BIOLOGICALLY ACTIVE PEPTIDES OF MEAT AND MEAT PRODUCT PROTEINS: A REVIEW

PART 2. FUNCTIONALITY OF MEAT BIOACTIVE PEPTIDES

Keywords: biologically active peptides, meat proteins, the functionality, ACE-I, antioxidant activity, antithrombotic activity, antimicrobial activity, anticancer effect, opioid effect, bioavailability

Abstract

Biologically active peptides (BAP) are regarded as the main products of protein hydrolysis. BAP activity depends on the amino acid sequence molecular weight and chain length, type and charge of an amino acid at the N-terminus and C-terminus, hydrophobic and hydrophilic properties, spatial structure. They positively influence many systems of the human body, including the blood circulatory, nervous, immune, gastrointestinal and other systems. The health-improving effect of bioactive peptides is formed due to their antioxidant, antihypertensive, antithrombotic, immunomodulatory, antimicrobial, anti-allergic, opioid, anti-inflammatory, hypcholesterolemic and anticancer properties. Angiotensin-I-converting enzyme (ACE) inhibitory peptides are most studied due to their effect on blood pressure regulation. Unlike synthetic preparations, biologically active peptides do not have side effects and, therefore, can be used as their alternative. There is a growing commercial interest in peptides generated from meat proteins in the context of health saving functional foods. The paper describes prospects, pros and cons of using bioactive peptides as functional food ingredients and biologically active food additives.

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Introduction

Native amino acid sequences and peptides generated during meat autolysis or enzymolysis can be functionally active. Bioactive peptides (BAPs) can be generated during thermal or other technological processing. Bioactive peptides with the antioxidant, antithrombotic, antimicrobial, hypotensive, immunomodulatory, opioid and other biological effects, which have curative or preventive impact on pathogenesis of several diseases, have been discovered and studied [1,2,3,4].

A significant number of peptides with different activities have already been isolated from meat raw materials. These peptides contain 2 to 9 (to 25 sometimes) hydrophobic amino acid residues in a strict sequence [5]. Many of them were obtained by both in vivo and in vitro proteolysis with enzymes of different origin [6].

At present, the interaction between the activity and structure of peptides has been studied. The peptide activity depends on the amino acid sequence, molecular weight and chain length, type and charge of an amino acid at the N-terminus and C-terminus, hydrophobic and hydrophilic properties, spatial structure, etc. Peptides with higher ACE inhibitory activity usually have aromatic or basic amino acids at the N-terminus, more hydrophobic and positively charged amino acids at the C-terminus [7].

Many natural BAPs structurally differ from those formed as a result of the protein post-translational modification. They contains non-protein amino acids (β-alanin, γ-aminobutyric acid), D-amino acids, alkylated amino acids. The H-peptide bonds and cyclic structures are the characteristic of low molecular weight peptides. Along with residues of pyroglutamic acid, they provide protection from proteases with substrate specificity to peptides from α-amino acids with normal bonds, which allows retention of peptide functionality up to the moment of their absorption [1,8].

Main part

1. Angiotensin-I-converting enzyme (ACE) inhibitory peptides

Angiotensin-I-converting enzyme (ACE) inhibitory peptides are the most studied meat BAPs, probably due to their effect on blood pressure regulation. ACE-I is an enzyme dipeptidyl carboxypeptidase, which converts angiotensin-I (decapetide) into angiotensin-II (octapeptide), which leads to constriction of arteries and, consequently, to an increase in blood pressure. In this connection, ACE inhibition can be linked with prophylaxis of cardiovascular diseases [9,10].

ACE-I inhibitory peptides are small peptides with 2 to 20 amino acids. Their function depends on a protein, conditions and a degree of hydrolysis, molecular weight and amino acid composition, as well as a position of amino acids in peptide sequences. ACE-I inhibitory peptides have hydrophobic amino acids and branched-chain amino acids in their structure [11,12].
Katayama et al. found two ACE-I peptides (KRQKYD, EKERERQ), which showed the hypotensive activity on rats. They were obtained by hydrolysis of pork with pepsin [13].

The other peptide with similar function, RMLGQTPTTK (44–52 position of troponin C) was also isolated by treatment of a porcine skeletal muscle with pepsin [14].

Using pepsin and pancreatin, 22 ACE-I peptides were isolated; peptide sequences KAPVA and PTPVP showed the highest antihypertensive effect [15].

Peptides with ACE inhibitory activity were found in the hydrolysate of a porcine skeletal muscle as a result of the complex digestion by eight enzymes — proteases. Peptides MNPPK and ITTNP showed the highest activity [16].

Peptides isolated from connective tissue were also identified as ACE inhibitors. Bioactivity of collagen peptides depend on the quantity of glycine and proline [17].

Properties with ACE-I effect were found in vitro and in vivo in peptides AKGANGAPIAGAPFGARPGS-GPQPGSGPP and PAGNPADGQPGAKANGAP isolated from a hydrolysate of bovine Achilles tendon collagen using bacterial collagenase. Both peptides showed the ACE-I activity in rats [18].

Peptides from beef tendons (GPRGF, SPLPPPE, EG-PQGPPPGVG and PGLIGARGPPGP), which showed high ACE- and renin-inhibitory activities, were also identified [19,20].

Peptides with the in vivo ACE-I activity were isolated from chicken collagen treated with protease from Aspergillus oryzae [21].

Peptides from protein hydrolysate of a mixture of chicken combs and wattles were obtained by enzymatic hydrolysis. The protein hydrolysate showed the anticoagulant capacity and high ACE-inhibitory activity. The peptides were identified by LC–MS sequencing. From the pool of sequenced peptides, three peptides were selected and synthesized based on their low molecular weight and the presence of amino acids with ACE-inhibitory potential at the C-terminus: peptide I (APGLPGPR), peptide II (Piro-GPPGPT), and peptide III (FPGPPGP). Peptide III demonstrated the highest ACE-inhibitory capacity [22].

ACE-inhibitory peptides were found in hemoglobin. For example, peptides LGFPTTKYFPHF and VVYPWT, which corresponded to the 34–46 fragment of the α-chain and the 34–39 fragment of the β-chain of porcine hemoglobin, were obtained by hydrolysis of pork blood with pepsin [23].

Over the last several years, many studies have been focused on antioxidant peptides obtained from fish, while studies on antioxidant peptides from hydrolysates of farm animal’s muscles are limited [25].

Two peptides that inhibited linoleic acid autoxidation in the model system were identified in the extract of chicken essence extract. The first peptide HVTEE with the induction period of 2.49 days, the second PVPVEGV had the induction period of 6.5 days [26].

The hydrolysate with the potent antioxidant activity was obtained after cleavage of venison with papain. Two peptides that inactivate hydroxyl, DPPH, superoxide, and peroxyl radicals were identified: APVPH I (MQIFVKTLTG) and APVPH II (DLSDGEGQGVL) [27].

Several peptides isolated from meat have the antioxidant activity due to their ability to inhibit lipid peroxidation, chelate metal ions, inactivate free radicals and reactive oxygen species [28,29].

The most important antioxidants natively existing in meat are dipeptides carnosine and anserine, which show their antioxidant activity chelating pro-oxidative metals [30].

Also, peptides with the antioxidant activity can be generated by hydrolysis of meat raw materials with certain proteases.

Saiga et al. [30] in studies in vitro of porcine myofibrillar proteins hydrolyzed with papain and actinase E, found five peptides (DSGVT, IEAEGE, EELDNALN, VPSIDDQEELM and DAQEKE), which showed antioxidant activity using the in the linolenic acid peroxidation system.

Three peptides (ALTA, SLTA and VT) obtained from actomyosin of porcine skeletal muscles actomyosin showed the antioxidant activity in rats not only in vitro but also in vivo in rats [31].

Li et al. [32] isolated four antioxidant peptides (QGAR, LQGM, LQGMH and LC) from pork collagen using animal, plant and microbial enzymes (pepsin, papain, protease from bovine pancreas and bacterial proteases from Streptomyces spp. and Bacillus polymyxa) in different combinations.

Banerjee and Shanthi [18] isolated a peptide from bovine tendon collagen that consisted of contains 36 amino acid residues. Peptide was able to inactivate free radicals and chelate metals, from bovine tendon collagen.

Peptides with the antioxidant activity can be found in finished meat products. For example, 27 antioxidant peptides from Spanish dry-cured ham were sequenced using LC–MS/MS [33]. The highest activity was revealed in peptides SAGPNP and GLAGA.

Broncano et al. [34] isolated two peptides FGG and DM with the antioxidant activity from pork «chorizo» sausages.

Xing et al. [35] described several antioxidant peptides from dry-cured Xuanwei ham. Peptide DLEE was with the highest antioxidant activity.

3. Peptides with antithrombotic properties

Peptides that inhibit aggregation of blood platelets are recommended as diet components facilitating prophylaxis of thrombosis, which often occurs in patients with
ischemic heart disease or other diseases of the blood circulatory system. One of the stages of platelet aggregation is non-covalent binding of the α- and β-fibrin chains (fibrin polymerization). The N-terminal fragment with the sequence GRP of the α-chain is the key factor of fibrin polymerization [36].

Luzak et al. [37] found that DGEA (Asp-Gly-Glu-Ala) sequence is type I collagen recognition motif, which significantly inhibited blood platelets adhesion, aggregation and release reaction by collagen. In particular, Shimizu et al. [39] examined antithrombotic activity both in vitro by the platelet function test using porcine muscle longissimus dorsi hydrolyzed with papain. In rat blood and in vivo by oral administration to mice at a dose of 70 mg/kg body weight. The in vivo results showed that meat the peptide significantly reduced carotid artery thrombosis and the platelet activity and the effect is comparable to aspirin treatment at a dose of 50 mg/kg body weight.

4. Hypocholesterolemic peptide

Increased cholesterol concentrations in plasma are linked with an increased risk of cardiovascular diseases. Cholesterol enhances lipid peroxidation, protein oxidation and generation of free radicals (H₂O₂), disrupts the antioxidant system (SOD, CAT, GPx and GSH), as well as the activity of ATPase and causes histopathological disorders. Bioactive peptides can be regarded as an alternative for prophylaxis or treatment of these disorders. Peptides identified as hypocholesterolemic include lactostatin (IIAEK), enterostatin (VPDPR), peptides DPR, LPYP and LPLPR [40]. It is assumed that peptides of this type have different mechanisms of action and can increase excretion of bile acids with feces, bind with phospholipids and exert the activity of the cholesterol level reduction in human serum.

Nagaoka et al. hydrolyzed bovine β-lactoglobuline with porcine trypsin to obtain peptide IIAEK [41]. This peptide had the highest hypocholesterolemic effect in rats compared to β-sitosterol, a plant microelement with the chemical nature similar to the cholesterol nature and well known for its therapeutic potential, for example, the antioxidant, anti-diabetic, anticancer, antihyperlipidemic, immunomodulatory and other effects [42].

When studying the protein profile of raw smoked sausages produced with starter cultures, including those that were capable to reduce the cholesterol level in the culture medium and finished meat products, a change in protein fractions and presumptive formation of BAPs were shown. A wide range of peaks of peptide masses that had certain differences was obtained by MS. Analysis of rat blood serum lipids revealed the highest decrease in the cholesterol concentration in the group with starter culture the composition from the collection of MGUPP (Lactobacillus sakei 104, Pediococcus pentosaceus 28 and Staphylococcus carnosus 108) compared to the control, mainly, due to a 3 fold decrease in LDL cholesterol (P<0.05) [43,44,45].

5. Peptides with antitumor activity

It is known that several peptides can also show the antitumor activity, inhibit cell proliferation and exert the cytotoxic action on cancer cells [46]. Jang et al. [47] studied the activity of four peptides isolated from beef sarcoplasmic proteins regarding breast, stomach and lung adenocarcinoma cells. The results showed that peptide GFHI had the highest cytotoxic effect on breast cancer cells and reduced viability of stomach adenocarcinoma cells. Peptide GLSDGEWQ showed the inhibitory effect on proliferation of stomach adenocarcinoma cells.

Yu et al. antitumor effect analyzed bioactive peptide-3 (ACBP-3) isolated from goat liver antitumor effect on gastric cancer stem cells (GCSCs) in vitro and in vivo. ACBP-3 decreased the percentage of CD44(+) cells, suppressed the proliferation of the SC (spheroid colonies) cells and inhibited their clone-forming capacity in a dose dependent manner. Tumor formation from GCSCs occurred substantially longer when the cells were treated with ACBP-3 in vivo [48].

6. Peptides with antimicrobial activity

Biologically active peptides with the antimicrobial properties were identified in microorganisms, animals and plants. Antimicrobial peptides of animal origin show the inhibitory activity towards much larger spectrum of microorganisms than those produced by bacteria, while the latter demonstrate higher effectiveness at extremely low concentrations even of the nano level. However, the antimicrobial peptides have certain common properties. A majority of antimicrobial peptides have 50 amino acids and less; about 50% of them are hydrophobic amino acids and often make amphipathic 3-D structures [49,50].

Several peptides with the antimicrobial activity were isolated from beef blood; however, only one study showed the presence of antimicrobial peptides obtained from meat [47]. In this study, Jang et al. [47] isolated four peptides (GLSDGEWQ, GFHI, DFHING and FHG) after hydrolysis of beef sarcoplasmic proteins with commercial enzymes. All peptides were then tested on the antimicrobial activity against six pathogens (Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus, Bacillus cereus and Listeria monocytogenes). The results showed different antimicrobial effect on one or several bacteria. All peptides were active against P. aeruginosa. Peptide GLSDGEWQ demonstrated the inhibitory action on E. coli, S. typhimurium, B. cereus and L. monocytogenes.

Peptides of the hemoglobin α-chain show the antimicrobial activity after treatment with pepsin. Adje et al. [51] described such action of peptides TKAVEHLLDLGALSELDLHAKHKLVDPVNFKLLHSL and LDDLPGALSELSDLHAKHKLVDPVNFKLLHSL of the 67–106 fragment of the hemoglobin α-chain regarding Kocuria luteus, Listeria
innocua, Escherichia coli and Staphylococcus aureus. Daoud et al. described the action of peptide VTLASHLPSPDT-PAVHASLDKFLANVSTVL obtained in the similar way against Micrococcus luteus A270, Listeria innocua, Enterococcus faecalis, Bacillus cereus, Staphylococcus saprophyticus and Staphylococcus simulans [52].

Catiau et al. determined a minimal number of amino acid residues in a peptide that showed the antimicrobial properties and was isolated from the hemoglobin α-chain. It was tripeptide KYR. The experiments demonstrated the inhibitory activity both against gram-negative microorganisms Escherichia coli, Salmonella enteritidis, and gram-positive ones — Listeria innocua, Micrococcus luteus, Staphylococcus aureus [53].

7. Opioid peptides

Opioid peptides received their name due to their ability to interact with opioid receptors and impact on the nervous system of the body [54]. They can reduce pain similar to opiates and affect an emotional condition. Opioid peptides are endogenous ligands of opiate receptors. There are three main classes of endogenous peptides. Enkephalins are neuropeptides with the morphine-like effect. Leucine-enkephalin H$_2$N-Tyr-Gly-Gly-Phe-Leu-COOH (with the molecular weight of 556) and methionine-enkephalin H$_2$N-Tyr-Gly-Gly-Phe-Met-COOH (with the molecular weight of 574) were discovered in 1975. Dynorphins are peptides that contain a high proportion (almost one third) of basic amino acid residues, in particular lysine and arginine. Dynorphins have up to 41% of hydrophobic residues [55]. The main action of dynorphins is associated with the activation of κ-opioid receptors. The dynorphin analgesic effect is six times higher than that of morphine [56]. Endorphins block transmission of pain impulses. The structural formula of endorphin is NH$_2$-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Ser-Gln-Thr-Pro-Leu-Lys-Lys-Gln-COOH (with the molecular weight of 556) and methionine-enkephalin H$_2$N-Tyr-Gly-Gly-Phe-Met-COOH (with the molecular weight of 574) were discovered in 1975. Dynorphins are peptides that contain a high proportion (almost one third) of basic amino acid residues, in particular lysine and arginine. Dynorphins are peptides with alterations in their native structure and can become a source of opioid peptides [58].

A method for quantitative assessment of opioid peptides dynorphin AI–8 and [Met$^5$]-enkephalin-Arg$^6$-Gly$^7$-Leu$^8$ (MERGL) was described. The quantity of prodynorphin and dynorphin AI–8 increased with the beginning of the inflammatory process in the spinal cord and did not reduce during the inflammation resulted from administration as the inflammatory agent, carrageenan. The quantity of the enkephalin precursor also increased, more as a result of nerve injury rather than as a response to inflammation [59].

An opioid effect of fragments 143AYFYPEL149 and 144YFYPEL149 of alpha S1 casein, which stimulated gene MUC5A expression, was revealed in the samples of ileum in the experiments on guinea pigs [60]. It was found that several food-derived peptides influence rodent behavior. These peptides can be formed in the process of food digestion and can affect opioid receptors and cross the gastrointestinal and blood-brain barriers without their injury. An increased consumption of peptides from wheat gluten (gliadorphin-7) and milk casein (β-casomorphins) can be a factor linked to the development or maintenance of schizophrenia symptoms. Also, peptides derived from spinach (rubiscolins) and soy (soymorphins) influenced spontaneous behavior and memory [61].

8. Bioavailability of bioactive peptides

For bioavailability assessment it is necessary to determine whether the BAP can reach its target site in an active form and sufficient amount to affect health. The action of gastrointestinal enzymes such as intestinal absorption, cellular uptake and action of blood plasma peptidases, can modify the peptide structure or hydrolyze them leading to losses, retention or an increase of bioavailability [62,63,64].

Small peptides are more resistant to destruction by intestinal enzymes and more easily absorbed [65]. Ohara et al. [66] found small peptides obtained from collagen in blood after oral administration of protein hydrolysate products.

On the other hand, cell models, such as heterogeneous monolayers of human epithelial colorectal adenocarcinoma cells (Caco-2 cells) are effective to study transepithelial transport mechanisms of peptides [67,68,69].

Shimizu et al. [70] reported that chicken collagen octapeptide GAXGLXGP can be transported through the human intestinal epithelium. Fu et al. [20] identified two peptides (VGPV and GPRGF) obtained from bovine collage and showed their ACE-I inhibitory activity on human intestinal epithelial Caco-2 cells highlighting bioavailability of these peptides.

An ability of peptides to withstand enzymatic splitting and be transported through intestinal cell membranes into the bloodstream depends on their characteristics, length and amino acid composition. Proline-rich peptides are more resistant to gastrointestinal enzymes. Di- and tripeptides can be absorbed intact by the peptide transport systems and hydrolyzed later [65]. The low transport ability of oligopeptides compared to di- and tripeptides is probably because of their length and may involves the paracellular route, while the hydrophobicity of peptides apparently does not influence the absorption [71].

In addition, the absorption of peptides can be influenced by co-existing peptides and food components, which can share the transport pathway or take part it its regulation [71].

Bioavailability of BAP can also be influenced by the conditions of their processing/storage and food matrix — peptide interactions, which can lead to the modification of peptides with alterations in their native structure and activity [72].
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