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β-Lactoglobulin as source of bioactive peptides

Review Article

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Summary. β-Lactoglobulin (β-Lg) is currently an important source of biologically active peptides. These peptides are inactive within the sequence of the precursor protein, but they can be released by in vivo or in vitro enzymatic proteolysis. Once released, these peptides play important roles in the human health, including antihypertensive, antioxidant and antimicrobial activities as well as opioid-like features and ability to decrease the body-cholesterol levels. Bioactive peptides derived from β-Lg are currently a point of intensive research. Their structure, biological significance and mechanism of action are briefly presented and discussed in this review.

Keywords: β-Lactoglobulin – Bioactive peptides – Physiological activity

1. β-Lactoglobulin: structure and function

β-Lactoglobulin (β-Lg) is the major whey protein found in the milk of cows and other ruminants. It is also found in the milk of some non-ruminants such as pigs, horses, dolphins and cats. Though some reports have suggested that minor amounts appear in human milk (Hambraeus and Lönnerdal, 2003), this protein has generally been reported to be absent from human breast milk and rodents (Hambling et al., 1992).

β-Lg is a small, soluble and globular protein, containing 162 amino acids in a single peptide chain with a molecular weight of 18.3 kDa (Brautnizer et al., 1973). The amino acid sequences of bovine, ovine and caprine β-Lg are shown in Fig. 1. The primary sequence reveals two intrachain disulphide bridges (Cys66-Cys160 and Cys106-Cys-119) and a free thiol group at Cys121 (Creamer et al., 1983). Five and four genetic variants of bovine and ovine β-Lg, respectively have been discovered, of which the phenotypes A and B are most predominant. Bovine β-Lg variant A differs from variant B by only 2 amino acids: Asp_{64} and Val_{118} in the variant A are substituted, respectively by Gly and Ala in the variant B (Eigel et al., 1984). Caprine β-Lg only shows one genetic variant.

The three-dimensional structure of β-Lg consists of nine strands of antiparallel β-sheet, eight of which form a hydrophobic barrel bordered on one side by an α-helix (Papiz et al., 1986). At the normal pH of milk, β-Lg exists in the solution as a dimer, with an effective molecular mass of about 36.6 kDa. The low content of amino acid Pro and the presence of Cys, Met and cistine residues have been described as responsible for low stability of β-Lg against the heat treatment. However, the globular structure of this protein is remarkably stable against the acids and proteolytic enzymes present in the stomach (Papiz et al., 1986).

The tertiary structure of bovine β-Lg shares strong homology with the plasma retinol-binding protein and other proteins (bilin-binding protein, hemoycianin-binding protein) involved in binding and transport of small hydrophobic ligands, all of which are playing important physiological roles (Dalgalarrondo et al., 1990). This finding suggests that the role of β-Lg may be connected with transport or accumulation of lipid-soluble biological components such as fatty acids and retinoids. The high stability of this protein to proteolytic action of digestive enzymes could be relevant to the biological function of β-Lg as a resistant carrier of retinol (a provitamin A) from the cow to the young calf (de Witt, 1998).

It has been reported that β-Lg is present in products that utilize colostrums as ingredient to improve immunity in

β-LG BOVINA A ^{1}**L** IVTQTMKGLDIQKVAGTW **Y**^{20} SLAMAASDISLLDAQSAPLR^{40} VYVEELKPTPEG **D** LEILLQK^{60}
β-LG BOVINA B ^{1}**L** IVTQTMKGLDIQKVAGTW **Y**^{20} SLAMAASDISLLDAQSAPLR^{40} VYVEELKPTPEG **D** LEILLQK^{60}
β-LG OVINA A ^{1}**I** IVTQTMKGLDIQKVAGTW **Y**^{20} SLAMAASDISLLDAQSAPLR^{40} VYVEELKPTPEG **N** LEILLQK^{60}
β-LG OVINA B ^{1}**I** IVTQTMKGLDIQKVAGTW **H**^{20} SLAMAASDISLLDAQSAPLR^{40} VYVEELKPTPEG **N** LEILLQK^{60}
β-LG CAPRINA ^{1}**I** IVTQTMKGLDIQKVAGTW **Y**^{20} SLAMAASDISLLDAQSAPLR^{40} VYVEELKPTPEG **N** LEILLQK^{60}

β-LG BOVINA A WEN **D** ECAQKKIIAEKTKIPA^{80} VFKIDALNENKVLVLDTDYK^{100} KYLLFCMENSAEPEQSL **V** CQ^{120}
β-LG BOVINA B WEN **G** ECAQKKIIAEKTKIPA^{80} VFKIDALNENKVLVLDTDYK^{100} KYLLFCMENSAEPEQSL **A** CQ^{120}
β-LG OVINA A WEN **D** ECAQKKIIAEKTKIPA^{80} VFKIDALNENKVLVLDTDYK^{100} KYLLFCMENSAEPEQSL **A** CQ^{120}
β-LG OVINA B WEN **D** ECAQKKIIAEKTKIPA^{80} VFKIDALNENKVLVLDTDYK^{100} KYLLFCMENSAEPEQSL **A** CQ^{120}
β-LG CAPRINA WEN **D** ECAQKKIIAEKTKIPA^{80} VFKIDALNENKVLVLDTDYK^{100} KYLLFCMENSAEPEQSL **A** CQ^{120}

β-LG BOVINA A CLVRTPEVD **D** EALEKFDKAL^{140} KALPMHIRL **S** FNPTQLE **E** QC^{160} H **I**^{162}
β-LG BOVINA B CLVRTPEVD **D** EALEKFDKAL^{140} KALPMHIRL **S** FNPTQLE **E** QC^{160} H **I**^{162}
β-LG OVINA A CLVRTPEVD **N** EALEKFDKAL^{140} KALPMHIRL **A** FNPTQLE **G** QC^{160} H **V**^{162}
β-LG OVINA B CLVRTPEVD **N** EALEKFDKAL^{140} KALPMHIRL **A** FNPTQLE **G** QC^{160} H **V**^{162}
β-LG CAPRINA CLVRTPEVD **K** EALEKFDKAL^{140} KALPMHIRL **A** FNPTQLE **G** QC^{160} H **V**^{162}

Fig. 1. Amino acid sequences of β-lactoglobulin of bovine, ovine and caprine species

the newborn. Several possible functions have been described for β-Lg, but one suggestion was its possible role in developing passive immunity with IgG (Sutton and Alston-Mills, 2006). Moreover, β-Lg is a rich source of Cys, an essential amino acid that appears to stimulate glutathione synthesis, an anticarcinogenic tripeptide produced by the liver for protection against intestinal tumors (Mcintosh et al., 1995).

The high nutritional and functional value of β-Lg is widely recognized and has made this protein an ingredient of choice in the formulation of modern foods and beverages (Chatterton et al., 2006). During last years, food scientists and technologists have focused their studies on bioactivities associated with β-Lg peptides. These peptides, inactive within the sequence of parent protein, are activated once released during gastrointestinal digestion or during food processing (Meisel, 2005). Once they are released in the body, bioactive peptides may act as regulatory compounds with hormone-like activity. These peptides usually contain 3–20 amino acid residues per molecule and their primary sequence defines their function. There are several articles about bioactive peptides from different origins published recently in *Amino Acids* (e.g. Janin, 2003; Henle, 2005; Krause et al., 2006; Siemion et al., 2005). This paper will deal with the biological properties of peptides derived from β-Lg, focusing special attention on antihypertensive, antioxidant, antimicrobial and immunostimulating peptides. Other activities such as opioid and hypocholesterolaemic will also be considered. Within each sub-section, attention will be paid to the sequence and activity of the peptides identified, to their structure/activity relationship and to their in vivo effects.

2. Angiotensin-I-converting enzyme inhibitory and antihypertensive peptides

Hypertension is an important problem in our society, given its high prevalence and its role in cardiovascular diseases, including coronary heart disease, peripheral arterial disease and stroke. The rennin-angiotensin system is a key factor in the maintenance of arterial blood pressure. One of the main components of this system is angiotensin-converting-enzyme (ACE) (EC 3.4.15.1) which catalyzes the conversion of angiotensin I, an inactive decapeptide, into angiotensin II, an octapeptide with a potent vasoconstrictor action (Skeggs et al., 1956). Moreover, ACE catalyzes the inactivation of bradykinin, which has an important vasodilatation activity. Therefore, ACE plays an important role in the regulation of arterial blood pressure, and inhibition of this enzyme can generate an antihypertensive effect. In fact, synthetic ACE inhibitors such as captopril, enalapril, alecepril and lisinopril are commonly used in the treatment of essential hypertension despite their side effects, such as hypotension, cough, increased

Table 1. ACE-inhibitory and antihypertensive peptides derived from β-lactoglobulin

Fragment	Sequence	IC_{50} (μM)	Origin	Changes in SBP (mmHg)	References
β-Lg f(9–14)	GLDIQK	580	Hydrolysis with trypsin	n.d.	Pihlanto-Leppälä et al. (1998)
β-Lg f(15–20)	VAGTWY	1682	Hydrolysis with pepsin + trypsin	n.d.	Pihlanto-Leppälä et al. (1998)
β-Lg f(22–25)	LAMA	1062	Hydrolysis with trypsin	n.d.	Pihlanto-Leppälä et al. (2000)
β-Lg f(32–40)	LDAQSAPLR	635	Hydrolysis with trypsin	n.d.	Pihlnato-Leppälä et al. (2000)
cβ-Lg f(58–61)	LQKW	34.7	Hydrolysis with thermolysin	-18.1 ± 3.5 (SHR)	Hernández-Ledesma et al. (2002, 2007a)
β-Lg f(78–80)	IPA	141	Hydrolysis with proteinase K	-31 ± 6.1 (SHR)	Abubakar et al. (1998)
β-Lg f(81–83)	VFK	1029	Hydrolysis with trypsin	n.d.	Pihlanto-Leppälä et al. (2000)
β-Lg f(94–100)	VLDTDYK	946	Hydrolysis with pepsin + trypsin + chymotrypsin	n.d.	Pihlanto-Leppälä et al. (2000)
β-Lg f(102–103)	YL	122	Chemical synthesis	n.d.	Mullally et al. (1996)
β-Lg f(102–105)	YLLF	172	Hydrolysis with trypsin	n.d.	Mullally et al. (1996)
cβ-Lg f(103–105)	LLF	79.8	Hydrolysis with thermolysin	-29.0 ± 9.5(SHR)	Hernández-Ledesma et al. (2002, 2007a)
β-Lg f(106–111)	CMENSA	788	Hydrolysis with pepsin + trypsin + chymotrypsin	n.d.	Pihlanto-Leppälä et al. (2000)
β-Lg f(142–145)	ALPM	928	Whey product	-21.4 ± 7.8 (SHR)	Murakami et al. (2004)
β-Lg f(142–146)	ALPMH	521	Hydrolysis with pepsin + trypsin + chymotrypsin	n.d.	Pihlanto-Leppälä et al. (2000)
β-Lg f(142–148)	ALPMHIR	42.6	Hydrolysis with trypsin	n.d.	Mullally et al. (1997)
β-Lg f(146–148)	HIR	953	Chemical synthesis	n.d.	Mullally et al. (1996)
β-Lg f(147–148)	IR	695	Chemical synthesis	n.d.	Mullally et al. (1996)

SBP Systolic blood pressure; *n.d.* Not determined; *c* Caprine protein; *SHR* spontaneously hypertensive rats

potassium levels, reduced renal function, angioedema, etc (Fitzgerald et al., 2004). In view of the role of the diet in the prevention and treatment of hypertension, efforts are being put into the production of foods with antihypertensive activity (Dawson et al., 2000). Milk-protein-derived ACE-inhibitory peptides are latent or encrypted within the primary sequences of the proteins (Meisel, 1998). Proteolysis of milk proteins may be used to generate a mixture of in vitro ACE-inhibitory peptides and in vivo antihypertensive peptides (Fitzgerald and Meisel, 2000; Pihlanto-Leppälä, 2001; López-Fandiño et al., 2006). These bioactive peptides may also be produced during the generation of fermented dairy products (Gobbetti et al., 2000; Seppo et al., 2003; Hernández-Ledesma et al., 2004; Quirós et al., 2005).

Concerning the whey proteins, several peptides derived from β-Lg by hydrolysis processes have been shown to have inhibitory activity against ACE (Table 1). The tryptic β-Lg peptide ALPMHIR, corresponding to residues 142–148, was characterized as a potent ACE inhibitor (IC_{50} of 42.6 μM) (Mullally et al., 1997). Pihlanto-Leppälä et al. identified two peptides with moderate ACE-inhibitory activity in β-Lg hydrolysates with digestive enzymes (Pihlanto-Leppälä et al., 1998). The release of fragment f(9–14) (IC_{50} of 580 μM) was related to tryptic activity while the release of fragment f(15–20) (IC_{50} of 1682 μM) is associated to the activity of pepsin and trypsin used in the hydrolysis. The peptide β-lactorphin YLLF, corresponding to fragment f(102–105) has shown ACE-inhibitory activity (Mullally et al., 1996) in addition to its opioid-agonist activity (Table 1). Several authors have reported a more efficient cleavage for the formation of potent ACE-inhibitory and antihypertensive peptides when microbial enzymes are being used to prepare the hydrolysates. The peptide IPA f(78–80), called β-lactosin A and derived from β-Lg hydrolysates by proteinase K, showed moderate ACE-inhibitory activity (IC_{50} value of 141 μM) but strong antihypertensive activity in spontaneously hypertensive rats (Abubakar et al., 1998). In a study where a caprine β-Lg hydrolysate was prepared using thermolysin as proteolytic enzyme, two potent ACE-inhibitory peptides, whose sequences were LLF and LQKW, were identified (Hernández-Ledesma et al., 2002). Recently, the antihypertensive effect of these two peptides in spontaneously hypertensive rats has been reported (Hernández-Ledesma et al., 2007a).

The structure-activity relationship of ACE inhibitory peptides from food proteins is not well studied. However, some general features have been found. The binding to ACE is strongly influenced by the C-terminal sequence, whereby hydrophobic amino acids are more active if present at each of the three terminal positions. In addition, the presence of the positive charge of Lys (ε-amino group) and Arg (guanidine group) as the C-terminal residue may contribute to the inhibitory potency. These structural char-

Table 2. Bioactive peptides derived from β-lactoglobulin

Fragment	Sequence	Origin	Activity	References
β-Lg f(42–46)	YVEEL	Hydrolysis with thermolysin	Antioxidant	Hernández-Ledesma et al. (2005)
β-Lg f(145–149)	MHIRL	Hydrolysis with thermolysin	Antioxidant	Hernández-Ledesma et al. (2005)
β-Lg f(19–29)	WYSLAMAASDI	Hydrolysis with thermolysin	Antioxidant	Hernández-Ledesma et al. (2005)
β-Lg f(15–20)	AGTWY	Hydrolysis with pepsin	Antimicrobial	Pellegrini et al. (2001)
β-Lg f(25–40)	AASDISLLDAQSAPLR	Hydrolysis with pepsin	Antimicrobial	Pellegrini et al. (2001)
β-Lg f(78–83)	IPAVFK	Hydrolysis with pepsin	Antimicrobial	Pellegrini et al. (2001)
β-Lg f(92–100)	VLVLDTDYK	Hydrolysis with pepsin	Antimicrobial	Pellegrini et al. (2001)
β-Lg f(102–105)	YLLF	Hydrolysis with pepsin + trypsin or pepsin + chymotrypsin	Opioid	Antila et al. (1991); Sipola et al. (2002)
β-Lg f(146–149)	HIRL	Hydrolysis with chymotrypsin	Opioid; antihypertensive; Analgesic Antinociceptive Hypocholesterolemic	Yoshikawa et al. (1991) Yamauchi et al. (2003a) Yamauchi et al. (2003b)
β-Lg f(71–75)	IIAEK	Hydrolysis with trypsin	Hypocholesterolemic	Nagaoka et al. (2001)
β-Lg f(9–14)	GLDIQK	Hydrolysis with trypsin	Hypocholesterolemic	Nagaoka et al. (2001)
β-Lg f(142–146)	ALPMH	Hydrolysis with trypsin	Hypocholesterolemic	Nagaoka et al. (2001)
β-Lg f(41–60)	VYVEELKPTPEGDLEILLQK	Hydrolysis with trypsin	Hypocholesterolemic	Nagaoka et al. (2001)

acteristics were also found, by Pripp and co-workers, as determinants for ACE-inhibitory activity of milk-derived peptides (Pripp et al., 2004). It has been postulated that the mechanism of ACE-inhibition also involves interactions with sub-sites not normally occupied by substrates or with an anionic inhibitor-binding site that is different from the catalytic site of the enzyme (Meisel, 1997). Recently, the relevance of the conformational structure of the peptide on the interaction with the active site of ACE has also been reported (Gómez-Ruiz et al., 2004).

3. Antioxidant peptides

Reactive oxygen species (ROS) are produced as a part of normal cell metabolism and are involved in a variety of normal in vivo regulatory systems. Some external agents (ultraviolet light, ionizing radiation, environmental toxins, etc.) can trigger ROS production. A high level of ROS can perturb the redox balance and promote ''oxidative stress'' in cells. It is well known that the free-radicals-mediated lipid oxidation is considered to be one of the main limiting factors for the quality and acceptability of foods during processing and storage. In addition, it is assumed that ''oxidative stress'' is implicated in the ethiology of age-associated chronic diseases such as cardiovascular diseases, diabetes, cataracts, neurodegenerative disorders, certain types of cancer and aging (Ames et al., 1993). Currently, synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are commonly used to act against free radicals in food and biological systems. However, the potential adverse effects of these synthetic additives have stimulated their replacement by natural antioxidants derived from dietary sources (Chen et al., 1992). The utilization of protein hydrolysates or peptides for the improvement of the antioxidant activity in functional foods presents additional advantages over other natural antioxidants, since they also confer an additional nutritional value, as well as, other desired functional properties. In past few years, the searching for whey-derived peptides with radical scavenging and lipid peroxidation inhibitory activities is receiving a special attention. Peña-Ramos and Xiong have described the capacity of whey-protein-isolate (WPI) hydrolysed by different enzymes to decrease the formation of thiobarbituric acid-reactive substances (TBARS) in a liposome system (Peña-Ramos and Xiong, 2001, 2003). Peptide fractions having a relatively high-antioxidant activity in an iron-catalysed liposomal system were related to the size (usually lower molecular weights) and the specific amino acid composition. In general, strong antioxidant ability was exhibited by peptides with high concentrations of Hys and hydrophobic amino acids (Peña-Ramos et al., 2004). Hernández-Ledesma and co-workers investigated the antioxidant activity of hydrolysates of the major whey proteins, β-Lg and α-lactalbumin, by commercial proteases (pepsin, trypsin, chymotrypsin, thermolysin and corolase PP) (Hernández-Ledesma et al., 2005). These authors found that corolase PP was the most appropriate enzyme to produce β-lg hydrolysates having high oxygen radical scavenging activity (ORAC-FL values). Several peptides identified in the 3 kDa-permeate were derived from these hydrolysates. The radical scaveng-

ing activity of one of these peptides, whose sequence was WYSLAMAASDI, was slightly higher (2.62 μmol Trolox equivs/μmol peptide) than that shown by BHA (2.43 μmol Trolox equivs/μmol BHA). Studies about the structure/activity relationship have demonstrated that the presence of certain amino acids, as well as their position within the peptide sequence, are the most determinant factors on the antioxidant activity of a peptide (Dávalos et al., 2004; Hernández-Ledesma et al., 2005). Trp and Tyr seem to be the main amino acids that contribute on the antioxidant activity, showing ORAC values of 4.65 and 1.57 μmol Trolox/μmol of amino acid, respectively. The high antioxidant activity of these amino acids may be explained by the capacity of the indolic and phenolic groups to serve as hydrogen donors. It is, therefore, likely that the oxygen radical quenches the Trp-indolic and Tyr-phenolic hydrogen (H^+), resulting in the formation of more stable indoyl and phenoxyl radicals. Recently, L-Tyr has been found to be an effective antioxidant in different in vitro assays including antilipid peroxidation, reductive ability, ABTS, DPPH and superoxide anion radical scavenging, hydrogen peroxide scavenging and metal chelating activities (Gulcin, 2007). It has been demonstrated that the presence of Trp and Tyr residues in the N-terminal or C-terminal positions of a peptide notably increases its radical scavenging activity (Hernández-Ledesma et al., 2007b). Other factors such as the synergistic/antagonistic effects among amino acids also contribute on the antioxidant activity of the peptides (Dávalos et al., 2004; Hernández-Ledesma et al., 2005).

4. Antimicrobial and immunomodulating activities

In the past two decades there has been a wide effort to identify and characterize antimicrobial peptides present in man and animals because they are considered to be important components in innate immunity. These peptides constitute a unique and diverse group of molecules and are divided into classes on the basis of their amino acid composition and structure. Most of the antimicrobial peptides that are a benefit to health are synthesised by the cells of the organism itself, and the others are from food-protein molecules, which are broken down by proteolytic enzymes in the digestive tract (Pellegrini, 2003).

The antibacterial properties of milk have been known for a long time. In addition to the naturally occurring antimicrobial proteins present in milk, such as lactoferrin and immunoglobulins, there are also a variety of antibacterial peptides encrypted within the sequence of milk proteins that are released upon its suitable hydrolysis. Although these milk proteins-borne antimicrobial peptides have been studied extensively, their mechanisms of action, as well as their effects on immune system, are still unknown (Brogden, 2005). These peptides might affect the microbial cell in many ways; for example, by activation of its autolytic enzyme system, by formation of transmembrane pore, by inhibition of its wall and/or nucleic acid synthesis, or when acting in synergy with other host innate immune molecules (Zhang and Falla, 2004; Brogden, 2005; Lohner and Blondelle, 2005).

Mercier and co-workers demonstrated that the addition of whey proteins products in cell culture media at a concentration of 100 μg/mL stimulated in vitro proliferation of murine spleen lymphocytes (Mercier et al., 2004). A notable decrease of the immunostimulating activity was found when these products were hydrolysed by trypsin/chymotrypsin. However, several fractions isolated from these hydrolysates were found to be much more active (0.5–500 μg/ml). This in vitro study supports the hypothesis that some short-chain (<5 kDa) and neutral/basic peptides from whey proteins are effective in stimulating immune cell proliferation.

Recently, the antimicrobial activity (the ability to activate the microbial autolytic system) and immunostimulatory activity (the ability to improve the phagocytic cell functioning) of β-Lg, among other food proteins, hydrolysed with four gastrointestinal proteinases (trypsin, chymotrypsin, pepsin and pancreatin) have been examined by Biziulevicius and co-workers (2006). The hydrolysates acted antimicrobially in vitro by stimulating the autolytic system of all 20 naturally autolyzing as well as four naturally nonautolyzing microbial (bacterial and fungal) strains were tested. All the β-Lg hydrolysates tested, when given to mice, were shown to enhance the phagocytosing capacity of peritoneal macrophages. These authors suggested that immunostimulating activity of β-Lg hydrolysates is a consequence of their antimicrobial activity (Biziulevicius et al., 2006).

β-Lg-derived fragments, generated through the action of alcalase, pepsin or trypsin, have been shown to be bacteriostatic against *Escherichia coli* and against pathogenic strains of *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* (Pihlanto-Leppälä et al., 1999; El-Zahar et al., 2004). Proteolytic digestion of bovine β-Lg by trypsin allowed the release of four peptide fragments [f(15–20), f(25–40), f(78–83), f(92–100)] with bactericidal activity against Gram-positive bacteria (Pellegrini et al., 2001). The charge, hydrophobicity and cationic/anionic character have been reported by these authors as important factors for bactericidal activity. The

negative charge of these peptides explains why they were only weakly effective against Gram-negative bacteria whose membranes contain lipopolysaccharide, a negatively charged molecule. Small-targeted modifications carried out by these authors in the sequence of these β-Lg peptides were found to be useful in expanding their antimicrobial function (Pellegrini et al., 2001).

5. Opioid activity

Opioid peptides are defined as peptides which have an affinity for an opiate receptor as well as opiate-like effects, inhibited by naloxone. Opioid peptides exert their activity by binding to specific receptors of the target cell. The individual receptors are responsible for specific physiological effects, e.g. the μ-receptor for emotional behaviour and suppression of intestinal mobility, the σ-receptor for emotional behaviour and the κ-receptor for sedation and food intake. The opioid activity of milk-derived peptides was the first biological property demonstrated. Since then, several types of opioid-agonist peptides have been characterized. These peptides were referred as exorphins to distinguish them from opioid peptides of endogenous origin and were called endorphins. The major whey proteins, β-Lg, α-La and bovine serum albumin, are precursors of several opioid peptides (Pihlanto-Leppälä et al., 2001). Digestion of bovine β-Lg with pepsin and trypsin, or trypsin and chymotrypsin yielded YLLF, corresponding to fragment f(102–105) and called β-lactorphin. A contracting effect of this peptide on smooth muscle on being antagonized by naloxone was determined in coaxially stimulated guinea pig ileum in vitro (Antila et al., 1991). Recent studies have demonstrated that β-lactorphin also improved the impaired vascular function in mesenteric rings of adults SHR (Sipola et al., 2002). This beneficial action was directed towards endothelial function and also enhanced endothelium-independent relaxation.

Structurally this tetra-peptide closely resembles endogenous opioid peptides. The common structural feature among endogenous and exogenous opioid peptides is the presence of a Tyr residue at the N-terminus, coupled with the presence of another aromatic residue, e.g. Phe or Tyr, in the third or fourth position. This is an important motif that fits into the binding site of the opioid receptors. The negative potential, localized in the vicinity of the phenolic hydrolxyl group of Tyr, seems to be essential for opioid activity (Silva and Malcata, 2004; Meisel, 2005).

β-Lactotensin (HIRL) is a peptide derived from residues f(146–149) of bovine β-Lg. It was isolated by Yoshikawa and co-workers from a chymotrypsin digest of this whey protein (Yoshikawa et al., 1991). This peptide has demonstrated to be a neurotensin agonist with high selectivity for the NT_2 receptor. Different activities, such as ileum-contracting, antihypertensive, analgesic and antinociceptive, have been reported for this peptide as result of its binding to this neurotensin receptor (Yamauchi et al., 2003a). A recent study has demonstrated that β-lactotensin has an antistress effect promoting the extinction of fear memory in the central nervous system (Yamauchi et al., 2006).

6. Hypocholesterolaemic activity

The whey proteins have been reported to exhibit a greater hypocholesterolaemic effect in comparison with casein or soybean proteins in rats (Nagaoka et al., 1991, 1992). A posterior study of these authors have provided the first evidence that β-Lg tryptic hydrolysate has a hypocholesterolaemic activity in rats (Nagaoka et al., 2001). This effect was at least partly due to an enhancement of fecal steroid excretion. By using Caco-2 cell screening, these authors identified four kinds of novel peptide sequences which inhibited cholesterol absorption in vitro, i.e., IIAEK, GLDIQK, ALPMH, and VYVEELKPTPEGDLEILLQK, which corresponded, respectively, to fragments f(71–75), f(9–14), f(142–146), and f(41–60) of bovine β-Lg. The peptide IIAEK have demonstrated in animal studies having a powerful influence on the serum cholesterol level. Its effect as hypocholesterolaemic in rats was greater than that shown by β-sitosterol (Nagaoka et al., 2001).

Recently, β-lactotensin has been found to show hypocholesterolaemic activity after administration for 2 days at a dose of 30 mg/kg (i.p.) or 100 mg/kg (p.o.) to mice (Yamauchi et al., 2003b). These authors have reported the relationship between this hypocholesterolaemic effect and the action of β-lactotensin as NT_2 neurotensin receptor agonist.

7. Future prospects

Whey is a natural by-product of cheese making process that contains all the essential amino acids and has the highest protein quality rating among other proteins. Estimates of the worldwide production of whey indicate that about 700,000 tons of true whey proteins are available as valuable food ingredients with important nutritional and functional properties. Currently, whey proteins are used as common ingredients in various products including infant formulas, specialized enteral and clinical protein supplements, sports nutrition products, products specific

to weight management and mood control. The major whey protein, β-Lg, provides the food industry with a unique ingredient material, a cost-effective protein with attractive properties in food functionality. The occurrence of many biologically active peptides encrypted in β-Lg is now well documented. An increasing number of in vitro and in vivo studies reveal that these peptides are released from β-Lg upon enzymatic hydrolysis. Once released, they can affect crucial physiological functions and modulate several regulatory processes in four main physiological systems: the cardiovascular system, the nervous system, the immune system and the nutrition system. Such bioactive peptides have been proven to possess antihypertensive, antioxidant, antimicrobial, immunomodulating, opioid and hypocholesterolaemic activities. The bioactivities reported for β-Lg-derived peptides provide an opening for the inclusion of β-Lg as the active ingredient in a range of functional foods, dietary supplements and pharmaceutical preparations. However, whether bioactive peptides are beneficial as food constituents or as drugs is dependent on the availability of rigorous scientific data demonstrating their efficacy and safety for human use. Most of the claimed physiological properties of β-Lg-derived peptides have been carried out in vitro or in animal model systems. It is important to confirm the hypothesis and demonstrate the health enhancing effect of bioactive peptides by human cell culture models as well as human studies. The adverse effects associated with bioactive peptides should be carefully considered and their molecular mechanisms should also be investigated.

In addition to focusing on their physiological aspects, the technological properties of the bioactive peptide fractions should be studied in order to develop model foods that contain them and retain their activity for a guaranteed period. The question of the cost of the process required to manufacture the desired bioactive peptide is also an important feature. There is a need to develop strategies, e.g., chromatographic and membrane separation techniques, to produce fractions enriched in bioactive peptides. The interaction of peptides with other food components, such as carbohydrates and lipids, as well as the influence of the processing conditions (specially heating) on peptide activity and bioavailability should be further investigated. On the marketing side, cost-effectiveness of the ingredient and its easy incorporation into a good-tasting end product are important. Claiming a message that can be understood by consumers or is allowed by legal authorities is a further prerequisite for successful market introduction.

Designing new health-promoting whey products containing biologically active peptides looks promising and would be attractive to consumers and producers alike. It is hoped that the functional properties of these peptides in the consumer's gut will exert a disease suppressing effect, making cheese whey an invaluable and cheap, functional food. This food may regulate specific body processes and would be suggested as a daily aid to maintain good health status.

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