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# BIOLOGICALLY ACTIVE PEPTIDES DERIVED FROM PROTEINS – A REVIEW

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Proteins play an important role in body functioning. They can also be a good source of peptides with different activities. Such peptides are defined as biologically active (bioactive peptides). Bioactive peptides interact with proper body receptors and such an effect can be beneficial or not. Biopeptides as components of food with desired features have become an interesting issue for scientific research. Many of bioactive peptides are found in milk and dairy products, plant, animal and microbial proteins. They function mainly as inhibitors of the angiotensin converting enzyme but there is a plenty of peptides derived from other sources that can even prevent chronic diseases.

This paper focuses on peptides derived from different sources and their physiological role in the body as well as functional aspects of their application in food production. In this article we concentrate on the peptides exerting the following activities: affecting blood pressure, prolyl endopeptidase inhibitors, coeliac toxic, immunomodulating and opioid.

## INTRODUCTION

In living organisms, during gastrointestinal digestion or food processing, small peptides can be released and may act as regulatory compounds, hormone-like substances and play important (beneficial or not) physiological role [Wang & Gonzalez De Meja, 2005].

According to contemporary knowledge about proteins, they may be a precursor of peptides with the biological activity (bioactive peptides) [Dziuba *et al.*, 1996; Karelin *et al*., 1998].

Peptides were isolated from milk, plant, meat and egg proteins and then well-characterised [Wang & Gonzalez De Meja, 2005]. Such peptides are known as the molecules involved in blood pressure reduction, prolyl endopeptidase inhibition, and immune system stimulation. Moreover, they can play a role of antithrombotic, antioxidative, antibacterial *etc*. agents [Meisel & Schlimme, 1994, 1996; Meisel, 1998; Dziuba *et al.*, 1999a, 2003, 2004]. Many of bioactive peptides were isolated from a single species using the genome fragment libraries [Watt, 2006]. The application of peptides for therapeutic purposes especially in the field of the treatment of cancer, infections, immunological system disorders, cardiovascular disorders is at present the focus of interest of many research groups [Latham, 1999].

Bioactive peptides are also reccommended as functional food components, *i.e.* food with designed properties [Arai, 1996; Korhonen & Pihlanto, 1998, 2001]. Research concerning bioactive peptides and application as physiological food components is still underway. It concerns especially the industrial scale production of food hormones based on antihypertensive peptides including phosphopeptides and immunostimulating peptides [Gobetti *et al.*, 2000].

## PEPTIDES AFFECTING BLOOD PRESSURE

Peptides with the above-mentioned activity are the most well-known group of biologically active peptides derived from food proteins [Ariyoshi, 1993; Yamamoto, 1997; Matsui *et al.*, 1997; Dziuba *et al.*, 1999a, b, 2003, 2004].

Many of them are inhibitors of angiotensin converting enzyme (ACE) (EC 3.4.15.1.). ACE catalyses the hydrolysis of inactive prohormone angiotensin I to angiotensin II. The angiotensin second is the key factor involved in blood vessels contraction which, in turn, results inblood pressure increases [Seweryn *et al.*, 2000; Roy *et al*., 2000]. This enzyme is also involved in the degradation of bradykinin as well as Met-enkephalin [Meisel, 1986; Meisel & Schlimme, 1996; Schlimme & Meisel, 1995]. ACE inhibitors were originally detected in snake poison. They were involved in smooth muscles contractions in the guinea pig ileum because of the bradykinin activity, thus the 1st ACE inhibitor was called a bradykinin potentiator [Kodera & Nio, 2006].

Although the structure-function relationship of ACE inhibitors is still not clear, some common similarities can be found between them [Ariyoshi, 1993; Meisel & Schlimme, 1996; Yamamoto, 1997]. They are rich in hydrophobic amino acids and many of them contain proline, lysine or arginine as C-terminal residues. Amongst the ACE peptide inhibitors the majority of them are di- or tripeptides, which are resistant to the digestive tract endopeptidases and can be easily absorbed to the blood as well. However, there are some exceptions, *e.g.* tetrapeptide YGGY can easily be hydrolysed by digestive enzymes and loose its activity [Saito *et al.*, 2000].

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Milk proteins are a rich source as the precursor of peptides reducing blood pressure. Bovine and human caseins as well as whey proteins are very rich in amino acid sequences that can form antihypertensive peptides [Saito *et al.*, 2000].

Some of ACE inhibitors have shown *in vivo* activity in spontaneously hypertensive rats. It was found out that the oral intake of tryptic casein hydrolysates reduced blood pressure in rats after few hours since intake [Miyoshi *et al.*, 1991; Saito *et al.*, 1994a, b]. Maruyama & Suzuki [1982] have proved that the intake of milk by the spontaneusly hypertensive rats (SHR) reduced their blood pressure whereas it did not cause any of such effects in rats with normal blood pressure. Similar effect were achieved in rats fed with sour milk [Xu, 1998]. Clinical tests with people involved showed that blood pressure significantly reduced with the patients suffering from hypertension after the intake of 95 mL of sour milk [Matsui *et al.*, 1997; Yamamoto, 1997].

Lo and co-workers [2006] applied the dynamic model for the *in vitro* digestion of isolated soy proteins (ISP). They concluded that ACE inhibitory activity was dependent on the time of the digestion and heat treatment of soy protein. Pepsin hydrolysis of ISP produced peptides with a higher ACE inhibitory activity due to the increased digestion time. Hydrolysis of ISP by pancreatin produced soy peptides with higher ACE--inhibitory activity as compared to pepsin hydrolysates, but decreasing inhibitory acitivity appeared after longer digestion time. These results suggest that at the longer digestion time pancreatin may have hydrolysed the peptides from pepsin digestion that had strong ACE-inhibitory activity, and then turned them into peptides with lower ACE-inhibitory activity.

Arihara and co-authors [2001] hydrolysed the porcine skeletal muscle proteins. The reaction of thermolysin (EC 3.4.24.27) with the heavy chain of myosin gave the so-called myopentapeptides A and B with the sequences MNPPK and ITTNP. These peptides as well as their shorter fragments like MNP, NPP, PPK, ITT, TTN, TNP were observed to lower blood pressure. The peptides with antihypertensive effect occurred also in other materials, *i.e.* wheat gluten hydrolysate, maize zein, rice proteins, soybean α-proteins cytochrome and haemoglobin. The computer analysis of amino acid sequences of wheat gliadins made by means of BIOPEP database [Iwaniak *et al.,* 2005; Dziuba & Iwaniak, 2006] showed the presence of fragments that are homological with the sequences regarded as antihypertensive peptides. They were: LQP (α,β and γ-gliadins), PYP (α-, β- and γ-gliadins), IPP (α-and β-gliadins), LPP (γ-gliadins) and LVL (γ-gliadins). The most frequently appearing one was the fragment with the sequence LQP. Clinical studies on the peptides from *Lactobacillus helveticus* milk fermented peptides with the sequences IPP and VPP showed that they reduce systolic and diastolic blood pressure in hypertensive patients. The blood pressure lowering effect was observed in the patients after 8 weeks of intervention with *Lb. helveticus* milk [Jauhiainen & Korpela, 2007].

## PROLYL ENDOPEPTIDASE INHIBITORS

In the mammalian organisms such prolyl endopeptidase inhibitors were found in the brain, womb, skeletal muscles, liver and kidneys. Different inhibitors of prolyl endopeptidases affect the memory and learning abilities, which was observed in rodents and primates in laboratory conditions [Schneider *et al.*, 2002]. PEP hydrolysed a substance P – peptide taking part in the processes of remembering [Huston *et al.*, 1993; Kimura *et al.*, 1997], and also oxitocin, vassopresin, tyroliberin, bradykinin, neurotensin, and dynorphin. Prolyl endopeptidases (PEP) (EC 3.4.21.26) hydrolyse many of biologically active peptides from plant, animal and microorganism resources [Yaron & Naider, 1993; Cunningham & O'Connor, 1997] and belong to the the serine proteases family [Rawlings *et al.*, 1991] *i.e.* enzymes family which evolved the arachea, prokaryota and eukaryota divergently between 2000 and 4000 million years ago. It means that members of the PEP family were found in the last universal common ancestor of all life forms [Venäläinen *et al.*, 2004].

PEP are also considered as soluble cytoplasmic proteins [García-Horsman *et al.*, 2007].

Inhibitors of prolyl endopeptidases were found in food proteins such as: γ-zein (HLPPV, a product of the hydrolysis of γ-zein by subtilisin) [Maruyama *et al.*, 1992, 1993] and peptide with the sequence LLSPWNINA isolated as the side product of sake production [Saito *et al.*, 1997].

Typical structural motif that occurs in the majority of peptide PEP inhibitors are repetitive proline residues. The activity of prolyl endopeptidase inhibitors derived from food proteins was determined *in vitro*. These inhibitors were obtained from food products as well as their by-products on the laboratory scale [Saito *et al.*, 1997; Dziuba *et al.*, 1999a].

## COELIAC TOXIC PEPTIDES

The production of non-coeliac toxic food is a global problem. Coeliac is also referred to as gluten enteropathy or non--tropical sprue and is an autoimmune-like systemic disorder in people who have a genetic expression of the HLA type II molecules DQ2 or DQ8 [Hausch *et al.*, 2002]. The characteristic symptom of this disease is the damage of the epithelium of small bowel and intolerance to wheat gluten [Cornell, 1996]. The products containing wheat proteins as well as barley and rye should be eliminated from the diet of people suffering from this disease [Cornell *et al.*, 1994; Cornell, 1996; Silano & De Vincenzi, 1999].

There a few theories about the reasons of coeliac disease induction [Cornell, 1996]: (1) enzymopathic hypothesis – assuming that incomplete proteolysis of the peptides evoke their abnormal concentration in the large intestine and then tissue lesions; (2) immunological hypothesis – suggesting the binding of gluten or its fragments by the intestine membrane and then being the substrate for the immunological reactions; (3) glycoprotein defect hypothesis – suggests the presence of abnormal glycoproteins which bind gluten in the cells of small bowel epithelium, which leads to cell damage; and (4) hypothesis of the expanded permeability of intestine membrane – causes lysozyme lesions and the release of enzymes damaging the cells.

Examples of the coeliac toxic peptides are fragments of gliadins with the sequences: QQPYPQ [Cornell, 1988] and YPQPQ [Graf *et al.*, 1987]. The characteristic motif of coeliac toxic peptides is the high content of glutamine, proline and

tyrosine. Proline-rich peptides, 12-mer, 19-mer and 33-mer, were produced by proteolytic enzymes and were toxic only to celiac patients, not to healthy subjects [Pyle *et al.*, 2005]. In the sequences of coeliac peptides the repetitive motifs are QQQP, PSQQ and QQY. They were released *in vitro* from gliadins by digestive tract enzymes such as pepsin (EC 3.4.23.4) and trypsin (EC 3.4.21.4). Fragments potentially regarded as coeliac-toxic, such as QQQP, QQPY, PSQQ, QPYP, are present in other non-toxic proteins [De Ritis *et al.*, 1988]. According to MacLachlan and co-workers [2002], it may be related to the so-called extended structural motif, a fragment possesing additional amino acid residues located at C- and N-end of the above-mentioned tetrapeptides.

Matysiak-Budnik and co-workers [2005] reported that endogenous prolyl oligopeptidases can play a role in the aetiology of coeliac disease. Some resistant alpha-gliadin peptides can be digested intraluminally by enterocytes both in healthy and treated celiac patients. In turn patients with active celiac have incomplete degradation of 33-mer peptides and protected intestinal transport of gliadin peptides. Prolyl endopeptidases activity in duoedenal mucosa was higher in patients with treated coeliac than in healthy or treated celiac subject.

Specific proteases can be used to degrade proline-rich gluten peptides into smaller fragments which do not stimulate the intesitnal T cells in patients with celiac disease. It is called a "protease therapy". Bacterial and fungal enzymes were found to degrade proline-rich toxic peptides but it needs to be mentioned that there are some disadvantages such as slow reaction rate, neccessity of using some additional enzymes, high concentrations, and high production costs. It may limit the practical application of the protease therapy [Hartmann *et al.*, 2006].

## IMMUNOMODULATING PEPTIDES

Peptides possessing such an activity are usually low molecular weight compounds. They are involved in *in vitro* cell interactions with various body cells of the immune system and *in vivo* exert or depress cell-mediated and humoral immune functions. Immunomodulating peptides include glycopeptides, hormones, peptidic fragments of immunoglobulins and peptides isolated from food proteins [Werner, 1987].

Oryzatensin (GYPMYPLPR) – a peptide contracting smooth muscles – was isolated from trypsin hydrolysate of rice protein. Probably it is derived from its albumin fraction [Takahashi *et al.*, 1994, 1996]. Similar effect is assigned to the shorter, C-terminal fragments of this peptide [Takahashi *et al.*, 1996, 1997]. Activity of such peptides was confirmed *in vitro* in fragments of guinea pig intestine. Non-separated tryptic hydrolysate of rice proteins shows the oryzatensin-like activity [Takahashi *et al.*, 1994]. Oryzatensin and its fragments are the cause of smooth muscles contraction by the stimulation of histamine production and the substance acting similarly as the prostaglandin. Immunostimulating peptide with the HCQRPR sequence obtained from the tryptic hydrolysate of soy proteins is another example of smooth muscle contracting peptide [Yoshikawa *et al.*, 1993]. It stimulates *in vitro* fagocytosis as well as the production of the murine cancer necrosis factor *in vivo*.

The source of immunostimulating peptides is casein and whey proteins from human and bovine milk. Here are the examples: LLY (precursor: β-CN, fragment 191-193), TTMPLW (precursor: $\alpha_{s1}$-CN fragment 194-199) and PGPIPN (precursor: β-CN, fragment 63-68) [Stepaniak *et al.*, 1996; Xiao *et al.*, 2000]. Immunopeptides produced during milk fermentation contributed to the antitumor effects observed in fermented milk products [Korhonen & Pihlanto, 2006].

Mercier and co-workers [2004] evaluated the effect of whey proteins digests on proliferation of lymphocytes isolated from murine spleen. The results indicated that microwaved whey protein isolates increased lymphocytes proliferation, whereas no such effect was observed for beta-lactoglobulin, alpha-lactalbumin, and glycomacropeptide. Milk-derived peptides with an immunomodulating effect may alleviate allergic reactions in humans and enhance mucosal immunity in the gastrointestinal tract. It means that such peptides may regulate the development of the immune system in newborn infants and moreover it has been suggested that immunopeptides which are formed during milk fermentation may contribute to the antitumor effects of fermented milk [Haque & Chand, 2006].

## OPIOID AND OPIOID AGONIST PEPTIDES

Opioid peptides are the ligands of opioid receptors. They affect the functioning of spinal cord, adrenal gland, digestive tract, pituitary gland and hypothalamus by the special receptors of central and peripheral nervous system. They are involved in a stress reaction. Pain, anaesthesia, sedation, cataphrenia, breathing depression, hypotension, fluctuating body temperature, lack of appetite, reduced secretion of digestive juices and the alteration of sexual behaviors may be the symptoms of action of opioid peptides in the body [Molina & Abumrad, 1994; Dziuba *et al.*, 1999a].

The characteristic structural motif of all endogenous opioid peptides such as enkephalins, endorphins, dynorphins is N-terminal sequence YGGF [Kupryszewski, 1985]. Endogenous opioid peptides are able to alter the functions as well as the growth of the central neural system cells in the adult and growing organism [Calvo *et al.*, 2000]. Several peptide fragments produced by hydrolysis of the proenkephalin A contain a number of enkephalin fragments. One of them is the adrenal medulla peptide 22 (BAM22) which implicates in diverse biological functions, including analgesia [Lembo *et al*., 2002].

The first as well as major object of research in the opioid peptides derived from food proteins were casein peptides [Brantl *et al.*, 1981]. Milk opioid peptides can be *in vitro* enzymatically released from bovine and human β-casein [Parish *et al.*, 1983; Schlimme & Meisel, 1995, Kostyra *et al.*, 2004]. These peptides are called casomorphins and were found in a chyme of guinea pig duodenum and human small intestine as the products of *in vivo* digestion [Maubois & Léonil, 1989]. Typical of milk opiod peptides is the casomorphin-β-7 sequence (precursor: β-CN, fragment 60-66), beginning with tyrosine – YPFPGPI. The physiological role of β-casomorphin peptides has not been fully recognized yet. Children are a group of patients sensitive to milk foods, possibly due to the opioid peptides present in dairy products [Kostyra *et al.,* 2004]. Opioid activity was also detected in the following

fragments of caseins: 90-96 of bovine $\alpha s_1$-CN, 40-44 of human β-CN, *i.e*. casomorphin-β, 50-53 of human and bovine α-lactoalbumin, 102-105 of bovine β-lactoglobulin (lactorphin) [Chiba & Yoshikawa, 1986] and 399-404 of bovine blood serum albumin (serorphin).

The high content of proline in β-casomorphins favours their resistance to a number of proteolytic enzymes like pepsin, trypsin and chymotrypsin [Muehlenkamp & Warthesen, 1996].

Apart from their opioid activity, β-casomorphins play a favourable role in the digestive tract. They extend the time of food transit through the digestive tract as well as prevent from diarrhoea. It is possible *via* the stimulation of water and electrolytes absorption in the small intestine. Casomorphins may also affect the absorption of food components and insuline secretion. Casomorphin agonist opioid peptides were isolated from the κ-casein hydrolysates. Such peptides are called casoxins and are involved in blocking specific neuroreceptors for casomorphins [Stepaniak *et al.*, 1996].

Another peptide with the sequence Tyr-Pro-Phe-Pro-$CONH_2$ is called a morphiceptin. It is the amide of a fragment of the milk protein beta-casein and shows morphinelike activity. This peptide is highly specific for morphine $\mu$ receptors but not for enkephalin δ receptors. It was proved that non-enyzmatic glycation (pH 7.8 and temp. of 37°C) of morphiceptin can take place in the digestive tract and change the immunogenicity of this peptide due to a new epitope produced [Krawczuk *et al.*, 2000].

The most well-known precursor of exorphins excluding the casein, is wheat gluten. The examples of wheat gluten exorphins are GYYPT, YGGW, YPISL. Exorphins show affinity with δ and $\mu$ receptors and lower the tension of guinea pig small intestine *in vitro*. Similar activity was observed in non-separated gluten hydrolysates. Exorphins differ between another in terms of amino acid sequence, but they contain mainly hydrophobic amino acid residues [Fukudome & Yoshikawa, 1992, 1997].

The bovine haemoglobin is also a source of opioid peptides. Peptides derived from this protein are called hemorphins. They are formed in the enzymatic hydrolysis of β-, κ-, δ- or ε-chain of hemoglobin. Examples of hemorphins are: VVYPWTQRF (VV-hemorphin-7), LVVYPWTQRF (LLV-hemorphin-7) [Nyberg *et al.*, 1997]. Identical fragments occur in human hemoglobin and can be classified as endogenous opioid peptides (endorphins) [Zhao *et al.*, 1997].

Apart from variety functions of opioid peptides, Scopsi and co-workers [1989] discovered that release of endogenous opioids has been found to stimulate the growth of experimental breast cancers and opiate receptor blockers have reduced the growth of chemically-induced rat breast tumors. Out of 61 premenopausal women analysed immunocytochemically for the presence of opioid peptide immunoreactivity, 34 of them (56%) were positively identified of the tumors. Opioid peptides may therefore play a role in human breast cancer.

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