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STRUCTURAL ADAPTATIONS OF CELL WALLS OF *CHLORELLA VULGARIS* Beij. (*CHLOROPHYTA*) THE ACTION OF IONS ZINC AND LEAD

Main specific and nonspecific cells responses and membrane structures participation in formation of cells resistance of *Chlorella vulgaris* in stress conditions, caused by heavy metals (zinc, lead) in toxic concentrations are analyzed. The cell membranes participation in adaptation to toxicants (formation of growths, multiplication membranes, fluidization, forming of aquaporin, apoptosis), which are first exposed to stressors, is discussed. Found specific and nonspecific reactions in membrane formation are proposed to use as biomarkers of toxicity.

K e y w o r d s : ions zinc and lead, cell wall, *Chlorella vulgaris*.

Introduction

The disclosure of the cellular mechanisms of water plants adaptation to toxicants is important in the decision of the problems of hydrocenosis stability under the conditions of the chronic pollution and the role of the biota in the formation of water quality (Gandzyura, Grubinko, 2008; Arsan, 2010).

It is known that the resistance of aquatic organisms to the unfavorable factors is determined by the rate of formation and the adequacy of the response to the acting factor of cells protective systems, among which cell membranes have the decisive importance (Findley, Evans, 1990; Pathological ..., 1975). In this context it is important to analyze both: the membrane cell response to the action of toxicants and their participation in the formation of general cellular responses.

The concept of the cell as a biological trigger allows dividing its responses into specific and nonspecific. It is believed that the specific relationships are any changes of relations characteristics at the beginning of the cell response within the tolerance zone (reaction norm) and nonspecific reaction is the process of transition to a new stable state when the power factor and frequency of exposure are above a threshold level and are accompanied by a radical restructuring of the system – dissipative continuum transition systems (Grubinko, 2010). The specific responses of cells to the toxic stress include: the activation of the antioxidant system; the growth of activity of adaptive, including detoxifying enzymes, such as ATP-ase, phosphatases, hydrolases, lipases; changes in fatty acid structure of the membranes, especially the ratio of their saturated and unsaturated forms, direction of energy processes (Mehrle, Bergmann, 2002). During chronic toxic effects and high concentrations of substances when the basic defensive resources of cells are exhausted the spontaneous not genetically determined flow of nonspecific phenomena take place: the uncontrolled permeability of membranes and membrane potential depolarization of the plasma membrane (Veselova et al., 1993), the entrance of calcium ions into the cytoplasm from the cell walls and intracellular compartments; acidification of the cytoplasm, the activation of microfilaments (Pachomova, 1995); the accelerated expenditure of ATP; the chaotic development of free-radical processes, the increasing of functioning of proton pump in plasma membrane with the simultaneous disconnection of oxidation and phosphorylation; the formation of the stress proteins; the increasing of the content of the proline and as a result – structural and functional modifications of proteins, etc. (Chirkova, 1997).

At the same time the problem is primarily in the effective characteristic of reception and the prediction of possible damage by nonspecific factor ("disaster signals") at the subcellular and cellular levels which can provide an early warning of pathology development in the cell and, consequently, the reduction in the productivity of ecosystems (Gandzyura, Grubinko, 2008). The above mentioned specific and nonspecific responses are the qualitative and quantitative changes in the cells that develop some time and show the pathological state of cells and therefore are not objective for monitoring (Gandzyura, Grubinko, 2008). Therefore it is advisable to view those responses of cells to stress that appear and identify as instant answers and are more objective markers of cell resistance to toxic contamination of aquatic organisms.

The purpose of the investigation was to determine the specific and nonspecific responses of water plants cells depending on the nature, concentration and time of action of toxic levels of zinc and lead ions.

Materials and methods

The *Chlorella vulgaris* were used during the investigation. Their choice is connected with the evolutionary and ecological peculiarities of nonspecific responses in unicellular and multicellular aquatic organisms. The alga was cultivated in Fitzgerald medium in Zehnde and Gorhem modification N 11 at 22–25 °C and 2500 lx light for 16 hours/days. There were added to the medium under experimental conditions aqueous salt solutions $ZnSO_4 \cdot 7H_2O$ and $Pb(NO_3)_2$ based on the number of ions: Zn^{2+} – 1.0 mg/dm^3, 2.0 and 5.0 mg/dm^3 and ions Pb^{2+}– 0.1 mg/dm^3, 0.2 and 0.5 mg/dm^3 – 1, 2 and 5 MPC. The selection of plant biomass was performed for 1, 3, 7, and 14 days of the experiment under the effect of metal ions. The control plants were those grown in a medium without toxicants.

The cell membranes that were received as it was previously described (Grubinko, Kostiuk, 2012) were studied. Membrane changes were fixed microscopically (MBI-15, followed by an integrating digital analysis on the complex “SSTU-camera Manual Vision SSD-color-WOYV00020”) after their coloring “chloro-zinc-iodine” reagent.

Results and Discussion

The cell membranes of aquatic organisms are the first barrier to toxicants into the cell and fix them by binding sites (Grubinko, 2010). Therefore, we revealed a number of membrane effects in the investigated plants depending on the concentration and duration of toxicant exposure. At short-term impact of toxicants in concentrations below the threshold initially damaged cell membranes are able to restore the damages quickly (Fig. 1), with the resumption of functional activity. This functional activity can be seen on the restoration of activity of membrane ATP-ase (Kostiuk, Grubinko, 2010a). In this case various external "growths" on cells may appear. They diminish with time and in consequence can disappear (Fig. 2).

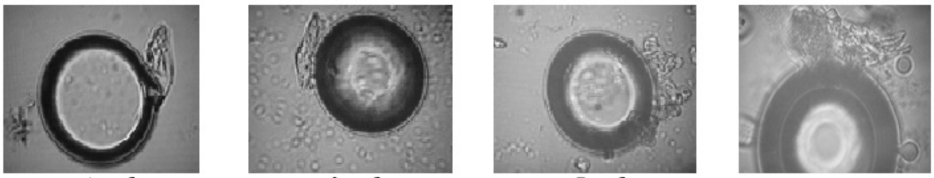


Fig. 1. Reparation of cellular membranes *Chlorella vulgaris* under the action of lead ion (0.2 mg/dm^3 – 2 MPC). In this and subsequent figures, an increase x9000

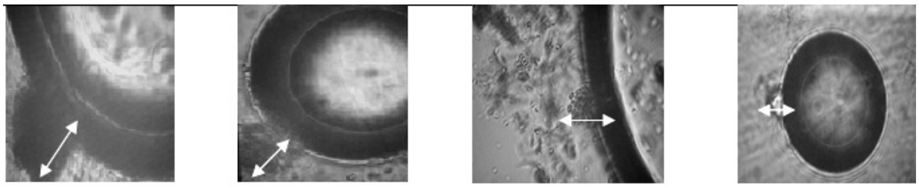


Fig. 2. Formation of outside "growths" on the cell walls of *Chlorella vulgaris* under the action of lead ions (0.2 mg/dm^3 – 2 MPC)

In case of chronic effects of toxicants in concentrations above the threshold cells are moving into a new discrete-functional state which is characterized by the active substance-induced formation of secondary concentric membranes (Grubinko, Kostiuk, 2012), constituting an additional protective system, conducive to the normalization of functional and metabolic homeostasis of cells under the toxic impact of their survival (Fig. 3).

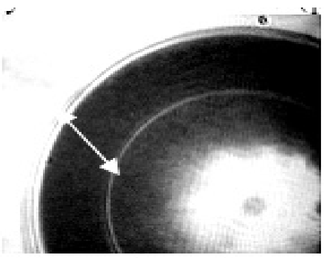
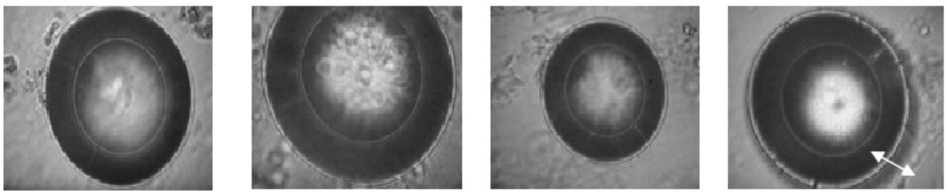


Fig. 3. The formation of secondary concentric membranes in the cells *Chlorella vulgaris* under the influence of zinc ions (5.0 mg/dm^3 – 5 MPC)

The process of a concentric double membrane system formation is universal, which confirms the general biological nature of the detected phenomena. It happens already in the beginning steps of stressors action regardless of their nature (biogenic zinc or toxic lead) (Grubinko, Kostiuk, 2012). We believe that a system of concentric membranes is one of the essential parts of a specific cellular response for damage and is especially important for the cells of aquatic organisms. We assume that the basis of the membrane adaptation to unfavorable factors lies in hyperplasia of endoplasmic reticulum, since it is proved that in the structures formed by smooth endoplasmic reticulum the content of enzymes responsible for the detoxification is increased (Pathoanatomy ..., 1975). The phenomenon of multiplication of membrane system in the cells of water plants revealed by us is consistent with the established for some organisms cells ability to adapt to the action of stressful factors due to thickening and multiplicative fragmentation of cell membranes. For example, as in a process of ascosporogenesis in *Arthroderma vanbreuseghemii* Takashio and *Arthroderma simii* Stockdale (Ito et al., 2000), dehydrated pollen *Pyrus communis* L. (Tiwari et al., 1990), while growing chlorella and micrococcus in radioactive according to deuterium water (Mosin, 1996) and testifies about its adaptive value. At the same time the destruction of the primary membrane is revealed. The base of this destruction may lie in the phenomenon of membranes fluidization (Fig. 4), which is also observed in high-and hypo-osmotic stress (Thewke et al., 2003).

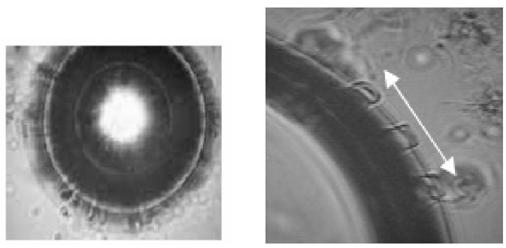


1 day 3 days 7 days 14 days

Fig. 4. Fluidization of membranes in cells of *Chlorella vulgaris* under the toxic effects of lead ions (0.5 mg/dm^3– 5 MPC)

Probably, the thicker the concentric secondary membrane is, the primary one is smaller and there are more concentric circles of divergence (spreading) of the lipid structures in it. On the basis of the study of the composition and functional parameters of the membranes we assume that the secondary membrane is a complete membrane formation, which functions as the primary after the loss of functions or degradation of the latter.

Fig. 5. The formation of multimembrane system, apoptosis (*a*) and aquaporins (*b*) of cells of *Chlorella vulgaris* under the action of the lead ion in concentration (5.0 mg/dm^3 – 5 MPC; 7 days)



a b

First of all, this is confirmed by its lipid and protein composition, as well as a manifestation of the specific enzymes activity (ATP-ase, alkaline phosphatase), whose activity after its initial decrease by the action of toxicants is resumed synchronously with the formation of a secondary membrane (Kostiuk, Grubinko, 2010a,b). Further the formation of multimembrane system is possible in the cells (Fig. 5).

However the following membranes are likely to be "defective" nonspecific formations and do not operate effectively. It can lead to the formation of pathological structures, and therefore this process is accompanied by apoptosis. As a result of the changes in the structure of the cell walls their permeability increases, the way out of substances from the cells is observed, ion balance is disturbed which leads to the inverse exit of potassium ions from the cell and the entrance the sodium ions (Chirkova, 1997). Ca^{2+} plays an essential role in the development of cellular adaptation reactions. Its participation in the generation of action potential, which may be one of the mechanisms of signal transduction of stress, is viewed (Chirkova, 1997). Thus aquaporins play the regulatory role in the stabilization of the ionic status of the cells and their pH (Blooma, 2006) (Fig. 5).

Due to this flow of water molecules and low molecular neutral compounds through the lipid matrix the cell resumes homeostasis.

Conclusions

The primary response of water plants cells to the action of toxicants is largely determined by changes in the cell wall. The primary reactions of the outer membranes to stress are: membranes ruptures and their reparation, multiplication and fluidization, the formation of aquaporins. Revealed by us the connection between the resistance of cells to various toxicants and the state of their outer walls makes it possible to assert that the stability of cell membranes is an integral factor in ensuring the stability of algae to adverse environmental conditions. The generation of membrane structures in algae cells can be used as a biomarker of disadvantage for the aquatic environment organisms. The advantage of the revealed effects as a biomarker is their early identification.

REFERENCES

Arsan O.M. New conception in a hydroecology: is new possibilities of system estimation of negative influences on the ecosystems // Hydrobiol. J. – 2010. – **46**(2). – P. 115–118.

Blooma D.A. Plants aquaporins // Physiol. and Biochem. Cult. Plants. – 2006. – **38**(5). – P. 396–404.

Chirkova T.V. Cellular membranes and stability of plants to stress influences // Soros. Obrazovat. J. – 1997. – (9). – C. 12–17.

Findley G., Evans U. Biological membranes. – Moscow: Mir Press, 1990. – 423 p.

Gandzyura V.P., Grubinko V.V. Concept of harmfulness in ecology. – Ternopil: Ternop. Nat. Ped. Univ., 2008. – 144 p.

Grubinko V.V. A regulator role of metals is in adaptation of aquatic organisms: evolutional and ecological aspects // Current problems of physiology and biochemistry of aquatic organisms. – Petrozavodsk: Inst. Biol. Karel. SC RAS, 2010. – P. 43–46.

Grubinko V.V., Kostiuk K.V. Structural Changes in the Cellular Membranes of the Aquatic Plants under the Impact of Toxic Substances // Hydrobiol. J. – 2012. – **48**(2). – P. 40–54.

Ito H., Hanyaku H., Harada T., Tanaka S. Fine structure in ascosporogenesis of freeze-substituted Arthroderma simii // Rev. Iberoamer. Micol. – 2000. – **699**(E-48080). – P. 13–16.

Kostiuk K.V., Grubinko V.V. Role of Membrane ATP-ases in Adaptation of Aquatic Organisms to Environmental Factors // Hydrobiol. J. – 2010a. – **46**(6). – P. 45–56.

Kostiuk K., Grubinko V. The effect of zinc, lead ion and diesel fuel on membrane lipid composition of aquatic plants // Visn. Lviv Univ. Biol. Ser. − 2010b. − **54**. − P. 257−264.

Mehrle P.M., Bergmann H.L. Biomarkers: Biochemical, Physiological, Histological Markers of Anthropogenic Stress. − Lewis: Boca Raton, 2002. − P. 211−234.

Mosin O.V. About the phenomenon of cellular adaptation to heavy water / Access mode: htth://www.gaudeamus.omskcity.com. 1996

Pachomova V.M. Substantive provisions of modern theory of stress and nonspecific adaptation syndrome at plants // Cytology. − 1995. − **37**(1/2). − P. 66−87.

Pathoanatomy and physiology / Ultrastructural pathology of cell / Ed. by N.G. Feroze / Access mode: http://www.nedug.ru/library/doc.aspx?item=34099. 1975.

Tiwari S.C., Polito V.S., Webster B.D. In dry pear (*Pyrus communis* L.) pollen, membranes assume a tightly packed multilamellate aspect that disappears rapidly upon hydration // Protoplasma. − 1990. − **153**(3). − P. 157−168.

Thewke D., Kramer M., Sinensky M.S. Transcriptional homeostatic control of membrane lipid composition // Biochem. Biophys. Res. Commun. − 2003. − **273**. − P. 1−4.

Veselova T.V., Veselovskiy V.A., Chernavskiy D.S. Stress at plants (Biophysical approach). − Moscow: RAS, 1993. − 144 p.

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АДАПТАЦИЯ СТРУКТУРЫ КЛЕТОЧНЫХ СТЕНОК *CHLORELLA VULGARIS* BEIJ. (*CHLOROPHYTA*) К ДЕЙСТВИЮ ИОНОВ ЦИНКА И СВИНЦА

Выявлены специфические и неспецифические ответы клеток и участие их мембранных структур в формировании сопротивляемости *Chlorella vulgaris* к действию ионов цинка и свинца. Обсуждается участие клеточных стенок в адаптации к токсикантам: образование наростов, мультипликация, флюидизация, формирование аквапоринов, апоптоз. Выявленные изменения в клеточных стенках предложено использовать в качестве биомаркеров токсичности.

Ключевые слова: ионы цинка и свинца, клеточная стека, *Chlorella vulgaris*.