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α-Amylase inhibitors are major components of sea anemone *Heteractis magnifica* mucus

Key words: sea anemone, venom, amylase, defensin, diabetes.

Sea anemones (phylum Cnidaria) are ancient sessile predators inhabiting the marine environment. Sea anemone mucus, due to its multiple and vital functions, is a valuable substance for investigation of new biologically active peptides. Pancreatic α-amylase inhibitors have been recently isolated from sea anemones. They have a great pharmacological potential for the treatment of type II diabetes, which accounts for 90% of all diabetes cases. The aim of the work is a searching of new pancreatic α-amylase inhibitors in mucus of sea anemone *Heteractis magnifica.* Compounds of *H. magnifica* mucus were separated by multistage liquid chromatography and resulting fractions were analyzed by MALDI-TOF MS. Peptide maps constructed according to the molecular masses and hydrophobicity showed presence of 326 both new and known peptides. Most fractions inhibited porcine pancreatic α-amylase, thus proteomic analysis revealed that α-amylase inhibitors along with proteinase inhibitors, pore forming toxins and neurotoxins are major components of *H. magnifica* mucus which play an important role in the successful existence of sea anemones. Magnificamide, the major α-amylase inhibitor of *H. magnifica,* was isolated and its amino acid sequence was determined. BLAST analysis of this sequence revealed only one sequence-based homolog (87.5%), which corresponded to helianthamide, α-amylase inhibitor from *Stichodactyla helianthus*. Magnificamide contains 44 amino acid residues; its molecular weight is 4770 Da. With the help of genetic engineering approaches, a recombinant analog of magnificamide was obtained. Artificial gene encoding the peptide was cloned into the pET32b vector, and expressed in *Escherichia coli* as part of a fusion protein. The fusion protein was isolated from the cell lysate by metal affinity chromatography, hydrolyzed by endoproteinase, and then the recombinant magnificamide was purified by RP HPLC. The average yield of the target peptide was 2 mg per liter of cell culture. The recombinant magnificamide inhibited porcine pancreatic and human saliva α-amylase. Thus we obtained functionally active recombinant analog of magnificamide, which will be used for further study of its biological activity as potential drug for treatment of type II diabetes.

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