Journal of Polymer Science Part A: Polymer Chemistry

Supporting Information

Radical-medicated end-group transformation of amphiphilic methacrylate random copolymers for modulation of antimicrobial and hemolytic activities

Enrico T. Nadres,[†] Haruko Takahashi,[†] and Kenichi Kuroda*

Department of Biologic and Materials Sciences, School of Dentistry, University of Michigan, Ann Arbor, MI 48109, USA.

* To whom should be correspondence addressed.

E-mail: kkuroda@umich.edu, Tel: (+1) 734-936-1440, Fax: (+1) 734-647-2805.

[†] These two authors contributed equally (E.T.N. and H.T.)

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SI 1. Experimental details of antimicrobial and hemolytic assays, and membrane depolarization.

MIC assay. Three gram-positive strains such as *Bacillus subtilis* ATCC®6633™, *Enterococcus faecalis* ATCC®29212™, *Staphylococcus aureus* ATCC®25923™, and four gram-negative bacterial strains such as *Acinetobacter baumannii* ATCC®17978™, *Escherichia coli* ATCC®25922™, *Pseudomonas aeruginosa* ATCC®27853™, and *Salmonella enterica* subsp. *enterica* serovar Typhimurium ATCC®14028™ were used for evaluation of minimum inhibitory concentration (MIC) of copolymers. Additionally, *S. aureus* strain LAC BB1263 was tested for community acquired methicillin-resistant *S. aureus* (CA-MRSA). The MICs of copolymers were determined in a standard microbroth dilution assay according to the Clinical and Laboratory Standards Institute guidelines with suggested modifications by R.E.W. Hancock Laboratory (University of British Columbia, Vancouver, British Columbia, Canada)[1] and Giacometti *et al.*[2] All bacteria cultured in Mueller Hinton Broth (MHB, BD and Company©) prepared according to manufacturer's instruction. Each copolymer was dissolved in 0.01 % acetic acid. An overnight (approximately 18 hours) culture of bacterial strains was regrown to exponential phase (OD_{600} of 0.5−0.7) and diluted with the MHB to give the bacterial suspension with approximately 4×10^5 CFU mL^{-1} as final concentration. After preparation of serial concentration of copolymer solutions (10 μL) on a 96-well sterile round-bottom polypropylene plate, the bacterial suspension (90 μL) was added and incubated at 37 °C for 18 hours. 0.01 % acetic acid was used as a solvent control. The MIC was defined as the lowest copolymer concentration to completely inhibit visible bacterial growth. Bacterial growth was detected at OD_{600} using WPA

S800 visible spectrophotometer (Biochrom). Each MIC assay was independently repeated at least three times using different stock solutions in triplicate on different days.

Hemolysis assay. Human red blood cells from healthy donor (RBCs; 1 mL) were suspended in 9 mL of PBS buffer (pH 7.4) and centrifuged at 660 × g for 5 minutes. The supernatant was removed by pipetting and RBCs were re-suspended in PBS. This procedure was repeated two additional times. The number of RBCs in suspension was counted by counting chamber and diluted in PBS to give the final concentration of 3.0×10^8 cells mL^{-1} as final concentration. Each copolymer solutions with serial concentration (10 μL) were mixed with the RBC suspension (90 μL) on a 96-well sterile round-bottom polypropylene plate and incubated at 37 °C with orbital shaking (180 rpm). Triton X-100 (0.1 % (v/v) in PBS) was used as the positive lysis control and 0.01 % acetic acid was used as negative control. The bee venom toxin melittin was also tested as reference standard. After 1 hour, the plate was centrifuged at 1,000 × g for 5 minutes and an aliquot of supernatant (6 μL) from each well was diluted within PBS buffer (100 μL) in a 96-well sterile flat-bottom polystylene plate. The absorbance of the released hemoglobin at 415 nm was measured using Varioskan Flash microplate reader (Thermo Fisher). The percentage of hemolysis was determined relative to the positive lysis control Triton X-100 (100 %) and negative control 0.01 % acetic acid (0 %). HC_{50} which was defined as the polymer concentration causing 50 % hemolysis, or hemolysis% at highest concentration if the hemolysis% showed below 50 % is reported. Each hemolysis assay was independently repeated at least three times using different stock solutions in triplicate on different days.

***S. aureus* membrane depolarization assay.**[3-6] Cytoplasmic membrane disruption was evaluated for copolymers against *S. aureus* using the membrane potential sensitive dye $DiSC_3$-(5). An overnight (approximately 18 hours) culture of *S. aureus* ATCC®25923™ was regrown to exponential phase (OD_{600} of 0.5−0.7) in MH broth. This bacterial solution was centrifuged at 1,700 × g for 10 minutes to give bacterial pellet and the supernatant broth was removed by pipetting. The pellet was suspended in buffer (5 mM HEPES, 5 mM glucose, 100 mM KCl, pH 7.2) and washed once by another centrifugation. The resulting bacterial suspension was diluted with buffer to OD_{600} of 0.05.

For time course measurement, *S. aureus* suspension (3 mL) in cuvette was stirred at room temperature. After 20 seconds, a stock solution of $DiSC_3$-(5) in DMSO (0.3 mM; 5 μL) was added to *S. aureus* suspension and stirred for 180 seconds until a stable reduction in fluorescence intensity was achieved due to quenching upon accumulation of dye on the *S. aureus* membrane. The final $DiSC_3$-(5) dye concentration is 0.5 μM. At 200 seconds, a solution of melittin (0.45 mg mL^{-1}; 40 μL) or copolymers (1.17 mg mL^{-1} of **P1**, 2.34 mg mL^{-1} of **P1a**, 4.69 mg mL^{-1} of **P1b**; 40 μL) in 0.01 % acetic acid was added to the bacterial suspension to give the final concentration of their MICs and monitored for 300 seconds. 0.01 % acetic acid (40 μL) was also tested as solvent control. The fluorescence intensity was monitored using FP-6200 spectrofluorometer (JASCO) with an excitation and emission wavelengths of 622 and 670 nm, respectively. As a control, mixtures of $DiSC_3$-(5) dye and copolymers in buffer showed no significant change in fluorescence intensity compared to only dye in buffer. The experiment was repeated at least 2 times from bacterial cell culture, and similar results were obtained. One of the data set was

presented as a representative data.

To evaluate the membrane depolarization in different concentrations of copolymers, a stock solution of $DiSC_3$-(5) in DMSO (0.3 mM; 33.3 μL) was added *S. aureus* suspension (OD_{600} of 0.05; 20 mL) in buffer (5 mM HEPES, 5 mM glucose, 100 mM KCl, pH 7.2) and incubated for 10 minutes with orbital shaking (180 rpm) until a stable reduction in fluorescence intensity was achieved due to quenching upon accumulation of dye on the *S. aureus* membrane. The final $DiSC_3$-(5) dye concentration is 0.5 μM. **P1**, **P1a**, and **P1b** copolymer solutions with serial concentration (10 μL) were added to the bacterial suspension with $DiSC_3$-(5) dye (90 μL) on a 96-well flat-bottom black plate and incubated for 5 minutes with orbital shaking (180 rpm). The fluorescence intensity was measured using Varioskan Flash microplate reader (Thermo Fisher) with an excitation and emission wavelengths of 622 and 670 nm, respectively.

SI 2. ^{1}H NMR spectra of copolymers.

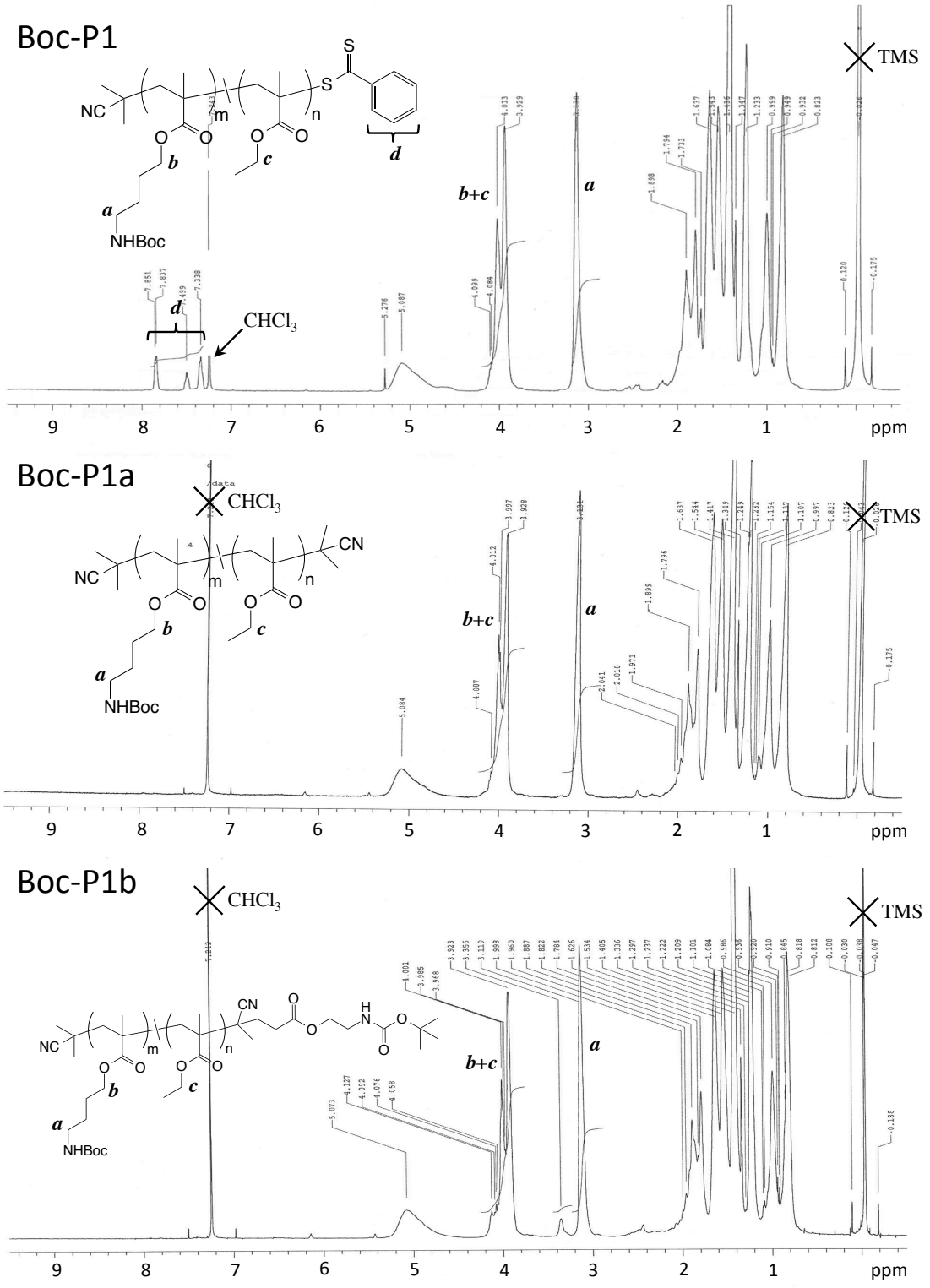

Figure S1. ^{1}H NMR spectra of **Boc-P1**, **Boc-P1a** and **Boc-P1b** in $CDCl_3$.

SI 3. GPC profiles of Boc-protected copolymers.

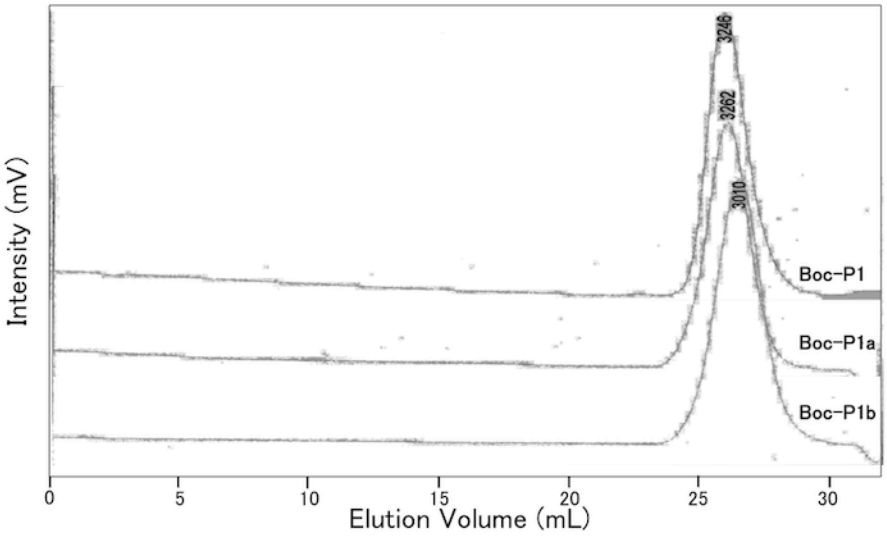

Figure S2. Gel permeation chromatographs of **Boc-P1**, **Boc-P1a** and **Boc-P1b** in THF.

SI 4. Solubility of copolymers in the M-H broth.

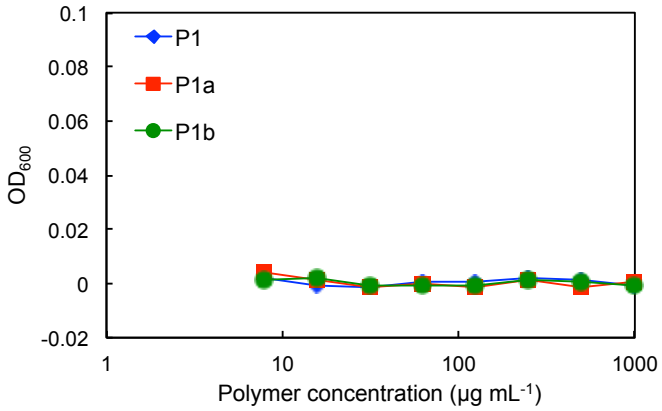

Figure S3. Solubility of copolymers in the M-H broth. Copolymer solutions with serial concentration (10 μL) were mixed with M-H broth (90 μL) on a 96-well sterile round-bottom polypropylene plate and incubated at 37 °C for 18 hours. After incubation, the solutions were mixed well and 50 μl of each aliquot was transferred to new 96-well sterile flat-bottom polystylene plate, then the OD_{600} was measured.

SI 5. Hemolysis dose-response curves of copolymers and melittin.

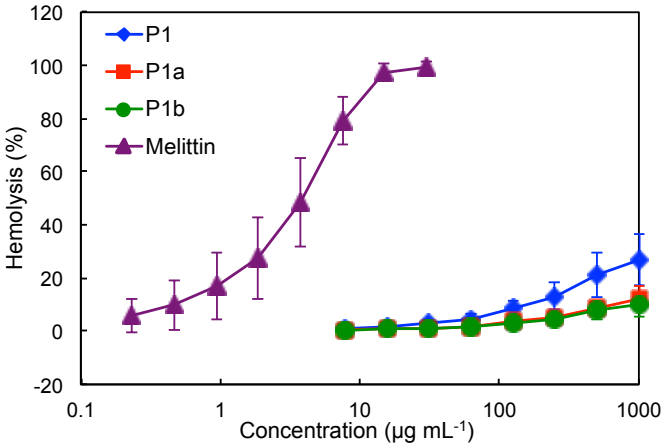

Figure S4. Hemolysis dose-response curves of copolymers and melittin.

SI 6. Fluorescence of $DiSC_3$-(5) dye in the presence of copolymers.

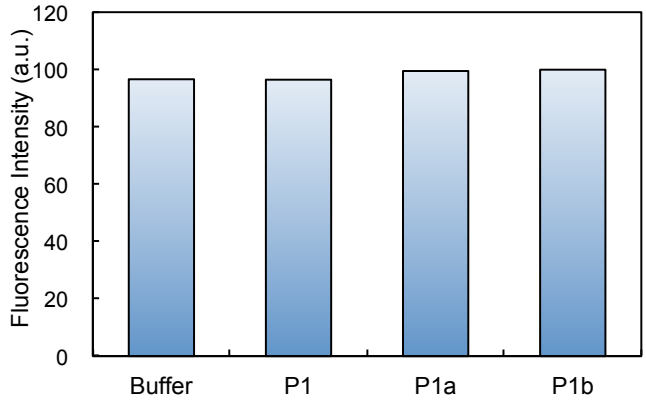

Figure S5. Fluorescence of $DiSC_3$-(5) dye in the presence of copolymers. The dye was mixed with each copolymers for 5 minutes at room temperature and fluorescence intensity at 660 nm was measured. [$DiSC_3$-(5)]= 0.5 μM and [copolymers]= 100 μg mL^{-1}.

References

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