Review Article

IJCRR
Section: Healthcare
Sci. Journal
Impact Factor
4.016

NISIN: PRODUCTION AND MECHANISM OF ANTIMICROBIAL ACTION

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ABSTRACT

Nisin is a heat stable lantibiotic consisting of 34 amino acids. Of these amino acids, there are several unusual amino acids including dehydroalanine, dehydrobutyrine, aminobutyric acid, lanthionine and β-methyllanthionine. It has antimicrobial activity against many species of Gram positive bacteria, but not Gram negative bacteria due to their outer membrane barriers. However, when used in combination with other chemical or physical treatments that destabilize the outer membranes, nisin can inhibit Gram negative bacteria. Nisin has been used as a food preservative in many food industries because it is legally approved as safe for use in food and beverage. The knowledge on the production and mechanism of antimicrobial action of nisin is important for the understanding how nisin contains unusual amino acids and how it kills sensitive bacteria. The knowledge may also be a factor for the successful application of nisin. Therefore, this review focuses on presenting these two aspects of nisin.

Key Words: Bacteriocin, *Lactococcus lactis*, lanbiotic, nisin

INTRODUCTION

Nisin is the antimcirobial peptide produced by *Lactococcus lactis* subsp. *Lactis*[1]. It is the only bacterioicin that have been legally approved as safe for use in food and beverage. Nisin was first commercially marketed in England in 1953. In 1969, a joint commission between the Food and Agriculture Organization of the United Nation (FAO) and The World Health Organization (WHO) recognized nisin as a safe and legal biological food preservative. In the United States, the use of nisin in food has been legally approved by the American Food and Drug Administration (FDA) since 1988[2]. The word "nisin" (Group N Inhibitory Substance + the suffix "in") was coined by Mattick and Hirsch[3] to distinguish nisin from the bacteriocin produced by *Lactococcus lactis* subsp. cremoris called diplococcin.

Nisin is a heat stable, cationic lantibiotic belonging to class I bacteriocin according to the classification criteria of Klaenhammer[4]. It consists of 34 amino acids. Of these amino acids, there are several unusual amino acids including dehydroalanine (Dha), dehydrobutyrine (Dhb), aminobutyric acid (Aba), lanthionine (Ala-S-Ala) and β-methyllanthionine (Aba-S-Ala). Nisin has antimicrobial activity against many species of Gram positive bacteria (Table 1), but not Gram negative bacteria due to their outer membrane barrier. Normally, nisin producer has immunity to its own produced nisin but not to other lantibotics. This is for protecting itself from being killed by its own nisin. Although it is a protein, it is not digested by all of the protein digesting enzymes. It is sensitive to chymotrypsin, but not to trypsin and pronase[5]. At present, several natural nisin variants have been reported including nisin A[1], nisin Z[6], nisin Q[7], nisin F[8], nisin U[8] and nisin U2[8]. Nisin A, nisin Z, nisin Q and nisin F are produced by *Lactococcous lactis* while nisin U and nisin U2 produced by *Streptococcus* sp.[8] Of the bacteriocins, only nisin A and nisin Z have been extensively studied. These variants differ in a single amino acid residue at position 27 which is histidine in nisin A and aspartic acid in nisin Z.

PRODUCTION AND MODIFICATION OF NISIN

Nisin is ribosomally produced from a structural gene as a prenisin having 57 amino acids (Fig. 1 A). It is an inactive form of nisin containing a leader peptide (having 23 amino acids) at the N terminus of the molecule. Modifications after nisin synthesis are dehydration (Fig. 1 B), cyclization (Fig. 1 C) and leader peptide digestion (Fig. 1 D). In the dehydration step, serine and threonine are

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Received: 11.12.2014 **Revised:** 25.12.2014 **Accepted:** 12.01.2015

dehydrated to dehydroalanine (Dha) and dehydrobutyrine (Dhb), respectively. In cyclization step, several thioether crosslinks (S) are formed between alanine and alanine and between aminobutyric acid and alanine. The thioether crosslink between alanine and alanine results in the formation of lanthionine (A-S-A) and that between aminobutyric acid (Abu) and alanine results in the formation of β-methyllanthionine (Abu-S-A). Upon export of nisin outside the cell, leader peptide is digested from the prenisin resulting in the active nisin that is containing 34 amino acids.

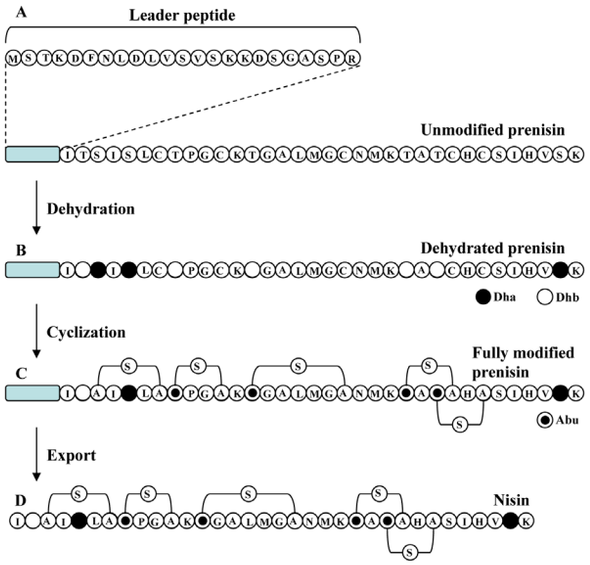

Figure 1: Modification of prenisin

MECHANISM OF ANTIMICROBIAL ACTION OF NISIN

For its killing activity, nisin does not require a membrane receptor on its target cell. This is unlike killing activity of many bacteriocins such as colicin that need membrane receptors on target cells.

There are two major steps for nisin to kill sensitive cells.

1. Passage through cell wall

To pass through target cell wall, nisin generally interactions (via hydrophobic or electrostatic interactions) with anionic components in the cell wall of sensitive cells such as teichoic acids, teichuronic acids and lipoteichoic acids, acidic polysaccharides or phospholipids[9].

2. Interaction with lipid II

Lipid II (Fig. 2) is a membrane anchored cell wall precursor that is essential for bacterial cell wall biosynthesis. It is composed of a membrane anchor of 11 polyisoprene residues to which, via a pyrophosphate, the basic building block of the cell wall (peptidoglycan monomer), N-acetylglucosamine-N-acetylmuramic acid(pentapeptide), is attached. It brings the peptidoglycan monomer from cytoplasm of the bacterial cell to incorporate into growing peptidoglycan network in the bacterial cell wall (Fig. 3).

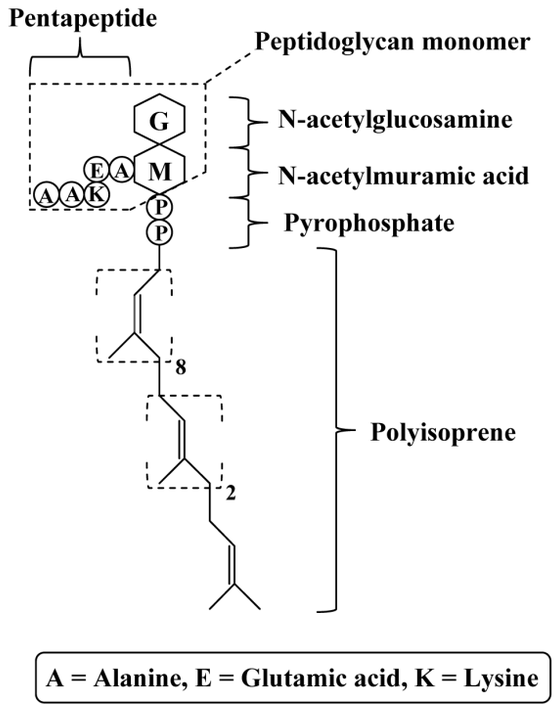

Figure 2: Structure of lipid II

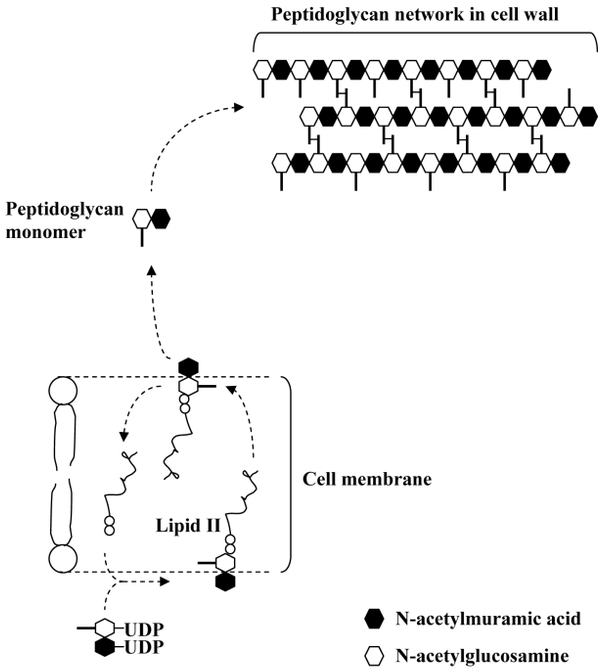

Figure 3: Involvement of lipid II in cell wall synthesis

Once nisin reaches cell membrane of the sensitive cells, it may perform one of these actions.

2.1. It binds to lipid II and prevents the peptidoglycan monomer to incorporation into the growing peptidoglycan network (Fig. 4).

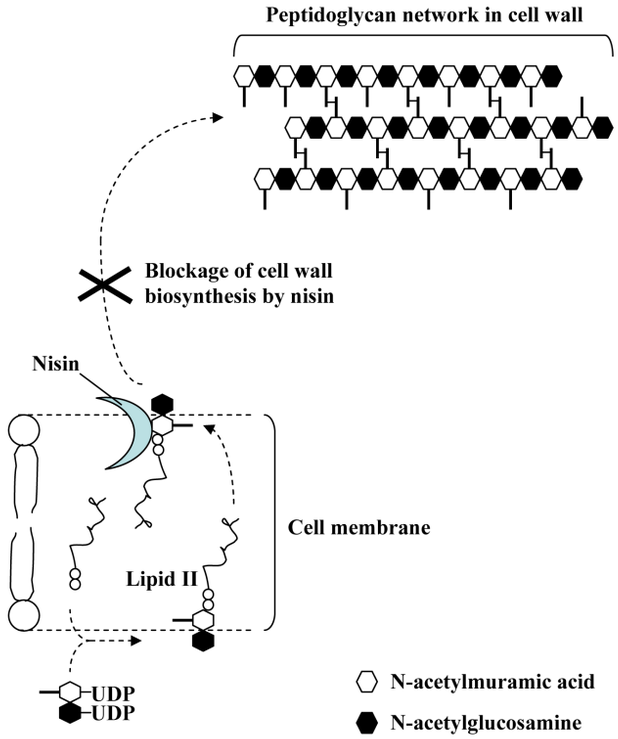

Figure 4: Inhibition of cell wall synthesis by nisin

2.2. It uses N-terminal binding motif to bind to the carbohydrate-pyrophosphate moiety of lipid II. This enables the C-terminal segment of nisin to insert into the cell membrane. Several nisin-lipid II complexes assemble to form a stable pore with diameter of 2 nanometers in cell membrane of target cells (Fig. 5)[10]. However, some reports state that the pore is formed by 4 nisin-lipid II complex and 4 nisin molecules (Fig. 6). Once the pore is formed in cell membrane, it can cause an increase in membrane permeability which can lead to the dissipation of the membrane potential, an efflux of small cytoplasmic contents such as amino acids, nucleotides and ions from the damaged cells[11,12]. Consequently, the damaged cells cannot produce energy as well as vital macromolecules resulting in cell death eventually[11,13].

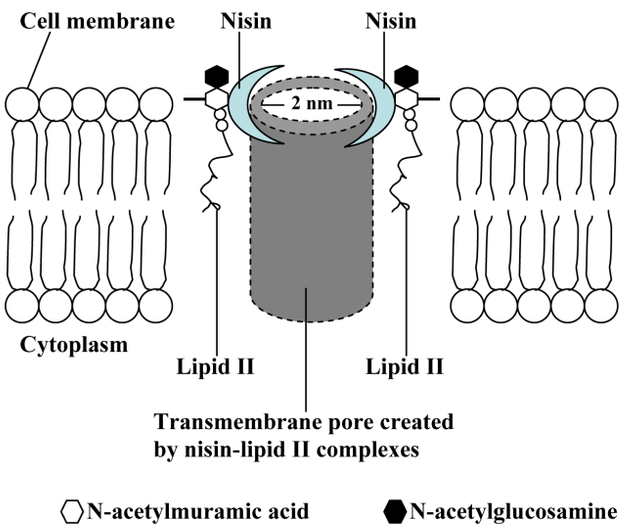

Figure 5: Formation of 2-nm pore in cell membrane by nisin

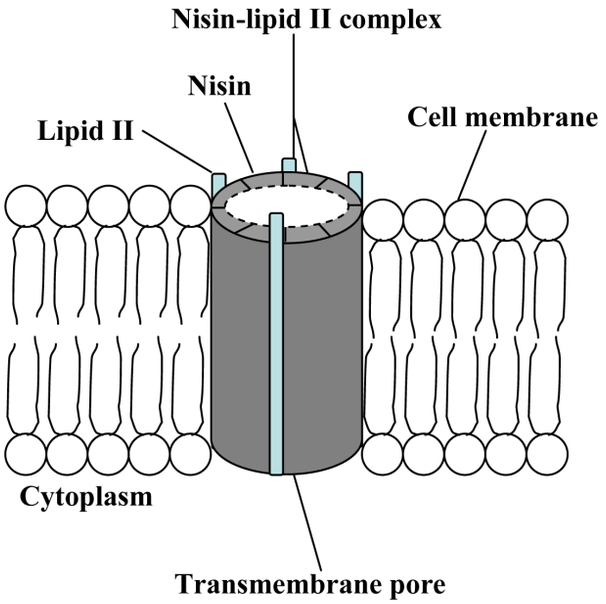

Figure 6: Pore formation in cell membrane by nisin

Besides the lipid II mediated mode of action, nisin can cause lysis of the cell wall of sensitive cells (Fig. 7), particularly in staphylococci.

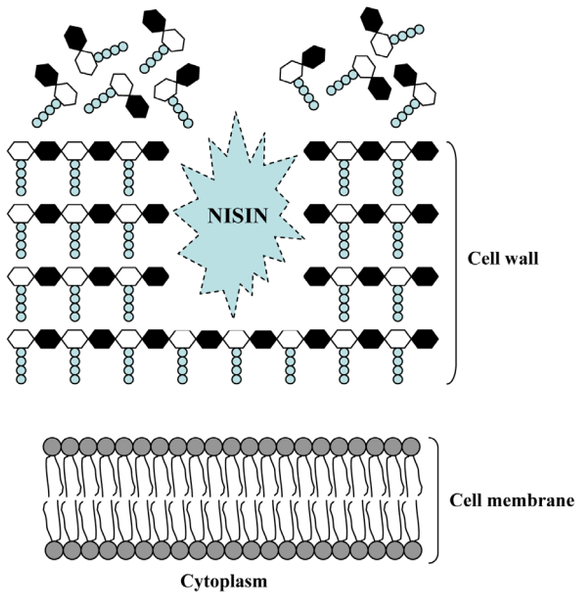

Figure 7: Lysis of cell wall by nisin

Table 1: Examples of Gram positive bacteria sensitive to nisin

Sensitive bacteria	Reference
Alicyclobacillus acidoterrestris	14
Bacillus cereus	15, 16
Bacillus subtilis	17
Clostridium botulinum	18
Clostridium difficile	19
Clostridium perfringens	20
Enterococcus faecalis	20
Enterococcus hira	20
Lactobacillus acidophilus	17, 20
Lactobacillus bulgaricus	17, 20
Lactobacillus casei	20
Lactobacillus curvatus	20
Lactobacillus delbrueckii	20
Lactobacillus sake	20
Lactococcus lactis	17, 20
Leuconostoc cremoris	20
Leuconostoc mesenteroides	20
Listeria monocytogenes	20, 21, 22, 23, 24
Listeria innocua	20, 25
Micrococcus luteus	26, 27
Pediococcus pentosaceus	20
Staphylococcus aureus	20, 22, 24, 28
Streptococcus pneumoniae	29
Streptococcus suis	30
Streptococcus thermophilus	17

Inhibition of Gram negative bacteria by nisin together with other treatments

As mentioned earlier, nisin has antimicrobial activity against some Gram positive bacteria but not against Gram negative bacteria because of their outer membrane barriers. However, many research works show that nisin can inhibit Gram negative bacteria when it is used in combination with substances or physical treatments that destabilize their outer membranes. Table 2 shows some examples of antimicrobial activity of nisin together with other substances or treatments against a variety of Gram negative bacteria both *in vitro* and in foods.

Table 2: Examples of antimicrobial activity of nisin with substances or treatments against a variety of Gram negative bacteria both in vitro and in foods.

Treatment	Sensitive bacteria	Treatment condition	Reference
Nisin + cinnamon	*Escherichia coli* O157:H7	in apple juice	31
	Salmonella Typhimurium	in apple juice	
Nisin + ρ-Cymene	*Salmonella enterica* Serovar Typhi	*in* vitro and on ready-to-eat food	32
Nisin + EDTA	*Escherichia coli*	*in vitro*	33
	Escherichia coli O157:H7	*in vitro*	34, 35
	Escherichia coli O157:H7	on beef	36
	Escherichia coli O157:H7	in ground beef	37
	Pseudomonas aeruginosa	*in vitro*	35
	Salmonella enteritidis	*in vitro*	35
	Salmonella species	*in vitro*	38
	Salmonella Typhmurium	*in vitro*	34
	Salmonella Typhmurium	on beef	36
Nisin + heat	*Escherichia coli* O157:H7	*in vitro*	39
	Salmonella enteritidis	in liquid whole egg	40
Nisin + heat + carvacrol	*Salmonella enteritidis*	*in vitro*	41
Nisin + high	*Escherichia coli*	*in vitro*	42, 43
hydrostatic pressure	*Escherichia coli*	in liquid whole egg	44

Table 2: (Continued)

Treatment	Sensitive bacteria	Treatment condition	Reference
	Escherichia coli	in meat	45
	Escherichia coli	in milk	46, 47
	Pseudomonas fluorescens	in milk	47
Nisin + maltol	E. coli	in vitro	48
Nisin + oregano essential oil	Salmonella Enteritidis	in minced sheep meat	49
Nisin + potassium sorbate	*Escherichia* coli O157:H7	in ground beef	37
Nisin + pulsed electric fields	*Escherichia* coli	in simulated milk ultrafiltrate media	40
	Escherichia coli O157:H7	in fresh apple cider	51
	Pseudomonas aeruginosa	in vitro	52
	Salmonella Typhimurium	in orange juice	53
Nisin + sodium citrate	Arcobacter butzleri	on chicken flesh	54
Nisin + sodium lactate	Arcobacter butzleri	on chicken flesh	54
Nisin + thyme essential oil	Escherichia coli O157:H7	In minced beef	55
Nisin + trisodium phosphate	*Escherichia* coli O157:H7	on chicken skin	56
	Salmonella enteritidis	on chicken skin	56
Nisin + eugenol + thymol	*Escherichia* coli	in vitro	57

CONCLUSION

Although the production and posttranslational modification of Nisin are now fully understood, its mechanism of antimicrobial action still requires further investigation. This may be because nisin has more than one mechanisms of antimicrobial action depending on several factors such as structural properties of target bacteria. Nisin, by itself, is only active against Gram positive bacteria. However, the use of Nisin together with outer membrane destabilizing treatments makes it become active against Gram negative bacteria. This finding broadens the application of Nisin as a food preservative.

ACKNOWLEDGEMENT

Authors acknowledge the immense help received from the scholars whose articles are cited and included in references of this manuscript. The authors are also grateful to authors / editors /publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

Source of funding: The Faculty of Science, Ubon Ratchathani University, Thailand

Conflict of Interest: The authors declare that there is no conflict of interest regarding the publication of this article

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