QUANTITATIVE ASSESSMENT OF PROTEOLYTIC ENZYME HOMOLOGY *HOMO SAPIENS* AND *BIOMPHALARIA GLABRATA* USING A RESOURCE BLAST

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It is now considered established that proteolytic enzymes and their inhibitors are conservative. These systems originated in eukaryotic cells using preliminary preparations of prokaryotic cells.

Proteases play a diverse role in regulating the biology of virtually all organisms. In fact, they make up about 5% of all genes in any given genome. Before protease had a molecular unit removal "garbage", which just degraded proteins are used to maintain general homeostasis. However, detailed studies have shown that the functions of proteolytic enzymes are much more complex because they play key roles as regulators of important cellular processes such as cell signaling, cell division, cell death, and metabolism. Extracellular proteins and some cell surface proteins are absorbed by endocytosis and degraded in lysosomes. Some cytosolic proteins are degraded in the lysosomes after uptake in autophagic vesicles that fuse with lysosomes. Chaperone-dependent autophagy is also known, in which the directed transport of partially denatured proteins from the cytoplasm through the lysosome membrane into its cavity occurs. However, in all tissues of living organisms, most intracellular proteins are degraded by ubiquitin (ubiquitin, Ub)-proteasome pathway (UPP). This adjustable type of proteolysis plays the most important role in cell signaling [1].

The relevance of the study of the degree of homology of proteolytic enzymes in different types of organisms is associated with the search for tissues and cells that can be sources of these enzymes for practical needs (biopharmaceuticals, cosmetics, food).

The aim of the research is a comparative analysis of the degree of homology of proteolytic enzymes of regulated and unregulated proteolysis in humans and pulmonary freshwater mollusks. As a possible source for obtaining proteolytic enzymes, mollusks widely distributed in the reservoirs of Europe – *Planorbarius corneus*, the closest relative of which is a well-studied *Biomphalaria glabrata*, are chosen [2].

Material and method. The paper uses bioinformatic approaches based on the use of different servers: search and selection of nucleotide sequences encoding human proteins was carried out on the server https://www.ensembl.org; the search for homologous sequences for molluscs was carried out on the server https://www.ncbi.nlm.nih.gov using the resource BLAST.

The following enzymes of unregulated proteolysis were used for the analysis: Prolyl oligopeptidase (EC 3.4.21.26); Furin (EC 3.4.21.75); Signal Peptide Peptidase; Aminopeptidase B (EC 3.4.11.2); Leucyl aminopeptidases (EC 3.4.11.1); Thimet oligopeptidases (EC 3.4.24.15); and regulated proteolysis (ubiquitin-proteasome pathway): Ubiquitin conjugating factor E4 B-like (EC 6.3.2.19); Ubiquitin conjugating factor E2 W-like; Ubiquitin carboxyl-terminal hydrolase L5; Ubiquitin-like modifier-activating enzyme 5. For comparison, two enzymes of purine metabolism important for the synthesis of nucleotides are taken: Amidophosphoribosyl transferase (EC 2.4.2.14); Adenylosuccinate lyase (EC 4.3.2.2).

Findings and their discussion. As a result of the work done, the following indicators have been established: Expected value (the accuracy of the alignment), Identities (the percentage of matching amino acids or nucleotides; the similarity percentage), Query coverage (what percentage of the length of the original sequence is aligned with the found sequence). Table 1 presents the established indicators of the enzymes of the mollusk *Biomphalaria glabrata* in comparison with human enzymes.

Table 1. Parameters of enzymes mollusks *Biomphalaria glabrata*.

Enzyme	Type of sequence	Expected value	Identities	Query coverage
Unregulated proteolysis				
Prolyl oligopeptidase	NS[1]	2e-88	66 %	61 %
	AAS[2]	0.0	62 %	99 %
Furin	NS	1e-105	69 %	22 %
	AAS	0.0	68 %	79 %
Signal Peptide Peptidase	NS	8e-71	67 %	23 %
	AAS	7e-177	68 %	94 %
Amino-peptidase B	NS	1e-66	66 %	38 %
	AAS	0.0	50 %	89 %
Leucyl aminopeptidases	NS	9e-48	66 %	35 %
	AAS	0.0	55 %	94 %
Thimet oligopeptidases	NS	9e-52	66 %	16 %
	AAS	0.0	63 %	94 %
Regulated proteolysis				
Ubiquitin conjugating factor E4 B-like	NS	7e-33	72 %	4 %
	AAS	0.0	49 %	71 %
Ubiquitin conjugation factor E2 W-like	NS	2e-57	75 %	14 %
	AAS	1e-87	74 %	99 %

Ubiquitin conjugation factor E2 E1	NS	3e-66	74 %	24 %
	AAS	2e-84	88 %	65 %
Ubiquitin carboxyl-terminal hydrolase L5	NS	3e-89	72 %	12 %
	AAS	4e-162	67 %	96 %
Ubiquitin-like modifier-activating enzyme 5	NS	2e-88	76 %	17 %
	AAS	2e-151	59 %	99 %
E3 ubiquitin ligase	NS	3e-91	69 %	31 %
	AAS	0.0	51 %	85 %
Purine exchange				
Amidophos-phoribosyl-transferase	NS	1e-61	68 %	60 %
	AAS	0.0	67 %	97 %
Adenylosuc-cinate lyase	NS	7e-19	64 %	20 %
	AAS	2e-139	60 %	84 %

[1] – nucleotide sequence; [2] – amino acid sequence.

From the analysis of table 1 it follows that the percentage of similarity of nucleotide sequences of unregulated proteolysis is in the range of 66–69 %, and regulated proteolysis – 69–76 %. The percentage of similarity of amino acid sequences for both types of proteolysis has lower values (except for a few cases). The percentage of coverage for amino acid sequences is greater than for nucleotide sequences, because they have a shorter length in comparison with nucleotide sequences of enzymes and do not contain introns. The high degree of homology of the enzymes of the ubiquitin-proteasomal pathway in human and mollusc associated with the formation of the close of the tertiary structures of proteins.

Conclusion. The homology of enzymes unregulated proteolysis in humans and pulmonary freshwater mollusks is in the range of 66–69%, and the ubiquitin-proteasomal path – 69–76%.

The practical importance of a high degree of homology of proteolytic enzymes in humans and freshwater pulmonary mollusks justifies the formation of aquaculture of molluscs, for obtaining from their tissues protein enzyme preparations of proteolytic action within the tasks of biopharmaceutics, cosmetics and food industry.

Reference list:

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