Terahertz Spectra of L-phenylalanine and Its Monohydrate

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Abstract

The low-frequency vibrational property of L-phenylalanine (L-Phe) and L-phenylalanine monohydrate (L-Phe·H₂O) has been investigated by terahertz time-domain spectroscopy (THz-TDS) at room and low temperature ranging from 0.5 to 4.5 THz. Distinctive THz absorption spectra of the two compounds were observed. Density functional theory (DFT) calculations based on the crystal structures have been performed to simulate the vibrational modes of L-Phe and L-Phe·H₂O and the results agree well with the experimental observations. The study indicates that the characterized features of L-Phe mainly originate from the collective vibration of molecules. And the characterized features of L-Phe·H₂O mainly come from hydrogen bond interactions between L-Phe and water molecules. L-Phe and L-Phe·H₂O were also verified by differential scanning calorimetry and thermogravimetry (DSC-TG) and powder X-ray diffraction (PXRD) examinations.

Key words: L-phenylalanine, monohydrate, terahertz absorption spectrum, low-frequency vibration, DFT

1. Introduction

L-phenylalanine (L-Phe) is important as an essential amino acid for human nutrition and is widely used as food or feed additive, in infusion fluids, and for chemical syntheses of pharmaceutically active compounds[1-3]. L-Phe has been found to have two solid forms, anhydrous form and monohydrate form which is known as polymorphism[4, 5]. Different pseudopolymorphs can have vastly different product characteristics such as bioavailability, solubility, mechanical behavior, stability, dissolution rate and shelf-life[6-8]. Incorporation of water of crystallization of L-Phe changes the molecular structure and also changes its active pharmaceutical ingredients (API) performance characteristics[9-11]. Water molecule has the capability to act both as hydrogen bond donor and acceptor and can therefore affect the intermolecular interactions and packing in L-Phe molecules[10]. And the forces holding these crystals together originate from hydrogen bonding, van der Waals interactions, and electrostatic interactions[12]. Therefore, it is critically important to understand and monitor the anhydrate and its hydrate forms of L-Phe.

Terahertz (THz) spectroscopy is an alternative and novel technique for study the structure and interactions of chemical materials[13, 14]. It is highly sensitive to the hydrogen bonding, lattice vibration and water of crystallization[10, 15]. Recently, Zeitler et al.[16] successfully distinguished the different hydrate and anhydrate forms of four pharmaceutical materials including lactose, carbamazepine, piroxicam and theophylline by the use of THz pulsed spectroscopy. Zheng et al.[17] investigated the THz spectra of anhydrous and monohydrated glucose by using THz spectroscopy and DFT theoretical calculation. They found the characterized features of monohydrated glucose mainly come from the intermolecular modes of water-glucose and glucose-glucose molecules, while those of anhydrous glucose origin from the interactions of glucose molecules. Liu et al.[18] found the THz spectra of pharmaceutical substances including caffeine, theophylline, D-glucose, and ampicillin exhibit different features from their hydrated forms, presumably due to their different intermolecular vibrational modes mediated by hydrogen-bonding. As for L-Phe,

Miyamaru et al.[19] observed one broad peak at 2.72 THz. Wang et al.[20] reported THz spectra of 20 alpha-amino acids including Phe. Li et al.[21] investigated the experimental THz spectrum of Phe in the frequency ranging from 0.2 to 2.8 THz and also simulated the vibrational modes based on the isolated molecule, **nevertheless, what kind of samples' chirality were not mentioned.** These works provide useful information of molecular recognition and intermolecular interaction in low-frequency. However, the characterization of L-Phe monohydrate (L-Phe·H₂O) form in THz region has not been reported and the analysis of vibrational modes of anhydrous and hydrous L-Phe is still insufficient. In this paper, we investigated the THz spectra of L-Phe and L-Phe·H₂O ranging from 0.5 to 4.5 THz. **And the temperature effect was investigated.** The hydrous and anhydrous forms of the samples were also verified by differential scanning calorimetry and thermogravimetry (DSC-TG) and powder X-ray diffraction (PXRD) examinations.

Quantum chemical calculations were performed to simulate the vibrational properties of L-Phe and L-Phe·H₂O molecules to help understanding the THz experimental observation and the molecular interactions. The simulations were carried out based on the crystal structure and multiple molecules are involved. DFT calculations were applied using the CASTEP program as a part of Materials Studio package from Accelrys and the results agree well with the experimental observations.

2. Materials and Methods

2.1 Materials

The sample of L-Phe was purchased from J&K Co. Ltd. (purity > 98%). Polyethylene powder (PE) (particle size 53-75 μm) was purchased from Sigma-Aldrich Co. LLC. The samples were used without further purification.

L-Phe·H₂O was obtained by recrystallization **from water**[22]. DSC-TG and PXRD were used for characterization the hydrate yielded.

2.2 Sample preparation

The powder of samples for THz detection were blended with PE powder at a mass

ratio of 1:5 and then pressed into disks with a hydraulic press at a pressure of 3.0 MPa. The thicknesses of the sample tablets were approximately 1.2-1.3 mm, and the diameter was 13 mm.

2.3 THz time-domain spectroscopy (THz-TDS)

THz spectra of L-Phe and L-Phe·H₂O were measured using a TAS7400TS THz-TDS system (Advantest Corporation, Japan) ranging from 0.5 to 4.5 THz. The frequency resolution is 1.9 GHz. The number of scans is 1024. The sample was placed into the THz system which was purged with drying air. The data was an average of three. PE was used as reference. The software used for the data evaluation was Origin.

2.4 Temperature controller

The pellets were placed into temperature controller (Specac Ltd. U.K., the accuracy ± 0.5 °C) which is equipped with high-density polyethylene windows. The liquid nitrogen was put into the variable temperature cavity. THz spectra of L-Phe and L-Phe·H₂O were recorded over the temperature range from -180 to -40 °C.

2.5 Differential scanning calorimetry and thermogravimetry (DSC-TG)

DSC-TG was performed using STA 449 F3 Jupiter (NETZSCH). Sample was put in a corundum crucible with a lip on it (L-Phe: 6.104 mg, L-Phe·H₂O: 6.512 mg), and heated ranging from 35 to 300 °C with a heating rate of 10 °C/min under Ar atmosphere (60 mL/min). The software used for the data evaluation was Origin.

2.6 Powder X-ray diffraction (PXRD)

PXRD patterns of L-Phe and L-Phe·H₂O were recorded using the X'Pert Pro MPD (Cu source, 40 kV voltage, 40 mA filament emission). The data were collected with a scan ranging from 10° to 90° (2 θ). The scan rate was 0.2°/min. The software used for the data evaluation was Xpert Highscore.

3. Quantum Chemical Calculation

The total energy optimization and the frequency calculations were performed based on the DFT using the CASTEP[23] program as a part of Materials Studio package from Accelrys. The simulation of L-Phe and L-Phe·H₂O were performed using the crystal

cell parameters taken from King et al. Ref[24] and Williams et al. Ref[25] respectively and the calculations of THz spectra were performed on the strength of the geometry optimized structure. The space group of L-Phe crystal is $P2_1$ ($Z=8$), with lattice dimensions of $a=8.7407 \text{ \AA}$, $b=6.0412 \text{ \AA}$, $c=31.3941 \text{ \AA}$, $\alpha=90^\circ$, $\beta=97.745^\circ$, $\gamma=90^\circ$. And the space group of L-Phe·H₂O crystal is $P2_1$ ($Z=4$), with lattice dimensions of $a=13.05 \text{ \AA}$, $b=5.44 \text{ \AA}$, $c=13.98 \text{ \AA}$, $\alpha=90^\circ$, $\beta=101.2^\circ$, $\gamma=90^\circ$. The calculations were performed on the crystalline state within the generalized gradient approximation (GGA) at PBE (Perdew-Burke-Ernzerhof correlation) functional[26]. Calculations were performed using norm-conserving pseudopotential. The quality of energy calculations were ultra-fine. The plane-wave cut-off energy was 830 eV. For L-Phe and L-Phe·H₂O, Brillouin zone sampling of electronic states were performed on $2 \times 2 \times 1$ and $1 \times 3 \times 1$ Monkhorst-Pack grid respectively, and the total energy was converged to 5.0×10^{-6} eV/atom and the maximum forces between atoms were less than 0.01 eV/Å. The grids for fast Fourier transform were $90 \times 60 \times 300$ and $125 \times 54 \times 135$ respectively.

4. Result and Discussion

4.1 THz analysis and temperature effect

The THz absorption spectra of L-Phe and L-Phe·H₂O at both 25 °C and -180 °C respectively are shown in Figure 1. At room temperature, L-Phe shows two strong peaks at 2.81 and 4.19 THz, two less pronounced bands at 1.17, 1.99 respectively. The L-Phe·H₂O has two strong peaks at 3.09, 4.03 THz, two obvious peaks at 0.93, 1.48 THz, and relative weaker peaks at 1.71, 1.92 THz. The absorption peak of L-Phe located at 1.99 THz is consistent with Wang and Li [20, 21]. Since the chiral amino acid molecules always present different crystalline structures between the enantiomers and the racemate[27], and the different crystal packing arrangements always show different responses to THz radiation[28]. Therefore, we infer that the sample of the Phe they used probably is not the racemate. Though both of L-Phe and L-Phe·H₂O present relative weak absorption peaks before 2 THz, their whole features are obviously different.

The absorption peaks usually become stronger and sharper at low temperature, owing to the temperature dependent change in the distribution of occupied energy states[29]. For L-Phe, a new peak arises at 2.30 THz. And the broad peak at 2.81 THz at room temperature split into three peaks at 2.85, 3.22, 3.41 THz at -180 °C. As shown in Figure 1, the absorption peaks blue-shifted with the temperature decreasing. Such a shift is usually attributed to the increased bond lengths due to thermal expansion with warming. And the degree of blue shift is different at each absorption peak, probably because different vibrational modes have different response degree to the temperature change[30]. The result indicates that THz spectra are sensitive to temperature. And the evolution of the THz spectra in the temperature ranging from -180 °C to -40 °C was measured (supplementary information).

The result indicates that THz spectroscopy is sensitive to the different forms of L-Phe, and the characteristic THz fingerprints can be used in detection and identification applications. Considering the different physical forms and the crystalline structures of a sample always show different responses to THz radiation, PXRD was carried out to characterize the samples in this study.

Figure 1. THz absorption spectra of L-Phe and L-Phe·H₂O.

4.2 DSC-TG analysis

Figure 2 shows the results of L-Phe and L-Phe·H₂O obtained by DSC-TG thermal analysis. The L-Phe shows three endothermic peaks at 263.7, 278.7 and 293.9 °C, corresponding to the simultaneous processes of melting and decomposition of the sample. And it can be confirmed from TG curve which is accompanied by a significant weight loss. As to L-Phe·H₂O, another endothermic peak occurs at 88.7 °C and corresponds to dehydration and has the accompanying weight change. The result is agree with previous report by Lu et al.[31].

Figure 2. The DSC-TG curves of L-Phe and L-Phe·H₂O.

4.3 PXRD patterns of L-Phe and L-Phe·H₂O

The crystal structure of L-Phe and L-Phe·H₂O were confirmed by PXRD measurements. The patterns are shown in Figure 3, and both of L-Phe and L-Phe·H₂O present specific diffraction patterns which are consistent with the previous published crystal database[24, 25]. Therefore, the DFT simulations were performed based on their crystal cell parameters. The significant differences of their crystal structures suggest the presence of water directly influence the spatial arrangement of L-Phe molecules. In L-Phe·H₂O, each water molecule is engaged in hydrogen bonding to L-Phe molecule and the hydrogen bonding network can lead to the change of the crystal lattice.

Figure 3. Powder X-ray diffraction patterns for L-Phe and L-Phe·H₂O.

4.4 Quantum chemical calculation

The interpretation and understanding of the experimental THz spectra are still a fundamental scientific challenge at present. Some theoretical simulations and calculations have been suggested as effective means to bridge the observed low-frequency vibrational modes and the molecular structures and interactions[14, 32-34]. And the simulations based on crystal cells which involve the intermolecular interactions have been suggested more reliable compared to isolated molecule[35].

The simulated THz spectra produced by solid-state DFT calculations are plotted against the experimental THz spectra of L-Phe and L-Phe·H₂O in Figure 4(a) and 5(a), the vibrational modes of L-Phe and L-Phe·H₂O were demonstrated in Figure 4(b) and Figure 5(b), respectively. The descriptions of the calculated vibrational modes of L-Phe and L-Phe·H₂O were presented in Table 1.

For L-Phe, The experimental spectral feature at 1.99 THz corresponds to the vibrational mode calculated at 1.91 THz, arising from translation of L-Phe. This peak was suggested as wagging of -NH₂ and ring structure out-of-plane in previous study based on an isolated molecule simulation[21]. The broad peak at 2.81 THz at 25 °C which is split into three peaks at -180 °C can be attributed to the calculated mode from 2.78 to 3.39 THz, arising from rotation and translation of L-Phe.

Figure 4. The experimental and calculated spectra of L-Phe. (a) THz spectra of L-Phe from simulated and the corresponding experiment. (b) The vibrational modes of L-Phe at 1.91 THz and 3.15 THz.

For L-Phe·H₂O, the measured feature at 0.93 THz is produced by the calculated mode at 0.97 THz, which originates from rotation and translation of L-Phe mixed with translation of water molecules. The observed peak at 3.09 THz is attributed to intermolecular hydrogen bond vibration besides torsion of L-Phe and translation of water molecules. And the strong absorption centered at 4.03 THz is much similar with that of 3.09 THz.

Figure 5. The experimental and calculated spectra of L-Phe·H₂O. (a) THz spectra of L-Phe·H₂O from simulated and the corresponding experiment. (b) The vibrational modes of L-Phe·H₂O at 0.97 THz and 3.30 THz.

The molecular vibration of L-Phe in THz region involves most of atoms even collective vibration of all molecules. The hydrogen bonding network involves intermolecular N-H···O hydrogen bonds between the carboxylate and amine groups.

Incorporation of the water molecules in the crystal lattice of L-Phe changes the dimensions, shape, symmetry and number of molecules of the unit cell. And additional bonds formation between the L-Phe molecules and the water molecules lead to the

change of intermolecular interactions. The change of unit cell leads to the significant difference of THz spectra.

In the L-Phe·H₂O structure, the two crystallographically distinct water molecules are engaged in O-H···O hydrogen bonding with each other. And one water molecule forms O-H···O hydrogen bond with L-Phe molecule. The other water molecule forms O-H···O hydrogen bond with carboxylate group of L-Phe molecule and N-H···O hydrogen bond with ammonium group of a different L-Phe molecule. These hydrogen bonds strengthen governing lattice vibration as an important component to maintain the stability of lattice pattern.

Table 1. The descriptions of calculated vibrational modes and corresponding experimental peaks of L-Phe and L-Phe·H₂O^a

Compound	Experimental peak (THz)		Calculated mode (THz)	Mode description
	25 °C	-180 °C		
L-Phe	1.17	1.33	1.24	Rotation
	1.99	2.08	1.91	Translation
		2.30	2.41	Rotation
		2.85	2.78	Rotation and translation
	2.81	3.22	2.87	Rotation
		3.41	3.15	Rotation of -Ph
			3.39	Rotation of -Ph
	4.19	4.35	3.86	Rotation of -Ph
			4.18	Rotation of -Ph
L-Phe·H ₂ O	0.93	1.01	0.97	Rotation and translation of L-Phe and translation of H ₂ O
	1.48	1.59	1.38	Rotation of L-Phe and translation of H ₂ O
	1.71	1.83	1.57	Rotation and translation of L-Phe and translation of H ₂ O
	1.92	2.04	1.98	Rotation of L-Phe
			2.60	Wagging of -Ph and translation of H ₂ O
	3.09	3.24	2.89	Wagging of -Ph and translation of H ₂ O
			2.97	Torsion of L-Phe and translation of H ₂ O
			3.11	Wagging of -Ph

		3.30	Torsion of L-Phe and translation of H ₂ O and HB vibration
		3.85	Torsion of L-Phe and translation of H ₂ O and HB vibration
		3.91	Wagging of -Ph
4.03	4.29	3.96	Torsion of L-Phe and translation of H ₂ O and HB vibration
		4.26	Torsion of L-Phe and translation of H ₂ O and HB vibration

^a “HB” stands for hydrogen bond; “-Ph” for -phenyl.

Although the quantum chemical calculation provides us with much valuable information, there are some offset compared with the experimental results. And the following reasons may be worth considering, such as the temperature effect of experiments and simulations, humidity of environment, system error of experimental measurements etc. In addition, the disparity of crystals situations in actual experiments and theoretical simulations also need to take into consideration, since the calculation is based on the perfect crystal structure, while the actual system is difficult to idealize.

Conclusions

In this work, L-Phe and L-Phe·H₂O were investigated by THz-TDS at room and low temperature. Different crystalline structures of them are found to be the main reason for the different THz spectral features. The result indicates that THz wave is sensitive to differences of crystal structure. And the absorption peaks become stronger and narrower at low temperature. DSC-TG and PXRD were performed to investigate the thermodynamic properties and molecular packing patterns of L-Phe and L-Phe·H₂O. The THz spectra were simulated by solid-state DFT, producing high-quality spectral simulations for L-Phe and L-Phe·H₂O. The study indicates that the characterized features of L-Phe mainly originate from the collective vibration of the molecules. And the characterized features of L-Phe·H₂O mainly come from hydrogen bond interactions between L-Phe and water molecules. This study demonstrates the feasibility of utilizing THz spectroscopy for pharmaceutical applications.

Additional Supporting Information may be found in the online version of this article.

Acknowledgments

This work was supported by the Main Direction Program of Knowledge Innovation; Open Project Program of Key Laboratory of Interfacial Physics and Technology, Chinese Academy of Sciences, China; and the National Science Foundation [No. 10574134, 61675151], National Basic Research Program of China [No. 2014CB339806].

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Graphical Abstract