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COMMUNICATIONS

Published on Web 09/16/2003

Helical Peptoid Mimics of Magainin-2 Amide

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Nature abounds with peptides that exert important biological activities. The possibility of harnessing these activities for new pharmaceutical and biomaterial applications has motivated research in which nonnatural mimics of bioactive peptides have been studied.[1] Helical peptidomimetic oligomers (foldamers) with a structure similar to that of linear, cationic, facially amphipathic helical antibacterial peptides such as magainins have garnered particular interest.[2,3] For example, certain amphipathic β-peptide helices are comparable to magainins in antibacterial activity and selectivity.[4,5] Facially amphipathic polyarylamides have also shown promising activity, but are hemolytic.[6] Yet, no other nonpeptide foldamer has demonstrated similar, let alone selective, activity.

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,[2,7−9] resist proteolytic degradation,[10] and are being developed for use in a variety of biological applications.[1,11] Peptoids are readily synthesized by a solid-phase "submonomer" methodology[12] 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, we have shown 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 $^1/_3$ α-chiral aromatic (*S*)-*N*-(1-phenylethyl)glycine (*N*spe) residues as part of a total of $^2/_3$ hydrophobic α-chiral residues.[9] All sequences include a lysine-like *N*-(4-aminobutyl)glycine (*N*Lys) 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 protocol[12] 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 *N*Lys, (*S*)-*N*-(*sec*-butyl) glycine (*N*ssb), and *N*spe monomers, respectively. Peptoids **5** and **6** are 12- and 17-mer variants, respectively, of a simple, repetitive sequence motif containing $^2/_3$ α-chiral aromatic *N*spe and $^1/_3$ achiral cationic *N*Lys. Due to relatively high *N*spe 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 $^2/_3$ 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]

Chart 1. Peptoid Mimics of Magainin-2 Amide

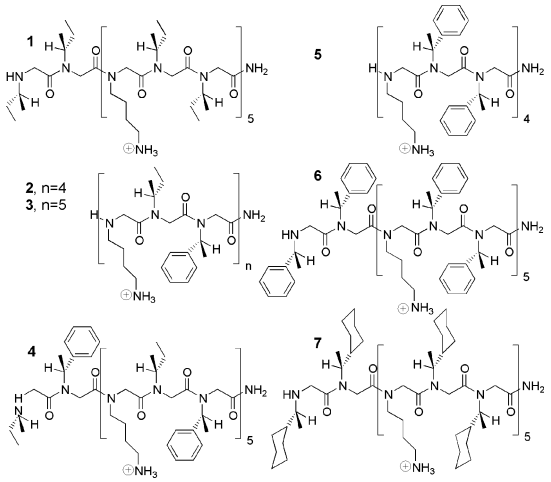

Table 1. Peptoid Antibacterial and Hemolytic Activities

	HPLC elution solvent[a]	*E. coli* MIC[b] μM (μg/mL)	*B. subtilis* MIC[b] μM (μg/mL)	hemolysis at *E. coli* MIC
1	38.9%	>100 (>200)	>100 (>200)	0%
2	39.2%	49 ± 8.3 (80)	7.8 ± 1.3 (13)	0%
3	42.0%	9.9 ± 2.5 (20)	4.4 ± 0.8 (8.9)	0%
4	45.5%	19 ± 4.9 (44)	1.4 ± 0.2 (3.2)	1.2%
5	46.2%	5.4 ± 0.9 (9.8)	0.82 ± 0.14 (1.5)	1.4%
6	57.5%	7.7 ± 1.3 (20)	1.2 ± 0.2 (3.1)	51%
7	83%	>75 (>200)	>75 (>200)	100%[c]

[a] Vydac C4 column. Percent solvent B in A. Solvent A: 0.1 vol % TFA in H_2O. Solvent B: 0.1% (v/v) TFA in CH_3CN. [b] With estimated uncertainty. [c] Reported at 200 μg/mL (~75 μM).

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 *N*spe-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 (Θ_{218}) than do **2**−**4**, which is likely due

10.1021/ja037320d CCC: $25.00

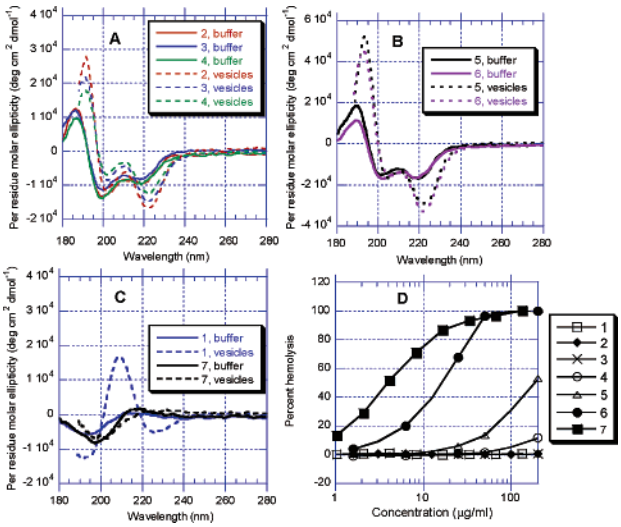

Figure 1. CD spectra and hemolytic activities of **1**−**7**. For CD, peptoids were ∼50 μM in either 5 mM Tris-HCl buffer, pH 7.0 (buffer), or 5 mM POPE:POPG (7:3) vesicles, 10 mM Tris-HCl buffer, pH 7.0 (vesicles). (A) CD of **2**−**4**, buffer and vesicles. (B) CD of **5** and **6**, buffer and vesicles. (C) CD of **1** and **7**, buffer and vesicles. Most peptoids exhibit intensification of molar ellipticity in lipid vesicles, consistent with increased helical structure. (D) Hemolytic activities of **1**−**7** as the percentage erythrocytes lysed following a 1 h peptoid incubation. The most lipophilic peptoids are also the most hemolytic. Conversely, the most hydrophilic peptoids **1**−**4** exhibit negligible hemolysis at concentrations as high as 200 μg/mL.

to their greater *N*spe 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 peptoid 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 (Table 1, Figure 1d). Selectivity among cationic magainins arises from both preferential electrostatic attraction toward an anionic bacterial cell membrane and low molecular hydrophobicity.[18] Similarly, we found that relatively hydrophilic compounds **1**−**4** show negligible hemolysis at concentrations as high as 200 μg/mL. Hemolytic activity increases among increasingly lipophilic peptoids. For instance, **6** and **7** are both quite hemolytic, even at low concentrations.

Peptoid 12mer **5** is a selective (nonhemolytic) antibacterial peptoid at low concentrations (<10 μg/mL), yet rapidly becomes hemolytic at higher concentrations (Figure 1d). In contrast, 15mer **3** causes negligible hemolysis at concentrations up to 200 μg/mL, yet retains low-micromolar MICs only slightly higher than **5**. There seems to be a length-dependence of antibacterial activity, as the 15mer **3** has a lower MIC against *E. coli* than either the analogous 12mer **2** or the 17mer **4**. Interestingly, against *B. subtilis*, the 17mer **4** is most effective. Moreover, 12mer **5** has slightly lower MICs than the related 17mer **6**. Thus, the optimal length for antibacterial activity seems to depend on bacterial identity and the peptoid sequence, but remains between 12 and 17 residues. These lengths correspond to peptoid helices about 24−34 Å long, which are 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 antibacterial β-peptides.[4] These readily synthesized, protease-resistant peptoids represent an important advance in peptide biomimicry, the development of nonpeptide foldameric analogues of antibacterial peptides.

Acknowledgment. We thank David M. Steinhorn, Samuel H. Gellman, Ronald N. Zuckermann, Cindy W. Wu, and Tracy J. Sanborn for their help. We acknowledge use of instruments at the Keck Biophysics Facility at Northwestern University. J.A.P. was supported by a NIH Molecular Biophysics Training Grant (NIH #5 T32 GM08382-10). We also acknowledge grants from Northwestern IBNAM and the Dreyfus Foundation, and a DuPont Young Investigator Award.

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.

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JA037320D