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Review

Dairy-derived antimicrobial peptides: Action mechanisms, pharmaceutical uses and production proposals

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Milk proteins are precursors of many different biologically active peptides including antimicrobial ones. These peptides have already been considered for application both as dietary supplements in "functional foods" and as drugs. This review focuses on the recent knowledge pertaining to antimicrobial peptides derived from major milk proteins (caseins and whey proteins) and the mechanism of action of these peptides. Possible applications in the pharmaceutical industry and processing technologies designed for the large-scale production of these protein fragments are also discussed.

Introduction

Dietary proteins are the source of physiologically active components which have a positive influence on the function of the body. In recent years, the role of protein in the diet and the potential for bioactive peptides to contribute to a healthier diet have been acknowledged worldwide (Haque, Chand, & Kapila, 2009).

Bioactive peptides of food origins can be defined as components (genuine or generated) of ready-to-eat foods which may exert a regulatory activity on the human organism beyond basic nutrition (Meisel, 2004). These peptides are produced by microbial fermentation, enzyme digestion, or proteolysis by enzymes *in vitro*, and can perform physiological activities in major body systems (Korhonen & Pihlanto, 2006). A wide range of activities has been described, including antimicrobial properties, blood pressure-lowering (Angiotensin Converting Enzyme, ACE-Inhibitory) effects, cholesterol-lowering ability, antithrombotic and antioxidant activities, enhancement of mineral bioavailability, cyto- or immunomodulatory effects, and opioid activities (Hartmann & Meisel, 2007).

To exert bioactivity, food peptides must be either ingested and then reach the intestine intact or be liberated *in situ* from their parent proteins to act locally, that is in the gut, or even systemically, i.e., through the blood stream. In recent years, a lot of interest has been given to identification and characterization of bioactive peptides. Many databases on bioactive peptides have been formed to store information such as sequence, molecular weight, activity, references to published work, EC50 (half maximal effective concentration), sources and more (Panchaud, Affolter, & Kussmann, 2012). BioPep is a database containing information especially on bioactive peptides ($n = 2594$) (Iwaniak, Dziuba, & Niklewicz, 2005). Panchaud *et al.* (2012) classified these peptides to identify sites of major bioactivity and reported that antibacterial peptides constitute the second-largest group of peptides identified, following ACE-inhibitory peptides. The Antimicrobial Peptide Database (APD) is another database of natural antimicrobial peptides with fewer than 100 amino acid residues ($n = 1773$) (Wang & Wang, 2004). The following activities were registered in this database: antiviral peptides; antifungal peptides; anticancer/tumor peptides; antibacterial peptides; anti-HIV peptides; antiparasital peptides; spermicidal peptides; and insecticidal peptides (link: http://unmc.edu/AP/main.php) (Panchaud *et al.*, 2012).

Although other food-derived proteins contain potential bioactive peptides, milk proteins are currently the main source of a range of biologically active components that exhibit various physiological activities (Haque *et al.*, 2009; Meisel, 1998). Depending on these activities bioactive peptides of milk origin are regarded as potential ingredients for various food and medical preparations. Some

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bioactive peptides of milk origin have been established for their antimicrobial roles and thus have the prospects of use as an ingredient in functional foods, nutraceuticals, and pharmaceuticals to control and prevent diseases. They are claimed to be health-enhancing ingredients for food and pharmaceutical preparations. Therefore, many studies have been done to detect, purify, and identify these peptides for application in industrial production (Agyei & Danquah, 2012; Meisel, 1997, 2004). Furthermore, as these peptides have the advantage of being obtained from a harmless and cheap material, they have excellent potential for use in the food and medicine industry (Benkerroum, 2010).

Milk proteins exert numerous physiological activities for the neonate, including strong protection against infection. Substances that provide this protection contain direct-acting antimicrobial factors, anti-inflammatory substances, and immunomodulators (López-Expósito & Recio, 2006, 2008). Direct-antimicrobial factors include substances of different origin, such as glycans (Morrow, Ruiz-Palacios, Jiang, & Newburg, 2005), and lipids (Isaacs, 2005; Isaacs, Kashyap, Heird, & Thormar, 1990) but among the most relevant because of their diversity and multifunctionality are proteins and peptides (Clare, Catignani, & Swaisgood, 2003). Although the antimicrobial activity of milk is mainly attributed to immunoglobulins and to non-immune proteins such as lactoferrin, lactoperoxidase and lysozyme, antimicrobial peptides are known as an important component of innate immunity (Haque & Chand, 2008). It has been demonstrated that some milk-derived antimicrobial peptides can reach intracellular targets (Haque *et al*., 2009).

These peptides of widely diverse sources show different structural characteristics and mechanisms of action (Clare *et al*., 2003). To show any antimicrobial activity, those milk-derived peptides have to be first released from their parent molecules. This can be provided either by hydrolysis of the parent molecules (caseins and whey proteins) by digestive proteases (Clare & Swaisgood, 2000) or by fermentation with selected proteolytic lactic acid bacteria (LAB) (Hayes, Ross, Fitzgerald, Hill, & Stanton, 2006). Milk acidification followed by heat treatment has also been reported to generate active peptides (Tomita, Wakabayashi, Yamauchi, Teraguchi, & Hayasawa, 2002; Zucht, Raida, Adermann, Magert, & Forssmann, 1995).

The present review will deal with the more recent knowledge of antimicrobial peptides derived from major whey proteins (α-lactalbumin and β-lactoglobulin) and casein fractions (α_{s1}-casein, α_{s2}-casein, β-casein, and κ-casein) excluding minor ones such as lactoferrin and lysozyme. Their antibacterial mechanisms of action, pharmaceutical uses, and production processes are also discussed.

Whey protein-derived antimicrobial peptides

Several antimicrobial peptides have been identified within the sequences of major whey proteins, α-lactalbumin and β-lactoglobulin (Chatterton, Smithers, Roupas, & Brodkorb, 2006; Kamau, Cheison, Chen, Liu, & Lu, 2010; López-Expósito & Recio, 2006) (Table 1).

α-Lactalbumin

Bovine α-lactalbumin (α-La), quantitatively the second-most important protein in whey, is one of the globular proteins. The concentration of α-La is 1.0−1.5 g L^{-1}, making up approximately 20% of the whey proteins in mature bovine milk, while human α-La is the dominant whey protein. Bovine α-La is made up of 123 amino acids including essential and branched chain amino acids, and exhibits a high affinity for metal ions, especially to calcium (Chatterton *et al*., 2006; Kamau *et al*., 2010).

The antibacterial activity of native α-La derived from bovine milk has been searched against several Gram-positive and Gram-negative bacteria. However, α-La exhibited no bactericidal effects against all the bacterial strains tested (Pellegrini, Thomas, Bramaz, Hunziker, & von Fellenberg, 1999). Different proteolytic enzymes were tested for hydrolyzing activity and bactericidal peptides were obtained from α-La after digestion with trypsin or chymotrypsin. Digestion with pepsin did not produce an active hydrolyzate (Pellegrini *et al*., 1999).

In contrast, α-La hydrolyzed with pepsin and trypsin inhibited the metabolic activity of *Escherichia coli* JM103 releasing some peptides (Pihlanto-Leppälä *et al*., 1999). The peptide concentration was 25 mg mL^{-1}, whereas unhydrolyzed α-La did not inhibit growth of *E. coli* JM103 at a concentration of 100 mg mL^{-1} (Pihlanto-Leppälä *et al*., 1999). The finding of Pellegrini *et al*. (1999) on the lack of antimicrobial activity in peptic hydrolyzates can be depend on the low assay dosage, low sensitivity of method used, or microorganism specificity.

On the other hand, α-La hydrolyzates obtained with alcalase did not have antimicrobial activity, although these hydrolyzates have a higher degree of hydrolyzation (DH) than pepsin and trypsin. This implies that pepsin and trypsin have higher specificity in releasing antimicrobial peptides from α-La. Although the DH of protein contributed to the antimicrobial activity, a higher DH did not necessarily correspond to a higher antimicrobial activity (Pihlanto-Leppälä *et al*., 1999).

The overall structure and charge distribution of the peptides may have critical importance for antimicrobial activity (Pihlanto-Leppälä *et al*., 1999). The antibacterial peptides derived from α-La were negatively charged at the pH of the antibacterial assay. This character may explain why they were weakly active against Gram-negative bacteria whose outer membranes contain negatively charged lipopolysaccharide as a major component (Pellegrini *et al*., 1999). Pellegrini *et al*. (1999) found that a disulfide linkage between the peptide components was necessary for bactericidal activity, as no antimicrobial activity was observed with mixtures of individual peptides. Since the peptide fragments were composed of two