An Experiment-Based Algorithm for Predicting the Partitioning of Unfolded Peptides into Phosphatidylcholine Bilayer Interfaces†

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ABSTRACT: Knowing the partitioning free energy of unfolded polypeptides into membrane interfaces is necessary for understanding membrane protein stability and for designing antimicrobial and other peptides. Experiment-based whole-residue free-energy (hydropathy) scales for amino acids in unfolded peptides, derived from the partitioning of host−guest pentapeptides (Ac-WLXLL) into the interfaces of phosphatidylcholine bilayers and into *n*-octanol, have been determined by W. C. Wimley, S. H. White, and colleagues [(1996) *Nat. Struc. Biol. 3*, 842; Wimley, W. C. et al. (1996) *Biochemistry 35*, 5109]. These scales offer the possibility of computing absolute partitioning free energies of unfolded peptides given only their amino acid sequences. However, the scales are incomplete, because partitioning free energies of N- and C-terminal groups are missing. To complete the scales, we have measured the pH-dependent partitioning of the host−guest pentapeptide variants AcWL-X-LL-NH_2 and WL-X-LL-NH_2 (X = G or W) into palmitoyloleoylphosphatidylcholine (POPC) bilayer interfaces and *n*-octanol. These measurements, in combination with the earlier ones, lead to hydrophobicity scale values for protonation, deprotonation, or acetylation of the N terminus and protonation, deprotonation, or amidation of the C terminus. A surprising finding is that a charged N terminus has a much smaller effect on bilayer partitioning than a charged C terminus. We present a simple algorithm for computing the absolute partitioning free energies of unfolded peptides into the phosphatidylcholine bilayer interface.

Reliable predictions of the free energy of polypeptide partitioning into membranes are crucial for understanding and predicting the folding of membrane-active peptides and proteins and especially for the rational design of antimicrobial peptides. The fundamental ingredient for accurate predictions from amino acid composition is a free-energy (hydrophobicity) scale. Scales that account accurately for the partitioning of the constituent amino acids of unfolded polypeptides into *n*-octanol and the membrane interfaces of palmitoyloleoylphosphatidylcholine (POPC)[1] bilayers were determined by Wimley, White, and colleagues (*1*−*3*) from measurements of the partitioning free energies of two families of small peptides: $AcWL_n$ ($n = 1-6$) and AcWL-X-LL (X is any of the 20 natural amino acids). An important feature of the scales is that they account for the dominating unfavorable energetics of peptide-bond partitioning. The Wimley−White scales are thus whole-residue scales, because they include both backbone and side-chain contributions to partitioning free energies. The scales do not, however, account for the composition of the N and C termini of polypeptides. We have therefore determined end-group contributions to the partitioning of unfolded pentapeptides into POPC bilayer interfaces and *n*-octanol.

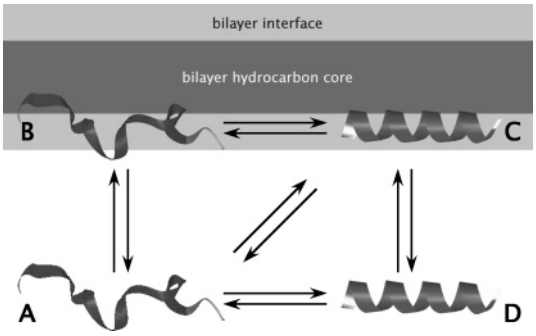

FIGURE 1: Thermodynamic cycle for describing the partitioning and folding of peptides into bilayer interfaces from water. Measurements of the partitioning of most biologically interesting peptides yield free energies for the A ⇆ C equilibrium, because the B state is much less populated than the C state. Nevertheless, the A ⇆ B equilibrium establishes an important reference state. The algorithm presented in this paper allows the A ⇆ B equilibrium free energy to be calculated with good accuracy.

A simplified version of the thermodynamic (*4*) cycle that forms the quantitative framework for describing partitioning and folding of peptides in the bilayer interface is shown in Figure 1 as an equilibrium between four states: unfolded peptide in water (A) and the membrane interface (B) and folded peptide in the membrane interface (C) and water (D). Determination of accurate free-energy scales for the complete cycle opens the way for predicting the partitioning and secondary structure of membrane-active soluble peptides from amino acid sequence. The energetics of the A ⇆ B leg of

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[1] Abbreviations: Ac, acetyl or acetylated; -NH_2, amidated; POPC, palmitoyloleoylphosphatidylcholine; LUV, extruded large unilamellar vesicles of 100 nm in diameter.

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the cycle are problematic, because peptides such as melittin (*5*), which fold upon partitioning [partitioning-folding coupling (*5*, *6*)], are dominated by the A ⇆ C equilibrium (*2*). This obscures the energetics of the A ⇆ B equilibrium, because the small population of peptides in the B state is not easily detected. The simplest way around this problem is to compute the energetics of the A ⇆ B equilibrium. The experiment-based hydrophobicity scale of Wimley and White (*2*) provides a starting point for this approach.

Because small peptides often have blocked N or C termini or both, the Wimley−White scales must be expanded to include values for common N- and C-terminal groups. We have accomplished this expansion by measuring the pH-dependent partitioning of variants of the Wimley−White pentapeptides into *n*-octanol and the POPC bilayer interface: AcWL-X-LL-NH_2 and WLXLL-NH_2 (X = G or W). These measurements, in combination with the earlier measurements (*1*, *2*), permit hydrophobicity values to be added to the Wimley−White scales for protonated, deprotonated, and acylated N termini and for protonated, deprotonated, and amidated C termini. These additional values lead to a simple algorithm for predicting the interfacial partitioning free energy of unfolded peptides that have any combination of N- and C-terminal groups.

MATERIALS AND METHODS

Peptide Synthesis. All peptides were synthesized on an amide resin using standard FMOC methodology (*7*) and were cleaved for 2 h under argon in 90% trifluoroacetic acid, 5% thioanisole, 3% ethanedithiol, and 2% anisole. The peptides were purified using C18 reverse-phase HPLC and water/acetonitrile gradients with 0.1% trifluoroacetic acid. All peptides were better than 99% pure in both solvent systems, as verified by molecular weights determined using MALDI−TOF mass spectrometry. Concentrations of stock solutions in methanol were determined by UV absorbance. Using criteria established in early publications (*1*, *2*), the peptides appeared to be monomeric in solution and to have no regular secondary structure in either water or octanol under the conditions of the partitioning experiments.

Partition Coefficients and Free Energies of Transfer. Volume-fraction octanol−water partition coefficients were measured using a buffer containing 10 mM HEPES, 2 mM MES, 2 mM glycine, 50 mM KCl, 1 mM EDTA, and 3 mM NaN_3, buffered over a wide range of pH values. As previously reported (*1*), the peptide was incubated in octanol-saturated buffer with buffer-saturated octanol overnight at 25.0 °C while rotating the sample vials at 20 rpm. The peptide concentration prior to and after octanol addition was measured by HPLC (*8*). The volume-fraction partition coefficient was calculated as $K_v = (V_o/V_b)\,(P_b)/(P_s - P_b)$, where V_o and V_b are the volumes of the octanol and buffer phases, respectively, and P_s is the concentration of the peptide in the buffer prior to octanol addition. Mole-fraction partition coefficients (K_x) were calculated from the volume fraction (K_v) values by $K_x = K_v(\upsilon_{wat}/\upsilon_{oct})$, where $\upsilon_{wat}/\upsilon_{oct} = 0.114$ is the ratio of the molar volumes of water and octanol.

Mole-fraction partition coefficients between the buffer and membrane interface were determined over a wide pH range using equilibrium dialysis and reverse-phase HPLC, described in detail previously (*8*, *9*). The principle of equilibrium dialysis is that two half-cells, one containing lipid solution and the other buffer, are separated by a membrane that is permeable to peptide but impermeable to lipid vesicles. Peptide was added to one half-cell, and after equilibrium was reached, the peptide concentration in the two compartments was assayed by HPLC. The difference in the two concentrations was due to peptides bound to the liposomes. The partition coefficient K_x was determined by the ratio of bound to free peptide and the lipid concentration, described in ref *2*. All free energies of transfer into octanol or the POPC interface were calculated in mole-fraction units according to $\Delta G = -RT \ln K_x$ (*9*).

Measurements of pK_a. The pK_a of the free N terminus of the peptides was determined by measuring Trp fluorescence. The rationale for this approach was that the fluorescence emission of Trp depends on the charge state of the adjacent N terminus.

Measurement of N-terminal pK_a in buffer were carried out using 10 μM peptide in buffer containing 10 mM HEPES, 2 mM MES, 2 mM glycine, and 50 mM KCl. This assured adequate buffering capacity over a wide pH range. The pH was adjusted to 2 and then gradually increased by adding 1−2 μL of concentrated KOH solution. After each addition of KOH, 10 fluorescence spectra were collected with an SLM Aminco 8100 spectrophotometer (Rochester, NY) and averaged. The pH of the buffer was measured with a microelectrode Mi-410 (Microelectrode, Inc., Bedford, NH). The fluorescence signal at 360 nm was plotted as a function of pH, and the pK_a of the free N terminus was determined by fitting a Boltzmann function to the data.

Measurement of N-terminal pK_a on the POPC bilayer could be determined only for WLWLL-NH_2, because only it partitioned avidly enough into the POPC interface (95% bound). For the same lipid concentration, only about 60% of WLGLL-NH_2 was bound, making it impossible to decouple the fluorescence of bound and unbound peptide. We found, however, that the wavelength maximum of Trp fluorescence intensity (λ_{max}) provided an approximate measure of the N-terminal pK_a. WLWLL-NH_2 or AcWLWLL-NH_2 (as the control) was added to 1.5 mM liposomal solutions and was equilibrated at different values of pH, and tryptophan fluorescence spectra were collected. We compared values of λ_{max} as a function of pH. The λ_{max} for each pH was determined by fitting the fluorescence spectra to log-normal distribution (data not shown). Plots of λ_{max} against pH were fitted with a Boltzmann function to find the apparent pK_a.

RESULTS

Table 1 summarizes the free energies of transfer at pH 3.5 and 8 for all of the peptides studied. The data presented in Figures 2−5 show that the amino termini of the peptides are protonated at pH 3.5 and deprotonated at pH 8. The free energies of transfer of the four peptides from *n*-octanol into water as a function of aqueous-phase pH are shown in Figure 2. As expected from the octanol hydrophobicity scale (*1*, *3*), the -W- homologues partition better in octanol than the -G- homologues. The partitioning of the acetylated peptides (AcWLGLL-NH_2 and AcWLWLL-NH_2, ● in parts A and B of Figure 2) do not depend on pH, as expected. In contrast, the partitioning of the peptides with free N termini in *n*-octanol (□ in parts A and B of Figure 2) does depend on

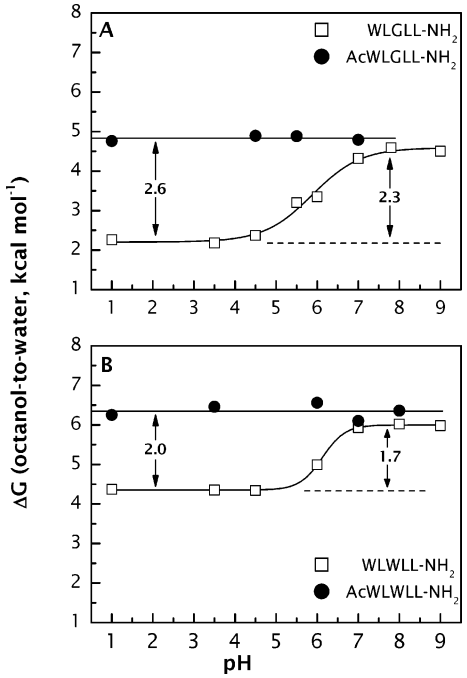

FIGURE 2: Free energies of transfer of the pentapeptides from *n*-octanol to water as a function of aqueous pH. (A) Results for AcWLGLL-NH_2 (●) and WLGLL-NH_2 (□). (B) Results for AcWLWLL-NH_2 (●) and WLWLL-NH_2 (□). While the results for the acetylated peptides do not depend on the pH, the free energies for those peptides with free N termini follow a Boltzmann curve. The pH dependence of partitioning has an apparent N-terminal pK_a of 6.5.

Table 1: Summary of Partitioning Free Energies from Water (kcal mol^{-1}) of Pentapeptides at Different Values of pH

	free energies of transfer (±SEM) to water from			
	n-octanol		POPC	
peptide	pH 3.5	pH 8.0	pH 3.5	pH 8.0
WLGLL-NH_2	2.27 (±0.06)	4.54 (±0.05)	5.58 (±0.09)	6.20 (±0.03)
AcWLGLL-NH_2	4.83 (±0.03)	4.83 (±0.03)	5.90 (±0.11)	6.10 (±0.02)
WLWLL-NH_2	4.35 (±0.01)	6.00 (±0.02)	7.54 (±0.10)	8.24(±0.04)
AcWLWLL-NH_2	6.35 (±0.08)	6.35 (±0.08)	7.98 (±0.10)	8.10 (±0.3)

pH and are accurately described by a Boltzmann distribution with pK_a ~ 6.5. The peptides partition weakly at low pH, because of protonation of the amino terminus. Deprotonation of the amino terminus improves partitioning of both peptides. The partitioning free-energy difference of the charged form relative to the uncharged form is 1.7 kcal mol^{-1} for the -W- peptide and 2.3 kcal mol^{-1} for the -G- peptide, giving a mean value of 2.0 (±0.3) kcal mol^{-1} (Table 1). Wimley and White (*1*) found the cost of C-terminal deprotonation upon octanol partitioning to be 4.8 kcal mol^{-1}. At 2 kcal mol^{-1}, the cost for N-terminal protonation upon partitioning is much less costly than deprotonation of the C terminal. Comparing the acetylated peptides and the nonacetylated peptides at high pH (N terminus deprotonated) shows that acetylation makes octanol partitioning more favorable by only 0.3 kcal mol^{-1} (Table 1). At low pH (N terminus protonated), however, acetylation makes octanol partitioning more favorable by 2.3 (±0.3) kcal mol^{-1}.

The free energies of transfer from POPC bilayers to water for the four peptides as a function of pH are shown in Figure 3. Consistent with the interfacial hydrophobicity scale (*2*), the partitioning free energy into the bilayer is more favorable

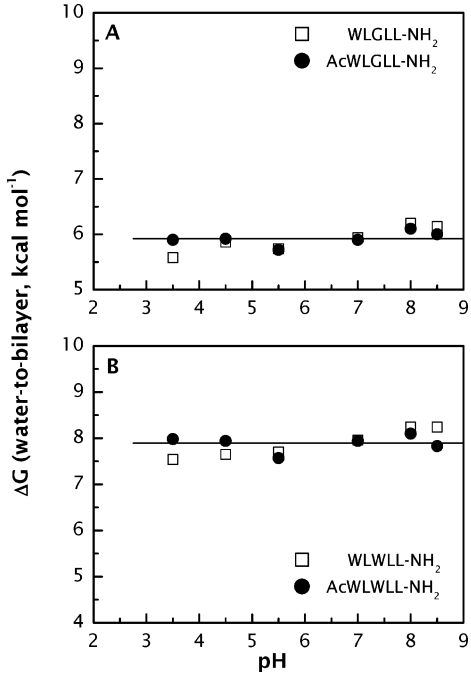

FIGURE 3: Free energies of transfer from water to the POPC bilayer interface as a function of pH. (A) Results for AcWLGLL-NH_2 (●) and WLGLL-NH_2 (□). (B) Results for AcWLWLL-NH_2 (●) and WLWLL-NH_2 (□). A surprise is that N-terminal modification has little effect on peptide partitioning into POPC bilayers over a pH range of 3−8.5.

for the -W- peptides than for the -G- peptides. A surprise is that acetylation of the peptides (● in Figure 3) improves partitioning into the bilayer interface only marginally at low pH. The mean improvement is 0.38 kcal mol^{-1} (Table 1). A comparison of the pH 3.5 and 8 partitioning free energies for the nonacetylated peptides shows that the mean value of the thermodynamic penalty for N-terminus protonation is only 0.66 kcal mol^{-1}. This result is also surprising, because the penalty for C-terminal deprotonation in the interface is about 2.7 kcal mol^{-1} (*2*).

These unexpected observations assume, of course, that the free N terminus is actually charged in buffer at low pH. To verify that the amino group is titratable, we took advantage of the fact that the charge state of the amino group affects the fluorescence of the adjacent Trp residue (see the Materials and Methods). The results for acetylated and nonacetylated peptides are shown in Figure 4 for both the -G- and -W- variants. The Trp fluorescence of the acetylated peptides depends somewhat on pH but not in the manner expected for a titratable group. The reason for this variation is unclear, but we observed the same behavior for the model compound acetyl tryptophan-amide (data not shown). In contrast, the fluorescence of the nonacetylated peptides depends strongly on pH, as expected. From fits of Boltzmann curves to the data of Figure 4, the pK_a values for the free amino termini in solution are 7.2 for the -G- peptide and 6.9 for the -W- variant. The model compound tryptophan-amide was found to have a pK_a of 7.5 (data not shown). We conclude that the amino terminus is titratable in the aqueous phase, as expected. But, is it titratable when partitioned into the bilayer interface?

We examined this issue using the -W- variants that bind avidly to POPC membranes. For large unilamellar vesicle (LUV) concentrations as low as 1.5 mM, more than 95% of

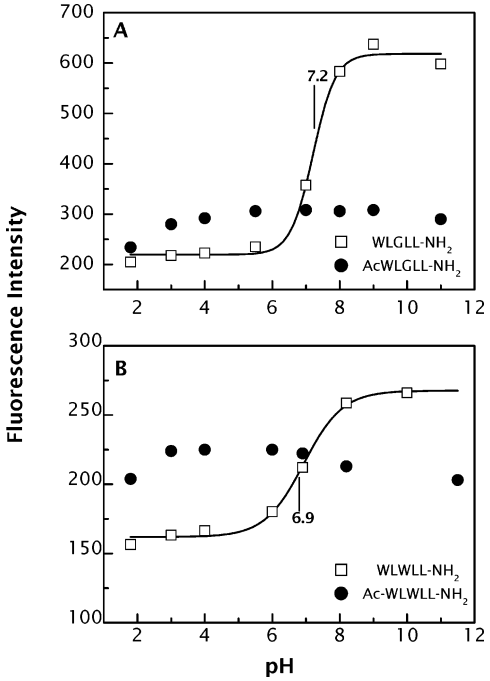

FIGURE 4: Dependence of tryptophan fluorescence intensity (360 nm) as a function of pH for acetylated and nonacetylated peptides in buffer. (A) Titration curves for WLGLL-NH_2 (□) and Ac-WLGLL-NH_2 (●). (B) Titration curves for WLWLL-NH_2 (□) and Ac-WLWLL-NH_2 (●). The fluorescence of Trp depends directly on the pH for both the acetylated and nonacetylated peptides, but only the nonacetylated peptides reveal titration of a charged group. The reason for the small pH dependence of the fluorescence of the acetylated peptides is unknown.

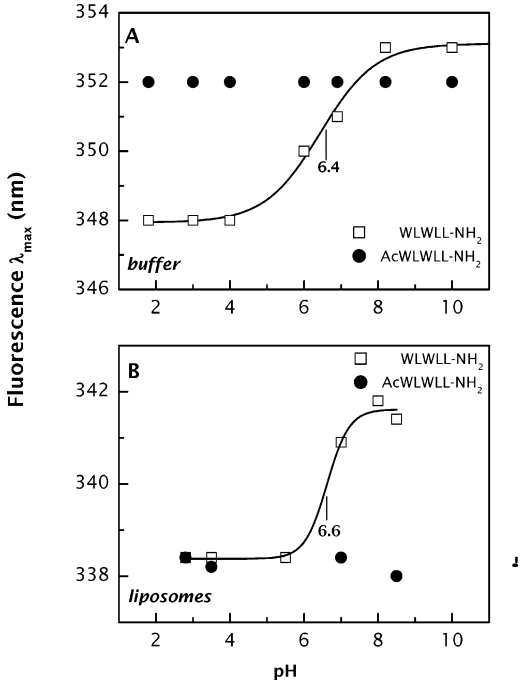

FIGURE 5: Wavelength maximum λ_{max} of tryptophan fluorescence for the Trp pentapeptides in buffer (A) and POPC LUVs (B) as a function of pH. More than 95% of the peptide is bound to POPC under the experimental conditions used. Although λ_{max} does not yield exact values of the pK_a value because it is not a linear response function, the data show that the apparent pK_a values of the WLWLL-NH_2 (□) in liposomes and buffer are identical. The lack of a pK_a shift is consistent with protonation of the peptide in both the buffer and bilayer interface. λ_{max} for the Ac-WLWLL-NH2 (●) peptide has little dependence on pH, as expected.

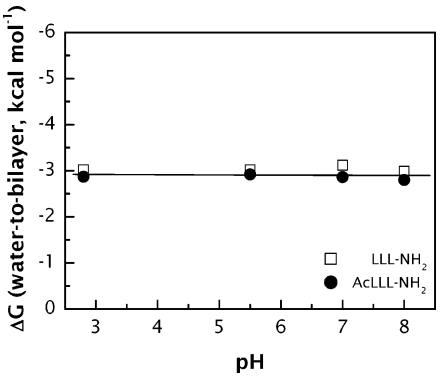

FIGURE 6: Free energies of transfer for the Ac-LLL-NH2 (●) and LLL-NH2 (□) peptides from POPC bilayers to water as a function of pH. The partitioning of the two peptides is essentially identical, meaning that the N-terminal group has little effect on binding to POPC LUVs. It also rules out the possibility of a strong amino-terminus interaction with the Trp of the pentapeptides used in this study.

AcWLWLL-NH_2 or WLWLL-NH_2 was bound. In contrast, only about 60% of WLGLL-NH_2 was bound for the same lipid concentration, which made it impossible to distinguish the fluorescence of the bound and unbound peptide. Because fluorescence intensities in buffer and in liposome could not be compared reliably under these circumstances, we compared instead the wavelengths of maximum Trp fluorescence intensity (λ_{max}) as a function of pH (see the Materials and Methods). We did this by measuring the Trp fluorescence spectra of both -W- analogues equilibrated at different pH values in buffer and in LUV suspensions. Plots of λ_{max} versus pH are shown in Figure 5 for the peptides in buffer (A) and in liposomes (B). Figure 5A demonstrates that λ_{max} can be used to estimate the pK_a of the amino terminus. Although the maximum of the W fluorescence cannot give the correct pK_a value because it is not a linear response function (compare Figure 5A with Figure 4), the data in Figure 5 show that the apparent pK_a in liposomes and buffer are practically identical (inflection points at pH 6.4 and 6.6 for buffer and vesicles, respectively). The lack of an apparent pK_a shift upon association with vesicles indicates that the amino terminus is protonated at low pH in both the buffer and bilayer interface.

The last two possibilities for explaining the unexpected behavior of the N terminus were a failure of the N terminus to partition into the interface or a cation$-\pi$ interaction (*10*−*12*) between the N terminus and the adjacent tryptophan. The first possibility was remote at the outset, because of the very favorable partitioning of Trp into interfaces. However, it was eliminated entirely by absolute-scale (*13*, *14*) X-ray diffraction measurements that revealed a deep interfacial location of AcWLWLL (data not shown). The second possibility was examined by the simple expedient of measuring the pH dependence of the partitioning of AcLLL-NH_2 and LLL-NH_2 peptides into POPC bilayers. Figure 6 shows that (1) AcLLL-NH_2 (●) and LLL-NH_2 (□) bind equally well to POPC vesicles and that (2), over the pH range of 3−8.5, the binding of the nonacetylated peptide does not change. It therefore appears that the unexpected behavior of the N terminus is not restricted to WLXLL-NH_2. Because partitioning measurements are challenging for partitioning free energies in the range of 2−3 kcal mol^{-1}, one cannot reliably

Table 2: Summary of Consequence of End-Group Substitutions on Partitioning Free Energy into *n*-Octanol and POPC Bilayers[a]

N term ($\Delta\Delta G$, kcal mol^{-1})		C term ($\Delta\Delta G$, kcal mol^{-1})	
POPC	*n*-octanol	POPC	*n*-octanol
$NH_3^+ \rightarrow NH_2$		$COO^- \rightarrow COOH$	
-0.7 ± 0.2	-2.0 ± 0.3	-2.7 ± 0.1	-4.8 ± 0.1
$NH_3^+ \rightarrow Ac-NH$		$COO^- \rightarrow CO-NH_2$	
-0.4 ± 0.3	-2.3 ± 0.3	-1.9 ± 0.1	-3.6 ± 0.2

[a] Results are from this study and data published by Wimley and White (*1*, *2*). See the text.

extract differences in free energy as a function of pH. Nevertheless, the data in Figure 6 are consistent with the minor effect of amino-terminal protonation on partitioning. However, when these data are taken at face value, they do not rule out entirely the possibility of a neighbor effect between tryptophan and the amino terminus.

DISCUSSION

Wimley and White found the energetic cost of deprotonating the C terminus of the Ac-W-L-X-LL peptides in *n*-octanol (*1*) and in the POPC interface to be 4.8 and 2.7 kcal mol^{-1}, respectively. Here, we have determined the effect of N-terminal acetylation and C-terminal amidation on peptide partitioning into *n*-octanol and POPC bilayer interfaces using the pentapeptide variants AcWL-X-LL-NH_2 and WL-X-LL-NH_2 (X = G or W). From data presented here and from the data of Wimley, White, and colleagues (*1*, *2*), the contributions of various end-group substitutions on the free energies of transfer of unfolded peptides can be established, as summarized in Table 2. Amidation of the C terminus ($COO^- \rightleftharpoons CO-NH_2$) makes partitioning more favorable by 3.6 kcal mol^{-1} for *n*-octanol and 1.9 kcal mol^{-1} for the POPC bilayer interface. Acetylation ($NH_3^+ \rightleftharpoons Ac-NH_2$) or deprotonation ($NH_3^+ \rightleftharpoons NH_2$) of the N terminus improves partitioning in *n*-octanol by 2.3 and 2.0 kcal mol^{-1}, respectively. These substitutions had very small effects on partitioning into the bilayer interface: -0.38 (± 0.28) kcal mol^{-1} for $NH_3^+ \rightleftharpoons Ac-NH_2$ and -0.66 (± 0.28) kcal mol^{-1} for $NH_3^+ \rightleftharpoons NH_2$. These marginal effects of amino-terminal changes are contrary to our expectations. We have no explanation for them at present. We can say only that they must result from a peculiar set of compensating interactions within the thermally disordered bilayer interface. A further study is required to elucidate the structural basis for this observation.

Our principal goal for a number of years has been an algorithm for predicting the binding free energy and secondary structure of peptides and proteins that partition into the lipid bilayer interface. As illustrated in Figure 1, we use the unfolded state (state B) as a thermodynamic reference state for describing interfacial folding (B $\leftrightarrows$ C) (*4*, *15*). This choice is necessary to account systematically for the energetics of secondary-structure formation. The problem is that most peptides and proteins of biological interest generally fold upon partitioning into interfaces (*4*−*6*) in a manner that causes the B-state population to be virtually undetectable relative to the C state. In only a few cases can the energetics of the A $\leftrightarrows$ B equilibrium be determined by direct measurement (*5*). One must therefore resort to a computational approach. The end-group data presented here and the Wimley−White (*2*) interfacial partitioning data allow the

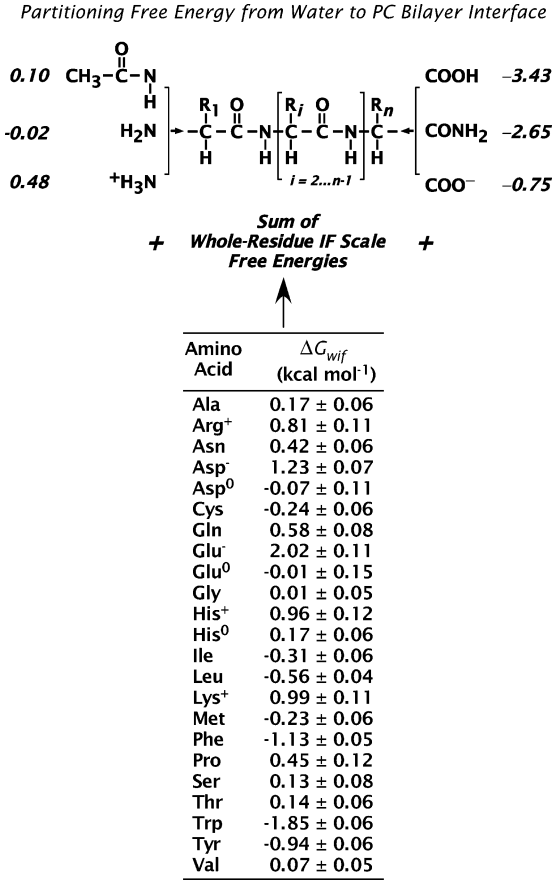

FIGURE 7: Algorithm for computing the partitioning of unfolded peptides from water into the POPC bilayer interface. The water−interface (IF) whole-residue hydrophobicity values are from Wimley and White (*2*). See the text.

interfacial hydrophobicity scale to be expanded to include N- and C-terminal groups. Before this can be done, however, absolute values for the partitioning of one N-terminal group and one C-terminal group must be known. As shown in the Supporting Information, a value for the COOH group can be obtained from the bilayer-partitioning free energies of the $AcWL_m$ ($m = 1-6$) peptides (*2*). The value obtained is -2.2 kcal mol^{-1} for water−bilayer partitioning. A simple calculation based on the partitioning free energies of a methyl group and the peptide bond yields $+0.1$ kcal mol^{-1} for the acetyl group (see the Supporting Information). From these values and data presented in Table 2, absolute values for the other terminal groups are easily obtained. The values obtained are incorporated into the algorithm presented in Figure 7 along with the Wimley−White whole-residue interfacial hydrophobicity scale (*2*). As presented, the algorithm accounts for the important contribution of the peptide bond to the partitioning of the *n*th residue plus the C-terminal group. The algorithm has been incorporated into the Totalizer tool of Membrane Protein Explorer (MPEx), which is available at http://blanco.biomol.uci.edu/mpex.

The consistency of the algorithm is demonstrated by Figure 8, in which measured values of peptide partitioning are plotted against values computed using the algorithm of Figure 7. Internal consistency is revealed by the Wimley−White (*2*) peptides (■) and the variants used in this study (△). The absolute consistency of the algorithm (then unpublished) was shown earlier by Ladokhin and White (*16*) from measure-

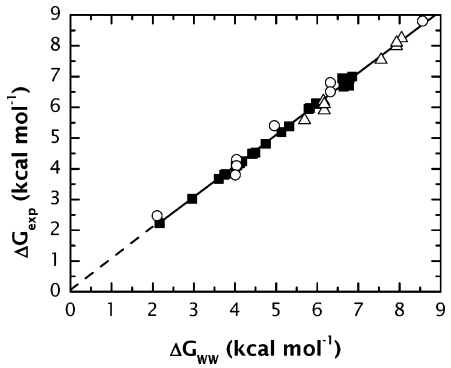

FIGURE 8: Plot of measured water−bilayer free energies of transfer (ΔG_{exp}) and the free energies of transfer (ΔG_{WW}) computed using the algorithm of Figure 7. The measured values for the peptides used in this study are shown as △. The measured values for the pentapeptides studied by Wimley and White (*2*) are shown as ■. ○ are values measured for indolicidin variants by Ladokhin and White (*16*). The best-fit linear curve through all of the data points has a slope of 1.01 ± 0.01 and an intercept of 0.06 ± 0.08. This shows that the algorithm of Figure 7 allows the partitioning of unfolded peptides to be computed with reasonable accuracy.

ments of the partitioning free energies of variants of the antimicrobial peptide indolicidin (*17*). This 13-residue peptide, which is rich in tryptophan and proline, has no regular secondary structure when partitioned into the POPC bilayer interface (*18*, *19*). The Ladokhin and White values are included in Figure 8 (○). The best-fit linear curve through all of the data points has a slope of 1.01 ± 0.01 and an intercept of 0.06 ± 0.08. We conclude that the algorithm of Figure 7 provides a means of computing with reasonable accuracy the free energy of partitioning of small unfolded peptides into the POPC bilayer interface.

We emphasize that the algorithm can only be used for computing the partitioning of peptides that have no regular secondary structure, i.e., the A ⇆ B equilibrium in the thermodynamic cycle of Figure 1. The computation of the free-energy changes associated with the other steps in the cycle requires knowledge of the number of residues that participate in secondary structure and the free energy reduction per residue that accompanies secondary structure formation, typically ∼0.5 kcal mol^{-1} (*5*, *6*). In principle, the contributions of the endgroups to partitioning are independent of this per-residue reduction, but we have no direct evidence that supports that hypothesis.

SUPPORTING INFORMATION AVAILABLE

A value for partioning the COOH group was calculated by two methods: (1) The $n = 0$ intercept of a linear curve fitted to a plot of free-energy values of $(CONH)_nCOOH$ as a function of n (Figure S1) and (2) the $n = 0$ intercept of a linear curve fitted to free-energy values of $AcWL_m$ plotted against the number of residues $n = m + 1$ (Figure S2). This material is available free of charge via the Internet at http://pubs.acs.org.

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