EXPERIMENTAL BIOLOGY

Correlation of Intracellular Ca^{2+}-Activated Proteinase Activity and Cholesterol Content in White Sea Mussel (*Mytilus edulis*) Membranes at Different Water Saltiness

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Correlation between changes in activity of intracellular Ca^{2+}-activated proteinases and cholesterol content in mussels in response to changes in habitat saltiness was detected in mollusks from littoral and sublittoral White sea zones. Calpain activity in mussels decreased in low water saltiness and increased in high saltiness in parallel with decrease in cholesterol content, which attests to decreased microviscosity and modification of permeability of biomembranes. A complex pattern of interactions between metabolic routes in mussels under conditions of different habitat saltiness was detected.

Key Words: *mussels; saltiness; cholesterol; Ca^{2+}-activated proteinases*

Sea mollusks, including representatives of the *Mytilidae* family, belong to typical poikiloosmotic organisms incapable of regulating osmotic pressure of the internal media. On the other hand, they exhibit appreciable euryhalinity and can live under conditions of the habitat saltiness varying from 4-5 to 75-80%.

Mussels are prevalent in the entire White sea, mainly in the littoral and shallow sublittoral water. The littoral (ebb and flow) environment is more variable in comparison with sublittoral because of greater variability of temperature, saltiness, oxygen content, and availability of food. One of the main parameters of euhaline sea mollusks adaptation to changes in environmental saltiness is high osmotic stability of cells due to reactivity of lipid and protein metabolism.

Along with phospholipids, cholesterol is one of the main lipid components and structural constituents of the plasma membrane; its membrane content varies greatly. Cholesterol regulates viscosity of membrane lipids [4,5]. By regulating microviscosity of the lipid bilayer cholesterol modulates conformation of membrane proteins, *e.g.* receptor and ionic channel subunits, thus regulating permeability of these channels. This is fully so for Ca^{2+}, effectors of intracellular Ca^{2+}-activated proteinases (calpains).

Microviscosity of plasma membranes can modulate activity of calpains. Ca^{2+}-activated proteinases were detected in all vertebrate tissues [8] and in some invertebrates [7,14], but the mechanisms regulating activities of these proteinases are not yet studied sufficiently well [12]. One of these regulators, along with Ca^{2+}, thiol groups, and calpastatin can be modification of the membrane microviscosity, which is largely determined by cholesterol.

We studied the ratio of cholesterol content in membrane lipids and the reactivity of intracellular Ca^{2+}-activated proteinases in the development of adaptive reactions in mussels in response to modification of the habitat saltiness.

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MATERIALS AND METHODS

Mytilus edulis were collected in the littoral and sublittoral zones at the "Kartesh" Belomorsk Biological Station of Zoological Institute, Russian Academy of Sciences. Mussels were placed for 12 days into aquariums with different concentrations of salt (from 5 to 45‰) and kept at constant temperature (10°C) and illumination. Mussels kept in sea water served as the control. Water saltiness in the White sea is 25‰. Lower salt concentrations were attained by sea water dilution, high concentrations by adding estimated quantity of Instant Ocean Aquarium System salt, identical to sea water salt. Homogenates of whole organs were prepared [15] and centrifuged at 100,000*g* for 1 h. Calpain activity was evaluated after preliminary gel chromatography of the samples on 2.5×95 cm columns packed with Sephacryl S-200 gel (Pharmacia) equilibrated with buffer A (10 mM Tris-HCl (pH 7.5) containing 50 mM NaCl, 4 mM EDTA, 5 mM mercaptoethanol) for separation of specific protein inhibitor (calpastatin) and for fractionation of individual molecular forms of the enzyme. Separation was carried out in a refrigerator at 4°C using standard LKB-Pharmacia equipment. The fractions were registered on a Uvicord II absorptiometer at 280 nm. The eluent fractions (4 ml) were collected using fraction collector; activity of Ca^{2+}-activated proteinases was measured in the fractions by the standard method (by casein hydrolysis [13]). The reaction mixture for both calpains included 0.4% casein, 5 mM dithiotreitol, 50 mM imidasole-HCl buffer (pH 7.5), and enzyme solution. Experimental samples were incubated with $CaCl_2$; control samples were incubated without calcium, which was added after incubation. After 30-min incubation at 30°C the reaction was stopped by adding an equal volume of 10% TCA. The concentration of acid-soluble hydrolysis products was measured by spectrophotometry (E_{280}). A unit of calpain activity was defined as alteration of optical density (dE_{280}) for 30-min incubation at 30°C [13]. Elution profile of water-soluble proteins in mussel homogenates (Fig. 1) is presented by three peaks with different molecular weights. Three protein fractions exhibiting Ca^{2+}-dependent proteolytic activity were detected. They can be identified as homologues of calpain II, calpain I, and catalytically active subunit of higher animal calpains.

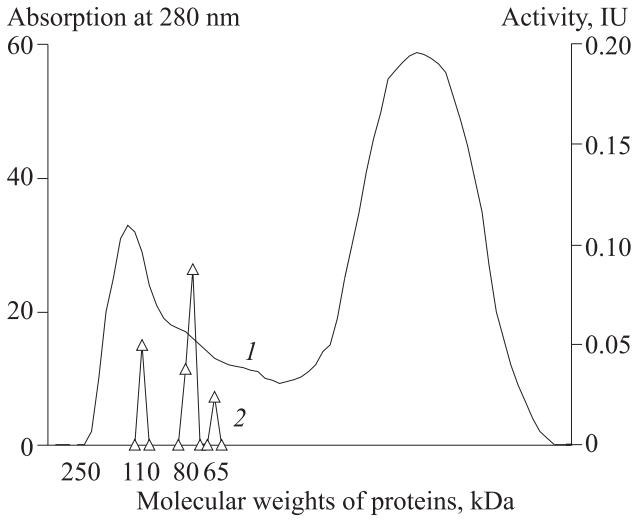

Fig. 1. Elution profile of water-soluble proteins and Ca^{2+}-dependent activity (E_{280}/g tissue/h) in homogenates of control *M. edulis* after gel chromatography. *1*) protein; *2*) activity.

Cholesterol was measured by Engelbrecht's method [11] after isolation by thin layer chromatography on Sylifor plates in a solvent (petroleum ether:sulfuric ether:acetic acid; 90:10:1).

RESULTS

Calpain activity in littoral and sublittoral mussels decreased appreciably as a result of saltiness decrease from 25 to 5‰ and increased almost 2-fold after increase of saltiness from 25 to 45‰ (Fig. 2) Calpain activation in mussels exposed to high saltiness and their suppression under conditions of low saltiness reflect the reaction of cell metabolism, presumably adaptive, because we observed no hyperactivation of calpain II activated by high (above physiological) concentrations of calcium [8,12] typical of pathological activation. A slight (1.3 times) increment in the subunit activity of calpains in experimental samples (except for 45‰ saltiness) indicates the absence of enzymatic protein labilization under the effect of the saltiness factor [10]. Calpain subunit activity increased in mussels kept in a medium with high salt concentration (45‰), which probably attests to increased lability of the initial forms of the enzyme. Relative stability of the enzymatic protein molecules in mussels partially confirms our data that euryhaline poikilosmotic invertebrates are characterized by a wide range of permissible saline concentrations at which native conformation of macromolecules is retained.

The level of summary activity of calpains in littoral mussels is much lower than in sublittoral ones. This suppression of Ca^{2+}-dependent proteolysis in the littoral mussels can be due to their adaptation to the coastline zone with variable temperature, salt and oxygen concentrations. Changes in activities of some enzymes affecting the rate of RNA and protein synthesis in mussels in response to external factors including changed saltiness were reported previously [1].

Cholesterol content in membranes of sublittoral mussels increases (1.5 times) with decreasing saltiness from 25 (normal level) to 5‰ and decreases with increasing saltiness from 25 to 45‰ (Fig. 3), that is, the

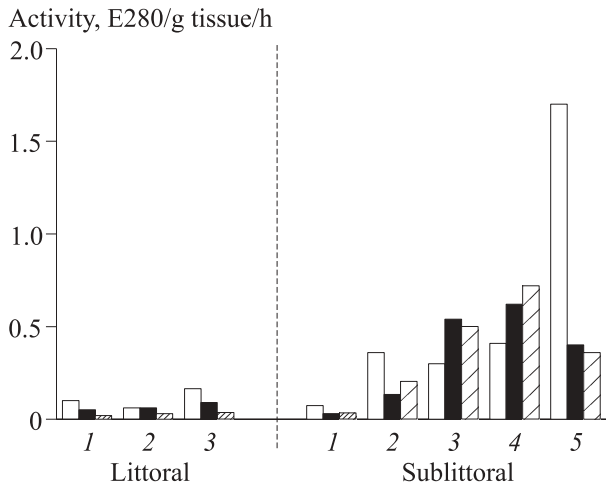

Fig. 2. Distribution of total Ca^{2+}-dependent activity between calpain molecular forms in homogenates of *M. edulis* from the littoral and sublittoral zones of the White sea during acclimatization to saltiness. Here and in Fig. 3: light bars: 60 kDa; dark bars: 80 kDa; cross-hatched bars: 110 kDa; *1*) 5; *2*) 15; *3*) 25 (control); *4*) 35; *5*) 45‰.

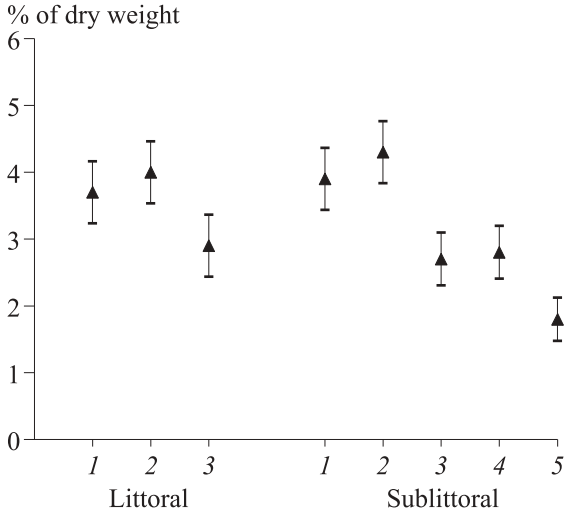

Fig. 3 Cholesterol content in *M. edulis* cell membranes from mussels of littoral and sublittoral zones of the White sea during acclimatization to saltiness.

increase in saltiness from 5 to 45‰ causes a significant decrease in cholesterol level in cell membranes. Changes in cholesterol content in mussels of the littoral and sublittoral zones are similar in low saltiness and exhibit a trend to increase. As was shown on developing *Coregonus albula L.* eggs, modification of salt concentration during egg incubation decreases membrane cholesterol content, which impairs egg membrane permeability [9].

A correlation between cholesterol content in mussel membranes and activity of Ca^{2+}-activated proteinases of the cytosol was detected. Cholesterol content in mussel membranes decreased with increasing the habitat saltiness above the normal level (Fig. 3), while activity of Ca^{2+}-dependent proteinases increased (Fig. 2). It was shown [6] that the decrease in cholesterol content above the normal increased ionic permeability of cell membranes, which led to increase of intracellular concentration of ions, including Ca^{2+}. The calpain—calpastatin (inhibitor) complex degraded with increasing Ca^{2+} concentration in the cytosol [2], this resulting in activation of calpains, which we observed in experiment on acclimatization of mussels to increased environmental saltiness. Hence, high concentrations of salt change membrane structure in mussels promoting calcium entry into cells and activation of Ca^{2+}-dependent proteinases (Fig. 2). The degree of calpain activation in mussels depends on salt concentration in water.

During acclimatization to low salt concentration membrane cholesterol content in mussels of the littoral and sublittoral zones increases (Fig. 3). Accumulation of cholesterol determines more compact packing of carbohydrate lipid chains, increase of microviscosity, and, hence, decrease in membrane permeability, which additionally decreases availability of deep membrane layers for ionogenic groups [3]. This is confirmed by the results of our experiments on acclimatization of mussels to low salt concentration. In this case cholesterol content in membranes increases significantly and activity of Ca^{2+}-activated proteinases decreases, which seems to be due to decreased Ca^{2+} entry in the cytosol.

The findings indicate adaptive changes in cholesterol content, essential for phasic status of biomembranes, which, in turn, is essential for activation of intracellular Ca^{2+}-dependent proteinases playing an important role in the regulation of cell metabolism, specifically, in osmoregulation.

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