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Is bacterial luminescence response to low-dose radiation associated with mutagenicity?

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ABSTRACT

Luminous marine bacteria are widely used in bioassays with luminescence intensity being a physiological parameter tested. The purpose of the study was to determine whether bacterial genetic alteration is responsible for bioluminescence kinetics change under low-dose radiation exposure. The alpha-emitting radionuclide ^{241}Am and beta-emitting radionuclide ^{3}H were used as the sources of low-dose ionizing radiation. Changes of bioluminescence kinetics of *Photobacterium phosphoreum* in solutions of $^{241}Am(NO_3)_3$, 7 kBq/L, and tritiated water, 100 MBq/L, were studied; bioluminescence kinetics stages (absence of effect, activation, and inhibition) were determined. Bacterial suspension was sampled at different stages of the bioluminescent kinetics; the doses accumulated by the samples were close or a little higher than a tentative limit of a low-dose interval: 0.10 and 0.85 Gy for ^{241}Am, or 0.11 and 0.18 Gy for ^{3}H. Sequence analysis of the 16S ribosomal RNA gene did not reveal a mutagenic effect of low-dose alpha and beta radiation in the bacterial samples. Previous results on bacterial DNA exposed to low-dose gamma radiation (0.25 Gy) were analyzed and compared to those for alpha and beta irradiation. It is concluded that bioluminescence activation and/or inhibition under the applied conditions of low-dose alpha, beta and gamma radioactive exposure is not associated with DNA mutations in the gene sequences tested.

1. Introduction

In recent years, there has been a growing interest in low-dose radiation impacts on the environment related to the escalating use of radioactive elements and concern about the increase of background radiation. Moreover, there has been a change in the radiobiological approach: investigations have become primarily targeted towards the environment as a whole with humans included as part of it. This explains the attention paid to microorganisms which are an essential part of the biosphere. In particular, their physiological responses to external exposures are widely used for monitoring environmental toxicity including radiation toxicity. This is evident in the case of luminous marine bacteria, used as a convenient tool in radiobiological and radioecological investigations. Bioluminescence intensity of the bacteria is the major parameter tested which can be easily measured with simple physics devices. The simplicity of the registration procedure is beneficial because it enables researchers to conduct a large number of experiments under comparable conditions ensuring adequate statistical treatment of the results. Over recent decades, bioluminescent bacteria-based assays have been widely applied for toxicity monitoring in water media including the effects of low-dose radiation (Roda et al., 2009; Girotti et al., 2008; Kudryasheva and Tarasova, 2015; Kudryasheva and Rozhko, 2015).

Radiosensitivity of organisms is usually evaluated as a dose-effect relationship, but there is a considerable uncertainty concerning low-dose exposures. Three models exist describing this relationship: linear, threshold, and hormesis models (Kudryasheva and Rozhko, 2015; Burlakova et al., 2004; Calabrese, 2014; Baldwin

Abbreviations: ROS, Reactive Oxygen Species; HTO, tritiated water.

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and Grantham, 2015). The hormesis hypothesis suggests that low-dose radiation can be favorable for living organisms. Probably, the hormesis model could be accepted as the basic one (Shi et al., 2016), while the other two (threshold and linear models) could be considered as simplified derivatives from the former coming into being under certain conditions.

The review by Kudryasheva and Rozhko (2015) summarizes the effects of exposure of luminous marine bacteria to chronic low-intensive ionizing radiation of alpha and beta types. The effects of model solutions of americium-241, uranium-235+238, and tritium were analyzed, nonlinear dose-response dependencies were demonstrated and attributed to the hormesis phenomenon. Three successive stages in bioluminescence response to ionizing radiation were demonstrated: (1) absence of effect (stress recognition), (2) activation (adaptive response), and (3) inhibition (suppression of the physiological function or radiation toxicity). The effects of alpha and beta emitting radionuclides were compared in (Selivanova et al., 2014); different effects were explained with the differences in the reactive oxygen species (ROS) concentration and the efficiency of biochemical redox processes (Alexandrova et al., 2011; Selivanova et al., 2013). Low-dose effects of gamma radiation on luminous bacteria were studied in (Kudryasheva et al., 2017). Gamma-radiation effects differed from the effects of ionizing radiation of alpha and beta types: bacteria demonstrated time/response dependence of threshold type and did not show bioluminescence activation. This peculiarity was explained with lower ionization ability and higher penetrability of electromagnetic gamma radiation. A number of research findings indicate that low-intensive gamma irradiation might induce a mutagenic effect in different organisms (Bolsunovsky et al., 2016; Sykes et al., 2006; Hussain and Ehrenberg, 1979); however, estimated probability of direct interaction of gamma-rays with bacterial cells is very low (Lampe et al., 2016).

The mechanism of bacterial bioluminescence response to low-dose radiation of different types might be related to mutations in bacterial DNA triggered by a series of events, such as water radiolysis, ROS formation, and penetration of elementary particles (electrons, protons, and neutrons) and gamma quanta into cells. The ability of ROS to interact with DNA directly leading to DNA alteration has been shown in (Kohen and Nyska, 2002). Similar effects are known to be caused by reactive nitrogen (Pauly et al., 2006) and chlorine (Mishra et al., 2016) species.

Alternatively, the results of low-dose exposures might be explained in terms of the novel “exposome” concept, where ‘exposome complements the genome and encompasses the totality of environmental non-genetic exposures’ (Rappaport and Smith, 2010; Wild, 2012). It has been discussed earlier that not only genetic mechanisms but membrane processes can be responsible for radiation induced changes of cellular functions in bacteria (Kudryasheva and Rozhko, 2015). Rozhko et al. (2016) made a conclusion on a ‘non-genomic’ mechanism of bioluminescence activation by tritium. It was supposed that tritium effects were caused by ionization of aqueous media followed by activation of cellular membrane processes. Hydrated electrons and ROS were considered to behave as biologically active particles in aerated water solutions.

This paper continues a series of investigations on effects of low-dose radiation of different types on luminous marine bacteria. The purpose of the work is to look into genetic aspects of radiation hormesis mechanisms in the bacteria.

Alpha and beta emitting radionuclides (americium-241 and tritium, respectively) were used as model sources of ionizing radiation. Both radionuclides can pose a threat to natural environments. Local nuclear incidents can increase tritium concentration dramatically; in addition, tritium contamination might be due to the forthcoming launch of controlled fusion reactors. Americium-241 is a long-living (half-life of 432,8 years) byproduct of plutonium radioactive decay with high specific radioactivity. Its ability to accumulate in the environment is associated with its binding by organic compounds, concentrating on the surface of cells and penetrating through the cellular membrane by siderophores, specific cellular proteins (Johnsson et al., 2009). Increased amounts of ^{241}Am in the biomass of aquatic plants were discovered in the waters of the Chernobyl zone (Gudkov et al., 2002). Currently, accumulation of americium-241 by sediments and aquatic plants in the Siberian river Yenisei is being examined (Bolsunovsky, 2010; Zotina et al., 2010).

Bacterial bioluminescence kinetics was studied under the conditions of chronic irradiation in americium-241 solutions and in tritiated water. To evaluate the probability of nonspecific DNA damage in irradiated bacteria, a sequence analysis was performed for the 16S ribosomal RNA gene. Mutational robustness of this gene, which had been earlier considered as highly conservative (Clarridge, 2004), was studied in (Kitaharaa et al., 2012) and its unexpected plasticity was discovered. On the other hand, this gene is universal for different taxonomic groups of bacteria and responsible for vital functions of bacterial cells (Clarridge, 2004). On these grounds, it was chosen for the first series of investigation of genome response to low dose radiation. DNA was isolated from irradiated and control samples of bacteria collected at the stages of bioluminescence activation and inhibition. The findings were compared to the results obtained earlier under similar conditions with low-dose gamma radiation. A conclusion was made on a role of DNA mutations in examined low-dose radiation effects on luminous bacteria.

2. Materials and methods

Intact luminous marine bacteria, strain *Photobacterium phosphoreum* 1883 IBSO (Kuznetsov et al., 1996), were used in a bioassay to monitor radiotoxicity of aquatic media. The strain was obtained from the collection of luminous bacteria at Institute of Biophysics SB RAS, Krasnoyarsk, Russia. Bacteria were cultivated at 22°C, pH 7.2–7.4 on a semisynthetic nutrient medium (1 L distilled water, 30 g NaCl, 1g KH_2PO_4, 0.5 g $(NH_4)_2HPO_4$, 0.2 g $MgSO_4 \cdot 7H_2O$, 10 g $Na_2HPO_4 \cdot 12H_2O$, 3 g glycine, 5 g peptone).

Bacterial suspensions were exposed to low-dose alpha and beta radiation. A solution of $^{241}Am(NO_3)_3$, and tritiated water, HTO, were used as sources of these radiation types, respectively.

Bioluminescence kinetics of the bacterial samples was studied in 3% NaCl solutions containing $^{241}Am(NO_3)_3$ and HTO of 7kBq/L and 100 MBq/L activity concentrations, respectively, and in control non-irradiated bacterial samples. All experiments were carried out at 20 °C.

Kinetics of the bioluminescence signal of all irradiated and control bacterial samples was registered using CL3606 Biochemiluminometer (SEDD “Nauka”, Russia). Bioluminescent intensity, I, was averaged from three parallel experiments with five replicates for all irradiated and control bacterial suspensions. The experimental error did not exceed 3–5%. An example of bioluminescent kinetics is presented in Fig. 1.

Relative bioluminescent intensity I^{rel} was calculated as

$$I^{rel} = \frac{I_{rad}}{I_{contr}},$$

where: I_{rad} is bioluminescence intensity in an irradiated bacterial sample; I_{contr} is bioluminescence intensity in a control (non-irradiated) sample measured under similar conditions. The error for I^{rel} did not exceed 10%.