Studying the Non-Thermal Effects of Terahertz Radiation on *E. coli*/pKatG-gfp Biosensor Cells

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Studies of the impact of terahertz radiation on living objects present a significant interest since its use for security systems is currently considered promising. We studied the non-thermal impact of terahertz radiation on *E. coli/pKatG-gfp* biosensor cells. The Novosibirsk free electron laser (NovoFEL), which currently has the world's highest average and peak power, was used as the source of terahertz radiation. We demonstrated that exposure to terahertz radiation at the wavelengths of 130, 150, and 200 μm and a power of 1.4 W/cm^2 induces changes in green fluorescent protein (GFP) fluorescence values and thus induces the expression of GFP in *E. coli/pKatG-gfp* biosensor cells. Possible mechanisms of the *E. coli* response to non-thermal exposure to terahertz radiation are discussed. Bioelectromagnetics © 2012 Wiley Periodicals, Inc.

Key words: terahertz free electron laser; non-thermal impact of terahertz radiation; biosensor; *Escherichia coli*

INTRODUCTION

The term "terahertz radiation" is usually applied to the wavelength range from 100 μm to 1 mm. In the last decade, this part of the spectrum has been widely used due to the emergence of new radiation sources. Novel sources of terahertz radiation, novel detection techniques, as well as new methods of scientific studies using terahertz radiation are being developed.

The importance of terahertz radiation for studying biological systems is caused by the fact that its frequencies correspond to the range of conformational oscillations of DNA and protein molecules, as well as by the coincidence of its quantum energies with the energies of hydrogen bonds and Van der Waals intermolecular interactions. Terahertz radiation is promising for fields such as diagnostic medicine, spectroscopy, security systems [Liu et al., 2008; Ashworth et al., 2009; Suen et al., 2009; Schirmer et al., 2010], and spectroscopy [Plusquellic et al., 2007; Cao et al., 2009; Shen and Ying, 2009]. These imply the increasing application of instruments using terahertz radiation and their contact with humans [Matsuura et al., 1998; Náfrádi et al., 2008; Tanabe et al., 2009; Hofmann et al., 2010; Yasui et al., 2010]. Despite the non-ionizing nature of terahertz radiation, studying its influence on living systems is necessary since some other effects can occur.

It was demonstrated that exposure to 0.1 THz results in increased genomic instability of human lymphocytes [Korenstein-Ilan et al., 2008]. Ramundo-Orlando et al. [2007] demonstrated that terahertz radiation alters the permeability of liposome membranes. No effect of terahertz radiation on human leukocytes in peripheral blood, keratinocytes, and nervous cells cultures was demonstrated [Bourne et al., 2008]. Based on mathematical modeling, it has been proposed that terahertz radiation could cause partial and even complete destruction of hydrogen bonds between complementary DNA strands [Alexandrov et al., 2010]. This impact may considerably alter DNA dynamics and chromatin organization with an impact on gene transcription,

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replication, and the cellular phenotype [Petrov et al., 2007; Alexandrov et al., 2010]. Over 10% of genes altered their expression level in response to prolonged (2–6 h) exposure to terahertz radiation in mice stem cells [Bock et al., 2010]. The growth of *Saccharomyces cerevisiae* microcolonies significantly increased after exposure to 0.2–0.3 THz radiation [Hadjiloucas et al., 2002]. Irradiation at the wavelength of 1 THz caused the formation of meristem cells in the hypocotyls of flax (*Linum usitatissimum*) seedlings. Analogous changes are induced by other weak interactions, such as the radiation emitted by mobile phones (Global System for Mobile Communications, GSM), mechanical irritation, and cold shock [Tafforeau et al., 2004].

The construction of a new, powerful, and tunable source of terahertz radiation at the Novosibirsk Scientific Center (Novosibirsk, Russia) opened up new possibilities for studying the impact of this radiation on biological objects in a wide range of wavelengths and power. In this work, we investigated the non-thermal impact of terahertz radiation on *Escherichia coli* cells. This bacterium is a convenient and thoroughly studied model object for molecular biology. There is a variety of biosensor cells based on this bacterium. Biosensors are an artificial genetic reporter system that contains a sensitive element—the promoter of a sensor gene that is activated in response to external impacts. A reporter gene, which is under regulatory control of the sensor gene and encodes the green fluorescent protein (GFP), is an important part of this system. Currently, there is a range of rather effective biosensor constructs based on promoters of *E. coli* genes, which are the key parts of bacterial response to stress. These are mainly biosensors sensitive to oxidative stress [Belkin et al., 1996; D'Souza, 2001] and heavy metals [Tauriainen et al., 1998, 1999; Virta et al., 1998]. There are also sensors that specifically respond to the damage of DNA, proteins, and cell membranes [Kim and Gu, 2003].

In this study we used biosensor constructs based on the stress-sensitive promoter of the catalase gene *katG* for studying cellular processes associated with the response to exposure to terahertz radiation.

MATERIALS AND METHODS

Terahertz Source

Experiments were performed using a terahertz free electron laser (TFEL) designed and constructed by the Institute of Nuclear Physics, Siberian Branch (SB) of the Russian Academy of Sciences (RAS, Novosibirsk, Russia), at the Siberian Center of Synchrotron and Terahertz Radiation [Gavrilov et al., 2007]. Parameters of the laser are given in Table 1.

TABLE 1. Parameters of the Novosibirsk Terahertz Free Electron Laser (TFEL)

Parameters	Value
Wavelength, μm	50–240
Pulse duration, ps	50
Pulse repetition frequency, MHz	2.8–11.2
Average power, W	Up to 400
Peak power, MW	Up to 1
Minimum relative width of the spectral line, $\Delta\lambda/\lambda$	3×10^{-3}
Using average power density, W/cm^2	1.4

In order to control the temperature of the culture medium under terahertz radiation, we used the highly sensitive TKVr-SVIT101 thermal imager produced by the Institute of Semiconductor Physics SB RAS (Novosibirsk, Russia), which has a sensitivity over 0.027 °C and can dynamically detect changes in temperature fields within the 10–42 °C range [Kuryshev et al., 1998].

Preparation of Biosensor Cells

We used recombinant *E. coli JM103* bacteria containing the *pKatG-gfp* plasmid (biosensor *E. coli/pKatG-gfp*) [Khlebodarova et al., 2007]. The GFP, which has reduced stability (shortened half life about 60 min long) and 20 times stronger fluorescence than wild-type protein, was used as the reporter protein (GFPaav—mutant proteins with the C-terminal extension sequences RPAANDENYAAAV) [Keiler et al., 1996; Andersen et al., 1998]. Cell cultures were grown overnight to the average logarithmic phase (optical density 0.3) in fresh Luria-Bertani (LB) medium with 100 μg/ml ampicillin. Since LB medium has its own fluorescence in the fluorescence range of the GFP, cells were transferred to minimal M9 medium containing 0.4% glucose, 0.2% casamino acids, 48 mM Na_2HPO_4, 22 mM KH_2PO_4, 18.7 mM NH_4Cl, 8.5 mM NaCl, 1 mM $MgSO_4$, 0.1 mM $CaCl_2$, and 100 μg/ml ampicillin. The cell culture in minimal medium was subsequently placed into a special cuvette for exposing the biological samples.

Exposure of Bacterial Cells Containing Biosensor Molecules to Terahertz Radiation

An aliquot (50 μl) of the culture was placed into a specially designed cuvette between two stretched polypropylene films, 40 μm thick. The thickness of the irradiated volume was also 40 μm

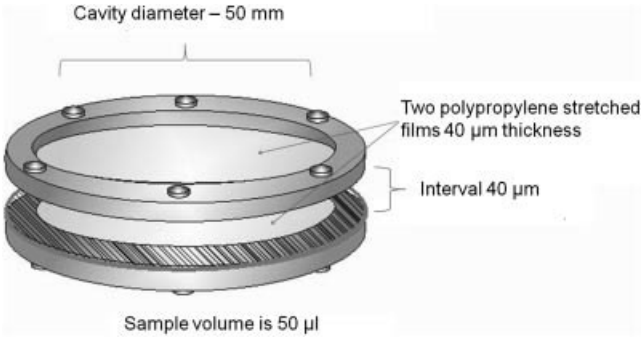

Fig. 1. Cuvette designed for terahertz irradiation of an aliquot of the culture placed between two polypropylene films. The cuvette was placed on a rotating table; an obturator located between the cuvette and the laser was used to control the emission power during the experiment.

(Fig. 1). The radiation cross-section was elliptical in the plain of the cuvette. The cuvette was rotated using a special mechanism in order to provide uniform exposure for the sample volume. To control the average emission power, we constructed an obturator consisting of two copper circles 20 cm in diameter, which were rotated around the common rotation axis using an electric motor. Each circle had a single sector aperture sized 1:30 of the circle area. By turning the circles relative to each other one can control the size of the aperture, thus changing the average emission power while leaving the peak power unchanged. To change the peak power values, the sample was positioned at various points of the emission lines behind the obturator. The temperature of the culture medium was kept at 35 ± 2 °C using the thermal imager and by changing the average emission power. The temperature was strictly controlled during the experiment; if the temperature of the samples exceeded the range of 35 ± 2 °C, observations were discarded.

To check the operability of the stress-sensitive biosensors, we induced the GFP by 2.5 mM hydrogen peroxide (positive control). Samples were subsequently transferred to a 96-cell, round-bottom plate in 100 μl aliquots. The fluorescence intensity was measured using a Perkin Elmer VICTOR3 fluorometer (1420-011 multilabel counter, Wallac, Turku, Finland) every 10 min for 300 min after the induction mix was added. The temperature of the biosensor cells was kept at 37 °C using a table "Duet" thermostat (PST-60HL thermo shaker, Biosan, Riga, Latvia). After the operability of a clone was checked, it was exposed to terahertz radiation as described above. The fluorescence intensity was also measured as described above. A cell culture, sham exposed to any impact except irradiation was used as a negative control; 50 μl of cells in a special cuvette was kept in the thermostat at 37 °C for the same time as the cells exposed to irradiation.

We also performed control experiments with the biosensor cells exposed to increased (heat shock, 42 °C for 5 min) and decreased (30 °C, 15 min) temperature. No increase in the GFP fluorescence signal was observed (data not shown). Therefore, temperature changes within the studied range (35 ± 2 °C) did not induce the fluorescence of biosensor cells.

Visualization of the Fluorescent Signal of Single Biosensor Cells Using Microscopy

A fluorescent Axioskop 2 plus microscope (Zeiss, Gottingen, Germany) was used to visualize the GFP in *E. coli/pKatG-gfp* biosensor cells. The cell culture in minimal medium (3 μl) was applied onto slides, covered with cover glass, and studied using phase contrast and fluorescence microscopy at an excitation wavelength of 488 nm.

Counting Cell Number on Agar Medium

To estimate the viability of cells in controls and after irradiation, aliquots were sowed onto solid agar medium containing 100 μg/ml ampicillin. After 18 h, the number of colonies was counted; the number of viable colonies turned out to be roughly the same in all samples.

RESULTS

The induction of the catalase gene promoter was initially demonstrated in microscopy studies of *E. coli/pKatG-gfp* biosensor cells after a 10 min exposure to terahertz radiation at the wavelength of 130 μm and 1.4 W/cm^2 power density. Microphotographs of irradiated and control cells obtained using phase contrast and excitation of the GFP at a wavelength of 488 nm are presented in Figure 2. As seen in this figure, the majority of cells exposed to terahertz radiation produced the GFP, while no signal was detected in unexposed cells.

In subsequent experiments, the intensity of GFP fluorescence was determined using a fluorometer. We performed a series of exposures of *E. coli/pKatG-gfp* biosensor cells to terahertz radiation at wavelengths of 130, 150, and 200 μm. Figures 3a, 4a, and 5a show GFP fluorescence values after a single 15 min exposure to terahertz radiation with 1.4 W/cm^2 power density and 130, 150, and 200 μm wavelengths, respectively. GFP fluorescence values in the same cells induced by hydrogen peroxide (positive control) and the basic fluorescence values in unexposed cells

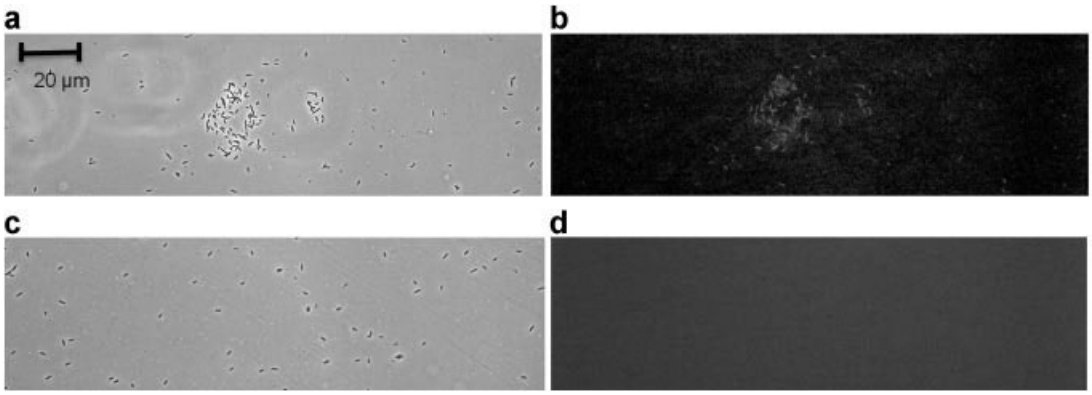

Fig. 2. *E. coli/pKatG-gfp* biosensor cells after exposure to terahertz radiation at a wavelength of 130 μm for 15 min. (**a**) Phase contrast picture of irradiated cells; (**b**) fluorescence microscopy picture of the irradiated cells; (**c**) phase contrast pictures of control cells; (**d**) fluorescence microscopy picture of control cells.

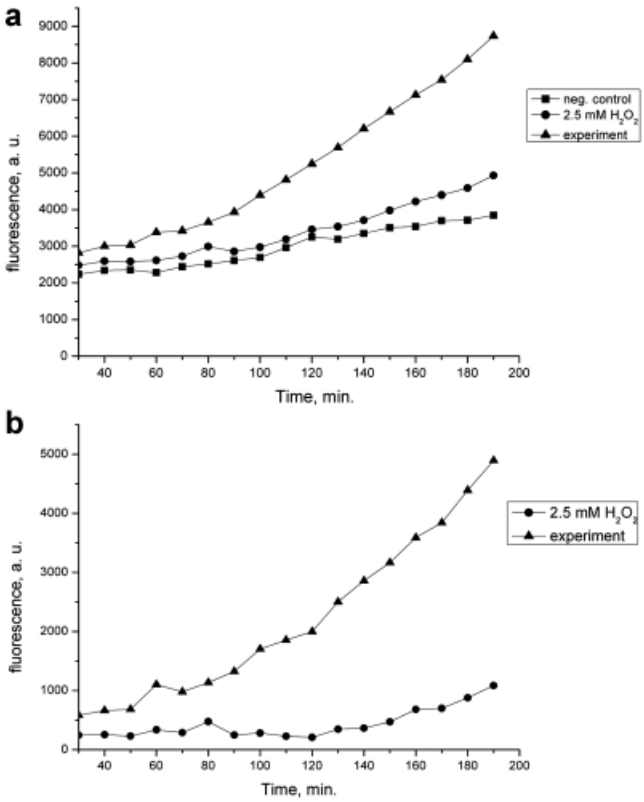

Fig. 3. **a**: Fluorescence intensity of the GFP in *E. coli/pKatG-gfp* biosensor cells after 15 min exposure to terahertz radiation at a wavelength of 130 μm. neg. control: unexposed cells; H_2O_2: inducer (positive control). **b**: Fluorescence intensity of the GFP in *E. coli/pKatG-gfp* biosensor cells after 15 min exposure to terahertz radiation at a wavelength of 130 μm, normalized to the background fluorescence values.

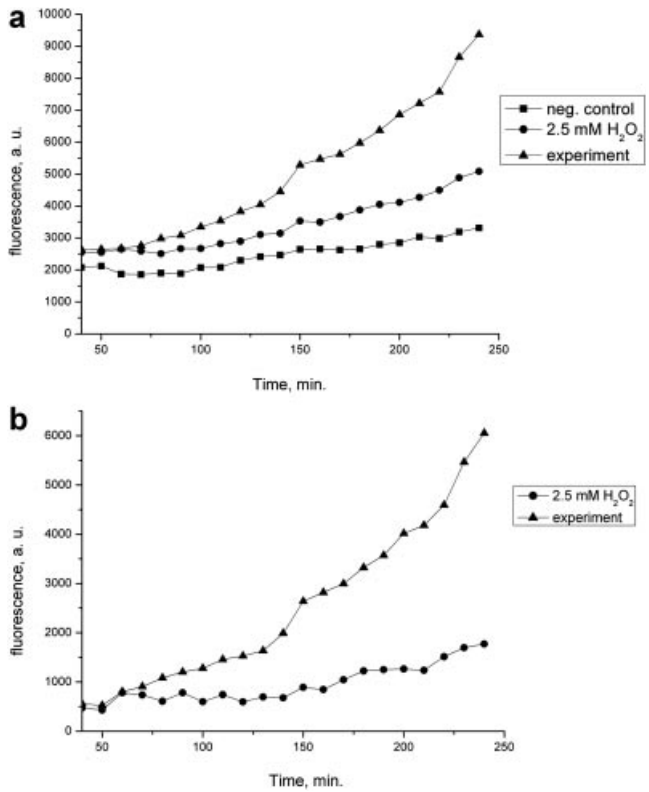

Fig. 4. **a**: Fluorescence intensity of the GFP in *E. coli/pKatG-gfp* biosensor cells after 15 min exposure to terahertz radiation at a wavelength of 150 μm. neg. control: unexposed cells; H_2O_2: inducer (positive control). **b**: Fluorescence intensity of the GFP in *E. coli/pKatG-gfp* biosensor cells after 15 min exposure to terahertz radiation at a wavelength of 150 μm, normalized to the background fluorescence values.

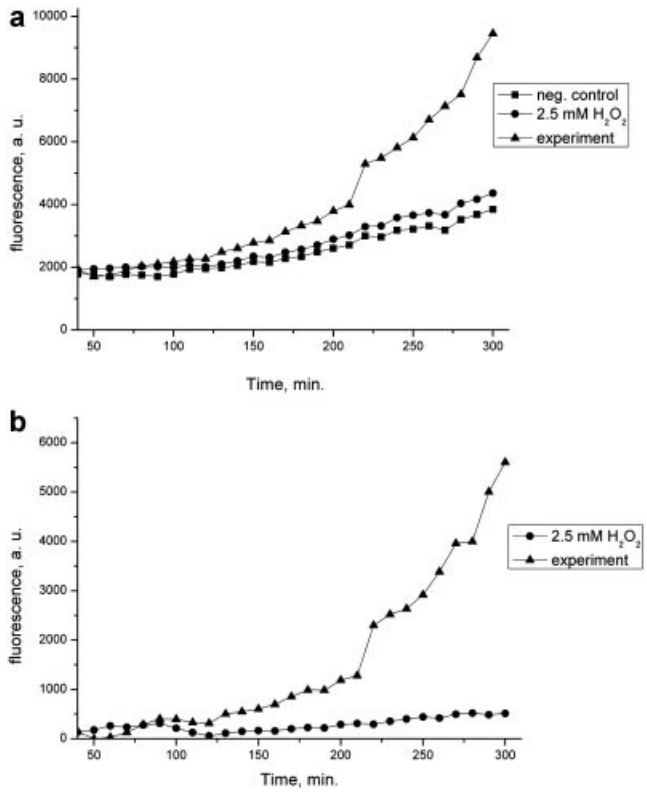

Fig. 5. **a**: Fluorescence intensity of the GFP in *E. coli/pKatG-gfp* biosensor cells after 15 min exposure to terahertz radiation at a wavelength of 200 μm. neg. control: unexposed cells; H_2O_2: inducer (positive control). **b**: Fluorescence intensity of the GFP in *E. coli/pKatG-gfp* biosensor cells after 15 min exposure to terahertz radiation at a wavelength of 200 μm, normalized to the background fluorescence values.

(negative control) are also shown. Figures 3b, 4b, and 5b show fluorescence values induced by terahertz radiation and hydrogen peroxide normalized to the background fluorescence values.

Three independent replications (including positive and negative controls and irradiation) were performed for each experiment. All data were analyzed using the linear regression method. The results of the experiments at 130 and 150 μm wavelengths were not converted; results of the experiment at 200 μm were converted according to the $y = x^{1/3}$ formula. Slope coefficients of each replication were then compared using Student's *t*-criterion. The differences between negative controls and experiment replicates were statistically significant for all experiments ($P < 0.001$; Table 2).

As illustrated in Figures 3–5, the 15 min exposure of the cells to terahertz radiation at all studied wavelengths induced the expression of the GFP in *E. coli/pKatG-gfp* biosensor cells. At the same time,

TABLE 2. Critical Values of Student's *t*-Criterion Calculated for the Experimental Results and Negative Controls for the Cells Exposed for 15 min at Wavelengths of 130, 150, and 200 μm

	t-Criterion		
	130 μm	150 μm	200 μm
Experiment—negative control	14.17649*	15.97146*	10.4565*

*$P < 0.001$.

exposure for 5 and 10 min did not evoke a pronounced increase in fluorescence intensity (data not shown). At the constant irradiation intensity of 1.4 W/cm^2, the induction of GFP synthesis did not occur after 5 min of irradiation, after 10 min it was observed only in some experiments, and after 15 min it was detected for all samples.

Figures 3–5 demonstrate that *E. coli/pKatG-gfp* biosensor cells react similarly to both the presence of various concentrations of hydrogen peroxide and to terahertz radiation. Moreover, exposure to terahertz radiation results in significantly higher fluorescence intensity, although this response takes longer to develop. We should note that different clones have different base fluorescence levels and different response intensities to exposure to hydrogen peroxide; for that very reason we give both original (Figs. 3a, 4a, and 5a) and normalized (Figs. 3b, 4b, and 5b) experimental data. As seen in Figures 3–5, the same clones have approximately the same fluorescence dynamics in response to terahertz radiation. The number of cells was roughly the same in all experiments, which indicates the increase of GFP synthesis in *E. coli/pKatG-gfp* biosensor cells. Biosensor cells express GFP for 5 h after exposure to terahertz radiation, which equals about eight life cycles.

DISCUSSION

In this study we demonstrated that terahertz radiation induces a stable expression of the reporter gene under the *katG* promoter for a significant time after irradiation. These results seem unexpected since it is known that the *katG* gene is sensitive to oxidative stress.

It is also known that the *katG* promoter contains binding sites for transcription factors such as the oxidative stress regulator (OxyR), and the fumarate and nitrate reduction factor (FNR). These regulators perform positive regulation of the *katG* gene. The OxyR regulon is known to play a key role in *E. coli* protection from oxidative stress associated with active

aerobic growth. Nine of about 30 proteins whose expression increases on exposure to hydrogen peroxide are controlled by OxyR, catalase (*katG*), Mn-superoxide dismutase, alkylhydroperoxidase (*ahpFC*), glutathione reductase (*gorA*), glutaredoxin I (*grxA*), DNA-binding protein (*dps*), as well as several heat shock proteins. The functions of the majority of these proteins are the protection of cells from agents of oxidative stress, destruction of H_2O_2 by catalase, and protection of DNA from oxidative stress [González-Flecha and Demple, 1995]. The binding site of the FNR transcription factor in the *katG* gene promoter was detected by its sequence similarity to other binding sites of this protein. Currently, there are no reliable data that confirm the role of the FNR transcription factor in *katG* gene expression. The transcription factor FNR is a global genomic regulator controlling the expression of over 120 *E. coli* genes. We should note that the activity of this transcription factor is regulated through the redox-sensitive Fe-S cluster directly by the presence of oxygen, which inactivates FNR; the active form is characteristic of anaerobic conditions. FNR activates the expression of anaerobic respiration enzymes that use alternative final electron acceptors such as dimethyl sulfoxide (DMSO), fumarate, or nitrate. In addition to activation of the expression of proteins specific for anaerobic conditions, FNR also represses aerobic respiratory enzymes including cytochrome oxidase (*cydA*) and nicotinamide adenine dinucleotide (NADH) II dehydrogenase. Besides respiration, FNR also regulates the transcription of many genes with other functions, such as resistance to acids, chemotaxis, etc. [Salmon et al., 2003; Kang et al., 2005].

We observed different dynamics of the biosensor response to hydrogen peroxide and terahertz radiation. The response graph to hydrogen peroxide has its maximum 100–150 min after the addition of the inducer [Belkin et al., 1996; Lu et al., 2005]. Since it was demonstrated that the concentration of cells in the negative and positive control samples was identical, the presence of the maximum is directly associated with biosensor induction and the increase in GFP quantity. After passing the maximum the fluorescence intensity decreases, which is caused by reduced GFP expression due to the processing of the inducer, as demonstrated in many experimental studies [Lu et al., 2005; Khlebodarova et al., 2007].

Kinetics of the response to terahertz radiation has a principally different look. We observed significant GFP quantities 150–180 min after the beginning of the experiment (Figs. 3–5). No reduction in GFP concentration was detected during the rest of the experiment. We assume that the response to terahertz radiation is mediated by other regulatory mechanisms rather than the response to hydrogen peroxide. The absence of a rapid response of irradiated cells may indicate that indirect activation of oxidative stress transcription factors may take place, and therefore, the response takes a longer time to manifest itself. Some specific mechanism of *katG* promoter activation in response to terahertz radiation will be the object of further studies.

Since there are no other binding sites of OxyR and FNR transcription factors in the *katG* gene promoter, we suggest that the *katG* promoter is induced through the oxidative stress pathway. The fact that the kinetics of response to terahertz radiation does not resemble the kinetics of response to hydrogen peroxide indicates that the FNR transcription site found within the *katG* promoter is active and activated when the promoter is induced by terahertz radiation.

CONCLUSIONS

We demonstrated that *E. coli/pKatG-gfp* biosensor cells synthesize the reporter GFP in response to exposure to terahertz radiation. At the power of 1.4 W/cm^2, the expression intensity depends on the duration of irradiation: GFP was always expressed after 15 min of exposure, after 10 min it was unstable, and no GFP was detected after 5 min. This dependence was observed for all wavelengths tested (130, 150, and 200 μm). In all experiments, the GFP was expressed for over eight cell generations after exposure to terahertz radiation.

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