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Vibrational spectra of nucleosides studied using terahertz time-domain spectroscopy

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Abstract

We demonstrate that terahertz (THz) time-domain spectroscopy is a powerful technique for measuring temperature-dependent and spatially-resolved vibrational spectra in the frequency range 0.2–3.0 THz. A number of well resolved absorption peaks are observed for poly-crystalline nucleosides, and we have mapped the evolution of these absorption features continuously between 4 and 295 K. The resonance frequency is found to shift with temperature and an empirical expression describing this frequency shift is given. In addition, we present spectrally-resolved THz images based on these vibrational modes, accessing both spatial and compositional information of subsurface biochemical materials.

Keywords: Terahertz (THz); Infrared; Adenosine; Vibrational spectrum; Time-domain

1. Introduction

For many years, Fourier-transform infrared (FTIR) spectroscopy has been used successfully to study the chemical composition of materials. More recently, with the introduction of focal plane array detectors, FTIR imaging systems have been developed and led to the acquisition of spatially-resolved spectral data in the mid-infrared frequency range [1–3]. In the far-infrared spectral range, however, the poor performance of FTIR spectrometers, owing to the lack of suitable sources, makes it impractical to develop FTIR imaging systems. Here we demonstrate that terahertz (THz) time-domain spectroscopy can be used to obtain spatially-resolved and temperature-dependent vibrational spectra in the far-infrared spectral range.

The THz region of the electromagnetic spectrum spans the frequency range between the mid-infrared and the millimeter/microwave. The relatively unexplored central part of the THz region (0.3–3 THz) comprises frequencies lower than those corresponding to most internal vibrations of isolated small molecules. Instead, spectra contain information on motions associated with coherent, delocalized movements of large numbers of atoms and molecules. Such collective modes are sensitive to perturbation by inter-molecular interactions with surrounding molecules as well as to changes of intra-molecular interactions through structural fluctuations [4,5]. The measurement of these low-frequency vibrations over a wide temperature range is thus important in understanding the structural and functional properties of biomolecules.

In this paper, we present THz time-domain spectroscopy [6–8] studies of the vibrational spectra of poly-crystalline nucleosides, monitoring the evolution of the resonance absorption features from 4 to 295 K. We also demonstrate that spectrally-resolved THz imaging is capable of accessing both spatial and compositional information of subsurface biochemical materials.

2. Experiment

Fig. 1 shows the THz time-domain spectroscopy apparatus used in our experiments [9]. In brief, a Ti:sapphire laser provides visible/near-infrared pulses of 12 fs duration at a center wavelength of 790 nm with a repetition rate of 76 MHz. The output is split into two parts: a 300 mW pump beam is focused onto the surface of a biased GaAs photo-conductive emitter for THz generation, and a 25 mW beam

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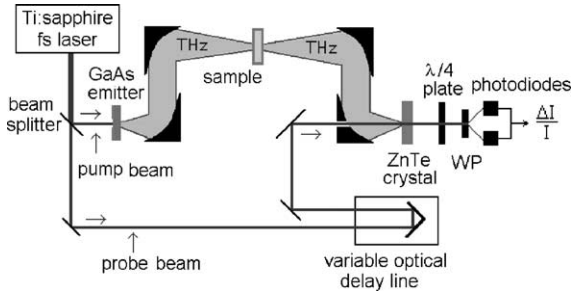

Fig. 1. Schematic representation of the experimental apparatus for coherent generation and detection of THz radiation.

serves as the probe beam for electro-optic detection [10,11] using a 1 mm thick ZnTe crystal. The variable delay stage, which provides the time delay between the THz pulse and the probe pulse, is scanned over a distance of 10 mm, providing a spectral resolution of 0.015 THz. Using a lock-in detection scheme, a signal-to-noise ratio of 10^5 was achieved.

All chemicals were purchased from Sigma-Aldrich and used without further purification. Adenosine (A) (Lot 91K1748), cytidine (C) (EC No. 2006109) and thymidine (T) (Lot 61K1274) had a purity of 99%. Guanosine (G) (Lot 11K2517) had a purity of 98%. X-ray diffraction measurements (Philips X-ray Diffractometer PW1050) revealed that all samples were poly-crystalline. Polyethylene (Lot 17410AO-061) had a spectrophotometric grade purity and was amorphous. Furthermore, it had negligible absorption in the frequency range of interest (0.2–3.0 THz) and was therefore suitable as a filling material for spectroscopic applications [8,12,13].

Nucleoside powders were finely milled and then mixed with polyethylene powder in a mass ratio of 1:10 [nucleoside:polyethylene]. For temperature-dependent measurements, samples were prepared by compressing the mixture with a specially-designed pellet maker into a copper ring of 8 mm diameter. The pellet samples were about 1.3 mm thick. For spectrally-resolved THz imaging, the mixtures containing nucleosides A, C, G and T, as crystalline powders, were filled into four slots, separated by 1.5 mm, on a polythene plate of thickness 3 mm. Each slot was 0.5 mm deep, 1.5 mm wide and 10 mm long. The samples were covered with a 140 μm thick cellulose nitrate membrane to prevent penetration of the visible/near-IR beam into the samples, and to demonstrate the capability of THz radiation for detecting subsurface biochemical substances.

3. Results and discussion

Fig. 2a shows a typical temporal THz waveform obtained from the apparatus shown in Fig. 1. Fourier-transforming this time-domain signal gives the frequency response of the THz spectroscopy system (Fig. 2b). The useful bandwidth is 0.1–3.5 THz, and is mainly limited by the frequency response of the 1 mm thick ZnTe detector. Using a 20 μm

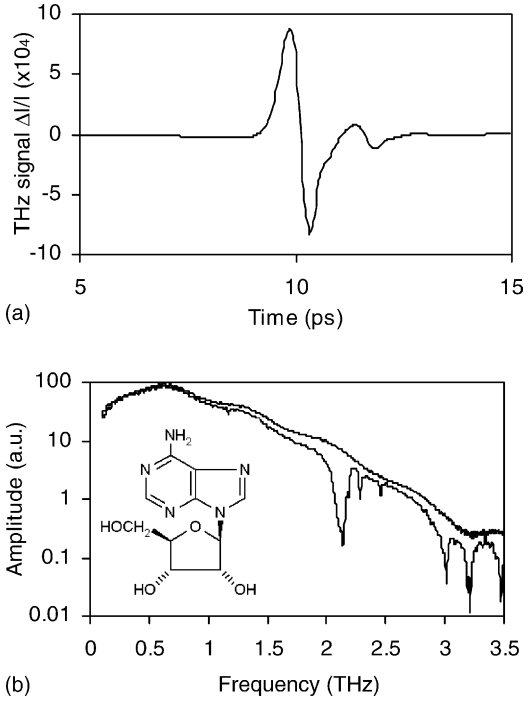

Fig. 2. (a) A typical temporal THz waveform, and (b) its corresponding Fourier-transform amplitude spectrum (upper trace), together with a spectrum measured in the presence of an adenosine sample at 4 K (lower trace). Inset shows the molecular structure of adenosine.

thick ZnTe crystal as detector, the useful bandwidth has recently been extended to over 20 THz [14], but at the expense of a reduced signal-to-noise ratio. Fig. 2b also shows the Fourier-transform spectrum of the THz signal measured after passing the radiation through an adenosine sample (at 4 K). A number of absorption features centred at 1.17, 2.12, 2.28, 2.45, 3.02 and 3.20 THz were observed.

The adenosine nucleoside comprises a ribose sugar attached to an adenine nucleic acid base through a glycosidic bond [15], as shown in Fig. 2b. The low-frequency vibrational modes of adenine have been studied previously and three modes centred at frequencies of 1.33, 1.79 and 2.28 THz (at 4 K) reported [12,13]. Since both sets of measurements were made on poly-crystalline samples, one would expect to see extensive inter- as well as intra-molecular vibrations in this frequency range, owing to the hydrogen-bonded networks [5]. In fact, all three vibrational modes of adenine observed in this frequency range were interpreted previously as originating from inter-molecular interactions mediated by hydrogen bonds [12,13]. The observation of a vibrational mode at 2.28 THz (at 4 K) for both adenine and adenosine may indicate that this particular THz mode of adenosine originates from the adenine base, rather than the ribose group. The ribose group in the adenosine molecule will prohibit the formation of some hydrogen bonds that would occur between adenine bases. This may explain why the 1.33 and 1.79 THz modes, which were observed in poly-crystalline adenine, are not seen in the THz spectrum of poly-crystalline adenosine.

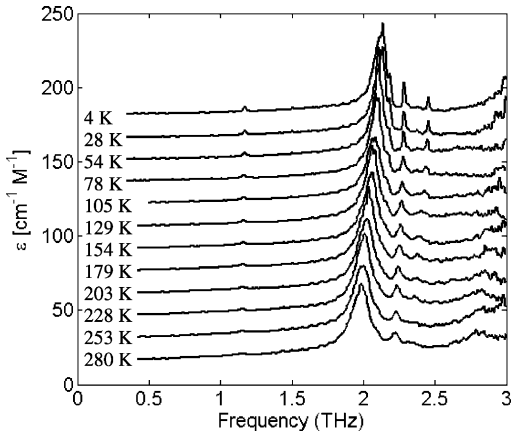

Fig. 3. Extinction coefficient spectra of the adenosine as a function of sample temperature. Spectra are vertically offset for clarity.

However, the ribose group in adenosine has additional sites for hydrogen bond formation, with either other adenine or other ribose groups. This is consistent with our observation that more vibrational modes exist for adenosine than for adenine in the THz range.

In order to study the temperature dependence of the vibrational spectra quantitatively, 124 spectra of adenosine were recorded over the temperature range 4–280 K during a 2 h warming-up process. Fig. 3 shows 12 such spectra measured at different temperatures. As the sample temperature increases, all absorption bands become broader and less intense. As a result, some bands could no longer be resolved at room temperature. Furthermore, most absorption bands shift to lower frequencies as the temperature is increased, and we found that the frequency shift can be quantitatively described by following empirical expression:

$$\upsilon(T) = \upsilon_0 - \frac{AT_C}{e^{T_C/T} - 1}$$

where A is a constant and υ_0 is the center frequency of the vibration mode at 0 K. T_C is the characteristic temperature related to the energy of the effective phonon [12]. Table 1 lists the center frequencies and the best-fitting parameters of the vibrational modes observed for the four nucleosides. The strong temperature dependence of these absorption features is thought to be related to the temperature dependence of the sample density and the average hydrogen-bond strength, as well as the anharmonic distribution of vibrational states. Detailed theoretical modelling is, however, required to understand these vibrational spectra fully.

THz time-domain techniques can also be used for spectrally-resolved imaging, i.e. they provide both spatial and spectral information of materials. Fig. 4a shows a typical THz image in the time-domain, with Fig. 4b showing the THz image in the frequency-domain, obtained by Fourier-transforming the THz time-domain data. The images were obtained at room temperature by scanning the sample across the THz focal point and simultaneously recording the temporal THz signal.

Table 1
Center frequencies of individual vibration modes and the best-fitting parameters for adenosine (A), thymidine (T), cytidine (C) and guanosine (G)

	υ_0 (THz)	T_C (K)	A (GHz/K)
Adenosine			
#1	1.169	26	0.08
#2	2.117	133	0.63
#3	2.281	174	0.30
#4	2.453	120	0.57
#5	1.60		
#6	2.06		
#7	3.02		
#8	3.20		
#9	3.48		
Thymidine			
#1	1.219	97	0.22
#2	1.274	126	0.34
#3	1.384	54	0.29
#4	1.506	93	0.53
#5	1.764	105	0.38
#6	1.89		
#7	2.15		
#8	2.34		
#9	2.54		
#10	2.72		
#11	2.88		
Cytidine			
#1	1.294	48	0.32
#2	1.679	85	0.45
#3	1.841	145	0.53
#4	2.18		
#5	2.70		
Guanosine			
#1	1.04	–	–

There is clear contrast in both time-domain and frequency-domain images between areas with and without the nucleoside/polythene mixture, although the whole sample was covered with cellulose nitrate membrane and so has no contrast in the visible spectral range. In the time-domain THz image, the contrast mainly comes from the fact that the refractive index of the nucleoside-filled area is smaller than that of the polyethylene plate. As a result, the THz pulses (time-domain) will have different arrival times hence giving clear contrast between areas with and without nucleoside samples. However, THz time-domain images alone cannot provide distinguishing contrast between the four areas filled with different nucleosides, although the THz time-domain signal contains all the necessary information. The frequency-domain image, on the other hand, shows different features for the different nucleosides (note that the signal from regions without a nucleoside sample has been used as reference). These features correspond to the vibrational modes of nucleosides. For example, adenosine has a distinct feature at 1.98 THz whilst thymidine has two features (2.2 and 2.5 THz), and cytidine has even more absorption features (1.65, 2.1, 2.5 and 3.0 THz). Therefore, spectrally-resolved THz imaging can provide both spatial and

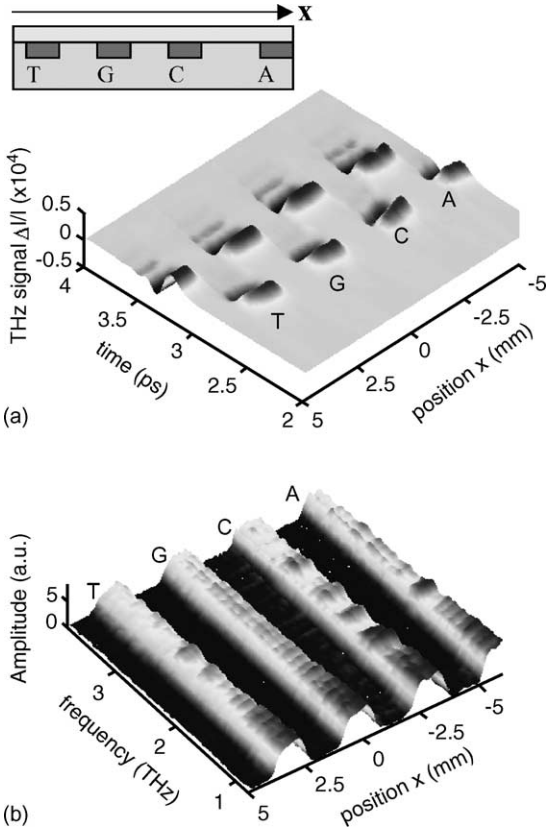

Fig. 4. THz images of nucleosides at room temperature; (a) time-domain and (b) frequency-domain. The inset of (a) shows the schematic structure of the sample for imaging.

biochemical composition information of the sample under investigation, without the need for extrinsic labelling or staining, which may perturb the system under investigation.

4. Conclusion

We have measured the low-frequency vibrational spectra of nucleosides in the temperature range 4–290 K, using THz time-domain spectroscopy. Sharp absorption features were observed in the frequency range 0.1–3.0 THz, and were used for spectrally-resolved THz imaging. In addition, a number of vibrational modes were found to become more intense and shift to higher frequencies as the temperature was reduced, and these were fitted by an empirical formula.

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References

[1] J.L. Koenig, S.Q. Wang, R. Bhargava, Anal. Chem. 73 (2001) 360A–369A.
[2] E.N. Lewis, P.J. Treado, R.C. Reeder, G.M. Story, A.E. Dowrey, C. Marcott, I.W. Levin, Anal. Chem. 67 (1995) 3377–3381.
[3] P. Colarusso, L.H. Kidder, I.W. Levin, J.C. Fraser, J.F. Arens, E.N. Lewis, Appl. Spectrosc. 52 (1998) 106A–120A.
[4] R.H. Garrett, C.M. Grisham, Biochemistry, Harcourt College Publishing, Fort Worth, 1999 (Chapter 1).
[5] M. Plazanet, N. Fukushima, M.R. Johnson, Chem. Phys. 280 (2002) 53–70.
[6] M.C. Nuss, J. Orenstein, in: G. Grüner (Ed.), Millimeter and Submillimeter Wave Spectroscopy of Solids, Berlin, Springer-Verlag, 1998, and reference therein.
[7] M.C. Beard, G.M. Turner, C.A. Schmuttenmaer, J. Phys. Chem. B 106 (2002) 7146.
[8] P.F. Taday, I.V. Bradley, D.D. Arnone, M. Pepper, J. Pharm. Sci. 92 (2003) 831–838.
[9] Y.C. Shen, P.C. Upadhya, A.G. Davies, E.H. Linfield, J. Biol. Phys. 29 (2003) 135.
[10] Q. Wu, X.-C. Zhang, Appl. Phys. Lett. 70 (1997) 1784.
[11] A. Leitenstorfer, S. Hunsche, J. Shah, M.C. Nuss, W.H. Knox, Appl. Phys. Lett. 74 (1999) 1516.
[12] Y.C. Shen, P.C. Upadhya, A.G. Davies, E.H. Linfield, Appl. Phys. Lett. 82 (2003) 2351.
[13] B.M. Fischer, M. Walther, P. Uhd Jepsen, Phys. Med. Biol. 47 (2002) 3807.
[14] Y.C. Shen, P.C. Upadhya, E.H. Linfield, H.E. Beere, A.G. Davies, Appl. Phys. Lett. 83 (2003) 3117.
[15] L.E. Bailey, R. Navarro, A. Hernanz, Biospect. 3 (1997) 47–59.