UDC 577.2 DOI: 10.25808/08697698.2018.202.6S.004

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Slow-binding irreversible inhibitors of recombinant alpha-galactosidase from marine bacteria *Pseudoalteromonas* sp. KMM 701 and its C494N mutant

Key words: Slow-binding irreversible inhibitors, alpha-galactosidase, marine bacteria Pseudoalteromonas sp, monanchomycaline B, monanhocicidin A and normonanhacidin A, sponge Monanchora pulchra

O-glycoside hydrolases play an important role in the existence of micro- and macro-organisms. In bacteria, yeasts and fungi, these enzymes are involved in the degradation of various plant poly- and oligosaccharides that serve as a source of carbon and energy for growth of the organism, as well as performing various functions in organisms, including an offensive-defensive function. Modification or blocking of these functions by powerful selective inhibitors underlies the treatment of a number of infectious diseases, malignant tumors and genetic disorders. Inhibitors of enzymes are molecules that reduce or complete blocking the catalytic activity of an enzyme, so causing either complete death of cell or modification in the metabolic pathways.

The marine sponges are important sources of inhibitors of different class enzymes. We focus our attention to metabolites of marine sponge as inhibitors of glycosidases from marine bacteria. The effect of monanchomycaline B, monanhocicidin A and normonanhacidin A isolated from the Far Eastern sponge *Monanchora pulchra* on the activity of recombinant alpha-galactosidase from the marine bacterium *Pseudoalteromonas* sp. KMM 701 and to its C494N mutant, as well as the recombinant alpha-N-acetylgalactosaminidase from the marine bacterium *Arenibacter latericius* KMM 426^T was investigated. It was shown that all compounds are slow-binding irreversible inhibitors of the alpha-galactosidase and its C494N mutant. Galactose that is a competitive inhibitor of the alpha-galactosidase protects this enzyme from the inactivation under the action of the alkaloids. These compounds have no effect on an activity of the alpha-N-acetylgalactosaminidase.

The inactivation rate constants (k, min^{-1}) have been defined. The dependence of k value on the inhibitor concentrations has been established. It is shown that the inhibitory ability of the alkaloids depends on their chemical structure, and the structural features of the active site of the enzymes.

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The work was supported partly by FEB RAS grant № 18-4-026.

The theoretical model of 3D-structure of the alpha-galactosidase from the marine gammaproteobacterium *Pseudoalteromonas* sp. KMM 701 was constructed with the use of Homology module of MOE package on the based of X-ray structure of *Lactobacilus acidophilus* alpha-galactosidase (PDB ID: 2XN2) as a template. The theoretical model of the structure of the complexes of the alpha-galactosidase molecule with monanchomycaline B, monanhocicidin A and normonanhacidin A were constructed with the method of molecular docking. Two binding sites for the alkaloids in the molecule of alpha-galactosidaseare shown. Apart from the carboxyl groups of the catalytic residues Asp451 and Asp516, the SH group of Cys494 in the active site of the alpha-galactosidase takes part directly in the interaction with anchor parts of the molecules of guanidine alkaloids. A possible mechanism of interaction of monanchomycalin B with the active center of alpha-galactosidase is discussed, as well as a biological role of the compounds in the life of sponges and symbionts. Detailed study of the new marine inhibitors will provide the basis for future research.