Chemosphere 165 (2016) 329–334

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Characteristics of water and ion exchange of *Elodea nuttallii* cells at high concentrations of lanthanides

Vladimir N. Vorob'ev [a, b], Samat I. Mirziev [b], Evgeniy A. Alexandrov [b], Timur A. Sibgatullin [a, *]

[a] Kazan Institute of Biochemistry and Biophysics, Kazan Science Center, Russian Academy of Sciences, P.O. Box 30, Lobachevsky st. 2/13, Kazan, 420111, Russia
[b] Kazan (Volga) Federal University, Kremlevsky st. 16, Kazan, 420008, Russia

HIGHLIGHTS

- La, Nd and Lu stimulate the leakage of electrolytes from cells and have no effect on the water permeability of membranes.
- Lanthanide treatment resulted in the decreased water diffusive permeability of the membrane lipid bilayer.
- Lanthanides facilitate the change in the spontaneous curvature of the membrane lipid layer.

ARTICLE INFO

Article history:
Received 29 April 2016
Received in revised form
9 September 2016
Accepted 12 September 2016
Available online 30 September 2016

Handling Editor: Martine Leermakers

Keywords:
Membrane permeability
Lanthanides
Rare-earth elements (REE)
Aquaporins
NMR-Diffusometry
Elodea nuttallii

ABSTRACT

Changes of diffusive permeability of membranes of *Elodea nuttallii* cells following a short-term (60 min) treatment with high concentrations of lanthanides were recorded by the ^{1}H NMR-diffusometry and conductometry methods. The 1-h infiltration of segments of *Elodea nuttallii* internodes in 10 mM solutions of nitrates of La, Nd and Lu resulted in the increased leakage of electrolytes from cells, but has no effect on a water diffusive permeability of membranes. In samples subjected to a 30 min pretreatment with a water channel inhibitor $HgCl_2$ the water diffusive permeability of membranes (P_d) drops down under the influence of lanthanides, as well as an outcome of electrolytes. To explain the observed effects the change of spontaneous curvature of membrane lipid layer has been taken into consideration. The interaction of lanthanides with lipids of plasmalemma leads to the negative spontaneous curvature of lipid layer at which membrane channels are unclosed. Blocking of the ionic and water channels by mercury ions compensate the effect of change of spontaneous curvature of lipid layer.

1. Introduction

An increased application of lanthanides in industry and agriculture, and, hence, a growing extraction of rare earth element (REE) containing minerals leads to amplification of concentration of elements of this group in the environment (Kulaksız and Bau, 2013). The majority of REEs is adsorbed by soil particles, but approximately 10% remain solvable (Pang et al., 2001). These solvable lanthanides can migrate through soil, getting to ground waters, and invoking the pollution of rivers and lakes. The extensive use of gadolinium in medical researches has led to the increase of its concentration in lakes, drowned rivers, neritic and groundwater, and also in tap water (Bau et al., 2006; Kulaksız and Bau, 2013, 2007; Lawrence, 2010; Möller et al., 2002; Morteani et al., 2006; Rabiet et al., 2009).

Submersed aquatic plants, including *Elodea nuttallii*, play a key role in the functioning of freshwater ecosystems being natural sorbents of heavy metal ions (Jeppesen et al., 1998). The sorption capacity of cell walls of *Elodea nuttallii* causes a high tolerance of this plant to mercury, cadmium and lanthanum (Larras et al., 2013; Zhang et al., 2015). Biological effects of metals appear after filling of sorption capacity of cell wall and are caused by interaction of metals with cell membranes.

The main function of plasma membranes is the regulation of

* Corresponding author.
E-mail addresses: timsdance@mail.ru, sibgatullin@kibb.knc.ru (T.A. Sibgatullin).

http://dx.doi.org/10.1016/j.chemosphere.2016.09.044
0045-6535/

water-ion homeostasis of a cell. It is obvious that the secondary-water plants would firstly modify the permeability of plasma membrane in the 'above-ground' organs to conform to the ion change caused by the altering of their inhabitancy place. Mineral nutrition of submersed macrophytes in most cases is provided by whole surface of a plant, whereas the root system can function only as an anchoring organ. Secondly, they adapted water exchange processes to the absence of one of the main driving forces - the hydrostatic pressure created by transpiration. It is known that in the absence of a pressure gradient water flow through membrane is determined by osmosis and described by the following equation (Steudle, 1994; Zimmermann and Steudle, 1998):

$$Jw = P(C_{in} - C_{ex}) \quad (1)$$

where P – membrane permeability coefficient, C_{in} and C_{ex} – concentration of osmotically active molecules inside and outside the cell.

The intercellular water exchange depends on the amount and functional activity of water channel proteins, known as aquaporins, and the permeability of lipid bilayer of cell membrane (Li et al., 2014; Maurel, 1997; Maurel et al., 2015). Currently it is pointed out that lipids play an important role in regulation of cellular metabolism not only on the biochemical level, but also through the changes of physical parameters of membranes. It is known that an increased cholesterol content in membranes of animal cells has a negative influence on water conductivity of aquaporins which is caused by a decreased lateral mobility of lipids in membrane bilayer (Tong et al., 2013, 2012).

In the majority of researches the main indicator of biological activity of lanthanides is the disbalance of various biochemical processes - development of oxidative stress, weakening of photosynthetic pigments, disruption of absorption of nutrients (Zhang et al., 2015). But the change of water permeability of cellular membranes was out of focus. Therefore the main objective of the present work is the assessment of the effect of lanthanide-membrane interaction on the integral water permeability of cellular membranes of *Elodea nuttallii* and the revealing of the underlying mechanism.

2. Materials and methods

2.1. Plant material and metal treatment

Vegetative shoots of *Elodea nuttallii* were grown in 5% Hoagland-Arnon solution in controlled laboratory conditions (illumination intensity - 114 μmol/m^2/s, a 14 h photoperiod, 25°C/20°C day/night temperature). After removal of leaves the shoots were cut into segments (15–20 mm length) and vacuum infiltrated at a pressure of 10 Pa during 60 min in: a) a nutrient solution for a Control; or one of the following lanthanide-containing 10 mM solutions: b) $La(NO_3)_3$ (La^{3+} 1.4 g L^{-1}); c) $Nd(NO_3)_3$ (Nd^{3+} 1.44 g L^{-1}); d) $Lu(NO_3)_3$ (Lu^{3+} 1.74 g L^{-1}).

To inhibit the functioning of water conducting channels the segments were vacuum infiltrated in $HgCl_2$ 100 μM (Hg^{2+} 20 mg L^{-1}) solution for 30 min prior to the infiltration with one of the lanthanide-containing solution or nutrient solution. In such a case the resulted overall duration of infiltration was 90 min.

2.2. Determination of lipid peroxidation

The level of lipid peroxidation in the segments of shoots (0.2 g) was determined by thiobarbituric acid reaction according to Heath and Packer (1968) just after the infiltration in control or one of lanthanide solutions. The concentration of MDA was calculated using 155 mM cm^{-1} as extinction coefficient in terms of nmol g^{-1} fresh weight. Measurements were performed on spectrophotometer Unico-2800 UV/VIS (USA) at wave length of 532 nm, as well as at 600 nm for an adjustment of nonspecific absorption (Hodges et al., 1999).

2.3. Determination of barrier properties of membranes

The barrier properties of cellular membranes were assessed by the extent of electrolyte release from the 0.2 g segments of shoots (Kholodova et al., 2005). After infiltration the segments were shaken for 15 min in 10 ml of distilled water – to remove any extracellular electrolytes and remnants of the cells damaged upon cutting. The segments were quickly dried with filter paper and placed into clean tubes with 20 ml of deionized water (Millipore Milli-Q, Germany), with their subsequent incubation during 120 min at 20°C. The concentration of electrolytes after the incubation indicated the extent of membrane leak. To extract the remaining electrolytes, the sections were boiled for 30 min in a new portion of water and then shaken for at least an hour. Electroconductivity of extracts was measured using a PWT (HI 98308) conductometer (HANNA Instruments, Germany). The extent of membrane leak was calculated as a percentage of the electrolytes released after the 1st, 60-min, incubation to the total cellular electrolytes extracted.

2.4. Cytoplasmic streaming

The rotational protoplasmic motion was registered by detecting the chloroplast motion velocity. The method of synchronous tracking (Vorob'ev et al., 2002) was used to register the chloroplast motion. The measurements were carried out on the cells of the leaf rear layer where the central rib begins at room temperature. There were no any signs of plasmolysis detected under lanthanide treatment.

2.5. Determination of water permeability of membranes by pulsed field gradient NMR

After infiltration the segments were gently wiped with a filter paper. 20–25 arranged in parallel segments were placed into a glass test-tube for NMR measurements. Experiments were carried out at 25°C on the time-domain ^{1}H NMR-analyzer “Spin Track” (Resonance Systems Ltd., Yoshkar-Ola, Russia) operating at 19.1 MHz and equipped with the permanent magnet. A three-pulse stimulated echo sequence with pulsed field gradient was used to measure the translational diffusion coefficient of water (Tanner, 1970). The pulsed magnetic field gradient was applied perpendicularly to the test tube. Thus the water self-diffusion in a cross direction of the segments (radial transport) was observed.

During the experiments the attenuation of spin echo signal as a function of the strength of magnetic field gradient pulse (g) changing up to 3 T/m with fixed values of the pulse duration (δ) of 350 μs and the diffusion time t_d of 700 ms was registered. An initial part of signal attenuation plot (at $g \rightarrow 0$) was fitted with Eq. (2) resulting in a mean water diffusion coefficient (D)

$$A(g)/A(0) = \exp\left[-\gamma^2\delta^2 g^2 t_d D\right] \quad (2)$$

where $A(0)$ is the echo amplitude in the absence of the magnetic field gradient; γ is the proton gyromagnetic ratio.

At t_d equal to 700 ms, the displacement of water molecules becomes comparable with the transverse size of *Elodea nuttallii* internodal cells which was measured to be about 50 μm. Thus, the