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An Alternative Bactericidal Mechanism of Action for Lantibiotic Peptides That Target Lipid II

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Lantibiotics are polycyclic peptides containing unusual amino acids, which have binding specificity for bacterial cells, targeting the bacterial cell wall component lipid II to form pores and thereby lyse the cells. Yet several members of these lipid II–targeted lantibiotics are too short to be able to span the lipid bilayer and cannot form pores, but somehow maintain their antibacterial efficacy. We describe an alternative mechanism by which members of the lantibiotic family kill Gram-positive bacteria by removing lipid II from the cell division site (or septum) and thus block cell wall synthesis.

In less than eight decades, Alexander Fleming’s discovery of penicillin in 1928 catalyzed a multibillion-dollar pharmaceutical industry, spawning a plethora of antibiotics, and saved countless lives. However, human deaths stemming from infectious diseases have steadily reemerged in parallel with the rise of antibiotic-resistant pathogens, and medical researchers urgently need to develop new classes of antibiotics. Here, we report our findings on an alternative mechanism for bactericidal activity in members of the lantibiotic family of small peptides. No protein receptor is required for the bactericidal activity of this class of antibiotics. These molecules kill bacteria by sequestering the essential cell wall biosynthetic molecule lipid II from the sites where bacterial cell wall synthesis occurs.

The cell wall of bacteria forms a large scaffold of linear polymers of alternating amino sugars that are cross-linked via peptide bridges (the peptidoglycan layer). This layer is essential for survival. Its composition is quite uniform throughout the eubacteria, and consequently cell wall biosynthesis is an important target for antibiotics (1, 2). One of the key molecules in bacterial peptidoglycan synthesis is lipid II, because it is essential for the transport of cell wall subunits across the bacterial cytoplasmic membrane (fig. S1A). This highly dynamic molecule is present in all eubacteria in relatively small amounts (3). Lipid II is assembled on the cytoplasmic side of the bacterial membrane and is composed of one peptidoglycan subunit linked via a pyrophosphate on a polysaccharide membrane anchor [GlcNAc-MurNAc-pentapeptide (fig. S1B)]. Subsequently, lipid II is transported across the plasma membrane, where it delivers its cargo to a multienzyme complex for polymerization and insertion into the preexisting cell wall (4).

![Image](https://example.com/image.jpg)

**Fig. 1.** Lipid II segregation caused by lantibiotics visualized by fluorescence microscopy. (A) Nisin in action. After the addition of 2 to 4 μl of a 2 mM nisin solution, the peptide started to diffuse into the field of view from the bottom right corner. This image is a snapshot of three vesicles that have either not yet encountered nisin (vesicle a), just encountered nisin (vesicle b), or already been exposed to nisin for some time (vesicle c). Scale bar, 60 μm. (B) GUV doped with NBD-labeled lipid II before treatment with mutacin 1140. (C) Mutacin 1140–induced segregation of NBD-labeled lipid II. (D) Snapshot of a GUV treated with mutacin 1140 just at the onset of the lipid II segregation; the interior remains black. The green fluorescence of the NBD-labeled lipid II and the red fluorescence of Texas Red were sequentially detected with the use of two lasers.
We visualized the effect of nisin on giant unilamellar vesicles (GUVs) doped with fluorescently labeled lipid II. The fluorescence of the 7-nitrobenz-2-oxa-1,3-diazol-4-yl (NBD)-labeled lipid II was initially spread homogeneously over the GUV surface, and the fluorescence of the soluble marker Texas Red remained extracellular (Fig. 1A). After the addition of nisin, the GUVs first filled up with red fluorescence as a result of pore formation (Fig. 1), and the green fluorescence originating from NBD-labeled lipid II then clearly segregated in the membrane (Fig. 1). Thus, wild-type nisin caused the segregation of lipid II in model membranes after the formation of pores.

The addition of mutacin 1140 to NBD-labeled lipid II–doped GUVs (Fig. 1B) resulted in hot spots of lipid II fluorescence appearing within the membranes (Fig. 1C). Mutacin 1140 did not increase the permeability of the membrane, because red fluorescence was not seen inside the GUVs (Fig. 1D and Fig. S2). A hinge-region mutant of nisin ([N20PM21P]-nisin) that is a potent bacterial killer but is unable to form pores (12, 13) gave results similar to those of mutacin 1140 (Fig. S3). Comparable fluorescent patches were observed on the GUVs (Fig. S4), when fluorescently labeled nisin [fluorescein was attached to the C terminus, where it does not affect the activity of the lantibiotic (fig. S5)] was added to GUVs doped with unlabeled lipid II. Hence, the patches contained both the lantibiotics and lipid II. These results suggest that the sequestration of lipid II is involved in the mode of action of these lantibiotics.

Peptidoglycan synthesis of bacteria has been shown to take place at defined positions on the bacterial membrane. During cell division, large amounts of peptidoglycan are synthesized at the bacterial membrane. During cell division, large amounts of peptidoglycan are synthesized at the bacterial membrane. After the addition of fluorescently labeled nisin to these bacteria, a different lipid II distribution pattern was observed (Fig. 2, D and E). The fluorescence was absent from the septum (Fig. 2D), and the helical threads were not seen. Instead, the fluorescence originating from the nisin molecules appeared to be clustered in patches on the bacterial membranes, as in the observations in the GUVs. The effects were observed for more than 80% of the bacteria (fig. S6). Similar results were obtained with fluorescently labeled [N20PM21P]-nisin (fig. S7), and control experiments showed that, under the conditions used, the fluorescently labeled nisins bound only to lipid II (fig. S8). Similar patches could be observed upon treatment of Lactococcus lactis IL1403 with fluorescently labeled nisin, demonstrating that the lipid II sequestration by nisin was not limited to rod-shaped bacteria (fig. S9). These results demonstrate that nisin segregates lipid II into abnormal domains not only in vitro but also in vivo.

Our results provide an explanation for previously observed effects of staphylococcin T (a lantibiotic similar to mutacin 1140) on Micrococcus sp. (10), which may now be explained by an uncoupling between cell wall synthesis and membrane synthesis during cell division. Recently, it has been shown that lantibiotics similar to mutacin 1140 (such as gallidermin and epidermin) are able to form pores only in model membranes composed of short-chain phospholipids that form thin membranes (17). Their high level of antibacterial activity can now be explained by the sequestration mechanism described here.

Based on these experiments, we propose that nisin and a broad range of at least 10 other lantibiotics (18–20) having similar A/B ring structures displace lipid II from its functional location in Gram-positive bacteria. The lantibiotics bind to the pyrophosphate of lipid II, and their effect depends on the combination of the length of the peptide and the thickness of the lipid bilayer. Lantibiotics resembling nisin kill the bacteria primarily by forming pores together with lipid II. Those that bear the pyrophosphate cage, but are short and cannot span a bacterial bilayer to form pores, still remove lipid II from its functional location. This sequestering effect is a distinct mode of bactericidal activity that is currently known only in the lantibiotics which does not appear to have any known candidate resistance mechanisms.

Fig. 2. Nisin segregates lipid II into nonphysiological domains in vivo. (A) GUV containing 0.5 mole percent wild-type lipid II 15 min after the addition of fluorescently labeled vancomycin. (B) B. megaterium cells that were incubated for 10 min with labeled vancomycin (2 μg/ml). The arrows point at newly formed division sites or older emplars. (C) B. subtilis stained with fluorescent vancomycin (4 μg/ml). (D) B. megaterium cells after incubation for 10 min with fluorescein-labeled nisin (0.5 μg/ml). The arrow marks where the bacteria has already divided. (E) B. subtilis cells after incubation with fluorescein-labeled nisin (4 μg/ml). The bottom image in (E) and the insets in (B) to (D) show Nomarski images.

References and Notes
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Materials and Methods
Figs. S2 to S9
References
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