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EXPERIMENTAL ARTICLES

Photoquenching of the Bioluminescence of the Genetically Engineered *Escherichia coli* TG1 (pXen7) Strain in the Presence of Photodithazine

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Abstract—The photoquenching of the bioluminescence of the genetically engineered *Escherichia coli* TG1 (pXen7) strain was studied in the presence of the photosensitizer photodithazine, a glucosamine salt of chlorin e_6. The photosensitized quenching of the bioluminescence was found to correlate with the colony-forming ability of the strain. The data obtained are discussed from the standpoint of using biosensor luminescent bacterial systems for the assessment of the efficiency of photosensitizers in antimicrobial photochemotherapy.

Key words: bioluminescence, bacteria, chlorin e_6, photosensitization, visible light.

Photodynamic chemotherapy is considered to be a potential alternative approach to cancer treatment [7, 10] and antimicrobial therapy (because of the ever-increasing antibiotic resistance of pathogenic microorganisms [14]). This approach is based on the inactivation of unwanted cells by active oxygen species generated by the photoexcited molecules of exogenously added or endogenous photosensitizers. The most frequently used photosensitizers are porphyrins and related compounds [4–6, 8, 9, 11, 12]. Search for new potent photosensitizers requires model cell systems (biosensors) which would allow the photocytotoxicity of screened compounds to be rapidly evaluated.

Luminescent biosensor systems are poduced under the trademarks Microtox, Toxitest, Mutatox, and Ecolum [1, 2, 13]. They have been only used for the assessment of the toxicity of various compounds and not for photosensitization experiments.

The aim of the present work was to evaluate the sensitivity of the bioluminescence of the genetically engineered *Escherichia coli* TG1 (pXen7) strain to visible light in the presence of the photosensitizer photodithazine, a glucosamine salt of chlorin e_6.

MATERIALS AND METHODS

Bacterial strains. Experiments were carried out with the genetically engineered *Escherichia coli* TG1 (pXen7) strain (the principal component of the Ecolum test system [1]), whose bioluminescence is due to the complete *lux* operon cloned from the luminescent soil enthomopathogenic bacterium *Photorhabdus luminescens* ZM1 [3].

Growth media and cultivation conditions. The strain *E. coli* TG1 (pXen7) was grown at 30°C on Luria–Bertani (LB) agar containing (g/l) bactotryptone, 10; yeast extract, 5; NaCl, 5; and agar, 20 (pH 7.0–7.2) or in shaken (220 rpm) 750-ml flasks with 100 ml of LB broth of the same composition but without agar. The media were supplemented with 100 μg/ml ampicillin.

Lyophilization. Bacterial cells from the late exponential growth phase were separated from the culture liquid by centrifugation at 4000 *g* for 20 min (4°C), washed with physiological saline solution, and resus-

The effect of photodithazine (PD) on the bioluminescence intensity of *E. coli* TG1 (pXen7) cells in the dark and under illumination with visible light

Preincubation with photodithazine	Bioluminescence of cells preincubated with PD, %	
	after 10 min of exposure to dark	after 10 min of illumination (30 kJ/m^2)
15 min without PD (control)	100 ± 5	105 ± 5
15 min with 7 μM PD	121 ± 8	80 ± 5
15 min with 15 μM PD	115 ± 6	53 ± 4

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pended in a protecting medium containing (%) gelatin, 1; sodium glutamate, 1; and sucrose, 10 in distilled water. The pH of this medium was adjusted to 7.5 using a concentrated solution of NaOH. Cells were lyophilized in small glass vials containing 0.5 ml of a bacterial suspension in the protecting medium. The suspension was frozen with liquid nitrogen. The lyophilization in a New Brunswick setup took 20 h. Lyophilized samples were stored at 4°C in the dark. During storage, the viability and bioluminescence of cells were tested at regular intervals.

Cell rehydration. Cells were used in experiments after their rehydration in cold (4°C) distilled water (pH 7.0), which was added to vials in a volume of 0.5 ml. After 20 min of incubation at 4°C, the suspension was diluted with distilled water to a density of $(3–4) \times 10^7$ cells/ml and incubated at 20–22°C for the next 20 min.

Optical measurements. The turbidity of bacterial suspensions was measured at 650 nm using a KF 77 photoelectrocolorimeter (Zalimp, Poland). Optical density was converted to cell density using a calibration curve.

Bacterial survival. The survival rate of rehydrated bacterial cells was estimated from the number of colonies grown on LB agar at 30°C within 24 h after plating the serial suspension dilutions.

Bioluminescence. The intensity of bacterial luminescence was evaluated using a Biotox-6 luminometer (Russia) and 1.5-ml cuvettes with 1 ml of the bacterial suspension tested. The luminescence was measured at 20–22°C and expressed in relative units.

Reagents. Photodithazine was produced by the ZAO Veta (Moscow, Russia). All other reagents were purchased from Sigma (United States).

Experimental design. A suspension (1 ml) of rehydrated bacterial cells was mixed with 20 μl of a photodithazine solution in distilled water (or 20 μl of distilled water in the control sample). The experimental and control samples were incubated in a temperature-controlled cuvette (20–22°C) for 25 min (this time was determined empirically) and then irradiated with visible light of wavelengths longer than 380 nm from a DRSh-1000 high-pressure mercury lamp equipped with a BS-8 light filter and a heat-absorbing water-filled trap. The samples were illuminated at an intensity of 50 W/m^2, which was measured with a high-sensitivity Hilger FT 16.1/622 thermocouple calibrated using an F116/1 galvanometer. The so-called "dark samples" were preincubated with photodithazine and exposed to the dark instead of being illuminated. The control samples that were preincubated without photodithazine were illuminated under the same conditions as the experimental samples.

All the experiments were conducted in quintuplicate.

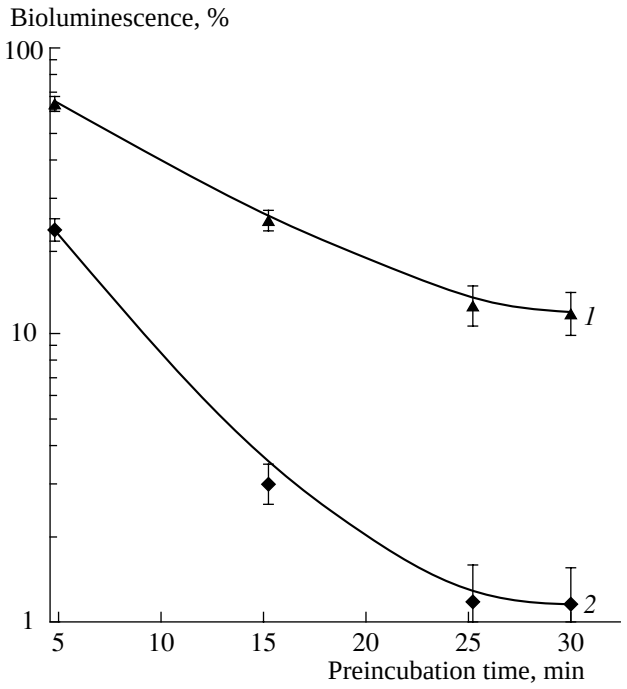

Fig. 1. The effect of the time of preincubation with (*1*) 7 and (*2*) 15 μM photodithazine on the sensitivity of the bioluminescence of *E. coli* TG1 (pXen7) cells to illumination at 30 kJ/m^2. The bioluminescence of cells was measured 20 min after their illumination. The bioluminescence of cells preincubated with photodithazine without subsequent illumination was taken as 100%.

RESULTS AND DISCUSSION

The conventional (dark) cytotoxicity of photodithazine was determined by measuring its effect on the bioluminescence of *E. coli* TG1 (pXen7) cells without illuminating them with visible light. As is evident from the table, photodithazine at concentrations below 15 μM is not toxic to bacterial cells in the dark. The illumination of the cells at an intensity of 30 kJ/m^2 in the absence of photodithazine did not affect their luminescence either. At the same time, the illumination of the cells preincubated with photodithazine quenched their bioluminescence.

Figure 1 shows the effect of the preincubation time with photodithazine on the sensitivity of bacterial luminescence to illumination. It can be seen that the photoinduced quenching of bioluminescence was maximum when bacterial cells were preincubated with photodithazine for 25–30 min. This preincubation time coincides with the preincubation time of the wild-type *E. coli* cells with chlorine e_6 that was earlier determined to be optimal via measuring colony-forming ability [4].

When *E. coli* TG1 (pXen7) cells were preincubated with 7 μM photodithazine for 25 min, the photoinduced quenching of cell bioluminescence increased within 15–20 min after illumination and then varied insignifi-

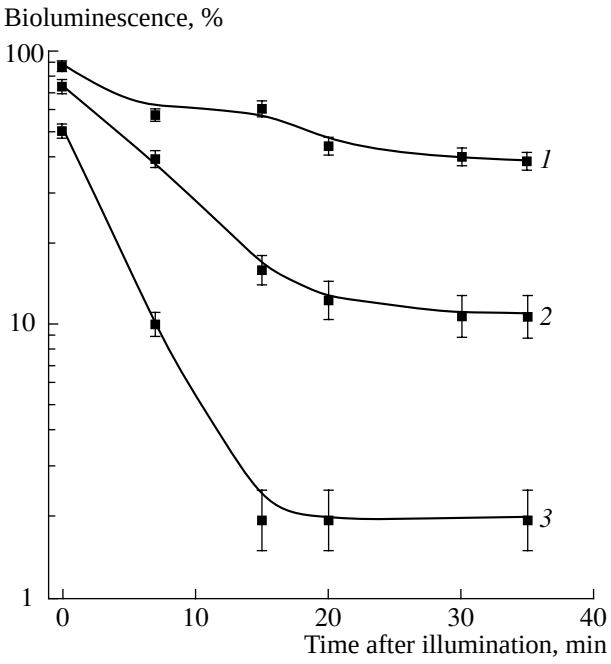

Fig. 2. Photoinduced quenching of the bioluminescence of *E. coli* TG1 (pXen7) cells preincubated with 7 μM photodithazine for 25 min and then illuminated at fluences of (*1*) 15, (*2*) 30, and (*3*) 45 kJ/m^2. The bioluminescence of cells preincubated with photodithazine without subsequent illumination was taken as 100%.

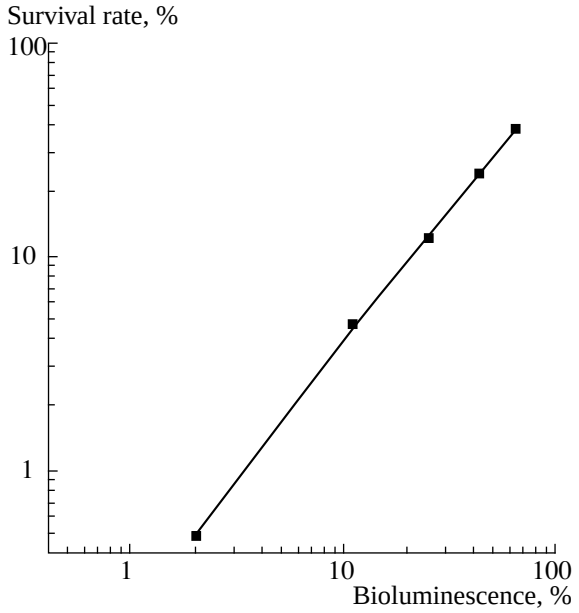

Fig. 3. Survival–bioluminescence relationship for *E. coli* TG1 (pXen7) cells preincubated with 7 μM photodithazine for 25 min and illuminated at fluences of 10 to 45 kJ/m^2. The illumination of control cells (unincubated with photodithazine) at these intensities did not influence their survival and bioluminescence.

cantly (Fig. 2). The degree of this quenching increased with the fluence of illumination, so that the bioluminescence of bacterial cells was almost completely quenched by illumination at a fluence of 45 kJ/m^2.

Bifactorial experiments showed that there is a good correlation between the intensity of the bioluminescence of *E. coli* TG1 (pXen7) cells incubated with photodithazine and irradiated with visible light and their viability (Fig. 3).

Thus, the luminescent biosensor system Ecolum is appropriate to the rapid assessment of the survival rate of luminescent bacteria subjected to photodynamic treatment. The decline in the survival rate of photosensitized bacterial cells is accompanied by a decrease in their bioluminescence, which may serve as a rapid and simple test for the assessment of the photocytotoxicity of analyzed compounds. This and other biosensor systems based on lyophilized bacterial cells with the cloned *lux* operon are very promising in the rapid search for efficient photosensitizers and in the determination of their activity against pathogenic microorganisms.

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