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Amphiphilic Polymethacrylate Derivatives as Antimicrobial Agents

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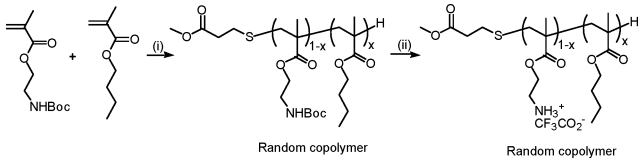
The membrane-disrupting ability of amphiphilic synthetic polymers has been utilized in preparing chemical disinfectants and biocides.[1] A number of polymeric disinfectants have been prepared using conventional synthetic polymers, including poly(vinyl pyridine)s,[2] poly(vinyl alcohol)s,[3] polyacrylates,[4] and polystyrenes.[5] Their amphiphilic structures disrupt cell membranes,[1] causing breakdown of the transmembrane potential, leakage of cytoplasmic contents, and ultimately cell death. The cooperative action inherent in polymeric structures enhances this disruption mechanism as compared to small amphiphilic molecules, such as surfactants.[6] However, one of the major drawbacks of polymeric disinfectants is a lack of selectivity for bacterial over human cells, limiting their clinical and medicinal utility.

In this report, we take steps toward the development of nontoxic antimicrobial synthetic polymers in our investigation of the structure−activity relationship of amphiphilic polymethacrylate derivatives as measured via antimicrobial and hemolytic activities. Free radical copolymerizations of *N*-(*tert*-butoxycarbonyl)aminoethyl methacrylate and butyl methacrylate (BMA) were conducted using mole percentages of BMA from 0 to 60% in the presence of methyl 3-mercaptopropionate (MMP) to give a precursor polymer protected with *tert*-butoxycarbonyl (Boc) groups (Scheme 1). In this polymerization, MMP served as a chain transfer agent to control the degree of polymerization (DP)[7] and allowed us to prepare low molecular weight (MW) polymers with relatively high yields, avoiding the necessity of time-intensive fractionation of polymers by column chromatography. The subsequent treatment of the Boc-protected polymer with TFA afforded the desired cationic random copolymer. Natural host defense peptides are believed to selectively target bacterial cells while remaining nontoxic to the host due to preferable charge interactions between the dense population of negatively charged lipids on bacterial cell surfaces and the cationic side chains of the peptides;[8,9] we have similarly incorporated cationic functionality into the polymer framework to improve selectivity. By alternating MMP concentrations, we obtained a series of polymers of three different MW ranges displaying a wide range of mole percentages of BMA (MP_{Bu}) (0−60%) (Table 1).[10]

Antimicrobial activity of the polymers was tested using turbidity-based assays in Mueller−Hinton broth with *Escherichia coli* D31, and the minimum inhibitory concentration (MIC) was determined as the lowest polymer concentration to completely inhibit bacterial growth after an 18 h incubation period at 37 °C. The MICs for all series decreased as MP_{Bu} increased up to ∼30%, at which point further increase of the butyl functionality did not affect the MIC (Figure 1). The smallest polymer series, **3**, further demonstrated the lowest MIC of 16 μg/mL above ∼30% MP_{Bu}, which is comparable with that of the bee venom toxin peptide, melittin (12.5 μg/mL). The increased activity with increasing MP_{Bu} suggests that the hydrophobicity of polymers is a major consideration in the antimicrobial action of polymers. As the polymers become more hydrophobic, incorporation of polymers to lipid membranes is enhanced, and thus the integrity of membrane is more efficiently disrupted. However, further increases in MP_{Bu} create hydrophobic polymers more likely to undergo a collapse of the polymer chain in water or irreversible aggregation with components of the assay medium, preventing antimicrobial action. It has also been reported that aggregation of antimicrobial peptides reduces their antimicrobial potency.[11]

To examine the effect of increasing hydrophobicity on the solution properties of the polymers, their solubility in the assay medium was measured using the growth media in the absence of

Scheme 1. Synthesis of Amphiphilic Polymethacrylate Derivatives[a]



[a] Conditions: (i) methyl 3-mercaptopropionate, AIBN, acetonitrile, 60 °C, overnight; (ii) neat TFA, rt, 1 h.

Table 1. Characterization of Amphiphilic Polymethacrylates

series	[MMP]/[monomers][a]	MP_{Bu}[b]	DP[b]	MW range[c]
1	0.05	0−57	32−46	7900−10100
2	0.10	0−53	19−31	4500−6000
3	0.50	0−47	5−9	1300−1900

[a] Concentration ratio of MMP to total amount of monomers in polymerizations. [b] Determined by ^{1}H NMR. [c] Calculated from MP_{Bu} and DP.

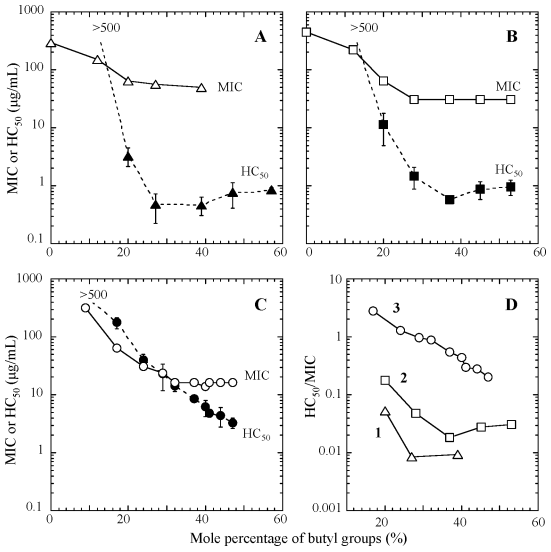


Figure 1. Antimicrobial and hemolytic activities of polymethacrylate derivatives: (A) polymer series **1**; (B) **2**; and (C) **3**. MICs and HC_{50}s presented as opened and closed marks, respectively. (D) Selectivity index (HC_{50}/MIC).

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bacteria.[10] The solubility limits of series **1** and **2** decreased from 60 to 16 μg/mL as MP_{Bu} increased from 20 to 60%. In contrast, polymer series **3** is soluble in the medium up to at least 60 μg/mL as MP_{Bu} varies from 0 to 50%. We speculate that the low molecular weight of polymer series **3**, coupled with its high solubility, not only increases the molar concentration (the number of molecules per unit volume) for any given weight concentration but also promotes the availability of the polymers to act on the bacterial inner membrane and cause cell death due to enhanced permeation of the polymers through bacterial peptidoglycan layers.

Important features of polymers useful as disinfectants are not only their antimicrobial activity but also the lack of toxicity to human cells, particularly for medical and clinical utility. Toward this end, their lytic activity against human red blood cells (hemolytic activity) was evaluated as HC_{50} for each series of polymers, which is the polymer concentration necessary for 50% lysis of cells. In each series, the HC_{50} for the polymers decreased as MP_{Bu} increased (Figure 1). In the high MP_{Bu} region (30−60%), the HC_{50} of the high MW polymers (series **1** and **2**) reached a plateau of <1 μg/mL, which is lower than that of melittin (1.24 μg/mL) and likely the minimum value (maximum toxicity) of the series of cationic random copolymers studied here. In contrast, the HC_{50} values for series **3** decrease monotonically with increasing MP_{Bu} and are 1 order of magnitude higher relative to those of larger MW polymer series **1** and **2** for the same MP_{Bu}. This result provides a window of efficacy from 10 to 30% MP_{Bu} in which series **3** is selectively toxic to bacterial cells with a maximum selectivity (HC_{50}/MIC) of **3** at 17% MP_{Bu} (Figure 1D). Previous studies on antimicrobial peptides showed that a lack of selectivity arises when hydrophobic lipid−peptide interactions overcome the electrostatic attraction to the bacterial cell surface.[9] Consistent with these studies, series **3** polymers showed selectivity (HC_{50} > MIC) in the low MP_{Bu} region, where the polymers are less hydrophobic. One conclusion that could be drawn from this result is that decreasing MP_{Bu} provides greater selectivity for bacterial over human cells. However, in contrast to the limited solubility of the polymers in the antimicrobial assay medium, the polymers are highly soluble in the hemolysis assay buffer (TBS = Tris-buffered saline) up to at least 500 μg/mL at all values of MP_{Bu} (0−60%), which may increase the number of polymers able to interact with cell membranes. The solubility difference observed in the assay media for the two experiments indicates not only that the interactions of polymers with lipids must be considered but also that environmental interferences and solution properties (conformations) of polymers must also be evaluated to correctly interpret the biological activities detected. Previous studies on peptides and synthetic polymers proposed models of membrane disruption mechanisms, in which polymer conformation and aggregation play an important role in pore formation on membranes.[9,12,13] We are currently screening a variety of polymer backbones and substituents to better understand the interplay of these factors on the antimicrobial activities of polymers and the mode of antimicrobial action.

Recent reports indicated that synthetic polymers composed of conformationally rigid polymer backbones coupled with regulated facially amphiphilic structures mimicking the natural host defense peptides acquire selectivity in their antimicrobial activity.[14,15] While this seems to be a promising approach for the preparation of nontoxic antimicrobial polymers, we demonstrate in this report that copolymers consisting of flexible polymer backbones and random amphiphilic sequences show antimicrobial activity comparable to that of natural peptides and relatively reduced toxicity compared to that of high MW polymers and the toxin melittin. Our results suggest that preorganized facial amphiphilicity is not necessarily required for antimicrobial activity in polymers, suggesting that the polymer interface can induce an amphiphilic conformation in a large enough population of the polymers to provide a potent antimicrobial effect. Shai and co-workers have come to a similar conclusion through the examination of diastereomeric peptides.[12]

In summary, antimicrobial and hemolytic activities of amphiphilic polymethacrylate derivatives can be tailored by alternating the content of hydrophobic groups and molecular weights. This class of synthetic polymers is inexpensive and easy to prepare, allowing the production of antimicrobial materials on industrial scales. Determination of the specific elements in polymers, which affect their biological activity, however, has previously been difficult due to the broad molecular weight distributions and random sequences characteristic of radical polymerization. Utilization of controlled polymerization methods[16] to produce well-defined polymers and selection of amphiphilic block or alternating polymer structures will provide us with greater insight into their antimicrobial mechanism.

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Supporting Information Available: Synthesis of copolymers, polymer characterization, solubility data in assay media, and assay protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

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