by receptors or translocators. The translocation domain of diphtheria toxin (Choe et al., 1992) and the channel-forming domains of bacterial toxin colicins from *Escherichia coli* insert into bilayers to perform their functions in the membrane (Cramer, 1990).

The interaction of a polypeptide with a bilayer is governed by complex energetics. However, the fact that most biomembranes contain negatively charged lipids (typically ~20%) and the notion that the charged membrane surface plays a role in membrane−peptide interactions make us conveniently divide energetics into two terms: (1) electrostatic contributions and (2) nonelectrostatic contributions. The nonelectrostatic contributions may arise primarily from the hydrophobic effect upon burial of the nonpolar residues on the polypeptide in the bilayer. On the other hand, the electrostatic terms come from interactions between the charged residues on the polypeptide and the negatively charged lipid headgroups in the membrane.

For the binding of a charged peptide to the membrane, the electrostatic interactions contribute to the transfer free energy. Supposing that the direct charge-pair interactions between positive charges on polypeptide and the negative charges on lipid, which would be similar to the salt-bridge interactions in proteins, are not significant, the electrostatic contribution to the free energy of transfer $\Delta G^{el}$ is approximated by

$$\Delta G^{el} = \Psi F z_p \tag{1}$$

where $\Psi$ is the electrostatic surface potential, $z_p$ is the net charge of the peptide, and $F$ is Faraday's constant. The surface potential $\Psi$ is often described by Gouy−Chapman−Stern theory, which is based on the mean-field smeared-charge approximation (McLaughlin, 1989).

Small charged peptides which bind reversibly to membranes have been widely used for the investigation of the energetics of membrane−polypeptide interactions. With these small peptides the electrostatic and hydrophobic components can be easily altered to probe specific interactions and the resultant change in the binding equilibrium is readily converted to the corresponding changes in transfer free energy.

In most previous studies with small peptides, however, it has been found that $\Delta G^{el}$ is much smaller than anticipated. Such small $\Delta G^{el}$ has been, in most cases, accounted for by arbitrarily introducing an effective $z_p$ ranging from $^1/_8$ to $^1/_2$ the full charge on the peptides (Beschiaschvilli & Seelig, 1990a,b; Seelig et al., 1993; Stankowski & Schwarz, 1990; Kim et al., 1991; Moisior & McLaughlin, 1992; Seelig & McDonald, 1989; Roise, 1991).

Here we report EPR studies of the binding equilibria of spin-labeled peptides derived from the first 25 residues of the presequence for subunit IV of cytochrome *c* oxidase to the phospholipid bilayer. This peptide contains five basic residues but no acidic residues except for one carboxyl group at the C-terminal end, and it has been previously inferred from immersion depth measurements that the charges are likely located right at the surface (Yu et al., 1994).

In this work it is found that the binding equilibrium constant is an explicit function of only one variable, the surface potential $\Psi$, and variations in ionic strength and surface charge density are all implicit in $\Psi$ in the Gouy−Chapman−Stern equation. The binding equilibrium is described by the linear dependence of $\Delta G^{el}$ on $\Psi$ in the region of low $\Psi$ ($<0.5RT/F$) and by the gradual saturation

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of $\Delta G^{el}$ as $\Psi$ increases. For the limiting case ($\Psi < 0.5RT/F$), the binding equilibrium is well described by a simple partition model where the surface potential is conveniently obtained from Gouy−Chapman theory, and the electrostatic component of the free energy of transfer is obtained from eq 1 using the full charge on the peptide.

## MATERIALS AND METHODS

*Materials.* 1-Palmitoyl-2-oleoylphosphatidylcholine (POPC),[1] 1-palmitoyl-2-oleoylphosphatidylglycerol (POPG), and cardiolipin were obtained from Avanti Polar Lipids. 4-Carboxy-TEMPO and *S*-(1-oxy-2,2,5,5-tetramethylpyrroline-3-methyl)methanethiosulfonate (MTSSL) were obtained from Aldrich Chemical Co. and Reanal (Hungary), respectively.

*Peptide Synthesis and Spin Labeling.* Peptides were synthesized by solid-phase peptide synthesis using (9-fluorenylmethoxy)carbonyl (Fmoc) amino acids and a Wang resin and purified using a Vydac $C_{18}$ HPLC column as described previously (Yu et al., 1994). The amino acid sequences for the two peptides used in this study were $NH_2$-MLSLRQCIRFFKPATRTLSSSRYLL-COOH (pcox7) and $NH_2$-MLSLCQSIRFFKPATRTLSSSRYLL-COOH (pcox-5). The purities and identities of peptides were confirmed by electrospray ionization mass spectrometry. The peptides were specifically labeled at the unique cysteine in 10 mM MOPS (pH 7.0) for 1 h at room temperature using about a 2-fold molar excess of MTSSL introduced by adding small amounts of a concentrated stock solution in acetonitrile. The spin-labeled peptides were purified as single peaks on the HPLC column using a 20−45% water/acetonitrile gradient containing 0.1% trifluoroacetic acid. Under these conditions the modified and unmodified peptides gave completely separated peaks, and fractions containing the spin-labeled peptide were collected, lyophilized, and stored dry at −20 °C. Electrospray ionization mass spectrometry confirmed the masses of the spin-labeled peptides and revealed no impurities. Immediately before experiments the spin-labeled peptides were dissolved to the desired concentration in buffer A (0.2 mM EDTA, 5.00 mM $NH_4OAc$, and 10.0 mM MOPS adjusted to pH 7.2 with NaOH). As even very small amounts of free spin label would interfere with the measurements, the exposure of solutions of spin-labeled peptides to room temperature was minimized (<2−4 h). No formation of free spin label was, however, detected when spin-labeled peptides were left at room temperature for 12 h or at 4 °C for 1 week.

*Vesicle Preparation.* Appropriate amounts of POPC, POPG, and cardiolipin were dissolved in chloroform and mixed to obtain the desired ratio of negatively charged lipid. The solvent was subsequently removed in a stream of nitrogen, and the dry lipid was placed in high vacuum for several hours to remove trace amounts of chloroform. The lipids were resuspended at 20 °C in buffer A to yield a total lipid concentration of 5.0−65 mM. Vesicles were prepared by extrusion through 100-nm pore size polycarbonate membranes using a LipsoFast extruder (Avestine, Canada) after five freeze−thaw cycles.

*Sample Preparation.* The effects of surface potential on the binding of peptides to bilayers were studied by varying both the amount of the negatively charged lipid and the salt concentration. The salt concentration of the sample was adjusted by adding buffer A containing various amounts (80−780 mM) of the membrane-permeable electrolyte ammonium acetate (Sundberg & Hubbell, 1986), typically in a 2:1 ratio. The spin-labeled peptide was introduced to the vesicle solutions and the resulting solutions were thoroughly mixed and allowed to stand for at least $^1/_2$ h at room temperature before EPR measurements. The EPR spectra are collected under equilibrium conditions since the binding is fast. In fact, EPR measurements at 10 min, 30 min, and after storage at 4 °C for 1 week following sample preparation yield partition constants that are identical within experimental error. Peptide concentrations in samples were determined by comparing the double integration of the first-derivative EPR spectra with that of TEMPO standards.

*Electron Paramagnetic Resonance Measurements.* EPR spectra were measured using a Bruker ESP300 EPR spectrometer (Bruker, Germany) equipped with a low-noise amplifier (Mitech) and a loop-gap resonator (Medical Advances). All experiments were performed at 20 °C.

*A Model for Partition Equilibria.* Previous studies have found that the binding of small peptides to lipid bilayers can be described using a simple partition model:

$$X_b = K_{app} P_f \tag{2}$$

where $X_b$ is the mole fraction of peptide in the lipid bilayer, $K_{app}$ is the apparent partition constant in units of liter per mole, and $P_f$ is the bulk peptide concentration in the aqueous phase. When there exists an electrostatic potential on the membrane surface due to the presence of negatively charged lipids, $K_{app}$ for a peptide with the valence $z_p$ is given by the product of $K_0$, the partition constant in the absence of the surface potential, and a Boltzmann factor accounting for the electrostatic accumulation of peptide in the surface layer:

$$K_{app} = K_0 \exp(-z_p F\Psi/RT) \tag{3}$$

where $\Psi$ is the surface potential and $F$ is Faraday's constant.

From EPR spectra we obtained the amounts of bound and free peptide ($P_b$ and $P_f$) (see Results section), allowing the estimation of the apparent partition constants:

$$K_{app} = (P_b/P_f)/(^5/_9\, C_L) = X_b/P_f \tag{4}$$

where $C_L$ is the total lipid concentration. The peptide does not cross the bilayer in the absence of a transmembrane potential (Roise, 1992), and the bound peptide stays in the outer layer of the vesicles. Thus, $X_b$ is the molar ratio of the bound peptide to the lipid in the outer leaflet of the vesicles. The outer leaflet contains $^5/_9$ of the total lipid in vesicles whose diameter is 100 nm.

The membrane surface potential was calculated using Gouy−Chapman−Stern theory, which is an approximate solution for the Poisson−Boltzmann equation, and it is based on the smeared-surface charge approximation (McLaughlin, 1989). Since the peptide is positively charged, the binding of the peptide to the bilayer will lead to the reduction of the

[1] Abbreviations: EPR, electron paramagnetic resonance; HPLC, high-performance liquid chromatography; MOPS, 3-(*N*-morpholino)-propanesulfonic acid; MTSSL, *S*-(1-oxy-2,2,5,5-tetramethylpyrroline-3-methyl)methanethiosulfonate spin label; NMR, nuclear magnetic resonance; PC, phosphatidylcholine; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; POPG, 1-palmitoyl-2-oleoylphosphatidylglycerol; TEMPO, 2,2,6,6-tetramethyl-1-piperidinyloxy.

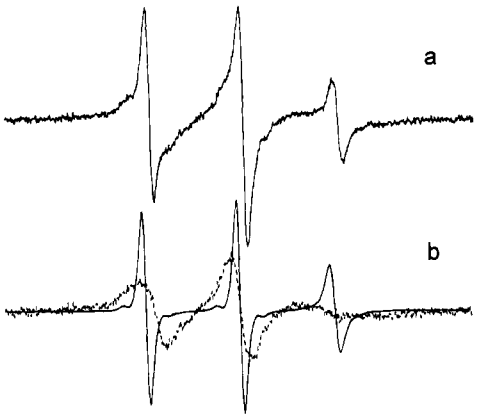


FIGURE 1: (a) First-derivative EPR spectrum for pcox7 in the presence of POPC vesicles containing 3 mol % POPG in 10 mM MOPS, pH 7.2. Scan width is 80 G. The total salt concentration was 111 mM (11 mM $Na^+$ and 100 mM $NH_4^+$). The peptide concentration was 25 $\mu$M and the final lipid concentration was 16.5 mM. (b) Sharp (solid line) and broad (dotted line) spectral components separated from each other by spectral subtraction. The spin concentration corresponding to the sharp component was 6 $\mu$M, and the spin concentration corresponding to the broad component was 19.4 $\mu$M.

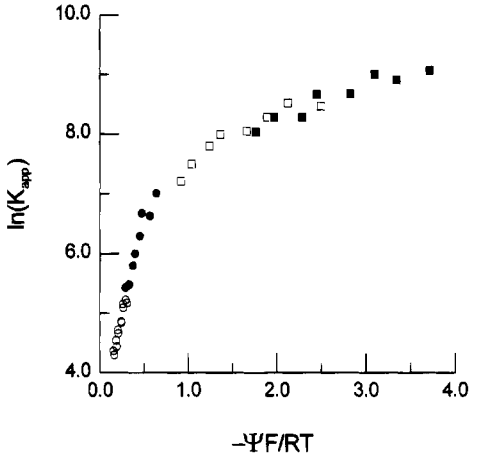


FIGURE 2: Effect of surface potential on the binding equilibrium of pcox7 to POPC vesicles containing 2.45% POPG (○), 5% POPG (●), 7.5% cardiolipin (□), and 30% POPG (■). For each lipid composition the salt concentration was varied in the range of 20–200 mM by adding the permeable electrolyte ammonium acetate. The final peptide concentration was 25 $\mu$M and total lipid concentration was 5 mM, except for 2.45% POPG (○), where total peptide and lipid concentrations were 50 $\mu$M and 32.5 mM, respectively.

membrane surface charge density from that for a given mole fraction of negatively charged lipid ($X_{neg}$) unless the charged residues are completely deprotonated on membrane binding. The charge density will then be given by

$$\sigma_0 = (-X_{neg} + X_b z_p')/[A_L(1 + (A_p/A_L)X_b)] \quad (5)$$

where $A_L$ and $A_p$ are the surface areas per lipid molecule and per peptide, respectively, and $z_p'$ is the net charge on the bound peptide. The surface charge density was in the units of charges per square angstrom, and $A_L$ = 70 Å$^{-2}$ (Castle & Hubbell, 1976) was used. We used 560/70 for the ratio of the surface areas for the peptide and lipid molecules ($A_p/A_L$), which was estimated from monolayer studies (Tamm, 1986). Furthermore, the specific binding of cations such as $Na^+$ and $NH_4^+$ to the charged phospholipid headgroups, which effectively lowers the surface charge density (Eisenberg et al., 1981), needs to be taken into account. Since the binding of cations is dependent upon the surface potential Ψ, Ψ was calculated by iterating the Gouy equation (valid for symmetric electrolytes and small Ψ)

$$\Psi = 2\,(RT/F)\,\text{asinh}\,(136\sigma/\sqrt{c}) \quad (6)$$

together with a Langmuir adsorption isotherm for ion binding

$$\sigma = \sigma_0/\{1 + (K_1[Na^+] + K_2[NH_4^+])\exp(-F\Psi/RT)\} \quad (7)$$

until Ψ converged. In eq 7, $K_1$ and $K_2$ are the association constants for 1:1 binding of $Na^+$ and $NH_4^+$ to POPG, respectively, and we used $K_1 = 0.6$ and $K_2 = 0.17$ (Eisenberg et al., 1981).

## RESULTS

Figure 1a shows the EPR spectrum for pcox7 in the presence of phospholipid vesicles. The spectrum contains two spectral components, a sharp fast-motional component from the spin-labeled peptide species in the aqueous phase and a broad relatively slow-motional component from the peptide species bound to the bilayer. The two spectral components were separated from each other using the spectral subtraction method and are shown overlaid in Figure 1b. The fraction bound to the bilayer was determined from the ratio of the concentrations of these two components. The absolute spin concentration was obtained in reference to the 100 $\mu$M TEMPO standard.

In Figure 2 the natural logarithms of the apparent partition constants $K_{app}$ for the binding of pcox7 to vesicles are plotted as a function of the surface potential Ψ. Each set of points (denoted by the same symbol) was obtained by varying the salt concentration for a given surface charge density while keeping total peptide and lipid concentrations constant. The surface potential was calculated using eqs 5–7 with $z_p'$ = 5. In Figure 2 it is interesting to find that all data points fall into one curve when ln $K_{app}$ is plotted with respect to the surface potential Ψ. This is also true for two very different kinds of negatively charged lipids used, POPG and cardiolipin. Cardiolipin is a dimeric phospholipid and carries two negative charges. On the other hand, POPG is a monomeric phospholipid and carries one negative charge. Despite such differences in molecular properties, data for vesicles containing 7.5% cardiolipin fall on a curve made of data for various POPG/POPC vesicles. In Figure 2 the molar lipid-to-peptide ratio was 200 for all samples except for vesicles containing 2.45% POPG, for which it was 650. To further check whether the universal behavior of $K_{app}$ vs Ψ is dependent on the lipid-to-peptide ratio or not, we measured $K_{app}$ at an additional five different ratios in the range of 200–1000 for vesicles containing 2% POPG (40 mM $NH_4OAc$ in buffer A). All the data fall on the smooth curve in Figure 2 (data not shown), indicating that at lipid-to-peptide ratios above 200 this behavior is independent of the lipid-to-peptide ratio. The results in Figure 2 suggest that it is the surface potential rather than the amount or identity of the negatively charged lipid that determines the electrostatic contribution to the binding.

Figure 2 reveals that ln $K_{app}$ increases linearly with surface potential (Ψ) at low potential (<12 mV) but levels off as the potential increases. The slope is approximately 6 when

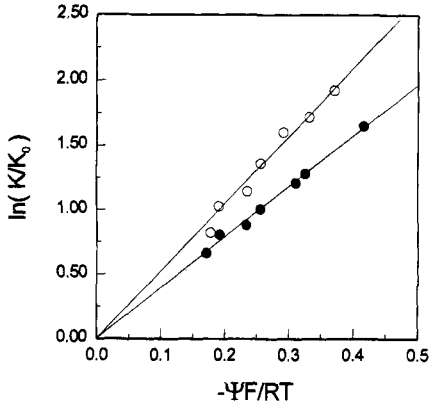


FIGURE 3: Comparison of the binding equilibria of pcox5 (●) and pcox7 (○) to POPC vesicles containing 3.0% POPG. The total lipid concntration was 16.4 mM in 10 mM MOPS, pH 7.2. Final peptide concentrations were 24−28 $\mu$M for pcox7 and 26−29 $\mu$M for pcox5. The salt concentration was varied using the permeable electrolyte ammonium acetate. The solid lines represent the best fits to the relationship $\ln K_{app} - \ln K_0 = -z_p F\Psi/RT$, with $z_p = 4$ for pcox5 and with $z_p = 5.4$ for pcox7. The nonelectrostatic partition constant ($K_0$) was similar for both peptides and approximately 40 $M^{-1}$.

$\Psi$ is less than 12 mV, which is close to the formal charge (+5) of the peptide at neutral pH. However, the slope is even less than 1 for POPC vesicles containing 30 mol % POPG.

There are four arginines and one lysine in pcox7. Assuming that $pK_a = 10$ and 12 for Lys and Arg, respectively, the free energy cost for deprotonation at pH 7.0 would be approximately +4 kcal/mol for each Lys and ~ +6.5 kcal/mol for each Arg. This relatively high free energy cost will disfavor deprotonation of these charged residues upon binding. However, the α-amino group at the N-terminus is more prone to deprotonation upon membrane binding. Therefore, one might expect a change in $K_{app}$ on shifting pH to well below the $pK_a$ value of the terminal amino group. However, $K_{app}$ values of the pcox presequence determined at two pH values, 5.0 and 8.1 (4% POPG, 160 mM salt), were nearly the same, suggesting that in this pH range membrane binding does not result in a change in the protonation state of the α-amino group. Thus, it is likely that the net charge of the peptide stays the same upon binding to the bilayer.

Figure 2 reveals that at low surface potentials the electrostatic effect is well described by eq 1 with the full formal peptide charge. To further investigate the limiting behavior at low surface potentials, $K_{app}$ values for the binding of another spin-labeled variant of the presequence, pcox5, to vesicles containing 3% POPG were compared to those of pcox7 under the same conditions. For pcox5 the spin-labeled cysteine residue replaces Arg at position 5, whereas for pcox7 it replaces a serine at position 7. Thus, pcox5 carries an overall charge of 4, which is one less than that of pcox7. In Figure 3 ln $K_{app}$ values for pcox7 and pcox5 are plotted with respect to the surface potential $\Psi$. The best fits to eq 1 yielded $z_p = 5.4$ for pcox7 and $z_p = 4.0$ for pcox5. These results make it clear that the electrostatic effect on the peptide binding to the bilayer is well accounted for by a simple partition model in eq 1 with the full peptide charge at low surface potentials.

In the calculation of the surface potential $\Psi$, $z_p'$ enters into eq 5. This accounts for the reduction of the surface charge density due to the positive charges from the bound peptide on the bilayer surface. To assess the effect of $z_p'$ in eq 5 on the final results, we also evaluated the valences of pcox7 and pcox5 on the assumption of $z_p' = 0$ in eq 5, which is equivalent to the case of complete neutralization of charges in the peptide on membane binding. From this analysis we obtained $z_p = 2.4$ for pcox5 and $z_p = 3.1$ for pcox7. On increasing $z_p'$ in eq 5, the differences between $z_p'$ in eq 5 and $z_p$ from the slope of ln $K_{app}$ vs $\Psi$ reach their minima near $z_p \sim 4$ for pcox5 and near $z_p \sim 5$ for pcox7, respectively, indicative of the self-consistency in the model.

## DISCUSSION

It is shown that the equilibrium partition constant $K_{app}$ for the binding of the basic peptide derived from the presequence of the precursor of COX IV to the bilayer is an explicit function of a unique variable, the membrane surface potential $\Psi$ (Figure 2). On the other hand, variations in other experimental parameters such as ionic strength and surface charge density are implicitly accounted for in the Gouy−Chapman−Stern theory (eqs 5 and 6). Such a simple description of the electrostatic effect is valid regardless of the identity of the negatively charged phospholipids used (Figure 2). This strongly suggests that the electrostatic effect is best described by the simple partition model of eq 1. According to eq 1 the main effect of electrostatics on the membrane binding is the decrease of the free energy of transfer from the bulk phase to the surface of the bilayer. In particular, for the limiting case where the surface potential is less than ~12 mV ($0.5RT/F$), ln $K_{app}$, or equivalently $-\Delta G/RT$, is linearly dependent upon the surface potential with a slope corresponding to the formal charge of the peptide. However, it levels off on going to higher surface potentials. A set of binding data for vesicles containing 15 mol % POPG in Figure 2 yields the slope corresponding to $z_p = 2.5$ and a set of data for 30 mol % POPG yields a best-fitting $z_p$ that is less than 1.

One of the primary sources for the deviation from the simple binding model in eq 1 at high potentials may be the large Boltzmann factors associated with the free energy of transfer. Since the peptide is pentavalent, the Boltzmann factors are 150 for $\Psi = 1.0RT/F$ (25 mV) and 22 000 for $\Psi = 2.0RT/F$ (50 mV). Thus, when the average peptide concentration in solution is 25 $\mu$M, the peptide concentrations in the surface layer would be as high as 4 mM for $\Psi = 25$ mV and as high as 0.2 M for $\Psi = 50$ mV if the Boltzmann law is obeyed. Such a high peptide concentration in the surface layer has several undesirable consequences. There would be a strong screening effect due to the high concentration of the pentavalent ion and we expect that this effect is strongly nonideal and nonlinear. Furthermore, the bulk concentration of the peptide would be overestimated, since we approximated the bulk concentration using the average concentration. The high accumulation of the peptide in the surface layer would make the bulk concentration less than the average concentration. It is also conceivable that the saturation stems from repulsive interactions between membrane-bound peptide molecules, in analogy to the dielectric saturation observed for small hydrophobic ions in membranes (Andersen & Fuches, 1975; Flewelling & Hubbell, 1986a,b).

Previously, attempts have been made to take into account the screening effect by charged peptides in analysis of the

membrane binding data for fluorescently labeled pcox presequences (Frey & Tamm, 1990; Tamm, 1991; Swanson & Roise, 1992), melittin (Stankowski & Schwarz, 1990; Beschiaschvili & Seelig, 1990a), substance P (Seelig & McDonald, 1989), cyclic somatostatin analogs (Beschiaschvili & Seelig, 1990b; Seelig et al., 1993), and cecrobin (Mchaourab et al., 1994). All these studies found that the effective charges are smaller than the full charges on the peptides. Obviously, for the screening of the membrane surface potential the effective charge of the peptide is expected to be dependent on the ionic strength and smaller than the formal charge due to the finite size of the peptide (Kim et al., 1991).

Our analysis is based on the assumption that the charges on the peptide are located right at the surface of the bilayer when bound to membranes. Our previous study of the topology of the bound pcox presequence found that the peptide backbone is buried at a depth of about 7 Å from the surface of the bilayer, or just above the boundary of the acyl chain region and the head group region and oriented parallel to the surface of the bilayer. The side chains of lysine and arginine are likely extended so that the charges are located within 1−2 Å from the surface (Yu et al., 1994). As we observed no pH dependence for the binding equilibrium, the net charge on the peptide likely remains unchanged upon membrane binding.

Now, what determines the intrinsic binding of the peptide to the bilayer whose partition constant is given by $K_0$ in eq 3? According to our topological model of the peptide in the membrane−bound state, the hydrophobic side chains are solvated by the hydrophobic acyl chains of phospholipid molecules. Thus, it is most likely that the main driving force for the intrinsic membrane binding, which is represented by $-RT \ln K_0$, is the hydrophobic effect.

The electrostatic contribution to the binding of charged biomolecules to membranes (and to receptors in membranes) can easily change apparent binding constants by several orders of magnitude. As previously mentioned, the analysis of the partition equiribrium of a peptide into the bilayer can be very complicated under the conditions in which the self-screening effect of the highly charged peptide becomes nonnegligible. When binding is measured in model systems using near-physiological lipid composition (10−30% negatively charged lipid), it is problematic to accurately account for the electrostatic contribution, since self-screening effects can produce misleading results. In the present work we have investigated the partition equilibrium in the limit of low surface potentials where the effect of the self-screening can be neglected. Under these limiting conditions we have found that the electrostatic effect on the equilibrium partitioning of the peptide into the bilayer is best described by a simple partition model (eq 1) with the full charge of the peptide.

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## REFERENCES

Andersen, O. S., & Fuchs, M. (1975) *Biophys. J. 15*, 795−830.
Beschiaschvili, G., & Seelig, J. (1990a) *Biochemistry 29*, 52−58.
Beschiaschvili, G., & Seelig, J. (1990b) *Biochemistry 29*, 10995−11000.
Castle, J. D., & Hubbell, W. L. (1976) *Biochemistry 15*, 4818−4831.
Choe, S., Bennett, M. J., Fujii, G., Curmi, P. M., Kantardjieff, K. A., Collier, R. J., & Eisenberg, D. (1990) *Nature 357*, 216−222.
Cramer, W. A., Cohen, F. S., Merrill, A. R., & Song, H. Y., (1990) *Mol. Microbiol. 4*, 519−526.
Eisenberg, M., Gresalfi, T., Riccio, T., & McLaughlin, S. (1979) *Biochemistry 18*, 5213−5223.
Flewelling, R. F., & Hubbell, W. L. (1986) *Biophys. J. 49*, 531−540.
Flewelling, R. F., & Hubbell, W. L. (1986) *Biophys. J. 49*, 541−552.
Frey, S., & Tamm, L. K. (1990) *Biochem. J. 272*, 713−719.
Kim, J., Mosior, M., Chung, L. A., Wu, H., & McLaughlin, S. (1991) *Biophys. J. 60*, 135−148.
Maduke, M., & Roise, D. (1992) *Science 260*, 364−367.
Mchaourab, H. S., Hyde, J. S., & Feix, J. B. (1994) *Biochemistry 33*, 6691−6699.
McLaughlin, S. (1989) *Annu. Rev. Biophys. Biophys. Chem. 18*, 113−136.
Mosior, M., & McLaughlin, S. (1992) *Biochemistry 31*, 1767−1773.
Newton, A. C. (1993) *Annu. Rev. Biophys. Biophys. Chem. 22*, 1−25.
Roise, D. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 608−612.
Sargent, D. F., & Schwyzer, R. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 5774−5778.
Seelig, A., & McDonald, P. M. (1989) *Biochemistry 28*, 2490−2496.
Seelig, J., Nebel, S., Ganz, P., & Bruns, C. (1993) *Biochemistry 32*, 9714−9721.
Stankowski, S., & Schwarz, G. (1990) *Biochim. Biophys. Acta 1025*, 164−172.
Sundberg, S., & Hubbell, W. L. (1986) *Biophys. J. 49*, 553−562.
Swanson, S. T., & Roise, D. (1992) *Biochemistry 31*, 5746−5751.
Tamm, L. K. (1986) *Biochemistry 25*, 7470−7476.
Tamm, L. K. (1991) *Biochim. Biophys. Acta 1071*, 121−148.
von Heijne, G. (1986) *EMBO J. 5*, 1335−1342.
Wickner, W. (1988) *Biochemistry 27*, 1081−1086.
Yu, Y. G., Thorgeirsson, T. E., & Shin, Y.-K. (1994) *Biochemistry 33*, 14221−14226.

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