Temperature-dependent low-frequency vibrational spectra of purine and adenine

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Terahertz time-domain spectroscopy has been used to measure the vibrational spectra of polycrystalline purine and adenine over the temperature range 4–290 K. A number of well-resolved absorption peaks were observed in the frequency range 0.2–3.0 THz, which are interpreted as originating from intermolecular vibrational modes mediated by hydrogen bonds. We find that as the temperature is reduced, the observed absorption bands resolve into narrower peaks and some shift towards higher frequencies. We explain the temperature dependence of the spectra by the anharmonicity of the vibrational potentials and give an empirical expression to describe the frequency shift. © *2003 American Institute of Physics.* [DOI: 10.1063/1.1565680]

Low-frequency vibrations play a crucial role in the biological functions of proteins and nucleic acids since they give rise to significant atomic rearrangements and conformational fluctuations.[1–3] Hydrogen-bonded networks are particularly important in this context since they are more easily broken than covalent bonds and facilitate structural changes.[4] The nucleic acid bases are the building blocks of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), and there have been numerous experimental investigations of nucleic acid bases and nucleosides using mid-infrared (IR) and Raman spectroscopy, as well as neutron inelastic scattering vibrational spectroscopy.[5–8] However, the extension of these spectroscopic investigations to the far-IR, which may give information about intermolecular vibrational modes mediated by the hydrogen bonds, has received much less attention. Over recent years terahertz (THz) time-domain spectroscopy has been demonstrated to give much better performance in the far-IR range than other spectroscopic techniques such as Fourier transform IR spectroscopy,[9,10] making it possible to study the temperature dependence of low-frequency vibrational spectra below $100\,\mathrm{cm}^{-1}$ (3 THz),[11] accessing information about the structure and vibrational dynamics of solids.

In this letter, we present temperature-dependent measurements of the far-IR vibrational spectra of purine and adenine, using THz time-domain spectroscopy. Adenine is one of two common purine bases and is found in both DNA and RNA. We continuously map the evolution of the absorption features from 4 to 290 K, and present an empirical expression describing the observed frequency shift as a function of temperature.

Samples were prepared by mixing purine or adenine polycrystalline powder with polyethylene powder in a mass ratio of 1:10 and then compressing the mixture with a specially designed pellet maker into a copper ring of 8 mm diam. The pellet was about 1.3 mm thick. The copper ring ensures adequate thermal contact while allowing the THz beam to pass through the sample. The sample was fixed via the copper ring into the cold finger of a cryostat equipped with Mylar windows (MicrostatHe, Oxford Instruments) for THz spectroscopy measurements. A calibrated rhodium–iron resistance thermometer mounted adjacent to the sample on the cold finger was used to monitor the sample temperature. All chemicals were purchased from Sigma-Aldrich and used without further purification. Adenine (lot 96H040625) and purine (lot 27114-079) have molecular weights of 135.10 and 120.12, respectively. Both had a purity of 99%. X-ray diffraction measurements (Philips x-ray diffractometer PW1050) revealed that both the adenine and purine samples were polycrystalline. The polyethylene (lot 17410AO-061) had a spectrophotometric grade purity and was amorphous. It was almost transparent in the frequency range 0.2–3.0 THz.[12,13] In addition, the grain size of these powder samples was much smaller than the THz wavelength, minimizing the effects of Mie scattering.

The THz time-domain spectroscopy[14] apparatus has been reported previously.[15] 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 250 mW beam is focused onto the surface of a biased semi-insulating GaAs photoconductive emitter[16] for THz generation, and a 25 mW beam serves as the probe beam for electro-optic[17] detection using a 1-mm-thick ZnTe crystal. In all measurements, 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 $0.5\,\mathrm{cm}^{-1}$ (15 GHz). Using a lock-in detection scheme, the signal-to-noise ratio achieved in our experiments was 10^5.

Figure 1(a) shows the measured absorption spectra of purine at room temperature and at 4 K. A number of well-resolved absorption peaks are seen in the far-IR region between 0.2 and 3.0 THz. In addition, the base line increases with frequency. As the temperature is reduced from room temperature to 4 K, all of the observed absorption bands

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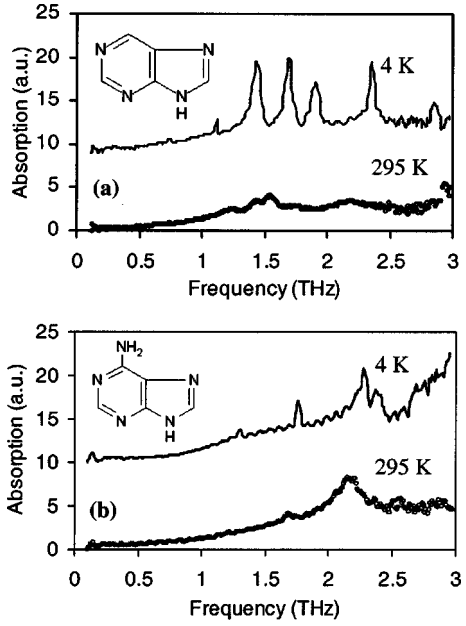

FIG. 1. Absorption spectra of (a) purine and (b) adenine at 4 and 295 K. 4 K spectra are vertically offset.

resolve into narrower peaks and most shift towards higher frequencies. In order to see if these effects are specific to purine, we examined the spectra of samples of four nucleic bases (adenine, guanine, cytosine, and thymine) prepared in an identical manner. In each case, a different series of spectral features was observed, but in general, as with purine, the frequencies and intensities of the features increased as the temperature was lowered. As an example, the spectra of adenine are shown in Fig. 1(b). The small-amplitude, ripple-like oscillations in the spectra result from multiple THz reflections in the sample and the detection crystal. By subtracting the weak features arising from reflections out of the raw time-domain data prior to the Fourier transform, these oscillations are minimized.

In order to study the temperature dependence of the vibrational spectra quantitatively, 120 spectra of purine were recorded over the temperature range 4–290 K during a 2 h warming-up process (Fig. 2). Figure 3 shows the frequency variation of the purine vibrational mode centered at 1.68 THz (at 4 K) as a function of temperature. The peak stays at a fixed frequency until 50 K, whereupon it progressively shifts to lower frequency approximately linearly with temperature.

Since our measurements were made on polycrystalline samples, rather than noninteracting molecules in, for example, the gas phase, one would expect to see extensive inter- as well as intramolecular vibrations in this frequency range[12,13] owing to the hydrogen-bonded networks.[5,8] The importance of the intermolecular bonding was confirmed by molecular modeling based on density functional theory (DFT) calculations. DFT calculations performed on a single adenine molecule and an adenine molecular dimer indicate that all vibrational modes in the frequency range investigated experimentally are a result of intermolecular interactions. Furthermore, DFT calculations on larger molecular clusters (tetramers, etc.) suggest that these vibrational modes are nonlocalized but are of a collective (phonon-like) nature.

If all the contributions to the frequency shift are mediated by phonons, one might expect that the temperature dependence of the vibrational modes will follow a Bose–Einstein distribution.[18] We fit the frequency shift for each of the observed absorption features by

$$\nu(T) = \nu_0 - AT_C/(e^{T_C/T} - 1), \tag{1}$$

where ν_0 is the center frequency of the vibration mode at 0 K and A is a constant. T_C is a characteristic temperature related to the energy of the mode. Figure 3 shows both the experimental and best-fit results of the purine peak centered at 1.68 THz at 4 K. The agreement is reasonably good over the entire temperature range, and the fitting parameters are shown in the inset.

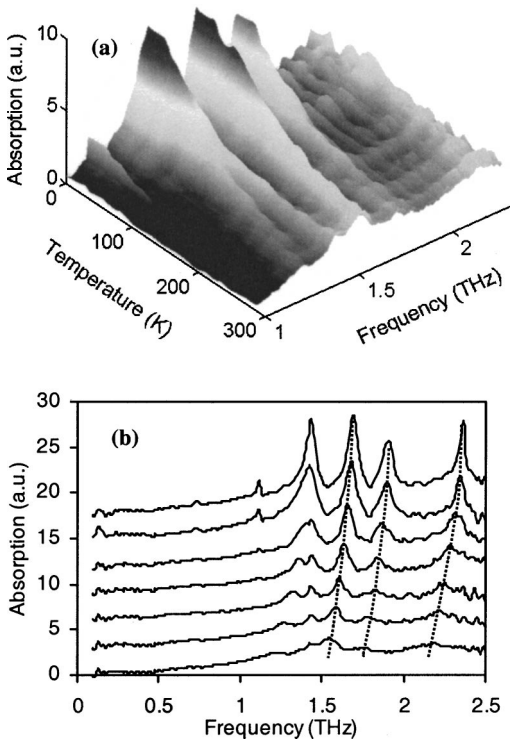

FIG. 2. (a) Evolution of the vibrational modes of purine in the temperature range 4–290 K. A total of 120 spectra were measured. For clarity, only spectral data in the 1.0–2.3 THz range are shown. (b) Absorption spectra of purine at 4, 54, 105, 153, 204, 253, and 295 K (from top to bottom). Spectra are vertically offset and the dotted lines are guides to the eye.

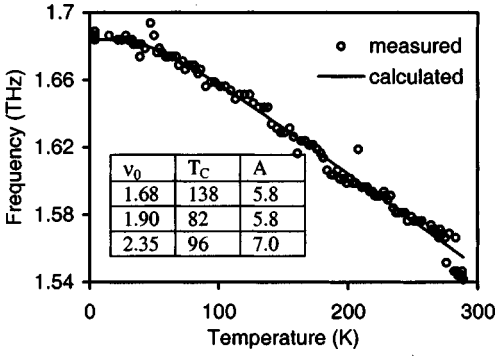

FIG. 3. Temperature dependence of the resonance frequency centered at 1.68 THz (at 4 K). Open circles are experimental data and the solid line is calculated using the empirical expression given in Eq. (1). Inset shows the best-fitting parameters in units of THz, K, and 10^{-4} THz/K for ν_0, T_C, and A, respectively.

The temperature-induced shifts for the purine peaks at 1.90 and 2.35 THz (measured at 4 K) have been measured and fitted in the same way, and the best-fit parameters are also listed in the Fig. 3 inset. It is important to note that the characteristic temperatures (T_C) for the 1.08, 1.90, and 2.35 THz peaks do not scale with their frequency. In addition, the center frequencies of two specific purine vibrational modes (1.11 and 1.43 THz) remain almost unchanged with temperature, although the intensities of all vibrational modes increase as the temperature is reduced. These observations indicate that a number of mechanisms are likely to be involved in the temperature dependence of the vibrational spectra of these samples in this low-frequency range.

For low-frequency vibrational modes, the ground state vibrational levels are significantly depopulated at room temperature ($290\ \mathrm{K} \equiv 6\ \mathrm{THz}$). In addition, the vibrational potentials are anharmonic, and therefore, the spacing between adjacent energy levels decreases for higher energy vibrational states.[3] This anharmonicity might explain the temperature dependence of the frequency shift observed in our experiments. At higher temperature, more molecules are in higher vibrational excited states, and hence, the decreased energy spacing results in the overall absorption envelope for the ensemble being shifted to lower frequency. As the temperature is lowered and more molecules populate the ground state, the average frequency of the absorption envelope would tend to increase. In addition, the distribution of the states should be narrower at lower temperature, which agrees well with our experimental results, since the absorption features sharpen as the temperature is reduced.

The different behavior seen at 1.11 and 1.43 THz possibly results from the complex nature of the hydrogen-bonded solids studied here. For example, the unit-cell volume of hydrogen-bonded solids may either increase or decrease with temperature.[19] In addition, the average hydrogen-bond strength may decrease with increasing temperature,[20,21] as can be inferred from the observation of the redshift of the hydrogen-bond (O–H···O) stretching frequency in the far-IR and low-frequency Raman spectra of water as the temperature is increased.[22,23] These effects change the shape of the potential energy curves, and hence, the vibrational energy levels in the hydrogen-bonded system, and this could lead to either an increase or decrease in peak position with changing temperature, or even leave it unaltered.

In conclusion, we have reported temperature-dependent measurements on the far-IR vibrational spectra of hydrogen-bonded purine and adenine, by THz time-domain spectroscopy. A number of vibrational modes were found to shift to higher intensities and frequencies as the temperature was reduced, and these were modeled by an empirical formula. Theoretical research will be needed to interpret fully the behavior observed.

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