Antimicrobial mechanism of lantibiotics

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Abstract

Lantibiotics are ribosomally synthesized antimicrobial peptides that commonly target the cell wall precursor lipid II during their antimicrobial mechanism and exert their inhibitory activity by (i) inhibition of cell wall biosynthesis, and (ii) stable pore formation in the target membrane. Type-A(I) (i.e. nisin) and two-component (i.e. lacticin 3147) lantibiotics initially interact with lipid II to stabilize the complex, which then proceeds to inhibit cell wall biosynthesis and pore formation. Type-A(II) (i.e. nukacin ISK-1) and type-B (i.e. mersacidin) lantibiotics also use lipid II as a docking molecule, but can only inhibit cell wall biosynthesis without forming pores. In the present paper, we review the antimicrobial mechanism of different types of lantibiotics, their current progress and future prospect.

Introduction

To survive in the competitive environment, many bacteria of different taxonomic branches and residing in various habitats produce antimicrobial substances that are active against other bacteria. Ribosomally synthesized antimicrobial substances from bacteria are traditionally known as bacteriocins [1]. Bacteriocins from Gram-positive bacteria are broadly classified into two main classes: (i) post-translationally modified bacteriocins, such as lantibiotics, and (ii) non-modified bacteriocins. Lantibiotics are characterized by the presence of post-translationally modified unusual amino acids, such as dehydroamino acids (dehydroalanine and dehydrobutryline) and the thioether amino acids (lanthionine and 3-methyl-lanthionine). Jung and Sahl [2] classified lantibiotics as type-A (linear peptides) or type-B (globular peptides), which are divided further into two subtypes, namely type-A(I) and type-A(II), on the basis of their structure (Figure 1). In addition, a third type, two-component lantibiotic, is now gaining recognition, wherein two structurally different peptides act synergistically to kill the target bacteria (Figure 1). Lantibiotics are intriguing because of their unique biochemistry, genetic regulation, range of biological functions and potential for engineering unique protein structures [3]. Many lantibiotics show promising activity towards a variety of pathogenic bacteria including MRSA (methicillin-resistant Staphylococcus aureus) and VRE (vancomycin-resistant Enterococcus) [4].

The mechanism of antimicrobial action of different lantibiotics generally follows one (i.e. mersacidin or nukacin ISK-1) or two (i.e. nisin or subtilin) of the following steps: (i) inhibition of cell wall biosynthesis by scavenging the peptidoglycan precursor lipid II, and (ii) lysis of the cell membrane by forming pores. The present review focuses on the recent development of antimicrobial mechanism and structure–function study of different lantibiotics.

Type-A(I) lantibiotics: nisin, subtilin and related peptides

Membrane interaction, insertion and pore formation

A typical type-A lantibiotic is a flexible elongated peptide with a net positive charge (Figure 1) [2]. Nisin and related lantibiotics of this group show bactericidal activity against a wide range of Gram-positive bacteria [5]. Using various physiological and artificial membrane systems, the detail of molecular mechanisms of membrane disruption by nisin has been investigated, and revealed that nisin forms poration complexes in target cell membranes through a multi-step process which includes binding and insertion [6–8] (Figure 2A). However, precisely how pore complexes are formed is not yet known.

The interaction between nisin and membrane components of sensitive cells is considered important for nisin’s mode of action. Anionic lipids in the membrane may serve as functional nisin-binding sites. The cationic nature of nisin allows it to bind to lipid bilayers through electrostatic interactions with phospholipid headgroups [9–12]. Studies with fragments of nisin [13] and nisin variants derived by mutagenesis and chemical modification [13–15] revealed that the C-terminus of the peptide plays a key role in the initial interaction as this region harbours most of the positive charges.

Nisin insertion into the membrane is mediated by the hydrophobic residues in the N-terminal part [16]. Its variants with N-terminal extensions or with minor changes in the first ring displayed reduced abilities to insert into the lipid.
Figure 1 | Representative examples of different types of lantibiotics

The shaded residues indicate the unusual amino acids, derived after post-translational modification. Abu-S-Ala, β-methyl-lanthionine; Ala-S-Ala, lanthionine; Dha, dehydroalanine; Dhb, dehydrobutyrine. Different rings of individual lantibiotic are indicated by capital letters. Residues within the large circles are proposed to be involved in lipid II binding.

monolayer and is paralleled by lower antimicrobial activity, whereas changes in the C-terminus hardly affected nisin insertion [14,17]. Membrane insertion and activity is also affected when the flexibility of the hinge region, which connects rings C and D, is reduced [15,18] suggested that this region might act as a twist that allows nisin to bend the lipid surface. Several studies have suggested that insertion is followed by aggregation of nisin monomers [10,19,20]. It is unknown how many monomers are required to form a pore, but it is probably a dynamic process in which peptides are joining and leaving the transmembrane oligomer pore complex [21].

Lipid II-mediated antimicrobial mechanism

Lipid II represents the central cell wall building block of the peptidoglycan biosynthesis that is structurally conserved among bacteria. Each lipid II monomer contains the disaccharide unit N-acetylmuramyl–N-acetylglucosamine (MurNAc-GlcNAc) and pentapeptide, as well as a bactoprenol carrier lipid (C55-P), which is linked to the disaccharide unit via a pyrophosphate bridge [22] (Figure 3).

Nisin shows significant affinity for lipid II. Incorporation of lipid II into artificial phospholipid vesicles increased the activity of nisin by three orders of magnitude (from micromolar to nanomolar concentration) [23,24]. Moreover, it has been shown that pores formed by nisin in membranes containing lipid II are much more stable than pores formed in the absence of the receptor [25,26] (Figure 2A). Recently, NMR investigations have provided molecular level insight into the interaction of nisin with lipid II [27]. They showed that nisin has a unique mode of binding to lipid II, entirely different from glycopeptide (i.e. vancomycin). A defined network of five intermolecular hydrogen bonds between nisin’s peptide backbone and the pyrophosphate moiety of lipid II are responsible for association. Interestingly, only the first ten N-terminal amino acids, containing lanthionine rings A and B, contribute to the pyrophosphate cage with which nisin binds lipid II [28] (Figure 1). The binding motif of nisin is conserved among a number of type-A(I) lantibiotics targeting lipid II, for example subtilin, epidermin, gallidermin and mutacin 1140 [29].

Surprisingly, nisin variants that do not form pores, but do bind to lipid II, were found to kill bacteria efficiently. Moreover, other lantibiotics (i.e. epidermin and gallidermin) that bind with high affinity via their N-terminus to lipid II kill bacteria without permeabilizing the membrane, demonstrating that the lantibiotics have another lipid II-mediated mechanism of cell killing [30]. This mechanism was uncovered via a fluorescence microscopy technique, which revealed that lantibiotics that bind lipid II cluster the molecules into patches when added to phospholipid bilayer, containing small amount of lipid II [30]. Since lipid II was found to be predominantly located into regions of the cytoplasmic membrane related to cell elongation and division, this suggested that the lantibiotic might relocate lipid II from its functional location and thereby block cell wall synthesis [31].
Figure 2 | Proposed model for the mechanism of action of different classes of lantibiotics

(A) Type-A(I) lantibiotics (i.e. nisin) initially interact with the membrane and then bind with lipid II, stabilizing the complex and making the poration complex in the target site. At the same time, they sequester lipid II that causes cell wall biosynthesis inhibition. (B) Type-A(II) (i.e. nukacin ISK-1) and -B (i.e. mersacidin) lantibiotics interact with lipid II, resulting in inhibition of cell wall biosynthesis. (C) Two-component lantibiotics (i.e. lacticin 3147) interact with cell membrane by A1 peptide and followed by binding with lipid II. This triggers a conformational change of A1 peptide, whereupon a high-affinity binding site is generated for the A2 peptide, which is followed by pore formation. They also inhibit cell wall biosynthesis.

This dual mode of action, in which inhibition of peptidoglycan synthesis and pore formation are most efficiently combined, makes nisin a very potent antimicrobial agent and impedes the emergence of resistant strains.

**Type-A(II) lantibiotics: lacticin 481, nukacin ISK-1 and related peptides**

Membrane interaction and pore formation

Type-A(II) lantibiotics consists of two domains: an N-terminal linear region, and a C-terminal globular region formed by lanthionine bridges [32] (Figure 1). The antimicrobial mechanism of action of this group of lantibiotics has not been extensively studied perhaps because of their inability to form pores in the target organisms or model membranes.

Asaduzzaman et al. [33] combined the site-directed mutagenesis and the use of various lantibiotic fragments to dissect nukacin ISK-1 activity and membrane-binding properties [33]. The three lysine residues, which are located in the linear region and are the only positively charged residues, were found to be essential for the strong affinity of this lantibiotic for the anionic lipid membrane. This indicated that electrostatic binding of nukacin ISK-1 to the bacterial membrane is an important step in its mechanism of action [33] (Figure 2B). However, this mode of interaction could be generalized to some, but not all, lantibiotics of this group: lacticin 481, streptococcin A-FF22, mutacin II and salivaricin A all have one or two positively charged residues. In contrast, some of the lantibiotics, e.g. variacin, salivaricin B and ruminococcin A, do not have any charged residues at these positions. So, it can be generalized that lantibiotics of this group share a similar global mode of action, but they might reach their target via different routes.

Few studies have addressed the pore formation of these peptides. Streptococcin A-FF22 forms unstable pores in the membrane, and these pores could disrupt the membrane.
potential, impairing ATP production, ultimately leading to cell death [34]. On the other hand, nukacin ISK-1 neither dissipates the membrane potential of *Bacillus subtilis* cells nor forms pores permitting the efflux of molecules or ions [35]. Using a model membrane system, as with nisin, a pore-formation study has not been conducted in detail for this peptide group. However, it might be that structural limitations of these peptides restrict them from forming pores and it is hoped in near future that it will be clarified.

**Lipid II interaction and inhibition of cell wall biosynthesis**

Type-A(II) lantibiotics possess a conserved sequence in their ring A, TXS/TXD/EC, that is very similar to the lipid II binding motif of mersacidin, LtnA1 of lacticin 3147, plantaricin C and some other peptides (Figure 1). We recently reported that type-A(II) lantibiotic directly interact with the lipid II and can inhibit cell wall biosynthesis [36]. Using different nukacin ISK-1 variants, direct interaction with lipid II molecule was investigated, which revealed that this peptide can interact with the peptidoglycan precursor via its ring A structure and a negatively charged residue Asp13 plays a critical role in this event [36]. However, more features of this interaction have to be clarified, such as, the binding site of lipid II and binding stoichiometry.

Inhibition of cell wall biosynthesis is a common phenomenon for lipid II-targeting antimicrobial agents. Using TEM (transmission electron microscopy) analysis, Asaduzzaman et al. [35] showed that nukacin ISK-1 significantly reduced cell wall thickness in *Bacillus subtilis*. Recently, we demonstrated that nukacin ISK-1-treated *S. aureus* SG511 accumulates the soluble lipid II precursor UDP-MurNAc-pp (UDP-N-acetylmuramyl pentapeptide) inside the cell, suggesting that nukacin ISK-1 might interfere with membrane-associated or extracellular process of peptidoglycan biosynthesis [36]. It can therefore be proposed that type-A(II) lantibiotics act on the cell wall by initially interacting with lipid II as the docking molecule, and then inhibit cell wall biosynthesis, which might lead to the cessation of cell growth (Figure 2B).

**Type-B lantibiotics: mersacidin, actagardine and related peptides**

**Lipid II binding and peptidoglycan synthesis inhibition**

The rigid globular type-B lantibiotics have no charge or are negatively charged (Figure 1). Mersacidin and actagardine inhibit cell wall biosynthesis which results in the cessation of growth and a slow induction of lysis rather than pore formation [37] (Figure 2B).

Brötz et al. [38] have shown that mersacidin interferes with the membrane-associated transglycosylation step, the first polymerization reaction in the peptidoglycan biosynthetic pathway, resulting in the accumulation of the lipid II precursor UDP-MurNAc-pp inside the cell [38] (Figure 2B). Later, it was defined that mersacidin forms a tight interaction with lipid II, and not with the transglycosylase enzyme [39].

Structural determinant of mersacidin, which is required for lipid II interaction, has been elucidated by Hsu et al. [40]. They found that the structure of mersacidin can be modulated by the environmental conditions and, when it binds with lipid II, it shows some flexible orientation. In a solution NMR study of a mersacidin–lipid II complex, a small hinge region (Ala12 and Abu13, where Abu is α-aminobutyric acid), which can open and close the ring structures, was identified which governs the conformational change (Figure 1). Upon binding to lipid II, the structural changes directly affect the exposure of the charge groups, suggesting that electrostatic interactions govern the binding mechanism despite the rather hydrophobic nature of mersacidin. Also, a change in the charge distribution of mersacidin probably plays a crucial role in the mersacidin–lipid II interaction. The side chain of Glu17 has been proposed to be the lipid II-binding site of mersacidin, and replacement of Glu17 by alanine by mutagenesis makes it inactive [39]. This result suggests that the charge of the side chain of Glu17 is required for binding with lipid II. Comparison of mersacidin with similar lantibiotics reveals a conserved sequence (CTLT/SHEC) that comprises residues 12–18, suggesting that these residues may form the core lipid II-binding motif [40] (Figure 1). Although the measured affinity of mersacidin for purified lipid II is much lower compared with other lipid II-binding peptides (i.e. nisin or vancomycin), it is still highly potent to inhibit bacterial growth, even exceeding the
activity of vancomycin, indicating additional factors likely to contribute to its overall antimicrobial mode of action.

**Two-component lantibiotics: lacticin 3147, haloduracin and related peptides**

Interaction with lipid II and pore formation

Two-component lantibiotics consist of two peptides that function synergistically to kill a range of Gram-positive bacteria (Figure 1). This type of lantibiotic induces cell death by making sensitive cell membranes permeable, allowing for the efflux of potassium and phosphate ions (Figure 2C).

Lacticin 3147 is one of the well-studied two-component lantibiotics that act synergistically at equimolar concentration (1:1 stoichiometry) [41]. Recently Wiedemann et al. [42] proposed a three-step model for the antibiotic activity of lacticin 3147: (i) the A1 component of lacticin 3147 associates with the membrane and lipid II; (ii) binding to lipid II induces or stabilizes a conformation of LtnA1 which facilitates the interaction with the LtnA2 and enables the formation of a two peptide–lipid II complex; and (iii) when bound to the LtnA1–lipid II complex, the LtnA2 peptide is able to adopt a transmembrane conformation which allows it to form a defined pore. The lacticin 3147 pore represents just a monomeric or dimeric complex with a 1:1:1 stoichiometry (lipid II/A1/A2) [42] (Figure 2C).

The interaction between LtnA1 and lipid II probably involves the mersacidin-like binding motif. The flexible hinge (Ala19–Abu20 in LtnA1 peptide) and the general architecture of the adjacent ring B and C are conserved in lacticin A1, haloduracin α, lichenicidin α and some other peptides, in particular ring C with the essential glutamate residue, and it is conceivable that the structural similarity is reflected in a similar course of events as found for mersacidin (Figure 1). The hinge controls a relative spatial arrangement of thioether ring B and C in solution, similar to mersacidin. Mutational study in this region for LtnA1 and haloduracin α also revealed that the ring C structure and the glutamate residue are highly conserved to retain the antimicrobial activity, suggesting that their involvement in lipid II binding is similar to mersacidin [43,44].

Two-component lantibiotics are excellent candidates for future application as antibiotics, owing to their exceptional antibiotic efficiency. However, its need to be addressed that such high activities in the nanomolar concentration range antibiotic efficiency. However, its need to be addressed that future application as antibiotics, owing to their exceptional activity, indicating additional factors likely to contribute to its overall antimicrobial mode of action.

Elucidation of the molecular mode of action of different lantibiotics will bring them into the clinical development for potential drugs. Most of the lantibiotics target lipid II in a variety of ways and this specific target now enables rational design strategies since lantibiotics can be modified by site-directed mutagenesis of their structural gene. By analogy, it may be possible to find some interesting lead structures for drug development among the numerous lantibiotic structural analogues identified with increased potentiality. Although certain pharmacokinetic obstacles remain to be fully addressed, the clinical development of lantibiotics may benefit from the information gleaned from the use of nisin in the food industry as a preservative, on issues relating to formulation, conditions of use and stability.

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