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DERIVATION OF TRYPsin-LIKE ENZYMES FROM ANTARCTIC MARINE ORGANISMS

Modern biotechnology provides continuous search for alternative sources of raw materials. The aim of this work was to isolate and characterize trypsin-like enzymes from tissues of Antarctic marine aquatic organisms (krill, starfish, nemertines). Trypsin fraction was obtained by column chromatography on benzamidine-Sepharose. Proteolytic activity in the resulting fraction was revealed. Analysis of protein fractions was performed by disc-electrophoresis in a 10% polyacrylamide gel. The presence of active hydrolyses with different molecular weights in the tissues of aquatic organisms was demonstrated. Protein bands with molecular weights below 10–14 kDa may be trypsin-like enzyme fragments that were subjected to autolysis process.

Key words: marine organisms, trypsin-like enzymes.

Introduction. Most modern biotechnology developments are focused on finding a variety of alternative sources of raw materials for the production of biologically active molecules with directed action, including marine and aquatic organisms [1]. The development of methods for the preparation of biologically active substances in order to use them to create original effective pharmacological agents with the most promising and important properties for academic research is the main problem of biotechnology. Decisive factor that motivates...
the need to find the target molecules of marine aquatic metabolites is the constantly rising price of new pharmacological agents [2], which are based on highly valuable biologically active substances of plant and animal origin. Scarcity and cost of marine aquatic organisms, making cost-effective production of marine biological resources, especially non-traditional, receiving pharmacological substances and creation of original effective biotechnological products. Great interest for pharmaceutical industry is active substances from metabolites of marine organisms – a group of cephalosporin antibiotics, nucleosides nereitoksin, eledoizin, glycosides, angiotensin-converting enzyme inhibitors, and many other substances with a different spectrum of pharmacological action. Development of optimal methodological approaches for obtaining and testing proteins of Antarctic organisms for further implementation of these proteins in practical biotechnology is the main interest of this study.

Object and research methods. Antarctic marine aquatic – krill (*Euphausia Superba*), starfish (*Odontaster validus*) and nemertine (*Parborlasia corrugata*) were used as an test objects. Animal tissues were homogenized in liquid nitrogen, followed by addition of the extraction buffer: 0.1 M Na-phosphate buffer containing 0.15 M NaCl and 0.15 mM (ethylenediaminetetraacetic acid) (EDTA), pH 7.4.) and were separated by centrifugation at 10,000 g at 4º C for 20 min. Supernatant was decanted and lyophilized for storage optimization. Lyophilized samples were dissolved in distilled water and precipitation of the proteins was performed using trichloroacetic acid (25%). Identification of potential trypsin-like protein fraction was performed by disc-electrophoresis in a 10% polyacrylamide gel with sodium dodecyl sulfate [3]. Trypsin fraction was obtained by column chromatography on benzamidine–Sepharose [4]. In order to separate solutions of proteins from non-protein fraction gel filtration chromatography using a Sephadex G-25 was performed [5]. Identification of trypsin-like protein fractions after chromatographic separation was performed by disc-electrophoresis in a 10% polyacrylamide gel with sodium dodecyl sulfate. Proteolytic activity of the studied fractions were determined using as substrate 4% solution of casein in 0.05 M phosphate buffer, pH 7.4.

Results and discussion. Analysis of total protein composition of organisms was performed by disc-electrophoresis polyacrylamide gel.

Electrophoretic analysis revealed the presence of proteins with a molecular weight from 3 to 126 kDa in tissues of marine animal. These results may indicate the presence of variety of enzymes, including trypsin-like enzymes in animal tissues. In order to analyze the amount of trypsin-like enzymes in the samples, we performed chromatographic separation by chromatography on benzamidine-Sepharose. The lyophilized material was dissolved in 2 ml of distilled water and transferred into a 50 mM Na-phosphate buffer, pH 7.4 using Sephadex G 25. Fraction was applied to 2 ml Sephadex G 25 equilibrated with 50 mM Na-phosphate buffer, pH 7.4 at 10 ml/min. Protein fractions separation and change of conductivity was monitored using an UV and conductivity sensors. Column with benzamidine-Sepharose was equilibrated with 20 mM Tris-HCl buffer, pH 8.0 using Sephadex G-25, immediately before the measurement of activity.

Measurement of protein content in fraction 2 gave the following result: *Euphausia superba* – 2.9 ± 0.3 mg/g sample, *Parborlasia corrugatus* – 1.7 ± 0.1 mg/g sample, *Odontaster validus* – 0.8 ± 0.3 mg/g, sample. Analysis showed the presence of trypsin-like activity in the fraction №2 and the complete absence of this activity in fractions №1 and №3. Measurement of trypsin-like activity showed results: krill – 4.1 ±04 c.u./ g sample, nemertines – 2.8 ± 0.3 c.u/g sample, starfish 1.8 ± 0.3 c.u/g sample.

Serine proteases are enzymes that cleave peptide bonds in proteins, in which serine serves as the nucleophilic amino acid at the active site. Trypsin-like serine proteases cleave peptide bonds following a positively charged amino acid (lysine or arginine) [7].

For more information about qualitative composition of trypsin-like enzyme fractions in the test samples, electrophoretic separation of the fraction №2 was performed using disc-electrophoresis in polyacrylamide gel with the addition of sodium dodecyl sulfate.
Fig. 2 – Chromatogram of separation of samples protein fractions on a column of benzamidine-Sepharose (A – nemertines – Parborlasia corrugatus; B – starfish – Odontaster validus; V – krill – Euphausia superba):
1 – not related stuff; 2 fraction of trypsin-like enzymes; 3 – fraction which was eluted with 1M NaCl

As it’s indicated in Figure 3, nine bands with different molecular weight were present in trypsin-like enzyme fraction. Protein bands with molecular weight below 10-14 kDa fragments may be trypsin-like enzymes that were subjected to autolysis.

Fig. 3. Electrophoregram of separation of trypsin-like fraction obtained from nemertines (A), krill (B) starfish (C) after chromatography on benzamidine-Sepharose:
1 – molecular weight markers (96, 67, 43, 30, 20, 14 kDa) 2 – fraction of trypsin enzymes

Conclusions. These findings suggest that development of optimal methodological approaches for obtaining and testing trypsin-like enzymes of Antarctic organisms might be used for the creation of potential biotechnological substances which can be used in modern
medical industry in order to create a new generation of pharmaceutical agents.

References