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Ionization-Dependent Behavior of Transmembrane Helices that Incorporate Glu or Tyr Residues
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GWALP23 (acetyl-GGALW^{5}LALALALALALW^{19}LAGA-amide) is a constructive low-dynamic model peptide for investigations of single-residue influence on protein-lipid interactions and the properties of membrane-spanning helices (J. Biol. Chem. 285, 31723). To investigate the pH dependence, ionization behavior and orientational constraints when potentially negatively charged glutamic acid side chains are present, we have substituted a single Leu residue with Glu at different positions and incorporated specific ^{2}H-Ala labels in the core GWALP23 or Y^{5}GWALP23. Solid state NMR experiments show well defined ^{2}H-Ala quadrupolar splittings for Y^{5}GWALP23-E14 over the pH range of 6 to 10, suggesting that the peptide helix is well oriented in DLPC and DOPC lipid bilayers. Spectral changes are evident above pH 11 (in ether-lipid bilayers), but the titration of the Glu is uncertain because the Tyr residue itself appears to titrate around pH 11.5. GWALP23-E14 shows no spectral changes over the pH range 6.0 to 11.5, yet a change in quadrupolar splittings is observed at pH 13. The combined results suggest that the Glu residue may titrate with a pK_a near 12. In bilayers of DLPC and DOPC, GWALP23-E16 shows similar trend and is suggested to have a pK_a of around 12. The rather modest shifts in the ^{2}H quadrupolar splittings, nevertheless, suggest that the orientation of the transmembrane helix actually may change rather little at high pH. It is conceivable that the close proximity of either E14 or E16 to W19 could provide stability to the neutral peptide helix and perhaps influence the results. We additionally are investigating the possibilities for helix unwinding near the ends of these peptides.

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Detection of Helix Fraying in Designed Transmembrane Alpha Helices
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Transmembrane helices of integral membrane proteins often have aromatic residues flanking them. The aromatic residues favor locations within the membrane–water interface of a lipid bilayer and may serve as anchors to aid the stabilization of a tilted transmembrane orientation. To further understand the influence of Tyr, Trp or Phe residues upon the properties of helical membrane proteins (see Biochemistry 53, 3637), we have investigated the possibility of partial unwinding near the ends of selected transmembrane helices. For this purpose, we substituted positions 4 and 5 with either two Phe or two Ala residues to generate F^{4,5}GWALP23 (acetyl-GGA**F**^{4}**F**^{5}(LA)$_6$LW^{19}LAGA-ethanolamide) or A^{4,5}GWALP23 (acetyl-GGA**A**^{4}**A**^{5}(LA)$_6$LW^{19}LAGA-ethanolamide), respectively. By incorporating specific ^{2}H-Ala labels at positions 3 and 21, we are able to compare the influence of interfacial Phe and Ala on the unwinding of the helix ends. Solid state NMR spectra of macroscopically aligned bilayer samples show well defined ^{2}H-Ala quadrupolar splittings for both F^{4,5}GWALP23 and A^{4,5}GWALP23, suggesting that the peptide helices are well oriented in DLPC, DMPC and DOPC lipid bilayers. We are also able to estimate the helix tilt from deuterium labeling of the core alanine residues. Geometric Analysis of Labeled Alanines (GALA) then shows unwinding at the terminals for both F^{4,5}GWALP23 and A^{4,5}GWALP23. It is conceivable that the helix fraying may be critical for the stability of the transmembrane helix orientation in the lipid bilayer membranes.

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Comparing Peptide-Lipid Interactions and Antimicrobial Activities of Peptides with Similar "Core" Lengths But Variable Arginine and Tryptophan Residues
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Antimicrobial peptides offer a potential alternative therapy option for the increasing problem of microbial drug resistance. To address this issue, ''hybrid'' peptides with total lengths intermediate between the surface-acting antimicrobial lactoferrin-derived LfB6 (RRWQWR-NH_2) and the inert model transmembrane α-helical peptide RWALP23 (acetyl-GRALW(LA)$_6$LWLARA-NH_2) have been designed with varying numbers of arginines, tryptophans and net positive charge, but with the same ''core'' length of five residues. The lipid interactions of the hybrid LfB-RWALP peptides having between 9 and 15 residues were investigated by circular dichroism, tryptophan fluorescence, and ^{31}P and ^{2}H solid-state NMR spectroscopy, and their antimicrobial activities assayed.

Peptide	Sequence
RRWWALP15	acetyl-GRRWWLALALWWRRA^{15}-NH_2
RRWWALP13	acetyl-RRWWLALALWWRR^{13}-NH_2
RWALP11	acetyl-GRWLALALWRA^{11}-NH_2
RWALP9	RWLALALWR^9-NH_2

Our results indicate that despite having the same core length, the peptides exhibit different conformations, different interactions with lipids, and different antimicrobial activities. While the 13-mer is α-helical in neutral and anionic lipid mixtures, the conformations of the shorter 11-mer and 9-mer vary considerably depending on the lipid environment. Fluorescence spectroscopy suggests that the tryptophan residues of all peptides are located at the membrane-water interface, with slightly varying depths of insertion into the lipid bilayer. Despite small perturbations to the phospholipid head groups, solid-state ^{31}P NMR spectra indicate that the lipids remain primarily in a bilayer phase. ^{2}H NMR spectra reveal that the two shorter peptides aggregate, while the two longer (and more helical) peptides align in neutral and anionic lipid mixtures. Although all four peptides exhibit similar antimicrobial activities, some variation is observed against gram negative and gram positive bacterial strains.

2810-Pos Board B240
Characterization of Maximin 3 Structure and Membrane Leakage
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Maximin 3 is a 27 amino acid cationic antimicrobial peptide that is derived from the skin secretions of the toad Bombina maxima. Maximin 3 has strong activity against a variety of bacteria (Gram-positive and Gram-negative), fungi, and viruses and is thought to cause toxicity by interaction with the plasma membrane. We previously quantified the binding affinity of Maximin 3 for lipid vesicles that mimic both bacterial and mammalian membranes and found that the peptide interacts more strongly with bacterial than mammalian models, in part due to the negative charge of these membranes.
In our current work we used Förster resonance energy transfer (FRET) and fluorescence leakage assays to further characterize this interaction. Specifically, we found that the end-to-end distance of the peptide as determined by FRET is consistent with an extended α-helical conformation, both in solution and when bound to bacterial and mammalian model membranes. Under the same experimental conditions, we found that Maximin 3 is capable of inducing leakage of rhodamine from vesicles that mimic bacterial membranes. These results are consistent with the hypothesis that the mechanism of Maximin 3 toxicity towards bacteria is due to a direct interaction between Maximin 3 and the outer or inner bacterial membrane. We further suggest that Maximin 3 adopts an extended α-helical conformation in the leakage-inducing state, though it is not yet clear if the peptide is monomeric or oligomeric under these conditions.

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Characterization of Membrane Interactions of Antimicrobial Lactoferricin Peptides with Central Residue Substitutions
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The rise in antibiotic-resistant bacteria has led to an active search for new and more effective antimicrobial drugs. A hexapeptide (LfB6: RRWQWR-NH_2) derived from the iron-binding protein lactoferrin exhibits antimicrobial activity (Tomita, *Acta Paediatr Jpn*, 1994, 36:585). A related heptapeptide produced in our lab, with 4 positively charged arginines and 2 methylated tryptophans (RR**MeW**Q**MeW**RR-NH_2; MeTrp-LfB7), exhibits enhanced activity against gram negative and gram positive bacteria. Substitutions of the central glutamine (Gln4;Q) residue that may alter peptide conformational flexibility are now being investigated. When Gln4 was changed to glycine (Gly;G) or proline (Pro;P), significant changes in peptide-membrane interactions were observed, although the antimicrobial activity was not increased. We now examine the effects of replacing Gln4 with gamma amino butyric acid (GABA), to introduce more flexibility; or D-Pro-Gly, to constrain the backbone into a β-hairpin turn (Stanger and Gellman, *J. Am. Chem. Soc.* 1998, *120:*4236). The increased positive ellipticity at ~230 nm observed in the CD spectrum of the D-Pro-Gly peptide in anionic membranes suggests significant Trp-Trp interactions. Tryptophan fluorescence emission spectra indicate that peptides with either GABA or D-Pro-Gly substitutions are more deeply buried in anionic membranes. Although the GABA and D-Pro-Gly peptides were both more active against gram negative (*E. coli*), compared to gram positive (*S. aureus*) bacteria, they were not as active as the Gln4 peptide. Investigations of the peptide-lipid interactions are being continued by means of solid-state ^{2}H and ^{31}P NMR spectra.