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# Membrane disruption ability of facially amphiphilic helical peptides

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*Received (in Columbia, MO, USA) 13th May 2002, Accepted 19th June 2002*
*First published as an Advance Article on the web 8th July 2002*

**A helical 14-residue peptide containing four polar, but uncharged, benzo-21-crown-7 side-chains aligned along one face induces significantly more vesicle leakage than analogous 21-mer or 7-mer peptides.**

Antimicrobial peptides are currently under active investigation as potential therapeutic agents.[1–3] Many of these peptides increase membrane permeability and appear to adopt α-helical conformations with polar peptidyl side-chains aligned along one face of the helix (*i.e.*, the helix is a facial amphiphile with a polar face and a non-polar face, as distinct to a head-tail amphiphile which has a polar head and a non-polar tail). Two limiting case mechanisms have been proposed to explain how leakage is achieved; namely the barrel-stave and the carpet-like mechanisms.[1,4,5] Briefly, the barrel-stave mechanism involves the perpendicular insertion and aggregation of a relatively small number of facial amphiphiles inside the membrane leading to a transmembrane pore or channel with a cylindrical, barrel-stave structure. The carpet-like mechanism requires the facial amphiphiles to initially associate, in a parallel orientation, with the outer membrane surface but after a concentration threshold is reached, toroidal-shaped pore formation occurs along with migration of some of the peptide to the membrane inner surface. At higher peptide concentrations, micellization occurs. In addition to pore shape, a carpet-like pore differs from a barrel-stave pore in that the polar face of the helical peptide remains associated with the polar lipid head groups that line the carpet-like pore surface. Although the literature contains more references to the barrel-stave model, recent studies indicate that the carpet-like mechanism may actually be the more common peptide-induced leakage pathway.[5] At present, a number of features remain to be clarified with each of these cooperative models and a better understanding of the structural features that promote either pathway is needed.

Here, we describe a series of synthetic helical peptides with uncharged polar residues along one face. Therapeutically, neutral peptides are rarely studied because antimicrobial activity is known to increase with cationic charge.† However, from a mechanistic perspective, an uncharged peptide family with membrane disruption activity has a number of useful features. In particular, it allows leakage studies to be conducted using peptide/vesicle systems that do not involve strong Coulombic interactions. This improves the chances of observing subtle structural effects that are due to other non-covalent interactions.

We have designed a series of synthetic helical peptides that are oligomers of a repeating unit with five leucine residues and two synthetic 21-crown-7-phenylalanines appropriately positioned so that the hydrophilic crown ethers align on one side of the helical axis (Fig. 1). Previously, we have shown that the helical 21-mer peptide ($n$ = 3) can span a bilayer membrane and act as an artificial ion channel.[6–9] The 7-mer peptide, consisting of one repeating unit ($n$ = 1), also transports cations but mechanistically it is an ion carrier and not an ion channel.[6] In this paper, we report that the 14-mer ($n$ = 2) interacts with bilayer membranes in a very different way; it is a powerful membrane disruption agent that promotes the leakage of large fluorescent dyes from vesicles, and hemoglobin from erythrocytes.

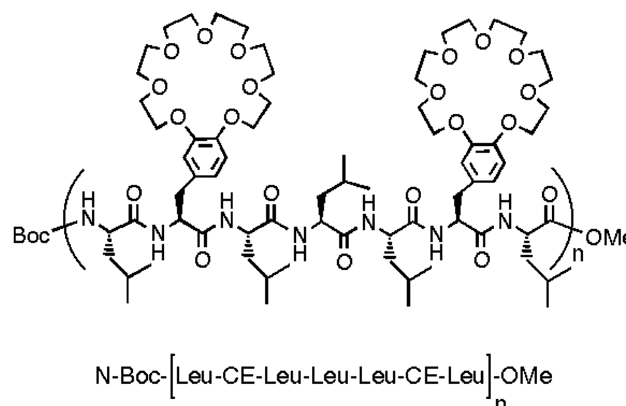


**Fig. 1** Two representations of the peptide structures, 7-mer ($n$ = 1), 14-mer ($n$ = 2), 21-mer ($n$ = 3), CE = 21-crown-7-phenylalanine.

Shown in Fig. 2 are the calcein leakage profiles induced by addition of 7-mer, 14-mer or 21-mer peptides to 100 nm unilamellar vesicles composed of egg phosphatidylcholine (egg-PC, lipid:peptide ratio is 70:1). As expected the 21-mer and 7-mer peptides induce very little calcein leakage but surprisingly the 14-mer induces rapid and significant leakage.

FTIR and CD spectra of the 21-mer[9] and 14-mer peptides in the presence of vesicles show that the peptides adopt helical conformations, and the following observations suggest that the 14-mer disrupts vesicle membranes *via* a carpet-like mechanism: (a) no leakage occurs until a 14-mer concentration threshold of 0.03 μM is reached after which leakage increases dramatically with peptide concentration. Also the leakage rate at any one peptide concentration slows with time (Fig. 2). Both kinetic features are signatures of the carpet-like mechanism, that is, a cooperative pore fomation process that dissipates as the peptide equilibrates across the membrane.[2] (b) Studies with deprotected versions of the 14-mer show that leakage is

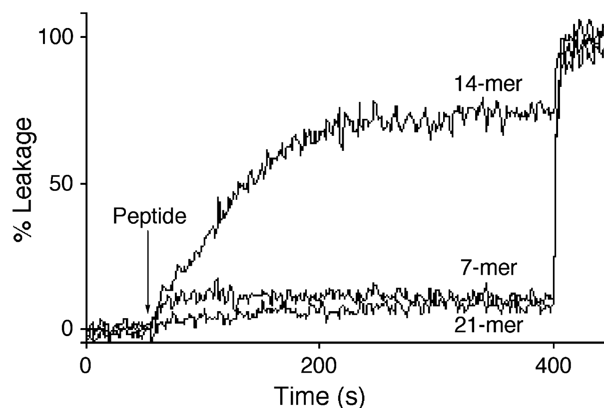


**Fig. 2** Calcein leakage induced by addition of 7-, 14-, or 21-mer peptides (0.72 μM) to egg-PC vesicles (50 μM lipid) in 10 MOPS/100 mM NaCl at pH 7.4. Vesicles lysed with detergent at 400 s.

DOI: 10.1039/b204640g


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essentially independent of charge at the peptide termini, suggesting that the peptide does not have to translocate through the lipophilic interior of the membrane. (c) The peptide works synergistically with the inverse-cone shaped lysophosphatidylcholine (LPC) to enhance leakage, whereas the cone-shaped 1-palmitoyl-2-oleoylphosphatidylethanolamine (POPE) dramatically inhibits leakage (Figs. 3 and 4).[10] (d) Dynamic light scattering shows that vesicle size does not change upon treatment with just enough 14-mer to induce leakage, suggesting that leakage is not caused by catastrophic destruction of the vesicles. (e) The calcein leakage rate hardly changes as the egg-PC concentration is increased from 50 μM to 500 μM (the phospholipid/peptide ratio maintained at 50 : 1). This suggests that leakage is not a vesicle contact mediated event, a conclusion that is confirmed by the lack of intervesicle membrane mixing seen with the fluorescent 1,2-dipalmitoyl-phosphoethanolamine-*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)/ 1,2-dipalmitoylphosphoethanolamine-*N*-(lissamine rhodamine B sulfonyl) (NBD-PE/Rh-PE) dilution assay.[11]

Most lytic peptides are composed of more than twenty amino acid residues, however, examples of shorter peptides with high activity are known.[3,12] Nonetheless, our finding that the 14-mer is much more disruptive than the 21-mer is notable. It appears that the difference in properties is due to a hydrophobic mismatch effect[13,14] that is unusually dominant in this uncharged peptide/phospholipid system. We propose that all three peptide lengths associate tightly with the vesicle membrane and that in each case a large fraction of the helical peptides adopt a

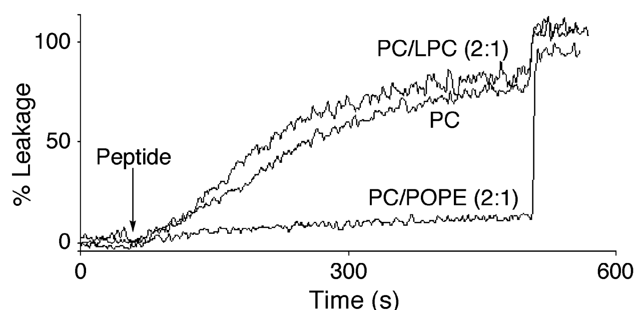


**Fig. 3** Calcein leakage induced by addition of 14-mer peptide (0.15 μM) to vesicles (50 μM lipid) in 10 MOPS/100 mM NaCl at pH 7.4. Vesicles lysed with detergent at 400 s.

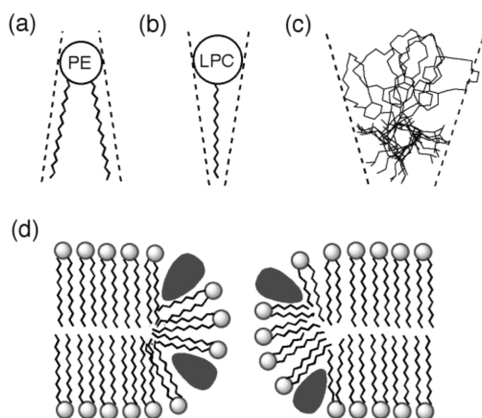


**Fig. 4** Cross-sectional views of: (a) Cone-shaped POPE, (b) Inverse-cone shaped LPC, (c) Inverse-cone shaped helical peptide with 21-crown-7-phenylalanine side-chains, (d) Toroidal-shaped pore formed during carpet-like leakage mechanism. The positive curvature of the pore surface is stabilised by inverse-cone shaped amphiphiles such as the 14-mer peptide or LPC.

parallel orientation along the membrane surface with their polar faces exposed to the aqueous solvent.[9,14] Although the peptides only contain one polar amino acid side chain per helix turn, the large size of the 21-crown-7 macrocycle imparts an inverse-cone cross-sectional shape which places positive curvature strain on the membrane (Fig. 4(c) and (d).[4] This helps drive a small fraction of the peptide population into the membrane interior. The 7-mer is short enough ( ~ 11 Å assuming a helix with 1.5 Å per residue) that it can be included inside the membrane (hydrophobic thickness of ~ 30 Å) without affecting lipid ordering. The helical 21-mer ( ~ 31 Å long) also does not greatly disturb the membrane but for a different reason; it is able to span the bilayer membrane and match its hydrophobic thickness. This allows the 21-mer peptide to function as an single ion channel.[6–9] In the case of the shorter 14-mer ( ~ 21 Å long) insertion into the membrane would lead to a significant peptide/membrane hydrophobic mismatch which is avoided by the membrane forming toroidal-shaped pores with the 14-mer oriented along the interior pore surface.[10] The positive curvature of the pore surface is stabilized by the inverse-cone cross-sectional shape of the amphiphilic peptide and is also sensitive to the shape of the constituent phospholipids (Fig. 4(d)).[2,4,15]

Our results illustrate how the combined effects of peptide/ membrane hydrophobic mismatch and peptide helix cross-sectional shape can determine the manner in which a facially amphiphilic helical peptide interacts with a bilayer membrane. A better understanding of these two supramolecular concepts should lead to improved designs of antimicrobial peptides.

This work was supported by the NIH (USA) and the NSERC of Canada. E. B. thanks the NSERC of Canada and the FCAR of Quebec for postgraduate scholarships.

## Notes and references

† Almost all known antibacterial helical peptides are positively charged which allows them to associate with the negatively charged bacterial membrane. Conversely, the outer monolayer of mammalian cells is composed primarily of zwitterionic phospholipids. It is not surprising that the 14-mer, an uncharged, amphiphilic peptide with moderate hydrophobicity, induces blood cell hemolysis (concentration threshold of 4 μM) and is unable to inhibit the growth of *E. coli*.

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