Terahertz irradiation effects on the morphology and dynamics of actin biopolymer

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Keywords: terahertz irradiation, actin, biopolymer

Abstract

Our recent experimental research on terahertz (THz) irradiation of actin filaments, which serve as representative biopolymer materials, is summarized in this review. We found that pulsed THz waves with energy density of $\sim 10^{-4}$ J cm\textsuperscript{-2} ($\sim 10^8$ W cm\textsuperscript{-2} at the peak) generate acoustic waves efficiently in the aqueous media. These acoustic waves propagated deeply into the water and demolished the actin filaments in living HeLa cells that were submerged into the cell culture medium. The results implied that THz pulsed irradiation affects the biomolecules in the tissues, even if these molecules are located a few millimeters away from the body surface. In contrast, irradiation using THz waves with lower peak power of the order of $\sim W$ cm\textsuperscript{-2} induces the elongation of the actin biopolymer without thermal or acoustic effects. The polymerization of actin molecules plays essential roles in cell motility, growth, differentiation and gene expression. Therefore, our results indicate that THz waves could be applicable to artificial manipulation of cellular functions.

1. Introduction

As a result of the recent developments in terahertz (THz) technologies, the applicability of these technologies has been demonstrated for a wide range of fields, including chemical sensing [1, 2], security imaging [3–5], motion sensing [6] and telecommunications [7–12]. For example, in the proposed sixth generation (6G) wireless communications that are intended to be ready for practical use in the 2030s, use of sub-THz electromagnetic waves is currently under consideration. Sub-THz devices are also being studied for use in acquisition of the high-precision position information required for radar systems for use in autonomous driving and motion sensors. Over the next few decades, THz wave devices will become miniaturized, more powerful and low in cost, and will thus become more familiar for use in daily life. To enable widespread practical THz applications, the safety of THz radiation for human health must therefore be guaranteed [13].

To date, the interactions between THz radiation and biological systems have been investigated by numerous researchers [13–18]. For example, the effects of THz radiation on DNA and gene transcription have been reported [19–23]. Radiation effects have been studied in a variety of human cells, including cancer cells [24], stem cells [20, 23], lymphocytes [21], Jurkat cells [25], stem cells from fibroblasts [26] and skin cells [22, 27], and they have also been studied in other faunal cells [28–30]. Many of the above studies reported significant THz radiation effects based on comparison of irradiated samples with controls. However, the mechanism behind these effects remains unclear because these phenomenological studies could not reveal the underlying physical and chemical origins of the effects in such complex biological systems.

The THz frequency range corresponds to the intra- and intermolecular vibration range of macromolecules and the intermolecular vibration and rotation range of water molecules. We can easily imagine that the irradiating THz photons excite the dynamics of the proteins and the DNAs, along with the surrounding water molecules. The results of molecular dynamics simulations suggest that THz
underdamped vibrational motion directs biochemical reactions in solutions [31]. However, only a few studies have succeeded in observing THz-induced structural changes [32–35]. One possible reason for this difficulty is the thermal relaxation that occurs at room temperature. The photon energy of the THz radiation corresponds to 48 K at 1 THz. Even if the THz radiation succeeds in inducing conformational change, the rapid relaxation at room temperature will then force the sample back to the thermal equilibrium condition. The THz irradiation effects are most likely to be observable in systems that never reach the equilibrium condition, e.g. biochemical reactions occurring in living cells.

To understand the mechanisms of biochemical reactions under THz irradiation, it is necessary to perform multiscale investigations over the range from the molecular scale to the cellular scale. In our recent studies, we focused on actin filaments, which is a representative biopolymer in cells. Actin takes two functional forms: monomeric globular (G)-actin and polymerized filamentous (F)-actin. F-actin form an elaborate network called the cytoskeleton, and play important roles in cell motility, growth, differentiation and gene expression [36]. The virtue of the actin protein is that actin polymerization can be reproduces in vitro with purified G-actin [37]. It is known that actin polymerization reaction consists of three phases: nucleation, elongation, and steady-state. These phases can be monitored using the fluorescence intensity of pyrene actin because the fluorescence of the polymerized pyrene actin is ten times higher than that of F-actin. In addition, the actin filaments in living cells can be observed directly via fluorescence microscopy by staining them with silicon-rhodamine (SiR)-actin [38]. Therefore, we can observe both the reaction and the morphology of the same protein molecule in vitro and in vivo, and can then understand whether or not the THz radiation affects the structure and function of the protein molecules directly.

Another difficult point in understanding of THz irradiation effects is the variety of light sources that can be used to perform the irradiation. THz sources by electron acceleration, such as the THz free-electron laser (THz-FEL) and the gyrotron, generate \( \sim \)mJ per pulse high-power fluxes. When using these sources, the THz power must be attenuated appropriately to avoid heating effects. In contrast, the thermal effect is negligibly small when using the THz radiation from nonlinear optics or from diode sources that generate energies of \( \sim \)\( \mu \)J per pulse or less. In addition, the pulse width of the THz beam must also be considered. Pulsed THz sources such as the THz-FEL and nonlinear THz sources generate ultrashort pulses with picosecond-scale durations. Such ultrashort pulses with high peak powers can induce nonlinear effects in molecular systems because of the high intensities of their electric fields (\( \sim \)MV cm\(^{-1}\)), which may then result in electron excitation or ionization processes that damage the biomolecules [39]. Our recent study also showed that irradiation by a pulsed THz wave generates acoustic waves in aqueous solutions, which can also cause changes in the biological samples [24, 40].

In this paper, our recent studies of THz irradiation experiments are reviewed [24, 33, 40]. Two different THz sources, a THz-FEL and a gyrotron, were used. The THz-FEL produces pulsed THz waves in the 3–6 THz frequency range with a duration of 2 ps. In early studies, morphological changes were observed in the macromolecules after THz irradiation, and these phenomena were believed to be the result of the THz photons acting on the molecular dynamics [32, 34]. However, our recent study revealed that the THz irradiation produces acoustic waves efficiently [40]. Therefore, the morphological changes observed could be due to the acoustic wave generated by the pulsed THz irradiation. To avoid both thermal and acoustic effects, we also performed an irradiation experiment using a gyrotron with lower peak power. As a result, an acceleration of the polymerization reaction of the actin proteins was observed [33].

2. Energy transport of the THz wave after irradiation

When the THz wave irradiates the biological samples, the radiation energy is transferred to become another type of energy. When the sample contains water, most of the input energy is initially absorbed by the rotational relaxation and the intermolecular vibration of the water molecules [41]. The THz light is absorbed very close to the water’s surface and has a penetration depth of less than 100 \( \mu \)m. Figure 1 shows the energy transport pathway after THz irradiation of the sample. When the THz wave is absorbed by the water, the energy will then be transformed into thermal energy and acoustic energy, while any remaining energy is absorbed by the protein molecules.

2.1. Heating effect

The heating effect is the most likely process to occur when high-power THz waves are used to irradiate biosamples. Under high irradiation conditions, biomolecules are wounded in a manner similar to the corresponding substances in the heated samples [14, 18]. For example, Wilmink et al reported that irradiation using molecular gas THz lasers (2.52 THz, 84.8 mW cm\(^{-2}\)) induced a cellular temperature increase of 3 °C, and a heat shock protein expression was also observed [42]. To avoid heating of the sample
and determine the nonthermal effect of the THz irradiation, the power of the THz source must be reduced appropriately.

To determine whether or not the observed phenomenon is a thermal effect, it is necessary to estimate the sample temperature during irradiation. If the radiation source is a continuous wave source and the irradiated spot size is sufficiently large, a thermocouple provides a simple and direct method to measure the temperature. Otherwise, computational simulations become necessary. For example, Kristensen et al developed a model using Kirchhoff's heat equation to estimate the temperature of a liquid sample under continuous wave THz beam irradiation [43]. An open-source program for Matlab software for this model is available online.

On the other hand, when a pulsed THz source is used to perform the irradiation, the sample temperature changes periodically by the adiabatic temperature rise and thermal diffusion. In many cases, these changes are too rapid to be measured using thermosensors. Numerical estimations are thus required.

Figure 2 illustrates the sample temperature characteristics during pulsed THz irradiation. When the THz pulse irradiates the sample, the water absorbs the energy and the sample temperature increases adiabatically throughout the pulse duration (ΔT). After the pulse ends, the sample temperature then decreases via thermal diffusion until the next pulse arrives. In total, the sample temperature increases by δT per second. If the thermal diffusion rate is negligibly slower than the rate of the temperature rise, ΔT can then be estimated as follows.

Figure 3 shows schematics of a sample with thickness dx and a surface area S, which is irradiated by a THz pulse with a pulse energy of I [J].

According to the Lambert–Beer law, the absorbed THz energy ΔI [J] can be written as follows:

$$\Delta I = -I_0 \alpha dx$$

(1)

where α is the absorbance of the sample. The increase in temperature at the surface of the sample, denoted by ΔT [°C], is:

$$\Delta T = \frac{\Delta I}{\rho S dx}$$

(2)
where $\rho$ [J °C$^{-1}$ cm$^{-3}$] is the specific heat of the liquid water. Introduction of the pulse energy density $P$ [J cm$^{-2}$] using the relationship $I = P \cdot S$ allows $\Delta T$ to be written as follows.

$$\Delta T = \frac{\log 10 \ast P [\text{J cm}^{-2}] \alpha [\text{cm}^{-1}]}{\rho [\text{J °C}^{-1} \text{cm}^{-3}]}.$$  \hspace{0.5cm} (3)

After THz irradiation, the sample temperature decreases via thermal diffusion. The rise in the average temperature $\delta T$ is dependent on the thermal diffusion rate. The finite element method can then be used to calculate the time-evolved temperature distribution numerically.

Figure 4 shows an example of a time-dependent study of the heat transfer in the fluids model in COMSOL Multiphysics® software. The 3D temperature distribution is simulated with respect to time. In the geometry of the simulation model, THz beam is vertically irradiated from top through the air and the polyethylene (PE) film, which corresponds to the sample dish, and heats up the surface of the water. To simplify the model, only the cylindrical area with a radius of 4 mm and thickness of 0.1 mm was heated up for 0.23 K at $t = 0$. The thermal distribution was simulated by 10 ms step. The simulated cross section of the thermal distribution at $t = 0$, 200 and 400 ms, respectively. The temperature at the surface of the sample drops down to 300.06 K at $t = 400$ ms.
Figure 5. Experimental setup used to generate photoacoustic waves and the optical layout used for the shadowgraph imaging system. The output of the THz-FEL containing a train of micropulses is irradiated on distilled water in the quartz cell. DW: polycrystalline diamond window. AT: THz attenuator. PM: off-axis gold-coated parabolic mirror with focal length of 50 mm. Probe: diode laser beam. Camera: image-intensified CCD camera. L1 and L2: BK7 uncoated spherical mirrors with focal lengths of 50 and 150 mm, respectively.

2.2. Acoustic effect

As another nonthermal effect, an acoustic wave is generated when an intense pulsed THz beam is absorbed by the water. The photon energy is transferred into the pressure wave via the thermoelastic effect [44]. We have observed the photoacoustic wave generated at the surface of water after THz pulse was irradiated [40]. As the THz light source, we used the THz-FEL on the L-band electron linear accelerator at the Research Laboratory for Quantum Beam Science of the Institute of Scientific and Industrial Research at Osaka University [32, 39, 45, 46]. The characteristics and the evaluation method used for the THz pulses from the FEL have been described elsewhere in the literature [46]. Linearly polarized THz macropulses were generated using the THz-FEL at a repetition rate of 5 Hz with pulse energy of 50 mJ. Each macropulse contains a train of approximately 220 micropulses separated at intervals of 36.9 ns (27 MHz repetition rate), as illustrated in figure 4. The highest micropulse energy was estimated to be 340 µJ. The temporal width of each micropulse was measured to be 1.7 ps using an electro-optic sampling technique. The center frequency is tuned at 4 THz range, which is close to the absorption band of intermolecular vibrations in liquid water.

Figure 5 shows a schematic diagram of the generation of the photo-acoustic wave and the observation system. The THz light from the FEL was loosely focused on a distilled water using an off-axis parabolic mirror. The spot size of the THz pulse at the surface of water was evaluated to be 0.7 mm using the knife-edge method. The input pulse energy was attenuated using THz attenuators (TYDEX). Shadowgraph technique was used to obtain a cross-section image of the photoacoustic wave. Continuous-wave (CW) probe light with the wavelength of 670 nm was horizontally irradiated to the cell, and imaged on the image-intensified charge-coupled device (CCD) detector (Princeton PI-MAX3). In the shadowgraph image, the signal intensity shows the density distribution in the liquid water, which depends on the second derivative of the refractive index.

Figure 6 shows a snapshot of the shadowgraph image with a macropulse energy of 2.6 mJ, which corresponds to micropulse energy of 18 µJ, i.e. a radiant exposure of 4.6 mJ cm\(^{-2}\). A background image obtained without THz light irradiation was subtracted from the original image, and the resulting image is shown in the figure 6. A stripe pattern shows the acoustic pulses that are induced by the micropulses in the output of THz-FEL. From the distance between the neighboring lines, the propagation speed of the photoacoustic wave was determined. The average spacing between the acoustic pulses is 55 µm, and the time intervals of the THz pulse train is 36.9 ns. Therefore, the speed of the photoacoustic wave was estimated to be 1491 m s\(^{-1}\), which is the same as the velocity of sound in distilled water at 23 °C. Since the diameter of THz beam is considerably larger than the thickness of the acoustic pulse, the photoacoustic waves propagate with a plane wavefront for long distance.
When the pressure wave is generated by the thermoelastic effect, the increase of the local pressure can be estimated using the relationship $p = \Gamma \alpha F$, where $F$ is the incident radiant exposure, $\Gamma = \gamma v^2/c_p$ is the Grüneisen coefficient of water, $\gamma$ is the coefficient of volume expansion and $c_p$ is the heat capacity at a constant pressure [44]. Under our experimental conditions, the increase of the local pressure was estimated to be 0.5 MPa at $\alpha = 800 \, \text{cm}^{-1}$.

Therefore, generation of the THz-induced acoustic waves is demonstrated. Photo-induced acoustic waves have been studied previously using ultrafast pulsed lasers, where tightly focused laser pulses generated a shockwave that followed plasma generation at the surface of the water [47]. In contrast with those methods, the energy of the THz light is directly transferred to the acoustic energy by the absorption of the intermolecular vibrational motion of the water molecules. THz-induced acoustic wave has the following features. First, the irradiated energy is completely absorbed by water and transferred to the thermal or the acoustic energy, which means photoexcitation does not occur at the samples inside water. Second, plane photoacoustic wave generated by the loosely focused THz beam propagates deeply into the water.

3. Demolition of actin filaments induced by pulsed THz irradiation

When THz wave is irradiated to a biological tissue, most of the energy is absorbed by water molecules because water is a major molecule that compose an organism, and the absorbance of water is much higher than that of biomolecules such as proteins [48]. Therefore, as demonstrated in the previous section, pulsed THz wave irradiation generates an acoustic wave that can propagate into tissues for depths of a few mm. Therefore, irradiation with THz waves may affect biological molecules located deep inside these tissues. To determine the effects of THz-induced shockwaves on biological systems, we observed the morphologies of the actin proteins both in vitro and in vivo [24].

3.1. Effects of pulsed THz irradiation on actin filaments in an aqueous solution

To observe the effects of pulsed THz irradiation on actin filamentation in vitro, the output of the THz-FEL was irradiated to a 1 mm thick aqueous solution of actin protein. THz pulses of 80 $\mu$J cm$^{-2}$ per micropulse were irradiated from the bottom of the dish during actin polymerization reaction. After 30 min of THz irradiation, a portion of the actin solution was collected and observed by a fluorescence microscope. To probe F-actin, SiR actin was applied. The fluorescence of SiR-actin increases by up to 100-fold when it binds to
Figure 7. Fluorescence microscopy images of actin filaments (a) with and (b) without THz pulsed irradiation (80 µJ/cm²). (c) Numbers of actin filaments counted from the fluorescence images. The data shown are the mean and the standard deviation of the data from three independent experiments. More than 300 actin filaments were counted in each of these experiments. The asterisk indicates a statistically significant difference (*P < 0.05). The number of actin filaments was counted in images from three independent experiments (140 × 140 µm) using Image-J software. Statistical significances were calculated by F- and T-test.

actin filaments, thus meaning that the actin filaments bound with SiR-actin could be analyzed quantitatively by wide-field microscopy. The irradiated sample (figure 7(a)) clearly lost brightness when compared with the control sample (figure 7(b)) and the number of actin filaments decreased by approximately 50% (figure 7(c)). Morphological deformation of the F-actin (including branching or molecular aggregation), which is characteristic of denatured F-actin, was not observed in the fluorescence image.

In the actin polymerization reaction, elongation progress until the reaction reaches to the equilibrium condition. At room temperature, 30 min is sufficient to reach equilibrium. Therefore, the reduction of the actin filaments is due to either a reduced elongation reaction or the collapse of the actin filaments. It is
known that acoustic waves can demolish the actin filaments. Therefore, we supposed that the acoustic wave generated by the pulsed THz wave induces the collapse of the actin filaments.

According to an adiabatic model (see section 2.1), the increase of the temperature at the surface of the solution is estimated as 0.035 °C per micropulse. The sample temperature measured at the end of the irradiation process was 1.4 °C higher than that of the control sample. Increasing the temperature by a few degrees Celsius does not explain the inhibition of the polymerization reaction because the rate of the actin polymerization does not change remarkably in this temperature range. In addition, the sample thickness is 100 times larger than the THz wave penetration depth. Even if the diffusion of the actin molecules is considered, the direct interaction of the THz wave at the surface of the irradiated spot does not explain the significant difference in the number of actin filaments. Therefore, we concluded that the actin filaments were...
demolished by the acoustic wave under the pulsed THz irradiation conditions, but not by the heat-induced inhibition of the actin filament elongation process.

### 3.2. Pulsed THz irradiation of the cellular actin filament

To investigate the in vivo effects of the acoustic wave generated by the THz pulse irradiation, the morphological changes of actin filaments in living cells were observed. HeLa cells were grown on a glass plate and sunk in a culture medium, and the THz pulses were applied from the bottom of the dish in order to avoid both direct interaction with the THz wave and the thermal effect at the surface of the medium (figure 8). The temperature of the culture medium was kept at 37 °C during the experiment.

HeLa cells were stained with SiR-actin to observe the actin filaments with a fluorescent microscope. Figure 9 shows live-cell images before (0 min.) and after (30 min.) irradiation with the THz micropulse energy of 0, 80, 160 and 250 µJ cm\(^{-2}\), respectively. The intact actin filament structure is clearly observed before THz irradiation in all samples, and maintained after 30 min in the control cells (figure 9(v)). On the other hand, the number of actin filaments was reduced and actin aggregates were observed in the cell cortex of the irradiated samples (figures 9(vi)–(viii), indicated by red arrows), which suggests that a layer of thin actin filaments are formed at the cell periphery. To observe detailed actin filament structure within the cell cortex, cells were immunostained with AlexaFluor 594 Phalloidin and observed by spinning-disk confocal microscopy after THz irradiation for 30 min (figures 9(ix)–(xii)). The samples after 80 and 160 µJ cm\(^{-2}\) THz irradiation show a dark area in the cell cortex (figures 9(x) and (xi)), indicating disassembly of the actin filaments. Actin aggregation is present near the cell periphery (figure 9(xii), red allow) after 250 µJ cm\(^{-2}\) irradiation. These results demonstrate that the acoustic wave generated by the THz pulse propagates about mm thick and changes the cellular protein morphology [24].

### 4. Actin polymerization by THz irradiation at lower peak power

To observe the nonthermal and nonacoustic effects of the THz irradiation, a gyrotron (Gyrotron FU CW GO-III 3, Fukui University) was used as an irradiation source and the polymerization reaction of the actin protein was observed in an aqueous solution [33]. The THz radiation generated at 0.46 THz was guided by a waveguide tube and used to irradiate the sample without focusing. The THz power incident at the sample is 0.6 W cm\(^{-2}\). To reduce the irradiation energy of the THz beam, the gyrotron's electron source was pulsed for a 1% duty cycle (i.e. 1 Hz repetition rate, 10 ms pulse duration). Under these conditions, the peak power of the THz beam is an eighth order of magnitude lower than that of the THz-FEL. Therefore, the generation of the acoustic waves is negligibly small. Additionally, the temperature rise at the sample as measured using a K-type thermocouple was less than 0.5 °C during irradiation, which is considered to be negligible for the
reaction for the aqueous actin proteins. Only the nonthermal and nonacoustic effect is thus observed under these conditions.

The experimental setup used for the actin polymerization reaction is the same as that described in section 3.1. The actin solution on a film dish was irradiated by THz beam from the bottom, and the fluorescence of pyrene fluorophores introduced into the actin molecule was monitored. The amount of F-actin was estimated by the fluorescence intensity of the pyrene actin which increases with the actin polymerization. The fluorescence of the pyrene actin solution was measured by a luminometer (EX: 365–395 nm; EM: 440–470 nm).

Figure 10(a) shows the fluorescence intensity of the pyrene actin during the polymerization reaction. The increase in fluorescence was enhanced significantly by the irradiation of the THz wave. Figure 10(b) shows the polymerization reaction after this steady state. The sample was grown for 1 h without THz irradiation to reach the steady state, in which the elongation reaction and depolymerization reaction is in equilibrium. In the steady state, the fluorescence intensity did not increase without THz irradiation. On the other hand, the fluorescence intensity an additional increase when THz wave was irradiated. This phenomenon suggests that the THz irradiation activates the actin polymerization process, at least in the elongation phase.

We also observed the actin filaments under a fluorescence microscope after THz irradiation (figures 11(a) and (b)). After the polymerization reaction of the actin solution had proceeded for 20 min, the sample was stained with SiR-actin. The filamentous structures in the microscope images show that the number of

![Figure 11. Fluorescence microscopy images of the actin solution (0.8 µM) and enlarged images of the actin filaments, (a) with and (b) without THz irradiation (0.6 W cm⁻²). (c) The numbers of actin filaments counted from the fluorescence images. The data shown are the mean and the standard deviation of the data from three independent experiments. More than 100 actin filaments were counted in each of these experiments. The asterisk indicates a statistically significant difference (*P < 0.05).](image-url)
filaments increased when the THz wave irradiated the sample during the polymerization reaction. No significant differences in the F-actin morphology were observed. These results confirm that the THz irradiation activates the actin polymerization process but does not induce either protein denaturation or aggregation.

In this study, we have demonstrated that THz irradiation at lower peak powers activates the elongation phase of the actin polymerization reaction without thermal or acoustic effects. The results presented here strongly suggest that the THz radiation excites dynamic motion of the proteins. Given that the photon energy of 0.46 THz corresponds to the vibration energy of the protein molecules, the rotational relaxation of the water molecules, and their collective motion, irradiation with intense THz waves affects the dynamics of these molecules. A single actin molecule consists of two major domains that are linked by a hinge domain. The structural difference between the G-actin and the F-actin is a relative rotation of the two major domains via the hinge domain, which is approximately 20° in the G-actin and flat in the F-actin [49]. One possible idea is that the THz irradiation excites the inter-domain flipping motion of actin molecules, which then leads to activation of the actin polymerization process. However, it is difficult to conclude the exact mechanism by which the THz irradiation influences the actin molecules in our work. Therefore, future studies will focus on investigating the structure of actin under THz irradiation in detail by varying experimental parameters such as the wavelength or intensity and by using actin mutants in which the characteristics of actin have been altered.

5. Conclusion

In this paper, our recent research on THz irradiation experiments is reviewed. We found that pulsed THz waves with energy density of \( \sim 10^{-4} \text{ J cm}^{-2} \) (\( \sim 10^8 \text{ W cm}^{-2} \) at the peak) generate acoustic waves efficiently in aqueous media. These acoustic waves propagate deeply into the water. In fact, fibrous actin proteins in HeLa cells that were submerged in the cell culture medium were demolished by the THz irradiation. The results presented here demonstrate that THz pulsed irradiation affects biomolecules in the tissues, even if they are located a few millimeters away from the body surface. These results must be considered when the safety guidelines for use of THz radiation are determined in the future. In contrast, THz wave irradiation with peak powers of the order of \( \sim \text{W cm}^{-2} \) produces different effects. This THz irradiation activates the elongation phase of the actin polymerization process without thermal or acoustic effects.

The polymerization of monomeric actin into filaments plays pivotal roles in cell motility, growth, differentiation and gene expression. Therefore, techniques for manipulation of actin polymerization have been developed to aid in understanding and regulation of multiple biological functions. THz irradiation technology, instead of actin-binding chemicals, should provide a safe and novel approach for regulation of the dynamics of actin polymerization in living cells. Our findings indicate the possibility of use of THz waves for artificial manipulation of biological phenomena through modulation of the functions and dynamics of various biomolecules.

Data availability statement

No new data were created or analysed in this study.

Acknowledgments

We would like to thank Professor Yuichi Ogawa, Professor Masaya Nagai, Professor Tositaka Idehara, Professor Goro Isoyama, Dr Chiko Otani, Dr Keiji Konagaya, Mr. Ginji Yokoyama and Mr. Yuya Ueno for their excellent technical assistance and advice. This work was supported by the Japan Society for the Promotion of Science (JSPS) KAKENHI under Grant Nos. JP25116009, JP15H04625, JP15K14706, JP16H05010, JP17H07365, JP19K15812, JP17J00302, JP18H02164, JP20K21261 and JP20H05378; the QST President’s Strategic Grant (Creative Research); and the JSPS Core-to-Core Program (Advanced Research Networks) entitled ‘Establishment of international agricultural immunology research-core for a quantum improvement in food safety’. This work was performed under the Cooperative Research Program of the Network Joint Research Center for Materials and Devices. This work was based on results obtained with the support of the RIKEN–AIST Joint Research Fund (Semi-full research).

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References

[1] Peiponen K-E, Zeitler A and Kuwata-Gonokami M 2013 Terahertz Spectroscopy and Imaging vol 171, ed K E Peiponen, A Zeitler and M Kuwata-Gonokami (Berlin: Springer)
[2] Ueno Y and Ajitoko K 2008 Analytical terahertz spectroscopy Anal. Sci. 24 185–92
[3] Mittleman D M 2017 Perspective: terahertz science and technology J. Appl. Phys. 122 230901
[4] Dean P et al 2014 Terahertz imaging using quantum cascade lasers—a review of systems and applications J. Phys. D: Appl. Phys. 47 574008
[5] Guillet J P, Recur B, Frederique L, Bousquet B, Canioni L, Manek-Hönninger I, Desbarats P and Mounaix P 2014 Review of terahertz tomography techniques J. Infrared, Millimeter, Terahertz Waves 35 382–411
[6] Lie T, Gallero G, Gasser M, Kallergi M, Amirtham P, Kohler C, Cefalas A C, Taday P, Clothey R H and Jepsen P 2011 Terahertz BRIDGE: A European project for the study of the interaction of terahertz radiation with biological systems Conf. Digest of the 2004 Joint 29th Int. Conf. on Infrared and Millimeter Waves and 12th Int. Conf. on Terahertz Electronics pp 817–8
[7] Leibeg N, Auvinen A, Dankar-hope H and Mild K H 2016 SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks), Potential health effects of exposure to electromagnetic fields (EMF), Scientific Committee on Emerging and Newly Identified Health Risks SCENIHR Opinion on Potential on health (https://doi.org/10.2772/75635)
[8] Romanenko S, Begley R, Harvey A R, Hool I and Wallace P V 2017 The interaction between electromagnetic fields at megahertz, gigahertz and terahertz frequencies with cells, tissues and organs: and potential of risk J. R. Soc. Interface 14 20170585
[9] Wilmink G J and Grundt J E 2011 Invited review article: current state of research on biological effects of terahertz radiation J. Infrared Millimeter, Terahertz Waves 32 1074–122
[10] Serdyukov D S, Goryachkovskaya T N, Mescheryakova I A, Bannikova S V, Kuznetsov S A, Cherkasova O P, Popik V M and Peltek S E 2015 Study on the effects of terahertz radiation on gene networks of Escherichia coli by means of fluorescent biosensors Biomed. Opt. Express 6 11528
[11] Bock J et al 2010 Mammalian stem cells reprogramming in response to terahertz radiation PLoS One 5 8–13
[12] Korenestillan A, Belk A, Hasin P, Efrain A, Gover A and Korenestin R 2008 Terahertz radiation increases genomic instability in human lymphocytes Radiat. Res. 170 228–34
[13] Titoiva J V, Ayesheshim A K, Golubov A, Fogen D, Rodriguez-Juarez R, Hegmann F A and Kovalchuk O 2013 Intense THz pulses cause H2A phosphorylation and activate DNA damage response in human skin tissue Biomed. Opt. Express 4 559
[14] Bogomazova A N, Vassina E M, Goryachkovskaya T N, Popik V M, Sokolov A S, Kolchanov N A, Lagarkova M A, Kiselev S L and Peltek S E 2015 No DNA damage response and negligible genome-wide transcriptional changes in human embryonic stem cells exposed to terahertz radiation Sci. Rep. 5 7749
[15] Yamazaki S, Harata M, Ueno Y, Tsuouchi M, Konagaya K, Ogawa Y, Isayama O, Canti and Hoshina H 2020 Propagation of THz irradiation energy through aqueous layers: demolition of actin filaments in living cells Sci. Rep. 10 9008
[16] Grundt J E, Cerna C, Roth C C, Ivey B L, Lipscomb D, Echchgadda I and Wilmink G J 2011 Terahertz radiation triggers a signature gene expression profile in human cells 2011 Int. Conf. on Infrared, Millimeter, and Terahertz Waves pp 1–2
[17] De Amarcis A et al 2015 Biological effects of in vitro THz exposure in human fetal fibroblasts Mutat. Res.—Genet. Toxicol. Environ., Mutagen. 793 150–60
[18] Yagashita N, Nokum S, Hayashi S and Kawase K 2018 Investigation of the non-thermal effects of exposing cells to 70–300 GHz irradiation using a widely tunable source J. Radiat. Res. 59 116–21
[19] Demidova E V, Goryachkovskaya T N, Mescheryakova I A, Malup T K, Semenov A I, Vinokurov N A, Kolchanov N A, Popik V M and Peltek S E 2016 Impact of terahertz radiation on stress-sensitve genes of E.Coli cell IEEE Trans. Terahertz Sci. Technol. 6 435–41
[20] Orovokova M A B, Ebreirakia M A S, Acheslav V, Eddarof E, Edykhy E G O R S, Aks V L I, Vexander A, Ichutin L, Almikova A L S and Hodzitsky M I K 2017 Investigation of terahertz radiation on rat cells Biomed. Opt. Express 8 39–44
[21] Alexander B S et al 2013 Specificity and heterogeneity of terahertz radiation effect on gene expression in mouse mesenchymal stem cells Sci. Rep. 3 1184
[22] Turton D A, Senn H M, Harwood T, Laphorn A J, Ellis E M and Wynn K 2014 Terahertz undamped vibrational motion governs protein-ligand binding in solution Nat. Commun. 5 3999
[23] Hoshina H, Suzuki H, Otani C, Nagai M, Kawase K, Iriyama A and Isayama G 2016 Polymer morphological change induced by terahertz irradiation Sci. Rep. 6 27180
[24] Yamazaki S, Harata M, Idhara T, Konagaya K, Yokoyma G, Hoshina H and Ogawa Y 2018 Actin polymerization is activated by terahertz irradiation Sci. Rep. 8 9990
[25] Kawasaki T, Tsuchiyama K and Irizawa A 2019 Dissolution of a fibrous peptide by terahertz free electron laser Sci. Rep. 9 1–8
[26] Greschner A A, Rappaport X, Kort M, Zuberi N, Perreault J, Razzari L, Ozaki T and Gauthier M A 2019 Room-temperature and selective triggering of supramolecular DNA assembly/disassembly by nonionizing radiation J. Am. Chem. Soc. 141 3456–69
[27] Pollard T D and Cooper J A 2009 Actin, a central player in cell shape and movement Science 326 1208–12
[28] Cooper J A, Walker S B and Pollard T D 1983 Pyrene actin: documentation of the validity of a sensitive assay for actin polymerization J. Muscle Res. Cell Motil. 4 253–62
[38] Lukinavičius G et al 2014 Fluorogenic probes for live-cell imaging of the cytoskeleton Nat. Methods 11 731–3
[39] Nagai M, Aono S, Ashida M, Kawase K, Irizawa A and Isoyama G 2017 Luminescence induced by electrons outside zinc oxide nanoparticles driven by intense terahertz pulse trains New J. Phys. 19 053017
[40] Tsubouchi M, Hoshina H, Nagai M and Isoyama G 2020 Plane photoacoustic wave generation in liquid water using irradiation of terahertz pulses Sci. Rep. 10 18537
[41] Shiraga K, Tanaka K, Arikawa T, Saito S and Ogawa Y 2018 Reconsideration of the relaxational and vibrational line shapes of liquid water based on ultrabroadband dielectric spectroscopy Phys. Chem. Chem. Phys. 20 26200–9
[42] Wilming G J, Rivest B D, Roth C C, Ibey B L, Payne J A, Cundin L X, Grundt J E, Peralta X, Mixon D G and Roach W P 2011 In vitro investigation of the biological effects associated with human dermal fibroblasts exposed to 2.52 THz radiation Lasers Surg. Med. 43 152–63
[43] Kristensen T T L 2010 WithawatWithayachumnankul, Jepsen P U and Abbott D 2010 Modeling terahertz heating effects on water Opt. Express 18 4727–39
[44] Vogel A and Venugopalan V 2003 Mechanisms of pulsed laser ablation of biological tissues Chem. Rev. 103 577–644
[45] Kawase K et al 2013 The high-power operation of a terahertz free-electron laser based on a normal conducting RF linac using beam conditioning Nucl. Instruments Methods Phys. Res. Sect. A 726 96–103
[46] Kawase K, Nagai M, Furukawa K, Fujimoto M, Kato R, Honda Y and Isoyama G 2020 Extremely high-intensity operation of a THz free-electron laser using an electron beam with a higher bunch charge Nucl. Instruments Methods Phys. Res. Sect. A 960 163582
[47] Stryker B D, Springer M M, Traverso A J, Kolomenskii A A, Kattawar G W and Sokolov A V 2013 Femtosecond-laser-induced shockwaves in water generated at an air-water interface Opt. Express 21 23772
[48] Yamamoto N, Ohta K, Tamura A and Tominaga K 2016 Broadband dielectric spectroscopy on lysozyme in the sub-gigahertz to terahertz frequency regions: effects of hydration and thermal excitation J. Phys. Chem. B 120 4743–55
[49] Oda T, Iwasa M, Aihara T, Maéda Y and Narita A 2009 The nature of the globular- to fibrous-actin transition Nature 457 441–5