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MicroCommentary

A lesson in efficient killing from two-component lantibiotics

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Summary

The combined activity of the constituents of two-component antibiotic systems is always significantly higher than the sum of the activities of the individual pieces. Understanding the principles behind this phenomenon might provide new ways to design new antibiotics. In this issue of Molecular Microbiology, Wiedemann and coworkers have made a big step towards understanding the mechanism of action of the two-component lanthionine-containing antibiotic lacticin 3147. It has now become clear that this two-component system specifically targets the bacterial cell wall precursor Lipid II. This makes this essential bacterial lipid one of the most sought-after targets in nature. Surprisingly, in view of its small size (MW 1875 Da), this is now the fifth different way that this key molecule is known to be targeted.

With the ever-increasing prevalence of antibiotic resistance and the almost empty antibiotic pipelines of the pharmaceutical industry, there is a great need for new antibiotics. In designing new antibiotics, it is very helpful to look at the antibiotics that bacteria themselves use in their fight for survival. Evolutionary forces have shaped and continue to shape these antibiotics into excellent weapons. By learning how they work, we might be able to design even better ones and keep ahead in the arms race against the resistant bacteria.

The family of lanthionine-containing antibiotics (lantibiotics) is one of the most widespread antibiotic families of bacterial origin in nature. This family can teach us some important lessons on how to kill pathogens. It includes a variety of peptides (for an excellent overview see Chatterjee *et al*., 2005), of which a surprising number is targeted to a lipid-like molecule called Lipid II that plays an essential role in bacterial cell wall synthesis. This molecule provides the bacterial cell wall synthesis machinery with substrates, as it carries the basic building block of the peptidoglycan across the bacterial plasma membrane, and is an ideal target for antibiotics (Breukink and de Kruijff, 2006). Until recently, only single-component lantibiotics were shown to target Lipid II, which they use in at least two different ways. Members of the lantibiotic type B subgroup bind to Lipid II and thus inhibit cell wall synthesis. Type A lantibiotics have an additional killing mode in which they use this lipid as a high-affinity binding site to form pores in the targeted membrane. The most prominent A-group lantibiotic is nisin (Breukink *et al*., 1999). In this issue of *Molecular Microbiology*, Wiedemann and coworkers show that a two-component lantibiotic, lacticin 3147, also kills bacteria by targeting Lipid II.

Although not common, synergism between two structurally different antibiotics that seem to have been deliberately designed as such has been observed before. For example, streptogramins usually consist of mixtures of two structurally dissimilar cyclic peptides that can act separately but have greater activity when mixed in the appropriate ratios. The best-known combination is that of quinupristin/dalfopristin, which is sold as Synercid and is used to treat infections by Gram-positive bacteria. The basis for their synergism is the enhanced affinity of one component for the 50S ribosomal subunit when the other component is already bound (Cocito *et al*., 1997), a situation not unlike that of the two-component lanticins, as discussed below.

The study by Wiedemann and coworkers revealed a sequence of events in which the A1 peptide of lacticin binds first to Lipid II to generate a binding site for the A2 peptide. The subsequent binding of the A2 peptide drives the system towards pore formation. As a result, the activity of the two peptides combined is significantly higher than the sum of the activities of the individual peptides. With this lantibiotic, it seems that nature has for some reason chosen to divide the two functionalities found in the type A lantibiotics (targeting followed by pore formation) between two components. To date, five homologues of lacticin 3147 are known, and this number is likely to increase. Their

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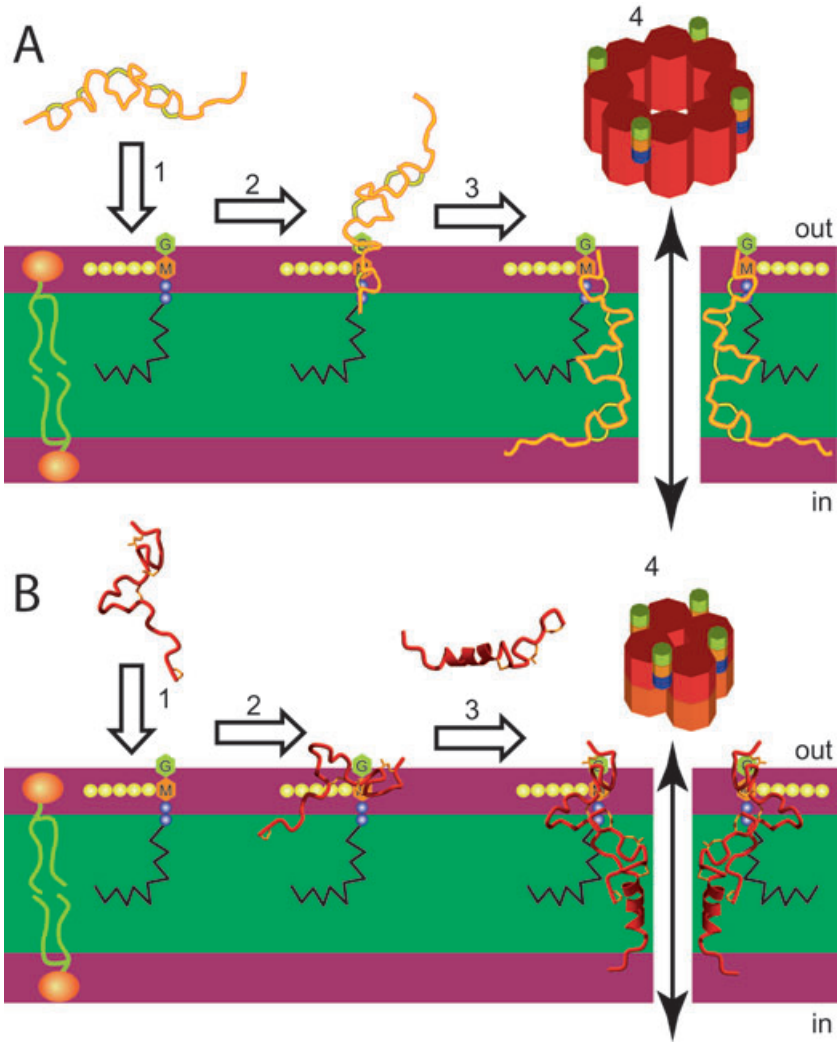

Fig. 1. Mechanism of action (MOA) of pore-forming lantibiotics.
A. MOA of nisin. Nisin first reaches the bacterial plasma membrane (1), where it binds to Lipid II via two of its N-terminal rings (2). This is then followed by pore formation (3), which involves a stable transmembrane orientation of nisin. During the pore-formation process, four other nisin molecules are recruited to form the pore complex with 8:4 (nisin over Lipid II) stochiometry, generating a 2 nm-diameter pore (4).
B. MOA of lacticin 3147. The A1 peptide first binds to Lipid II, probably followed by insertion, to a certain extent, of the peptide into the bilayer (1,2). This triggers a conformational change in the A1 peptide whereupon a high-affinity binding site is generated for the A2 peptide, which is followed by pore formation (3). The pore complex might have a stochiometry of 4:4:4, with a significant smaller pore size (0.6 nm) compared with the nisin-Lipid II pore. This figure is reproduced/adapted with permission from Nature Reviews Drug Discovery (Breukink and de Kruijff, 2006) © (2006) Macmillan Magazines Ltd. The lacticin 3147 structures were kindly provided by Dr N.I. Martin.

apparent rarity is probably due to the fact that scientists were primed to look for only one active compound. Intriguing questions that arise are why bacteria have chosen to develop two-component lantibiotics and what the benefits are. The answers are probably not a lower frequency of acquired resistance compared with one-component antibiotics, because the separate peptides do not have high activities and so inactivation of one component would be enough to confer resistance. Maybe the answers can be found when comparing the difference in activities towards different strains, for instance between nisin and lacticin 3147. However, both peptides are produced by *Lactococcus lactis* bacteria that come from similar niches, so the reason for target differentiation remains obscure.

It is interesting to compare the results of Wiedemann and coworkers on the mode of action of lacticin with those on the mode of action of nisin. Nisin also uses Lipid II to form stable pores in the bacterial membrane (the current model for this pore-formation mechanism is depicted in Fig. 1A). Eight nisin and four Lipid II molecules constitute a pore complex with a diameter of 2 nm (Hasper *et al*., 2004; Wiedemann *et al*., 2004). The initial complex of nisin with Lipid II, of which the NMR-structure has been solved (Hsu *et al*., 2004), has a stochiometry of 1:1. This implies that binding of the first nisin molecule to Lipid II generates a binding site for another nisin molecule (Hasper *et al*., 2004), much like the early events proposed for the mechanism of action of lacticin 3147 by Wiedemann and coworkers. Pore formation by nisin requires a flexible hinge region, which likely facilitates the transfer of the C-terminus across the membrane such that nisin adopts a *trans*-membrane orientation. This is likely to be a cooperative process requiring several nisin molecules that might be achieved by first forming a pre-assembled complex of the 8:4 stochiometry followed by the cooperative insertion and translocation of the C-termini to obtain the final *trans*-membrane orientation (Hasper *et al*., 2004). Lacticin 3147 and related two-component lantibiotics are likely to operate in much the same way. Translocation of the positively charged C-terminus of the A2

peptide (or β-peptide in the case of the other two-component systems) upon pore formation would be energetically more favourable in a cooperative action together with other peptides simultaneously with pore formation. It would be energetically unfavourable for the highly charged C-terminus of the plantaricin W β-peptide, containing seven positively charged residues to traverse the membrane on its own even in the presence of a membrane potential. Alternatively, the C-terminus of the A2 peptide could be the recognition site for the A1 peptide, with the N-terminus being translocated across the membrane, which would explain the presence of the lanthionine rings in this part of the peptide. The D-Ala residues that are present in the N-terminus of the A2 peptide and were shown to be important for the activity (Cotter *et al*., 2005), might then be required for the interactions between the peptides in the pore complex. This reasoning can easily be reversed, pointing to the need for further experiments to understand this fascinating system.

The stochiometry of the pore complex of lacticin 3147 might also resemble that of nisin, with the roles of two nisin molecules being performed by the A1 and 2 peptides, leaving a pore complex composed of 4:4:4 molecules (A1 : A2 : Lipid II) (Fig. 1B). Such a stochiometry would also imply a smaller pore size, as the size of the two lacticin peptides together is much less than the sum of two nisin molecules.

The α-peptides of the two-component systems, including the A1 peptide of lacticin 3147, share a completely conserved region with members of the Lipid II-interacting type B-lantibiotics of which mersacidin is the most well known. This region is likely to be responsible for the interaction with Lipid II. An interesting observation of Wiedemann and coworkers is that mersacidin could not functionally replace the A1 peptide in the two-component system. Mersacidin might have been member of a two-component system from which one peptide has been lost, eliminating the need to maintain the ability to interact with a second peptide. Apparently, the interaction between the two lacticin 3147 peptides is very specific, but it also raises an interesting question about the absence of an interacting partner for mersacidin. Did the producer strain of mersacidin lose the second peptide?

It would be interesting to know the structural requirements for the interaction of the two-component lantibiotics with each other and with Lipid II. At first sight, the D-Ala residues in lacticin 3147 might be required for these interactions, as modification of these residues significantly reduces activity (Cotter *et al*., 2005). However, other two-component lantibiotics do not have residues in the D-configuration. The recent progress in the *in vitro* synthesis of lantibiotics, which ensures access to far more variants than can be generated via genetic manipulation, together with the elucidation of the crystal structures of the modifying enzymes (Xie *et al*., 2004; Li, *et al*., 2006) will be helpful tools for resolving the structural requirements of the mutual interactions within these two-component systems.

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