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Effects of Selenite Ions on a Luminescence Enzymatic System

This paper elucidates biochemical and physicochemical aspects of toxicity of a redox-active compound in organisms. Sodium selenite (Na_2SeO_3) was chosen as a model redox-active compound; a coupled enzymatic system from luminous marine bacteria was applied to imitate a biochemical process. We demonstrated that Na_2SeO_3 suppressed bioluminescence of the enzyme system; the effective inhibition concentration was 10^{-2} M. Besides that, Na_2SeO_3 decreased the content of reactive oxygen species (ROS) in aqueous solutions (enzyme-free media) at $>10^{-3}$ M. Addition of enzymes and their substrates to these solutions enhanced this decrease. Correlations between dependencies of the ROS content and bioluminescence intensity on the concentration of Na_2SeO_3 were positive and high, confirming the ROS involvement in the bioluminescence suppression in Na_2SeO_3 solutions. Hence, we observed the disturbance of the native biochemical oxidative functions of dissolved oxygen derivatives under exposure to redox-active toxicants. The effects of Na_2SeO_3 on the bacterial enzyme system should be further compared with those on bacterial cells, which are traditionally used as a toxicity bioassay. Additionally, the use of natural microorganisms is perspective due to their ability of redox transformation of toxic selenium oxoanions to elemental selenium. This ability is important as it provides: (1) natural detoxification of water ecosystems, and (2) biosynthesis of selenium nanoparticles.

Keywords: selenite ions, ecological monitoring, toxicity mechanism, bacterial enzymatic assay, reactive oxygen species, redox processes, luminescence, enzymatic system, sodium selenite.

Introduction

Luminous marine bacteria are a basis of a well-known bioassay which is widely applied in ecological investigations. This method implies that the bacterial luminescence is a physiological function inherent in this microorganism; and, hence, the bioluminescence intensity can be used as a quantitative characteristic of environmental toxicity. Due to a high sensitivity, simplicity, and high rates of analyses, the bacterial bioassay has been used for more than 60 years for toxicity monitoring [1–4]. One more important advantage of the bioluminescence assay is concerned with detailed investigation of effects of various compounds on the bacteria and related systems, e.g., enzymatic reactions, cellular membranes, etc. [5–7].

Study of effects of toxic compounds on enzymatic reactions elucidates the mechanisms of toxic impacts on living organisms.

Application of enzymatic bioluminescent reactions is a fairly new direction in toxicology. In 1990, an enzyme system of bacterial reactions was proposed as a bioluminescence toxicity assay [8]. This bioassay is based on the system of coupled enzymatic reactions catalyzed by enzymes — bacterial luciferase and NAD(P)H:FMN-oxidoreductase:

$$NADH + FMN \xrightarrow{\text{NAD(P)H:FMN-oxidoreductase}} NAD^+ + FMNH^-, \quad (1)$$

$$FMNH^- + RCHO + O_2 \xrightarrow{\text{luciferase}} FMN + RCOO^- + H_2O + h\nu\,(490\text{ nm}). \quad (2)$$

The enzyme system is sensitive to redox-active compounds and depends on their concentration and redox parameters [5, 6, 9]. Both of the bioluminescence assays, enzymatic and bacterial, were previously applied to characterize antioxidant [10–14] and prooxidant [15–16] effects of bioactive compounds, as well as to reveal the activity of reactive oxygen species (ROS) in these effects.

ROS are a group of compounds that contain oxygen with unpaired electrons. The group of ROS includes, but is not limited to, the following compounds: superoxide anion radicals ($O_2\cdot-$), hydrogen peroxide (H_2O_2), hydroxyl radical ($\cdot OH$), singlet oxygen ($^1O_2$), hypochlorite (HOCl), hydroperoxyl radical ($HOO\cdot$), and others. ROS are involved into physiological reactions in organisms, and thereby they are always present in physiological concentrations in organisms [17, 18]. ROS serve as multifunctional compounds in organisms: they can act as pleiotropic signaling agents [19], apoptosis factors [20–23], amplifiers of the cytotoxic

effect of drugs in tumor cells [24, 25]. The formation of ROS occurs both in pathological and physiological conditions. Processes of oxidation of biological molecules are usually accompanied by the ROS generation [26, 27]. At concentrations above the physiological levels, ROS are highly toxic for biological systems, as they initiate degradation of nucleic acids, structural proteins, and lipids of cell membranes [28]. Hence, the physiological role of ROS in cellular processes is essential; ROS are involved in natural functions of biological systems, being produced or utilized in metabolic processes. Maintaining the ROS balance is a vital function of organisms. However, ROS can suppress physiological functions under the conditions of their excessive production. In solutions of redox-active exogenous compounds, ROS initiate primary physicochemical processes followed by changes of rates of various intracellular processes, including bioluminescence reactions. Therefore, a role of ROS in bioluminescence enzymatic systems (reactions 1 and 2) exposed to redox-active compounds is of interest.

Bioluminescence assay systems, both enzymatic and cellular, have not been applied yet to study bioeffects of selenium compounds. Complex functions of selenium in natural environments are well-known: selenium is a vital microelement at low concentrations and a highly toxic element at higher concentrations. On the one hand, selenium is a component of enzymatic processes, it protects cells from oxidative stress. On the other hand, an acute toxicity is inherent in selenium oxoanions — selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}). An excessive selenium content in aquatic environments leads to various defects, disorders of the immune system, in the development of embryos in juvenile and adult fish, as well as to a decrease in fertility. Excessive selenium disrupts the stability of aquatic ecosystems and has a counterproductive effect on fish farming [29–31]. The consequences of excessive selenium for humans are heart disease, dermatitis, joint pain, and brittle nails [32].

Natural selenium pollution occurs due to weathering of selenium-rich soils, rocks, and volcanic eruptions. Additionally, selenium is released during industrial activities — oil production, metal smelting [33], burning of fossil fuels, mining, and the use of nuclear fuel. Selenium enters the environment in the form of oxides, then they are hydrolyzed and dissolved in water bodies in the form of oxoanions.

There exists a biogeochemical cycle of selenium compounds in nature, and microorganisms play a key role in this cycle. Bacteria can reduce selenium oxoanions to elemental selenium and form selenium nanoparticles. Bacterially synthesized selenium nanoparticles can be used as food additives, drug delivery agents, as well as adsorbents in the human body. Selenium nanoparticles have an advantage over metal-containing nanoparticles, due to potential inactivation of metal ions [34]. Properties of bacterially synthesized selenium nanoparticles have intensively been studied [34–42].

The current work aimed to study the effects of sodium selenite (Na_2SeO_3) on the bioluminescence system of enzymatic reactions that is responsible for glowing of luminous marine bacteria. We analyzed changes in the ROS content and bioluminescence intensity under exposure to Na_2SeO_3; the exposure time and selenite concentration were varied. Priority attention was paid to redox processes in the bioeffects of Na_2SeO_3.

Experimental

Bioluminescence enzymatic assay

Bioluminescence enzymatic assay was applied to evaluate the inhibition ability of Na_2SeO_3 in biochemical processes. The composition and construction of this system are presented in section "Bioluminescence Enzymatic Assay" in [16].

Luminol chemiluminescence assay

To determine the role of ROS in the inhibition ability of Na_2SeO_3, the ROS content was studied in the absence and presence of Na_2SeO_3; distilled water and bioluminescence enzyme system solutions were used as media for the measurements. The chemiluminescence intensity was registered just after the bioluminescence signal detection. The ROS concentration in the experimental samples was evaluated using a calibration curve [16].

Reagents for this assay are presented in section "*Luminol Chemiluminescence Assay*" in [16].

A 3×10^{-5} M alkaline luminol solution was applied. The chemiluminescence reaction was initiated by 1.2×10^{-4} M $K_3[Fe(CN)_6]$.

Measurements of bioluminescence/chemiluminescence intensities and statistical processing

A biochemiluminometer Luminoskan Ascent (Thermo Electron Corporation, USA) was applied to carry out all luminescence measurements at 25 °C [16]. Maximal values of chemi- and bioluminescence intensities

were registered and analyzed. All solutions of Na_2SeO_3 were "non-colored" and do not have an effect of "optic filter" [16]. All measurements were carried out in 5–10 replicates with an injector system. I^{rel} and ROS^{rel} were calculated as described in [16]. The SD values for I^{rel} and ROS^{rel} did not exceed 0.2 (GraphPad Prism 8, GraphPad Software, Inc., USA).

Correlation coefficients r between I^{rel} and ROS^{rel} were calculated [16, 43].

Results and Discussion

Bioluminescence kinetics was registered in solutions of sodium selenite, Na_2SeO_3. Figure 1 presents the time courses of bioluminescence intensity at different concentrations of Na_2SeO_3, during 120 min of observation. It can be seen that lower concentrations of selenite ($<5\times10^{-3}$ M) do not significantly change the bioluminescence intensity, while higher concentrations inhibit it. The results of the same experiment are presented in Figure 2A as the bioluminescence intensity, I^{rel}, vs. the concentration of Na_2SeO_3. The absence of the effect (I^{rel} is close to 1) and higher-concentration inhibition ($I^{rel}<1$) are evident from this Figure as well. The concentration at which bioluminescence was inhibited by 50 % appeared to be ca. 10^{-2} M for all times of exposure to Na_2SeO_3.

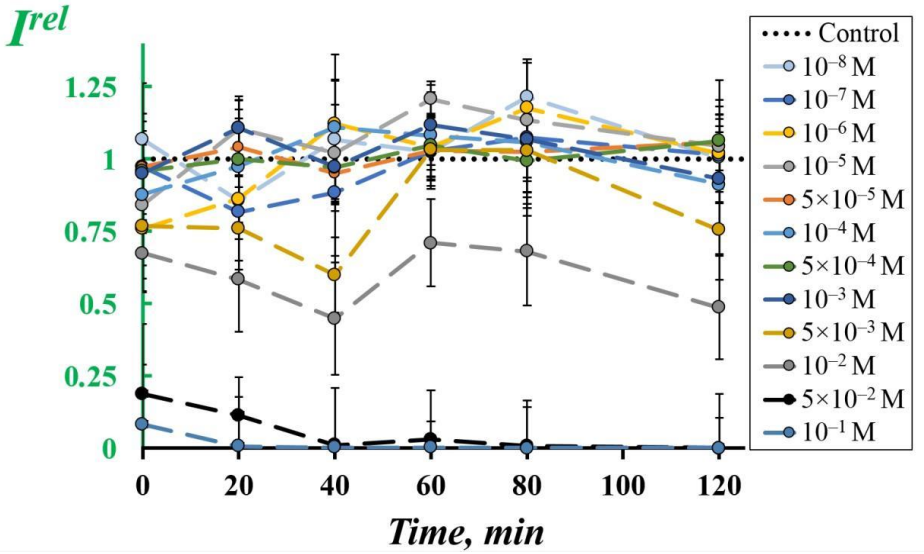

Figure 1. Relative intensity of bioluminescence of the enzyme system, I^{rel}, at various concentrations of Na_2SeO_3. "Control" — in the absence of Na_2SeO_3

The reason why the low-concentration interval of Na_2SeO_3 was analyzed is related to our intention to compare effects of Na_2SeO_3 on the enzymatic system and bacterial cells (the latter result has not been published yet) at similar conditions, as well as to conclude on the involvement of enzymatic processes in bioeffects of Na_2SeO_3 in cellular systems. It is known [5–8] that bacterial cells are often more sensitive to toxic compounds than enzyme reactions due to the involvement of additional processes and structures (cell membranes and others).

We analyzed the content of ROS, ROS^{rel}, in the solutions of the enzyme preparation and compared it to the bioluminescence intensity in those solutions, I^{rel} (Fig. 2B and Fig. 2A, respectively).

Figure 2B presents the ROS content in the absence and presence of the enzyme preparation. The dependence of the ROS content on the concentration of Na_2SeO_3 in enzyme-free solutions is presented with a dark-red curve. It can be seen that Na_2SeO_3 does not significantly affect the ROS content at $<10^{-3}$ M and suppresses it ($ROS^{rel}<1$) at higher concentrations. The suppression can be explained by oxidation of selenite to selenate (i.e., SeO_3^{2-} to SeO_4^{2-}); ROS can serve as oxidizing agents in this process.

It is evident from Figure 2B that the addition of the enzyme system to Na_2SeO_3 (enzyme-free) solutions decreases, as a rule, the ROS level at all Na_2SeO_3 concentrations. This effect could be explained by ROS consumption in the oxidative reaction catalyzed by bacterial luciferase (reaction 2) [44, 45].

To confirm the involvement of ROS in bioluminescence inhibition, we calculated correlation coefficients r between the dependencies of ROS^{rel} and I^{rel} on the concentration of Na_2SeO_3 (Fig. 2A and Fig. 2B). The r-values appeared to be positive and of high value: 0.88, 0.72, 0.89, 0.81, 0.82, and 0.87 at times of ex-

posure: 0, 20, 40, 60, 80, and 120 min, respectively. This result indicates that a lack of ROS in the solutions of Na_2SeO_3 initiates bioluminescence inhibition; the result confirms that ROS participate directly in the bioluminescence enzymatic reactions. Similar conclusions were made earlier using other bioactive compounds [12, 13, 16, 46].

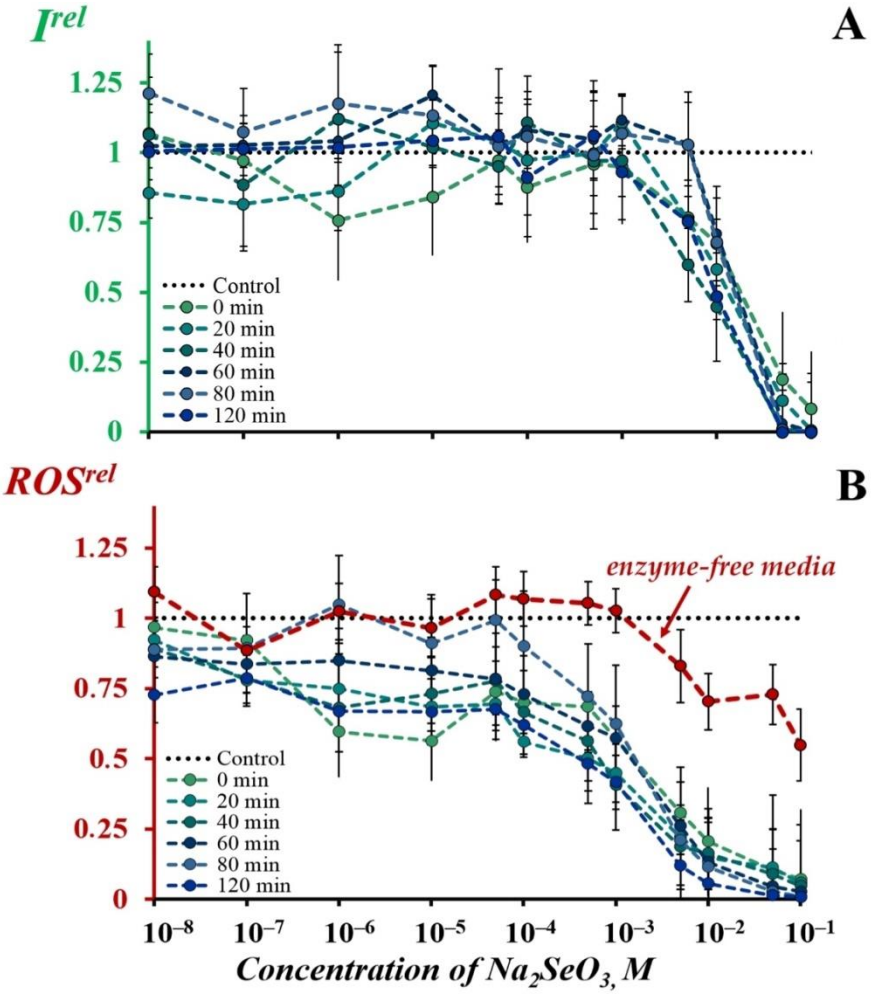

Figure 2. The dependence of relative bioluminescence intensity, I^{rel} (A,) and relative ROS content, ROS^{rel} (B), on Na_2SeO_3 concentration in enzymatic solutions. "Control" was measured in the absence of Na_2SeO_3. The content of ROS in the control enzyme solution was 4.6×10^{-6} M, in enzyme-free solution — 4.7×10^{-7} M

Therefore, bioluminescence of the enzymatic system from a luminous marine bacterium is sensitive to Na_2SeO_3. The inhibition activity of Na_2SeO_3 is characterized by the effective concentration $EC_{50} = 10^{-2}$ M. Na_2SeO_3 decreases the ROS content in aqueous solutions (enzyme-free media) at $>10^{-3}$ M. Addition of enzymes and their substrates to these solutions enhanced this decrease. Correlations between the dependencies of ROS^{rel} and I^{rel} on the Na_2SeO_3 concentration were found, confirming the ROS involvement in the bioeffects of Na_2SeO_3 on the bacterial enzymatic system.

Conclusions

The purpose of this article is related to the biochemical aspect of the toxicity of redox-active compounds. Sodium selenite (Na_2SeO_3) was chosen as a model of an inorganic redox-active compound due to its toxicity in the environment and the ability of microorganisms to biotransform it into neutral selenium. The enzymes from luminous marine bacteria were applied to imitate a biochemical process. Inhibition of the enzymatic activity by Na_2SeO_3 was evaluated and related to the activity of reactive oxygen species (ROS) in aqueous media, thus demonstrating the disturbance of vital oxidative functions of dissolved oxygen derivatives under exposure to redox-active toxicants. Hence, a physicochemical approach to the study of the toxic effects of redox compounds in organisms was presented.

Our further research will be aimed at studying the effect of Na_2SeO_3 on the bacterial cells and comparing its effect on bacterial enzymes. In general, the importance of bacterial biotransformation of toxic selenium oxoanions into elemental selenium is due to the fact that it provides detoxification of natural aquatic ecosystems contaminated with selenium compounds. Additionally, bacterial biosynthesis of selenium nanoparticles is very promising for biotechnology, biomedicine, pharmacology, dietetics and electronics.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. CRediT: **Ekaterina Sergeevna Sushko** methodology, investigation, formal analysis, visualization, writing-review & editing; **Andrei Valerievich Zenkov** investigation, formal analysis, visualization, writing-review & editing; **Nadezhda Stepanovna Kudryasheva** conceptualization, formal analysis, funding acquisition, project administration, supervision, validation, writing-original draft, writing-review & editing.

Conflicts of Interest

The authors declare no conflict of interest.

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