Journal of Environmental Radioactivity 169-170 (2017) 64–69

Contents lists available at ScienceDirect

Journal of Environmental Radioactivity

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

Exposure of luminous marine bacteria to low-dose gamma-radiation

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ARTICLE INFO

Article history:
Received 16 October 2016
Received in revised form
18 December 2016
Accepted 3 January 2017

Keywords:
Low-dose gamma-radiation
Luminous marine bacteria
Bioassay
Radiotoxicity
Mutagenic effect
Temperature dependence

ABSTRACT

The study addresses biological effects of low-dose gamma-radiation. Radioactive ^{137}Cs-containing particles were used as model sources of gamma-radiation. Luminous marine bacterium *Photobacterium phosphoreum* was used as a bioassay with the bioluminescent intensity as the physiological parameter tested. To investigate the sensitivity of the bacteria to the low-dose gamma-radiation exposure (≤250 mGy), the irradiation conditions were varied as follows: bioluminescence intensity was measured at 5, 10, and 20°C for 175, 100, and 47 h, respectively, at different dose rates (up to 4100 μGy/h). There was no noticeable effect of gamma-radiation at 5 and 10°C, while the 20°C exposure revealed authentic bioluminescence inhibition. The 20°C results of gamma-radiation exposure were compared to those for low-dose alpha- and beta-radiation exposures studied previously under comparable experimental conditions. In contrast to ionizing radiation of alpha and beta types, gamma-emission did not initiate bacterial bioluminescence activation (adaptive response). As with alpha- and beta-radiation, gamma-emission did not demonstrate monotonic dose-effect dependencies; the bioluminescence inhibition efficiency was found to be related to the exposure time, while no dose rate dependence was found. The sequence analysis of 16S ribosomal RNA gene did not reveal a mutagenic effect of low-dose gamma radiation. The exposure time that caused 50% bioluminescence inhibition was suggested as a test parameter for radiotoxicity evaluation under conditions of chronic low-dose gamma irradiation.

1. Introduction

Rapid development of nuclear energy and nuclear medicine has increased the background levels of radiation exposure of people and other living organisms. Microorganisms play the fundamental role in the biosphere, and their physiological parameters are traditionally used to monitor environmental toxicity, including radiation toxicity. Marine luminous bacteria are an appropriate tool for such investigation, as they are highly sensitive to the presence of toxic compounds. Bioluminescence intensity, the main physiological parameter tested, can be easily measured instrumentally. It is also important that luminous bacteria-based assays are simple and not time consuming due to high rates of bioluminescence response. Hence, bioluminescent assays provide a large number of experimental results under comparable conditions, which is essential for their statistical treatment. These are the reasons why luminous bacteria, as well as their enzymes, have been used as toxicity bioassays for several decades (Roda et al., 2004; Girotti et al., 2008; Tarasova et al., 2012; Kudryasheva and Tarasova, 2015; Kratasyuk and Esimbekova, 2015; Kudryasheva et al., 2017). Physicochemical basis for toxic effects in bioluminescent systems was elaborated in studies by Kudryasheva (2006) and Nemtseva and Kudryasheva (2007).

Bacterial bioluminescent assay based on recombinant *Escherichia coli* has been previously used to test biological effects of high-dose gamma-radiation exposures; doses accumulated by the bacteria were 2.6 Gy (Ptitsyn et al., 1997) and 1–200 Gy (Min et al., 2003). The last decade has seen the application of luminous bacteria to monitor biological effects of low-dose ionizing radiation (Kudryasheva and Rozhko, 2015).

Radiosensitivity of living organisms is usually expressed as a dose/effect relationship, and considerable uncertainty exists concerning low exposure doses. In addition to the linear dose/effect relationship, low-dose studies might be based on a threshold dose/

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http://dx.doi.org/10.1016/j.jenvrad.2017.01.002
0265-931X/

effect relationship or the hormesis phenomenon (Burlakova et al., 2004; Calabrese, 2014; Baldwin and Grantham, 2015; Kudryasheva and Rozhko, 2015; Rozhko et al., 2016). The hormesis hypothesis suggests that low dose radiation can be favorable for living organisms. In Shi et al. (2016), the hormesis model is suggested to be accepted as a basic one, while the other models (threshold and linear) can be considered as simplified derivatives from the former, coming into being under definite conditions.

In contrast to 'deterministic' effects of high doses, low-level radiation produces "stochastic" effects. They are described in terms of 'randomnicity' and 'probability', and assume that the low dose exposures, below 100–200 mSv, do not produce heritable effects in direct proportion to an equivalent dose.

A bioassay system based on luminous marine bacteria is a good candidate for monitoring the stochastic effects of low-dose radiation, due to high rates and simplicity of the assay procedure, as well as availability of reagents and devices. Modern microplate biochemiluminometers provide a technical support for such investigations. A review by Kudryasheva and Rozhko (2015) summarized the study of the effects of model solutions of alpha- and beta-emitting radionuclides (americium-241, uranium, and tritium) on marine bacteria under conditions of chronic low-dose irradiation. Non-linear dose-effect dependences were demonstrated. Three successive stages in the bioluminescent response to americium-241 and tritium were found: (1) absence of effects (stress recognition), (2) activation (adaptive response), and (3) inhibition (suppression of physiological function, i.e. radiation toxicity). The effects were attributed to the radiation hormesis phenomenon.

So far, the luminescent bacteria-based assay has not been used to evaluate low-dose effects of gamma-radiation. However, the gamma component of low-content radioactive contaminations might be extremely important, producing a harmful impact on living organisms due to its high penetrability and high energy. This type of radiation has energy of a few hundred keV and can reach up to 10 MeV. Additionally, gamma-rays are less ionizing and more penetrative than alpha- or beta-particles: the maximal energy of tritium beta-particles is 5.7 keV, the maximal range of their path is about 1 cm (in the air, at 20 °C), and specific ionization ability is 2.2×10^6 ions per cm (Selivanova et al., 2013).

Natural sources of low-intensive gamma irradiation include naturally occurring radioisotopes such as potassium-40 and atmospheric interactions with cosmic rays or particles. Natural exposure to gamma rays is about 1–2 mSv per year, and the average total amount of radiation received in one year per inhabitant in the U.S. is 3.6 mSv UNSCEAR, 1993). Artificial sources of gamma rays include radioactive decay in nuclear reactors, and high energy physics experiments, such as neutral pion decay or nuclear fusion.

Comparison of low-dose biological effects of alpha-, beta-, and gamma-radiation in model experiments using the bacteria-based luminescent assay is a question of interest from both fundamental and applied points of view. The purpose of the present study was to determine the effects of low-dose gamma-radiation on *Photobacterium phosphoreum* and compare them with the effects of alpha- and beta-emitting radionuclides. The applied aspect of the work is the usage of the bacteria as a cellular bioassay to monitor toxicity of the gamma-radiation low-dose exposure. Irradiation conditions (temperature, dose rate, and exposure time) were varied. Radioactive ^{137}Cs-containing particles from the Yenisei River, which is affected by the operation of the Mining-and-Chemical Combine of Rosatom, were used as model sources of gamma-radiation. The radioactive isotope ^{137}Cs has the following characteristics: $E\gamma = 661.7$ keV, $T_{1/2} = 30.07$ y.

2. Materials and methods

2.1. Objects

Microbiotest 677F, preparation of lyophilized *Photobacterium phosphoreum* 1883 IBSO (Kuznetsov et al., 1996), was used as a bioassay to monitor toxicity of aquatic media exposed to gamma radiation. The preparation was obtained from the Institute of Biophysics SB RAS, Krasnoyarsk, Russia.

^{137}Cs-containing radioactive hot particles were used as the point sources of external gamma radiation. The particles were extracted from the floodplain soils and sediments of the Yenisei River in the area affected by the operation of the Mining-and-Chemical Combine of Rosatom (Bolsunovsky and Tcherkezian, 2001; Chuguevsky et al., 2010). Two hot particles were used in the experiments with luminous bacteria. Radioactivity of the particles, their distances from the bacterial samples, and the corresponding dose rates at these distances are given in Table 1.

2.2. Experimental procedure

A radioactive particle was placed in the center of the experimental chamber. Eppendorf tubes with bacterial suspension in 1.5% NaCl were placed around the radioactive particle, at different distances from it. Dose rates of gamma-irradiation ranged from 0.2 to 137 μGy/h and from 122 to 4100 μGy/h for Particle 1 and Particle 2, respectively (Table 1). The average background exposure dose for the control bacterial samples was 0.1 μGy/h. The dose rate calculations were based on the activity of the ^{137}Cs source; they were additionally verified by direct measurements with a DKG-02U dosimeter (SPC "Doza", Ltd, Russia).

Bioluminescence kinetics of the bacterial samples (control and irradiated ones) was measured in 3% NaCl solutions using a CL3606 Biochemiluminometer (SDTB "Nauka" KSC SB RAS, Russia). Bacterial suspensions were exposed to the radiation in three experiments: at 5, 10, and 20 °C. Fig. 1 shows bioluminescence kinetics of the control bacterial suspensions at the different temperatures.

Bioluminescent measurements of the control and irradiated samples were carried out and compared as described in the section below.

A mutagenic effect of low-dose gamma radiation was examined using sequence analysis of 16S ribosomal RNA gene of *P. Phosphoreum*. The analysis was performed on the samples of bacterial suspensions exposed to gamma radiation (4100 μGy/h, 20 °C); it was compared to that of the control bacterial suspensions.

2.3. Evaluation of radiotoxicity of the test samples

Radiotoxicity of a bacterial sample was assessed by relative bioluminescent intensity, I^{rel}, calculated as

$$I^{rel} = I_{rad}/I_{contr}$$

here, I_{rad} is the average value of bioluminescence intensity in the bacterial sample exposed to gamma radiation, and I_{contr} is the average value of bioluminescence intensity in the control sample. The average values were obtained in four parallel experiments with five measurements for all irradiated and control bacterial suspensions. Experimental error did not exceed 10%.

Values of I^{rel} were calculated for all samples at different times of exposure. Times for control sample measurements corresponded to those of the exposed samples.