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SALTWATER MUSSELS (FAMILY MYTILIDAE) – PROSPECTIVE SOURCE OF HIGH-ACTIVE HYDROLYTIC ENZYMES

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An economically and environmentally pressing challenge of today is waste-free sustainable utilization of natural biological resources, a substantial part of which is extracted from the World Ocean. Researchers' attention to marine macro- and microorganisms remains high, since they are a source of physiologically active substances used in human and veterinary medicine, food industry, agriculture and other sectors. Marine flora and fauna supply substances employed in genetic engineering, as fungicides, immunity stimulators, anti-radiation products, diagnostic and health food preparations. Widely used alongside with antibiotics, toxins, pigments, polysaccharides, and proteins are enzymatic preparations, including hydrolases, which decompose natural biopolymers to easily digestible and metabolisable components. Particular focus is on proteolytic enzymes (Mukhin and Novikov, 2001). Little studied in terms of applied enzymology are glycosidases from marine organisms. Since hereditary deficit of acid glycosidases causes lysosomal storage disorders, such studies would promote development of means and methods to fight this disease.

The present study aimed to investigate the activity of lysosomal glycosidases in different organs of the bivalve *Mytilus edulis* L. Mussels are typical inhabitants of the littoral and sublittoral zones of the White Sea. Besides, they are widely used in mariculture, both in Russia and in many European countries.

The material for the study was collected at the Kartesh facility of the RAS Zoological Institute's White Sea Biological Station. Mussels *M. edulis* were captured from the sublittoral zone in the Chupa Bay, Gulf of Kandalaksha. Mussels of one size were placed in 16 litre aquaria with salt water, natural temperature and light regimes, and forced aeration. After the mussels had grown acclimated to laboratory conditions they were subjected to biochemical study. Mussel organs were frozen, taken to the laboratory and stored at – 80°C until analysis. The material was then homogenized in 0.25 M sucrose solution with 0.001 M EDTA and 0.1% triton X-100 detergent, pH 7.4. The tissue/sucrose ratio was 1:9. The homogenates were clarified by centrifugation in K-24 centrifuge at 12 000 rpm. Lysosomal enzyme activity and protein content were determined in the supernatant fluid using techniques adjusted to the study object (Vysotskaya and Nemova, 2008). β-glycosidase (EC 3.2.1.21) activity was determined using the sodium para-nitrophenyl-β, D-glucopyranoside substratum with 0.15 M citrate-phosphate buffer (pH 5.0). β-galactosydase (EC 3.2.1.23) activity was determined by its reaction with para-nitrophenyl-β, D-glucopyranoside with citrate buffer (pH 4.0).

Enzyme activity was calculated as μM of para-nitrophenol formed through the reaction per 1 g of wet weight of tissue per minute and per 1 mg of protein.

The study showed the activity of lysosomal glycosidases in organs of mussels from the White Sea to be quite high (Table). Notably high is the β-glycosidase activity in the mussels' digestive gland. We have never observed such high glycosidase activity in our studies of lysosomal enzyme activity in other aquatic organisms. Absolute values of acid glycosidase and galactosydase in the digestive gland of the mussels are an order of magnitude higher than in fish, even in lysosome-rich organs such as kidneys and liver (Vysotskaya and Nemova, 2008).

Table. Glycosidase activity in organs of the mussel *Mytilus edulis* (μM para-nitrophenol / g wet weight / min), n = 7

Organ	β-glycosidase	β-galactosidase
Digestive gland	1.48–1.57	0.474–0.640
Gills	0.022–0.041	0.123–0189
Foot	0.012–0.013	0.040–0.048

The data obtained have to do with specific characteristics of the biochemical organization of carbohydrate and energy metabolism in mussels inhabiting the tidal zone – one of the most stressful environments. Being exposed to frequent and abrupt alternations of ambient conditions, the enzyme systems of saltwater mussels have evolved to acquire a number of features (such as low sensitivity to changes in the microenvironment, high activity, thermal stability, etc.) maintaining their catalytic ability in a wide range of conditions. Lysosomal enzymes, including glycohydrolases, perform many physiological functions connected not only with substrate destruction but also with secretion processes and metabolism regulation (Vysotskaya and Nemova, 2008). Thus, glycosidases are involved in these processes as they hydrolyse glucosidic bonds and through transglycolysation reactions. Among glycohydrolases, acid β-glycosidase (human β-glucocerebrosidase) is the most common enzyme, present in all living organisms from bacteria to humans. It can be used in various fields of biotechnology: from enzyme replacement therapy to cellulolysis for renewable energy production (Turan, 2008). β-glucocerebrosidase deficiency leads to glucosylceramide storage in lysosomes and triggers the inherited disorder – Gaucher disease. Other lysosomal storage disorders, as well as neurodegenerative diseases, ageing processes and many pathological states are attributed to the deficiency of other acid glycosidases. Injections of the deficient enzymes are widely practiced in the therapy of human lysosomal storage diseases (Pupyshev, 2006). As a rule, preparations made of human cells are used. Research now focuses on finding cheaper sources of the enzymes, as well as on investigating the mechanisms to deliver the injected enzymes to the destination, i.e. lysosomes (Aerts et al., 2003; LeBowitz et al., 2004). In addition to the enzyme replacement therapy one is developing gene therapy methods based on most recent scientific findings (Pupyshev, 2006; Desnick, 2004). A prerequisite for success in this undertaking is comprehensive study of the properties and distinctive patterns of lysosomal glycosidase functioning in different taxa and different ecological circumstances (Barkalova and Yershova, 2009; Xie and Chen, 2004; Turan, 2008). A vivid example of the mussels' adaptation to the ambient conditions is the high activity of carbohydrate metabolism enzymes, including lysosomal glycosidases.

Thus, our studies demonstrated high β-glycosidase and β-galactosidase activity in the digestive gland of the White Sea mussels, wherefore this object is commendable as a source of acid glycosidases. The hydrolases can be used both in the research meant to reach a deeper understanding of these important lysosomal enzymes, and to address various applied tasks involving hydrolysis of glucosidic bonds.

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