Tryptophan Anchors in Transmembrane Peptides

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Abstract

Geometric considerations prescribe that embedded proteins may be regarded as "defects" in lipid bilayers (unless there is "perfect" structural matching). A critical accumulation of such defects could mandate a lipid phase transition, and such macroscopic effects may lend insight into individual molecular lipid-protein interactions. To investigate these issues, we are using two types of model transmembrane peptides: gramicidins and designed membrane-spanning alpha-helices. Both models are anchored at each membrane/water interface by multiple tryptophans, which appear to be important for modulating the lipid phase behavior.

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Introduction

Lipid bilayers vary in hydrophobicity from the polar exterior to the non-polar membrane interior, with the hydrophobicity difference being bridged by the derivatized glycero-phosphate groups. The fluid-mosaic model of the cell membrane provided a way to incorporate proteins into this picture, based on the principle of maximizing hydrophobic and hydrophilic interactions (Singer and Nicolson, 1972). That embedded proteins might influence the host lipids was not considered. Molecular packing considerations (Israelachvili, 1977), nevertheless, reveal that– even in the absence of specific intermolecular interactions between lipids and proteins–the structure or mobility of lipids near a protein must differ from those in the rest of the bilayer. Moreover, geometric constraints impose a structural coupling between proteins and neighboring lipids such that the proteins do not "float

freely" in a sea of lipids (Israelachvili, 1977).

Embedded proteins therefore may be regarded as "defects" within otherwise pure bilayers, which raises questions about how different amino acid sequences will insert, orient and interact with lipids. Depending on their hydrophobicities, different side chains will seek the membrane interior, the aqueous phase or the membrane/water interface. Mismatches in the hydrophobic lengths of lipids and membrane-spanning peptide segments will introduce strain. A low density of defects will increase the bilayer energy marginally and cause local perturbations that could be important for large-scale dynamic processes such as endocytosis, membrane division or fusion. In the extreme, a critical accumulation of packing defects may mandate a phase transition. To address some of these issues, we have made use of membrane-spanning peptides of defined sequence.

The Questions We Wished to Elucidate Were:

A. How do protein sequences fit into the hydrophobicity gradient of the membrane?

B. What determines the "hydrophobic length" of a particular peptide?

C. What are effective anchoring residues for transmembrane segments of proteins? How many such residues are needed per segment?

D. What determines the transmembrane orientation or tilt of embedded protein segments?

Peptide Models

In order to be useful for understanding these questions, peptide models will need to:

(A) adopt defined, non-random, folded structures; and

(B) assume defined orientations within lipid bilayer membranes.

Single-span transmembrane peptides, such as gramicidin channels and non-aggregating α-helical peptides, may satisfy these demands (Killian, 1992; Koeppe and Andersen, 1996; Killian et al., 1996).

The structure and organization of the peptides and lipids will be influenced by:

(1) the relative lengths of the peptides and lipids,

(2) the hydrophobicity of the internal part of the peptide sequence, and

(3) the identity and placement of anchoring residues.

Anchoring residues will seek the aqueous phase or the membrane/water interface (head group region). Charged residues are good anchors, and several transmembrane peptide models have been developed using charged residues as anchors (e.g., Davis et al., 1983; Zhang et al., 1995). Charged anchors are not the only possible anchor residues, however. Peptides that have no side-chain or end-group charges and that fit entirely within the span of the bilayer can use tryptophan indole rings as anchors (Figures 1, 2).

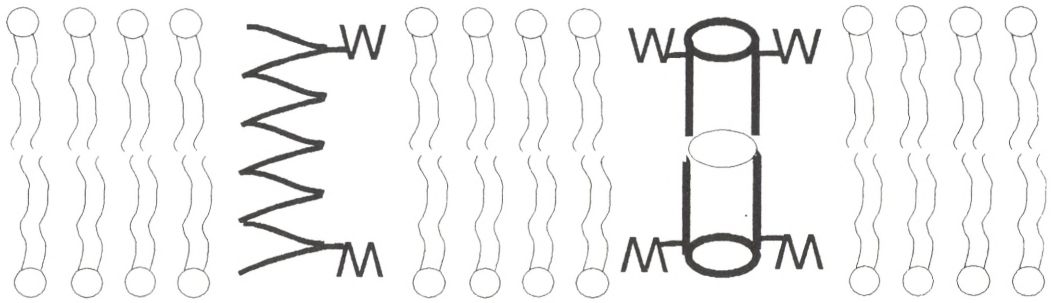

Figure 1. Schematic drawings of a 30-residue gramicidin dimer (channel) and a 17-residue transmembrane α helix, each with tryptophan anchors depicted as "W". Each model peptide has a defined folded conformation and transmembrane orientation. (For clarity, only 50% of the tryptophans are shown.)

gA HCO-VGALAVVVWLWLWLW-$NHCH_2CH_2OH$

WALP17 HCO-AWWLALALALALALWWA-$NHCH_2CH_2OH$

Figure 2. Sequences of the peptide models in Fig. 1: gramicidin A (gA, with D-residues underlined), which spans a bilayer as a $\beta^{6.3}$- helical dimer with W's at both ends; and WALP17 (Killian et al., 1996), which spans a bilayer as an α-helical monomer with all L- residues, and W's at both ends.

Lipid Influence on Peptide Conformation

The linear gramicidins require a lipid bilayer (or bilayer-like environment) to fold properly. If the phospholipid acyl chains are less than eight carbons long, the channel fold is not observed (Figure 3); if the acyl chains are too long, the membrane-spanning channels are destablilized. When the phospholipid acyl chains are lengthened from 16 to 20 carbons, the average channel duration decreases from ~5,000 to ~50 ms (N. Mobashery, C. Nielsen and O. S. Andersen, unpublished observations). If the acyl chain is increased to 22 carbons, the standard gramicidin channels become so destabilized that new phenotypes predominate (Nielsen et al., 1997). These results highlight the importance of hydrophobic matching between peptides and lipids (Owicki et al., 1978; Engelman and Zaccai, 1980; Mouritsen and Bloom, 1984; Mouritsen and Bloom, 1993). Taken together with the results of O'Connell et al. (1990), these results show that the tryptophan indole rings act as anchors that prefer the lipid head groups (over both the aqueous phase and the membrane interior).

In the case of alpha-helical WALP peptides and when the phospholipid and peptide lengths are matched (so that the peptides can span the acyl chain region), hydrophobic WALP peptides such as WALP17 (Figure 2) fold into α-helices with the helix axis in a transmembrane orientation (Figure 4).

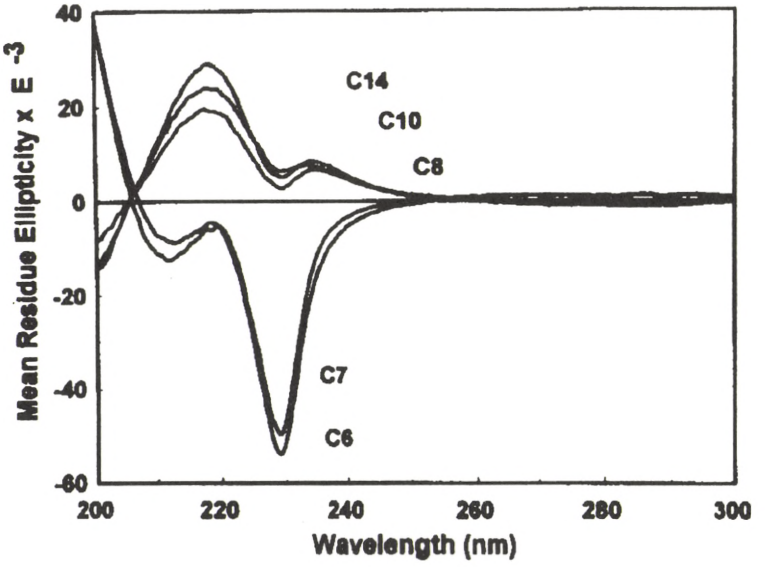

Figure 3. Circular dichroism spectra of aqueous gA/di-Cn-PC dispersions, showing characteristic RH SS $\beta^{6.3}$ channel spectra for $n = 8$, 10 or 14, and LH DS spectra for $n = 6$ or 7 (1 mM gA, 28 mM lipid, 55°C). Reproduced from Greathouse et al. (1994).

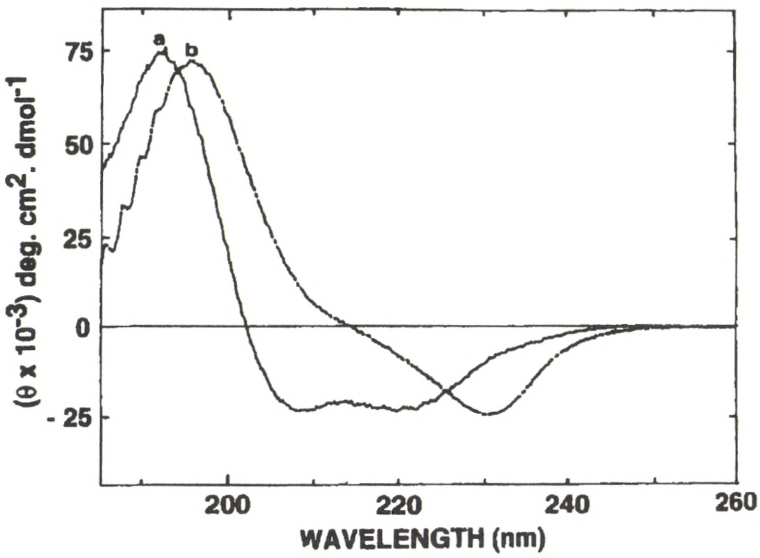

Figure 4. CD spectra of WALP17 in di-C14:0-PC at 1/25 peptide/lipid in (a) sonicated vesicles in excess water, and (b) oriented bilayers. From Killian et al. (1996).

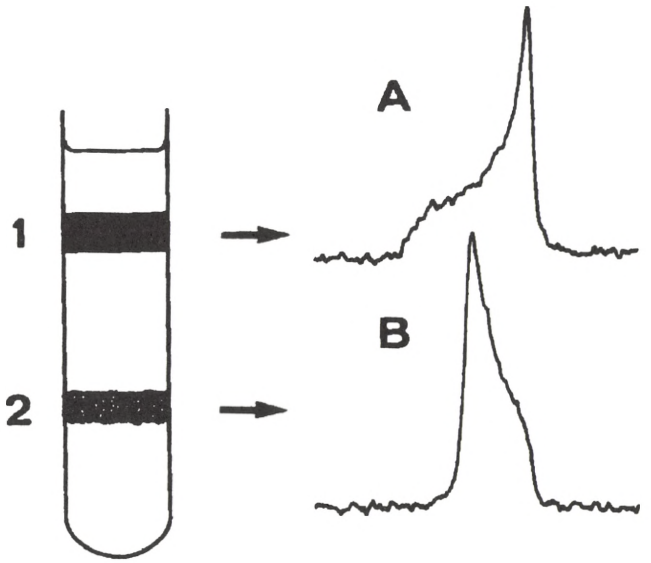

Figure 5. Illustration of the separation by sucrose density gradient centrifugation of a mixture of di-C18:1*c* PC and WALP16 into bilayer (A) and H_{II} components (B). From Killian et al. (1996).

Peptide Influence on Lipid Organization

Peptide-lipid interactions are reciprocal. When gramicidin or WALP17 is incorporated in phosphatidylcholine (PC) bilayers at high peptide/lipid molar ratios, the bilayer responds to a hydrophobic mismatch (in which the peptides are shorter than the lipid acyl chains) by forming non-bilayer structures (Van Echteld et al., 1982; Killian et al., 1989; Killian 1992; Killian et al., 1996). The separation of a peptide/lipid mixture into bilayer and inverted H_{II} phase components is illustrated for a WALP peptide in Figure 5.

Similar phase transitions have not been observed for other hydrophobic peptides in PC systems. The effect is therefore attributed to the presence of multiple tryptophans near the lipid/water interfaces (Killian et al., 1996; c.f., Figure 1). But we cannot at this time exclude that the peptide-induced phase transition is due to a combination of the anchoring effect of hydrogen bonding between the indole NH group and water, and the bulk of the indole ring *per se*, which may perturb the lipid packing.

Significance and Future Questions

The local lipid phase behavior around embedded proteins will affect biological processes that involve membrane rearrangement, fusion or separation. The mechanisms that regulate such processes remain enigmatic.

The tryptophan-anchored peptides, both the gramicidins and the WALP peptides, provide evidence that a build-up of bilayer energy, due to hydrophobic mismatch, can affect the structural organization of both lipids and the imbedded peptides. Remaining questions include:

A. What boundaries or sequence elements determine the effective peptide hydrophobic length? Is the length related to the distance between the two innermost amphipathic or polar side chains?

B. Will embedded helices tilt relative to the membrane normal– particularly, when the effective hydrophobic length of a transmembrane segment is longer than that of the lipids in the bilayer? How will that affect lipid-peptide interactions?

C. What is the minimum number of tryptophans required to induce the lipid phase transitions?

D. What is the relation between the density of membrane- spanning inclusions (proteins or peptides), the hydrophobic mismatch, and the propensity for the lipids to adopt nonbilayer structures?

E. To what extent, if any, is membrane protein function affected by the energetic cost of a hydrophobic mismatch? Again, experiments with model systems that adopt defined conformation will be important. The gramicidin derivative gLW, for example, assumes three different functional membrane-spanning conformations (Koeppe and Andersen, 1996). More examples will be needed.

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