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Comparison of Effects of Uranium and Americium on Bioluminescent Bacteria

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Effect of $UO_2(NO_3)_2$ on bioluminescent bacteria P.Phosphoreum was studied. It was compared with the effect of solutions of the more active radionuclide - $^{241}Am(NO_3)_3$ studied earlier (Rozhko et al., 2007). Bioluminescence inhibition was observed under uranyl concentrations exceeding 10^{-7} M (30 Bq/l); and bioluminescence activation was not observed under all radionuclide concentrations and exposure times in the experiment. Effect of uranyl was attributed to chemical component of its impact, not radiation one. It was shown that solutions of Americium were detoxified by humic substances (0.25 mg/ml), but solutions of uranyl – are not.

Keywords: Radiotoxicity, biomonitoring, bioluminescence.

Introduction

Increase of radioactive contamination in the environment makes the effects of low-level radiation on living organisms very important. Detailed investigations of the effect of low dose radiation on living organisms were conducted using simple assay systems such as microorganisms, bacteria, plant and mammal cells, tissues of laboratory mice, drosophila flies, freshwater crustaceans, etc (Lyutykh, 1998; Nikolsky and Koterov, 1999; Spitkovsky, 1999; Koterov and Nikolsky, 1999). Assay systems based on luminous bacteria are good candidates for such investigations. Bacterial bioluminescent assays are widely used to monitor environmental toxicity (Kratasyuk and Gitelson, 1987). The tested parameter is luminescent intensity of bacteria, which can be easily measured instrumentally. The advantages of the bioluminescent assays are their rapidity, sensitivity, simplicity, and availability of the devices for toxicity registration (Kratasyuk and Gitelson, 1987; Kudryasheva et al., 1998).

Bioluminescent bacteria have been used as a bioassay for almost a half of a century. The bioassay was described in its current form in 1969 (Grabert and Kossler, 1997). Later, it was modified by different researchers and adapted for their specific purposes; numerous applications of the bacterial bioassay are known (Stom et al., 1992; Wood and Gruber, 1996; Natecz-Jawecki et al., 1997; Roda et

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al., 2004; Kudryasheva, 2006). In 1990 the in *vitro* assay based on bioluminescent system of enzymatic coupled reactions was suggested as a toxicity assay (Kudryasheva et al., 1998); its applications were developed (Kudryasheva et al., 2002, 2003). Now, the bioluminescent ecological assay is a traditional and important biotechnological application of the bioluminescence phenomenon (Roda et al., 2004).

General radiotoxicity was already investigated using bioluminescence of the recombinant *Escherichia coli* strains exposured to gamma-ray irradiation (Min et al., 2003). Bioluminescent bioassays have been already used to study the effect of low-level alpha-ray irradiation in (Rozhko et al., 2007). In this work, three bioluminescent assay systems were applied: intact bacteria, lyophilized bacteria, and bioluminescent system of coupled enzyme reactions. Solutions of $^{241}Am(NO_3)_3$ were used in this paper as a source of α-radiation. The activation processes were shown to predominate in all three bioluminescent assay systems subjected to short-term exposure (to 20-55 hr) and inhibition processes – in the systems subjected to longer-term exposure to radiation. Fig. 1 demonstrates the effect of $^{241}Am^{3+}$ on the bioluminescent intact bacteria as an example. The absence of the effect of nonradiative Americium analogue – Eu – under similar experimental conditions is demonstrated, too. The comparison of two curves here brings up the conclusion that the effect of $^{241}Am^{3+}$ was caused by its radiation component. In the paper mentioned (Rozhko et al., 2007), the impact of $^{241}Am^{3+}$ was shown to depend on its concentration, the level of organization and integrity of bioluminescent assay system. Bioluminescent assay system *in vivo* (bioluminescent bacteria) was found to be more sensitive to $^{241}Am^{3+}$ (down to 10^{-11} M, 300 Bq/l).

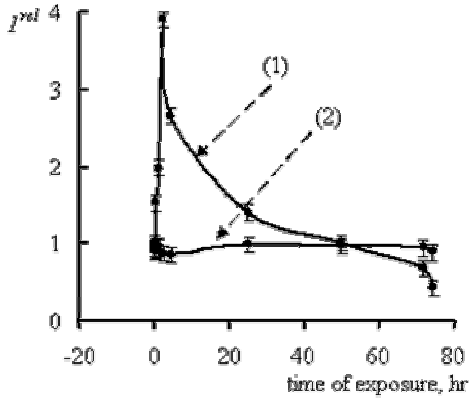

Fig. 1 Bioluminescent intensity (I^{rel}) vs time of exposure (t, hours) in solutions of $^{241}Am^{3+}$ ($C = 2 \cdot 10^{-10}$ M, A = 6000 Bq/l) (1) and Eu^{3+} ($C = 2 \cdot 10^{-10}$M) (2), from: (Rozhko et al., 2007)

The effects of radionuclides of various α-activity on the bioluminescent assay system are of interest. In this paper, two α-radionuclides of different specific activity were chosen – Uranium and Americium. The following data compare radioactivity of these radionuclides: the activity of 3000 Bq/l corresponds to 10^{-3} M solution of Uranium and 10^{-10} M solution of Americium. The purpose of this study was to monitor the effect of Uranium on the bioluminescent bacteria, to compare the effects of Uranium and Americium, and to compare the detoxification activity of humic substances in the solutions of these radionuclides.

Materials and Methods

1. Objects

Cell suspension of 16-h *P.phosphoreum* 1883 IBSO culture from the Collection of Luminous Bacteria CCIBSO was applied as a bioassay system. Solutions of $UO_2(NO_3)_2$ of basic concentration 10^{-3} M in 1.1M $NaNO_3$ (pH 6.5-7.0) were used as a source of ionizing radiation. The activity of this solution was 3000 Bq/l. Bioluminescent bacteria were exposed to solutions of $UO_2(NO_3)_2$ of the following concentrations: 10^{-3} M, 10^{-4} M, 10^{-5} M, 10^{-7} M, 10^{-9} M, 10^{-10} M, and 10^{-11}M.

To investigate the effect of uranyl nitrate, the radioactive and control samples were prepared.

Radioactive samples: solutions of $UO_2(NO_3)_2$ in 1.1 M $NaNO_3$ were added to bioluminescent bacteria. Control samples: 1.1 M $NaNO_3$ solutions were added to bioluminescent bacteria. Radioactive and control samples were maintained at constant temperature 4°C for 25 hours. Periodically, the test samples (radioactive and control) were prepared and the bioluminescent intensity was recorded at 20°C applying the standard procedure (Kuznetsov et al., 1996). Measurements were carried out in 0.5-10 hr with increasing time-intervals between samplings.

Compositions of the test samples (radioactive or control) were following: 50 μl of radioactive (or control) samples and 500 μl of the 3% NaCl solution.

The Gumat-80 ("Gumat", Irkutsk) was used as a source of humic substances. It was produced by non-extracting treatment of coal (Levinsky, 2000).

2. Evaluation of radiotoxicity of the test samples

Kinetics of bioluminescence signal was studied using a CL3606 Biochemiluminometer (SKTB "Nauka", Russia). Relative bioluminescent intensity I^{rel} was calculated:

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

where:

I_{rad} is bioluminescence intensity in the radioactive test sample,

I_{contr} is bioluminescence intensity in the control test sample.

Values of I^{rel} were plotted vs time of exposure to radionuclides. I^{rel} was evaluated in the absence and in the presence of 0.25 mg/ml humic substances in solutions of $UO_2(NO_3)_2$ (10^{-3} -10^{-11} M), $Am(NO_3)_3$ ($2\cdot10^{-10}$ – 10^{-13} M), and $Eu(NO_3)_3$ (10^{-3} – 10^{-13} M).

3. Detoxification of radioactive solutions by humic substances

Values of I^{rel} vs time of exposure to $Am(NO_3)_3$ or $UO_2(NO_3)_2$ were compared in the absence and presence of humic substances (C=0,25 mg/ml).

Results and Discussion

It was found that only higher concentrations of $UO_2(NO_3)_2$ (10^{-3}-10^{-7} M) inhibited ($I^{rel} < 1$) bioluminescence. Dependence of I^{rel} vs time of exposure is presented in Fig. 2(A) for three concentrations of Uranium as an example. Thus, the effect of Uranium on the bioluminescent bacteria was observed at much higher concentrations than that of Americium (Rozhko et al., 2007): $>10^{-5}$ M (30 Bq/l) and $> 10^{-11}$ M (300 Bq/l) respectively. Second difference is the absence of activation period under all concentrations of $UO_2(NO_3)_2$ and exposure times in the experiment.

The impact of Uranium on the bioluminescent assay system can include a radiation component and a chemical one. To isolate the chemical component, we compared the effect of Uranium to that of model nonradioactive heavy metal – Europium. The effect of several concentrations of $Eu(NO_3)_3$ on the bacterial bioluminescence is demonstrated in Fig. 2(B). The bioluminescence kinetics was approximated with linear fit:

$$I^{rel} = -a \ln t + 1 \qquad (1)$$

Angular coefficients a for of $UO_2(NO_3)_2$ and $Eu(NO_3)_3$ of two concentrations are presented in Table.

It is seen from the Table that a-values are of the same order for Uranium and Europium. This shows that effects of Uranium and Europium are similar. Hence, all the effects of $UO_2(NO_3)_2$ studied above can be ascribed to the chemical component.

It is known that humic substances serving as natural complexing agents are able to detoxify solutions of metallic salts (Takahashi et al., 1997;

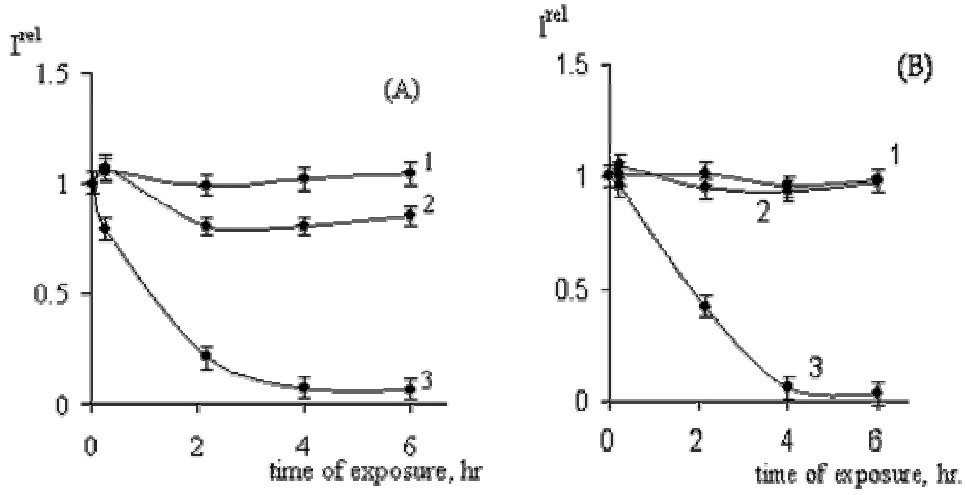

Fig. 2. Bioluminescent intensity (I^{rel}) vs time of exposure (t, hours) in solutions of $UO_2(NO_3)_2$ (A), and $Eu(NO_3)_3$ (B). Concentrations of the salts: 1 - 10^{-11}, 2 - 10^{-7}, 3 - 10^{-3} M

Table. Angular coefficient *a* and approximation values A in equation (1) in solutions of $UO_2(NO_3)_2$ and $Eu(NO_3)_3$

Concentration, M	$UO_2(NO_3)_2$		$Eu(NO_3)_3$	
	a	*A*	*a*	*A*
10^{-3}M	0,17	0,93	0,13	0,94
10^{-5}M	0,02	0.97	0,01	0,71

Provenzano et al., 2004). Detoxification ability of humic substances in solutions of $Am(NO_3)_3$ and $UO_2(NO_3)_2$ was studied using luminescent bacteria as assay system. Conditions of experiment (radionuclide concentration and incubation time) were chosen revealing time-decay of I^{rel}. For instance, Fig. 3 demonstrates detoxification of Americium solution (10^{-10} M, 3000 Bq/l). It is seen that humic substances decrease time-decay of I^{rel}, approaching it to control, thus decreasing toxicity of the radioactive solution.

We found that humic substances do not change time-decay of I^{rel} in solutions of uranyl under all conditions inhibiting bioluminescence (incubation time till 25 h, and uranyl concentrations $10^{-3} \div 2 \cdot 10^{-7}$M) (Fig.2 (A). Hence, these solutions are not detoxified at the conditions of the experiment.

Conclusion

Contributions of radiation and chemical components to the effect of the radionuclides on bacterial bioluminescence depend on specific radioactivity of the radionuclide. The effect of Uranium on bioluminescent bacteria is conditioned by chemical, but not radioactive properties of this radionuclide. The effect of Americium was due to its radioactive component only.

The effect of Uranium was found at higher concentrations (>10^{-7} M or >0.3 Bq/l) than that of Americium (>10^{-11} M or >300 Bq/l). The effect of Americium included activation and inhibition of bioluminescence at different times of exposure.

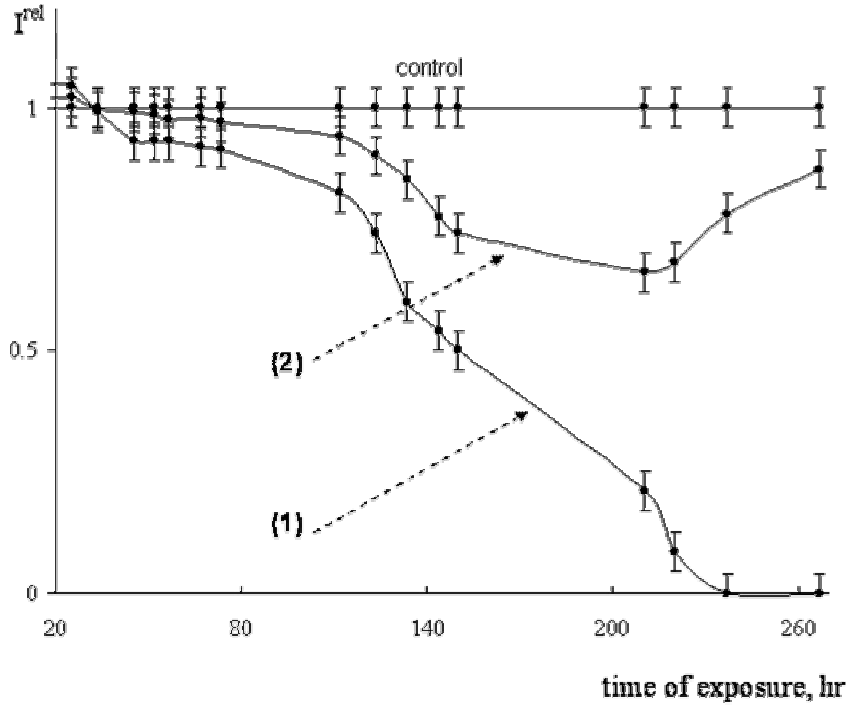

Fig. 3. Bioluminescent intensity (I^{rel}) vs time of exposure (t, hours) in solutions $^{241}Am(NO_3)_3$ (10^{-10} M, 3000 Bq/l) in the absence (1) and in the presence (2) of humic substances. Control – bioluminescent intensity in the absence of $^{241}Am(NO_3)_3$

Only inhibition of bioluminescence was observed in the presence of Uranium. Humic substances (C = 0.25 mg/ml) detoxified solutions of Americium and did not detoxify solutions of Uranium.

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