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EFFECT OF HYPOXIA ON IMMUNE SYSTEM OF BIVALVE MOLLUSCS

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Over the past decades, research on bivalve immune system is focused on studying the effect of environmental factors on the basal status of defense systems. The immune system of bivalves is greatly affected by abiotic factors, and the most significant ones are water temperature, salinity, and level of dissolved oxygen. Hypoxia is widespread in the coastal waters of the World Ocean since the 1950s. Hypoxic zones (with dissolved oxygen concentration < 0.5 mL $O_2 \cdot L^{-1}$) occur in shelf areas for a long time corresponding to the life cycle of many hydrobionts. Being benthic organisms, bivalve molluscs often experience reduced dissolved oxygen concentrations. This group of aquatic invertebrates both plays an important role in aquatic ecosystem functioning and is actively used in aquaculture. The efficiency of bivalve cultivation directly depends on its immune status determining resistance to diseases. The immune system of bivalve molluscs is based on a complex of nonspecific reactions of cellular and humoral components. Hemocytes circulating in the hemolymph are the key effectors of the cellular immune response which, along with the barrier tissues of molluscs, synthesize humoral factors with a wide spectrum of antimicrobial activity. The hemolymph of various bivalve species contains different cell types differing by size, morphology, and granulation of cytoplasm. Most bivalve species have 2 types of hemocytes – granular and agranular ones; those can be subdivided into morphotypes depending on number and color of granules, size of the nucleus, and presence of organelles in the cytoplasm. Granulocytes are considered the main immune cells that perform phagocytosis and (or) encapsulation of infectious agents, as well as their subsequent neutralization by releasing reactive oxygen species, lysing enzymes, and humoral antimicrobial proteins. Moreover, the complex of defense systems includes an antioxidant system which is closely related to mollusc immunity since it neutralizes reactive oxygen species releasing during cellular immune mechanism activation. An excess of these compounds damages mollusc cells by oxidizing proteins, cytoplasmic membrane lipids, and DNA. This article provides data on an oxygen deficiency effect on the cellular and humoral components of the immune system, as well as the tissue antioxidant complex of bivalve molluscs.

Keywords: bivalve molluscs, immunity, hemocytes, hypoxia, antimicrobial proteins, antioxidant enzymes

Deep hypoxia is typical for aquatic ecosystems where oxygen minimum zones are formed (Diaz & Breitburg, 2009). The reasons for hypoxia formation in many aquatic ecosystems are natural, but an increased anthropogenic load on coastal areas of water bodies has led to a significant spread of hypoxia and anoxia in the World Ocean (Gallo & Levin, 2016). At the same time, a sufficient amount of dissolved oxygen is a factor determining survival of organisms in the aquatic environment. Hypoxia causes significant alterations in the structure of communities resulting in a change in their species composition and transformation in the abundance and biomass of populations (Diaz & Breitburg, 2009). Lack of oxygen greatly affects physiology of benthic macroorganisms (Wang Y. et al., 2012).

Bivalve molluscs are mass representatives of coastal marine water bodies. Many species are cultivated and have a high commercial value (Wijsman et al., 2019). Moreover, molluscs play a key role in marine ecosystem functioning (Hartmann et al., 2016). High biological and economic significance of these organisms determines the interest of researchers in studying the peculiarities of the functioning of their immune system and estimating the effect of negative environmental factors on an ability of the immune system to resist infectious agents of various nature (Anderson, 2001).

The immune defense of bivalves is primarily based on biological barriers (shell and mantle), as well as on nonspecific reactions of innate immunity (Donaghy et al., 2012). Bivalve immunity includes cellular and humoral components (Anderson, 2001). Molluscs have an open circulation system. Hemocytes are the main agents providing the cellular immune response (Donaghy et al., 2012). The mechanisms of the cellular component of the mollusc immune system include phagocytosis and (or) encapsulation of pathogenic microorganisms followed by their destruction by enzymatic cleavage or release of reactive oxygen species (hereinafter ROS) (Pauletto et al., 2014). The humoral response is expressed in affecting pathogens with a complex of molecules, *inter alia* antimicrobial proteins (Rodrigues et al., 2010), C-type lectins (Wang S. et al., 2010), peptidoglycan recognition proteins (Ikuta et al., 2019), and several other compounds (Wootton et al., 2003).

Some authors consider the antioxidant (hereinafter AO) tissue complex as another mechanism of immune defense in molluscs since ROS are produced by hemocytes during the response to pathogens and adverse environmental conditions (Donaghy et al., 2012). In this case, the reduction of oxygen to a superoxide anion results in the occurrence of many highly ROS, *inter alia* hydroperoxides, singlet oxygen, or hydroxyl radicals (Lambert & Brand, 2004). Their excess can damage cellular structures (Valko et al., 2006). However, the balance in ROS production and neutralization is achieved by maintaining a high activity of tissue AO enzymes (catalase, superoxide dismutase, *etc.*) which split hydroperoxides into less active gaseous oxygen and water (Monari et al., 2007). Thus, the internal mechanism of organism protection from the damaging effect of ROS is carried out during the immune system functioning.

Being predominantly bottom-dwelling organisms, molluscs often experience periodic or constant hypoxia; this led to emergence of a wide spectrum of adaptive mechanisms to survive in an environment with a minimum oxygen content (Sokolov et al., 2019). This article provides an overview of modern concepts on the reaction of the cellular and humoral immune response, as well as the response of the AO complex of bivalves to oxygen deficiency.

1. Cellular immune response

1.1. The ratio of hemocyte types and total number of hemocytes in the hemolymph. As known, the cellular composition of the hemolymph and the functional peculiarities of hemocytes are not the same in representatives of bivalve molluscs. Differences in hemocyte classification in different species (and sometimes even in the same one) depend on the method of analysis and the principle underlying the cell classification (Hine, 1999). Summarizing the existing classifications, two main cell types can be distinguished – agranular ones (blast-like cells, agranulocytes, and hyalinocytes) and granular ones (Andreyeva et al., 2019 ; Hine, 1999). Granular cells are more involved in the implementation of the immune response (Wang W. et al., 2017); therefore, the ratio of cell types in the hemolymph is an important diagnostic indicator of the functional state of the mollusc body. A decrease in the total number of hemocytes or a change in their ratio in the hemolymph can alter the effectiveness of the immune response.

A decrease in the total number of hemocytes under oxygen deficiency in various mollusc species was shown. Specifically, incubation of mussels *Mytilus coruscus* Gould, 1861 for 3 days under hypoxia led to a decrease in the relative number of granular cells in the hemolymph (Sui et al., 2016). In the mussel *Perna perna* (Linnaeus, 1758), incubation in air for 48 hours resulted in a decrease in the number of circulating hemocytes by 73 % (Nogueira et al., 2017). A drop in the total number of hemocytes after incubation under hypoxia was noted in *Perna viridis* (Linnaeus, 1758) (Wang Y. et al., 2011) and *Chamelea gallina* (Linnaeus, 1758) (Matozzo et al., 2005). A decrease in the total number of hemocytes is associated with impaired proliferation, migration of hemocytes to other tissues, and increase in the rate of apoptosis and mortality (Mydlarz et al., 2006). As shown, incubation under oxygen deficiency leads to a rise in the mortality rate of *P. viridis* hemocytes (Wang Y. et al., 2011) and *M. coruscus* hemocytes (Sui et al., 2016). According to other studies, hypoxia can result in a decrease in the total number of hemocytes without an increase in their mortality rate (Nogueira et al., 2017). Interestingly, in *Ch. gallina* subjected to daily hypoxia, after 96 hours of incubation under normoxia, the number of hemocytes recovered to the control level (Matozzo et al., 2005). At the same time, exposure to oxygen deficiency for more than 24 hours led to irreversible changes in the number of hemocytes (Pampanin et al., 2002).

1.2. Phagocytosis. Hemocytes are capable of phagocytizing heterogeneous particles and pathogenic microorganisms penetrating into the mollusc body. The process of phagocytosis includes recognition, binding, and inactivation of the pathogen (Canesi et al., 2002). As a rule, hypoxia results in a suppression of phagocytic activity (Ellis et al., 2011); its decrease was recorded in *P. viridis* after a daily incubation under hypoxia (Wang Y. et al., 2011) and in *Mytilus galloprovincialis* Lamarck, 1819 after 12 and 24 hours of incubation in air (Mosca et al., 2013), as well as in *M. coruscus* (Sui et al., 2016), *Chlamys farreri* (K. H. Jones & Preston, 1904) (Chen J. et al., 2007), and *Ch. gallina* (Matozzo et al., 2005). The changes depended on the incubation duration: the longer hypoxia, the lower intensity of phagocytosis (Sui et al., 2016). A decrease in phagocytic activity during hypoxia is associated with a decrease in the number of hemocytes (Pampanin et al., 2002). Insufficient production of adenosine triphosphate under oxygen deficiency results in a decrease in the ability to migrate and phagocytize heterogeneous particles. At the same time, air incubation of *P. perna* contributed to an increase in phagocytic activity of its hemocytes (Nogueira et al., 2017).

1.3. Spontaneous production of reactive oxygen species. ROS are produced by hemocytes for antimicrobial protection (Anderson, 2001). Typically, granular cells in bivalves are more capable of generating an oxidative burst than agranular ones (Pauletto et al., 2014). As believed, the main source of ROS in hemocytes is mitochondria (Donaghy et al., 2013), and a decrease in ROS production may be caused by inhibition of enzymes involved in the generation of the oxidative burst (Andreyeva et al., 2019). Inhibition of ROS production was registered in *P. viridis* hemocytes (Wang Y. et al., 2011). A decrease in ROS production is associated with the mechanisms of metabolic adjustment with the hypoxia-inducible factor (HIF) involved (Michiels et al., 2002). On the other hand, in *M. galloprovincialis*, short-term hypoxia induces an increase in the ability to produce ROS in agranulocytes and a decrease in granulocytes (Andreyeva et al., 2019). Similar results were obtained by other authors (Chen J. et al., 2007 ; Sui et al., 2016). Probably, in some species, hypoxia induces reorganization of the mitochondrial respiratory chain, and this leads to a rise in ROS production (Chandel et al., 2000).

2. Humoral immunity and response to hypoxia

Bivalve molluscs are endowed with a complex of humoral immune factors that are activated in response to the invasion of pathogenic microorganisms and (or) the negative effect of the environment. The main classes of compounds involved in the humoral immune response of molluscs are antimicrobial proteins, cytokines, complement system factors, AO enzymes, and acute-phase proteins (Rodrigues et al., 2010). Effectors of humoral nonspecific immunity have a wide spectrum of activity against gram-positive and gram-negative bacteria, protozoans, yeasts, fungi, and viruses. One of the first identified and described antimicrobial proteins were those in *Mytilus edulis* Linnaeus, 1758 and *M. galloprovincialis* (Charlet et al., 1996 ; Mitta et al., 2000). In bivalves, defensins are the most common group of antimicrobial proteins; however, other classes of compounds with antimicrobial properties are described as well – histones (Dorrington et al., 2011), lysozymes, *etc.* (Wang Q. et al., 2013). As shown, the lysozyme family in molluscs is represented by a large number of proteins which are mostly expressed in mucosal tissues (mantle, gills, and hepatopancreas) (Wang Q. et al., 2013). Obviously, the spectrum of compounds involved in the humoral immune response of molluscs is much wider than currently known. The research principle is based on the search for analogies with the already described factors of nonspecific immunity in vertebrates; with such an approach, mollusc-specific compounds remain undescribed. The specific role of most of the identified factors of humoral immunity is still unexplored due to complexity in setting up experiments and choosing methods. Most authors, however, agree on the primary role of these compounds in the immune response of molluscs. This assumption is based on rapid changes in the expression levels of humoral factors in response to experimental immunization of hemocytes (Suárez-Ulloa et al., 2013).

Since there is no base of fundamental knowledge on the humoral immunity functioning in bivalves, it is extremely difficult to characterize the degree of negative effect of oxygen deficiency on this part of the immune system. Transcriptomic studies indicate the activation of a whole complex of genes involved in several immune signaling pathways that implement the response to bacterial invasion in molluscs kept under hypoxia (Zhang et al., 2019). As shown, hypoxia has a depressing effect on the humoral immunity of molluscs. The expression level of defensin in the gills of *Brachidontes pharaonis* (P. Fischer, 1870) decreased by 5–20 times after 6 days of exposure to low oxygen concentrations (Parisi et al., 2015). Other representatives of the Mollusca phylum showed significant changes in the expression of immune genes as well. Specifically, in the Pacific abalone, the expression of 6 genes associated with the immune response was inhibited under oxygen deficiency (Shen et al., 2019). On the other hand, incubation of the abalone (*Haliotis discus discus* Reeve, 1846) under hypoxia for 8 hours caused a significant increase in the transcription of proteins involved in the regulation of cytokine activity. This evidences the activation of the latter, as well as of some other proteins involved in the immune response of vertebrates (De Zoysa et al., 2009).

Direct responses of humoral immunity resulting from changes in gene expression remain the subject of discussion, and the results only fix the fact of changes in the expression of humoral immunity factors. The real physiological role of the observed changes is still unexplored. Apparently, such reactions of the immune system are negative since some species showed a decrease in the expression of humoral immunity factors in response to stimulation by pathogenic organisms during incubation under hypoxia (Sun et al., 2016).

3. The effect of hypoxia on the antioxidant complex of bivalves

The AO complex of mollusc is the key system of its nonspecific protection from oxidative stress (hereinafter OS) and to a great extent determines its resistance to the effect of adverse environmental factors (Gostyukhina & Andreenko, 2018 ; Soldatov et al., 2014). Oxygen deficiency is one of the most significant damaging factors that the AO system protects against. Increased release of ROS in molluscs during hypoxia increases the risk of OS (Tomanek, 2015). In molluscs, AO protection is provided by AO enzymes, such as catalase, superoxide dismutase, and glutathione peroxidase (hereinafter CAT, SOD, and GP, respectively), as well as by a number of low molecular weight antioxidants (Livingstone, 2001). Several components of the AO complex not only protect against hypoxia, but also serve as important humoral factors of immune defense. Specifically, the activity of CAT, SOD, and GP correlates well with the immune competence of mollusc cells (Liu et al., 2004 ; Sui et al., 2017). High activity of different parts of the AO system in eurybiont molluscs allows them to withstand a long-term oxygen deficiency (Irato et al., 2007 ; Soldatov et al., 2014), adapt to it, and occupy their own econiche (Dovzhenko, 2006).

3.1. Species specificity in the reactions of the antioxidant complex to hypoxia. Despite the universal, nonspecific nature of the AO complex functioning, it can have specificity depending on species, tissue, evolutionary, and ecological peculiarities of molluscs (Gostyukhina & Andreenko, 2018 ; Dovzhenko, 2006 ; Istomina, 2012 ; Gostyukhina & Andreenko, 2019 ; Livingstone, 2001 ; Soldatov et al., 2014). Thus, under experimental hypoxia and anoxia, three types of reactions were revealed: an increase in the activity of AO enzymes (in species tolerant to hypoxia), a decrease in their activity, and a constancy of the state of the AO complex (Istomina et al., 2011). An increase in the activity of SOD and glutathione reductase was found in *Spisula sachalinensis* (Schrenck, 1862) and *Littorina mandshurica* (Schrenk, 1862) – molluscs experiencing oxygen deficiency due to environmental conditions. Time to time, *S. sachalinensis* burrows into sediments and stays under hypoxia. *L. mandshurica* inhabiting the littoral zone is daily subjected to fluctuations in oxygen level at high and low tides. These reactions are associated with the ability of AO enzymes of these molluscs to respond quickly to an increase in a ROS level. In *S. sachalinensis* hepatopancreas, a decrease in the content of reduced glutathione (hereinafter GSH) was noted as well (Istomina, 2012), which also reflects an active AO role of GSH and a rapid depletion of its resource during hypoxia. In OS-resistant mollusc species, hypoxia increases the activity of AO enzymes, primarily SOD and CAT. Thus, the reaction to short-term and long-term critical hypoxia in the mollusc *Astarte borealis* (Schumacher, 1817) was expressed in the activation of CAT, SOD, and GP (Abele-Oeschger & Oeschger, 1995). In hepatopancreas and gills of the hypoxia-resistant species *Scapharca inaequivalvis* (Bruguière, 1789), high activity of CAT, SOD, and GP was recorded as well (Irato et al., 2007).

For a stenooxybiont species *Patinopecten yessoensis* (Jay, 1857), the activity of AO enzymes decreased which is due to its lower resistance to hypoxia. This is associated with the fact that the mollusc lives under relatively stable conditions and is capable of avoiding hypoxia (Istomina et al., 2011). For the OS-sensitive mollusc *Tapes philippinarum* (A. Adams & Reeve, 1850), a decrease in SOD and CAT activity was shown under hypoxia; this may indicate the OS in tissues (Irato et al., 2007). Moreover, for the scallop *Mizuhopecten yessoensis*, a significant increase in a GSH level – by 6 times – was shown under hypoxia (Istomina, 2012). This reflects the leading role of GSH in protecting OS-sensitive molluscs.

Apparently, the third mode of response – constancy of AO activity – is associated with resistance to oxygen deficiency as well. Lack of significant changes in the AO complex during hypoxia was shown for *Crenomytilus grayanus* (Dunker, 1853) and *Tegula rustica* (Gmelin, 1791) – species inhabiting the sublittoral, leading an attached or sedentary lifestyle, and not being subjected to frequent oxygen deficiency, in contrast to littoral or burrowing molluscs (Istomina et al., 2011). Moreover, the Gray mussel *C. grayanus* is capable of maintaining constant SOD and CAT activity for a long time under oxygen deficiency against the backdrop of a constant level of lipid peroxidation products (Istomina, 2012). Such an AO defense strategy is probably due to an evolutionary adaptation of mytilids to prolonged hypoxia/anoxia (Hicks & McMahon, 2005). However, a decrease in glutathione reductase activity and GSH level was noted (Istomina, 2012). This proves that the main contributors to the AO protection of *C. grayanus* during hypoxia are the key enzymes – SOD and CAT. In the tissues of *Anadara kagoshimensis* (Tokunaga, 1906), even more OS-resistant species than *M. galloprovincialis*, a higher activity of AO enzymes and a significantly increased GSH resource were shown (Gostyukhina & Andreenko, 2018). This gives *A. kagoshimensis* an advantage for living and surviving under hypoxic environmental conditions.

3.2. Sensitivity of individual components of the antioxidant complex to oxygen deficiency. In the response to lack of oxygen, the specificity of the reactions of individual components of the AO complex is recorded as well. First of all, an increase in SOD and CAT activity is detected during hypoxia (Chen J. et al., 2007 ; Chen X. et al., 2014 ; Sui et al., 2017). Out of AO systems, SOD provides the front line of defense – the most significant one (Sui et al., 2017). Under oxygen deficiency, a rapid increase in SOD activity is often recorded – in the scallop *Ch. farreri* hemocytes (Chen J. et al., 2007), in *S. inaequivalvis* hepatopancreas and gills, in the oyster *Pteria penguin* (Röding, 1798) (Gu et al., 2020), in *S. sachalinensis* and *L. mandshurica* (Istomina et al., 2011), and in *M. coruscus* gills and hemolymph (Sui et al., 2017). As a rule, SOD is one of the first to react, but mainly at the initial stages of hypoxia. With further oxygen deficiency, a decrease in enzyme activity is observed; for example, in *Ch. farreri*, a decrease in SOD activity occurred after 7, 14, and 21 days which indicates that prolonged hypoxia can lead to inactivation of the main protective enzymes (Chen J. et al., 2007). Importantly, in a highly resistant to hypoxia bivalve *A. borealis*, a reaction to short-term and long-term critical hypoxia was expressed in the activation of both SOD and CAT and GP (Abele-Oeschger & Oeschger, 1995). This evidences the joint action of different parts of the AO complex, *inter alia* during prolonged lack of oxygen. Apparently, this AO strategy determines high resistance of *A. borealis* to hypoxia.

Increased SOD activity results in a high rate of superoxide anion radical dismutation into H_2O_2, and this stimulates CAT activity which catalyzes the breakdown of H_2O_2 and hydroperoxides and also protects the body from high amounts of hydroxyl radicals (Hermes-Lima, 2004). As shown, CAT activity often increases after a rise in H_2O_2 under OS (Hermes-Lima, 2004). This is consistent with the results of (Sui et al., 2017): for *M. coruscus*, CAT activity increases in response to low oxygen and pH levels.

In some cases, CAT activity decreases under oxygen deficiency in water – for example, in *M. galloprovincialis*. At the same time, other AO enzymes, in particular glutathione transferase (hereinafter GT), can play a more significant role than catalase in protecting mussels from hypoxia (Woo et al., 2013). Such a diversity of responses reflects the species specificity of the reactions of the AO complex during hypoxia and indicates their complexity and variability, as well as the plasticity of the AO system in protecting molluscs from hypoxia/anoxia.

The prevalence of low molecular weight antioxidants, primarily GSH, in the AO defense of molluscs was shown as well. As a rule, this is observed in species that are more sensitive to OS. Thus, in the stenooxybiont species, the scallop *P. yessoensis*, a decrease in the activity of AO enzymes under hypoxia was recorded (Istomina et al., 2011); apparently, this is determined by their lower resistance to oxygen deficiency. However, against this backdrop, a significant increase in a GSH resource is noted in the scallop which ensures the mollusc protection from ROS under conditions of reduced enzyme activity. This can be confirmed by the ability of GSH to inactivate the superoxide anion radical, thus partly duplicating SOD function (Hermes-Lima, 2004). Under hypoxia, such mutual substitution can contribute to effective protection and survival of the mollusc: with an increase in a ROS level, it allows to provide a fast and effective AO response with the help of low molecular weight glutathione without activating SOD (not requiring time and energy) (Gostyukhina & Andreenko, 2018).

An important protective role of GSH is also demonstrated by a comparison of AO reactions of molluscs with different resistance to oxygen deficiency. In a number of tissues of the Japanese scallop *M. yessoensis*, which is sensitive to hypoxia, not only increased activity of AO enzymes was shown, but also a higher GSH resource. In the Gray mussel *C. grayanus*, only an increase in SOD activity in gills was found. This is a species-specific reaction determining the resistance of these species to hypoxia (Belcheva et al., 2016).

3.3. Tissue-specific features of the antioxidant complex under hypoxia. The responses of the AO system of molluscs to hypoxia are tissue-specific as well. As a rule, the highest activity of AO enzymes is detected in hepatopancreas and gills (Gostyukhina & Andreenko, 2018 ; Dovzhenko, 2006), but there are some exceptions. Specifically, for *M. coruscus*, higher CAT, SOD, and GP activity was shown under hypoxia in hemocytes than in gills (Sui et al., 2017). This is associated with a more important role of hemolymph in immune defense. Similar features are registered in other species with lack of oxygen – higher values of CAT activity in hemocytes of *M. galloprovincialis* than in gills (Katsumiti et al., 2015) and higher values of SOD and GT activity in hemocytes of *Venerupis philippinarum* (Adams & Reeve, 1850) (Chen X. et al., 2014). For *A. kagoshimensis*, maximum values of a GSH level and GP activity were recorded in a foot. This proves active participation of GSH in the work of this enzyme as a cofactor; at the same time, this indicates its own active AO role. The content of other low molecular weight antioxidants (glucose, amino acids, and urea) was the highest in hepatopancreas and gills (target organs), and the lowest in a foot (Gostyukhina & Andreenko, 2019). Apparently, these low molecular weight antioxidants contribute much to the AO defense of the mollusc under hypoxia. Other authors came to the same conclusion. For *Anadara* experiencing anoxia and reoxygenation, a conclusion was made on the key role of a low molecular weight link of the AO system in ROS detoxification since low molecular weight antioxidants are less dependent on the intensity of metabolism and energy sources (Istomina et al., 2011).

3.4. Features of the antioxidant system functioning while in the atmosphere. Several studies were devoted to the effect of anoxia on molluscs when exposed to air. Specifically, anoxia in combination with different temperatures in the scallop *Ch. farreri* led to a complex of reactions – an increase in ROS production, a decrease in acid phosphatase activity and in a number of phagocytic hemocytes, and an increase in hemocyte mortality. At the same time, SOD activity did not depend significantly on air temperature but depended on anoxia duration (Chen J. et al., 2007).

Under anoxia in air at +25 °C for 2 hours, SOD activity in scallop hemocyte lysate significantly increased against the backdrop of a simultaneous significant rise in ROS production (Chen J. et al., 2007). This proves a protective effect of SOD with an increase in an oxidative load under these conditions. In scallops at +5 °C, ROS production was significantly higher than the initial values under this anoxia regime; however, SOD activity in hemocyte lysate remained at the control level (Chen J. et al., 2007). Such reactions testify to the important protective role of SOD under different oxygen deficiency regimes and different temperatures. At the same time, the authors believe that scallops showed a relatively low anoxia tolerance at high temperatures. This is consistent with the opinion of a number of researchers that the effect of anoxic and hypoxic loads can alter the immune reactions of a mollusc and lead to increased susceptibility to diseases (Matozzo et al., 2005 ; Monari et al., 2005 ; Pampanin et al., 2002). Apparently, this type of AO protection is more characteristic of stenooxybiont molluscs.

The leading role of SOD in mollusc protection from hypoxia is also noted for *P. penguin* when exposed to air (Gu et al., 2020). SOD activity increased after exposure to air and then stabilized in oyster hepatopancreas and hemolymph, which resulted in a constant MDA level during 6 and 9 hours of hypoxia. In contrast to SOD activity, CAT and GP activity, as well as total AO capacity, increased rapidly and then gradually decreased after 6 hours of oyster exposure to air. Perhaps, the reason for this was an increase in MDA level in hemolymph after 9 and 12 hours of exposure to hypoxia.

3.5. Reversibility of hypoxia effect on the antioxidant complex during reoxygenation. A number of studies were devoted to the response of the AO system of mollusc to hypoxia and subsequent reoxygenation. In *M. galloprovincialis* gills, under conditions of 48-hour exposure to air followed by 48-hour reoxygenation, an increase in gene expression and a rise in SOD, CAT, and GT activity were observed. A recovery to normoxic levels was registered during reoxygenation (Giannetto et al., 2017). Such reactions reflect a preventive increase in the AO potential of the mollusc to strive the oxidative burst during further reoxygenation.

Under the effect of different hypoxia regimes in *M. galloprovincialis*, a decrease in CAT activity (SOD activity did not change), an increase in GT activity, and its subsequent decrease against the backdrop of a rise in the lipid peroxidation level were established. This reflects the transcriptional stability and selective changes in the genes of individual AO enzymes that protect the mussel under various oxygen deficiency regimes (Woo et al., 2013). Moreover, different reactions were identified for individual isoforms of enzymes. Specifically, under anoxia effect on the immune response of *Ch. gallina* hemocytes, Cu/Zn-SOD activity decreased, while Mn-SOD activity increased significantly. Mn-SOD activity is especially high during reoxygenation which is probably due to high inducibility of this isoform and its important role in protection against ROS when recovering to normoxia (Monari et al., 2005).

Conclusion. The results of recent studies made it possible to significantly deepen and expand knowledge on hypoxia effect on the immune system of molluscs at tissue, cellular, and molecular levels. Further analysis of adaptive mechanisms of mollusc hemocytes will allow assessing and predicting possible negative consequences of hypoxia effect on the cellular component of the immune system. Molecular genetic studies will help in assessing the degree of an oxygen deficiency effect on the humoral component. In turn, the degree of hypoxia effect on the immunity of bivalves will depend on a combination of climatic changes in the environment (global warming, changes in wind characteristics and currents, *etc.*) and on prospects for the use of coastal areas in economic activity. The reactions of the antioxidant complex of bivalve molluscs to hypoxia are species-specific, and so is the immune response. An AO response involves components of both enzymatic and low molecular links of the AO system in various combinations. Changes in AO activity during hypoxia are often caused by preparation for subsequent reoxygenation and are often reversible. This reflects the functional plasticity of the AO system and its significant role in the defense mechanisms, as well as in the formation of nonspecific immune reactions in the body of bivalves under oxygen deficiency in the environment.

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ВЛИЯНИЕ ДЕФИЦИТА КИСЛОРОДА НА ИММУННУЮ СИСТЕМУ ДВУСТВОРЧАТЫХ МОЛЛЮСКОВ

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В течение последних десятилетий исследования иммунной системы двустворчатых моллюсков сфокусированы на изучении влияния факторов внешней среды на базальный статус защитных систем организма. Иммунная система моллюсков чувствительна к действию абиотических факторов, среди которых наиболее существенны температура, солёность воды и уровень растворённого кислорода. Гипоксия широко распространена в прибрежных водах Мирового океана с 1950-х гг.; гипоксические зоны (с концентрацией кислорода менее 0,5 мл $O_2 \cdot л^{-1}$) сохраняются на шельфе в течение длительного времени, соответствующего продолжительности жизненного цикла многих гидробионтов. Двустворчатые моллюски, являясь бентосными организмами, часто попадают под воздействие пониженной концентрации растворённого кислорода. Данная группа водных беспозвоночных играет важную роль в функционировании водных экосистем, при этом двустворок активно используют для аквакультурного выращивания. Эффективность культивирования этих организмов напрямую зависит от их иммунного статуса, определяющего устойчивость к заболеваниям. Основу иммунной системы двустворчатых моллюсков составляет комплекс неспецифических реакций клеточного и гуморального компонентов. Гемоциты, циркулирующие в гемолимфе, являются ключевыми эффекторами клеточного иммунного ответа, которые, наряду с барьерными тканями моллюсков, осуществляют синтез гуморальных факторов с широким спектром антимикробной активности. Гемолимфа моллюсков различных видов содержит разные типы клеток, которые отличаются по размерам, морфологии и наличию включений в цитоплазме. Большинство видов двустворок имеет два типа гемоцитов — гранулярные и агранулярные гемоциты; они могут подразделяться на морфотипы в зависимости от числа и окраски гранул, размеров ядра и наличия органелл в цитоплазме. Считается, что гранулоциты являются основными иммунными клетками, осуществляющими фагоцитоз и (или) инкапсуляцию инфекционных агентов, а также их последующую нейтрализацию путём выделения активных форм кислорода, лизирующих ферментов и гуморальных антимикробных белков. Также в комплекс защитных систем организма входит антиоксидантная, тесно связанная с иммунитетом моллюсков, поскольку эта система осуществляет нейтрализацию активных форм кислорода, выделяющихся в процессе активации клеточных иммунных механизмов. Избыток этих веществ оказывает повреждающее действие на клетки моллюсков путём окисления белков, липидов цитоплазматической мембраны и ДНК. В настоящем обзоре приведены данные о влиянии недостатка кислорода на клеточный и гуморальный компоненты иммунной системы и на тканевый антиоксидантный комплекс двустворчатых моллюсков.

Ключевые слова: двустворчатые моллюски, иммунитет, гемоциты, гипоксия, антимикробные белки, антиоксидантный комплекс