Mechanisms of Low-Dose Effects on Bacterial Cells

Subjects: Biophysics

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Mechanisms of low-dose responses of bacteria can be considered at the biochemical, chemical, and physico-chemical levels.

low-dose radionuclides radiotoxicity radiation hormesis bioassay

1. Changes in the Rates of Intracellular Enzymatic Processes under Exposure to Radionuclides

The effects of alpha- and beta-emitting radionuclides (americium-241 and tritium) on the bioluminescence system of coupled enzyme reactions catalyzed by bacterial luciferase and NADH:FMN-oxidoreductase (see in Introduction) were studied in [1][2][3]. Bioluminescence activation and inhibition were observed. A monotonic dependence on the concentration of tritiated water is evident from **Figure 1**. However, the authors [4] did not find similar monotonic dependence in a wide concentration range of tritiated water, and bioluminescence activation was only registered within the range of tritium radioactivity concentration of 0.005–200 Mbq/L.

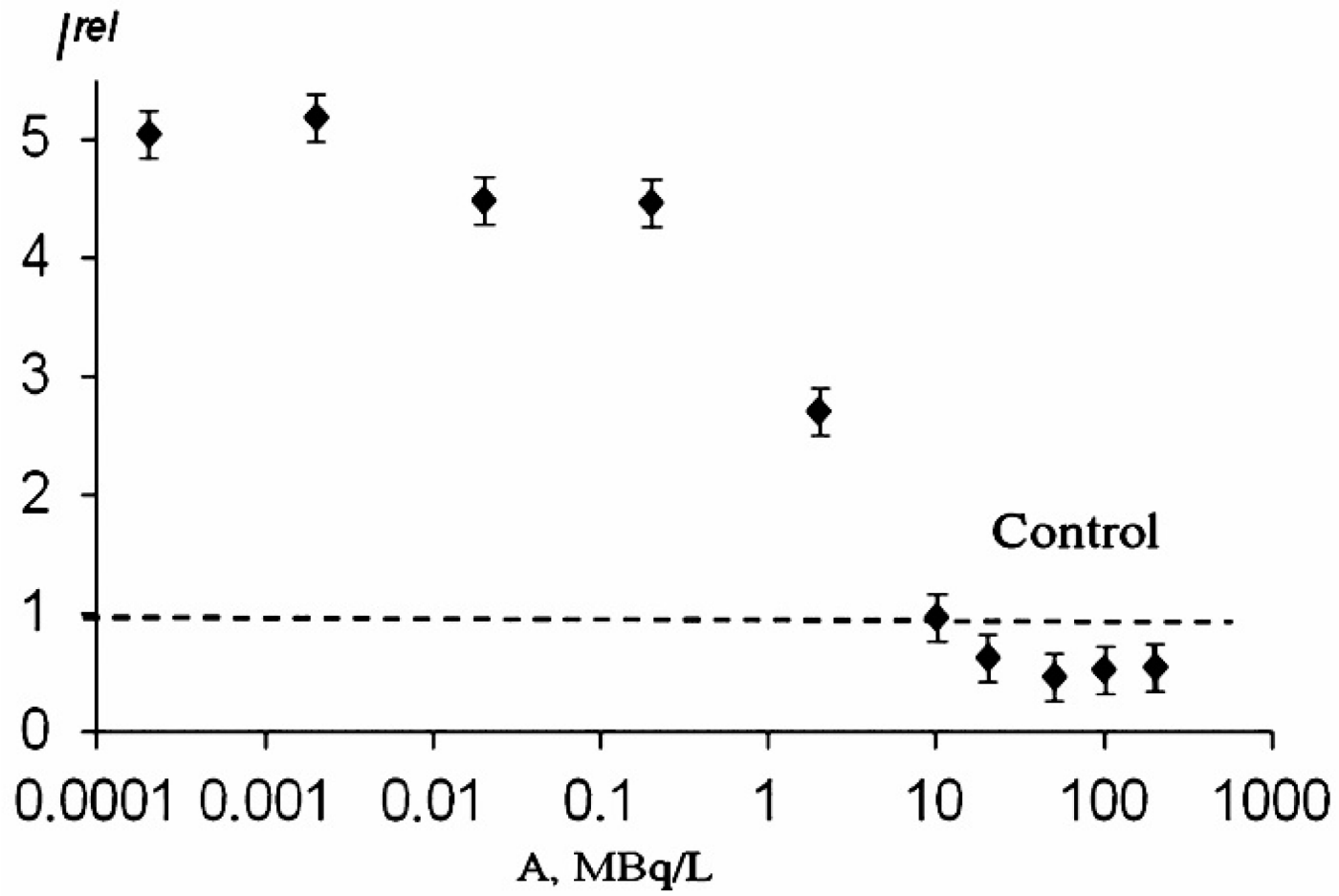

Figure 1. Bioluminescent intensity of the enzyme system, I^{rel}, vs. radioactivity concentration of tritiated water, A, MBq/L [1][2][3][5].

Similar to the luminous bacterial cells, a low-concentration increase in the bioluminescence intensity was observed in the enzymatic system under low-concentration exposure to thorium-232 [6], thus revealing the hormetic phenomenon in the enzymatic assay system in thorium solutions.

2. Consumption of An Intracecllular Reducer, NADH

NADH is an organic intracellular reducer; it can be considered an indicator of the reduction activity in enzymatic and cellular systems, which is involved into complex metabolic processes in organisms.

The rates of NADH oxidation were studied in solutions of the components of the bioluminescent enzyme system: the enzyme preparation and FMN [7]. The data obtained in are shown in **Table 1**. The rates of NADH oxidation were determined in the presence and absence of thorium-232.

Table 1. Rates of NADH oxidation (V) in the solutions of different composition. The wavelength of optical density registration was 340 nm. The concentration of $Th(NO_3)_4$, was 10^{-7} M [6].

Number of Solutions	Components of Solutions	V·10^8, M	
		without Th	with Th
1	NADH	2.43	4.05
2	NADH + enzyme preparation	4.05	6.07
3	NADH + FMN	14.20	20.60
4	NADH + FMN + enzyme preparation	16.20	26.70

e samples (1–4, **Table 1**), with this increase being equal to 1.5–1.7. This result demonstrates that thorium-232 increases the efficiency of the reduction process involving enzymes and biologically important molecules (FMN and NADH). Hence, thorium can both (1) increase the bioluminescence intensity by accelerating the enzymatic processes and (2) decrease the bioluminescence intensity by accelerating the non-enzymatic processes and removing low-molecular components out of active enzymatic centers. The balance between these two processes depends on the peculiarities of the enzyme environment and these should be taken into consideration while explaining the activation or inhibition effects of radionuclides.

3. Active Role of Reactive Oxygen Species

Molecular mechanisms of the radionuclide bioeffects are conventionally attributed to reactive oxygen species (ROS) which are generated in water bodies in the presence of dissolved molecular oxygen [1][8][9][10]. On the other hand, ROS are native products of metabolic oxidative processes in living organisms [11][12][13]. It was demonstrated that luminous marine bacteria naturally increase the ROS content in aquatic media, and intensify the ROS production upon the addition of tritium [4][14].

Chemically, ROS are products of the partial reduction of oxygen; the ROS group includes hydroxyl radicals (OH•), hydrogen peroxide (H_2O_2), superoxide anion (O_2•−), etc. [15].

According to modern approaches, ROS are able to produce both damaging and signal bioeffects [16][17]; they regulate vital functions, such as cellular protective or apoptosis responses [9]. ROS are responsible for migration, proliferation, and differentiation [18][19]; they are known as stimulators of cell division [20][21] and cell death—apoptosis, necrosis, and autophagy [22][23]. The signal function of ROS is now being discussed [20][24][25][26]. It should be noted that ROS can serve both as inter- and intra-cellular messengers [27][28][29]. Both reactive oxygen and nitrogen species [30] released by cells can serve as signal particles which initiate the radiation-induced ‘bystander effect’ [31][32]. It is stated in [33][34][35] that ROS are responsible for both inhibiting (toxic) and activating bioeffects. It is noted in the papers mentioned before that the lack of ROS can suppress biological functions, similarly to the excess of ROS, but only the latter is widely and conventionally stated and discussed in biomedical literature. The reason for both effects is the disturbance of the ROS balance in bacterial suspensions.

Rozhko et al. [36] explained the decrease in the ROS content in bacterial suspensions in tritiated water by consumption of ROS in the bacterial bioluminescence reaction followed by the formation of a reaction intermediate–peroxide flavin derivative [37][38]. An increase in the ROS content was also observed, which was

explained by the intensification of complex metabolic processes in bacterial cells under radioactive exposure to tritiated water, similarly to the explanation presented in [36]. Direct correlations between the time-dependences of the ROS content and the bacterial bioluminescence intensity were found in the studies by Rozhko [14][36], presenting the basis for the explanation of the bioluminescence activation or inhibition under exposure to the radionuclide.

The luminescent marine bacteria naturally increased the ROS content in aqueous media, and additionally increased the ROS production up to 300% in the presence of tritium [36]. Hašler et al. [39] confirmed that tritiated water can stimulate the ROS production in another type of bacteria, *Pseudendoclonium basilense*, a bacterial strain from standing water. The 300% activation of luminescence of marine bacteria by tritiated water was attributed to the 'bystander effect' [36]. The result was explained by the 'trigger' effect of tritium decay products, and by the signaling function of ROS.

The time-course of the ROS content in the bacterial suspension in the presence of the alpha-emitting radionuclide thorium-232 is presented in **Figure 2** as curve 2, according to [6]. A moderate decrease in the ROS content (compared to the nonradioactive control sample) with a tendency to its restoration was found. Negative correlations between the ROS content and the bioluminescence intensity were found, thus demonstrating inverse relations between the bacterial physiological functions and the ROS concentration in the environment. It was concluded that the consumption of ROS contributes to the bioluminescence activation under low-dose exposure to thorium-232. It is assumed that the role of ROS should be taken into consideration in studying molecular mechanisms of the 'hormesis' approach [40][41][42][43].

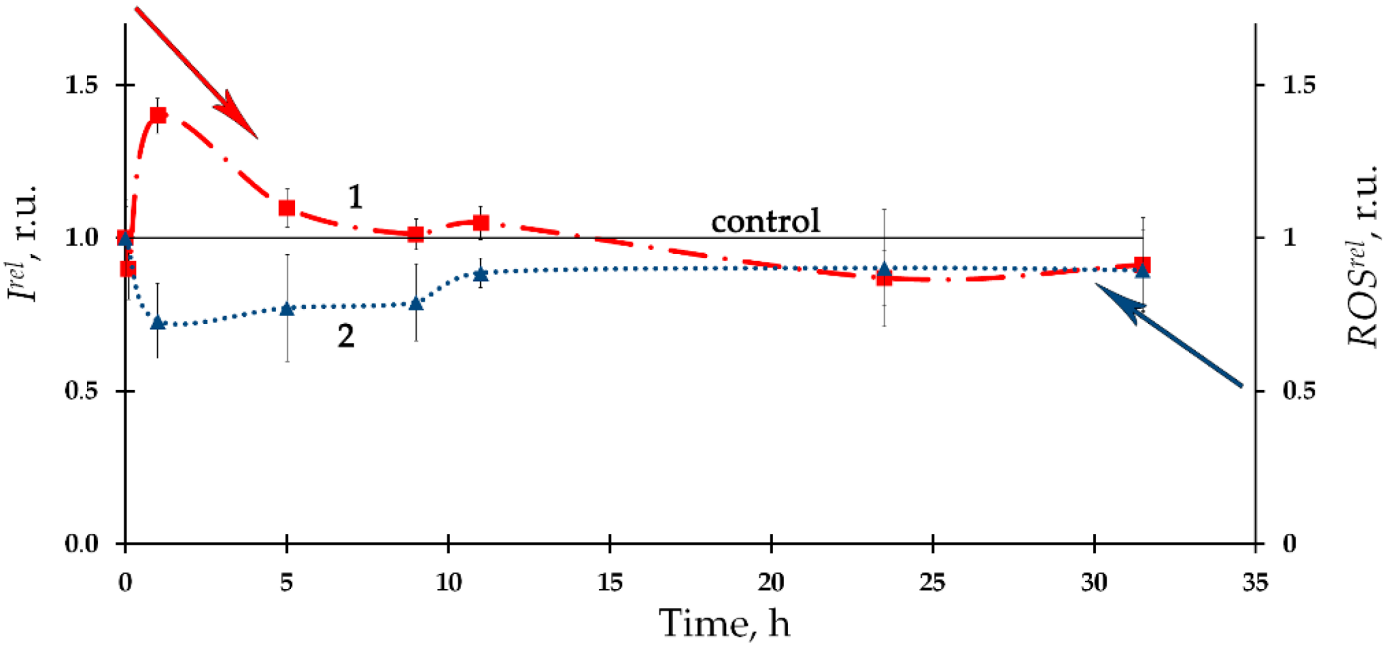

Figure 2. (1) Kinetics of bacterial bioluminescence, I^{rel}, and (2) ROS content, ROS^{rel}, in the presence of thorium-232, 10^{-7} M [6].

Hence, one should indicate the differences in the correlations for the effects of the alpha and beta emitting radionuclides (americium-241 and tritium, respectively): correlation coefficients between the time-dependences of the bioluminescence intensity and the ROS content differed in their sign. This fact reflects the complexity of the ROS-dependent processes occurring in the biological systems under exposure to radionuclides of different types.

4. Repair of DNA Damage

The first hypothetical mechanism of the hormesis phenomenon is based on repairing DNA damage [44][45][46]. The involvement of non-genetic mechanisms into low-dose chronic radioactive effects in luminous bacteria was proved earlier by Rozhko et al. [47][48] with the reference to the activation of membrane processes as a result of ionization of water media in radioactive solutions [49][50].

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