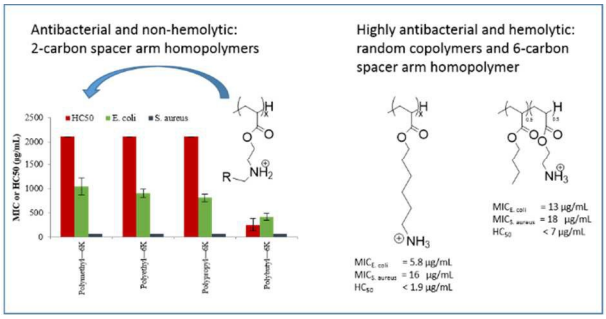
RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances



Effects of the variation in topographical position of cationic center and hydrophobic segments on antibacterial and hemolytic activities of polyacrylates

Journal Name

ARTICLE

Structure-property relationships of antibacterial amphiphilic polymers derived from 2-aminoethyl acrylate

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Ashish Punia,[a] Priya R. Debata,[b] Probal Banerjee,[b] and Nan-Loh Yang[a]†

Findings from investigation on an ensemble of amphiphilic polymers derived from 2-aminoethyl acrylate establish significant effects of the variation in topographical position of cationic center and hydrophobic segments on their biological activities. For example, the isomeric polymer pair of poly(6-aminohexylacrylate) and poly(2-(butylamino)ethyl acrylate) shows striking difference in their biological activities, with the former having biological activities orders of magnitudes higher. The trend of activities of alkyl tails attached to the charge center shows an abrupt increase in biological activity at butyl length in the series of methyl to butyl tail. The distribution and interaction of charge center in the chain domain is one of the main parameters in influencing polymer activities. Within the 2-aminoethyl acrylate system of homo and copolymer, the homopolymer with its cationic centers closely distributed along the amphiphilic macromolecular chain with proximity to the backbone leading to rigid conformations not conducive to attachment of polymer to the cell surface. In copolymers, the incorporation of uncharged counits increases the distance between the cationic centers, resulting in significant reduction of charge repulsion and thus enhancing the flexibility of chain conformation conducive for polymer-cell association, leading to a remarkable surge in orders of magnitude in biological activities but with low selectivity against bacteria over red blood cells.

Introduction

The steady increase in infections involving multi-drug resistant bacteria (superbugs) have now threatened to upend a century of medical advances in antibiotics.[1] A recent report has projected 10 million deaths due to antimicrobial resistance annually by 2050, which will surpass the number of deaths caused by cancer.[2] The biopolymers antimicrobial peptides (AMPs) are considered capable of destroying bacteria with very low likelihood of bacterial resistance development.[3] Large scale therapeutic applications of AMPs have been impeded by their costly synthesis and challenging drug administration due to proteolysis.[4] On the other hand, synthetic amphiphilic polymers, mimicking the fundamental principles of AMPs, have emerged as a promising antibacterial candidates because of their facile synthesis and ease of structural tunability.[5] We report here our findings from our ongoing investigation in structure-property relationships for antibacterial synthetic amphiphilic polymers. The polymers described here are based on 2-aminoethyl acrylate monomer leading to their cationic centers only 2-carbon away from the chain backbone.

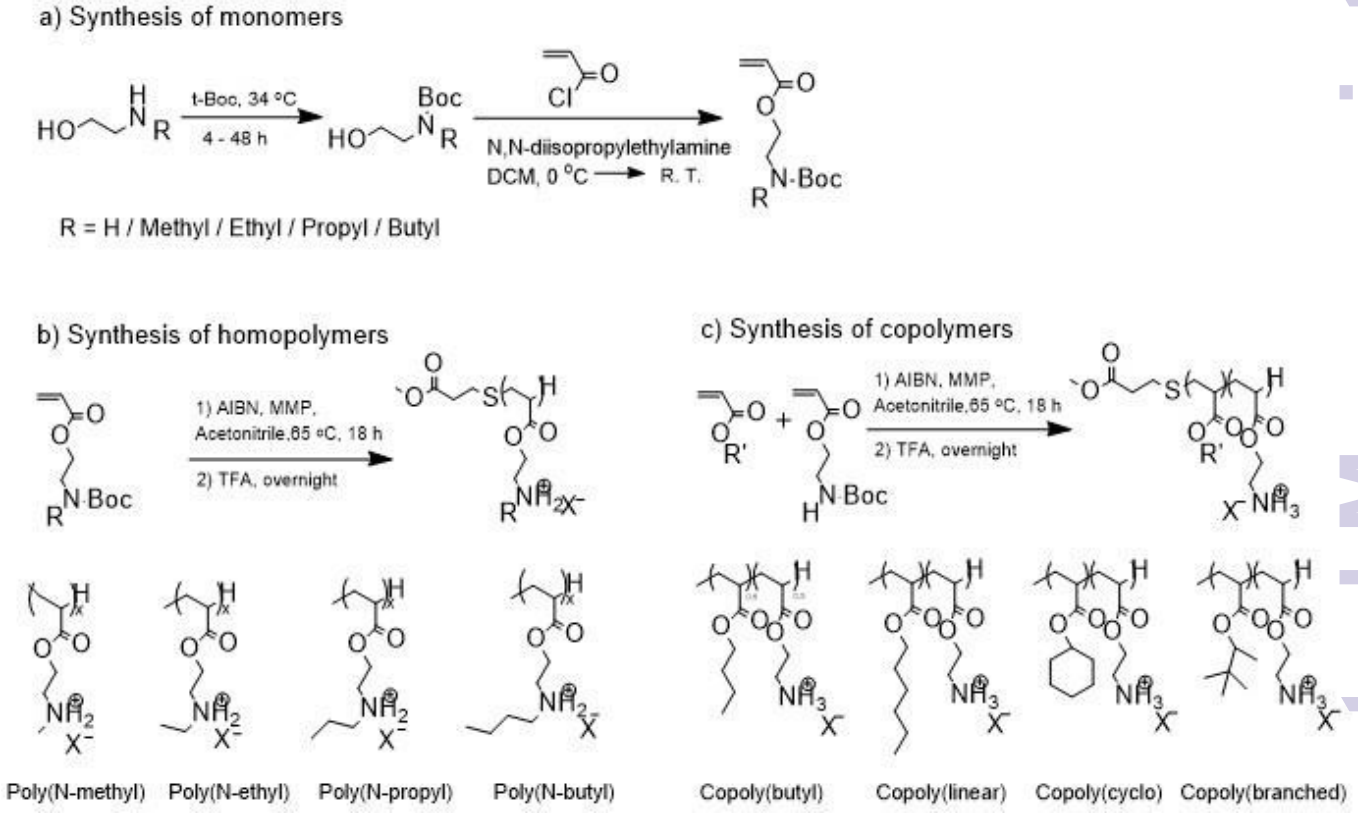
For therapeutic applications, it is highly desirable for synthetic amphiphilic polymers to selectively attack bacteria while exhibiting minimal toxicity towards mammalian cells. To this end, a number of studies have been focusing on various structural determinants of antibacterial and hemolytic activity of amphiphilic polymers including the effects of amphiphilic balance,[6] identity of amine functionality,[7] block versus random copolymer architecture,[8] charge density,[9] counter-ion effect,[10] and inclusion of poly(ethylene glycol) (PEG) side groups.[11] Hemolytic activity of polymers towards red blood cells (RBCs) has been widely used as a benchmark to assess the toxicity of polymers towards mammalian cells.

Our investigation in the area of synthetic antibacterial polymers has been focusing on polyacrylate systems with the goal to further our understanding on the effect of controlled amphiphilicity of the polymer. Recently we reported our findings on systems based on aminoethyl and n-aminohexyl acrylates.[12] The two counits led to copolymer side chains having two and six carbons spacer arm (distance from polymer backbone to cationic center), facilitating the study of the effect of controlled topography of cationic charge distribution in connection with polyacrylate polymer main chain backbone. A 2-carbon spacer arm homopolymer displayed low activity against *Escherichia coli (E. coli)* and low hemolytic activity while a 6-carbon spacer arm homopolymer displayed high antibacterial but with very high hemolytic activity.[12(a),13] We found polymers with high antibacterial and low hemolytic activity by utilizing the high antibacterial activity of the 6-carbon spacer arm component while mitigating its very high

[a] Center for Engineered Polymeric Materials and Department of Chemistry, College of Staten Island and Ph.D. Program in Chemistry at The Graduate Center of the City University of New York, New York, United States.
[b] Center for Developmental Neuroscience and Department of Chemistry, College of Staten Island, City University of New York, New York, United States.

† Nanloh.yang-cepm@csi.cuny.edu
Electronic Supplementary Information (ESI) available: NMR Spectra, calculation of DP. See DOI: 10.1039/x0xx00000x

Scheme 1. Synthesis of monomers and polymers (X^- is CH_3COO^-)



hemolytic effect through incorporation of counits with designed characteristics. In one approach, we tuned the polymer amphiphilicity through copolymerizing the 6-carbon spacer arm monomer with non-ionic poly(ethylene glycol) methyl ether methacrylate monomers.[12(b)] Through control of polymer composition and length of PEG side groups, a copolymer was synthesized with >100 times selectivity (Hemolytic activity/antibacterial activity) towards *E. coli* over RBCs. In another strategy, control of spacer arm lengths through various combinations of 6-carbon spacer arms and 2-carbon spacer arms led to high antibacterial activity with concomitant low hemolytic activity.[12(a)] Copolymerization of just 10 mole% of smaller 2-carbon spacer arm counits led to drastic reduction in hemolytic activity while retaining the high antibacterial activity, leading to >200 times selectivity towards *E. coli* over RBCs. These results illustrate that the incorporation of 2-carbon spacer arm acrylate comonomer units can be an effective tool to obtain antibacterial polymers with dramatically reduced hemolytic activity.

Thus, the comonomer 2-aminoethyl acrylate is of keen interest as a comonomer with short spacer arm from polymer backbone to cationic center. The focus of the present study is on this acrylate monomer and its derivatives as cationic building units for antibacterial homo- and copolymers. The amphiphilic balance of 2-carbon spacer arm homopolymers was varied by controlling the length of alkyl side tail attached to cationic centers. These polymers were synthesized at two molecular weight levels. For copolymers of 2-aminoethyl acrylate with alkyl acrylate, the number of cationic and hydrophobic groups on each polymer chain can affect the initial interactions of polymers with bacterial and mammalian cell surfaces and further steps in the cell membrane penetration. Random copolymers with butyl side chains and cationic groups on separate repeating units were synthesized to investigate their biological activity for comparison with similar homopolymers having cationic groups and hydrophobic groups on the same repeating units. Furthermore, the effect of shapes of 6-carbon alkyl isomers on the biological activities of random copolymers was explored. The antibacterial activities of polymers were evaluated against *E. coli* and *Staphylococcus aureus (S. aureus)*, and the toxicities of polymers against mammalian cells were evaluated in terms of hemolytic activity of polymer against mouse RBCs.

Experimental

Materials and methods

(Methylamino)ethanol, 2-(Ethylamino)ethanol, 2-(Propylamino)ethanol,2-(Butylamino)ethanol, dichloromethane (anhydrous), N,N-diisopropylethylamin acetonitrile (anhydrous), AIBN, methyl 3-mercaptopropionate, 1-hexanol, cyclohexanol, 3,3-dimethyl-2-butanol, hexane, and diethyl ether were purchased from Sigma-Aldrich and used without further purification. Acryloyl chloride from Sigma-Aldrich was distilled prior to use. Butyl acrylate was stirred with inhibitor remover for 20 minutes and filtered before use. Di-tert-butyl dicarbonate and trifluoroacetic acid were

Table 1. Characterization and biological activities of homopolymers and copolymers

Polymer	[MMP]/ [Monomer]	M_w (GPC)	PDI	DP[a]	MIC, µg/mL (E. coli)	MIC, µg/mL (S. aureus)	HC_{50} (RBCs)	Selectivity (HC_{50}/MIC)	
								E. coli (b)	S. aureus (c)
Poly(N-methyl)-1.6K	0.20	1646	1.54	6.9	>2000	>2000	>2000	1	1
Poly(N-ethyl)-1.6K	0.20	1730	1.5	5.7	>2000	>2000	>2000	1	1
Poly(N-propyl)-1.6K	0.20	1723	1.5	6.0	>2000	809	>2000	1	>2.5
Poly(N-butyl)-1.6K	0.20	1637	1.8	6.6	2000	62	42	0.02	0.68
Poly(N-methyl)-6K	0.05	7359	1.33	19	1048	62	>2000	>2	>32
Poly(N-ethyl)-6K	0.05	6299	1.31	23	905	62	>2000	>2.2	>32
Poly(N-propyl)-6K	0.05	5314	1.37	23	810	62	>2000	>2.5	>32
Poly(N-butyl)-6K	0.05	6380	1.47	24	417[d]	62[d]	253	0.61	4.1
Copoly(butyl)-1.6K	0.20	1420	1.57	4.1	13	18	16	1.2	0.9
Copoly(butyl)-6K	0.05	6493	1.5	24	13	34	<7	<0.5	<0.2
Copoly(linear)-6K	0.05	6316	1.26	----	16	18	26	1.6	1.4
Copoly(cyclo)-6K	0.05	5478	1.48	16	21	18	<7	<0.3	<0.4
Copoly(branched)-6K	0.05	5350	1.34	22	16	18	<7	<0.4	<0.4

Degree of polymerization (DP) is calculated from ^{1}H NMR (ESI)

purchased from VWR. ^{1}H NMR spectra were obtained on 300 MHz and 600 MHz Varian NMR spectrometers using $CDCl_3$ or DMSO-d_6 as solvents. Molecular weights (M_w and M_n) of Boc protected polymers, and their molecular weight distributions (M_w/M_n, PDI) were obtained on Waters alliance GPCV 2000 using linear polystyrene as standard. Tetrahydrofuran (HPLC grade) was used as an eluent at a flow rate of 1mL/min. OD_{600} was obtained to measure bacterial growth on an Agilent 8453 spectrophotometer (using 1 cm path length plastic cuvette). OD_{595} and OD_{414} were obtained on SpectraMax 340 PC microplate reader (Molecular devices).

N-Boc protection of alkylamino alcohols[12(a),14]

Di-tert-butyl dicarbonate (26 g, 119 mmol) was added in a 250 mL single neck round bottom flask, already charged with 2-(Methylamino)ethanol (8.6 mL, 108 mmol) and H_2O (110 mL). The reaction mixture was then stirred at 34 ^{0}C for 6 hours. After 6 hours, the reaction mixture was extracted with ethyl acetate (3×125 mL) and dried with sodium sulfate. After filtration, the ethyl acetate was evaporated using a rotary evaporator to yield pure product (90 % yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.45 (s, 9H), 2.92 (s, 3H), 3.38 (s, 2H), 3.73 (s, 2H). N-Boc protection of 2-(Ethylamino)ethanol, 2-(-(Propylamino)ethnaol, and 2-(Butylamino)ethanol was carried out following a modified work-up procedure (ESI).

Synthesis of amine functionalized monomers[6(c),12(a)]

A representative monomer synthesis is as follows. 2-(N-Boc-butylamino)ethanol (12.84 g, 60 mmol) was added into a 500 mL, 3-neck round bottom flask, loaded with N,N-diisopropylethylamine (17.4 mL, 100 mmol) and dichloromethane (100 mL). Acryloyl chloride (5.52 mL, 68 mmol) was then added drop-wise at 0 ^{0}C, under nitrogen atmosphere. The reaction mixture was allowed to warm to room temperature, and stirred overnight. After 18 hours, reaction mixture was washed with distilled water (3 times), 10% citric acid (2 times), 10% potassium carbonate (2 times), and saturated sodium bicarbonate solution (3 times). Organic layer was separated and dried over sodium sulfate, followed by removal of excess solvent using the rotary evaporator. The resultant liquid was purified by silica gel column chromatography, using hexane/ethyl acetate (1:1) as eluent. 60% yield (Monomer 4). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.92 (t, 3H), 1.30 (m, 2H), 1.48 (bs, 11H), 3.23 (s, 2H), 3.48 (s, 2H), 4.3 (s, 2H), 5.86 (d, 1H), 6.14 (m, 1H), 6.43 (d, 1H). A simil procedure was followed for the synthesis of all other monomers.

Synthesis of polymers[6(c),12(a)]

A representative synthesis procedure is as follows. A 3 neck 100 mL flask was charged with Monomer 2 (2.25 g, 9.25 mmol), AIBN (15.2 mg), Methyl-3-mercaptopropionate (MMP, 0.205 mL), and Acetonitrile (10 mL). The reaction mixture was degassed with dry nitrogen for 20 minutes and stirred at 65 ^{0}C for 18 hours. Acetonitrile was then evaporated using the rotary evaporator, and a small amount (1 mL) of dichloromethane was added into reaction mixture, followed by precipitation into hexane (3 times). The resultant polymer was dried under reduced pressure. By varying the mole ratios of MMP and Monomer, two series of molecular weights were obtained (M_w approximately 6k g/mol and 1.6k g/mol).

Removal of Boc protecting groups

For the deprotection of Boc protecting group, Polybutyl was dissolved in minimum quantity of dichloromethane, and excess quantity of trifluoroacetic acid (TFA) was added. The mixture was stirred for 4 hours at room temperature. TFA was removed under reduced pressure using the rotary evaporator. Small quantity of acetonitrile (2 mL) was added and th

mixture was repeatedly precipitated into diethyl ether to obtain amphiphilic polymer. The polymer was dried under reduced pressure and lyophilized. All other cationic amphiphilic polymers were similarly obtained.

Preparation of polymer dilutions for antibacterial and hemolytic activity determination

Each polymer was dissolved in DMSO to prepare a stock solution with 20 mg/mL concentration. Further two-fold dilutions were prepared by adding deionized water. Control solutions (without polymers) were prepared in a similar way. As described below, a ten-fold dilution of polymer concentrations would take place in assays for antibacterial and hemolytic activity determination.

Determination of antibacterial activity[6(c),12]

Antibacterial activities of polymers were studied by following a slightly modified literature protocol. To assess the antibacterial activities of polymers against gram negative bacteria, *E. coli* TOP 10 (ampicillin resistant) were incubated overnight at 37 ^{0}C in Luria Bertani (LB) broth (containing ampicillin, 100µg/mL). OD_{600} was obtained on an Agilent 8453 spectrophotometer using plastic disposable cuvettes (1 cm path length) to measure bacterial cell growth. This cell suspension was diluted to obtain OD_{600} = 0.1, by adding fresh LB broth. The cell suspension was allowed to grow at 37 ^{0}C (under shaking) for approximately 1.5 hours, and OD_{600} increased to around 0.5 (log phase growth). Final stock cell suspension with OD_{600} = 0.001 was obtained by a final dilution with fresh LB broth. To each well of a 96 well sterile tissue culture plate (REF 353916, BD falcon, flat bottom), 90 µL of cell stock suspension (with ampicillin) was added followed by the addition of 10 µL polymer solutions, or control solutions. Each polymer concentration was added in triplicate, and the assay plates were incubated at 37 ^{0}C for 18 hours. OD_{595} values were then obtained using a SpectraMax 340 PC micro plate reader, and minimum inhibitory concentration (MIC) is defined as the lowest polymer concentration required to completely inhibit the bacterial cell growth. Antibacterial activities of polymers against *S. aureus* ATCC 25923 were examined by following the similar protocol as described above for *E. coli*, except Mueller-Hinton (MH) broth was used in place of Luria-Bertani (LB) broth. MIC values reported in this study are the averages of three independent MIC values obtained on different days under similar conditions.

Determination of hemolytic activity[6(c),12(a)]

Freshly drawn RBCs were obtained by centrifuging mice blood, and discarding the white blood cells and plasma (as supernatant). 4.5 mL of TBS (Tris buffer, 10 mM, pH=7, 150 mM NaCl) was added to 0.5 mL RBCs. In 250 µL of this cell suspension, 10 mL of TBS was further added to obtain a final stock cell suspension (40 fold dilution, 0.25% RBCs). 130 µL of this stock solution was added to a 600 µL centrifugation tube containing 15 µL polymer solution (or control solution) and TBS (15 µL). Centrifugation tubes were incubated at 37 ^{0}C for 1 h, and then centrifuged for 4 minutes at 4000 rpm. The supernatant (30 µL) was obtained and diluted with TBS (70 µL) in a 96 well sterile assay plate (in triplicate). Hemoglobin concentration as optical density at OD_{414} was obtained on the microplate reader (SpectraMax 340 PC). 1% triton X-100 was used as a reference for 100 % hemolysis (positive control), and the control solutions were used as a reference for 0% hemolysis (negative control). Percent hemolysis corresponding to each polymer concentration was obtained by using the following formula:

$$\%\ Hemolysis = \left(\frac{OD_{414}Polymer - OD_{414}negative\ Control}{OD_{414}Triton - OD_{414}negative\ Control}\right) \times 100$$

HC_{50} values reported here are the averages of 3 independent experiments conducted on different days.

Results

Synthesis of polymers

The strategy employed for the synthesis of monomers and amphiphilic polymers is as shown in Scheme 1. Homopolymers were synthesized by free radical polymerization of N-Boc protected monomers (Scheme 1b). Likewise, N-(tert-butoxycarbonyl)aminoethyl acrylate was copolymerized with butyl acrylate (1:1, feed mole ratio, Scheme 1c) to synthesize a random copolymer in order to compare its antimicrobial activity with a similar homopolymer. Two series of molecular weights (Mw~ 6k g/mol, degree of polymerization (DP) ~ 22) and 1.6 g/mol (DP ~ 6)) were synthesized for each homopolymer and copolybutyl. Data from ^{1}H NMR (600 MHz or 300 MHz) was used to estimate DP of products and to ascertain the copolymer composition. Gel permeation chromatography (linear polystyrene standards) was employed to estimate molecular weights and polydispersities (PDI) of Boc protected polymers. Poly(N-methyl)-6k represents the homopolymer with methyl chain attached to amine group and having a molecular weight of approximately 6k g/mol. Scheme 1c represents the synthesis of copolymers designed to evaluate the shape effect of 6-carbon hydrophobic side chain on antibacterial and hemolytic activity of amphiphilic copolymers. Mole percentage feed of hydrophobic comonomer was kept at 40%.

Antibacterial activity

Antibacterial activities of polymers were determined in terms of minimum inhibitory concentration (MIC) against *E. coli* TOP 10 (ampicillin resistant) and *S. aureus* ATCC 25923 (Figure 1 and Table 1). Homopolymers with one to three carbon tail in 6k g/mol series displayed high and similar activity against gram positive *S. aureus* (Figure 1a, MIC=62 µg/mL). However, this homopolymer series displayed much lower antibacterial activity towards *E. coli* than with *S. aureus* (Figure 1a). With an addition of one more carbon to the three carbon tail, the homopolymer poly(N-butyl) showed a marked increase in biological activities towards both bacteria and RBCs. A conspicuous surge in orders of magnitude in biological activities was observed in going from homopolymers to copolymers. Random copolymer copoly(butyl)—6k displayed high antibacterial activity against both *S. aureus* (MIC=34 µg/mL) and *E. coli* (MIC=13 µg/mL), as shown in Figure 1c and Table 1. Similarly,

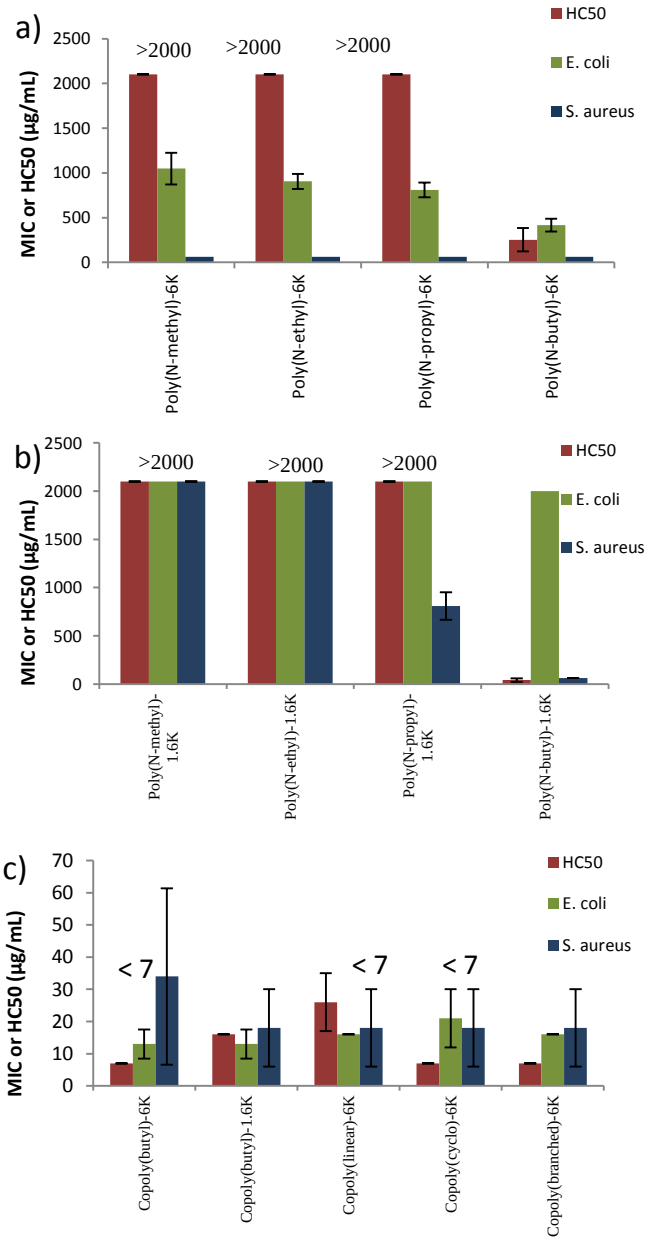


Figure 1. Antibacterial and hemolytic activities of a) 6k g/mol series homopolymers; b) 1.6k g/mol homopolymers; and c) antibacterial and hemolytic activities of random copolymers. Error bars represent standard deviation.

the three copolymers with six-carbon alkane groups attached to the charge center: copoly(linear)—6K, Copoly(cyclo)—6K, and Copoly(branched)—6K, displayed high activity against both *S. aureus* and *E. coli* (Figure 1c and Table 1). The role of molecular weight of homopolymers on their biological activity is apparent from Figure 1 and Table 1. Smaller molecular weight homopolymers were inactive against *E. coli* (Figure 1b), in contrast to moderately active 6K g/mol series homopolymers. Similarly, 1.6k g/mol series homopolymers, except poly(N-butyl)-1.6k, did not show activity against *S. aureus*, whereas 6k g/mol series homopolymers demonstrated high activity against *S. aureus*. The 1.6k g/mol series with low DP of ~ 6 has a much lower likelihood of association with cell surface at initial contact than the 6k g/mol series. In contrast to homopolymers, the effect of molecular weight on the antibacterial activity of random copolymer was not observed as both Copoly(butyl)—6K and Copoly(butyl)—1.6K manifested very high and similar activities against *E. coli* and *S. aureus*.

Hemolytic activity

To ascertain the toxicity of polymers towards mammalian cells, hemolytic activity (HC_{50}) of polymers was measured against mouse RBCs. HC_{50} is defined as the minimum concentration of polymer solution required to cause lyses in 50 % of RBCs within an incubation period of 1 h at 37 ^{0}C. Our study found that all of our homopolymers, except Poly(N-butyl), are non-hemolytic (HC_{50} >2000 µg/mL), whereas Copoly(butyl), the random copolymer, was highly hemolytic at both 6k g/mol and 1.6k g/mol molecular weight levels (Figure 1 & Table 1). Copoly(linear)—6K, Copoly(cyclo)—6K, and Copoly(branched)—6K displayed similar and high hemolytic activity despite having different shapes of hydrophobic side groups.

Selectivity of polymers

Selectivity is defined as the ratio HC_{50}/MIC of polymers. Polymers with selective activity against bacteria over RBCs are highly desired. Poly(N-methyl)-6K, Poly(N-ethyl)-6K, and Poly(N-propyl)-6K demonstrated a selectivity of greater than 32 times for *S. aureus* over RBCs. Furthermore, 6k series homopolymers displayed selectively higher activity against *S. aureus* over *E. coli*. Thus, these homopolymers are doubly selective: *S. aureus* over RBCs and *S. aureus* over *E. coli*. In contrast to homopolymers, random copolymers in this study did not demonstrate selective activity against bacteria over RBCs, and showed similar activity against both *S. aureus* and *E. coli.*

Discussion

Antibacterial activity of polymers

All homopolymers in 6k series displayed high activity against *S. aureus* but lacked high activity against *E. coli*. *S. aureus* has a single plasma membrane surrounded by a negatively charged peptidoglycan layer (~15-80 nm), whereas *E. coli* has a double membrane structure in which the negatively charged peptidoglycan layer (~8 nm) is sandwiched between an outer membrane and an inner membrane.[15] Thus, the double membrane structure of *E. coli* cell surface can be considered more challenging to lyse than the single membrane structure of *S. aureus*. In contrast to homopolymers, copolymers did not demonstrate selectivity between the types of bacteria and showed high activity against both *E. coli* and *S. aureus*. The ability of polymers to selectively attack *S. aureus* over *E. coli* can be advantageous in treating *S. aureus* and potentially other gram positive infections without negatively affecting the gut microbiome consisting of gram negative bacteria.[16]

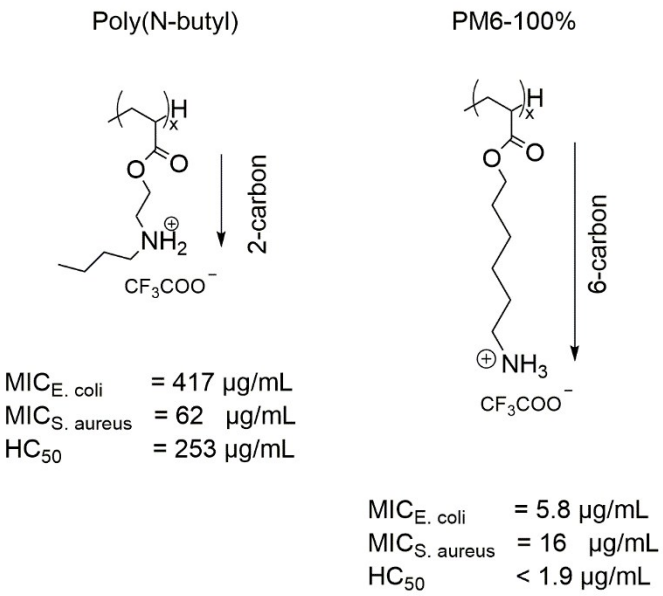


Figure 2. A structural isomeric polymer pair: poly(2-(butylamino)ethyl acrylate), i.e. Poly(N-butyl) and poly(6-aminohexylacrylate), i.e. (PM6-100%). Effect of spacer arm, locations of hydrophobic segments, and proximity of cationic center to the chain backbone on the antibacterial and hemolytic activities of polyacrylate homopolymers.

The significantly different levels of antibacterial activities between the homopolymers and the copolymers mainly arise from the topographical variation of the locations of hydrophobic side segments, cationic center, and the chain backbone conformation. The homopolymer macromolecule carries a positive charge on each repeat unit while copolymer molecule has positive center on every other counit. The distance between positive centers in Poly(N-butyl)-6K is about half than that in the copoly(butyl)-6k; its activity against *E. coli* is 32 times lower than that of Copoly(butyl)-6k. High cationic charge density of homopolymers leads to rigid conformation due to charge repulsion and thus may hinder the initial process of polymer - cell surface association and their permeabilization through the hydrophobic core of lipid bilayer, especially in *E. coli* with a double bilayer structure. Furthermore, the short 2-carbon spacer arm of these polymers can also be a deterring factor behind their low activity against *E. coli*. Poly(N-butyl)-6k and PM6-100% (cationic poly(6-aminohexylacrylate) homopolymer) are isomeric in that both contain six hydrophobic alkane carbons on their side chains (Figure 2). The structural difference is that the former polymer has four carbon alkane tail attached as a tail to the charge center while the latter with all six carbon on the spacer arm. Both polymers have similar number of cationic charges and hydrophobicity, but PM6-100%, with 6-carbon long spacer arm, is highly antibacterial ($MIC_{E. Coli}$ = 5.8 µg/mL; $MIC_{S. aureus}$ = 16 µg/mL) and hemolytic (HC_{50} < 1.9 µg/mL) as compared with Poly(N-butyl)-6k ($MIC_{E. Coli}$ = 417 µg/mL; $MIC_{S. aureus}$ = 62 µg/mL; and HC_{50} = 253 µg/mL).[12(a)] The striking difference in biological activities can be attributed to the topology of their amphiphilicity arrangement.

In poly(N-butyl), a hydrophobic butyl tail is attached to the positive center, thus may hamper the ionic interaction to with cell surface but can also assist in hydrophobic interaction within the double layer of cell membrane PM6 100% has a longer spacer arm with four more carbon. A long spacer arm enhances the snorkel effect in which the cationic center can attach to the cell surface and the polymer backbone can then permeabilize through the cell membrane core.[13] On the other hand, the shorter 2-carbon spacer arm hinders the permeabilization of polymer backbone through the hydrophobic core of cell membrane. Molecular weight has substantial effect on the antibacterial activity of these homopolymers. As compared with higher molecular weight polymers, the lower molecular weight polymers (DP ~ 6) will have lower number of attached points per chain to the surface of bacterial cells leading to lower binding ability to the cell surface of bacteria. This effect may be especially exacerbated in case of homopolymers with cationic groups that are sterically hindered due to presence of hydrophobic tail on the cationic center. Copoly(linear)—6K, Copoly(cyclo)—6K, and Copoly(branched)—6K displayed similar activity against both *E.coli* and *S. aureus*. Thus the shape of hydrophobic alkyl tail of six carbon in these polymer has no significant effect on their antibacterial activity, indicating the spatial resolution of recognition for biological agent by the cell surface not sensitive down to the six carbon structural level.

Hemolytic activity of polymers

The toxicity of polymers towards mammalian cells has been a major concern hindering the therapeutic applications of amphiphilic polymers. All our homopolymers, except Poly(N-butyl), demonstrated non-hemolytic activity, as opposed to random copolymers with very high hemolytic activity. The outer surface of RBCs' cell membrane lacks net negative charge and the hemolytic activity of amphiphilic polymers is believed to primarily result from the insertion of hydrophobic alkyl tail of polymers into the hydrophobic domain of lipid bilayer.[4] The presence of a cationic group along with every alkyl side tail in homopolymers and the high cationic charge density would not favor hydrophobic interactions of homopolymers with lipid bilayer of RBCs. However, in copolymers cationic charges and hydrophobic alkyl side groups are placed on separate repeat units, thus hydrophobic side groups can more readily insert into lipid membrane of RBCs. Copoly(linear)—6K, Copoly(cyclo)—6K, and Copoly(branched)—6K showed similar hemolytic activity, suggesting that the shape of hydrophobic alkyl tail on a six carbon level in these polymer does not substantially affect their hemolytic activity.

Conclusions

The findings here on the biological activities of a series of amphiphilic copolymers and homopolymers derived from 2-aminoethyl acrylate comonomer can serve as significant references for further development of structure-property relationships in the subject of synthetic antibacterial polymers. The biological activities of the structural isomeric pair (Figure 2): poly(6-aminohexylacrylate) and poly(2-(butylamino)ethyl

acrylate), clearly shows the strong impact of spatial arrangement of the hydrophobic segments and charge center from the two polymers with the same total composition. Increase in spacer length and keeping cationic center unhindered for ionic interaction leads to pounced rise in antibacterial and hemolytic activities. The distribution and interaction of charge center in the chain domain is one of the main parameters. In homopolymer, cationic centers are closely distributed along the amphiphilic macromolecular chain with proximity to the backbone leading to rigid conformations not conducive to attachment of polymer to the cell surface. In the copolymer, incorporation of non-charged counits doubles the distance between the cationic centers, resulting in significant reduction of charge repulsion and thus in enhancing the flexibility of chain conformation. The position and properties of hydrophobic alkyl side group with respect to cationic center has substantial effect on biological activities. Acrylate homopolymers with hydrophobic groups directly attached to the cationic center showed low antibacterial activity against *E. coli* and low hemolytic activity against RBCs. High charge density and short cationic spacer arm can hinder the permeability of homopolymers through the hydrophobic core of outer and inner cell membranes in *E. coli* and lipid bilayer of RBCs.

In contrast to doubly selective homopolymers (*S. aureus* over RBCs and *S. aureus* over *E. coli*), random copolymers having hydrophobic segment and cationic groups on separate repeating units, displayed very high but non-selective activity against bacteria and RBCs.

Acknowledgements

We acknowledge the support from Center for Engineered Polymeric Materials, Department of Chemistry at the College of Staten Island of CUNY; Ph.D. Program in Chemistry at the Graduate Center of CUNY and CUNY RF 68464-00 46.

Notes and references

1 (a) D. Jabes, *Curr. Opin. Microbiol*., 2011, 14, 1; (b) H. W. Boucher, *Clin. Infect. Dis*., 2010, 50, S4; (c) www.cdc.gov/drugresistance/threat-report-2013/;(d) www.independent.co.uk/life-style/health-and-families/health-news/superbugs; (e) A. Y. Peleg and D. C. Hooper, *N. Engl. J. Med*., 2010, 362, 1804; (f) R. M. Klevens, J. R. Edwards, C. J. Jr. Richards, T. C. Horan, R. P. Gaynes, D. A. Pollock and D. M. Cardo, *Public Health Rep*, 2007, 122, 160; (g) H. C. Kung, D. L. Hoyert, J. Xu and S. L. Murphy, *Natl. Vital. Stat. Rep*., 2008, 56, 1; (h) P. W. Stone, E. C. Hedblom, D. M. Murphy and S. B. Miller, *Am. J. infect. Control*, 2005, 33, 542.

2 http://amr-review.org/

3 (a) M. Zasloff, *Nature*, 2002, 415 (6870), 389; (b) K. A. Brogden, *Nat. Rev. Microbiol*., 2005, 3, 238.

4 K. Kuroda and G. A. Caputo, *WIREs Nanomed. Nanobiotechnol*., 2013, 5, 49.

5 (a) A. M-Bonilla and M. F-Garcia, *Prog. Polym. Science*, 2012, 37, 281; (b) A. C. Engler, N. Wiradharma, Z. Y. Ong, D. J. Coady, J. L. Hedrick and Y. Yang, *Nano Today*, 2012, 7, 201; (c) M. S. Ganewatta and C. Tang, *Polymer*, Doi: 1016/j.polymer.2015.03.007.

6 K. Lienkamp and G. N. Tew, *Chem. Eur. J.*,2009, 15, 11784; (b) B. P. Mowery, A. H. Linder, B. Weisblum, S. S. Stahl and S. H. Gellman, *J. Am. Chem. Soc*., 2009, 131, 9735; (c) K. Kuroda and W. F. DeGrado, *J. Am. Chem. Soc*., 2005, 127, 4128; (d) J. Tejero, D. Lopez, F. L. pez-Fabal, J. L. Gomez-Garce and M. Fernandez-Garcia, *Biomacromolecules*, 2015, 6(6), 1844; (e) B. P. Mowery, S. E. Lee, D. A. Kissounko, R. F. Epand, R. M. Epand, B. Weisblum, S. S. Stahl and S. H. Gellman, *J. Am. Chem. Soc.* 2007, 129, 15474.

7 E. F. Palermo and K. Kuroda, *Biomacromolecules*, 2009, 10, 1416.

8 (a) Y. Oda, S. Kanaoka, T. Sato, S. Aoshima and K. Kuroda, *Biomacromolecules,* 2011, 12(10), 3581; (b) Y. Wang, J. Xu, Y. Zhang, H. Yan and K. Liu, *Macromol. Biosci.,* 2011, 11, 1499.

9 Z. M. AL-Badri, A. Som, S. Lyon, C. F. Nelson, K. Nusslein and G. N. Tew, *Biomacromolecules*, 2008, 9, 2805.

10 K. Lienkamp, A. E. Madkaur, K. N. Kumar, K. Nusslein and G. N. Tew, *Chem. Eur. J*., 2009, 15, 11715.

11 (a) B. C. Allison, B. M. Applegate and J. P. Youngblood. *Biomacromolecules*, 2007, 8, 2995; (b) T. R. Stratton, J. Howarter, B. C. Allison, B. M. Applegate and J. P. Youngblood, *Biomacromolecules*, 2010, 11, 1286.

12 (a) A. Punia, E. He, K. Lee, P. Banerjee and N. Yang, *Chem. Commun.*, 2014, 50, 7071; (b) A. Punia, A. Mancuso, P. Banerjee, N-L. Yang, *ACS Macro Lett*., 2015, 4, 426.

13 E. F. Palermo, S. Vemparala and K. Kuroda, *Biomacromolecules*, 2012, 13, 1632.

14 S. V. Chankeshwara, A. K. Chakraborti*, Org. Lett.*, 2006, 8, 3259.

15 K. Lienkemp, K.-N. Kumar, A. Som, K. Nusslein and G. N. Tew, *Chem. Eur. J*., 2009, 15, 11710.

16 L. M. Thoma, B. R. Boles and K. Kuroda, *Biomacromolecules*, 2014, 15(8), 2933.