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Preview

Fracturing Rings to Understand Lantibiotics

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Haloduracin is a bacterially produced antibiotic system of two alkali-stable peptides (Halα and Halβ) that have extensive posttranslational modifications, including lanthionine rings. Now, Cooper et al. (2008) revise the structure of Halβ and demonstrate that some of the lanthionine rings are not essential for bioactivity.

Bacterial infections are a growing concern in human health, due to food-borne illnesses as well as infectious diseases that show resistance to currently available antibiotics. The emergence of virulent strains such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Staphylococcus aureus* (VRSA), and vancomycin-resistant *Enterococci* (VRE) has led to the outbreak of many hospital-acquired infections, often with life-threatening results. Common food pathogens, including *Escherichia coli*, *Listeria monocytogenes,* and *Salmonella typhimurium*, can cause gastrointestinal illnesses upon ingestion of contaminated food. These issues have prompted scientists to search for new antimicrobial agents to fight bacterial infections. In this line of research, antimicrobial peptides have gained attention as a promising class of possible therapeutic agents (Cotter et al., 2005).

Lantibiotics are a class of antimicrobial peptides produced by Gram-positive bacteria and display activity against other Gram-positive organisms. These peptides are ribosomally synthesized, undergo extensive posttranslational modifications, and, upon cleavage of a leader peptide, yield the biologically active mature peptides (Chatterjee et al., 2005; Willey and van der Donk, 2007). Lantibiotics are characterized by the presence of unusual amino acids, such as lanthionine (Lan), β-methyllanthionine (MeLan), 2,3-didehydroalanine (Dha), and didehydrobutyrine (Dhb) (Chatterjee et al., 2005; Willey and van der Donk, 2007). Nisin, the oldest and most-studied lantibiotic, has been used in the preservation of meat and dairy products for over 40 years without the appearance of significant resistance (Cotter et al., 2005). Studies have shown that some lantibiotics exert a dual mode of action against sensitive strains. They bind to lipid II, a precursor for the biosynthesis of bacterial cell wall, thereby interfering with bacterial growth. In addition, some have a pore forming ability that leads to the leakage of cellular contents and subsequent cell death (Wiedemann et al., 2006). Lantibiotics are usually orders of magnitude more potent than conventional small-molecule antibiotics, such as penicillin and vancomycin, and are often active against virulent strains such as MRSA and VRE (Cotter et al., 2005). The low toxicity of many lantibiotics toward mammals makes them an attractive class of compounds for food preservation and clinical applications (Cotter et al., 2005).

Two-component lantibiotic systems, such as lacticin 3147 (Ryan et al., 1996), consist of two structurally distinct peptides that are secreted by a single producer strain. The two peptides act synergistically to exert bioactivity against sensitive strains (Garneau et al., 2002; Ryan et al., 1996). It is believed that the mode of action of the two-component lantibiotics resembles that of the one-component lantibiotics (Wiedemann et al., 2006). The 3-dimensional structure of these peptides is believed to be crucial for bioactivity. Their conformation is governed not only by the amino acid sequence, but also by the presence of the lanthionine rings. Current research examining the biosynthesis and the functional role of posttranslational modifications in lantibiotics provides insights into how these antimicrobial peptides interact with target cells at the molecular level (Cotter et al., 2006). Ultimately, this knowledge may allow researchers to develop simpler structures through biological or synthetic means that retain bioactivity.

Recently, a novel two-component lantibiotic, haloduracin, was isolated from the alkaliphile *Bacillus halodurans* C-125 (Lawton et al., 2007; McClerren et al., 2006). The mature peptides have been named haloduracin α (Halα) and haloduracin β (Halβ) (Figure 1). Interestingly, *B. halodurans* grows at pH > 9.0, indicating that haloduracin is stable under alkaline conditions (McClerren et al., 2006). This gives haloduracin an important advantage over other known lantibiotics, which are often unstable at pH > 7.

In this issue of *Chemistry & Biology*, Cooper et al. (2008) have reported the structure-activity relationship (SAR) studies of haloduracin. This work has demonstrated the efficacy and benefits of in vitro biosynthetic methods for mutational studies of lantibiotics. After preparing a number of analogs of Halα and Halβ precursors via mutagenesis, various in vitro enzymatic transformations were performed to investigate the dehydration and cyclization modifications of these analogs. This led the authors to revise the initially proposed structure of Halβ and also allowed them to explore the importance of particular amino acid residues and structural modifications for activity. During their study, Cooper et al. (2008) systematically mutated the serine(Ser)/threonine(Thr) residues to alanine and examined the dehydration pattern of the mutants. In Halβ, it was initially believed, by sequence homology to other lantibiotics, that Thr18 escaped dehydration, whereas Ser22 was dehydrated to Dha (McClerren et al., 2006). However, the mutational studies have revealed that actually Ser22 escaped dehydration and Thr18 is dehydrated to Dhb. These results led to the revision of the originally proposed structure for Halβ and highlighted a limitation of the initial approach to haloduracin structural analysis. As the authors suggested, the use of sequence homology alone is not enough to allow for the structural elucidation of novel lantibiotics (Cooper et al., 2008).

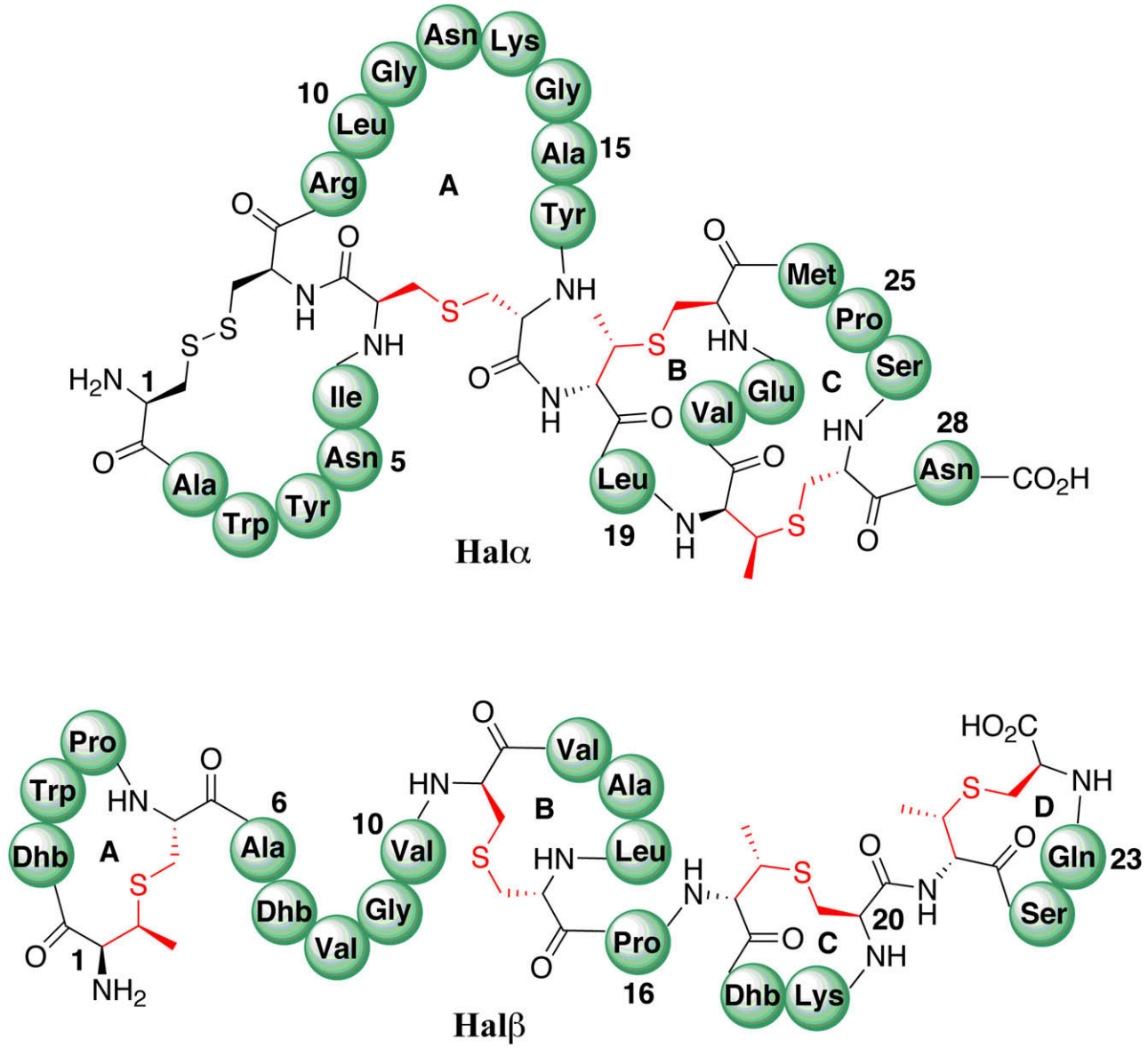

Figure 1. Structures of Haloduracin, Halα and Halβ
The thioether-linkage is highlighted in red.

Cooper et al. (2008) also studied the importance of the thioether rings of Halα and Halβ. By sequentially mutating each cysteine residue in the primary sequence of Halα and Halβ to an alanine, they could prevent the formation of a specific thioether ring. In Halα, it was found that thioether ring B is not necessary for bioactivity, whereas ring C is essential and ring A is an important contributor. In Halβ, ring A did not play a key role in bioactivity; however, disturbing the formation of ring C and D affected the activity significantly. An important finding from this work demonstrates that disrupting the formation of ring B in Halβ affects the formation of other rings. Further investigations may reveal whether there is a preference for the cyclization enzyme to act on conformationally-restricted substrates during formation of other rings. Also, knowing that ring A in Halβ is not important for bioactivity may allow researchers to design simpler analogs of Halβ. The authors also found that Glu22 in Halα is required for biological activity. This position shows sequence homology to mersacidin, suggesting that this residue may have a similar role in binding to lipid II. One could ask whether this observation can be generalized to other lantibiotics, such as lacticin 3147 A1, which has a Glu at position 24.

A unique feature of Halα is the presence of a N-terminal disulfide ring system. Cooper et al. (2008) have explored the role of this ring and their results suggest that this ring is not crucial for bioactivity. However, the authors claim that this ring system may protect the peptide from proteolytic cleavage by extracellular proteases. If the N-terminal ring does indeed serve such a function, it would be interesting to explore if such rings in other lantibiotics also provide proteolytic stability.

This work by Cooper et al. (2008) explores the functions of posttranslational modifications in lantibiotics by a combination of site-directed mutagenesis and in vitro enzymatic transformation, a versatile technique that can be extended to other lantibiotics. Although it is generally accepted that lanthionine rings and other posttranslational modifications are important for the mode of action of lantibiotics, structure activity relationship studies are needed to reveal the specific role of each modification. From the findings by Cooper et al. (2008), it is clear that not all posttranslational modifications are required for bioactivity. However, they may serve other key functions in stabilizing the peptide. By eliminating the functional moieties that do not interfere with the mode of action, new lantibiotics may be engineered or synthesized that retain bioactivity but are structurally simpler. This work offers a detailed evaluation of a two-component lantibiotic system and it opens the door for investigating the structure-activity relationships of other lantibiotics.

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