Mimicry of Antimicrobial Host-Defense Peptides by Random Copolymers

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The eukaryotic innate immune response to bacterial infection includes the production of peptides that kill prokaryotic invaders.1 These “host-defense” peptides can be grouped into several structural classes, and their mechanisms of antibacterial action are varied. Many host-defense peptides are thought to act by disrupting bacterial membranes. Members of one widely studied class are induced by target membranes to adopt α-helical folding patterns.2 These conformations are globally amphiphilic: discrete patches of lipophilic and hydrophilic side chains are projected from opposite sides of the helix (Figure 1A). Examples include cecropins3 (from insects), magainins4 (from amphibians) and cathelicidins5 (from mammals). The antibacterial activity of these helix-forming peptides appears to depend on the overall spatial segregation of lipophilic and cationic side chains rather than on the specific identities of the side chains, a characteristic that has inspired the exploration of analogues containing nonproteinogenic α-amino acid residues6 or subunits other than α-amino acid residues.7 All of these oligomers have been synthesized in step-by-step fashion, so that the sequence of hydrophilic and lipophilic subunits would give rise, upon adoption of a specific and regular conformation, to a globally amphiphilic molecular surface (Figure 1A). Host-defense peptide mimics have considerable therapeutic potential as complements to conventional antibiotics because it is difficult for bacteria to evolve resistance to the membrane-disruption mode of action;1 however, the cost of producing sequence-specific oligomers represents a significant stumbling block to their use.1c

Here we show that functional host-defense peptide mimics can be created on the basis of a conformational hypothesis that is quite different from classical helix-induction. Instead, we propose that flexible, sequence-random oligomers or polymers containing cationic and lipophilic subunits can be induced by a bacterial membrane surface to adopt irregular conformations that result in global amphiphilicity (Figure 1B). This hypothesis represents a significant expansion in our understanding of structure–activity relationships among antibacterial oligomers and polymers. In addition, this hypothesis has important practical consequences because it is far easier to prepare random copolymers than to synthesize sequence-specific oligomers. We show that materials generated via ring-opening copolymerization of β-lactams (±)-1 and (±)-2 (Scheme 1) match or exceed the growth-inhibiting effects of host-defense peptides toward several bacteria, including human pathogenic strains resistant to conventional antibiotics. These polymers can be tuned to display very low lytic activity toward human red blood cells (“hemolysis”) while retaining antibacterial potency, a profile that is characteristic of host-defense peptides. β-Lactam 1 can be prepared in large quantities via reaction of chlorosulfonyl isocyanate (CSI) with cyclohexene,8 and an analog-
Antibacterial and hemolytic activities of polymers 3, as a function of $y$ (i.e., the proportion of cationic subunits derived from $\beta$-lactam 2). The region with the greatest selectivity for bacteria relative to human red blood cells, between 60 and 65% cationic subunit, is shown in the expansion at the right. The minimum inhibitory concentration (MIC) is defined as the lowest polymer concentration that completely inhibits bacterial growth. The minimum hemolytic concentration (MHC) is defined as the lowest polymer concentration at which hemolysis is detected. The lines connecting the points are intended merely to guide the eye.

Table 1. Activities of Polymer 3$\alpha$ and Selected Peptides against Four Bacteria and Human Red Blood Cells

<table>
<thead>
<tr>
<th>Polymer</th>
<th>E. coli</th>
<th>B. subtilis</th>
<th>S. aureus</th>
<th>E. faecium</th>
<th>MHC ((\mu)g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>polymer 3$\alpha$</td>
<td>12.5</td>
<td>3.1</td>
<td>25</td>
<td>12.5</td>
<td>100</td>
</tr>
<tr>
<td>magainin 2</td>
<td>100</td>
<td>200</td>
<td>$&gt;400$</td>
<td>$&gt;400$</td>
<td>$&gt;400$</td>
</tr>
<tr>
<td>cecropin A</td>
<td>0.78</td>
<td>400</td>
<td>$&gt;400$</td>
<td>$&gt;400$</td>
<td>$&gt;400$</td>
</tr>
<tr>
<td>magainin-Ala$_3$</td>
<td>6.2</td>
<td>6.2</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

*represents averages for five independently synthesized batches, with at least six assays for each sample. GPC analysis of these five polymer samples indicated that they contained an average of 18 residues ($M_n$ varied between 3000, corresponding to ~16 residues, and 3800, corresponding to ~20 residues); PDI for the five samples fell in the range 1.3–1.4. MALDI MS data for the five samples were consistent with these GPC-based conclusions. The physical properties and biological activities of the five samples of 3$\alpha$ show that the polymerization reaction provides reproducible materials.

Table 1 shows that the three host-defense peptides are not active against the clinically derived strains of *S. aureus* and *E. faecium* we evaluated. The magainin derivative, on the other hand, is quite active against these pathogenic bacteria, consistent with the original report. Polymer 3$\alpha$ is comparable to this modified magainin in activity against the pathogens as well as against *B. subtilis* (a nonpathogenic species that is related to *B. anthracis*) and *E. coli* (nonpathogenic strain). Neither polymer 3$\alpha$ nor the magainin derivative achieves the high activities of the cecropins against *E. coli*, which is the only Gram-negative species in our panel. Overall, the MIC data indicate that random copolymer 3$\alpha$ is comparable in antibacterial activity to representative host-defense peptides, especially for Gram-positive species. Polymer 3$\alpha$ is superior to the magainin derivative in terms of hemolytic activity (MHC = 100 vs 25 \(\mu\)g/mL), but both are significantly more hemolytic than the natural host-defense peptides. Model studies involving large unilamellar vesicles (LUVs) with varying lipid content showed that 3$\alpha$ very effectively disrupts LUVs that mimic bacterial membranes but not LUVs that mimic red blood cell (RBC) membranes. Overall, these results are consistent with the hypothesis that polymer 3$\alpha$ selectively targets bacterial cells relative to RBCs, behavior that is a hallmark of host-defense peptides.

The data in Figure 2 suggest that hemolytic activity is very sensitive to copolymer composition when the cationic subunit proportion is 50–70%. We compared 3$\alpha$ to copolymers 3$\alpha_1$, 3$\alpha_2$, 3$\alpha_3$, 3$\alpha_4$, and 3$\alpha_8$ (Figure 2, expansion on right side) to identify an optimal balance of MIC and MHC. The MIC for *E. coli* rose from 12.5 to 100 \(\mu\)g/mL as the proportion of cationic subunit derived from 2 rose from 60% to 65%, and the MHC rose from 100 to 800 \(\mu\)g/mL, but the antibacterial activities for the three Gram-positive species showed little variation within this set. Polymer 3$\alpha_3$, with an MHC/MIC ratio of 32 for the pathogenic bacteria, demonstrates that substantial membrane selectivity can be achieved with this system. These results show that biological activities can be tuned, in some cases independently, via easily implemented modifications in polymer structure. The biological impact of other structural...
variables, including the identity of the monomers, average size, and N-terminal capping group, remains to be explored.

A $\beta$-lactam unit embedded within an imide occurs at the C-termini of polymers 3. Since some $\beta$-lactams exert antibacterial effects, we evaluated the activity of $\beta$-lactam imide (e)-4, generated by acylating the ring nitrogen and deprotecting the side chain of 2, against our panel of bacteria. In addition, we evaluated monomer 1. Neither compound displayed significant antibacterial activity up to a concentration of 400 µg/mL, and imide 4 decomposes rapidly. These observations suggest that the antibacterial activities of polymers 3 do not arise from the C-terminal imide/lactam unit.

Our results show that random copolymers can mimic the cell-type selectivity manifested by host-defense peptides. Specifically, the favorable activity profile displayed by copolymers 3 with 1:2 proportions near 40:60 suggests that the nontraditional hypothesis illustrated in Figure 1B constitutes a valid basis for design of host-defense polymers. The results reported here are consistent with the hypothesis that a polar polymer backbone is important for minimizing hemolytic activity.23a In this study, we evaluated the activity of polymers 3 with C-termini of polymers 3 containing either natural or unnatural $\beta$-lactams exert antibacterial effects, we evaluated the activity of $\beta$-lactam imide (e)-4, generated by acylating the ring nitrogen and deprotecting the side chain of 2, against our panel of bacteria. In addition, we evaluated monomer 1. Neither compound displayed significant antibacterial activity up to a concentration of 400 µg/mL, and imide 4 decomposes rapidly. These observations suggest that the antibacterial activities of polymers 3 do not arise from the C-terminal imide/lactam unit.

Acknowledgment. This work was supported by a Collaborative Research in Chemistry grant from the NSF (Grant CHE-0404704), and by the UW-Madison Nanoscale Science and Engineering Center (Grant NSF DMR-0425880). We thank Dr. M. F. Ilker for performing preliminary studies, Nathaniel J. Fredin for assistance with GPC measurements, and Alexandra H. Dillon for help with preparation of materials and bioassays.

Supporting Information Available: Polymer and peptide synthesis procedures and characterization data, U/V results, and bioassay protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(10) Manuscript in preparation.
(16) Many researchers quantify hemolytic activity in terms of the concentration required for 50% hemolysis (HC50); MHC is a more conservative parameter that we believe to be more appropriate. Polymer 3A induces ~20% hemolysis at 400 µg/mL, and the HC50 must be considerably higher. See Supporting Information.
(17) See Supporting Information.
(20) (a) Shai, Y. Biochim. Biophys. Acta 1999, 1462, 55–70.

JA077288D