Terahertz Pulse Spectroscopy of Biological Materials: L-Glutamic Acid

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Abstract. We report the terahertz pulse spectra of L-glutamic acid. There are a number of well-resolved transitions in the 1.75–2.5 THz ($58\text{--}83\text{ cm}^{-1}$) region. These are compared with published theoretical data on intra and intermolecular transitions. We could not find any correlation with the theoretical values. However, it was noted that the theoretical model did not include any crystalline or hydrogen-bonding effects.

Key words: L-glutamic acid, Terahertz pulse spectroscopy

1. Introduction

Studies of the vibrational spectra of materials is a powerful technique for the investigation the structures and dynamics of molecules. The Terahertz Pulse Spectroscopy (TPS) technique has recently being applied to the study of DNA [1,2] and low-frequency torsional vibrational modes in long chain carotenoids [3]. Since very little experimental work has been done in this region, there are correspondingly few theoretical calculations. Hopefully, with recent activity in the field theoretical molecular models will develop and test their models against our results. Walther et al. [4] have reported the THz-absorption spectra of the isomers of hydroxy-benzoic-acid (salicylic acid), showing the power of the technique to determine different conformers of a molecule.

We report on the progress in understanding the terahertz pulse absorption spectrum of the amino acid L-glutamic acid. The structure of the molecule is shown in Figure 1. The crystal structure of L-glutamic acids shows that the molecules are linked to form infinite chains along one direction by O-H...O hydrogen bonds, and these chains are linked together in the other directions by N-H...O hydrogen bonds [5, 6].

Glutamic acid plays a crucial role in nitrogen metabolism in biological systems. It plays an active role in parental nutrition; studies have shown that human milk contains 1.2% protein of which 20% is bound glutamic acid [7]. The molecule is also known to be a neurotransmitter for the central nervous system and is a major degradation product in tumour cells.

Figure 1. Molecular structure of L-glutamic acid.

The mid-infrared and Raman spectra of glutamic acid has been reported elsewhere [9, 10]. We report here for the first time room temperature and cooled (5 K) terahertz pulse absorption spectra for glutamic acid. The data obtained was also compared with the published theoretical models of the vibrational structure of glutamic acid.

2. Experimental

The samples were obtained from Sigma-Aldrich and used without any further purification. The L-glutamic acid was lightly crushed and mixed with polyethylene (PE) powder at a percentage weight of 25%. The mixture was compressed into a 1-mm thick disc with a compression of about 2-tons. PE is transparent in the THz region. Thus making it a suitable material as a matrix for spectroscopy in this region.

The apparatus employed for terahertz pulse spectroscopy (TPS) has been reported elsewhere [11]. But, briefly here the laser used in these experiments was a Coherent Vitesse Kerr-lens that produces 75-fs near-bandwidth limited pulses. The laser beam is split into two; one beam is used to generate the THz-radiation. The other beam is used as a probe to detect the THz-radiation using electro-optic sampling (EOS). The useful bandwidth of detection is from about 100 GHz to about 3 THz (6–100 cm^{-1}). Figure 2 shows the diagram of the experimental arrangement used and Figure 3 shows a typical output of the system.

The sample was placed in a helium-cooled cryostat and held at temperatures as low as 4 K. It should be noted, however, at this stage of the development, the temperature, at the sample position has not been accurately determined. All temperatures stated in this report are recorded at the heat exchanger of the cryostat.

Figure 2. Schematic diagram of the TPS spectrometer. WP is a Wollaston prism. $\lambda/4$ is a quarter waveplate at 800-nm. OAP is an off-axis parabolic mirror.

3. Results and Discussion

The THz-field transmitted through a sample is modified by dispersion and the absorption of the media under examination. The ratio of the electric field strength before E_r and after transmission, E_s , is given by

$$\frac{E_s^2}{E_r^2} = T(n) \exp \left(-\alpha d + \frac{i n \omega d}{c} \right)$$

where d is the thickness of the sample, ω the frequency of the radiation, c the speed of light in vacuum and $T(n)$ is the reflection loss at the sample surface. Both the refractive index n and the absorption coefficient α can be determined from the ratios of the measured THz-fields. We only show the absorption curves for the data obtained, as the refractive index data gives little or no extra information about the bands observed.

Figure 4 shows the terahertz pulse absorption spectra of L-glutamic acid at 5 K (black) and 300 K (grey). Shown for comparison is the stick spectrum for calculation values by, (a) Lopez Navarrete et al. [9], (b) Chhiba et al. [10] and (c) Ramirez and Lopez Navarrete [12]. In references 9 and 12 the authors only considers intramolecular vibrations of isolated L-glutamic acid molecule. While Chhiba et al. [10] considers the vibrations due to the L-glutamic acid dimer, these maybe inter or intramolecular vibrations, but it should be noted only a very limited model was used. One can easily see that there is no good agreement between our measurements and the calculated values. This is not surprising, as the theoretical computer models have not been tested in this region to date; this has been mainly due to the lack of experimental data. At this point we can only make general

Figure 3. (a) Representative time-domain interferogram obtained from the system used. (b) Frequency-domain spectrum of the time-domain interferogram shown in Figure 3(a).

observation regarding the spectra obtained. The terahertz absorption spectra of L-glutamic acid show three strong bands that the transitions at 2.53, 2.24 and 1.74 THz at 5 K. Not surprisingly the bands shift to lower frequencies when warmed to 300 K. But, perhaps more surprising is the band at 2.53 THz shifts to 2.46 THz and becomes sharper at 300 K. This is counterintuitive, as one expects bands to broaden when heated. This observation is not fully understood. More work is being undertaken to understand the observation.

Table I. Calculated and observed frequencies of L-glutamic acid. The calculated values and assignments are taken from reference [9]

Assignment	Peak position / THz (cm^{-1})		
	Predicted (ref 9)	5 K	300 K
st O...H	3.90 (130)		
Def. skel.	3.69 (123)		
Def. skel.	2.76 (92)		
Def. skel.	2.37 (79)	2.53 (84)	2.46 (82)
		2.24 (75)	2.22 (74)
To C-C	1.94 (65)		
To C-C	1.80 (60)	1.74 (58)	1.67 (56)
Def. skel.	1.50 (50)		
Def. skel.	1.14 (38)		
Def. skel.	0.84 (28)		
Def. skel.	0.54 (18)		

Abbreviations: Def. skel. deformation skeleton; To torsion;
st, stretching.

Figure 4. Shows the terahertz pulse absorption spectra of L-glutamic acid at 5K (black trace) and 300 K (grey trace). Shown for comparison is the stick spectrum for calculation values by, (a) Lopez Navarrete et al. [9], (b) Chhiba et al. [10] and (c) Ramirez and Lopez Navarrete [11].

4. Conclusions

We have obtained the temperature dependant terahertz spectra of L-glutamic acid. These were compared to a theoretical model, but no good agreement could be found. The model used by Chhiba et al. [10] was very limited as it did not include any crystalline effects or no explicit hydrogen bonding potential energy function. Since we know that the terahertz absorption spectra is very sensitive to the crystalline structure [11]; the theoretical models will need to be improved. Using density functional theory (DFT) on large collections of molecules may confirm the number of modes and may give frequencies within a factor of two. DFT calculations will be investigated and reported later.

Other molecules of biological interest are have been investigated. The data from these spectra are currently been analysed and will be reported in the future.

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