PORCINE MYOFIBRILLAR PROTEINS AS A SOURCE OF BIOACTIVE PEPTIDES - AN *IN SILICO* STUDY

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Abstract – This paper presents the results of a computer analysis (*in silico*) applied for the hydrolysis of selected sequences of porcine proteins to obtain biologically active peptides. The sequences of myofibrillar proteins were derived from the UniProtKB. For the *in silico* hydrolysis, digestives enzymes: pepsin, trypsin and chymotrypsin has been used. The *in silico* proteolysis of porcine myofibrillar proteins was carried out with the use of "Enzyme(s) action" tool in the BIOPEP database. Identified peptides were analyzed for their potential toxicity using the ToxinPred tool. It has been shown that the tested sequences of pig myofibrillar proteins are a potential source of a total of 253 peptides with the activities such as inhibiting enzyme, antioxidative, hypotensive, stimulating or regulating various body functions and antiamnestic. None of the identified peptides showed toxicity. The results indicate that pork myofibrillar proteins are a promising source of peptides with biological activity.

Key Words - BIOPEP database, functional food, meat proteins.

I. INTRODUCTION

In addition to meat-based bioactive substances studied so far, e.g. carnosine, anserine, L-carnitine, conjugated linoleic acid, glutathione, taurine and creatine, protein-derived bioactive peptides are another group of promising functional compounds of meat [1]. These peptides remain latent within the sequence of parent proteins until released by enzyme-catalyzed protein hydrolysis. This process can occur naturally within the gastrointestinal tract during normal metabolism of dietary proteins. The same happens during fermentation or ageing in food processing [2, 3]. Therefore, meat proteins have possible bioactivities beyond a nutritional source of amino acids alone [4].

Bioactive peptides usually contain 2-20 amino acid residues and depending on their inherent composition and sequence, may exert a number of different activities *in vivo*, affecting, e.g., the cardiovascular, endocrine, digestive, immune and nervous systems [5, 6]. To date, immune-modulatory, antimicrobial, antithrombotic, opioid agonistic, mineral binding, antioxidative, antihypertensive, hypocholesterolemic and other effects have been discovered in a range of foods [7, 8, 9]. While most of physiologically active peptides derived from animal sources are generated from milk, meat being a major source of high quality proteins, offer huge potential as a novel source of bioactive peptides [3, 5, 6].

The most widely studied meat protein-derived bioactive peptides are angiotensin I-converting enzyme (ACE) inhibitory peptides [10, 11]. These peptides have attracted much attention because of their ability to prevent hypertension, the risk factors for the development of cardiovascular diseases, one of the most common chronic lifestyle-realted diseses nowadays. ACE inhibitory peptides could be used as potent functional food additives and would constitute a natural and healthier alternative to hypertension drugs. Apart from the best-known antioxidants found in meat: carnosine (β-alanyl-L-histidine) and anserine (N-β-alanyl-1-methyl-L-histidine) [12], there are many antioxidant peptides isolated from meat sources recently reviewed by Di Bernardini *et al.* [13] and Lafarga *et al.* [14].

Peptides derived from meat proteins offer a promising approach to prevent, control and even treat lifestyle-related diseases through a regulated diet. However, information on bioactive peptides generated from meats is still limited. The first step is the identification of such peptides in the parent protein sequence. As *in vitro* determination of bioactive peptides is costly and time consuming, the bioinformatics analysis can be an effective tool for the evaluation of meat proteins as a source of peptides with biological activity. New solutions in the bioinformatics based on computer-aided methods (*in silico*) offer such possibilities.

The results obtained by an *in silico* method may partially correspond to the results of laboratory experiments [15].

II. MATERIALS AND METHODS

Eight sequences of porcine myofibrillar proteins were analyzed (Table 1). Proteins were selected based on their abundance in pork meat and the availability of sequence information for the proteins. The sequence and molecular weight for each protein were derived from the UniProt Knowledgebase (UniProtKB) [16]. For the analysis of amino acids composition constituting the protein sequences, the ProtParam tool has been used [17].

Table 1 List of porcine myofibrillar proteins available in the UniProtKB used in the *in silico* analysis

Protein	Amino acid chain length	Mass (Da)	Entry name/ Accession Number
Actin, alpha skeletal muscle	377	42,051	ACTS_PIG / P68137
Myosin-2	1939	223,150	MYH2_PIG / Q9TV63
Tropomyosin alpha-3 chain	284	33,058	TPM3_PIG / A1XQV4
Troponin C, skeletal muscle	159	18,025	TNNC2_PIG / P02587
Troponin T, fast skeletal muscle	271	32,176	TNNT3_PIG / Q75NG9
Troponin T, slow skeletal muscle	262	31,243	TNNT1_PIG / Q75ZZ6
Titin (fragment)	572	66,978	Q29117_PIG / Q29117
Nebulin	1691	192,330	Q3Y5G4_PIG / Q3Y5G4

The high potential of the selected protein sequences for releasing peptides were confirmed by determining the profile of their biological activity (see "Profiles of potential biological activity"). The value of selected porcine myofibrillar proteins as bioactive peptide precursors was evaluated based on the occurrence frequency of bioactive fragments in the protein chain (see "A, B, Y calculation") in BIOPEP database [18].

The selected protein sequences were simultaneously subjected to hydrolysis *in silico* by using digestive enzymes of the gastrointestinal (GI) tract: pepsin (EC 3.4.23.1) which is hydrolysing the bindings of selected aromatic amino acids, trypsin (EC 3.4.21.4) for which the preferential cleavage sites can be found after arginine or lysine and chymotrypsin (EC 3.4.21.1) which is cleaving the peptide bond formed by the carboxyl group of tyrosine, tryptophan, phenylalanine or leucine [16]. The *in silico* proteolysis of porcine myofibrillar proteins was carried out with the use of "Enzyme(s) action" tool in the BIOPEP database [18]. The peptides identified in this study were analyzed for their potential toxicity using the ToxinPred tool [19]. The SVM (support vector machine) based prediction method and SVM threshold value of 0.0 were selected for toxicity prediction [20].

III. RESULTS AND DISCUSSION

Eight sequences of porcine myofibrillar proteins were analyzed in order to determine their potential to generate biopeptides. The results of computer analysis of the profile of potential biological activity supported by information about the abundance of bioactive fragments (parameter A) in a protein sequence, confirmed the high potential of this type of pork meat proteins for bioactive peptides generation. For example, profile of potential biological activity of actin suggested that it is a potential source of up to 266 biopeptides, however due to *in silico* hydrolysis with selected enzymes (pepsin, trypsin, chymotrypsin) only 17 of these peptides can be released from the parent protein sequence [18]. This is probably due to the fact that the endogenous proteases selected for use in this study have specific cleavage sites, and can release only a limited number of peptides [14].Not all of the peptides released from the protein sequence will exhibit health benefits. The biological activity *in vitro* is not sufficient for confirming the physiological benefits for human health. The peptides provided by the ingestion must be resistant to degradation by endogenous enzymes. They should be also absorbed directly from the GI tract into the blood serum by enterocytes, and then they must reach the target site intact in order to exhibit the biological activity [21, 22]. To increase the likelihood of beneficial interactions of peptides *in vivo*, a combination of enzymes naturally occurring in mammals was used, thereby reducing the risk of degradation of the amino acid sequence during the transit in the gastrointestinal (GI) tract.

Table 1. Percentage of peptides of defined biological activity resulting from hydrolysis in silico [%]

Protein	Activity of peptides								
	ACE Inhibitor	DPP IV inhibitor	Antioxidative	Stimulating	Regulating	Inhibitor	Hypotensive	Antiamnestic	Total
Actin, alpha	52.94	29.41	11.77	5.88	0	0	0	0	100
Myosin-2	54.08	21.43	13.27	6.12	1.02	2.04	2.04	0	100
Tropomyosin alpha-3	40.00	20.00	30.00	10.00	0	0	0	0	100
Troponin C,	53. 85	15.37	11.54	11.54	0	3.85	3.85	0	100
Troponin T,	75.00	0	25.00	0	0	0	0	0	100
Titin (fragment)	62.50	18.75	0	6.25	0	6.25	6.25	0	100
Nebulin	52.44	24.39	13.41	3.66	2.44	1.22	1.22	1.22	100

As a result of an *in silico* analysis a total of 253 peptides with diverse biological activity were identified. Among them were distinguished those possessing: the inhibitory action of ACE (angiotensin converting enzyme inhibitors, ACE-I) - a total of 136 fragments, the inhibitory action of dipeptidyl peptidase IV enzyme (dipeptidyl peptidase IV inhibitors, DPP-IV) - 55 fragments, antioxidant - 33 fragments, stimulating various body functions (e.g. glucose uptake) - 15 fragments, regulating (e.g. the flow of ions) - 3 fragments, enzyme inhibitors other than ACE or DPP (e.g. CaMPDE inhibitor - calmodulin-dependent phosphodiesterase) - 5 fragments, hypotensive (e.g. renin inhibitor) - 5 fragments and antiamnestic - 1 peptide. Individual proteins differ from one another when it comes to quota of peptides of a specific activity that they consist of (Table 1). Thus for example, peptides possessing ACE-inhibitory activity accounted for more than half of the total number of peptides released by digestion of proteins (with the exception of tropomyosin). Biopeptides with cardioprotective properties are given most attention in the literature. In 2014, a number of 645 scientific papers devoted to angiotensin-converting enzyme inhibitory peptides were published [23]. All the sequences obtained were analyzed in view of their toxicity to the body. *In vivo* scientific data from toxicological studies of bioactive peptides is lacking [24]. Based on the obtained results, it was found that none of the identified peptides showed toxicity, and could potentially be used as functional ingredients in food. Among the identified peptides, mainly dipeptides (96.44%) were found, only 9 peptides were tri-amino acid residues. Many bioactive peptides have common structural features, including a relatively short amino acid chain length. According to Dziuba *et al*. [25], short sequences, mainly di- and tripeptides are often released from animal proteins.

The relationship between the amount of released peptides with biological activity and chain length of a protein they are derived from was observed. Most of the peptides were obtained by digestion of myosin and nebulin - proteins that have the largest number of amino acids in the sequence. It was also noted that more than one activity can be assigned to a given peptide sequence (e.g. a fragment EK which has inhibitory activity toward both ACE as well as DPP-IV). It was also true in the case of dipeptides IR and EF, which as a result of *in silico* analysis were deemed to have simultaneously inhibiting and hypotensive properties.

IV. CONCLUSION

In silico hydrolysis of studied sequences of porcine myofibrillar proteins revealed that under the influence of a combination of digestive enzymes, peptides potentially affecting human body may be released. The *in silico* research may be of great importance for the prediction of bioactive peptides from meat sources.

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