

STRESS PROTEIN SYNTHESIS IN MUSSELS MYTILUS EDULIS

P. Tykhonov, L. Tarasenko, V. Naida, V. Rud, N. Stepanova

Odesa State Agrarian University

The synthesis of heat shock proteins in vivo and in vitro in mussels Mytilus edulis under the impact of a lignosulfonate drilling mud was investigated. It was established that a stress protein with a molecular mass of 68 kD can be used as a biomarker of sea water pollution by lignosulfonate drilling muds at ultra-low concentrations.

Key words: mussels *Mytilus edulis*, stress proteins.

Introduction. Prokaryotes and eukaryotes respond to the temperature impact (high or low) and other adverse environmental factors or internal factors (infectious and non-infectious diseases) by synthesizing so-called stress proteins, or heat shock proteins [1]. Enhanced expression of genes encoding these proteins is part of the cellular response to adverse factors [2]. Under physiological conditions, some of these proteins function as intracellular molecular chaperones or proteases. Chaperones are known to be involved in the stabilization and translocation of oligomeric proteins, while proteases are involved in the degradation of damaged proteins [3,4]. Some heat shock proteins are synthesized under normal physiological conditions, but their intracellular concentration may increase in various pathological conditions of the organism. Mussels *Mytilus edulis* are filtrators in which contaminants can accumulate. Therefore, they are often used as test organisms for chemical pollution of waters [5].

The aim of our research was to determine the effect of lignosulfonate drilling mud at ultra-low concentrations [6] on the synthesis of stress proteins in mussels *Mytilus edulis*.

Materials and methods. Mussels 3-6 cm in length were acclimatized to aquarial conditions for a month. The aquarial part of the experiment was performed according to Gudimov [6], the biochemical part was performed as described earlier [7].

Results and discussion. Under normal physiological conditions (pure sea water), a set of polypeptides in the range of molecular weights from 10 to 92 kD is synthesized in mussel tissues. Polypeptide with a molecular weight of 68 kD is present in trace amounts (Fig.1, Lane 2). Drilling mud at a concentration of 0.0001% did not lead to significant changes in the electrophoretic spectrum of proteins compared with that under normal physiological conditions (Fig., Lane 3).

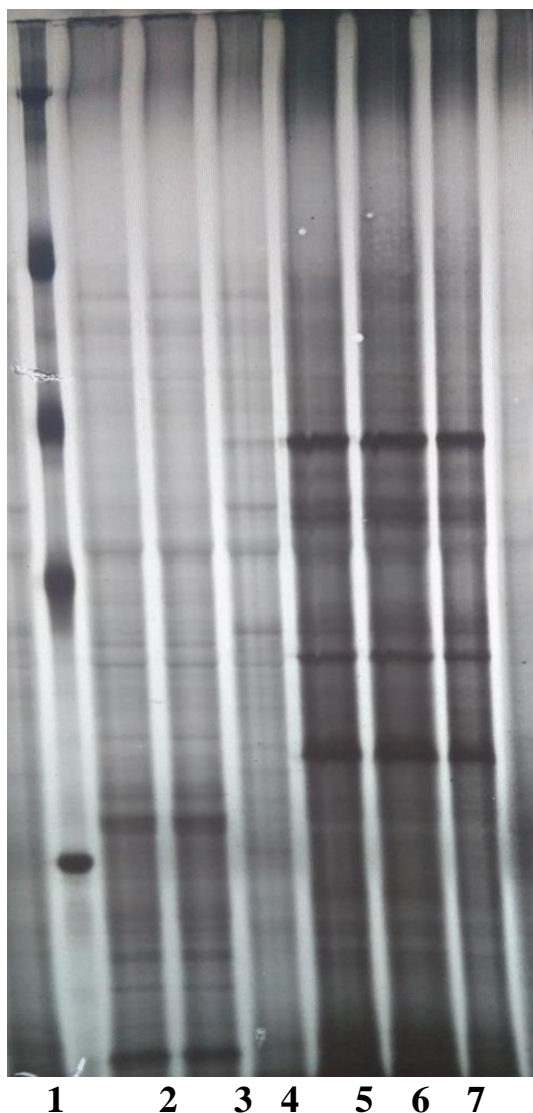


Fig.1. SDS polyacrylamide gel electrophoresis (12.5%) of ^{35}S -methionine-labeled mussel proteins synthesized in vivo (lanes 2, 3, 4) and in vitro (lanes 5, 6, 7). Lane 1 - molecular weight markers (in kD) (from bottom to top): 30 - carbonic anhydrase, 46 - ovalbumin, 69 - bovine serum albumin, 92.5 - phosphorylase B, 200 - myosin. Lanes 2, 5 - proteins of mussels that were in clean sea water. Lanes 3, 6 - proteins of mussels that were in seawater with drilling mud at a concentration of 0.0001%. Lanes 4, 7 - proteins of mussels that were in seawater with drilling mud at a concentration of 0.0005%.

Increasing the concentration of drilling mud to 0.0005% led to changes in the electrophoretic spectrum of proteins (Fig., Lane 4). The content of the component with a molecular weight of 68 kD increased significantly. Also increased the relative content of components with molecular weights of 40 and 55 kD, decreased - components with molecular weights of 45-46 kD, as well as in the range of 10-38 kD. Some polypeptides with molecular weights of 15-17 kD and 32-33 kD have disappeared, and some with molecular weights of 34-35 kD have appeared. The relative content of the component with a molecular weight of 48 kD remained the same in all cases, which may indicate the constitutive nature of the synthesis of this protein. The same properties were shown by proteins in the range of molecular

weights of 70-90 kD. The electrophoretic spectra of polypeptides synthesized *in vitro* when used as a template mRNA from mussel tissues in a wheat-germ and rabbit-reticulocyte lysate cell-free systems were similar. The figure shows the electrophoretic spectra of proteins synthesized in a wheat-germ cell-free system. Protein with a molecular weight of 68 kD was detected among the translation products of mRNA from mussels, which were under physiological conditions (Fig., Lane 5) and under the influence of drilling mud in concentrations of 0.0001% and 0.0005% (Fig., Lanes 6, 7). At the same time, *in vivo*, the relative intensity of this component increased significantly only under the influence of drilling mud at a concentration of 0.0005% (Fig., Lane 4). In all *in vitro* mRNA translation products, the relative content of components with molecular weights of 35, 42, 53-55 kD increases in comparison with those *in vivo*, and the relative content of the component with molecular weight of 32 kD decreases. These data suggest that the mussels *Mytilus edulis* have constitutive and inducible heat shock proteins. The results also suggest that genes encoding heat shock proteins with a molecular weight of 68 kD are transcribed into mussels under normal physiological conditions and under the influence of drilling mud at a concentration of 0.0001%. However, a small amount of this protein is synthesized *in vivo* under these conditions. When increasing the concentration of drilling mud to 0.0005%, the intensity of synthesis of this component increases significantly. That is, this protein is synthesized constitutively, and its synthesis increases significantly with increasing concentration of drilling mud.

Conclusion. Thus, the protein with a molecular weight of 68 kD can be used as a biomarker of seawater contamination by lignosulfonate drilling muds in ultra-low concentrations.

REFERENCEC

1. Hartl F. U., Bracher A., Hayer-Hartl M. (2011). Molecular chaperones in protein folding and proteostasis. *Nature*, V. 475(7356), p. 324–332. doi: 10.1038/nature 10317.
2. Akerfelt M., Morimoto R. I., Sistonen L. (2010). Heat shock factors: integrators of cell stress, development and lifespan. *Nature reviews. Molecular cell biology*, V. 11(8), p. 545–555. doi: 10.1038/nrm2938.
3. Toshihide Takeuchi, Mari Suzuki, Nobahiro Fujikake, H. Akiko Popiel, Hisae Kikuchi, Shiron Futaki, Keiji Wada, Yoshitaka Nagai. (2015). Intercellular chaperone transmission via exosomes contributed to maintenance of protein homeostasis at the organismal level. *Proc. Natl. Acad. USA*, V. 112(19), p. 2497–2506. doi.org/10.1073/pnas.1412651112.
4. Rita Marreiros, Andreas Muller-Schiffman, Svenja V. Trobach, Ingrid Prikulis, Sebastian Hansch, Stefanie Weidkamp-Peters, Ana Raquel Moreira, Shriya Sahu, Irina Soloviev, Suganya Selvarajah, Vishwanath R. Lingappa, Carsten Korth. (2020). Disruption of cellular proteostasis by H1N1 influenza A virus causes α -synuclein aggregation. *Proc. Natl. Acad. USA*, V. 117(12), p. 6741–6751. doi.org/10.1073/pnas.1906466117.
5. Kurelec B., Krca S., Lucic D. (1996). Expression of multixenobiotic resistance mechanism in a marine mussel *Mytilus galloprovincialis* as a biomarker of exposure to polluted environments. *Comparative Biochemistry and Physiology C –*

Pharmacology, Toxicology and Endocrinology, V. 113C. p. 283–289.

6. Gudimov A.V. Bioassay of drilling muds by physiological responses on bottom invertebrates. (1993). Arctic seas: bioindication of the environmental status, bioexamination and technology of destruction of pollutants, Murmansk marine biological institute, Kola Science Centre, Russian Academy of Sciences, Apatity, p. 36–44 (in Russian).

7. Tykhonov P.S. (2016). Induction of a major stress protein synthesis in mussels *Mytilus edulis*. Agrarian Bulletin of the Black Sea Littoral, Issue 79, p. 57–60.

СИНТЕЗ БЕЛКОВ ТЕПЛОВОГО ШОКА У МИДИЙ MYTILUS EDULIS

Тихонов П., Тарасенко Л., Найда В., Рудь В., Степанова Н.

*Исследован синтез белков теплового шока in vivo и in vitro у мидий *Mytilus edulis* при влиянии бурового раствора лигносульфонатного типа. Установлено, что стрессовый белок с молекулярной массой 68 кД можно использовать как биомаркер загрязнения морской воды буровыми растворами лигносульфонатного типа в сверхнизких концентрациях.*

Ключевые слова: мидии *Mytilus edulis*, стрессовые белки.

СИНТЕЗ БІЛКІВ ТЕПЛОВОГО ШОКУ У МІДИЙ MYTILUS EDULIS

Тихонов П., Тарасенко Л., Найда В., Рудь В., Степанова Н.

*Досліджено синтез білків теплового шоку in vivo та in vitro у мідій *Mytilus edulis* за впливу бурового розчину лігносульфонатного типу. Встановлено, що стресовий білок з молекулярною масою 68 кД можна використовувати як біомаркер забруднення морської води буровими розчинами лігносульфонатного типу у наднизьких концентраціях.*

Ключові слова: мідії *Mytilus edulis*, стресові білки.