Spectrochimica Acta Part A 100 (2013) 171–175

Contents lists available at SciVerse ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

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

Effects of americium-241 and humic substances on *Photobacterium phosphoreum*: Bioluminescence and diffuse reflectance FTIR spectroscopic studies

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ARTICLE INFO

Article history:
Received 15 May 2012
Accepted 5 June 2012

Keywords:
Bioluminescence
Americium-241
Humic substances
FTIR (DRIFT) spectroscopy
Poly-3-hydroxybutyrate
Photobacterium phosphoreum

ABSTRACT

The integral bioluminescence (BL) intensity of live *Photobacterium phosphoreum* cells (strain 1883 IBSO), sampled at the stationary growth stage (20 h), was monitored for further 300 h in the absence (control) and presence of ^{241}Am (an α-emitting radionuclide of a high specific activity) in the growth medium. The activity concentration of ^{241}Am was 2 kBq l^{-1}; [^{241}Am] = 6.5 × 10^{-11} M. Parallel experiments were also performed with water-soluble humic substances (HS, 2.5 mg l^{-1}; containing over 70% potassium humate) added to the culture medium as a possible detoxifying agent. The BL spectra of all the bacterial samples were very similar (λ_{max} = 481 ± 3 nm; FWHM = 83 ± 3 nm) showing that ^{241}Am (also with HS) influenced the bacterial BL system at stages prior to the formation of electronically excited states. The HS added per se virtually did not influence the integral BL intensity. In the presence of ^{241}Am, BL was initially activated but inhibited after 180 h, while the system ^{241}Am + HS showed an effective activation of BL up to 300 h which slowly decreased with time. Diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy, applied to dry cell biomass sampled at the stationary growth phase, was used to control possible metabolic responses of the bacteria to the α-radioactivity stress (observed earlier for other bacteria under other stresses). The DRIFT spectra were all very similar showing a low content of intracellular poly-3-hydroxybutyrate (at the level of a few percent of dry biomass) and no or negligible spectroscopic changes in the presence of ^{241}Am and/or HS. This assumes the α-radioactivity effect to be transmitted by live cells mainly to the bacterial BL enzyme system, with negligible structural or compositional changes in cellular macrocomponents at the stationary growth phase.

1. Introduction

Bacterial bioluminescence (BL) [1,2] assays are widely used to monitor environmental toxicity of various contaminants [2–6]. In such assays, the bioluminescent intensity of bacterial cells or bacterial enzyme (luciferase) based preparations (see, e.g. [7] and references reported therein) is the tested parameter which can easily be measured instrumentally.

The currently increasing environmental contamination with radionuclides, including low-level radiation of different kinds, is of growing environmental concern (see, e.g. [8–13] and references reported therein). Therefore, relevant studies involving luminescent bacterial cells are of significant interest both for applied fields such as optimisation of BL-based assays [7,8] and for basic research on elucidating bacterial metabolic responses to such hazardous environmental factors as radionuclide traces [9–13].

Previously, several bioluminescent assay systems in vivo and in vitro were shown [7] to be sensitive to solutions of the α-emitting radionuclide americium-241 (^{241}Am) in the activity range 0.16–6.67 kBq l^{-1}. Adding ^{241}Am salt to bacterial assay systems was found to result in an initial activation of the BL intensity, which was followed by its inhibition. In addition, humic substances (HS) were reported to be capable of detoxifying various contaminants as demonstrated by bioluminescent monitoring (see, e.g. [14–16] and references reported therein), including their detoxification effects on low-level α-radiation [16].

In the present work, the integral BL intensity of live *Photobacterium phosphoreum* cells, sampled at the stationary growth stage (20 h), was monitored for further 300 h in the absence (control) and presence of ^{241}Am. Parallel experiments were also performed with water-soluble humic substances added to the culture medium as a possible detoxifying agent (reported to be effective against

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http://dx.doi.org/10.1016/j.saa.2012.06.003

α-radioactivity [16]). BL spectra of the samples were monitored to control possible impacts of ^{241}Am and/or HS on the formation of electronically excited states [17,18]. Diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy was used to check the possibilities of any macroscopic changes in bacterial cell composition and/or fine structural rearrangements of cellular macrocomponents as metabolic responses to the external factors [19,20].

2. Materials and methods

Ph. phosphoreum (strain 1883 IBSO) was taken from the Collection of the Institute of Biophysics SB RAS, Krasnoyarsk, Russia (CCIBSO 863). The bacterium was cultured at 22 °C on a rotary shaker (130 rpm) as described earlier [21]. The aqueous nutrient medium used for bacterial growth included (g l^{-1}): NaCl, 30; KH_2PO_4, 1; $(NH_4)_2HPO_4$, 0.5; $Na_2HPO_4{\cdot}12H_2O$, 6; $MgSO_4{\cdot}7H_2O$, 0.2; peptone, 5; with 3 ml l^{-1} glycerol. Bacterial suspensions both for BL intensity and for DRIFT spectroscopic measurements were sampled at the stationary stage of growth (20 h).

Along with the control samples, those with ^{241}Am and/or water-soluble humic substances (HS, 2.5 mg l^{-1}; containing over 70% potassium humate) were separately studied. As a source of HS, the Gumat-80 preparation ("Gumat", Russia) produced by non-extracting treatment of coal with alkali [14,15] was used.

The radioactivity of ^{241}Am-containing samples was measured with a Wallac Wizard 1480 gamma-counter (PerkinElmer, Finland). The activity concentration of the culture medium, 2 kBq l^{-1} ([^{241}Am] = 6.5 × 10^{-11} M), was created by adding $^{241}Am^{III}$ nitrate.

The integral BL intensity was measured at room temperature (20 °C) by a standard procedure [3,6] using a TriStar Multimode Microplate Reader LB 941 (Berthold Technologies, Germany). Optical density of bacterial suspensions was registered using a KFK-2MP colorimeter (Russia). The number of cells was counted by a Zeiss Axioskop 40 fluorescence microscope with a Filter Set 02 (C. Zeiss, Germany).

After specified periods of time, samples (0.2 ml each) of the bacterial suspension (kept at 4 °C between BL measurements) were placed into microplates, incubated for 5 min at room temperature (20 °C), and then the stabilised values of their integral BL intensity were measured at 20 °C. Relative bioluminescence intensity (I_{rel}) was calculated as follows:

$$I_{rel} = \frac{I_{rad}/N_{rad}}{I_{contr}/N_{contr}}.$$

Here, I_{rad} and I_{contr} are the bioluminescent intensities of radioactive and control samples, respectively; N_{rad} and N_{contr} are the numbers of cells in the radioactive and control samples, respectively. Parallel measurements of BL intensity were performed in quadruplicate; mean values of I_{rel} with standard errors were plotted as a function of time.

Bioluminescence spectra were measured using a Fluorolog 3-22 spectrofluorimeter (Horiba Jobin Yvon, France) with the option of single photon counting.

For DRIFT spectroscopic measurements, the bacterial suspensions for each of the four samples (control, with HS, with ^{241}Am, and with HS + ^{241}Am) were repeatedly centrifuged (6000 × *g*, 4 °C, 15 min) and washed three times from the culture medium (and from ^{241}Am when applicable) with 2% NaCl aqueous solution. The final centrifuged cell pellets were dried under vacuum (6.67 Pa; 0.05 mmHg) at room temperature for 2 h and, prior to measurements, powdered in an agate mortar. DRIFT spectra were recorded and processed as reported elsewhere [19,20] using a Nicolet 6700 Fourier transform infrared (FTIR) spectrometer (Thermo Electron Corporation, USA) with a DRIFT accessory (DTGS detector; KBr beamsplitter; with 100 accumulated scans and a resolution ±4 cm^{-1}) using a Micro sampling cup (Spectra-Tech Inc., USA).

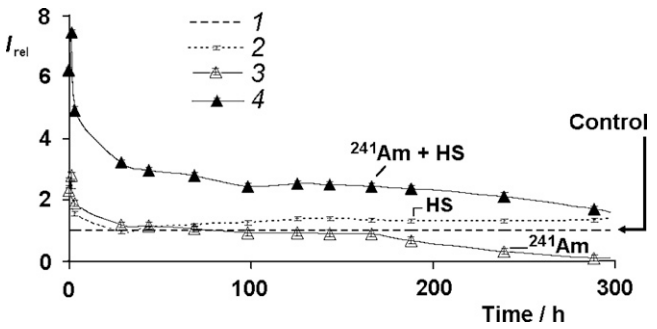

Fig. 1. Relative bioluminescence intensity (I_{rel}) as a function of time for *Photobacterium phosphoreum* (strain 1883 IBSO) sampled at the stationary growth stage (20 h of growth): (*1*) control cells (I_{rel} normalized to unity) and in the presence of (*2*) humic substances (HS), (3) americium-241 (^{241}Am), (*4*) ^{241}Am + HS.

3. Results and discussion

3.1. Bioluminescence studies

The values of relative bacterial BL intensity (I_{rel}) as a function of time for control cells, as well as in the presence of HS only, ^{241}Am only or HS + ^{241}Am, are presented in Fig. 1. In all the cases with the additives, as can be seen from curves 2 to 4, the period of the first few hours is characterized by a sharp leap in BL intensity as compared to the control, with an abrupt decrease. This is typical for initial periods of short exposures of luminous bacterial cells to low-level radioactivities and to HS and can be ascribed to the first steps of gradual uptake of ^{241}Am by cells or other applied moderate stresses [7,9,16]. This phenomenon (hormesis), attributed to triggering cell defense responses under the influence of low concentrations of toxic compounds, low-dose radiation and other stressors, is known for various organisms (see, e.g. [9] and references therein). For the sample with HS alone (curve 2), this leap of I_{rel} was the weakest, and further the addition of HS at its low concentration applied (2.5 mg l^{-1}) resulted in a weak activation of the BL intensity by up to maximum 40%.

In the presence of americium only (2 kBq l^{-1}; curve 3), after a short activation period, the BL intensity was close to that of the control (cf. line 1) and, after 180 h, was gradually inhibited virtually down to zero. However, interestingly, in the presence of both ^{241}Am (2 kBq l^{-1}) and HS (2.5 mg l^{-1}; see curve 4), an effective BL activation was observed (up to 750% within the initial period) throughout the whole period of measurements (300 h), gradually and slowly decreasing with time. Thus, even this very low concentration of HS can efficiently detoxify the radiological effect of ^{241}Am.

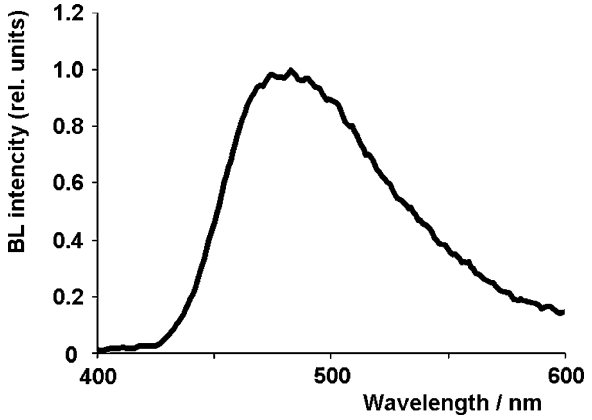

Fig. 2. Bioluminescent spectrum for live cell suspension of *Photobacterium phosphoreum* (strain 1883 IBSO).