Antibiotics

Non-haemolytic β-amino-acid oligomers

Pathogenic bacteria are becoming increasingly resistant to common antibiotics, stimulating an intensive search for new ones. Knowing that a class of medium-sized peptides (magainins[1]) are widely used by host organisms as a defence against microbial invasion[2], we developed a β-amino-acid oligomer (β-peptide) that mimics these natural antibiotics and tested it for antimicrobial activity. We find not only that the activity of our β-peptide is comparable to that of a magainin derivative but also that it is effective against four bacterial species, including two pathogens that are resistant to common antibiotics.

Natural peptide antibiotics are highly diverse in terms of size, sequence and conformation[2]; they are cationic and often adopt amphiphilic secondary structures. One common class, exemplified by the magainins[1], features 20- to 30-residue peptides that form amphiphilic α-helices (hydrophobic side chains on one side of the helix and cationic side chains on the other) which are attracted to the negatively charged surfaces of bacteria. These helices then somehow disrupt the bacterial membrane[3–5].

β-peptides are promising antimicrobial candidates because they offer a choice of secondary structures[6–8] and because the unnatural β-peptide backbone is resistant to protease degradation[9], in contrast to the α-amino-acid backbone of conventional peptides. Three distinct helical conformations have been identified among β-peptides, with the helix type being determined by the substitution pattern on the β-amino-acid residues[6–8]. Oligomers of (*R*,*R*)-*trans*-2-aminocyclopentanecarboxylic acid (ACPC) adopt a helix defined by a 12-membered ring formed as a result of hydrogen-bonding between each backbone carbonyl group and the amide proton of the third residue in the carboxy-terminal direction (a 12-helix)[10].

To test our amphiphilic versions of the β-peptide 12-helix for antimicrobial activity, we used (*3R,4S*)-*trans*-4-aminopyrrolidine-3-carboxylic acid (APC), together with ACPC, to prepare a β-17 oligomer (Fig. 1a). The APC residue should be cationic at pH 8 or below, by virtue of ring-nitrogen protonation. Because the 12-helix has about 2.5 residues per turn, the 12-helical conformation of β-17 should be amphiphilic, with all hydrophilic APC residues on one side of the helix and all hydrophobic ACPC residues on the other (Fig. 1b).

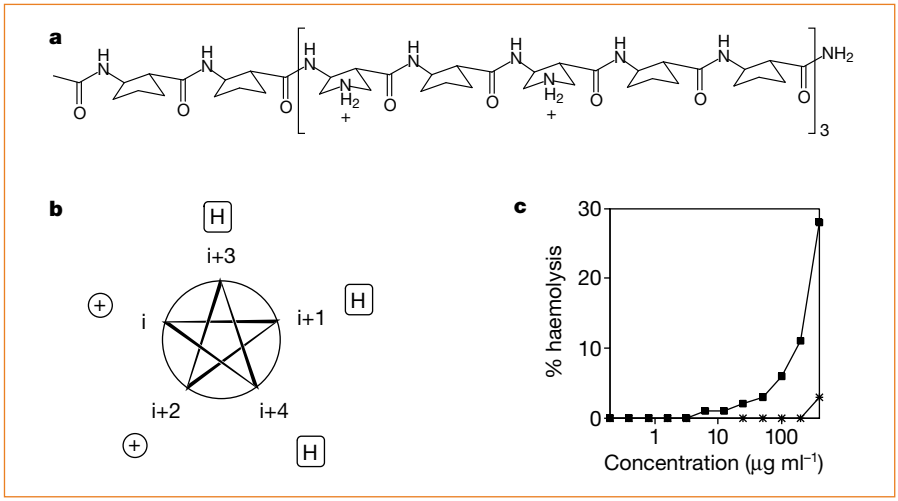

Figure 1 Structure and haemolytic activity of β-17. **a,** Chemical structure of β-17. **b,** Axial projection of the β-peptide 12-helix, highlighting the ~5-residue repeat that results from there being approximately 2.5 residues per 12-helical turn. The repeating pentad in β-17, +H+HH (+, cationic APC residue; H, hydrophobic ACPC residue), is shown. **c,** Haemolytic activity of β-17 (squares), the magainin derivative (crosses), and melittin (circles). Human red blood cells (hRBC, 1% suspension in PBS buffer) were incubated at room temperature for 1 h with a twofold serial dilution of peptide in PBS buffer. Release of haemoglobin was determined from the absorbance at 415 nm of the supernatant after centrifugation. Controls, hRBC suspended in PBS (0% hydrolysis) or in 1% SDS (100% haemolysis).

We compared the activities of β-17 and the synthetic magainin derivative[11] GIGK-FLHAAKKFAKAFVAEIMNS-NH_2 against four bacteria (Table 1). The bacterium *Enterococcus faecium* A436 (which is vancomycin resistant) and *Staphylococcus aureus* 5332 (methicillin resistant) are clinical isolates, whereas *Bacillus subtilis* BR151 and *Escherichia coli* JM109 are non-pathogenic strains commonly used in the laboratory for genetic construction. We find that the activity of β-17 is comparable to that of the magainin against all four species of bacteria.

To be useful therapeutically, this antimicrobial action must be effective in the presence of human cells. We therefore tested the effect of our β-peptide on red blood cells, knowing that although magainins themselves are only weakly haemolytic (cause red blood cells to break open), other natural cationic helix-forming peptides, such as melittin[12], are strongly haemolytic . Figure 1c compares haemolysis by melittin (a positive control), the magainin derivative, and β-17: the β-peptide has even less haemolytic activity than the magainin. Another class of β-peptides composed of acyclic residues are also potent against *E. coli*, but these are highly haemolytic[13], limiting their therapeutic application.

We have devised a hetero-oligomer with an unnatural backbone that can be used to mimic a specific and useful biological activity displayed by a naturally defensive, medium-sized peptide. The chemical and conformational stability of β-peptides may lead to the creation of a new class of antimicrobial agents, which will add to their other unusual clinical applications[14].

Emilie A. Porter*, Xifang Wang*, Hee-Seung Lee*, Bernard Weisblum†, Samuel H. Gellman*

**Department of Chemistry and †Department of Pharmacology, University of Wisconsin, Madison, Wisconsin 53706, USA*

e-mail: gellman@chem.wisc.edu

Table 1 Bacteriostatic and bactericidal activities of β-17 and Ala8,13,18-magainin II amide

		E. coli	*B. subtilis*	*E. faecium*	*S. aureus*
β-17	MIC	6.3	1.6	12.5	3.2
	MBC	6.3	3.2	~50*	~12.5*
Magainin	MIC	3.2	1.6	25	25
	MBC	3.2	3.2	100	>100

Minimal inhibitory concentration (MIC, in μg ml^{-1}) is defined here as the lowest concentration of peptide required for complete inhibition of growth, as determined from the absorbance at 590 nm. Bacteria in BHI medium (~10^6 CFU ml^{-1}) were incubated for 6 h at 37 °C with a twofold-dilution series of peptide in medium in a sterile 96-well plate. Microbial growth was determined by the increase in A_{590} over the 6-h period. Minimal bactericidal concentration (MBC, in μg ml^{-1}) refers to the lowest concentration of peptide required for the absence of viable colonies. The bactericidal activity of the peptide was measured at the MIC, MIC × 2 and MIC × 4. The well solution was diluted and plated to allow a maximum of ~10^3 colony-forming units to grow on the agar plate. Plates at zero time contained ~10^3 colonies; they were incubated overnight at 37 °C, and the colonies counted for determination of bactericidal activity.
*Peptide concentration of 100 μg ml^{-1} revealed 10 colonies or less (~99% killed).

1. Zasloff, M. *Proc. Natl Acad. Sci. USA* **84,** 5449–5453 (1987).
2. Boman, H. G. *Annu. Rev. Immunol.* **13,** 61–92 (1995).
3. Saberwal, G. & Nagaraj, R. *Biochim. Biophys. Acta* **1197,** 109–131 (1994).
4. Oren, Z. & Shai, Y. *Biopolymers* **47,** 451–463 (1999).
5. Wu, M., Maier, E., Benz, R. & Hancock, R. E. W. *Biochemistry* **38,** 7235–7242 (1999).
6. Seebach, D. & Matthews, J. L. *J. Chem. Soc. Chem. Commun.* 2015–2022 (1997).
7. Gellman, S. H. *Acc. Chem. Res.* **31,** 173–180 (1998).
8. DeGrado, W. F., Schneider, J. P. & Hamuro, Y. *J. Peptide Res.* **54,** 206–217 (1999).
9. Hintermann, T. & Seebach, D. *Chimia* **51,** 244–248 (1997).
10. Appella, D. H. *et al. Nature* **387,** 381–384 (1997).
11. Chen, H.-C., Brown, J. H., Morell, J. L. & Huang, C. M. *FEBS Lett.* **236,** 462–466 (1988).
12. Habermann, E. *Science* **177,** 314–322 (1972).
13. Hamuro, Y., Schneider, J. P. & DeGrado, W. F. *J. Am. Chem. Soc.* **121,** 12200–12201 (1999).
14. Werder, M., Hauser, H., Abele, S. & Seebach, D. *Helv. Chim. Acta* **82,** 1774–1783 (1999).