Studies on Functional Evaluation of Lipid II Binding Moieties of Lantibiotic Nukacin ISK-1

カレッド, モハメド サミル イルサイド アビドイルカリム

Name：Khaled Mohamed Samir Elsayed Abdelkarim

Title：Studies on Functional Evaluation of Lipid II Binding Moieties of Lantibiotic Nukacin ISK-1

(ランチビオティック Nukacin ISK-1 の標的分子 Lipid II との相互作用領域の解析に関する研究)

Category：Kou

Thesis Summary

Over the past several decades, there has been an increased interest in bacteriocins due to their high potential as food preservatives, and as future antibiotics against multidrug resistant, medically important pathogens. Generally, bacteriocins are divided into two main groups, class I and class II. Lantibiotics are class I bacteriocins. Currently, more than 95 lantibiotics have been isolated and characterized. The lipid II cycle is a critical step in the process of peptidoglycan synthesis for cell wall formation and it represents a good target for antimicrobials. Roughly one third of identified bacterial membrane acting lantibiotics target the lipid II cycle.

Nukacin ISK-1 is a Type A(II) lantibiotic that is produced by *Staphylococcus warneri* ISK-1. It consists of 27 amino acids including a dehydrobutyrine, 2 molecules of lanthionine (Lan), and a 3-methyllanthionine (Melan). Nukacin ISK-1 contains an N-terminal linear region and a C-terminal globular region containing these unusual amino acids (Figure 1). Previously, nukacin ISK-1 was reported to possess a bacteriostatic mode of action due to its ability to inhibit cell wall biosynthesis through binding to lipid II as a docking molecule. However, still little is known about the lipid II binding moieties of nukacin ISK-1. The techniques to analyse peptide-lipid II interactions such as isothermal titration calorimetry, crystal structural analysis, and NMR require high amounts of lipid II whose synthesis remains a challenge. Therefore, in this study, we applied the *Bacillus subtilis* two-component system, LiaRS (lipid II cycle interfering antibiotic response regulator and sensor), which is known to respond only to antibiotics interfering with the lipid II cycle, for peptide-lipid II interaction. We used this simple method to evaluate the lipid II binding activity of known bacteriocins and to identify lipid II binding moieties in nukacin ISK-1. Using the LiaRS reporter assay, we confirmed that the Melan ring (Ring A) in nukacin ISK-1 is crucial for lipid II binding as previously indicated. Moreover, we further identified that the N-terminal linear region, specifically K1-3 and G5, and the Lan rings (Ring B and Ring C) are highly involved in the lipid II binding process because their mutagenesis led to

a significant reduction in lipid II binding affinity and consequently their antimicrobial activities. Therefore, on the basis of the LiaRS reporter assay results and the antimicrobial activities of the variants, we suggested that the whole structure of nukacin ISK-1 binds to lipid II via its N-terminal linear region, Ring A, Ring B and Ring C leading to the inhibition of cell wall biosynthesis.

Furthermore, through our NMR study, we reported that the hydrophobic residues in the Ring C region in nukacin ISK-1 are involved in lipid II binding, probably through hydrophobic interactions with the hydrocarbon chains of the lipid II molecules. We also reported that Ring A is involved in the specific interactions with lipid II, probably through the electrostatic interactions with the pyrophosphate group of lipid II. Moreover, in this study we suggested that there are two different states of nukacin ISK-1 that are probably present due to the *cis-trans* isomerization of the Ile7-Pro8 peptide bond. Our data provided herein brought useful information about the structure-function relationship of nukacin ISK-1 that can open the door for the engineering of future lantibiotics with increased potency.

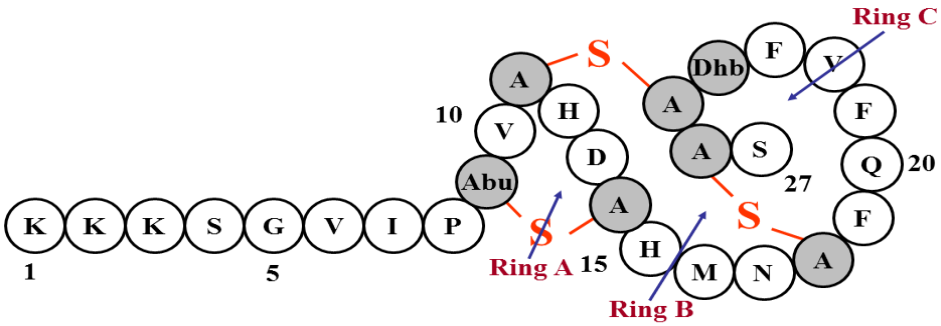

Figure 1. Proposed structure of nukacin ISK-1. Shaded residues indicate unusual amino acids generated by post-translational modification. Dhb, dehydrobutyrine; A-S-A, lanthionine; Abu-S-A, 3-methyllanthionine.