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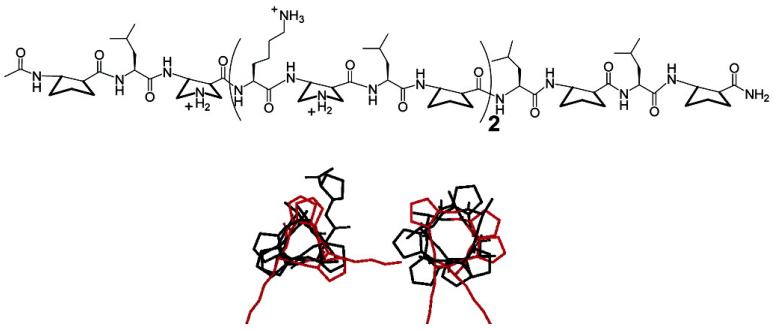

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Unexpected Relationships between Structure and Function in α,β-Peptides: Antimicrobial Foldamers with Heterogeneous Backbones

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The development of synthetic foldamers, oligomers with discrete folding propensities, provides an excellent opportunity to explore relationships among covalent structure, molecular shape, and function.[1] Oligoamide foldamers with alternating α-amino acid residues and cyclic β-amino acid residues have recently been reported by Reiser et al. and by us to adopt helical conformations.[2,3] Here we describe an effort to generate antimicrobial α/β-peptides based on this folding behavior. Our findings suggest that common design assumptions in this field may exclude productive possibilities.

Natural host-defense peptides such as the magainins and cecropins are potent antibiotics that can adopt globally amphiphilic α-helical conformations, with lipophilic side chains segregated along one side of the helix and hydrophilic side chains along the other side.[4,5] These host-defense peptides are cationic, which causes attraction to the anionic surfaces of bacterial cells. Lipophilic peptide surfaces are thought to interact with hydrocarbon portions of lipids, thus disrupting the bilayer and compromising the bacterial membrane barrier. Many unnatural α-peptide sequences display antimicrobial activity,[4−6] as do designed β-peptides,[7] peptoids (*N*-alkyl glycine analogues),[8] and other molecules that have been designed to display globally amphiphilic conformations. [9−11]

Knowledge of helical residue periodicity is required to design an α/β-peptide sequence that will form a globally amphiphilic helix. Our previous work[3] indicated that β-residues with a five-membered ring constraint lead to short α/β-peptides that equilibrate between two internally H-bonded helices: the 11-helix (the numeral indicates the number of atoms in the H-bonded ring), with ca. 3 residues per turn, and the 14/15-helix, with ca. 4.5 residues per turn (Figure 1). We designed three sequence isomers, **1**−**3**, as potential antibiotics; similar lengths and cationic/lipophilic proportions have been successful among β-peptides.[7] For **1**, 11-helix formation would lead to discrete lipophilic and hydrophilic surfaces, but the 14/15-helix would not display global amphiphilicity (Figure 2). The situation is reversed in **2**: the 14/15-helix would be globally amphiphilic, but the 11-helix would not. In **3**, neither helical conformation would display global amphiphilicity.

Reversed-phase HPLC was used for initial assessment of designs **1**−**3**. Longer retention time is expected to correlate with a greater propensity to adopt a globally amphiphilic conformation. Such correlations have been observed among helical α-peptides,[12] and we have demonstrated similar behavior among helical β-peptides.[13] α/β-Peptides **1**−**3** were very well resolved by RP-HPLC.[14] As expected, the "scrambled" isomer **3** was least retained. The large difference between **1** and **2**, with **2** more strongly retained, suggests that the 14/15-helix is preferred relative to the 11-helix at this α/β-peptide length.

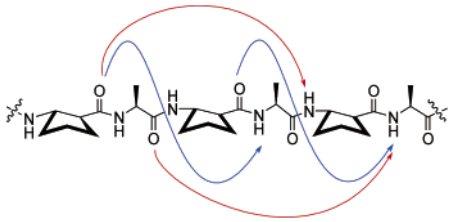

Figure 1. Hydrogen-bond patterns that define the helical secondary structures used to design potential antimicrobial peptides, with hydrogen bond from carbonyl groups to amide protons in the C-terminal direction. Blue arrows define the 11-helix; red arrows define the 14/15-helix.

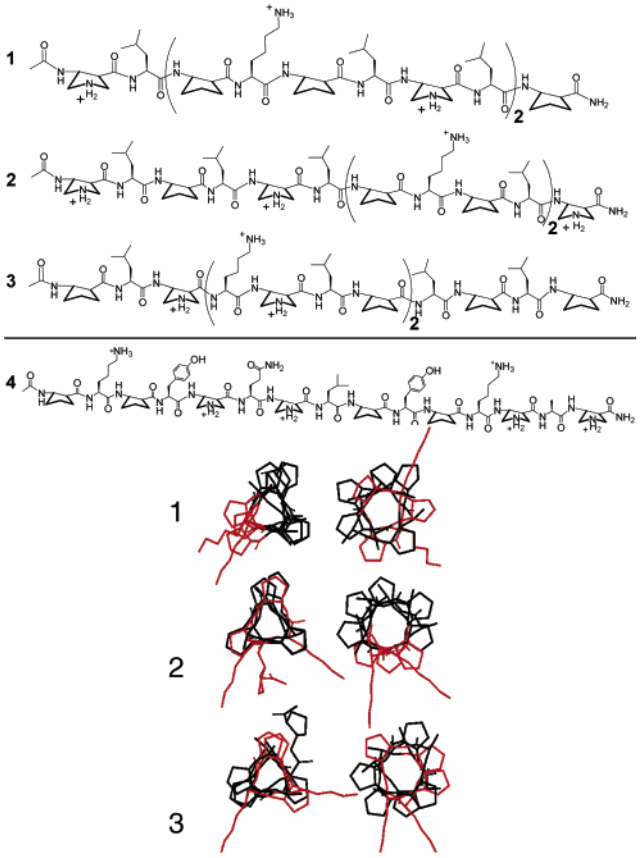

Figure 2. Axial views of predicted conformations (11-helix, left; 14/15-helix, right) for designed antimicrobial peptides **1**−**3** are shown. Residues colored red are positively charged.

The antimicrobial properties of **1**−**3** were assessed with four species (Table 1), including pathogens resistant to conventional antibiotics.[15] A modified host-defense peptide, Ala[8,13,18]-magainin-II amide, served as a positive control.[16] We were surprised to discover that **2**, designed to be globally amphiphilic in the 14/15-

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Table 1. Antimicrobial and Hemolytic Activities of Ala[8,13,18]-Magainin II Amide and α/β-Peptides (μg/mL)[13]

	E. coli	*B. subtilis*	*E. faecium*	*S. aureus*	max. concn without hemolysis
magainin	12.5	3.1	50	50	25
1	12.5	3.1	3.1−6.3	3.1	3.1
2	>100	6.3	25	50	1.6
3	6.3	6.3	6.3−12.5	12.5	50

helical conformation, is least active among the three α/β-peptide isomers. Both **1**, designed to be globally amphiphilic in the 11-helical conformation, and **3**, designed *not* to be globally amphiphilic in either helical conformation, are more active than **2** against the one Gram-negative species in our panel, *Escherichia coli*, and against the pathogenic *Enterococcus faecium* and *Staphylococcus aureus* strains.[17−19] The relatively high activity of **3** (comparable or superior to the magainin derivative against all four species) is particularly noteworthy in light of literature precedents on "scrambled" sequences among α- and β-peptides. Giangaspero et al. compared a 19-residue α-peptide designed to adopt a globally amphiphilic α-helix with a scrambled isomer; the latter displayed diminished activity relative to the former against a wide range of bacteria.[20] We have found even starker differences between β-peptides designed to form globally amphiphilic helices and their scrambled isomers, with the latter much less active.[7f,13]

Host-defense peptides are generally selective for killing prokaryotic cells relative to eukaryotic cells; in contrast, other peptides capable of forming amphiphilic α-helices, such as melittin, display indiscriminant toxicity.[21] Eukaryotic cell toxicity is often evaluated by monitoring human red blood cell lysis ("hemolysis"). We found that α/β-peptides **1** and **2** are at least as hemolytic as melittin, but scrambled isomer **3** is much less hemolytic and comparable to the magainin analogue in this regard (Table 1). This hemolysis trend, in contrast to the antimicrobial activity trend among **1**−**3**, parallels the effects of sequence scrambling on hemolytic behavior among helix-forming α- and β-peptides.[7f,13,20]

The unexpected finding that **2**, the sequence designed to be globally amphiphilic in the 14/15-helix conformation, is less toxic toward most bacteria than is the scrambled sequence, **3**, could indicate that the preference for 14/15-helical secondary structure inferred from RP-HPLC data (vide supra) is incorrect. NMR analysis of **1**−**3** was unsuccessful because of poor ^{1}H resonance dispersion, but nearly all backbone resonances of 15-mer **4** could be assigned in water and in methanol. Numerous *i*,*i*+*3* NOEs were observed for **4**, many of which are consistent with either the 11-helical or the 14/15-helical conformation. However, molecular modeling suggests that α-residue C_αH (*i*) → β-residue C_αH (*i*+*3*) NOEs should be observed only for the 14/15-helix (i.e., not for the 11-helix).[3] Five of the seven possible NOEs of this type are observed for **4** in CD_3OH, a structure-promoting solvent, which suggests that the 14/15-helix is the preferred conformation. These NOEs are seen in water as well, but poorer resonance dispersion renders identification ambiguous in some cases.[22] Furthermore, α-residue C_αH (*i*) → α-residue NH (*i*+*2*) NOEs are not expected for the 14/15-helix but should appear for the 11-helix (these NOEs are observed for shorter analogues of **1**−**4** (ref 3)); such *i*,*i*+*2* NOEs are not observed for **4** in methanol or water, which argues against significant population of the 11-helix. The conclusion that **4** (and by extension **1**−**3**) favors the 14/15-helix relative to the 11-helix is supported by observation of a few *i*,*i*+*4* NOEs that are predicted only for the 14/15-helix.[22] The contrast between these data and previous NMR studies of shorter α/β-peptides suggests that lengthening favors the 14/15-helix relative to the 11-helix.[3] This behavior supports the previously proposed analogy between helix formation in this class of α/β-peptides and in α-peptides,[3] because increasing length favors the α-helix (13-membered ring H-bonds) relative to the 3_{10}-helix (10-membered ring H-bonds).[23]

Among α/β-peptides **1**−**3** the most favorable behavior, high antimicrobial activity plus low hemolytic activity, is observed for scrambled isomer, **3**, and the least favorable behavior is observed for the isomer that is globally amphiphilic in the preferred 14/15-helical folding pattern, **2**. This result stands in contrast to the effects of sequence scrambling in α- or β-peptides.[7f,13,20] Antimicrobial oligomer design strategies have largely focused on the generation of globally amphiphilic conformations,[7−9] but our findings suggest that such approaches may unnecessarily limit the scope of the structures that are selected for evaluation.

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Supporting Information Available: NMR data and biological assay procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) HPLC conditions: C_8-silica analytical column; solvents: A: H_2O/0.1% TFA, B: 80% acetonitrile/20% H_2O/0.1% TFA; gradient: 1%B/min (20−60% B over 40 min); elution profile: **1**: 46.1% B; **2**: 55.4% B; **3**: 38.8% B.
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