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Mechanisms of the interaction of α-helical transmembrane peptides with phospholipid bilayers

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Abstract

The synthetic peptide acetyl-K_2-G-L_{24}-K_2-A-amide (P_{24}) and its analogs have been successfully utilized as models of the hydrophobic transmembrane α-helical segments of integral membrane proteins. The central polyleucine region of these peptides was designed to form a maximally stable, very hydrophobic α-helix which will partition strongly into the hydrophobic environment of the lipid bilayer core, while the dilysine caps were designed to anchor the ends of these peptides to the polar surface of the lipid bilayer and to inhibit the lateral aggregation of these peptides. Moreover, the normally positively charged N-terminus and the negatively charged C-terminus have both been blocked in order to provide a symmetrical tetracationic peptide, which will more faithfully mimic the transbilayer region of natural membrane proteins and preclude favorable electrostatic interactions. In fact, P_{24} adopts a very stable α-helical conformation and transbilayer orientation in lipid model membranes. The results of our recent studies of the interaction of this family of α-helical transmembrane peptides with phospholipid bilayers are summarized here.

Keywords: α-Helical transmembrane peptide; Phospholipid bilayers; Lipid–protein interactions; Hydrophobic mismatch; Phosphatidylcholine; Phosphatidylethanolamine

1. Introduction

Lipid–protein interactions are of fundamental importance for both the structural integrity and for the various functions of all biological membranes (see Refs. [1,2]). In particular, the chemical composition and physical properties of the host lipid bilayer can markedly influence the activity, thermal stability, and the location and disposition of a large number of integral membrane proteins in both the model and biological membrane systems (see Refs. [1–5]). For these reasons, many studies of the interactions of membrane proteins with their host lipid bilayers have been carried out in both biological and reconstituted model membrane systems, employing a wide range of different physical techniques (see Refs. [6–10]). However, our understanding of the physical principles underlying lipid–protein interactions remains incomplete and the molecular mechanisms whereby the associated lipids actually alter the activity, and presumably also the structure and dynamics, of integral membrane proteins are largely unknown. One reason for this situation is the fact that most transmembrane proteins are relatively large, multidomain macromolecules of complex and often unknown three-dimensional structure and topology that can interact with lipid bilayers in complex, multifaceted ways (see Refs. [1–10]). To overcome this problem, a number of workers have designed and synthesized peptide models of specific regions of natural membrane proteins and have studied their interactions with model lipid membranes of defined composition (see Refs. [11,12]). Physical studies of such relatively tractable model membrane systems have already significantly advanced our understanding of the molecular basis of lipid–protein interactions.

The synthetic peptide acetyl-K_2-G-L_{24}-K_2-A-amide (P_{24}) and its analogs have been successfully utilized as a model of the hydrophobic transmembrane α-helical segments of integral membrane proteins (see Refs. [12,13]). These peptides contain a long sequence of hydrophobic leucine residues capped at both the N- and C-termini with two positively charged, relatively polar lysine residues. Moreover, the normally positively charged N-terminus and the negatively charged C-terminus have both been blocked in order to provide a symmetrical tetracationic peptide, which will more faithfully mimic the transbilayer region of natural membrane

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proteins. The central polyleucine region of these peptides was designed to form a maximally stable α-helix which will partition strongly into the hydrophobic environment of the lipid bilayer core, while the dilysine caps were designed to anchor the ends of these peptides to the polar surface of the lipid bilayer and to inhibit the lateral aggregation of these peptides. In fact, circular dichroism (CD) [13] and Fourier transform infrared (FTIR) [14–16] spectroscopic studies of P_{24} have shown that it adopts a very stable α-helical conformation both in solution and in lipid bilayers, and X-ray diffraction [17], fluorescence quenching [18] and FTIR [14–16], and deuterium nuclear magnetic resonance (^{2}H-NMR) [19,20] spectroscopic studies have confirmed that P_{24} and its analogs assume a transbilayer orientation with the N- and C-termini exposed to the aqueous environment and the hydrophobic polyleucine core embedded in the hydrocarbon core of the lipid bilayer when reconstituted with various phosphatidylcholines (PCs) [15]. ^{2}H-NMR [21] and electron spin resonance (ESR) [22] spectroscopic studies have shown that the rotational diffusion of P_{24} about its long axis perpendicular to the membrane plane is rapid in the liquid-crystalline state of the bilayer and that the closely related peptide acetyl-K_2-L_{24}-K_2-amide (L_{24}) exists at least primarily as a monomer in the liquid-crystalline PC bilayers, even at relatively high peptide concentrations.

2. Materials and methods

Detailed descriptions of the material and methods utilized in the studies reviewed below can be found in the references indicated. Briefly, large multilamellar peptide-containing phospholipid vesicles were prepared by codissolving the phospholipid and peptide in methanol at the required molar ratio and evaporating the solvent under a stream of nitrogen. After the removal of the traces of methanol in vacuo overnight, the phospholipid–peptide film was hydrated by vigorous vortexing with aqueous buffer at a temperature well above the gel/liquid-crystalline phase transition temperature of the phospholipid. This procedure results in a quantitative incorporation of the α-helical peptides P_{24}, L_{24}, and $(LA)_{12}$ (but not A_{24}) into the host phospholipid bilayer in a transmembrane orientation. The reader is referred to the original publications for details of the DSC, and the CD, FTIR, NMR, and ESR spectroscopic techniques, utilized in these studies.

3. Results and discussion

High-sensitivity differential scanning calorimetry (DSC) and FTIR spectroscopy were used to study the interaction of a synthetic model transmembrane peptide P_{24} and members of the homologous series of *n*-saturated PCs [15]. In the low range of the peptide mole fractions, the DSC thermograms exhibited by the lipid/peptide mixtures are resolvable into two components (see Fig. 1). One of these components is fairly narrow, highly cooperative, and exhibits properties which are similar to but not identical with those of the pure lipid. In addition, the fractional contribution of this component to the total enthalpy change, the peak transition temperature, and cooperatively decrease with an increase

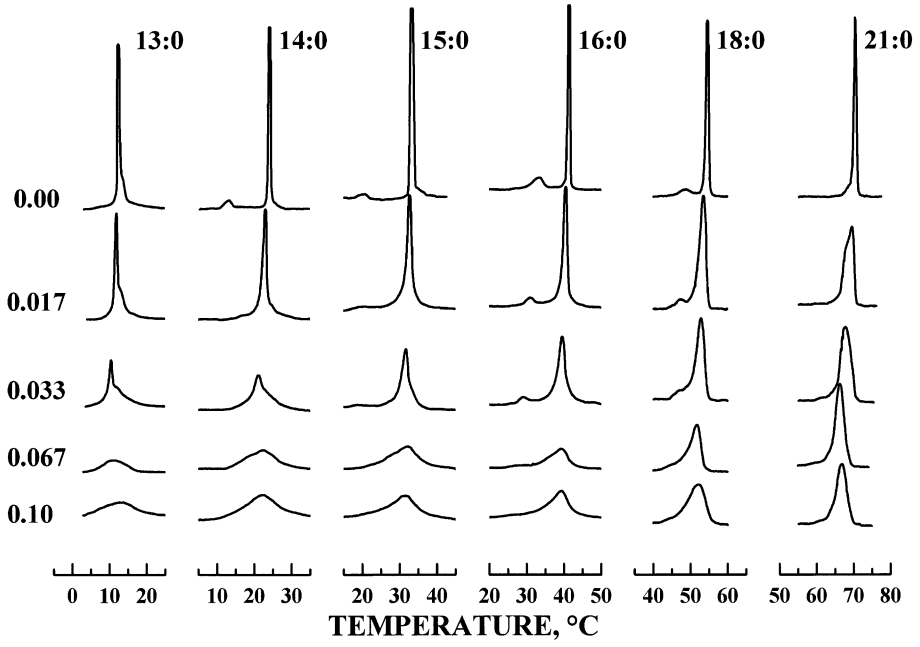

Fig. 1. The effect of increasing quantities of the peptide P_{24} on the DSC heating thermograms of a series of *n*-saturated diacyl-PCs. Thermograms are shown as a function of the acyl chain length (*N*:0) of the lipids, and the approximate P_{24}/lipid ratios are indicated on the column of numbers printed on the left side of the figure.

in the peptide concentration, more or less independent of the acyl chain length. The other component is very broad and predominates in the high range of peptide concentration. These two components have been assigned to the chain-melting phase transitions of populations of the bulk lipid and peptide-associated lipid, respectively. Moreover, when the mean hydrophobic thickness of the PC bilayer is less than the peptide hydrophobic length, the peptide-associated lipid melts at higher temperatures than the bulk lipid and vice versa. In addition, the chain-melting enthalpy of the broad endotherm does not decrease to zero even at high peptide concentrations, suggesting that this peptide reduces but does not abolish the cooperative gel/liquid-crystalline phase transition of the lipids with which it is in contact. Our DSC results indicate that the width of the phase transition observed at high peptide concentration is inversely and discontinuously related to the hydrocarbon chain length and that gel phase immiscibility occurs when the hydrophobic thickness of the bilayer greatly exceeds the hydrophobic length of the peptide. The FTIR spectroscopic data indicate that the peptide forms a very stable α-helix under all of our experimental conditions but the small distortions of its α-helical conformation are induced in response to any mismatch between peptide hydrophobic length and bilayer hydrophobic thickness (see Fig. 2). These results also indicate that the peptide alters the conformational disposition of the acyl chains in contact with it and that the resultant conformational changes in the lipid hydrocarbon chains tend to minimize the extent of mismatch of the peptide hydrophobic length and bilayer hydrophobic thickness.

High-sensitivity DSC and FTIR spectroscopy were also used to study the interaction of the synthetic α-helical hydrophobic transmembrane peptide P_{24} and members of a homologous series of *n*-saturated diacylphosphatidylethanolamines (PEs) [23]. In the lower range of peptide molecular fractions, the DSC endotherms exhibited by the lipid/peptide mixtures again consist of two components. The temperature and cooperativity of the sharper, higher-temperature component are very similar to those of the pure PE bilayers and are almost unaffected by variations in the peptide/lipid ratio. However, the fractional contribution of this component to the total enthalpy change decreases with increases in the peptide concentration, and this component completely disappears at higher peptide molecular fractions. The other component, which is less cooperative and occurs at a lower temperature, predominates at higher peptide concentrations. These two components of the DSC endotherm can be attributed to the chain-melting phase transitions of the peptide-nonassociated and peptide-associated PE molecules, respectively. Although the temperature at which the peptide-associated PE molecules melt is progressively decreased by increases in the peptide concentration, the magnitude of this shift is *independent* of the length of the PE hydrocarbon chain. In addition, the width of the phase transition observed at higher peptide concentrations is also relatively insensitive to the PE hydrocarbon chain length, except that peptide gel-phase immiscibility occurs

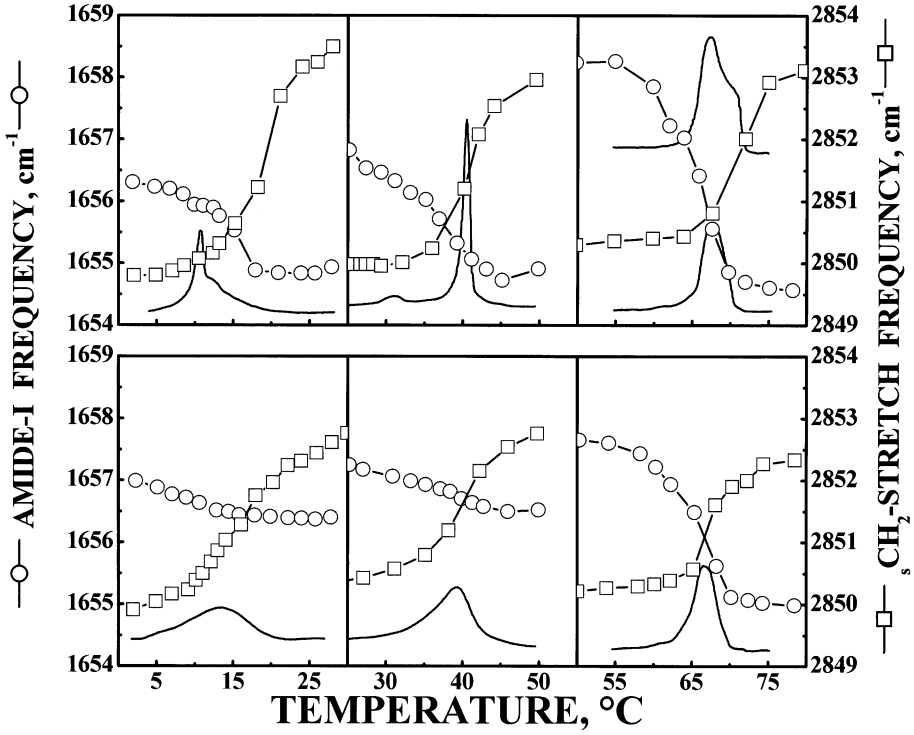

Fig. 2. Combined plots of CH_2 symmetric stretch (□), peptide amide I band (○), and calorimetric thermograms as a function of temperature for systems of P_{24}/13:0, 16:0, and 21:0. The peptide/lipid molar ratios are 0.03 (top panel) and 0.1 (bottom panel).

in very short- or very long-chain PE bilayers. Moreover, the enthalpy of the chain-melting transition of the peptide-associated PE does not decrease to zero even at high peptide concentrations, suggesting that this peptide does not abolish the cooperative gel/liquid-crystalline phase transition of the lipids with which it is in contact. The FTIR spectroscopic data indicate that the peptide remains in a predominantly α-helical conformation but that the peptide α-helix is subject to small distortions coincident with the changes in the hydrophobic thickness that accompany the chain-melting phase transition of the PE bilayer. These data also indicate that the peptide significantly disorders the hydrocarbon chains of the adjacent PE molecules in *both* the gel and liquid-crystalline states relatively *independently* of the lipid hydrocarbon chain length. The relative independence of many aspects of the PE–peptide interactions on the hydrophobic thickness of the host bilayer observed in the present study is in marked contrast to the results of our previous study of peptide PC model membranes [15], where strong hydrocarbon chain length-dependent effects were observed. The differing effects of peptide incorporation on the PE and PC bilayers are ascribed to the much stronger lipid polar headgroup interactions in the former system. We postulate that the primary effect of transmembrane peptide incorporation into the PE bilayers is the disruption of the relatively strong electrostatic and hydrogen-bonding interactions at the bilayer surface and that this effect is sufficiently large to mask the effect of the hydrophobic mismatch between the lengths of the hydrophobic core of the peptide and its host bilayer.

The interactions of the hydrophobic helical transmembrane peptide Ac-K_2-$(LA)_{12}$-K_2-amide [$(LA)_{12}$] with a series of *n*-saturated PCs and PEs were also studied by high-sensitivity DSC and FTIR spectroscopy [24–26]. In general, the effects of $(LA)_{12}$ on the phospholipid thermotropic phase behavior are similar to those observed with P_{24}, except that $(LA)_{12}$ decreases the temperature and enthalpy of the gel to liquid-crystalline phase transition of the host phospholipid to a greater extent, indicating a more substantial reduction in the organization of the gel-state bilayers. The FTIR spectra of $(LA)_{12}$ in these phospholipid bilayers indicate that this peptide also retains a predominantly α-helical conformation in both the gel and liquid-crystalline phases of the short to medium chain phospholipids studied. However, when incorporated into the bilayers composed of the longer chain phospholipids, $(LA)_{12}$ undergoes a reversible conformational change at the gel/liquid-crystalline phase transition of the mixture. In the liquid-crystalline phase, the amide I regions of the FTIR spectra of these mixtures are indicative of a predominantly α-helical peptide conformation. However, upon freezing of the lipid hydrocarbon chains, populations of $(LA)_{12}$ giving rise to a sharp conformationally unassigned band near 1665 cm^{-1} are formed, indicating a greater degree of conformational flexibility than for P_{24}. A comparison of the results of these calorimetric and FTIR spectroscopic studies with similar studies of a polyleucine-based analogue of $(LA)_{12}$ suggests that the thermodynamics of the interaction of hydrophobic transmembrane helices with lipid bilayers can be influenced by factors such as the polarity and topology of the helical surface, factors which are dependent upon the amino acid sequence of the helix. Also, possible adjustments to a hydrophobic mismatch between the peptide and its host lipid bilayer cover a spectrum of possibilities which can include changes in the degree of conformational disorder in the lipid chains and/or significant conformational changes on the part of the peptide.

We have also studied the conformation amide proton exchangeability of the model transmembrane peptide acetyl-K_2-A_{24}-K_2-amide (A_{24}) and its interaction with phosphatidylcholine bilayers by a variety of physical techniques [27]. A combination of the CD and FTIR spectroscopic results indicate that when dissolved in methanol or deposited from methanol as a dried film, A_{24} is predominantly α-helical. Upon dissolution in aqueous media, rapid H–D exchange of A_{24} amide protons occurs, indicating that the peptide is sufficiently conformationally dynamic that all amide protons are fully exposed to the solvent. Our CD and FTIR spectroscopic techniques also reveal that A_{24} exists primarily as a mixture of helical (but probably not α-helical) and β-sheet structures in aqueous media at room temperature. Upon heating, A_{24} converts reversibly primarily to unordered structures in unbuffered water but irreversibly to antiparallel β-sheet structures in the phosphate-buffered saline. Our studies also indicate that although A_{24} exists primarily as a membrane α-helix when incorporated into phospholipids in the absence of water, the hydration of that system results in a quick exchange of all amide protons. Also, when dispersed with PC in the aqueous media, the conformation and thermal stability of A_{24} are not significantly altered by the presence of the phospholipid, its phase state or its gel/liquid-crystalline phase transition. Our DSC and ESR spectroscopic studies indicate that A_{24} has relatively minor effects on the thermodynamic properties of the lipid hydrocarbon chain-melting phase transition, that it does not abolish the lipid pretransition, and that its presence has no significant effect on the orientational order or rates of motion of the phospholipid hydrocarbon chains. We conclude from these results that A_{24} has sufficient α-helical propensity but insufficient hydrophobicity to maintain a stable transmembrane association with the phospholipid bilayers in the presence of water. Instead, A_{24} exists primarily as a dynamic mixture of helices, β-sheets and other conformers and resides mostly in the aqueous phase, where it interacts weakly with the bilayer surface or the interfacial regions of phosphatidylcholine bilayers. Thus, the polyalanine-based peptides are not good models for the transmembrane α-helical segments of natural membrane proteins.

We have investigated the effects of the model α-helical transmembrane peptide L_{24} on the thermotropic phase behavior of the aqueous dispersions of 1,2-dielaidoylphos-

phatidyl-ethanolamine (DEPE) to better understand the interactions between the lipid bilayers and the membrane-spanning segments of integral membrane proteins [28]. We studied, in particular, the effect of L_{24} and the three derivatives thereof on the liquid-crystalline lamellar (L_α)–reversed hexagonal (H_{II}) phase transition of DEPE model membranes by DSC and ^{31}P-NMR. We found that the incorporation of L_{24} progressively decreases the temperature, enthalpy, and cooperativity of the L_α–H_{II} phase transition, as well as induces the formation of an inverted cubic phase, indicating that this transmembrane peptide promotes the formation of the inverted nonlamellar phases, despite the fact that the hydrophobic length of this peptide exceeds the hydrophobic thickness of the host lipid bilayer. These characteristic effects are not altered by truncation of the side chains of the terminal lysine residues or by replacing each of the leucine residues at the end of the polyleucine core of L_{24} by a tryptophan residue. Thus, the characteristic effects of these transmembrane peptides on the DEPE thermotropic phase behavior are independent of their detailed chemical structure. Importantly, significantly *shortening* the polyleucine core of L_{24} results in a *smaller* decrease in the L_α–H_{II} phase transition temperature of the DEPE matrix into which it is incorporated, and *reducing* the thickness of the host phosphatidylethanolamine bilayer results in a *larger* reduction in the L_α–H_{II} phase transition temperature. These results are not those predicted by the hydrophobic mismatch considerations or reported in previous studies of other transmembrane α-helical peptides containing a core of an alternating sequence of leucine and alanine residues. We thus conclude that the hydrophobicity and conformational flexibility of the transmembrane peptides can affect their propensity to induce the formation of the inverted nonlamellar phases by mechanisms not primarily dependent on the lipid–peptide hydrophobic mismatch.

The molecular organization and dynamics have been investigated in membranes consisting of 1-palmitoyl-2-oleoyl-L-α-phosphatidylcholine (POPC) and various ratios of a transmembrane α-helical peptide L_{24}, in order to gain insights into how the transmembrane portions of membrane proteins are mixed with phospholipids and organized in biological membranes [22]. Particular attention was paid to membranes with high peptide concentrations. The molecular organization and dynamics were studied in the ps-to-μs regime using the various spin-labeling techniques. Conventional ESR spectra, as well as the saturation–recovery curves measured in both the presence and the absence of molecular oxygen, showed that the PC spin-labels detect the existence of a single homogeneous environment, indicating that both L_{24} and POPC are undergoing fast translational diffusion in L_{24}–POPC membranes of up to 9 mol% peptide. Since 16–18 molecules of phosphatidylcholine are required to surround a transmembrane α-helical peptide, L_{24} must form L_{24}-rich regions at the P/L ratio of 1:10 instantaneously. However, these results suggest that the lipid exchange rates among the bulk, boundary, and L_{24}-rich regions are fast, and that the L_{24}-rich regions must be forming and dispersing rapidly in a time scale shorter than 0.1 μs, the conventional ESR spin-label time scale and the electron spin-lattice relaxation time scale in the presence of molecular oxygen. Although this does not exclude the possibility of the formation of small, stable oligomers of L_{24}, it is unlikely because L_{24} lacks the features that would favor their formation. L_{24} increases the hydrophobicity of the central part of the POPC membrane from the level of 1-decanol to that of pure hexane and also increases the hydrophobicity near the membrane surface from the level of 2-propanol to that of 1-decanol. The effect of L_{24} on the order parameter profile is similar to that of decreasing the temperature by ~ 8 °C between 10 and 55 °C. We conclude that L_{24} is highly miscible in POPC membranes even at high concentrations in the phospholipid bilayer.

We have studied the effects of the incorporation of the α-helical transmembrane peptides (L_{24}) and $(LA)_{12}$ on the thermotropic phase behavior of 1,2-dipalmitoyl-d_{62}-*sn*-glycero-3-phosphocholine (DPPC-d_{62}) and 1-palmitoyl-d_{31}-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC-d_{31}) lipid bilayer model membranes by DSC and the conformational and orientational order of the phospholipid chains by the FTIR spectroscopy and ^{2}H-NMR spectroscopy, respectively [29]. Our DSC and FTIR spectroscopic studies indicate that the peptides L_{24} and $(LA)_{12}$ both decrease the temperature and enthalpy of the gel/liquid-crystalline phase transition of the DPPC-d_{62} bilayers, with $(LA)_{12}$ having the greater effect in this regard, as reported previously. An examination of the FTIR spectroscopic frequencies of the CH_2 and CD_2 symmetric stretching bands of the infrared spectra of the liquid-crystalline states of the peptide-free and peptide-containing DPPC-d_{62} and POPC-d_{31} samples, and a comparison with the orientational order as measured by the ^{2}H-NMR spectroscopy, as well as with the chain order as measured by the ESR spectroscopy, lead us to conclude that the CH_2 (or CD_2) stretching frequencies of the lipid hydrocarbon chains are not a reliable measure of the chain conformational order in the lipid bilayers containing significant amounts of peptides or other lipophilic inclusions. In contrast, the results of our ^{2}H-NMR spectroscopic studies present a consistent picture in which both L_{24} and $(LA)_{12}$ increased in a similar way the time-averaged orientational order of the lipid chains of their liquid-crystalline lipid bilayer hosts, although the ordering by L_{24} in the DPPC bilayers is greater. The comparison of the effects L_{24} and $(LA)_{12}$ on PC bilayers indicates that the gel-to-liquid-crystalline phase transition appears to be more sensitive to small changes in the transmembrane peptide surface topology than is hydrocarbon chain orientational order in the liquid-crystalline state.

4. Conclusions

Our studies have shown that the simple peptide models of the transmembrane segments of integral membrane pro-

teins affect the thermotropic phase behavior and organization of the PC and PE bilayers in different ways, and that the physical properties of these host lipid bilayers, in particular the hydrophobic mismatch, can also affect the peptide conformation and state of aggregation. As well, our studies have shown that the transmembrane peptides must possess a sufficient hydrophobicity, as well as α-helical propensity, to form a stable transbilayer complex with a phospholipid membrane. Future studies will extend this work to other structural variations in both the polar capping and hydrophobic core regions of these transmembrane model peptides.

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