Helical Peptoid Mimics of Magainin-2 Amide

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We report the design and synthesis of several helical, cationic, facially amphipathic peptoid (oligo-N-substituted glycine) mimics of magainin-2 amide (Chart 1). Certain compounds have potent and, in some cases, selective (nonhemolytic) antibacterial activity (Table 1). Peptoids are nonnatural, sequence-specific peptidomimetic oligomers based on a protein-like backbone, but with a side chain appendage at the amide nitrogen. Additionally, peptoids can adopt a stable helical structure, resist proteolytic degradation, and are being developed for use in a variety of biological applications.1,3 Peptoids are readily synthesized by a solid-phase submonomer protocol12 and were all purified to homogeneity by RP-HPLC. The mass of each purified compound was confirmed by ESI-MS. Oligomers are numbered in order of formation by peptoids with bulky-α-chiral aromatic side chains.14 This helix has a structure similar to that of the type-I polyproline helix, with cis-amide bonds, ~3.0 residues per turn, and 6.0 Å pitch.15 Peptoids are readily synthesized by a solid-phase “submonomer” methodology12 and are amenable to combinatorial approaches.13

It was previously found that peptoids containing certain bulky α-chiral aromatic side chains exhibit remarkably stable helical structure.2,7–9 Recently, several similar helices are also formed by peptoids with bulky α-chiral aliphatic side chains.14 This helix has a structure similar to that of the type-I polyproline helix, with cis-amide bonds, ~3.0 residues per turn, and 6.0 Å pitch.15 To predispose peptoid magainin mimics to adopt a helix, most sequence designs incorporate $\frac{1}{3}$ α-chiral aromatic (S)-N-(1-phenylethyl)glycine (Nspe) residues as part of a total of $\frac{2}{3}$ hydrophobic α-chiral residues.9 All sequences include a lysine-like N-(4-aminobutyl)glycine (Nly) at every third position to provide a cationic, facially amphipathic helix and water solubility.

Peptoids 1–7 were synthesized on Rink amide resin via a solid-phase submonomer protocol12 and were all purified to >97% homogeneity by RP-HPLC. The mass of each purified compound was confirmed by ESI-MS. Oligomers are numbered in order of increasing HPLC elution times, indicating increasing molecular hydrophobicity (Table 1). To most closely mimic the spatial arrangement of side chains exhibited by magainin-2,16 2–4 include three types of helical faces (cationic hydrophilic, aliphatic lipophilic, and aromatic lipophilic faces) created by incorporating a repetitive sequence of Nly’s, (S)-N-(sec-butyl) glycine (Nsnb), and Nspe monomers, respectively. Peptoids 5 and 6 are 12- and 17-mer variants, respectively, of a simple, repetitive sequence motif containing $\frac{2}{3}$ α-chiral aromatic Nspe and $\frac{1}{3}$ αchiral cationic Nly’s. Due to relatively high Nspe content, 5 and 6 were anticipated to be most predisposed toward helix formation.7,9 In contrast, 1 and 7 contain no aromatic groups, yet they still include $\frac{2}{3}$ bulky α-chiral side chains and retain the same residue patterning as 2–6 and were anticipated to be helical. We studied peptoids between 12 and 17 monomers in length because their helical conformation was predicted to be similar in length to the α-helical conformation of magainin-2 (i.e., 24–34 Å).15

We used circular dichroism (CD) spectroscopy to assess the folded structure of 1–7 both in aqueous buffer and in bacterial membrane-mimetic lipid vesicles (Figure 1a–c).17 In neutral buffer, 2–6 exhibit spectra characteristic of Nspe-containing peptoid helices.9 1 and 7 give rise to weak CD spectra in buffer, reminiscent of that of a peptide random coil. In vesicles, the spectrum of 7 is similar to that in buffer, but 1 gives a more intense spectrum that resembles that of a polyproline type-I helix and peptoid helices with α-chiral aliphatic side chains.14 We are currently conducting a more comprehensive investigation of the folding behavior of 1 and 7 in the presence of a variety of lipid vesicles and organic solvents to better explain these results. Overall, 5 and 6 exhibit more intense CD signals (θ215) than do 2–4, which is likely due...
to their greater Nspc content.9 Similar to magainins, spectra of 1–6 are more intensely helical in vesicles than in aqueous buffer alone.

Antibacterial activities of 1–7 were measured using a broth dilution assay, in which Gram-negative E. coli JM109 and Gram-positive B. subtilis BR151 were cultured in LB media (Table 1). We defined the minimum inhibitory concentration (MIC) as the lowest peptide concentration to completely inhibit bacterial growth during a 12 h incubation at 37 °C. Overall, peptoid 12mer 5 exhibits the most potent antibacterial activity against both species of bacteria tested, with low-micromolar MICs. Interestingly, 1 and 7, which exhibit weak CD spectra in buffer, exhibit no detectable antibacterial activity. Effective antibacterial peptoids (2–6) are all more active against Gram-positive bacteria, similar to magainin-mimetic antibacterial β-peptides.4

We also determined peptoid selectivity as gauged by their hemolytic activity against both Gram-positive and Gram-negative bacteria. Similar in length to the magainin-2 helix (~34 Å) and the length necessary to span a POPE/POPG lipid bilayer. We are currently investigating more compounds, 6–17 monomers in length, to more fully characterize the length effect. In any case, there may be a minimum length of about 12 residues for appreciable antibacterial activity, as 6mer and 9mer analogues of 2 were ineffective (E. coli MICs > 200 μM, data not shown).

In conclusion, this is the first report of water-soluble, helical peptoid mimics of magainin antibacterial peptides and, more generally, the first report of a structured, bioactive peptoid. Certain short (12–17mer) peptoids exhibit selective, potent antibacterial activity against both Gram-positive and Gram-negative bacteria. Antibacterial and hemolytic activities of peptoids 3 and 5 are comparable to previously reported results using a synthetic magainin analogue and bacterial β-peptides.4 These readily synthesized, protease-resistant peptoids represent an important advance in peptide biology, the development of nonpeptide foldameric analogues of antibacterial peptides.

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Supporting Information Available: Synthesis and HPLC information, vesicle preparation, assay protocols (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References