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Effects of Cyclic vs. Acyclic Hydrophobic Subunits on the Chemical Structure and Biological Properties of Nylon-3 Co-Polymers

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Abstract

Nylon-3 co-polymers containing both hydrophobic and cationic subunits can mimic the activity profile of host-defense peptides, if subunit identity and proportion are carefully selected. These sequence- and stereo-random co-polymers inhibit bacterial growth at relatively low concentrations, apparently via disruption of bacterial membranes, but they are relatively non-disruptive toward eukaryotic cell membranes (low hemolytic activity). In all previous examples, the hydrophobic subunits have contained cycloalkyl groups that incorporate the backbone C α -C β bond. Here we have explored the effects of using analogous acyclic hydrophobic subunits. The results indicate that the replacing cyclic with acyclic hydrophobic subunits has a modest influence on biological properties. This influence appears to arise from differences in subunit flexibility.

Increasing bacterial resistance to conventional antibiotics has drawn considerable attention toward the development of new types of antibacterial agents. Host-defense peptides are attractive in this regard because these natural antibiotics can display broad-spectrum activity, and resistance to these peptides seems to be difficult for bacteria to develop.¹ Host-defense peptides are very diverse in terms of sequence, but most feature a combination of hydrophobic and basic amino acid residues.² Many of these peptides appear to disrupt bacterial membranes.^{1,3} A strong preference for prokaryotic vs. eukaryotic cell membranes is typically observed, apparently as a result of Coulombic attraction between these cationic peptides and the net negative charge that is characteristic of prokaryotic cell surfaces.⁴ Numerous synthetic peptides and related oligomers have been reported to display activity/selectivity profiles comparable to those of host-defense peptides;⁵ however, the practical application of natural or designed peptides is limited by the cost associated with step-wise synthesis, which is necessary if amino acid sequence is to be controlled.

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ASSOCIATED CONTENT

Supporting Information: Experimental details and characterization data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Some time ago we proposed that the membrane-selective activity profile of host-defense peptides could be mimicked with *sequence-random* co-polymers containing both hydrophobic and cationic subunits if key physical characteristics, such as cationic:hydrophobic proportion and net hydrophobicity, were properly controlled.⁶ Validation of this prediction would be useful because random co-polymers are much easier to synthesize than are sequence-specific peptides. In recent years diverse cationic-hydrophobic co-polymers have been evaluated for antimicrobial function;^{7,8} earlier reports described antibacterial homopolymers but did not include comparisons with natural peptides.⁹ Our efforts have focused on nylon-3 materials because the subunits are β -amino acid residues and the polymer backbone therefore bears an intrinsic similarity to that of conventional polypeptides (α -amino acid residues).⁸ We have identified specific hydrophobic/cationic subunit identities and proportions that lead to substantial growth inhibition for several bacterial species but low propensity for eukaryotic membrane disruption, as monitored by lysis of red blood cells ("hemolysis"). Optimal nylon-3 co-polymers (Figure 1) display biological activities comparable to those of helix-forming host-defense peptides.⁸

All nylon-3 co-polymers examined for antibacterial activity to date contain cycloalkane-based hydrophobic subunits.⁸ Here we examine the effects of replacing these cyclic subunits with analogous acyclic subunits.

Nylon-3 materials are synthesized by anionic ring-opening polymerization of β -lactams;¹⁰ use of an electrophilic co-initiator determines the group appended to the N-terminus of each polymer chain.^{8,11} We have previously used β -lactams DM β and MM β to generate cationic subunits (after Boc deprotection), and CH β and CO β to generate hydrophobic subunits.⁸ For the present study β -lactams HE β ¹² and OC β ¹³ (Figure 2) served as alternative sources of hydrophobic subunits. HE β and OC β are analogues of CH β and CO β , respectively, lacking the carbocycle; these β -lactams were prepared from the corresponding *cis*-alkenes. Each β -lactam was used in racemic form, and the resulting co-polymers are therefore random in terms of both the sequence and stereochemistry. New polymers in the DM+HE and DM+OC series were prepared in THF.^{8,11a,b} (Note: "DM β " designates a β -lactam, while "DM" designates the resulting nylon-3 subunit.) Use of 5 mol % acid chloride co-initiator (relative to total β -lactam) provided materials with 20–30 subunit average chain length and polydispersity indices (PDI) of 1.01–1.41.¹⁴ Attempts to prepare MM+HE copolymers in THF, however, resulted in insoluble gels, and these materials were therefore synthesized in dimethylacetamide (DMAc). Attempts to prepare MM+OC copolymers were problematic in either THF or DMAc.

Antibacterial activities were initially assessed by measurement of minimum inhibitory concentrations (MIC) for four bacteria, *Escherichia coli*,¹⁵ *Bacillus subtilis*,¹⁶ methicillin-resistant *Staphylococcus aureus* (MRSA),¹⁷ and vancomycin-resistant *Enterococcus faecium* (VRE)¹⁸.

Antibacterial activities for selected co-polymers are summarized in Table 1. Figure 3 shows the extent of hemolysis caused by selected co-polymers as a function of their concentration. Direct comparisons between analogous polymers reveal that those containing cyclically constrained hydrophobic subunits (CH or CO) display somewhat more desirable properties than do those containing analogous acyclic hydrophobic subunits (HE or OC). Thus, for example, DM₅₀CH₅₀ shows moderately lower MIC values than does DM₅₀HE₅₀, and the CH-containing co-polymer induces less hemolysis at a given concentration than does the HE-containing polymer. A similar trend in antibacterial activities is observed for DM₅₀CO₅₀ vs. DM₅₀OC₅₀, with the cyclic CO subunit providing lower MIC values than the acyclic OC subunit; however, both of these co-polymers are highly hemolytic.¹⁴ For the pair containing

MM, in a 3:2 ratio with hydrophobic subunit CH or HE, the MIC values are identical for the three Gram positive bacteria, but the polymer containing CH is more active against *E. coli* (Gram negative) than is the polymer containing HE. In this case use of the cyclic CH subunit results in substantially less hemolytic activity than does use of the acyclic HE subunit.

Why do nylon-3 co-polymers prepared with cyclic β -lactam CH β or CO β display different and somewhat more favorable biological properties than do co-polymers prepared with analogous acyclic β -lactam HE β or OC β ? At least three factors could underly the activity variations, individually or in combination: (1) differences in subunit conformational propensity, (2) differences in subunit hydrophobicity and/or (3) differences in subunit distribution along the co-polymer chains. Within each cyclic-acyclic pair, CH-HE or CO-OC, overall subunit hydrophobicities are expected to be similar because the number of carbon atoms is identical; however, reverse phase HPLC comparison of β -lactams indicates that CH β is somewhat less hydrophobic than HE β , which is consistent with the lower aqueous solubility of n-hexane relative to cyclohexane.¹⁹ Lipophilic dye solubilization measurements indicated similar critical aggregation concentration (CAC) values for representative pairs of polymers in aqueous solution (identical hydrophobic:cationic proportion for each pair).¹⁴ Since self-association of these co-polymer chains in water is presumably driven by the hydrophobic effect, the similarity in CAC values suggests that the replacing CH with HE, or CO with OC, does not lead to a large change in net co-polymer hydrophobicity.

We have previously shown that variations in β -lactam structure can cause differences in polymerization reactivity, which leads to a compositional drift along the resulting co-polymer chains.^{11b} In order to assess whether variations in compositional drift arise from replacing CH β with HE β in a polymerization reaction, we monitored subunit incorporation into nylon-3 co-polymers generated from DM₅₀CH₅₀ and DM₅₀HE₅₀ as a function of β -lactam conversion (Figure 4). The results suggest that both co-polymers are subject to compositional drift, and that the extent and nature of this drift differ between the two materials. For DM₅₀CH₅₀, analysis of remaining β -lactam after varying extents of polymerization indicates that CH β is incorporated a little more readily than DM β into the growing polymer chains. Therefore, after complete conversion and side chain deprotection there should be a slightly higher incidence of hydrophobic subunits near the N-terminus relative to the C-terminus, and vice versa for the cationic subunits, for a typical polymer molecule. In contrast, the data for 1:1 co-polymerization of HE β and DM β indicate that DM β is incorporated substantially more readily than HE β into growing polymer chains. In this case, after complete conversion and side chain deprotection there should be a preponderance of cationic subunits near the N-terminus and a corresponding preponderance of hydrophobic subunits near the C-terminus of the average polymer molecule. It should be noted that the chain ends of these nylon-3 co-polymers are not functionally equivalent,^{8d} since all N-termini bear a hydrophobic p-*t*-butylbenzoyl unit, derived from the co-initiator, while either a unit derived from CH β or a unit derived from DM β can occur at the C-termini. The data in Figure 4 suggest that differences in compositional drift between nylon-3 co-polymers containing cyclic vs. acyclic hydrophobic subunits could potentially contribute to variations in biological activity profiles among such materials.

To test the hypothesis that differences in compositional drift contribute to activity differences between DM₅₀CH₅₀ and DM₅₀HE₅₀, we synthesized co-polymers designated rDM₅₀HE₅₀ by introducing HE β +DM β monomer mixtures in two portions, with differing monomer proportions, to the polymerization reaction.¹⁴ The initial monomer mixture was enriched in HE β , either 2:1 or 3:1, in order to diminish the predominance of DM near the N-termini of the polymer chains; the second monomer batch had the opposite proportion, so that the overall 1:1 subunit proportion was maintained. The rDM₅₀HE₅₀ and DM₅₀HE₅₀

polymers displayed similar biological activity profiles,¹⁴ which suggests that variations in subunit distribution along the DM₅₀HE₅₀ vs. DM₅₀CH₅₀ polymer chains do not have a strong influence on the antibacterial and hemolytic activities of these nylon-3 materials.

As a further examination of functional differences between nylon-3 co-polymers containing constrained vs. flexible subunits, we evaluated the activities of DM₅₀CH₅₀ and DM₅₀HE₅₀ against several pathogenic bacteria including uropathogenic *E.coli* (CFT073)²⁰ and *P. aeruginosa* PA1066 (cystic fibrosis isolate), in terms of both MIC and minimum bactericidal activity (MBC) (Table 2). Both polymers were bactericidal at or near the concentration necessary to inhibit growth (i.e., the MIC). In these tests there were no significant differences between polymers with the cyclic and acyclic subunits, in contrast to the modest differences manifested in Table 1. Moreover, the MIC values in these cases are somewhat higher than those in Table 1. In this regard, it should be noted that the Gram negative bacterium in Table 1 is a laboratory strain of *E. coli*, while clinically isolated bacteria, which tend to be harder than laboratory strains, were used for Table 2. In addition, differences in composition of the growth medium and time of incubation could account for differences in the MIC values in the two Tables.¹⁴ We found that none of the bacteria represented in Table 2 was susceptible to growth inhibition by the host-defense peptide Magainin 2.¹⁴

Antibacterial random co-polymers are of potential utility because they are much easier to prepare than are sequence-specific peptides. Although subunit sequence is not controlled in a random co-polymerization reaction, the properties of the resulting materials can be modulated by changing subunit identity and proportion. Previous efforts to vary hydrophobic subunit properties in nylon-3 co-polymers have focused on altering side chains, e.g., adding or subtracting carbon atoms to tune hydrophobicity, or modifying the nature of the cationic group.⁸ Here we have evaluated a different type of polymer tailoring, involving the use of cyclic vs. acyclic hydrophobic subunits. Our results indicate that a cyclic hydrophobic subunit (CH or CO) can confer improved properties relative to an analogous acyclic subunit (HE or OC). In principle, this trend could arise from (1) alterations in subunit distribution along the polymer chains, (2) changes in subunit hydrophobicity, and/or (3) changes in local backbone flexibility as a result of replacing cyclic with acyclic hydrophobic subunits; however, experiments described above suggest that the first two factors do not exert strong effects on biological activity profiles. Thus, changes in local backbone flexibility between cyclic and acyclic hydrophobic nylon-3 subunits seem to have the largest influence on polymer properties.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. (a) Zasloff M. *Nature*. 2002; 415:389–395. [PubMed: 11807545] (b) Hancock REW, Sahl HG. *Nat. Biotechnol.* 2006; 24:1551–1557. [PubMed: 17160061] (c) Marr AK, Gooderham WJ, Hancock REW. *Curr. Opin. Pharmacol.* 2006; 6:468–472. [PubMed: 16890021]

2. (a) Steiner H, Hultmark D, Engström Å, Bennich H, Boman HG. *Nature*. 1981; 292:246–248. [PubMed: 7019715] (b) Zasloff M. *Proc. Natl. Acad. Sci. U.S.A.* 1987; 84:5449–5453. [PubMed: 3299384] (c) Durr UHN, Sudheendra US, Ramamoorthy A. *Biochim. Biophys. Acta*. 2006; 1758:1408–1425. [PubMed: 16716248]
3. (a) Shai Y. *Biochim Biophys Acta*. 1999; 1462:55–70. [PubMed: 10590302] (b) Tossi A, Sandri L, Giangaspero A. *Biopolymers*. 2000; 55:4–30. [PubMed: 10931439]
4. Axelsen PH. *Biophys J*. 2008; 94:1549–1550. [PubMed: 18065456]
5. (a) Wade D, Boman A, Wählin B, Drain CM, Andreu D, Boman HG, Merrifield RB. *Proc. Natl. Acad. Sci. U.S.A.* 1990; 87:4761–4765. [PubMed: 1693777] (b) Dathe M, Schumann M, Wieprecht T, Winkler A, Beyermann M, Krause E, Matsuzaki K, Murase O, Bienert M. *Biochemistry*. 1996; 35:12612–12622. [PubMed: 8823199] (c) Oren Z, Shai Y. *Biochemistry*. 1997; 36:1826–1835. [PubMed: 9048567] (d) Li C, Budge LP, Driscoll CD, Willardson BM, Allman GW, Savage PB. *J. Am. Chem. Soc.* 1999; 121:931–940. (e) Hamuro Y, Schneider JP, DeGrado WF. *J. Am. Chem. Soc.* 1999; 121:12200–12201. (f) Sharon M, Oren Z, Shai Y, Anglister J. *Biochemistry*. 1999; 38:15305–15316. [PubMed: 10563816] (g) Porter EA, Wang X, Lee H-S, Weisblum B, Gellman SH. *Nature*. 2000; 404:565–565. [PubMed: 10766230] (h) Liu D, DeGrado WF. *J. Am. Chem. Soc.* 2001; 123:7553–7559. [PubMed: 11480975] (i) Tew GN, Liu D, Chen B, Doerksen RJ, Kaplan J, Carroll PJ, Klein ML, DeGrado WF. *Proc. Natl. Acad. Sci. U.S.A.* 2002; 99:5110–5114. [PubMed: 11959961] (j) Porter EA, Weisblum B, Gellman SH. *J. Am. Chem. Soc.* 2002; 124:7324–7330. [PubMed: 12071741] (k) Raguse TL, Porter EA, Weisblum B, Gellman SH. *J. Am. Chem. Soc.* 2002; 124:12774–12785. [PubMed: 12392424] (l) Aravinda S, Shamala N, Desiraju S, Balaram P. *Chem. Commun.* 2002:2454–2455. (m) Oren Z, Ramesh J, Avrahami D, Suryaprakash N, Shai Y, Jelinek R. *Eur. J. Biochem.* 2002; 269:3869–3880. [PubMed: 12180963] (n) Patch JA, Barron AE. *J. Am. Chem. Soc.* 2003; 125:12092–12093. [PubMed: 14518985] (o) Matile S, Som A, Sorde N. *Tetrahedron*. 2004; 60:6405–6435. (p) Schmitt MA, Weisblum B, Gellman SH. *J. Am. Chem. Soc.* 2004; 126:6848–6849. [PubMed: 15174837] (q) Papo N, Shai Y. *Biochemistry*. 2004; 43:6393–6403. [PubMed: 15157073] (r) Nusslein K, Arnt L, Rennie J, Owens C, Tew GN. *Microbiology (Reading, U.K.)*. 2006; 152:1913–1918. (s) Li X, Li Y, Han H, Miller DW, Wang GJ. *J. Am. Chem. Soc.* 2006; 128:5776–5785. [PubMed: 16637646] (t) Schmitt MA, Weisblum B, Gellman SH. *J. Am. Chem. Soc.* 2007; 129:417–428. [PubMed: 17212422] (u) Radziszewsky IS, Rotem S, Bourdetsky D, Navon-Venezia S, Carmeli Y, Mor A. *Nat. Biotechnol.* 2007; 25:657–659. [PubMed: 17529972]
6. Gelman MA, Weisblum B, Lynn DM, Gellman SH. *Org. Lett.* 2004; 6:557–560. [PubMed: 14961622]
7. (a) Ilker MF, Nusslein K, Tew GN, Coughlin EB. *J. Am. Chem. Soc.* 2004; 126:15870–15875. [PubMed: 15571411] (b) Kuroda K, DeGrado WF. *J. Am. Chem. Soc.* 2005; 127:4128–4129. [PubMed: 15783168] (c) Fuchs AD, Tiller JC. *Angew. Chem., Int. Ed.* 2006; 45:6759–6762. (d) Sellenet PH, Allison B, Applegate BM, Youngblood JP. *Biomacromolecules*. 2007; 8:19–23. [PubMed: 17206783] (e) Sambhy V, Peterson BR, Sen A. *Angew. Chem., Int. Ed.* 2008; 47:1250–1254. (f) Lienkamp K, Madkour AE, Musante A, Nelson CF, Nusslein K, Tew GN. *J. Am. Chem. Soc.* 2008; 130:9836–9843. [PubMed: 18593128] (g) Palermo EF, Kuroda K. *Biomacromolecules*. 2009; 10:1416–1428. [PubMed: 19354291] (h) Palermo EF, Sovadinova I, Kuroda K. *Biomacromolecules*. 2009; 10:3098–3107. [PubMed: 19803480] (i) Li P, Poon YF, Li WF, Zhu HY, Yeap SH, Cao Y, Qi XB, Zhou CC, Lamrani M, Beuerman RW, Kang ET, Mu YG, Li CM, Chang MW, Leong SSJ, Chan-Park MB. *Nat. Mater.* 2011; 10:149–156. [PubMed: 21151166] (j) Nederberg F, Zhang Y, Tan JPK, Xu KJ, Wang HY, Yang C, Gao SJ, Guo XD, Fukushima K, Li LJ, Hedrick JL, Yang YY. *Nat. Chem.* 2011; 3:409–414. [PubMed: 21505501]
8. (a) Mowery BP, Lee SE, Kissounko DA, Epand RF, Epand RM, Weisblum B, Stahl SS, Gellman SH. *J. Am. Chem. Soc.* 2007; 129:15474–15476. [PubMed: 18034491] (b) Epand RF, Mowery BP, Lee SE, Stahl SS, Lehrer RI, Gellman SH, Epand RM. *J. Mol. Biol.* 2008; 379:38–50. [PubMed: 18440552] (c) Mowery BP, Lindner AH, Weisblum B, Stahl SS, Gellman SH. *J. Am. Chem. Soc.* 2009; 131:9735–9745. [PubMed: 19601684] (d) Zhang J, Markiewicz MJ, Mowery BP, Weisblum B, Stahl SS, Gellman SH. *Biomacromolecules*. 2012; 13:323–331. [PubMed: 22168316] (e) Zhang J, Markiewicz MJ, Weisblum B, Shannon S, Stahl SS, Gellman SH. *ACS Macro Lett.* 2012; 1:714–717. [PubMed: 23355958] (f) Liu R, Masters KS, Gellman SH. *Biomacromolecules*. 2012; 13:1100–1105. [PubMed: 22455338] (g) Liu R, Chen X, Hayouka Z, Chakraborty S, Falk SP,

- Weisblum B, Masters KS, Gellman SH. *J. Am. Chem. Soc.* 2013; 135:5270–5273. [PubMed: 23547967]
9. (a) Ikeda T, Tazuke S, Suzuki Y. *Makromol. Chem.* 1984; 185:869–876. (b) Kawabata N, Nishiguchi M. *Appl. Environ. Microbiol.* 1988; 54:2532–2535. [PubMed: 3202632] (c) Senuma M, Tashiro T, Iwakura M, Kaeriyama K, Shimura Y. *J. Appl. Polym. Sci.* 1989; 37:2837–2843. (d) Li GJ, Shen JR, Zhu YL. *J. Appl. Polym. Sci.* 1998; 67:1761–1768. (e) Tiller JC, Liao CJ, Lewis K, Klibanov AM. *Proc. Natl. Acad. Sci. U.S.A.* 2001; 98:5981–5985. [PubMed: 11353851]
10. (a) Graf R, Lohaus G, Börner K, Schmidt E, Bestian H. *Angew. Chem., Int. Ed.* 1962; 1:481–488. (b) Moriconi EJ, Kelly JF. *J. Org. Chem.* 1968; 33:3036–3046. (c) Hashimoto K. *Prog. Polym. Sci.* 2000; 25:1411–1462.
11. (a) Zhang J, Kissounko DA, Lee SE, Gellman SH, Stahl SS. *J. Am. Chem. Soc.* 2009; 131:1589–1597. [PubMed: 19125651] (b) Zhang J, Gellman SH, Stahl SS. *Macromolecules.* 2010; 43:5618–5626. (c) Dane EL, Grinstaff MW. *J. Am. Chem. Soc.* 2012; 134:16255–16264. [PubMed: 22937875]
12. Moriconi EJ, Kelly JF. *J. Org. Chem.* 1968; 33:3036–3046.
13. Chen L, Lei Y, Shilabin AG, Delaney JD, Baran GR, Seiburth S. *McN. Chem. Commun.* 2012; 48:9604–9606.
14. Please see the supporting information.
15. Yanisch-Perron C, Vieira J, Messing. *J. Gene.* 1985; 33:103–119.
16. Young FE, Smith C, Reilly BE. *J. Bacteriol.* 1969; 98:1087–1097. [PubMed: 4977981]
17. Nicas TI, Wu CYE, Hobbs JN, Preston DA, Allen NE. *Antimicrob. Agents Chemother.* 1989; 33:1121–1124. [PubMed: 2528940]
18. Weisblum B, Demohn V. *J. Bacteriol.* 1969; 98:447–452. [PubMed: 5784204]
19. Tsonopoulos C, Wilson JM. *AIChE, J.* 1983; 29:990–999.
20. Mobley HL, Green DM, Trifillis AL, Johnson DE, Chippendale GR, Lockatell CV, Jones BD, Warren JW. *Infect. Immun.* 1990; 58:1281–1289. [PubMed: 2182540]

Heterochiral

Figure 1.

Representative nylon-3 co-polymer containing subunits derived from β -lactams MM β and CH β (both racemic) in a 60:40 ratio. The polymer inhibits the growth of several bacteria at relatively low concentrations but exhibits only weak hemolytic activity.

Figure 2.

(a) β -lactams used in this study. (b) Random nylon-3 co-polymer containing cis-hexyl subunit.

Figure 3.
Hemolytic activity of nylon-3 co-polymers.

Figure 4.

(a) Composition of co-polymers (fraction derived from CH β or HE β , as deduced by measuring residual β -lactam by GC at various extents of reaction, at room temperature) versus total conversion of the monomers; (b, c) cartoons meant to illustrate different subunit distributions in typical co-polymer molecules that result from replacing the cyclic CH unit with the acyclic HE unit in the polymer backbone. The open balls represent CH or HE subunits, and the filled balls represent DM subunits. The green ball represents the N-terminal *p-t*-benzoyl group.

Table 1

Nylon-3 Co-Polymer Antibacterial Activities

Polymers	MIC ^a (μ g/mL) of copolymers			
	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. faecium</i>
DM ₅₀ CH ₅₀	6.25	3.13	6.25	6.25
DM ₅₀ HE ₅₀	25	6.25	25	25
DM ₅₀ CO ₅₀	6.25	1.6	6.25	6.25
DM ₅₀ OC ₅₀	100	6.25	25	25
MM ₆₀ CH ₄₀	50	3.13	12.5	25
MM ₆₀ HE ₄₀	>200	3.13	12.5	25

^a MIC = Minimum inhibitory concentration for bacterial growth.

Table 2Bactericidal activity of DM₅₀CH₅₀ vs. DM₅₀HE₅₀.

Bacteria	DM ₅₀ CH ₅₀		DM ₅₀ HE ₅₀	
	MIC (μ g/mL)	MBC ^a (μ g/mL)	MIC (μ g/mL)	MBC ^a (μ g/mL)
<i>E. coli</i> (CFT073)	25	50	50	50
<i>S. enterica</i> (LT2)	50	100	50	100
<i>B. cereus</i> (ATCC14579)	100	100	100	100
<i>P. aeruginosa</i> (PA01)	25	25–50	50	50–100
<i>P. aeruginosa</i> (PA1066)	25	50	50	100

^aMBC = Minimum bactericidal concentration.