Facially Amphiphilic Architectures as Potent Antimicrobial Peptide Mimetics: Activity and Biophysical Insight

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Introduction

In many cases, natural proteins exhibit elaborate self organization and precise placement of side chains. We have focused on the incorporation of cationic side chains into macromolecular architectures as an alternative to the many anionic systems reported. When the cationic and nonpolar side chains are positioned to give facially amphiphilic structures, these molecules are mimics of a class of naturally occurring peptides. These natural peptides belong to the host defense class and are the first line of defense for most organisms and the only defense of insects including moths. We report here new facially amphiphilic structures based on phenylene ethynylene (PE) backbones that contain polar side chains with primary amine functions and capture the essential biochemical properties of these host defense peptides [5].

An important consideration in the design of facially amphiphilic polymers is to match the hydrophobic period of the sequence with the conformation adopted by the backbone. Figure 1 illustrates two examples of how we design a polymer backbone to favor adoption of a facially amphiphilic structure. The top structure in Figure 1 adopts an extended conformation based on patterning polar (P) and nonpolar (NP) groups with a repeat of two. These structures are very reminiscent of β-strands and were found to organize at the air-water interface into assemblies similar to β-sheets. For example, these molecules stand with the aromatic ring perpendicular to the water surface and bury the charged amine group into the water while positioning the NP group away from the surface. They pack at a distance of ~4.0 Å by π-π stacking which would be replaced in β-sheets with hydrogen bonding.

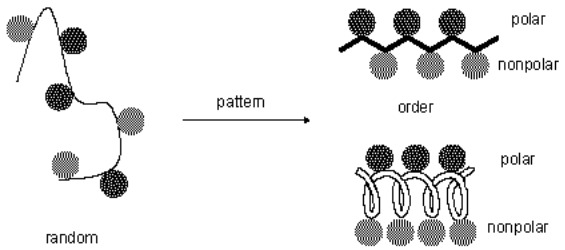

Figure 1. Placing P (blue) and NP (green) groups in the correct pattern to match the secondary structure of the polymer leads to facially amphiphilic structures.

Results and discussion

We recently designed a series of polymeric phenylene ethynylenes with both good activity and selectivity [1]. This report demonstrated that abiotic structures, and even polymeric materials, could be designed with antibacterial activity yet also be non-hemolytic. Polymeric systems with average molecular weight of 1,600 Daltons had an MIC of 50 μg/mL and HC_{50} of 540 μg/mL compared to the potent magainin analog, MSI-78, which had an MIC of 12.5 μg/mL and HC_{50} of 120 μg/mL. Although the polymeric analog was not quite as potent as MSI-78, it was just a selective based on a comparison of the HC_{50} and MIC values. This notion is now extended to discrete, small molecule oligomers with increased potency, better selectivity, broad spectrum activity, and significantly decreased bacterial resistance compared to ciprofloxacin and norfloxacin. These results demonstrate that the phenylene ethynylene oligomer has excellent biological activity and maybe a promising candidate for potential treatment of antibiotic resistant bacterial infections.

AMP mimic **1** was screened against a series of antibiotic-resistant bacteria and the results demonstrated potent activity of **1** was against antibiotic resistant *S. aureus, E. faecium, E. faecalis,* and *S. pneumoniae*. This includes potent activity against MRSA and VRE. The ability of **1** to remain potently active against a large series of antibiotic resistant bacteria is extremely encouraging.

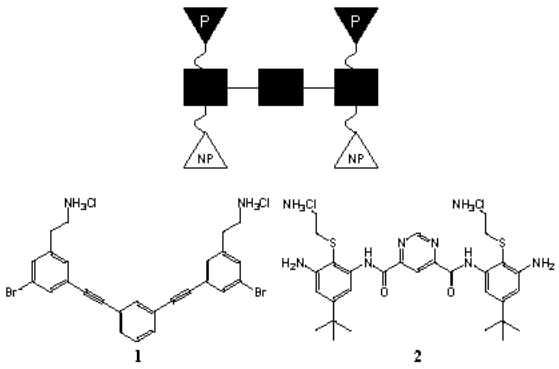

Figure 2. Design and molecular structure of facially amphiphilic foldamers.

Conclusions

The development of novel antibacterial agents is critically important. Designing abiotic oligomers with distinct polar and non-polar facers, similar to AMPs, is a powerful approach to creating broadly active antimicrobial agents. A unique phenylene ethynylene oligomer is reported here which shows activity against bacterial, yeast, and fungus. Because it is a non-natural backbone without amide or ester functionality, it will not undergo proteolytic degradation from enzymes, like natural AMPs. This oligomer is active against antibiotic-resistant bacteria and shows a lower propensity toward developing resistance in *S. aureus* than ciprofloxacin. The interaction of novel FA biomimetic molecules with phospholipid bilayers showed that the lipid headgroup and the concentration of lipid molecules within the bilayer have a significant impact on overall activity. PG-PE vesicles are significantly more sensitive to these molecules than the corresponding PS-PC vesicles, although the overall net charge is identical. Both leakage and lipid movement were more sensitive for PG-PE compared to PS-PC vesicles. *In vitro* experiments on *S. aureus* showed that these molecules are active against living bacterial membranes at the MIC concentrations and active on similar time scales to the vesicle experiments. This provides good support for using model membranes to investigate lipid interactions of these novel molecules. Integrating the physiochemical studies with *in vivo* activity is a significant challenge; however, a deeper understanding of the molecular mechanism will provide critical clues to this problem. Bringing a variety of analytical tools to this problem is sure to provide new, fundamental insight. The broad spectrum activity, potency, and selectivity of **1** strongly suggest this structure is a promising AMP mimic.

References

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