Comparison of chronic low-dose effects of alpha- and beta-emitting radionuclides on marine bacteria

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Abstract: Effects of Americium-241 (²⁴¹Am), alpha-emitting radionuclide of high specific radioactivity, and tritium (³H), beta-emitting radionuclide, on luminous bacteria Photobacterium phosphoreum were compared. Bioluminescence intensity served as a marker of bacterial physiological activity. Three successive stages in the bioluminescence response to ²⁴¹Am and ³H were found under conditions of low-dose irradiation: (1) absence of effects, (2) activation, and (3) inhibition. They were interpreted in terms of bacterial response to stress-factor as stress recognition, adaptive response/syndrome, and suppression of physiological function (i.e. radiation toxicity). Times of bioluminescence activation (TBA) and inhibition (TBI) were suggested as parameters to characterize hormesis and toxic stages in a course of chronic low-dose irradiation of the microorganisms. Values of TBA and TBI of ²⁴¹Am were shorter than those of ³H, revealing higher impact of alpha-irradiation (as compared to beta-irradiation) under comparable radiation doses. Increases of peroxide concentration and NADH oxidation rates in ²⁴¹Am aquatic solutions were demonstrated; these were not found in tritiated water. The results reveal a biological role of reactive oxygen species generated in water solutions as secondary products of the radioactive decay. The study provides a scientific basis for elaboration of bioluminescence-based assay to monitor radiotoxicity of alpha- and beta-emitting radionuclides in aquatic solutions.

Keywords: Luminous bacteria • Am-241 • Tritium • Hormesis • Radiotoxicity • Peroxides

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1. Introduction

Microorganisms are the simplest and most basic part of the biosphere, and their physiological indices can serve as indicators of the state of the biosphere on the whole. Hence, microorganisms can be used as bioassays to monitor environmental toxicity, including radiotoxicity. These bioassays are humane, as they do not apply to high organisms. Regularities of ionizing radiation influence on the microorganisms can be applied to analyze the radioactive impact on higher organisms.

The assay system based on luminous marine bacteria is a good candidate for radiotoxicity monitoring. This assay was described in its current form in 1969 [1]. In the late 1980s, the test was standardized in Germany as a method for pollutant detection. Later, it was modified by different researchers and adapted for their specific purposes [2-6]. Now, the bacterial bioluminescent assay is a traditional and important biotechnological application of the bioluminescence phenomenon [7-11]. The tested physiological parameter here is luminescence intensity that can be easily measured instrumentally. The advantages of the bioluminescent assays are their high sensitivity, simplicity, rapidity (1-3 min), and availability of devices for toxicity registration [12].
For the first time, the bacterial bioluminescence was used to monitor radiation toxicity in [13]. In this work, the effect of gamma-ray irradiation on a recombinant Escherichia coli strain was studied. Later, the bacterial bioluminescence was used to monitor toxicity of alpha-emitting [4,5,14] and beta-emitting [15] radionuclides.

Radiotoxicity of alpha-emitting radionuclides of high and low specific radioactivity was compared [14] with Americium-241 and Uranium-(235+238) taken as examples, respectively. The effect of Uranium-(235+238) on luminous bacteria was attributed to a chemical component of its impact, not a radiation component.

The effects of alpha- and beta-emitting radionuclides have not yet been compared. Americium-241 and tritium are the examples of radionuclides of these types, respectively.

Americium-241 (241Am), alpha-emitting radionuclide of high specific radioactivity, is a by-product of plutonium radioactive decay. It is known to be increasingly accumulated by the environment. Observations in the waters of the Chernobyl zone contaminated with radioactive fallout demonstrated that aquatic plants accumulate 241Am in their biomass in a species-specific manner [16]. Accumulation of Am-241 by sediments and aquatic plants in the Siberian river Yenisei is currently under study [17-19].

Tritium (3H), a beta-emitting radionuclide, is permanently generated by space radiation at the rate of 1200 atoms s⁻¹ m⁻² in the top layers of Earth’s atmosphere [20]. In the environment, it is mostly presented as a component of tritiated water (HTO). Until the 1950s, the tritium concentration in natural waters was low – one tritium per 10¹⁸ hydrogen atoms. However after atmosphere nuclear tests it increased 1000-fold. Since a half-life of tritium is 12.32 years, its concentration eventually decreased, though local rise of tritium content took place around nuclear power plants. Local nuclear incidents increase tritium concentration dramatically. In the future, controlled fusion reactors can bring an additional threat of contamination with tritium.

Tritium is considered to be one of the less dangerous isotopes. Maximal energy of its beta-particles is low (18.59 keV) and the maximal range of their path is short (5.8 mm at 20°C). Beta-particles are entirely absorbed by the skin’s surface layers; this is why tritium is not a radiation component. Tritium is capable of penetrating into cell organelles and can substitute hydrogen (protium) atoms in organic molecules. This is why tritium irradiation can produce local damage. Tritium specific ionization ability (2.2×10⁶ ions per cm) exceeds that of other beta-emitting radionuclides. Hence, tritium toxicity is a challenging problem for researchers working in related fields [21,22].

Due to the low energy of beta-decay, tritium presents a convenient subject for studying a protective response of organisms to low-dose radiation. Radiation hormesis (intensifying of physiological functions of organisms under low-dose irradiation) can be considered as an illustration of the protective response. Understanding molecular mechanisms of radiation hormeses and radiation toxicity is important for ecological and radiobiological investigations.

Bioluminescent kinetics was already studied in solutions of Am(NO₃)₃ [4,5,14], tritium-labeled aminoacid valine [23], and in tritiated water, HTO [15]. Reactive Oxygen Species (ROS), produced as secondary products of radioactive decay in aquatic solutions, were suggested to be responsible for biological effects of 241Am [24]. An increase in the content of beta-structured proteins in bacterial cells exposed to HTO was demonstrated by diffuse reflectance FTIR spectroscopic studies in [25,26]. These changes were interpreted in terms of the stress response of the bacterial cells to the low-dose chronic radioactivity. Similar changes were not found in the cells exposed to 241Am [27].

The present paper aimed at comparison of low-dose effects of alpha- and beta-emitting radionuclides under comparable radiation doses delivered to luminous marine bacteria; 241Am and 3H were chosen as representatives of alpha- and beta-emitting radionuclides, respectively. Bioluminescence kinetic parameters (activation and inhibition times) in solutions of the radionuclides are compared; the role of peroxides and stability of endogenous reducer (NADH) are under consideration.

2. Materials and Methods

Luminous bacteria Photobacterium phosphoreum from the Collection of the Institute of Biophysics SB RAS (CCIBSO 863), strain 1883 IBSO, were applied. Nutrient media used for bacterial growth included: 1 L H₂O, 30 g NaCl, 1 g KH₂PO₄, 12H₂O, 0.5 g (NH₄)₂HPO₄, 0.2 g MgSO₄·7H₂O, 6 g NaH₂PO₄, 3 ml glycerol, and 5 g peptone. Reagents included potassium ferricyanide, 3% H₂O₂ solution, luminol (Molecular probes Inc.), and NADH (ICN, USA). Solutions of 241Am(NO₃)₃ and tritiated water (HTO, radiochemical purity 98%) were used as sources of ionizing radiation.

Bacteria were grown in 30 ml nutrient media with the radionuclides involved. Specific radioactivities (A) of HTO were 0.0002, 0.002, 0.02, 0.2, 2, 10, 20, 50, 100, and 200 MBq L⁻¹. Specific radioactivities of 241Am(NO₃)₃ solutions were 0.2, 0.3, 0.7, 1.7, 3.0, 6.7 kBq L⁻¹.
Bioluminescence intensity ($I$) was measured in bacterial suspensions sampled at two growth stages: 15, and 22 h. The samples were placed into microplates; the time course of $I$ was measured in solutions of 3% NaCl at $+20^\circ$C [15,24]. Between the measurements, microplates with the radionuclides and control samples were kept under the same conditions of temperature (4°C). Bioluminescent intensities of control and radioactive samples were examined and compared. The measurements were carried out in four replications; SD values were calculated.

Figure 1 presents bacterial bioluminescent kinetics in control and radioactive samples. Radioactive media with HTO was taken here as an example. Specific radioactivity (A) of tritium ($^{3}$H) in HTO were 2 MBq L$^{-1}$.

Relative bioluminescent intensity $I_{rel}$ was calculated as:

$$I_{rel} = \frac{I_{rad}}{I_{contr}}$$

where $I_{rad}$ and $I_{contr}$ are bioluminescent intensities in radioactive and control samples, respectively. Parallel measurements of bioluminescent intensity were performed in quadruplicate; mean values of $I_{rel}$ with standard errors were plotted as a function of time. The data were processed by program equipment Mikrowin 2000.

Peroxide component in solutions of $^{241}$Am(NO$_3$)$_3$ and in HTO was determined by chemiluminescent method [28]. Luminol chemiluminescence was triggered by addition of potassium ferricyanide. Bioluminescent and chemiluminescent intensities were measured by TriStar Multimode Microplate Reader LB 941, Berthold Technologies. Optical density of bacterial suspension was registered by colorimeter KFK-2MP, Russia.

To study the rates of NADH oxidation, the optical density, $D$, of 1.3·10$^{-4}$ M L$^{-1}$ NADH solutions was registered at 340 nm by double-beam spectrophotometer UVIKON-943 (KONTRON Instruments, Italy).

3. Results and Discussion

3.1. Bioluminescent kinetics in solution of $^{241}$Am(NO$_3$)$_3$ and in HTO.

Bioluminescence kinetics of the bacteria was studied in aqueous media in the presence of HTO and Am(NO$_3$)$_3$.

Figure 2 presents $I_{rel}$ vs. time of the bacteria exposure to the radionuclides. The 10 MBq L$^{-1}$ of $^{3}$H and 0.3 kBq L$^{-1}$ of $^{241}$Am specific radioactivities (A) were chosen here to demonstrate the changes in bioluminescence kinetics.

Referring to the Figure, the exposure to both radionuclides resulted in three successive stages of bioluminescence time-courses: (1) absence of the effect ($I_{rel} \approx 1$), (2) bioluminescence activation ($I_{rel} > 1$), and (3) bioluminescence inhibition ($I_{rel} < 1$).

The stages of bioluminescence activation in Figure 2 can be ascribed to hormesis phenomenon [29-31]. Activation of vital functions of various organisms is a well-known effect, common to all living organisms. Hormesis is attributed to triggering of cell defense response under the influence of low concentrations of toxic compounds, low dose radiation, and other stressors. Examples of hormesis are nonspecific adaptive syndrome of plants [32] and stress reaction in animals [33].

The stages of bioluminescence inhibition in Figure 2 are evidence of suppression of the physiological function of the bacteria by the radionuclides, i.e. toxic effect of the radioactive media to the microorganisms.

![Figure 1](image_url). Bioluminescence intensity, $I$, of *Photobacterium phosphoreum* vs. time of exposure to $^{3}$H (2 MBq L$^{-1}$). Control sample – 15-h bacterial culture without radionuclides.
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The times at which bioluminescence reached the activation and inhibition stages are marked in Figure 2 as TBA (Time of Bioluminescence Activation) and TBI (Time of Bioluminescence Inhibition), respectively. The TBA\textsubscript{Am} and TBI\textsubscript{Am} were 6 h and 19 h. The TBA\textsubscript{T} and TBI\textsubscript{T} were 24 h and 59 h.

The doses of appr. 0.0004 Gy were delivered by \(^{241}\text{Am}\) to the bacterial cells by TBA\textsubscript{Am}, and 0.001 Gy – by TBI\textsubscript{Am}. The doses of appr. 0.0005 Gy were delivered by \(^{3}\text{H}\) to bacterial cells by the TBA\textsubscript{T} and 0.0015 Gy – by TBI\textsubscript{T}. These values are much lower than a tentative limit of a low-dose interval (0.1 Gy, as reported for tritium in [34,35]). Hence, first and second stages in bioluminescence kinetics (threshold effect and bioluminescence activation, Figure 2) are attributed to low-dose effects.

The calculations show that the doses delivered to bacteria by the time of bioluminescence activation/inhibition are comparable for \(^{241}\text{Am}\) and \(^{3}\text{H}\). However, as seen from Figure 2, the values of TBA and TBI are lower for \(^{241}\text{Am}\), i.e. its effects were observed earlier than for \(^{3}\text{H}\). This difference might be explained by peculiarities of alpha- and beta- irradiation – different secondary products of radioactive decays in aquatic \(^{241}\text{Am}\) solutions and in HTO.

The question of our interest deals with the molecular mechanism of bioluminescence activation and inhibition under exposure to radiation. Bioluminescence of bacteria is considered here as a simple model process for understanding activation and inhibition of physiological functions of organisms.

Radiolysis of water is a known process occurring under ionizing radiation [37,38]. Secondary products of ionizing radiation (ions and radicals) can affect water ecosystems and their inhabitants. Peroxide compounds are an important group among the secondary products of ionizing radiation. With regard to metabolic processes, peroxides are intermediates in a series of metabolic reactions, mainly, oxidation of organic

**Figure 2.** Dependence of relative bioluminescent intensity \(I^\text{rel}\) on time of exposure to \(^{3}\text{H} (A = 10 \text{ MBq L}^{-1})\) and \(^{241}\text{Am} (A = 0.3 \text{ kBq L}^{-1})\).
compounds. Depending on concentrations, they can either activate or inhibit the metabolic processes [39,40]. It was hypothesized [24] that ROS can contribute to activation and inhibition of the bioluminescence function of luminous bacteria; the effects of the hydrogen peroxide on luminous bacteria \( P.\text{phosphoreum} \) were compared to those of \( ^{241}\text{Am(NO}_3\text{)}_3 \), thus elucidating the role of peroxides in activation and inhibition of the bioluminescence. Peroxide content was determined in \( ^{241}\text{Am} \) solutions using chemiluminescent luminol method for the first time in [24].

To compare the role of ROS in bioluminescence kinetics changes in solutions of \( ^{241}\text{Am(NO}_3\text{)}_3 \) and HTO, the peroxide concentrations were compared in the radionuclide solutions of different specific radioactivities (Figure 3).

One can see that the increase of the peroxide concentration follows the rise of \( ^{241}\text{Am} \) specific radioactivity. At \( A^{(241}\text{Am}) = 5 \text{ kBq L}^{-1} \), the concentration of peroxides exceeds their background concentration up to 18 times. Probably, the increase of peroxide concentration takes place in solutions of all alpha-radionuclides of high specific radioactivity.

Hence, the peroxides produced in the solution of \( ^{241}\text{Am} \) can be a reason of the bioluminescence kinetics changes shown in Figure 2. Thus, the data presented in Figure 3 support the hypothesis [24] on the involvement of peroxides, secondary products of alpha-decay of \( ^{241}\text{Am} \) in water solutions, into functioning of bioluminescent system of luminous bacteria.

Bioluminescence activation by peroxides is supposed to deal with the increase of rates of metabolic oxidative reactions. One of them is the bioluminescence reaction which includes a peroxide compound (peroxymhiacetal) as an intermediate [41]. The excess amount of reaction components is known to inhibit all the reactions and quench electron-excited states of fluorofores [42], thus inhibiting bioluminescence and other metabolic processes. Previously in [43], activation and inhibition of bacterial luminescence by hydrogen peroxide was also reported, with \( \text{Vibrio harveyi} \) strain as an example.

However, one can see (Figure 3) that the increase of HTO specific radioactivity does not result in the rise of peroxide concentration. Hence, bioluminescence activation and inhibition by beta-emitting radionuclide, tritium (Figure 2), is not concerned with peroxide formation in water solutions. Probably, another type of particles, hydrated electrons, can be responsible for the change of bioluminescence intensity, too. They can be involved in the electron transfer chain of coupled metabolic redox reactions in organisms, thus increasing or decreasing the rates of metabolic processes.

### 3.3. Effects of radionuclides on endogenous reducer NADH.

NADH, organic reducer, is a component of many biochemical reactions including those of the bacterial bioluminescent enzyme system [44]. Change of rate of NADH oxidation under influence of exogenous compounds serves as an indicator of intensification (or slowdown) of redox processes in the assay system. Effects of \( ^{241}\text{Am} \) and \( ^3\text{H} \) on NADH solutions were studied.

Figure 4 presents the decays of NADH concentration in the solutions of \( ^{241}\text{Am(NO}_3\text{)}_3 \). The slight decay of NADH concentration in the control sample is concerned with NADH autooxidation in aqueous solutions in the presence of molecular oxygen [45].

The rates of NADH oxidation \( (V) \) in the control sample and in the presence of the radionuclides were calculated and presented in Table 1. The exposure to \( ^{241}\text{Am} \) increased the rate of NADH oxidation, probably.

![Figure 3. Comparison of peroxide concentrations in solutions of Am(NO_3)_3 and HTO. A - specific radioactivity.](image-url)
Comparison of chronic low-dose effects of alpha- and beta-emitting radionuclides on marine bacteria due to ROS accumulation in the solutions. HTO did not change the rates as compared to the control sample (Table 1).

The intensification of the NADH oxidation by $^{241}$Am can contribute to bioluminescence inhibition under exposure to this radionuclide (Figure 2). However, bioluminescence inhibition by HTO (Figures 1, 2) is not concerned with NADH concentration change.

4. Conclusion

Recent years have seen a change in the approach in radioecological studies: biota in toto is considered now as a target of radiation impact, with the human included as a part of biota and integrated into the biosphere by a multiplicity of functional interrelations. As microorganisms are the most basic and numerous part of biosphere, their physiological indices are useful for monitoring the state of the biosphere on the whole. This paper demonstrates advantages of luminous marine bacteria as a bioassay for monitoring microorganisms’ responses to low-dose radiation of alpha- and beta-emitting radionuclides. Additionally, due to advanced experience in effects of exogenous compounds on luminous bacteria and their enzyme reactions, the bacteria is a convenient tool for study physico-chemical mechanisms for toxic and hormesis effects of the radionuclides.

This paper compares the effects of Americium-241 ($^{241}$Am), alpha-emitting radionuclide of high specific activity, and tritium ($^3$H), beta-emitting radionuclide, on luminous bacteria Photobacterium phosphoreum under the conditions of low-dose irradiation. Bioluminescence intensity serves as a marker of physiological activity of the bacteria. Three successive stages in the bioluminescence response to $^{241}$Am and $^3$H were found: (1) absence of the effect, (2) activation, and (3) inhibition. They were interpreted in terms of reaction of organisms to stress-factor i.e. stress recognition, adaptive response/syndrome, and suppression of physiological function. Since the term “toxicity” is defined as a suppression of biological functions of organisms, the third stage can be attributed to radiation toxicity.

Hence, the bacterial response to the alpha- and beta-emitting radionuclides under conditions of low-dose irradiation is unified.

However, quantitative characteristics of the bioluminescent kinetics in solutions of $^{241}$Am and $^3$H appeared to be different under similar radiation doses delivered to the bacteria. Times of bioluminescence activation (TBA) and inhibition (TBI) were suggested as parameters to characterize hormesis and toxic stages in a course of chronic low-dose irradiation of microorganisms. The values of TBA and TBI of $^{241}$Am were shorter than those of $^3$H, revealing higher impact of the alpha-irradiation (as compared to the beta-
irradiation) under similar doses delivered to the bacteria.

Additionally, the paper compares the role of radioactive decay products in biological effects of alpha- and beta-emitting radionuclides. Increases of peroxide concentrations and rates of NADH (i.e. endogenous reducer, component of bacterial bioluminescent enzyme system) oxidation under exposure to $^{241}$Am were demonstrated; these were not found under exposure to $^3$H. The result attributes the biological effects of $^{241}$Am to reactive oxygen species generated in water solutions as secondary products of the radioactive decay.

Role of membrane processes, possible genetic mutations, composition and structure of intracellular components, in biological effects of alpha- and beta-emitting radionuclides are questions of our further interest.

The study provides a scientific basis for adaptation the bioluminescent bacteria-based assay for monitoring the radiotoxicity of alpha- and beta-emitting radionuclides in aquatic solutions.

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