

Fragmentomics: a New Insight into Structures and Functions of the Natural Oligopeptide Diversity

ALEXANDER A. ZAMYATNIN^{1,2}

¹Computer Biochemistry Group

A.N.Bach Institute of Biochemistry, Russian Academy of Sciences

33 Leninsky prosp., Moscow 119071

RUSSIAN FEDERATION

aaz@inbi.ras.ru, <http://erop.inbi.ras.ru>

²Departamento de Informática

Universidad Técnica Federico Santa Maria

1680 av. España, Valparaiso V-110

CHILE

alexander.zamyatnin@usm.cl

Abstract: - More and more facts appear showing that there are oligopeptides in different organs and tissues of living organisms that are not formed from specialized precursors, but are rather natural fragments of well studied proteins. However, up to the present time practically no systemic structural and functional investigations of protein fragments were have been carried out. Therefore, we have carried out a theoretical structure-function analysis of all possible fragments of several meat and milk proteins in order to identify their primary structure regions that could serve as potential sources of regulatory oligopeptides and to reveal their possible previously unknown functions. The analysis was performed by comparing the primary structures of all possible bovine hemoglobin, cytochrome *b*, casein, α -lactalbumin, β -lactoglobulin, and lactoferrin fragments with the amino acid sequences of presently known natural regulatory oligopeptides. The oligopeptide structures were extracted from the EROP-Moscow database, contained data on the structures and functions of more than 9000 natural oligopeptides. The structure-function analysis revealed that a lot of different di-, tri-, and tetrapeptide protein fragments were precise copies of known regulatory oligopeptides obtained from members of all biological kingdoms. Functional analysis of these natural non-meat/milk oligopeptides has shown that they exhibited a broad spectrum of functional activities, such as neuropeptide, hormone, enzyme inhibitor, transporter, etc. The new terms “*fragmentomics*” and “*fragmentome*” were grounded and defined and practical applications of food protein fragments was considered on the basis of these results.

Key-Words: - Fragment, Fragmentomics, Fragmentome, Oligopeptide, Food Protein, Meat, Milk, Structure, Function, EROP-Moscow Database.

1 Introduction

For more than a century, natural oligopeptides have attracted scientific attention as important biochemical regulators. The first described oligopeptide, carnosine (β -ala-his), was discovered in Russia by V.S.Gulevitch and S.Amiradzhibi in 1900 [1] and its chemical structure was determined in 1918 [2]. Since that time, thousands oligopeptide regulators have been described, and now ~600 new natural oligopeptides emerge annually, out of a literature of >70 000 publications each year on oligopeptide/fragment chemistry and biology according to PubMed database (<http://www.ncbi.nlm.nih.gov>).

It is known now that relatively small naturally occurring regulatory oligopeptides generally do not exceed ~50 amino acid residues [3] and they differ substantially from larger polypeptides (proteins) in their physicochemical and biological properties.

These substances attract ever increasing attention owing to their key role in the functioning of various regulatory systems in the organism. Oligopeptides control various parts of the nervous (neuropeptides), immune (immunoregulators), and endocrine (hormones) systems and possess many other functions [4, 5]. It should be noted that the same oligopeptide structure is capable of participating in various types of regulatory processes [6, 7], which makes us consider them polypotential. The spectrum of their functional activities is diverse, but regulators of nervous and endocrine systems along

with antimicrobial (antibacterial, antifungal, and antiviral) oligopeptides make up the bulk of known oligopeptides.

Most of the information on the structure and function of endogenous molecules of the chemical peptide class at present contained in different databases formed mainly on the basis of amino acid (e.g., SwissProt/TrEMBL database, <http://ca.expasy.org>) and/or nucleotide sequences (e.g., GenBank database, <http://www.ncbi.nlm.nih.gov/Genbank>). There are a lot of other databases for different naturally occurring peptide structures and those specialized for certain structural, functional and other features.

2 Problem Formulation

Regulatory peptide molecules usually excised from specialized precursors [8]. But more and more facts appear showing that there are also oligopeptides in different organs and tissues of living organisms that are not formed from specialized precursors, but are rather natural fragments of common proteins. Functional role in an organism is not established for most of them. Studies on functions of some of such fragments and of those obtained by experimental proteolysis showed that they can serve as oligopeptide regulators. Thus, a number of fragments of meat proteins (bovine hemoglobin [9-15] and cytochrome *b* [16, 17]) as well as of milk proteins (casein [18-21], α -lactalbumin, β -lactoglobulin, and lactoferrin [22, 23]) are able to exhibit opioid activity, i.e. they are neuropeptides. It is also known that some hemoglobin [24-28] and lactoferrin [29-31] fragments also exhibit antimicrobial activity.

To date the largest number of fragments has been experimentally revealed for hemoglobin (144 fragments) and casein (84) and the functional properties have been determined for some of these fragments. In addition to already mentioned functions, these fragments can serve as hormones [32, 33], bradykinin-potentiating structures [34, 35], enzyme inhibitors [35], hemopoietic agents [15, 26] and other regulators [26, 35, 37]. Multipotency was found for some of them. Thus, hemorphin-9 (site 31-39 of bovine hemoglobin β -chain) exhibits properties of both neuropeptide and enzyme inhibitor [11]. However, up to the present time practically no systemic structural and functional investigations of protein fragments were have been performed.

Therefore, we have carried out a theoretical structure–function analysis of all possible fragments of several meat and milk proteins in order to

identify their primary structure regions that could serve as potential sources of regulatory oligopeptides and to reveal their possible functions not previously known for meat and milk oligopeptides.

3 Problem Solution

The analysis was performed by comparing the primary structures of all possible meat (hemoglobin, cytochrome *b*) and milk (casein, α -lactalbumin, β -lactoglobulin, and lactoferrin) protein fragments with the amino acid sequences of all presently known natural regulatory oligopeptides.

The oligopeptide structures were extracted from the EROP-Moscow (Endogenous Regulatory OligoPeptides) database (<http://erop.inbi.ras.ru>) [38, 39], which at the time of analysis contained data on the structures and functions of 9003 natural oligopeptide regulators.

A specialized software package was performed for the structure-function analysis [40, 41]. The input data were the complete amino acid sequences of the proteins used as a source of fragments with a specified length. Then the initial sequence was fragmented in a stepwise manner. For example, in the case of dipeptide fragments, this procedure produced fragments with the following numbers of amino acids from the N-terminus: 1-2, 2-3, and so on until the fragment that started at the second residue from the C-terminus. The cases when the amino acid sequence of a fragment coincided with part of the primary structure of a natural oligopeptide were recorded in the table of results, automatically formed by a program in the software package. At the final stage, the table of results was analyzed according to the specified characteristics. Then these data were processed using standard programs for sorting and choosing groups of molecules according to certain structural and functional characteristics.

It has been shown (see Table) that di-, tri-, and tetrapeptide protein fragments were precise copies of known regulatory oligopeptides obtained from members of all biological kingdoms (animals, plants, bacteria, and fungi).

Functional analysis of these natural non-hemoglobin/casein oligopeptides has shown that they exhibit a broad spectrum of functional activities, such as neuropeptide, hormone, enzyme inhibitor, transporter, etc.

Small oligopeptide molecules can perform the most studied functions. For example, natural molecules as small as dipeptides can display neuropeptide, antimicrobial, and hormonal

properties. In particular, the global peptidome contains now 11 dipeptide neuropeptides, 5 hormones, and 2 antimicrobial agents. Therefore, small food protein fragments are very likely to be active and display such activities. Taking into account this probability can suggest which particular testing type should be used for studying functions of not yet studied meat and milk protein fragments. In addition, this approach makes it possible to considerably reduce the search for various structures for minimal active site of regulatory molecules.

To act as regulators, protein fragments must be formed in a natural manner, have access to the objects of their action (targets, for example, receptors), and possess the ability to interact with them. However, no specialized studies have determined the complete oligopeptide composition of the gastrointestinal tract after consumption of food containing meat, milk, and other proteins. Nonetheless, it is evident that the proteins entering the body with food are naturally cleaved by proteolytic enzymes into exogenous oligopeptides. Meat and milk proteins are among these structures; they are degraded into numerous different fragments and are the potential source of different regulatory oligopeptides.

It is also unknown which particular targets in the gastrointestinal tract are accessible to individual regulatory oligopeptides and whether these substances are able to penetrate into other organs and tissues. However, it is evident that such targets are accessible to putative antimicrobial protein fragments, which contact the gastrointestinal microflora and can thus manifest their activity. Therefore, such fragments of food proteins can be involved in this process, which is a component of the immune regulation [42].

Thus, in addition to the endogenous oligopeptides, the natural protein fragmentation in the body forms a pool of exogenous oligopeptides. Note that small fragments can be produced in considerable amounts owing to the repetition of their amino acid sequences in the initial protein molecule.

Use of this approach earlier allowed us to demonstrate that fragments of the enzyme bromelain could also display the properties of regulatory oligopeptides [43]. This suggests that the fragments of a protein molecule belonging to different functional classes can be involved in oligopeptide regulation.

The data obtained show that meat and milk protein fragments can have one or more functions not specific to the original molecule and exhibit functional diversity. Natural fragmentation of these

proteins can result in formation in the organism of a dynamically developing pool of exogenous regulatory oligopeptides whose functions may change during formation of smaller peptide structures. This results in more or less gradual transitions of biological activity spectra providing for any admissible combinations of effects on the organism's functions. The existence of the endogenous/exogenous pool of regulatory molecules makes it possible to expand the sense and content of a hypothesis concerning the functionally continuous population (continuum) of natural oligopeptides [44].

4 Conclusion

A new term "fragmentomics" has recently appeared. It has been used to characterize the protein fragments that are potential biological markers of cancer diseases [45]. However, this definition of fragmentomics is rather limited, as there are numerous biological processes where fragments of larger molecules are involved. A natural fragmentation of proteins, nucleic acids, carbohydrates, and other natural molecules is well known. For example, the peptide molecules excised from specialized precursors are fragments. This is characteristic of both the oligopeptide regulators and large protein structures.

Fragmentation has long been used in experiments, for example, when determining protein primary structure (Edman degradation [46-48]), mass spectrometry [49-50] and in theoretical analysis, in particular, when detecting homologies in both nucleic acids [51-53] and proteins [54-56].

A possible definition for fragmentomics could be the research field that studies the structure and function of a set of molecular fragments constituting a fragmentome. Thus, in the context of peptide structures, fragmentomics is some interlink between proteomics (proteome) and peptidomics (peptidome).

Correspondingly, performing a computer analysis of the structures and functions of all possible protein fragments we studied the protein fragmentome, in the context of theoretical fragmentomics.

According to different databases the primary structures of several million proteins and over 9000 natural oligopeptides are known so far. Their sets are the global proteome and global peptidome, respectively. The number of possible fragments of these substances is tremendous; however, the examples of their structural and functional application are rather small. The number of

experimentally obtained fragments of even a single protein is only a small fraction of all its possible fragments (its fragmentome), and only some of them are assayed for their functional properties.

It is clear that our basic conclusion about the functional role of the fragments of food proteins can presently be considered only as a hypothesis. Much remains to be done in order to elucidate the details of biochemical [8] and biophysical [57] regulation of physiological processes with the participation of both endogenous and exogenous regulators. It is necessary to synthesize the food protein fragments found in the natural oligopeptides and to examine their expected activity. These experiments require additional costs, but these costs may be defensible owing to promising practical applications.

Enrichment of food products with preliminary prepared fragments of food (and, possibly, inedible) proteins is an example of such a practical application. Elaboration of the technology of such an enrichment and its manufacturing application should result in enhancement of the immune status of the consumer. In addition, fragments with antimicrobial effects can be considered as natural preservatives of food products. Their addition will increase the shelf life of these products without any dangerous side effects due to their natural origin. In particular, food proteins and, hence, their fragments are safe, nontoxic, and free from side effects.

Active natural fragments of proteins are promising, for new drug creation, dietology, hygiene, cosmetics, etc. For example, use of antimicrobial peptide structures as components of hygienic and cosmetic preparations could result in the creation of effective toothpastes and creams of a new generation...

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Table. Structures and functions of the natural oligopeptides identical to food protein fragments.

EROP №	Oligopeptide name	Function	Sequence *	HB	CH <i>b</i>	CS	α -LA	β -LG	LF	SUM
E01599	Bioactive peptide 2 (ground squirrel)	transporter	+AL-	6	3	3	2	4	3	21
E06287	Transporter DtpT 1 (<i>Listeria monocytogenes</i>)	transporter	+LA-	4	7	1	1	2	6	21
E02539	Serum amyloid A peptide 6 (human)	hormone, transporter	+LP-	2	3	8	1	1		15
E01831	Bitter peptide (soybean)	taste, enzyme inhibitor	+LF-		6	1	1	1	5	14
E01337	ACEI 7 (Japanese sardine) **	enzyme inhibitor	+YL-		1	6		1	3	11
E04097	ACEI 1 (Tatarian buckwheat)	enzyme inhibitor	+VK-	4		3			3	10
E01800	Bradykinin [6-7] (bovine)	neuropeptide	+SP-		3	3			4	10
E01003	ACEI 5 (snake: jararaca)	enzyme inhibitor	JQK-			9				9
E04099	ACEI 6 (Tatarian buckwheat)	enzyme inhibitor	+YQ-			8				8
E00596	Peptide DE (rat)	neuropeptide	+DE-	1		2		1	4	8
E04069	ACEI 4 (wheat)	enzyme inhibitor	+AF-	1	2	1			3	7
E01336	ACEI 6 (Japanese sardine)	enzyme inhibitor	+LY-		4	2			1	7
E00285	Hypothalamic peptide IV (pig)	hormone	+YF-	1	2	1			3	7
E01334	ACEI 4 (Japanese sardine)	enzyme inhibitor	+RY-		1	4			1	6
E01598	Bioactive peptide 1 (ground squirrel)	neuropeptide	+FK-	2				1	3	6
E01333	ACEI 3 (Japanese sardine)	enzyme inhibitor	+MF-	1	2	1	1			5
E00244	TRH [2-3] (pig) ***	hormone	+HP-		1	4				5
E04068	ACEI 1 (wheat)	enzyme inhibitor	+TF-	1	2	1				4
E01331	ACEI 1 (Japanese sardine)	enzyme inhibitor	+VF-			2		1	1	4
E00823	TRH-related peptide (human)	hormone	JEPz			2			2	4
E04098	ACEI 3 (Tatarian buckwheat)	enzyme inhibitor	+AY-		1	2				3
E06385	ACEI WL (soybean)	enzyme inhibitor	+WL-		1		2			3
E01801	Bradykinin [8-9] (bovine)	neuropeptide	+FR-	1	1				1	3
E02182	APGWamide related peptide (common cuttlefish)	neuropeptide	+GWz		1				2	3
E02335	Regulating peptide Hym311 (hydra)	development regulator	+FW-	1	1					2
E04540	ACEI (human)	enzyme inhibitor	+AW-	1		1				2
E07046	ACEI IPP (bovine)	enzyme inhibitor	+IPP-			2				2
E01434	ACEI 3.1 (bonito)	enzyme inhibitor	+IW-		1				1	2
E01332	ACEI 2 (Japanese sardine)	enzyme inhibitor	+KW-		1			1		2
E06533	ACEI 6 (oriental sesame)	enzyme inhibitor	+LSA-	1	1					2
E01600	Bioactive peptide 3 (ground squirrel)	neuropeptide	+DY-				1	1		2
E07052	Antihypertensive peptide 2 (<i>Chlorella vulgaris</i>)	neuropeptide	+FAL-			1			1	2
E07055	Antihypertensive peptide 1 (<i>Spirulina platensis</i>)	neuropeptide	+IAE-					1	1	2
E07057	Antihypertensive peptide 2 (<i>Spirulina platensis</i>)	neuropeptide	+VAF-						2	2
E01340	ACEI 11 (Japanese sardine)	enzyme inhibitor	+AKK-				1			1
E01341	ACEI 12 (Japanese sardine)	enzyme inhibitor	+RVY-					1		1
E05453	ACEI (edible mushroom)	enzyme inhibitor	+GEP-			1				1
E01431	ACEI 1.1 (bonito)	enzyme inhibitor	+IKP-		1					1
E01438	ACEI 6.1 (bonito)	enzyme inhibitor	+LKP-					1		1
E01439	ACEI 7 (bonito)	enzyme inhibitor	+IY-						1	1
E04101	ACEI 8 (Tatarian buckwheat)	enzyme inhibitor	+PSY-			1				1
E04102	ACEI 9 (Tatarian buckwheat)	enzyme inhibitor	+LGI-		1					1
E04103	ACEI 10 (Tatarian buckwheat)	enzyme inhibitor	+ITF-		1					1
E06530	ACEI 3 (oriental sesame)	enzyme inhibitor	+LVY-			1				1
E06531	ACEI 4 (oriental sesame)	enzyme inhibitor	+LQP-			1				1
E07045	ACEI VPP (bovine)	enzyme inhibitor	+VPP-						1	1
E07062	ACEI m (marine shrimp)	enzyme inhibitor	+YLLP-					1		1
E06239	Gonadin Q (rat)	hormone	+EQPz			1				1
E02538	Serum amyloid A peptide 5 (human)	hormone	+GLP-			1				1
E00284	Hypothalamic peptide III (pig)	hormone, enzyme inhibitor	+VW-						1	1
E07050	Antihypertensive peptide (pig)	neuropeptide	+PPK-			1				1
E07051	Antihypertensive peptide 1 (<i>Chlorella vulgaris</i>)	neuropeptide	+AFL-			1				1
E06395	Antihypertensive peptide (human)	neuropeptide	+ALPM-					1		1
E04738	Neuropeptide-like peptide NLP-32-4 (nematode)	neuropeptide	+GYGG-				1			1
E00983	ECUM inhibitory tripeptide (human)	neuropeptide	+HGK-						1	1
E00798	Nuropeptide II (yellow fever mosquito)	neuropeptide	+TRFz		1					1
E06288	Transporter DtpT 2 (<i>Listeria monocytogenes</i>)	transporter	+LGG-		1					1
Total:		6 functional classes	58 different sequences	26	50	77	11	19	54	237

* Standard one-letter code is used. Additional abbreviations: J – pyroglutamic residue, “+” – N-terminal (+NH₃– group), “-” – C-terminal (–COO⁻ group) and “z” – amide (–NH₂). ** ACEI – angiotensin-converting enzyme inhibitor; *** TRH – thyrotropin-releasing hormone (thyreoliberin); HB – hemoglobin, CH *b* – cytochrome *b*, CS – casein, α -LA – α -lactalbumin, β -LG – β -lactoglobulin, and LF – lactoferrin.