milk proteins are considered the most important source of bioactive peptides and an increasing number of bioactive peptides have been identified in milk protein hydrolysates and fermented dairy products.

FUNCTIONALITY OF PEPTIDES

Biologically active peptides derived from food proteins with an affinity to modulate blood pressure (BP) have been thoroughly studied. Angiotensin I-converting enzyme (ACE) plays a major role in the control of BP. ACE inhibition leads to a decrease of the vasoconstrictory peptide, angiotensin II and to an increase of the vasodilatory peptide, bradykinin, and therefore may result in an overall reduction in BP. ACE-inhibitory peptides have been derived from numerous food sources, including tuna muscle, fish, casein, whey, egg, cereals and soy. ACE-inhibitory peptides are usually short peptides two to nine amino acids, are rich in hydrophobic amino acid residues and have a proline, lysine or arginine as a C-terminal amino acids. Most peptides are di- or tripeptides, which are resistant to the action of digestive-tract endopeptidases (6). The activities have been analyzed with various methods, which make the comparison of different peptides difficult. The initial ACE activity in an assay results contributes to ACE-inhibition (IC50-values) in different studies (7). Opioid peptides derived from food proteins have affinities to bind to opiate receptors and express similar opiate activity, which in turn can be reversed by an opioid antagonist. Opioid peptides are regarded as ligands of opioid receptors. Structurally, exogenous and endogenous opioid peptides vary in the N-terminal sequence of the peptide, such as having a tyrosine in the N-terminal site. Food peptides with opioid activity can be derived from hydrolysis of wheat gluten, caseinates, whey proteins and hemoglobulin (8). Opioid peptides may influence the gastrointestinal function by affecting smooth muscles, or by affecting the intestinal transport of electrolytes. The actual physiological effects of dietary opioid peptides remain to be confirmed. Food derived peptides can enhance immune cell functions, measured as lymphocyte proliferation, antibody synthesis and cytokine regulation (9). Immunomodulatory peptides derived from bovine casein have both suppressive and enhancing effects on immune variables (10). The protective effect of a casein-derived immunopeptide on resistance to microbial infection by Klebsiella pneumoniae has been demonstrated in mice (11). Cytomodulatory peptides derived from casein inhibit cancer cell growth or stimulate the activity of immunocompetent cells and neonatal intestinal cells (5). Also, it has been suggested that immunomodulatory milk peptides may alleviate allergic reactions in atopic humans, enhance mucosal immunity in the gastrointestinal tract and may regulate the development of the immune system in response.
newborn infant (2). Antimicrobial peptides have been identified from milk proteins hydrolysates, edible plants, fish and eggs. The most studied are the lactoferricins, derived from bovine and human lactoferrin which exhibit antimicrobial activity against various Gram-positive and -negative bacteria, yeasts and filamentous fungi. The disruption of normal membrane permeability is at least partly responsible for the antibacterial mechanism of lactoferricins (12). Antioxidative peptides can be released from caseins, soybean and gelatin in hydrolysis by proteolytic enzymes (2). The antioxidative activity of soybean derived peptides was attributed to those peptides with a Leu-Leu-Pro-His-His sequence (13). Milk derived antioxidative peptides include hydrophobic amino acids, proline, histidine, tyrosine or tryptophan in the sequence. Antioxidant activity of the hydrolysates seems to be inherent to the characteristic amino acid sequences of peptides derived, depending on the protease specificity (14). Specific casein derived CPPs can be found around 60-70 of β-casein show immunostimulatory, opioid and ACE-inhibitory activities (2).

MEANS TO PRODUCE FUNCTIONAL PEPTIDES

Biologically active peptides can be produced from precursor proteins in the following ways: (a) through enzymatic hydrolysis by digestive enzymes (b) through fermentation (c) through the action of proteolytic enzymes derived from microorganisms or plants, (d) through chemical or enzymatic synthesis (e) with recombinant DNA technology. Enzymatic hydrolysis has been the most common route to produce functional peptides - and pancreatic enzymes, especially trypsin, have been associated with efforts toward production, characterization and identification of many known peptides (Table 1). The release of various bioactive peptides from milk proteins through microbial hydrolysis has been reported (2, 4, 17, 18). Lactobacillus helveticus strains capable of releasing ACE-inhibitory peptides, in particular, have been demonstrated. The best known ACE-inhibitory peptides, Val-Pro-Pro and Ile-Pro-Pro, have been identified in milk fermented with Lb. helveticus strains (19, 20). Proteinases from Lb. helveticus CP790, Aspergillus oryzae, Lb. helveticus JCM1004 and an endopeptidase from Lb. helveticus CM4 have been used to generate antihypertensive peptides (4, 18).

The bioactive peptides derived from food proteins are often intermediates and are isolated from very complex peptide hydrolysates in which their concentrations are often low. The preparation of such peptide or peptide-containing fractions generally requires time-consuming chromatographic steps. Membrane separation techniques have provided the best technology for the enrichment of peptides with a specific molecular weight range. Ultrafiltration membrane reactors have been applied to produce antithrombotic, emulsifying and opiod peptides. Stepwise ultrafiltration have been found useful for separating small peptides from high molecular mass residues and for enrichment of bioactivities (2). Ion exchange chromatography has emerged as a promising technique for the enrichment of cationic antibacterial peptides and negatively charged phosphopeptides (21). One of the most important advances in the separation of small charged biomolecules, was the development of nanofiltration membrane (2, 22).

OCCURRENCE OF BIOACTIVE PEPTIDES IN FOODS

Various bioactive peptides can be found in various cheese varieties and fermented milks, e.g. yoghurt and sour milk. Proteolysis during cheese ripening may lead to the formation of different bioactive peptides, e.g. ACE-inhibitory, and the occurrence of bioactivity appears to be dependent on the ripening stage of the cheese (23, 24). Moreover, bioactive peptides are likely to be formed in the gastrointestinal tract upon ingestion of a piece of cheese (25). Many studies have employed Lb. helveticus strains for the production of antihypertensive peptides (18). At present, at least two fermented sour-milk products containing the ACE-inhibitory tripeptides Val-Pro-Pro and Ile-Pro-Pro have been launched commercially in Japan and Finland, respectively. The Japanese product “Calpis” (Amelco S) is fermented with Lb. helveticus strains (19, 20). Proteinases from Lb. helveticus strains (19, 20). Proteinases from Lb. helveticus strains have been demonstrated. The best known ACE-inhibitory peptides, Val-Pro-Pro and Ile-Pro-Pro, have been identified in milk fermented with Lb. helveticus strains (19, 20). Proteinases from Lb. helveticus CP790, Aspergillus oryzae, Lb. helveticus JCM1004 and an endopeptidase from Lb. helveticus CM4 have been used to generate antihypertensive peptides (4, 18).

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CONCLUSIONS

The potential health benefits of milk protein-derived peptides have been a subject of growing commercial interest in the context of health-promoting functional foods. Antihypertensive, mineral-binding and anticariogenic peptides have been most studied for their physiological effects. A few commercial developments have been launched on the market and this trend is likely to continue alongside with increasing knowledge about the functionalities of the peptides. The optimal exploitation of bioactive peptides for human nutrition and health poses an exciting scientific and technological challenge, while at the same time offering potential for commercially successful applications. Bioactive peptides can be incorporated in the form of ingredients in functional and novel foods, dietary supplements and even pharmaceuticals with the purpose of delivering specific health benefits. Such tailored dietary formulations are currently being developed worldwide to optimize health through nutrition. Many scientific, technological and regulatory issues must, however, be resolved before these substances can be optimally harnessed to this end.

REFERENCES AND NOTES