ChemComm

Cite this: *Chem. Commun.*, 2012, **48**, 892–894

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**COMMUNICATION**

# Mimicking an antimicrobial peptide polymyxin B by use of cyclodextrin†

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*Received 13th October 2011, Accepted 17th November 2011*
**DOI: 10.1039/c1cc16369h**



**Cyclodextrin derivatives prepared to mimic a membrane active antibacterial peptide polymyxin B strongly permeabilized bacterial membrane and inhibited bacterial proliferation.**

Polymyxin B is a membrane active cyclic peptide that strongly permeabilizes both the outer and cytoplasmic membranes of gram-negative bacteria (Fig. 1) and has been recognized as a promising agent against multidrug-resistant bacteria.[1] The peptide contains five diaminobutanoic acid residues whose side chain amino groups form a cationic region of an amphiphilic peptide structure. In order to disrupt the bacterial membrane, the polycationic region is considered to interact with the anionic phosphate moiety of the membrane, while the hydrophobic fatty acid tail interacts with the fatty acid moiety of the lipid membrane.[2] Polymyxin B nonapeptide, a proteolytic product of polymyxin B lacking the fatty acid tail, possesses no antibacterial activity but retains the high outer membrane-permeabilizing activity, and is expected to be of clinical importance for the development of bacterial outer membrane permeabilizers.[3] Studies to mimic the antibiotic peptide may lead not only to understanding of the relationship between their structures and activities but also to the development of new antimicrobial drugs.

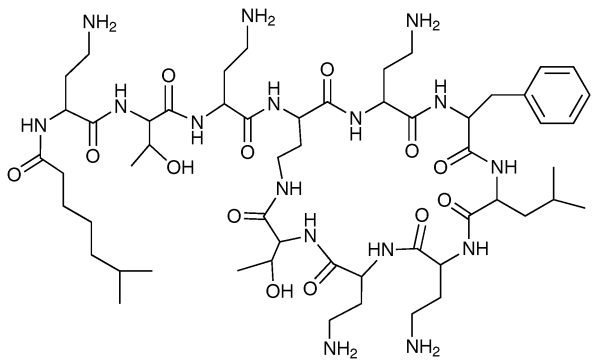

**Fig. 1** Structure of polymyxin B.

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c1cc16369h

In this study we used a cyclic oligosaccharide called cyclodextrin (CD) for this purpose.

The oligosaccharide consists of a D-glucose residue, with numbers six, seven or eight called α-, β-, or γ-CD, respectively. It has a truncated cone structure in which one side of the cone is rimmed by primary 6-OH groups of glucose residues and the other by secondary 2-and 3-OH groups. Its size (diameter) is *ca.* 1nm, almost the same as that of polymyxin B. We believed that a regiospecific chemical modification of the CD molecule[4] would allow the CD to realize a polymyxin B-like structure with arranged functional groups on its cyclic structure. An additional advantage of the CD to mimic polymyxin B is the rigidity of its cyclic structure due to hydrogen bonds between the residues. In analogue peptide studies, substitution and insertion of amino acid residue(s) often causes changes in conformation of the peptide that resulted in less antibacterial activity.[5]

Firstly, as an amino-group assembly on the CD mimicking the polycationic region of polymyxin B to interact with an anionic bacterial membrane, we prepared polyamino CD derivatives possessing amino groups on C-6 and whose number of amino groups varied from six to eight, namely hexamino-α-CD **1**, heptaamino-β-CD **2** and octaamino-γ-CD **3**, respectively (Fig. 2).[6] Each was derived from conversion of the corresponding poly-6-*O*-sulfonyl-CD[7] to an azide followed by reduction to the amine according the method of Lehn *et al.*[8]

Activity of the amino CDs **1–3** to permeabilize a bacterial membrane was examined. The CD acted on bacteria and $K^+$ efflux from bacterial cell cytosol was measured by a $K^+$ electrode (Fig. 3).[9] The presence of CDs did not interfere with the measurement of the electrode. The compound **3** possessing eight amino groups caused the most $K^+$ efflux from both *Staphylococcus aureus* FDA 209P and *Escherichia coli* K12 strain W3110. Membrane activity of the heptaamino derivative **2** was moderate and that of hex-amino derivative **1** was the

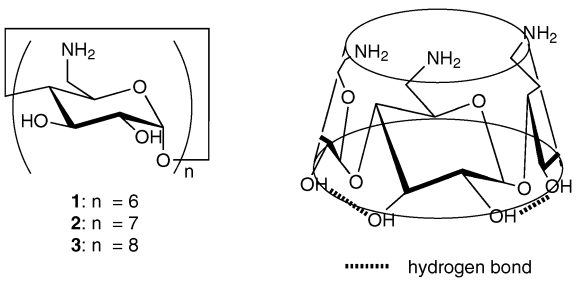



**Fig. 2** Structures of cyclodextrins **1–3**.

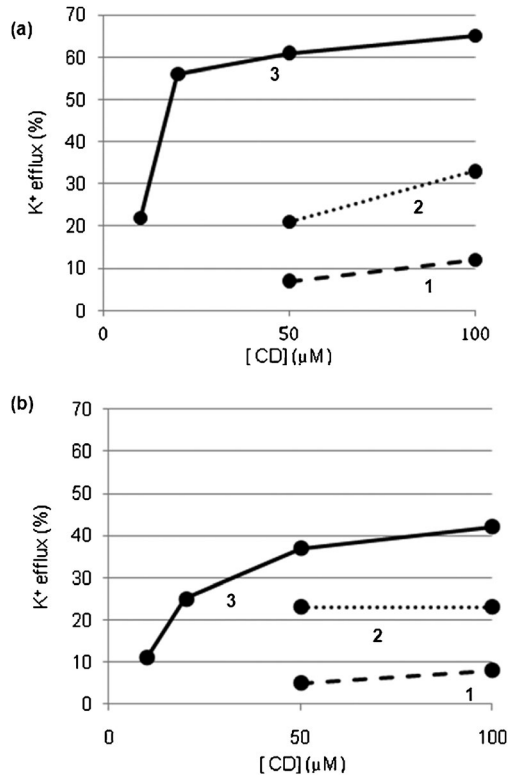
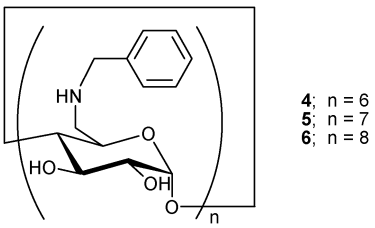



**Fig. 3** $K^+$ efflux from *S. aureus* (a) and *E. coli* (b) caused by polyamino CDs **1–3**.

least, while other derivatives with less than five amino groups were ineffective (data not shown). The observed dependence on the number of amino groups of the CD molecule demonstrated the importance of the number of amino groups on the molecule to effectively interact with a bacterial membrane.‡

The observed $K^+$ efflux from *E. coli* (gram negative) was smaller than that from *S. aureus* (gram positive), which may be due to the differences in membrane structure between the bacteria. Presumably the outer membrane in a gram negative strain may obstruct CD. However the $K^+$ efflux from *E. coli* suggested that not only the inner cytoplasmic membrane of the bacteria but also its outer membrane were permeabilized. This was confirmed by the synergistically high antimicrobial activity of the combination of the CD and the probe antibiotics (Fig. 4). The probes, novobiocin and erythromycin, exhibited low activity when dosed alone due to the outer membrane acting as a barrier. Upon addition of the amino CDs, both antibiotics inhibited the growth of *E. coli*. In particular, the effect of octaamino **3** almost equaled to that of polymyxin B nonapeptide as a positive control, while none of the amino CDs **1–3** themselves showed significant antimicrobial activity (MIC $>128$ μg $mL^{-1}$). Also, none of them showed hemolytic toxicity ($\sim$0%, CD 100 μM). These results are also similar to the effects of polymyxin B nonapeptide.




Next we added to the amino CDs benzyl groups as hydrophobic moieties, creating an amphiphilic property, because appropriate amphiphilicity is important to enhance antimicrobial activity.[10] Substitution of the corresponding CD poly-6-*O*-sulfonates by benzylamine gave polybenzylamino derivatives **4–6**.§

Their values of MIC against *E. coli* were moderate (32–64 μg $mL^{-1}$), while those against *S. aureus* were strong (4–8 μg $mL^{-1}$) (Table 1). These values were similar to those of gramicidin S, an antibacterial peptide against gram-positive bacteria.[11] Furthermore, we observed that **4–6** caused complete loss of $K^+$ in *S. aureus* (Fig. 5), whose concentrations giving 50% $K^+$ efflux (5–20 μM) were similar to that of gramicidin S (5–10 μM),[11] demonstrating that the benzylamino CDs caused higher disruption of the bacterial membrane than polyamino derivatives **1–3**, with the prime target being the cytoplasmic membrane. Hemolytic toxicity of **6** at 100 μM was 94% while that of gramicidin S was 100%. It is interesting that the addition of hydrophobic benzyl groups to polymyxin B nonapeptide-like amino CDs led to gramicidin S-like membrane activity.

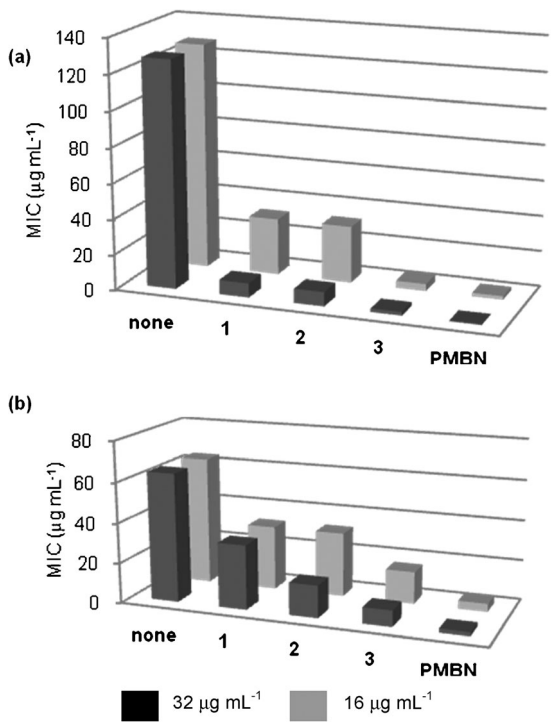



**Fig. 4** Synergistically antimicrobial activity of the combination of polyamino CDs **1–3** with novobiocin (a) and erythromycin (b). None: the antibiotics only. PMBN: polymyxin B nonapeptide.

**Table 1** MIC of polybenzylamino CDs **4–6**

| | MIC/μg $mL^{-1}$ | |
|---|---|---|
| Compounds | *S. aureus* | *E. coli.* |
| **4** | 4 | 64 |
| **5** | 4 | 64 |
| **6** | 8 | 32 |
| gramicidin S[a] | 4 | 32 |
| polymyxin B[b] | 64 | 0.5 |

[a] See ref. 11. [b] See ref. 5*a*.

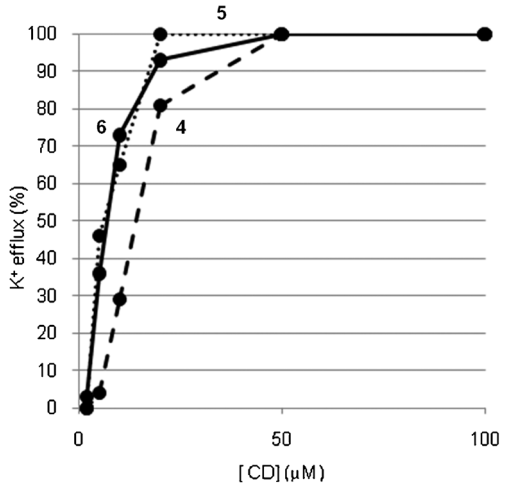



**Fig. 5** $K^+$ efflux from *S. aureus* caused by polybenzylamino CDs **4–6**.

In conclusion, we prepared amino group-containing CD derivatives to mimic membrane active antibacterial peptide polymyxin B. The derivatives effectively disrupted bacterial membranes. It is noteworthy that the CD derivatives with relatively simple substituents realized similar activities to those of peptides with relatively complex structures. Preliminary experiments showed that both the MIC values of **6** against methicillin-resistant *S. aureus* (MASA) and vancomycin-resistant *Enterococci* (VRE) were 12.5 μg $mL^{-1}$. All the results shown here demonstrated that our chemistry to mimic antibacterial peptides by CD molecules is promising. Also, the method to construct a nano-scale antibacterial site may lead to novel antimicrobial modifications for material surfaces. Further studies are on the way. The authors wish to thank Japan Maize Products Co. Ltd for the generous gift of CD.

## Notes and references

‡ The observed higher activity of octaamino γ-CD **3** than those of hexaamino α-CD **1** and heptaamino β-CD **2** may be due not only to the larger number of amino groups but also to its ring structure. Additional experiments by use of not-fully-aminated γ-CD and aminated linear oligosaccharides would be performed to address it.

§ **4**; $^1$H NMR (300 MHz, $D_2O$) $\delta$ 3.05–3.30 (12 H, H6), 3.44 (6H, t, $J$ = 8.5 Hz, H4), 3.53 (6H, br d, $J$ = 10.5 Hz, H2), 3.60 (6H, d, $J$ = 12.5 Hz, benzyl $CH_2$), 3.73 (6H, d, $J$ = 12.5, benzyl $CH_2$), 3.91 (6H, t, $J$ = 9.0 Hz, H3), 4.25 (6H, m, H5), 5.01 (6H, br s, H1), 7.10–7.30 (30H, phenyl CH), **5**; $^1$H NMR (300 MHz, $D_2O$) $\delta$ 3.07–3.17 (14H, H6), 3.43 (7H, t, $J$ = 9.0 Hz, H4), 3.51 (7H, d, $J$ = 12.5 Hz, benzyl $CH_2$), 3.55 (7H, dd, $J$ = 9.5, 3.0 Hz, H2), 3.75 (7H, d, $J$ = 12.5, benzyl $CH_2$), 3.87 (7H, t, $J$ = 9.5, H3), 4.23 (7H, m, H5), 5.03 (7H, d, $J$ = 3.0 Hz, H1), 7.10–7.17 (35H, phenyl CH), **6**; $^1$H NMR (300 MHz, $D_2O$) $\delta$ 3.10–3.20 (16H, H6), 3.57 (8H, t, $J$ = 9.5Hz, H4), 3.69 (8H, dd, $J$ = 10.0, 3.0 Hz, H2), 3.75 (16H, br s, benzyl $CH_2$), 3.98 (8H, t, $J$ = 9.5, H3), 4.35(8H, m, H5), 5.22 (8H, d, $J$ = 3.5 Hz, H1), 7.25–7.31 (40H, phenyl CH).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