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Antimicrobial Peptides from Milk Proteins: A Prospectus

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ABSTRACT

Milk is a highest quality source of well-balanced nutrients and also displays a range of biological activities that affects digestion, metabolic responses to absorbed nutrients, growth and development of specific organs, and resistance to disease. Bioactive peptides have been identified within the amino acid sequences of native milk proteins. Hydrolytic reactions, such as those catalyzed by digestive enzymes, result in their release. These peptides directly influence numerous biological processes evoking behavioral, gastrointestinal, hormonal, immunological, neurological, and nutritional responses.. The size of active sequences may vary from two to twenty amino acid residues. The total antimicrobial effect in milk is greater than the sum of the individual contributions of immunoglobulin and nonimmunoglobulin defense proteins such as lactoferrin, lactoferricin , and other peptides. A variety of naturally formed bioactive peptides have been found in fermented dairy products, such as yoghurt, sour milk and cheese. Bioactive peptides have the potential to be used in the formulation of health-enhancing nutraceuticals, and as potent drugs with well defined pharmacological effects.

Keywords: milk proteins, bioactive peptides, antimicrobial peptides

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INTRODUCTION

Milk is a highest quality source of well-balanced nutrients and shows a various range of biological function. Biological functions of milk are mainly due to milk peptides and proteins. Milk protein include of approximately 20% whey and 80% casein. Whey contains five major proteins, including α-lactalbumin, glycomacropeptide, β-lactoglobulin, proteose peptone 3, immunoglobulins, and serum albumin, which together make up 85% of whey protein. Casein contains αs1-casein, αs2- casein, β-casein and κ-casein [1,2]

However, some of the biological function of milk protein ingredient is hidden, and is liberated upon proteolytic action. Releases of biologically active milk peptides occur usually during digestion of milk proteins in the gut, during enzymatic hydrolysis, chemical processes and fermentation. These bioactive peptides have important effect on body function and promote body health. These peptides decrease risk factor of some disease and/or to prevent disease development result of their hormone-like properties [3]. Upon oral administration, because of gastrointestinal processes, bioactive peptides released and may influence the various body systems such as cardiovascular, digestive, immune and nervous systems depending on their amino acid sequence. These health promoting effects may be because of several known peptide sequences exhibiting antimicrobial, antioxidative,

antithrombotic, antihypertensive and immunomodulatory activities [4]. Bioactive peptide functions depend on their amino acid composition and sequence. The size of active peptides may vary from 2 to 20 amino acid residues [5].

Studies have been shown that several milk peptides exhibit multifunctional properties. The antimicrobial properties of milk have been widely recognized for many years. The first time, in 1930, it was described that milk has active inhibitors that decrease growth of streptococcal bacteria [6]. The antimicrobial activity of milk is mainly attributed to immunoglobulins, and to non-immune proteins, such as lactoferrin (LF), lactoperoxidase and lysozyme [7]. In addition, it has been demonstrated that breast-feeding of infants supplies protection from a wide range of respiratory and entric infections. Antibacterial peptides are known as an important ingredient of innate immunity, especially at mucosal surfaces such as the lungs and small intestine that are constantly exposed to a range of potential pathogens [8, 9].

It has been demonstrated that a positive charge and amphiphilic of these peptides as major structural motifs determining the interaction with bacterial membranes is their mechanism action. It has been recognized that some antibacterial milk peptides can reach intracellular targets. More recently, it has been demonstrated several whey proteins such as lactoferrin, lactoferricin, α- Lactalbumin, β- lactoglobulin have antimicrobial activity [10]. Antibacterial fragments also have been derived from α_{s1}-casein, α_{s2} -casein, β-casein and κ-casein. Studies have been shown that these peptides have antimicrobial activity against a wide range of pathogenic organisms (e.g. Escherichia, Helicobacter, Listeria, Salmonella and Staphylococcus), yeast and filamentous fungi [11].

Lastly, biologically active peptides have been chemically synthesized to prove the physiological properties associated with a specific amino acid sequence. There is significant evidence that many bioactive peptides serve in multifunctional roles and often share common structural features based on a defined, biospecific role [11].This review will deal with the antimicrobial properties of milk protein derived peptides including peptides obtain from enzymatic proteolysis and microbial fermentation. In vitro and in vivo studies of antimicrobial peptides will be presented.

Whey proteins derived antibacterial peptides

Lactoferrin

Lactoferrin (Lf), a single-chain iron-binding glycoprotein, is the most important protein present in whey, due to the numerous and diverse array of therapeutic properties it displays. Lactoferrin is able to influence on some strains of Streptococcus mutans, Vibrio cholerae, Escherichia coli and Legionella pneumophila. Lf is a natural defense protein present in most secretions commonly exposed to normal flora such as milk, colostrum, tears, pancreatic juice, intestinal mucus, nasal secretions, saliva, bile, and genital secretions. Antimicrobial peptides such as lactoferrin have are partly short (less than 100 amino acids), amphiphilic and positively charged. Cationicity, hydrophobicity, and secondary structure have been implicated in the antimicrobial effect [12] .

Some studies have shown that human lactoferrin can directly destroys the outer membrane of gram-negative bacteria, releases lipopolysaccharide (LPS) molecules from the membrane and enhances bacterial susceptibility to hydrophobic antibiotics and human lysozyme. It has been suggested that the interrupt of the outer membrane structure is most likely not the primary factor leading to cell death. The lactoferrin interact with and cross both the outer and cytoplasmic membranes, and cause cell death by a multihit mechanism that involves action on more than one ionic target [12].

Lactoferrin-derived peptide

Lactoferricin

Lactoferricin is a potent bactericidal peptide specifically generated by enzymatic degradation of lactoferrin, also exhibit antimicrobial activity against both Gram-positive and Gram-negative microorganisms. Lactoferricin B obtained from bovine lactoferrin and lactoferricin H obtained from human lactoferrin. The fragments were characterised and named human (H) and bovine (B) lactoferricin [13]. Some studies have shown that lactoferricin B has higher bacteriostatic and bactericidal activity than lactoferrin. Antimicrobial activity rather than lactoferrin.

It has been demonstrated that Lactoferricin starts electrostatic interaction with the negatively charged membranes of bacteriaIn initial binding, lipopolysaccaride and teichoic acid as binding site in Gram-negative and Gram-positive bacteria have been identified. It has been demonstrated the peptide approach the cytoplasm and suppress the bacterial protein synthesis that exact mechanism is not clear [14]. The structure–activity relation of lactofericcin

fragment has been studied during last year's. Some studies have been shown that antimicrobial, antifungal, antitumor, and antiviral properties of lactofericcin can be associated to tryptophan/Arginine-rich proportion of the peptide. Also the anti inflammatory and immunomodulating properties are associated to a positively charged region of the molecule [15].

Lactoferrampin

Lactoferrampin is an antimicrobial cationic domain in the N1-domain of lactoferrin. It includes amino acids 268-284 of bovine lactoferrin. It has been demonstrated that lactoferrampin display higher candidacidal activity than the lactoferrin. Furthermore, lactoferrampin has antimicrobial activity against of Bucillus subtilis, Escherichia coli, and Pseudomonas aeruginosa , but not against of the fermentingbacteria, Actinomyces naeslundii , Porphyromonas gingivalis , Streptococcus mutans and Streptococcus sanguis. Lactoferrampin plays a critical role in membrane-mediated activities of lactoferrin [16].

β-lactoglobulin derived peptides

β-Lactoglobulin exhibit around half of the whole protein in bovine whey, while human milk contains no β-lactoglobulin. Proteolytic digestion of bovine β-lactoglobulin by trypsin bears four peptide segments with bactericidal activity. These peptides corresponded to β-lactoglobulin *f*(15–20), *f*(25–40), *f*(78–83) and *f*(92–100). These peptides only effect on Gram-positive bacteria and inhibit them. The amino acid sequence of peptide fragment *f*(92–100) is alterd by replacing of Asp with Arg and the substitute of a Lys residue at the C-terminal end to enhance the bactericidal activity to Gram-negative bacteria [17] .

α-lactalbumin derived peptides

α-Lactalbumin is one of the primary proteins determined in human and bovine milk. It includes approximately 20-25 percent of whey proteins and contains a wide variety of amino acids, such as essential and branched chain amino acids. Some studies have been shown that α-lactalbumin provided bactericidal peptides after digestion with trypsin and chymotrypsin, but not with pepsin. It has bactericidal activity against of Gram-positive bacteria [18].

Casein derived antibacterial peptides

α_{S1}-Casein derived peptides

Caseins contain antimicrobial peptides. The first time Jones and Simms revealed the antimicrobial properties of milk [19]. They identified a substance capable of inhibiting the growth of Streptococci called lactenin. Subsequently, some studies identified a group of basic high molecular weight polypeptides, called casecidins. Casecidin, obtained by chymosin digestion of casein at neutral pH, and exhibited antimicrobial activity in vitro against some pathogenic bacteria such as *Staphylococcus*, *Sarcina*, *Bacillus subtilis*, *Diplococcus, pneumoniae*, and *Streptococcus pyogenes* [19].

Isracidin, is that N-terminal segment of αs1-CN that inhibit the in vitro growth of Lactobacilli and other Gram-positive bacteria. This peptide also protects sheep and cows against mastitis. Isracidin has a strong protective effect against *S. aureus, Streptococcus pyogenes* and *Listeria monocytogenes* when administered at low dose as 10 µg per mouse prior to bacterial challenge [20].

α_{S2}-Casein derived peptides

Bovine α_{S2}-Casein was also shown to be a precursor of various peptide fragments with antibacterial activity. Zucht *et al*. [21] describe the isolation and characterization of an antibacterial peptide, from bovine milk and called casocidin-I. The peptide includes amino acids 165-203 of bovine caseinαS2. Casocidin-I (bovine milk), is a cationic α_{s2}-CN derived peptide that suppress growth of Gram-negative (*E. coli*) and Gram-positive (*Staphylococcus carnosus*) bacteria. Recio and Visser [22] have recognized two antibacterial domains from bovine α_{S2}-casein, one of this is [*f*(164–279)] being included in the previously isolated fragment of α_{S2}-casein [*f*(150–188)] and a new antibacterial fragment at C terminus of the molecule [*f*(183–207)] with more strong antibacterial activity.

Recently, researchers have recognized new antibacterial peptides from a chymosin digest of bovine sodium caseinate, all of them originated from the C-terminal of bovine αs2-casein *f*(181–207), *f*(175–207), *f*(164–207). The peptides have antimicrobial activity against a wide variety of Gram-positive and Gram-negative bacteria with low concentration [23].Hydrolysis of bovine αS2-casein also, induces four antibacterial peptides. These peptides corresponded to amino acid residues 165–170, 165–181, 184–208 and 203–208 of bovine αs2-casein. Fragments

165–181, 184–208 were homologous to those previously identified in the bovine protein and *f*(165–181) showed potent antibacterial activity [23].

κ-Casein derived peptides

Kappacin

Liepke et al. [24] identified first antimicrobial peptide from *κ*-casein of human milk. This peptide was non-glycosylated portion of human *κ*-casein *f*(63–117) and it was obtained after acidification of human milk and incubation with pepsin. Kappacin is an antimicrobial peptide that obtained from *κ*-casein [25]. Kappacin corresponds to the non-glycosylated, phosphorylated form of caseinomacropeptide (CMP) which exhibited growth inhibitory activity against Gram-positive (*Streptococcus mutans*) and Gram negative (*Porphyromonas gingivals*) bacteria. It has been demonstrated that active component of this peptide that exhibit growth inhibitory activity against Gram-positive (*Streptococcus mutans*) and Gram negative (*Porphyromonas gingivals*) bacteria is phosphorylated and non-glycosylated. Also it has been demonstrated that non-phosphorylated and glycosylated forms don't exhibit any activity against *Streptococcus mutans* [25].

The mechanism that by this kappacin limit gastrointestinal tract infection in the growing neonate, may be release of kappacin in stomach that by which increase sensitivity of bacteria to gastric acid by collapsing important transmembrane cation gradients. Molecular modeling suggests that the reason why the glycosylated forms of kappacin don't have antimicrobial activity because of suguer moieties would block pore formation [26].

κ-Casecidin

κ-casecidin is a pentapeptide with antimicrobial activity that was isolated from trypisin digest of bovine *κ*-casecidin. It can suppress growth of some pathogenic bacteria such as *S. aureus*, *E.coli* and *S. typhimurium. Also* this peptide display cytotoxic activity against some mammalian cells such as human leukemic cell lines. Cytotoxic effect of this peptide may be inducing apoptosis [27].

Caseinomacropeptide

Caseinomacropeptide (CMP) derive from *κ*-casecidin interacts with toxins, viruses and bacteria, thus it can promote health. Glycosylated CMP suppresses the binding of cholera toxins to their oligosaccharide receptors on cell walls and defends cells from infection induced by influenza virus. CMP also suppresses the adhesion of cariogenic bacteria such as Streptococcus mutans, S.sanguis and S.sobrinus to the oral cavity and regulates the composition of the dental plaque microbiota. This could help to influence acid formation in the dental plaque, in turn reducing hydroxyapatite dissolution from tooth enamel and promoting remineralisation. For this, it has applied for oral care products to prevent dental caries [28].

β-Casein derived peptides

Studies have been shown that antimicrobial peptide derived from *β-Casein* suppress a large spectrum of Gram-positive and Gram-negative bacteria including potentially pathogenic bacteria of clinical interests such as *Enterococcus faecium, Bacillus megaterium, E.coli K-12, Staphylococcus aureus, Yersinia enterocolitica*. This peptide have 26 amino acids, contain a higher content of nonpolar hydrophobic residues, lower positive charge and some pro residues at C-terminal which makes it resistant to further digestion by trypsin and chymotrypsin [29]. Fractions of human β-casein have also a protective effect against Klebsiella pneumoniae in mice. The immunomodulatory peptide derived from bovine β-casein , viz. f193-209 , was shown to enhance the antimicrobial activity of mouse macrophages [30].

Lysozyme-derived peptides

It has been shown that milk lysozyme has antibacterial activity against both Gram-positive and some Gram-negative bacteria that are completely tolerant to egg white lysozyme. Amino acid residues 87-114 of chicken lysozyme and 87-115 of human- milk lysozyme were potently bactericidal strongly Gram-positive and Gram-negative bacteria. Both lysozyme fragments can be used for the design of novel antimicrobial peptides [26].

In vivo studies on milk-derived antibacterial peptides

There are several documents about antimicrobial activity of bioactive milk peptides but very limited studies have done in vivo using animal model or in human clinical trials. It has been demonstrated that that bioactive milk peptides may modulate the intestinal microflora when they formed during milk digestion in vivo and protect host against pathogen microorganisms [31].

Some researchers have shown in vivo activity of isracidin that is derived from α_{S1}-casein *f*(1–23) [20]. Isracidin is required in much smaller quantities in vivo to employ a protective effect against bacterial than in vitro. In mice it exerts a protective function against *Listeria monocytogenes*, *Streptococcus pyogens* and *Staphylococcus aureus*. In cows with mastitis, isracidin was successful in treatment of chronic streptococcal infection.

Future perspectives for antimicrobial peptides from milk proteins

Antibiotic – resistant bacteria is a great concerns that treat both animal and human health. Thus the necessarily of developing compatible antibiotic alternatives for feed and medical industries has been arisen. Many of researches have been paid attention to antimicrobial peptides derived from animals and plants because of their effective antimicrobial activity against pathogen microorganism compared with current antibiotics [32]. Antimicrobial peptides obtained from food proteins such as milk have more advantage to those derived from harmless substances, therefore they could have used with safety in medicine and food industry. Antibacterial domains of such proteins may have a potential use drug therapy, functional foods, or infant formulas [33].

Application of applicable protocols such as membrane processing and chromatographic isolation may also be an area of future interest in the extraction of potent biofunctional peptides from fermented dairy products and their subsequent utilization as functional food ingredients [11]. Developments in nano-encapsulation and nanoemulsion technology, as well as research into delivery systems, may finally lead to the generation of specific formulations containing bioactive peptides with anti-microbial activity. Antimicrobial peptides from food protein merit attention due to their mechanism of activity, which makes microbial resistance doubtful, and for increasing the functional values of foods. Bioactive peptides have the potencial to be used in the formulation of health-enhancing nutraceuticals, and as potent drugs with well defined pharmacological effects [4,34].

CONCLUSION

In summary, milk contains various peptide sequences that influence vital physiological functions and regulate many biochemical processes. The interest on bioactive milk peptides is increasing because milk proteins are available in great amounts with high degree of purity at low price which, under a technological aspect, make them attractive in the search of bioactive peptides. The potential physiological effects of these peptides have been examined to supply a better understanding of their effects.

Further work is also required to synthesize modified peptide sequences in order to find pharmacologically active peptides with higher potency and longer duration of action. Furthermore, there is a need to develop technologies by means of which active peptide fractions can be produced and enriched.

The future applications of bioactive peptides look promising in the field of food industry as food preservatives and nutraceuticals and also as natural drug in pharmaceutical industry.

Milk proteins are not only essential to supply nutrition and specific immunological protection to the neonate but that they also provide bioactive peptides which defened the organism against infectious agents. These antibacterial peptides havebeen derived from lactoferrin, $\alpha_{S\ 1}$,$\alpha_{S\ 2}$,β, and κ-casein, α-Lactalbumin, β-lactoglobulin, proteosepeptone-3 and lysozyme. Antibacterial domains of milk proteins may have a potential use in therapy, functional foods, or infant formulas.

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