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2.3 THz radiation: Absence of genotoxicity/mutagenicity in *Escherichia coli* and *Salmonella typhimurium*

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ABSTRACT

The mutagenicity and genotoxicity in bacteria of 2.3 THz radiation (THz) produced by a free-electron laser (NovoFEL) were evaluated; exposures were 5, 10, or 15 min at average power 1.4 W/cm^2. Two Ames mutagenicity test strains of *Salmonella typhimurium*, TA98 and TA102, were used. For the genotoxicity test, we measured SOS induction in *Escherichia coli* PQ37. No significant differences were found between exposed and control cells, indicating that THz radiation is neither mutagenic nor genotoxic under these conditions. Nevertheless, a small increase in total cell number of *S. typhimurium* after 15 min exposure, and an increase in β-galactosidase and alkaline phosphatase activities in *E.coli* PQ37, were observed, indicating some effect of THz radiation on cell metabolism. We also examined the combined effect of 4-NQO (8 μM; positive control) and THz exposure (5 min) on genotoxicity in *E.coli* PQ37. Unexpectedly, THz radiation decreased 4-NQO genotoxicity.

1. Introduction

Terahertz electromagnetic radiation (THz EM) has been little studied, because of the absence of natural sources, leading to the so-called 'THz gap'. However, the appearance of new radiation sources and detection methods has recently attracted increased attention. Sources of THz radiation have started to find practical applications in several fields, and exposure of organisms to THz radiation has become more likely. The need for a comprehensive study of the effects of THz radiation on organisms has been recognized [1–4]. Whether low-energy (non-ionizing) EM affects biological systems has been debated for a long time [5] and contradictory experimental results remain to be resolved, ranging from generation of free radicals and effects on enzyme activities to genotoxicity and cell cycle aberrations [6–11]. THz EM cannot alter the primary structure of biopolymers, but it may affect hydrogen bonds, major contributors to the organization of higher-order structures of protein and nucleic acids. Furthermore, genotoxicity can be mediated by indirect mechanisms: altered DNA repair, generation of active oxygen species, etc. In our earlier studies, we demonstrated that exposure of *Escherichia coli* to THz EM leads to a stress response mediated by the oxidative stress gene network [12].

In 2001–4, the European Community undertook the "BRIDGE" project ("Terahertz radiation in Biological Research, Investigation on Diagnostics and study of potential Genotoxic Effects") investigating the biological effects of THz EM [13]. The final report states that the effects of THz EM were detected only under the following conditions: high radiation power, long exposure, or at a particular wavelength. Since then, the number of studies on the effects of THz EM has increased significantly [1,14,15]. However, large variations in experimental conditions make it difficult to draw definitive conclusions. For example, in ref. [16], the authors found that exposures of human peripheral blood leukocytes to EM at frequencies 0.12 and 0.13 THz did not alter cell cycle kinetics or cause chromosome damage, micronucleus formation, or DNA damage as assessed by the comet assay. A study assessing the effect of THz EM on chromosome damage, cell proliferation, and DNA damage in skin cells *in vitro* found no evidence of genotoxic effects at 0.380 and 2.520 THz and power levels 0.03–0.9 mW/cm^2 [17]. On the other hand, an investigation of the effects of intense THz pulses (amplitude spectrum peak, 0.5 THz) on human skin tissue models found a significant exposure-dependent increase in phosphorylation of

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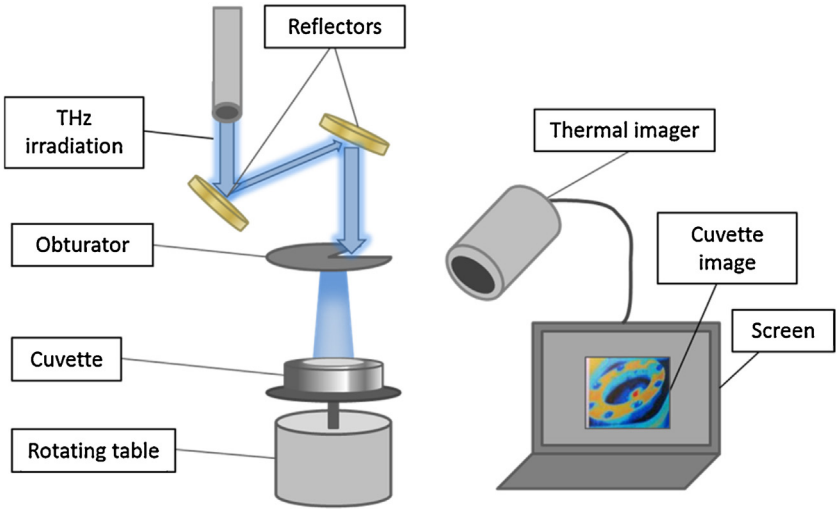

Fig. 1. Scheme of experimental station for sample irradiation.

histone H2AX, indicating that THz pulse radiation may cause damage to double-stranded DNA in exposed skin tissue [18]. In addition, the authors detected upregulation of certain cell-cycle genes, which indicates that THz EM may lead to genome instability [18]. In agreement with these data, a study by Korenstein-Ilan et al. [19] reported changes in chromosome number and synchronism of centromere replication in dividing human lymphocytes, as assessed by FISH, in response to continuous-wave 0.1 THz EM (0.031 mW/cm^2).

Most studies of the biological effects of THz EM use low-intensity emitters. Therefore, biological effect may be due to the very low radiation doses used. Nevertheless, even low-intensity THz EM sources may lead to non-thermal effects at the level of regulation of gene expression [12].

In the present study, we investigated genotoxicity and mutagenicity of 2.3 THz EM produced by the free-electron laser (NovoFEL) [20,21], using two bacterial tests: the Ames test and the SOS chromotest. The Ames test can detect mutagenicity of chemical compounds [22–25] and EM [26], while the SOS chromotest detects genotoxicity [27]. We used two *Salmonella typhimurium* Ames test strains, TA102 (*hisG428*) and TA98 (*hisD3052*), which are sensitive to base substitutions [22] and frameshifts [23], respectively. The SOS chromotest measures induction of SOS response genes, in particular the sfiA (sulA) gene, in response to DNA damage [27–30]. *E. coli* strain PQ37, used in the SOS chromotest, carries a *lacZ* reporter gene that is activated by the SOS response.

2. Materials and methods

2.1. Irradiation of bacterial cells

Radiation experiments were performed at the biological station of the Siberian Center for Synchrotron and Terahertz Radiation at the Budker Institute of Nuclear Physics SB RAS (Fig. 1), where THz EM is produced by the free-electron laser (NovoFEL) [20,21]. The NovoFEL wavelength range is 50–240 μm, which corresponds to 6–1.25 THz. Parameters of the NovoFEL radiation are presented in Table 1.

In our experiments, we used THz EM with λ = 130 μm (2.3 THz) and average power 1.4 W/cm^2. Samples (50 μL) were irradiated in a special cuvette with polypropylene walls which are transparent to THz EM, between two 40 μm polypropylene films. The thickness of the irradiated layer was 40 μm. To ensure uniform exposure of the entire sample, the cuvette was mounted on a rotating table. The average power was regulated using an obturator consisting of two 20-cm copper disks, having a common rotation axis, driven with an electric motor. Each disk has a sector aperture 1/30 of its area. The relative positions of the disk apertures set the area of the opening and thus regulate the average power of exposure. The temperature of the medium was kept at 35 ± 2°Ñ and controlled using a TKVr-SVIT101 thermal imager [31].

Table 1
Parameters of the NovoFEL radiation.

Parameter	Value
Wavelength, μm	50–240
Pulse duration, ps	50
Pulse repetition period, ns	180
Average power, W	up to 400
Relative linewidth, Δλ/λ	3.10^{-3}

2.2. Ames test

The Ames test was performed in strains TA98 and TA102 [22]. Mutagenicity is measured by counting revertant colonies [23]. In this study, the Ames test was carried out by plating bacteria onto selective bilayer agar, according to [23], modified to implement the protocol at the NovoFEL experimental station. The modification concerned the manner of exposing the cells to THz EM. We included an additional step of concentrating the cell suspension (grown to exponential phase) from 2 × 10^8 cells/mL to 2 × 10^9 cells/mL before exposure to THz, in order to adjust to the small (50 μL) volume of the experimental cuvette. This allowed us to obtain sufficient His$^+$ revertants for evaluation of mutagenecity. Consequently, the test was done as follows: LB medium (100 mL) was inoculated with overnight cultures of *S. typhimurium* (TA98 and TÀ102), 1 mL, with subsequent incubation at 37°C, and grown to OD_{600} = 0.45 (2 × 10^8 cells/mL), followed by centrifugation at 4000*g*. Pelleted cells were resuspended in 10% glycerol (10 mL), frozen, and stored at −70 °C.

Cell suspension from the frozen stock (50 μL, 2 × 10^9 cells/mL) was transferred to the cuvette and exposed to varying doses of THz EM (5, 10, or 15 min). Negative control cells were not exposed to radiation. Positive controls were incubated with 4-NQO, 0.25 μM. After exposure, the cultures were diluted × 10 with LB medium and incubated on a shaker at 37C for 1 h. An aliquot (100 μL; 2 × 10^7 cells) of the culture was then added to molten 0.6% top agar, 2 mL, mixed, and transferred to Petri dishes with a lower layer of 1.5% agar (minimal medium with 0.2% glucose), to form a uniform top layer. The dishes were incubated at 37C for 48 h and the numbers of His+ colonies were counted.