THz transmittance measurements of nucleobases and related molecules in the 0.4- to 5.8-THz region using a GaP THz wave generator

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Abstract

An automatic measurement system for terahertz (THz) transmission spectroscopy based on difference-frequency generation (DFG) of widely tunable coherent THz waves via excitation of a phonon–polariton mode in GaP was constructed and improved to give it a wider frequency measurement range and four times greater S/N ratio by using the double-beam method. In this paper, we present spectroscopic measurements of nucleobases, nucleosides, deoxynucleosides, and nucleotides, all of which are the components of RNA and DNA molecules, in the range of 0.4–5.8 THz in order to compare their characteristic features in the wider terahertz frequency region.

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1. Introduction

In 1963, Nishizawa [1,2] predicted coherent terahertz electromagnetic wave generation via the resonance of phonons and molecular vibrations. Following this proposal, Nishizawa and Suto [3–5] realized a semiconductor Raman laser using a GaP crystal, and a terahertz (THz) wave with a frequency of 12 THz was generated from a GaP Raman laser containing a GaAs mixing crystal [6]. Loudon [7,8] also proposed terahertz wave generation, but based on a uniaxial crystal. Nishizawa promoted the development of THz wave generation via the resonance of phonons and
proposed that a wavelength-tunable THz wave would be applicable to detect and treat of cancers [9]. At the same time, under Nishizawa’s guidance, Kawase and Ito [10,11] realized high-power frequency-tunable terahertz wave generation from LiNbO$_3$. Its narrow line width (100 MHz) enabled the spectral measurement of sharp water vapor lines with high resolution. In addition, Tanabe et al. [12–15] reported high-power frequency-tunable terahertz wave generation via the excitation of phonon–polaritons in GaP, with a maximum peak power as high as 4.8 nJ/pulse (800 mW) at 2.5 THz, and frequency-tunable in the range of 0.5 to 7 THz without missing frequencies.

Recently, we constructed a GaP terahertz wave generator with automatic scanning control in the range of 0.8–5.4 THz and made spectral measurements of terahertz vibrations of sugars, including glucose, deoxyglucose, fructose, and sucrose [16]. THz spectra of some sugar molecules have been measured [17] using terahertz time domain spectroscopy (THz-TDS) based on photoconductive switches [18,19], which were developed by several groups [20–40]. Terahertz spectra measured using a GaP frequency-tunable THz generator have revealed high frequency portions (above 3.5 THz) of resonance peaks that are hidden using conventional THz-TDS. The time domain THz wave radiation mentioned above has a pulse form. By contrast, the frequency-tunable THz wave obtained using the phonon–polariton mode of GaP or LiNbO$_3$ has tunable quasi-CW frequency characteristics.

Based on the THz-TDS, THz waves were applied for imaging of biological tissues and electronics [41–47]. Jepsen et al. [48–52] measured THz spectra of biomolecules using THz-TDS. Recently, Kawase et al. [53] applied THz imaging to the non-destructive detection of illicit drugs using spectral fingerprints and a LiNbO$_3$ tunable THz wave source.

In this paper, we present spectroscopic measurements of important biomolecules in the range of 0.4–5.8 THz using an improved GaP tunable-frequency THz wave source. These biomolecules are the essential components of RNA and DNA: the five nucleobases (adenine, guanine, cytosine, uracil, and thymine) and their nucleosides, deoxy-nucleosides, and nucleotides. These spectra differ markedly within this THz region, facilitating the identification and discrimination of biomolecules. Only limited data have been available on the resonance behavior of these molecules in the THz frequency region. Thus, the first step in the spectroscopic analysis requires the construction of a database of THz spectra of such kinds of biomolecules.

Our spectrometer was improved by introducing a sample and reference beam path configuration (double-beam method) to decrease the effect of THz power fluctuations, as shown in Fig. 1. Single-frequency THz wave radiation from the GaP THz wave generator has a 10-ns pulse form with a repetition frequency of 10 Hz. Consequently, the main source of noise in the spectrometer arises from the fluctuation in the THz intensity. This
fluctuation was as much as 10% between each pulse for the single-beam method and arose mainly from fluctuations in the source pulse power. By introducing the double-beam method, the noise during transmission spectrum measurement was reduced to about one-fourth that of the single-path method. Moreover, the frequency-tunable range has been decreased to 0.4 THz in order to search for the lowest frequency peaks.

Some THz spectra of biomolecules have been measured using THz-TDS [52, 54], far-infrared Fourier transform spectroscopy with a continuous wave source (mercury-arc lamp) [55–61], and low-frequency Raman spectroscopy [62]. By comparison with these methods, the results using the GaP frequency-tunable source reveal spectral features in most of the important THz region where intermolecular forces, such as hydrogen bonds, van der Waals forces, and other weak interactions, are dominant compared with the interactions that appear in near- and middle-infrared absorption and Raman spectra. Terahertz-frequency vibrational modes result from the geometric arrangement of macromolecules that have molecular interactions like hydrogen bonding in their structures [52, 62, 63]. Unfortunately, most of these peaks cannot be identified at present. Nevertheless, their strikingly different characteristic spectral patterns in this frequency region are sufficient for identifying these molecules, as long as they are in the crystalline state and not in an amorphous state [17].

2. Experimental method

Our previous papers described in detail the difference-frequency generation (DFG) of widely tunable coherent THz waves via excitation of a phonon–polariton mode in a GaP crystal [12–15]. Based on this method, an automatic measurement system for THz transmission spectroscopy was constructed [16]. In this study, the system was rebuilt with sample and reference beams (double-beam method) in order to reduce the effect of THz power fluctuation caused by laser-light power fluctuations. The S/N ratio in the double-beam method is about four times that in the single-beam method [16]. Fig. 1 illustrates the system. The 1064-nm fundamental beam of a Q-switched Nd:YAG laser was used as the signal beam for DFG, and the 355-nm third harmonic beam was used to pump a β-BaB2O4 (BBO)-based optical parametric oscillator (OPO). The pump beam for DFG, fed from the OPO laser, was tuned in the 1048–1062 nm range, which corresponds to the difference frequency range of 5.8–0.4 THz. The optical frequency of the OPO was locked at each THz frequency during a sweep. Both laser beams were shaped to 3-mm diameters using holes in metal plates, and the energies of the pump and signal pulses were attenuated to 3 mJ using a polarizer and half-waveplate configuration. The two beams were combined using a cubic polarizer placed on a rotating stage, which automatically produced a very small angle between the two beams to fulfill the phase-matching condition. Spatial overlap was realized automatically at the input surface of the GaP crystal for any angles of the beams by adapting the translational movement of the beam combiner. The undoped semi-insulating GaP crystal was 5 mm long. The incident beams were almost parallel to the (110) crystal direction of the GaP. The polarizations of the pump and signal lights were adjusted in the (001) and (110) directions, respectively, so that the polarization of the THz wave was horizontal. Both the input and output faces of the crystal were coated with Al2O3 using EB-evaporation (JEOL) after mechanical polishing and chemical etching, in order to avoid an interference effect and to increase the damage threshold. The generated THz wave was nearly a Gaussian beam [64], and the pulse width was 6 ns.

In our earlier study, we noticed that the available THz frequency range depends on the incident angle \( \alpha \) of the signal beam from the normal of the GaP input surface, owing to the total reflection of the THz wave [13, 14]. In this experiment, the frequency range was extended by selecting \( \alpha = 0^\circ \) for the range from 0.4 to 1.5 THz and \( \alpha = 20^\circ \) for the range from 1.5 to 5.8 THz. The output direction of the generated THz wave depends on its frequency [13]. Using a pair of off-axis parabolic reflectors, in which one moved linearly on a stage, the THz wave was adjusted automatically so that it always took the same path to a fixed
detector. Then, the THz beam was divided into sample and reference beams using a wedge-shaped silicon plate in order to avoid the effect of interference, as shown in Fig. 1. The THz wave path was purged with dry N₂ to eliminate water vapour absorption. Pyroelectric DTGS detectors operating at room temperature were used to detect the THz powers of the two beams. Although the $D^*$ of DTGS is only $10^{-3}$ times that of a Si-bolometer, the sufficiently high power from the GaP tunable THz wave source enables the use of DTGS over a wide range of THz wave frequencies. Black polyethylene film was used to cut off near-IR light.

The automatic scanning system control and the method of determining accurate THz frequencies were the same as described previously [16]. The absolute THz frequency accuracy was better than 5 GHz for the entire THz frequency region. The measured THz wave linewidth was 3.2 GHz (at 1.01 THz) using a far-infrared Fabry–Perot interferometer [64].

The transmittance spectra were measured at room temperature in the 0.4–5.8 THz (13–193 cm⁻¹) frequency region in 5-GHz steps using the GaP terahertz wave generator. At each THz frequency, 16 pulses were integrated so that the integration time was 1.6 s. It takes 10–70 min to acquire one transmission spectrum from 0.4 to 5.8 THz. The samples of nucleobases (adenine, C₅H₅N₅; guanine, C₅H₅N₅O; cytosine, C₄H₅N₃O; uracil, C₄H₄N₂O₂; and thymine, C₅H₆N₂O) were used without further purification or recrystallization. Each sample was milled for 10 min with polyethylene (PE) powder with a 5-μm diameter, which is probably sufficient for homogeneous mixing, and pressed into a 1- to 2-mm-thick pellet (20 mm diameter) under vacuum by applying a pressure of 2000 kg. Approximately 300 mg of PE powder was used for each sample. PE is a suitable filling material for terahertz spectroscopy because it is very transparent between 0.4 and 5.8 THz. The concentrations of the samples were 2–10 wt%. Each sample spectrum was normalized using the spectrum of a reference pellet made of pure PE.

3. Results and discussion

Fig. 2(a)–(e) shows the THz transmittance spectra of the five nucleobases (adenine, guanine, cytosine, uracil, and thymine) at room temperature, in the range of 0.4–5.8 THz. They revealed fuller characteristic spectral structures than those observed in a limited region below 3.5–4 THz using conventional THz time-domain spectroscopy (THz-TDS) [52]. For example, guanine showed peaks at 2.57, 3.00, 4.31, 4.84, and 5.44 THz, of which the 3.00 and 4.84 THz peaks were very strong. For each of the other nucleobases, we also observed two strong absorption bands with peaks at 2.85 and 3.39 THz (cytosine), 2.29 and 3.00 THz (thymine), 3.05 and 4.18 THz (adenine), and 3.34 and 3.84 THz (uracil).

The reported frequencies of absorption peaks for nucleobases are summarized in Table 1(a), which includes our data. Referring to Table 1, our spectral peaks for cytosine, uracil, and thymine are in good agreement with those of far-infrared Fourier transform spectroscopic studies [55,57] for which FTIR data are available. The high-power terahertz wave generator using GaP crystals also enables the measurement of terahertz spectra over a wide frequency range, with high resolution. Our spectral data were not signal-processed to smooth the curves, as is usually done for FTIR spectra, because we wanted to find small sharp peaks that were not noise.

Figs. 3 and 4(a)–(e) show the THz transmittance spectra of nucleosides (adenosine, guano-
sine, cytidine, uridine, and thymidine) and deoxy-
nucleosides (2'-deoxyadenosine monohydrate,
2'-deoxyguanosine hydrate, 2'-deoxycytidine
hydrochloride, 2'-deoxyuridine, and 3'-deoxy-
thymidine), respectively. The frequencies of the
reported absorption peaks for nucleosides and
deoxy nucleosides are summarized in Table 1(b)
and (c) and, which include our data. Generally,
the gradual increase in absorption with THz fre-
quency was more prominent in these samples than
in nucleobases, while the resonant absorption
peaks were weaker, except in adenosine, deoxya-
denosine, and thymidine, which had strong
absorption peaks at 1.96, 3.05, and 2.56 THz,
respectively. In nucleosides and deoxy nucleosides,
a ribose or deoxyribose molecule replaces one of
the hydrogen atoms in the nucleobase molecule,
respectively, making these molecules much larger
and more anisotropic than are nucleobases. Con-
sequently, the forces between each molecule
should be easier to distort, and the absorption
bands should broaden or split into weaker peaks.
In fact, most of the nucleosides and deoxy nucle-
sides had many weaker peaks, such as in cytidine
(1.71, 2.10, 2.44, 2.56, 3.00, and 5.32 THz), or
broad absorption bands, such as in guanosine (ob-
served around 3.92 THz). Our results are in good
agreement with far-infrared Fourier transform
spectroscopic studies for which FTIR data are
available (adenosine [57, 58], cytidine [56, 59], and
uridine [56]), as shown in Table 1(b).

Note that in the case of guanosine, low-
frequency Raman spectroscopy showed that there
was a sharp peak at around 0.6 THz, called S
mode, which was assigned as the resonance peak
originating from base pairs stacked around their
long axis (c-axis). However, we did not observe a
spectral peak below 1 THz in the THz spectrum
of guanosine in Fig. 2(b). This mode should be
infrared inactive.

The tendency towards spectral broadening ob-
served in nucleosides and deoxy nucleosides was
more prominent in nucleotides (adenosine 5'-
monophosphate, guanosine 5'-phosphate diso-
dium salt hydrate, cytidine 5'-monophosphate
disodium salt hydrate, and uridine 5'-monophos-
phate disodium salt hydrate) as shown in Fig.
5(a)–(d). The frequencies of the absorption peaks
are summarized in Table 1(d). We did not find
FTIR data for nucleotides, so only our data are
listed. In nucleotides, a phosphoric acid molecule

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**Fig. 2.** Transmittance spectra of nucleobases: (a) adenine (0.707 mol/L), (b) guanine (0.316 mol/L), (c) cytosine (0.172 mol/L), (d) uracil (0.426 mol/L), and (e) thymine (0.379 mol/L).
Table 1
Infrared vibrational frequency (THz) of nucleo-bases at room temperature

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Infrared vibrational frequency (THz) of nucleosides at room temperature

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### Infrared vibrational frequency (THz) of deoxy-nucleosides at room temperature

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### Infrared vibrational frequency (THz) of nucleotides at room temperature

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Fig. 3. Transmittance spectra of nucleosides: (a) adenosine (0.357 mol/L), (b) guanosine (0.169 mol/L), (c) cytidine (0.079 mol/L), (d) uridine (0.196 mol/L), and (e) thymidine (0.197 mol/L).

Fig. 4. Transmittance spectra of deoxynucleosides: (a) deoxyadenosine monohydrate (0.380 mol/L), (b) deoxyguanosine hydrate (0.179 mol/L), (c) deoxycytidine hydrochloride (0.072 mol/L), (d) deoxyuridine (0.209 mol/L), and (e) deoxythymidine (0.211 mol/L).
replaces a hydrogen atom of the nucleoside molecule, and the unit molecule is therefore larger and more anisotropic than the corresponding nucleoside or deoxynucleoside. In adenosine phosphate, uridine phosphate, and guanosine phosphate, the absorption bands appeared to be smeared, and a gradual increase in absorption with frequency dominated. Nevertheless, small peaks were still observed in adenosine monophosphate (Fig. 5(a)) and uridine monophosphate disodium salt hydrate (Fig. 5(d)). Spectral measurement with higher contrast, which can reveal weaker peaks, will be necessary for analyzing these peaks.

There are few published analyses of the THz vibrational modes of these molecules. A recent computational analysis of thymine showed that, for a tetramer of four thymine molecules, the hydrogen bonds between each molecule cause four resonance modes in the narrow region from 2.1 to 2.4 THz caused by in-plane and out-of-plane bending and stretching motions [52]. This should correspond to the unresolved 2.29 THz bands shown in Fig. 2(e).

THz-TDS is found to be a powerful tool for linear spectroscopy in the range below 3 THz. By contrast, the superiority of the GaP frequency-tunable THz source is its wide frequency range. Another advantage is its high-energy density within a narrow frequency width available at each tuned frequency. We have shown that the GaP source can deliver peak power of 4.8 nJ/pulse (800 mW) [15]. This should prove advantageous for spectroscopy subjected to strong attenuation, such as measurements made through fiber transmission lines; it will also prove advantageous for imaging at a single specified THz frequency. Chemical or thermal reactions at a resonance THz frequency are also a promising field of researches using this THz frequency.

4. Conclusion

A THz spectrometer using a GaP terahertz wave generator with an automatic scanning control was built, and its measurement range was widened by rotating the GaP crystal. The S/N ratio was increased using a double-beam method. The THz spectra of nucleobases, nucleosides, deoxynucleosides, and nucleotides in crystalline states were measured in the 0.4–5.8 THz frequency region, in 5-GHz steps. The molecules had quite different characteristic spectral patterns in this frequency region, and the patterns were sufficient for identifying and discriminating these molecules [22].
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