Low-frequency vibrational modes of glutamine*

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High-resolution terahertz absorption and Raman spectra of glutamine in the frequency region 0.2 THz–2.8 THz are obtained by using THz time domain spectroscopy and low-frequency Raman spectroscopy. Based on the experimental and the computational results, the vibration modes corresponding to the terahertz absorption and Raman scatting peaks are assigned and further verified by the theoretical calculations. Spectral investigation of the periodic structure of glutamine based on the sophisticated hybrid density functional B3LYP indicates that the vibrational modes come mainly from the inter-molecular hydrogen bond in this frequency region.

Keywords: vibrational modes, THz time-domain spectroscopy, Raman scattering, B3LYP, amino acid

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1. Introduction

As an efficient spectral measurement tool in the far-infrared region, terahertz time-domain spectroscopy (THz-TDS) has been widely used in the areas of biophysics and biochemistry since it can yield high resolution spectra with high signal-to-noise ratio (SNR). Particularly, a THz pulse can penetrate bio-specimens safely due to its low radiation energy and it may be used to measure molecular vibrations and inter-molecular modes for molecular crystals.[1−3] It is noted that the characteristic THz spectra of many bio-specimens, such as amino acids, proteins, DNA, RNA and other organic molecules have been reported successively.[4−20] However, how to interpret the features of THz spectral peaks accurately is still an emerging and challenging topic.

Theoretical modeling combined with measured data is usually used to assign the vibrational modes for specific structural features of bio-molecules. For solid phase bio-molecules, there exists an inter-molecule hydrogen bond between molecules, so the incorporation of periodicity is a key to the correct description of the system.[21−26] The most difficult task in the theoretical calculation of the vibrational spectrum of a solid, especially in the low-frequency region, is the tight accuracy which may be influenced by the quantum chemistry method for describing the system, the optimized structure for calculating frequencies and the convergence criterion on total energy.[27,28]

Recently, the development of the Raman spectrometer has made a breakthrough at the limitation in the low-frequency Raman signals of the material measurement. A three-grating Raman spectrometer, which inhibits elastic scattering, can measure only a few low-frequency Raman shifts of the molecules. In the THz-TDS, the interpretation of the THz absorption spectrum is similar to that of the Fourier transform infrared spectrum. Although the selection rules and equipment used in THz spectroscopy and Raman spectroscopy are completely different, these two techniques can complement each other so that the low-frequency spectrum properties of the molecules could be well represented.[10]

Amino acids constitute the smallest structural unit of a protein. Study of the low frequency vibrations of amino acids is of great significance because it gives information about the weak inter-molecular interactions that take place in enzyme reactions, the processes of the above-molecular self-organization and

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intracellular interactions.[29] Twenty standard amino acids have been studied by THz spectroscopy since 2003.[7,10−17] However, only a few amino acids and some peaks are identified. Glutamine is a source of nitrogen in the biosynthesis of many compounds, including amino acids and nucleic acids. So far, low-frequency vibrations of glutamine have not been discussed in detail yet.

In this paper, the THz-TDS and Raman spectroscopy are used to investigate low frequency vibrational modes of glutamine, separately. The infrared active and/or Raman active spectral peaks are compared and distinguished as A or B symmetry in the frequency region 0.2 THz–2.8 THz. Furthermore, a theoretical model is used to assign the vibrational modes. Also, the IR intensities obtained from the localized Wannier functions[30] are presented for comparing the theoretical and experimental spectra.

2. Experimental and computational details

The THz-TDS system developed in Ref. [17] is used in our experiment, where a mode-locked Mai–Tai laser is used to generate laser pulses with a repetition rate of 82 MHz, a central wavelength of 810 nm, a pulse width of 100 fs and a pulse power of 980 mW. An InAs wafer with ⟨100⟩ orientation is used to emit the THz beam and a ZnTe wafer with ⟨110⟩ orientation to detect THz signals. The sample of glutamine is polycrystalline powder (purity > 98%) mixed with pure polyethylene (PE) powder in mass ratio 2:1 and then is pressed into 1.0-mm pellet for THz-TDS. Amino acids are normally stored and clinically applied at room temperature environment. Test results at room temperature can help us to understand corresponding conformation and dynamic characteristic of the amino acids, as well as future practical applications in real-time monitoring and spectral analysis. Therefore, our experiments are performed at room temperature. The sample and the optical path are masked in a box, which is purged with dry nitrogen to keep the humidity less than 2%, to minimize the vapour effects and thus enhance the SNR.

The Raman spectroscopy used in the experiment is a JY-T64000 Raman spectrometer. Excitation wavelengths of argon-ion laser are 488 nm and 514.5 nm for distinguishing Raman scattering and fluorescence signal. The laser power is less than 0.5 mW. With 180-degree back-scattering experiment configuration, the powder samples are used directly for Raman measurements.

In order to increase the calculation accuracy, several strategies are applied. It is essential to make an applicable comparison between the theoretical and the experimental results especially for a complex system with multi-vibrational modes. Density functional theory is widely used in solid physics. With consideration of electron correlation energy, the density functional theory can give better result than the Hartree–Fock method. Generalized gradient approximations, including not only the electron density as a local density approximation, but also the gradient of electron density, are adopted by many scientists. Unfortunately, the exact correlation functional is unknown, so only approximate functionals are used. The sophisticated hybrid density functional B3LYP[31,32] is a generalized gradient approximation functional mixed with a portion of exact exchange (Hartree–Fock). The careful combination gives an outstanding description of geometry, energy and the relevant properties of chemical molecules in this work. The well-accepted accurate B3LYP functional used in this work is also helpful to reduce the shift of peak positions. A standard basis set 6-21G (d, p) included in the CRYSTAL06 program[33] is also used. Incorporating a polarized function into the basis set is beneficial to the description of the hydrogen bonds in amino acids.

Structural optimization is needed before the frequency calculation. If the structure is not well optimized, the system is not located at the local minimum on the potential surface. This will affect the vibrational frequencies obtained with the harmonic approximation and some imaginary frequencies normally with a wavenumber up to several tens of cm^{-1} will occur. For the ordinary functional group assignment of organic molecules, this is not so serious because the frequency concerned is quite large, usually larger than 1000 cm^{-1}. However, in the THz region, an imaginary frequency implies that one mode will disappear in this region. This will cause confusion in the multi-modes assignment, so the tight optimization convergence criterion on the root mean square of the gradient is set to be 10^{-5} Hartree/Borh (1 Hartree=27.21 eV) instead of the default 3×10^{-4} Hartree/Borh[33] to obtain well-optimized structures and then to ensure that there is no imaginary frequency in the calculation.

If the accuracy in self-consistent field calculation

is not high enough, there could also exist imaginary frequencies, and this will affect the positions of the spectral peaks. The self-consistent field convergence criterion on total energy is set to be 10^{-9} Hartree instead of the 10^{-5} Hartree in order to evaluate the energy accurately, because 10^{-5} Hartree is more than 0.06 THz, which is noticeable in the THz region. 55 radial and 974 angular points (default 434 angular points) are adopted for an atomic grid to evaluate the density functional numerical integrations in order to obtain a highly accurate frequency.[27,28]

3. Results and discussion

High-resolution terahertz absorption and Raman spectra of glutamine in 0.2 THz~2.8 THz are obtained, respectively, as shown in Fig. 1. It can be seen that there are 3 far IR active and 5 Raman active vibration modes in this range.

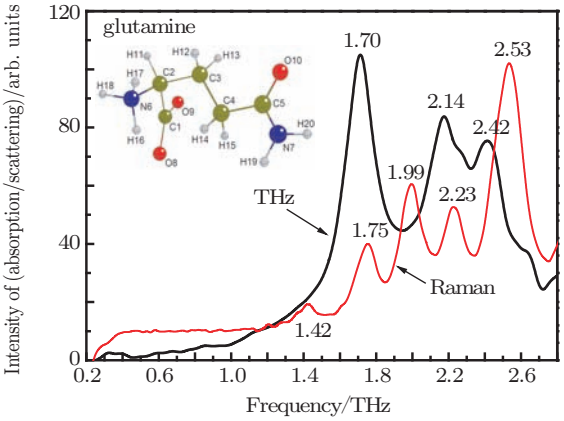


Fig. 1. (colour online) THz absorption and Raman spectra of glutamine. The inset shows the molecular structure.

The cell parameters a, b, c for glutamine are 16.020, 7.762, 5.119 Å (1 Å=0.1 nm), respectively. Even if the crystal has a size of 1 micron meter, it still has billions of cells. Therefore, we could analyse the features of the IR and the Raman active vibration modes based on the symmetry types of the space group $P2_12_12_1$ for glutamine. There are three types of symmetry normal modes, B_1, B_2 or B_3 symmetry that will be both IR and Raman active, and one type of symmetry normal mode A, which corresponds to only Raman active. By directly comparing THz absorption spectrum and the Raman experimental spectrum in Fig. 1, we can see that the absorption peaks at 1.70 THz and 2.14 THz are so close to those of Raman peaks at 1.75 THz and 2.23 THz, suggesting two peaks are for B_1, B_2 or B_3 symmetry vibrational modes. The Raman peaks at 1.42, 1.99, and 2.53 THz can be identified as A symmetry modes because they do not appear in the THz spectrum. There is no single infrared active for $P2_12_12_1$, so that the peak at 2.42 THz in the THz spectrum is suggested to be of mode B, in spite of the fact that it was not observed in the Raman spectrum. It is because weak scattering intensity results in difficult observation.

Each THz or Raman peak is explained by theoretical calculations in Table 1, while all the calculated vibrational modes have their corresponding experimental peaks. The calculated frequencies are smaller than the experimental results, but the largest difference is only 0.23 THz (less than 10 cm^{-1}). It is noticeable that the IR peak at 2.14 THz or Raman peak at 2.23 THz is contributed by two vibrational modes. Theoretical study suggests that the peaks at 2.02 THz and 2.04 THz are too near to be distinguished in the experiment. The experimental IR intensity at 1.70 THz is the largest peak, while that at 2.42 THz is the smallest peak among the three IR peaks. The IR intensity also matches well with high-accuracy theoretical calculation.

Table 1. Assignments of vibrational frequencies for glutamine.

Experiment		Calculation		
THz	Raman	Frequency/THz	Symmetry	Vibrational mode
	1.42	1.41	A	H_2NH–O–HNH rock
1.70	1.75	1.49	B_2	O–HNH_2 stretch, C_3–C_4torsion
	1.99	1.96	A	H_2NH–O–HNH rock, C_4–C_5 torsion
2.14	2.23	2.02, 2.04	B_1, B_3	H_2NH–O–HNH rock, H_2NH–O^a–HNH_2 rock
2.42		2.19	B_2	H_2NH–O–HNH rock
	2.53	2.45	A	H_2NH–O^a–HNH_2 rock, C_1–C_2 torsion

[a] Oxygen atom is in the COO–, and the rest oxygens in this table are in the –$CONH_2$.

Although the difference between the calculated and experimental frequencies is quite small and has no influence on the assignment, it can be attributed to the anharmonic and temperature effects. The anharmonicity appears especially in the low frequency region, because there are usually torsional or rocking modes with soft potential energy surfaces.[21] This effect is estimated to be about 0.18 THz (6 cm^{-1}) in the THz region,[26] which is a reason why the calculated frequencies are smaller than the experimental ones. Another reason is that the quantum chemistry method used here has not considered the temperature contribution to the frequencies, although the thermodynamic functions can be obtained with the consideration of temperature. An empirical expression[34] can give a good solution to this temperature effect, and the effect is smaller than 0.3 THz (10 cm^{-1}) in the experiments.[26,34] Generally, these small effects should affect similar vibrational modes to a similar extent and the order of the frequencies should not change, which facilitates the assignment of this system with many spectral peaks. The high-accuracy strategies (density functional, optimization and self-consistent field convergence criterion) are the key factors for the theoretical assignment.

Besides the torsional or rocking modes, some stretching modes involving hydrogen bonds also exist in this region, because the hydrogen bonds are weaker than traditional covalent bonds and the corresponding vibrational modes have low frequencies. Our theoretical result shows that for glutamine, the vibrational modes in the THz region are similar. The intra-molecular vibrations are mostly due to the torsion of carbon skeleton, such as C_3–C_4 torsion for 1.49 THz, C_4–C_5 torsion for 1.96 THz, and C_1–C_2 torsion for 2.45 THz. The vibration of carbon chain can inevitably change inter-molecular distance. The dominant linking pattern among amino acids in solid is hydrogen bond, so the inter-molecular modes are those involving hydrogen bonds. Since the vibration mode is always mixed with other modes, only the largest one or two modes are listed in Table 1. The prominent nature of the vibration in the THz region is the inter-molecular hydrogen bond rock and stretch, which cannot be found in single molecule calculations. The vibrational mode for 1.49 THz is mainly the hydrogen bond stretch of the oxygen atom in $–CONH_2$ and the hydrogen atom in NH_3^+, while the intra-molecular part is the torsion of C_3–C_4 carbon skeleton. The vibrational mode for 2.19 THz is mainly the rocking mode of the hydrogen atom in NH_3^+, the oxygen atom in $–CONH_2$, and the hydrogen atom in the amino group. The calculated vibrational vectors for 1.49 THz and 2.19 THz are shown in Fig. 2 with MOLDRAW program.[35,36]

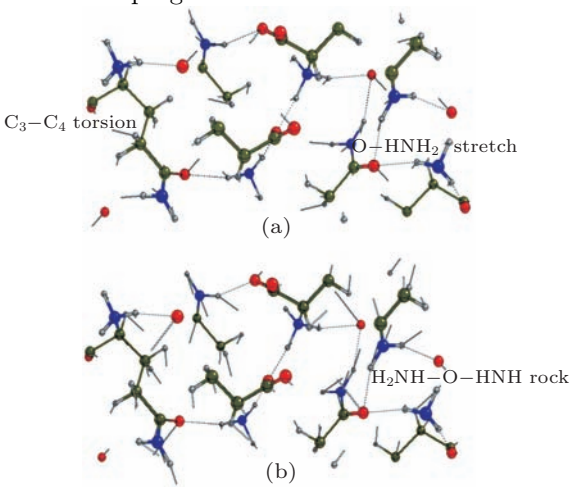


Fig. 2. (colour online) Calculated vibrational vectors for (a) 1.49 THz and (b) 2.19 THz.

4. Conclusion

The low-frequency vibrational modes of the glutamine in the frequency region 0.2 THz–2.8 THz are studied by using THz-TDS, Raman spectroscopy and quantum chemistry calculations. The spectral peaks with THz and Raman active, corresponding to A, B_1, B_2, and B_3 symmetry types, are identified successfully. The results show that the most important vibrational mode comes from the inter-molecular hydrogen bond, which cannot be found in the single molecule calculations. The theoretical investigation gives a powerful tool for the assignment of the vibrational modes.

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