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Proteolysis-Antiproteolysis System and Possible Mechanism of the Divergence of *Lymnaea stagnalis* and *Planorbarius corneus*

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Aim of the study: The aim of the study was to determine the divergence time of these two species of mollusks, the possible ancestor of hemocyanin, and the search for common targets in human and mollusks trypsin molecules for the antimetabolite - ethionine.

Material and Methods: To determine the divergence time, the www.timetree.org database was used, the NODE TIME search mode (search for divergence time for two species of mollusks). In the MEGA 5.2 program 64 nucleotide sequences of histone H4 organisms of various taxonomic groups were introduced. The dendrogram was constructed using the Neighbor-Joining method. There is an assumption that the hemocyanin originated from tyrosinase. Therefore, on the server www.weblogo.berkeley.edu/logo.cgi, logos of motifs from the amino acid sequences of the hemocyanin of mollusks and tyrosinases *Biomphalaria glabrata* and *Planorbarius corneus* were generated and compared. With the help of molecular docking technology, the correspondence of the L-ethionine ligand to two structures of the trypsin protein (*Homo sapiens* and *Biomphalaria glabrata*) on the server www.dockingserver.com was studied. In the hemolymph and hepatopancreas of pulmonary freshwater mollusks, the activity of trypsin-like proteinases was determined, as well as the content of proteinase inhibitors (α1-antiprotease inhibitor and α2-macroglobulin) using N-α-benzoyl-D,L-arginine paranitroanilide as a substrate at pH values of incubation media were 3.0, 3.6, 3.8, 6.1, 7.2, 8.0 and 9.0. The preparation of ethionine was administered at a concentration of 1 mg/g of mollusk mass.

Results: The divergence time of *Lymnaea stagnalis* and *Planorbarius corneus* was 182 million years. This time refers to the Toar era (182.7 - 174.1 million years ago). Then, as a result of volcanic eruptions, a lot of carbon dioxide got into the atmosphere, the average annual temperature of the Earth increased. Algae and bacteria were rapidly multiplying, consuming oxygen dissolved in water, which was manifested by the formation of black shales. As a result, a long period of relative oxygen deficiency arose for living organisms, including pulmonary mollusks. It can be assumed that *Lymnaea stagnalis* had some advantages in transporting oxygen compared to *Planorbis corneus*. When aligning the sequences of hemocyanins and tyrosinases, a motif (H - - - WHR), which is present in hemocyanins and tyrosinases, was found. H (His) corresponds to 6 amino acids on the logotypes of hemocyanins and tyrosinases, WHR (Trp, His, Arg) - 14, 15 and 16, respectively. The presence of this general motive may support the assumption of the origin of hemocyanins from tyrosinases, when the amount of bioavailable oxygen in the environment has decreased. When comparing the results of 2 docking, it was found out that 6 amino acids of trypsin in *Homo sapiens* and in *Biomphalaria glabrata* bind to ethionine. Amino acids for *Homo sapiens*: Asp 189, Ser 190, Gln 192, Ser 195, Val 213, Cys 220. Amino acids for *Biomphalaria glabrata*: Asp 224, Ser 225, Gln 227, Ser 230, Val 248, Cys 254. High values of trypsin-like activity were detected at pH values of the incubation medium in the range of 3.6-9.0, and the amounts of the α_1-antiprotease inhibitor at pH 3.6-3.8 and α_2-macroglobulin at pH 3.0. Ethionin had a different effect on the proteolysis-antiproteolysis system, which probably depended on the type of oxygen transport in pulmonary freshwater mollusks.

Acknowledgments: to Associate Professor V.V. Khrustalev for consultations on methods of bioinformatics *in silico*.

Keywords: *Lymnaea stagnalis*, *Planorbarius corneus*, proteolysis, *in silico.*