Terahertz Time-Domain Spectroscopy of Four Hydroxycinnamic Acid Derivatives

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Abstract The well-resolved absorption spectra of the hydroxycinnamic acid (HCA) derivatives, caffeic acid, ferulic acid, sinapic acid and chlorogenic acid, were measured over the frequency region from 0.3 to 2.0 THz at 294 K with terahertz time-domain spectroscopy (THz-TDS). Theoretical calculation was applied to assist the analysis and assignment of the individual THz absorption spectra of the HCA derivatives with density functional theory (DFT). The distinctive spectral features were originated from the collective motion of molecules held together by hydrogen bonds. The real and imaginary parts of dielectric function of the four HCA derivatives were also obtained.

Key words terahertz time-domain spectroscopy · hydroxycinnamic acid derivatives · collective vibration

1 Introduction

During the past 20 years, terahertz time-domain spectroscopy (THz-TDS) has grown into a popular spectroscopic tool in the frequency range of 0.1 to 10 THz. Its unique characteristics such as its high brightness, stable, single-cycle pulse generation, and coherent, time-gated detection make it a complementary technique to Fourier transform infrared (FT-IR) spectroscopy and Raman spectroscopy. THz-TDS is also capable of obtaining the absorption coefficient and the refractive index directly without using the Kramers–Kronig relation. These features make it a very suitable technique to study various materials [1].

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Recently, a wide variety of biological molecules such as benzoic acid and its derivatives [2, 3], glucose and uric acid [4], retinal isomers [5], nucleosides [6, 7], DNA and bovine serum albumin [8] were successfully investigated with this promising technique. These researches laid the foundations for an increasing number of studies on biological molecules using THz-TDS and pointed out some potential future applications, such as chemical recognition, time-resolved structural studies and diagnostics.

The hydroxycinnamic acid (HCA) derivatives produced from L-phenylalanine or L-tyrosine via the shikimate pathway [9], including caffeic acid, ferulic acid, sinapic acid and chlorogenic acid, are the most widely distributed phenolic components in plant tissues. Their structures are shown below (Figure 1). Chlorogenic acid can mimic animal iron-binding proteins such as transferrin and protect plants by withholding iron from pathogens. Caffeic acid, which is known to be absorbed into the bloodstream, can inhibit *in vitro* human low-density lipoprotein oxidation [10, 11]. The HCA derivatives are also reported to be more effective radical scavengers even than vitamins C and E *in vitro* [12, 13]. They protect against free-radical damage to DNA and proteins through charge transfer interactions. These processes had been investigated with laser flash photolysis in our lab. But the complicated spectroscopic lines in the mid-IR made analysis difficult. Now, with THz technology the modes measured are often unique to a particular molecule. Optical pump/THz probe spectroscopy was reported to be applied to monitor these charge/electron transfer processes [14, 15]. A thorough understanding of the interaction between HCA derivatives and DNA or proteins will provide more profound information about the essence of such biological processes.

In this letter, we have performed THz-TDS on a series of similar molecules in crystalline form to explore some characteristic features of their THz spectra, which are related with their low-energy vibrational modes. Theoretical calculation based on density functional theory (DFT) was carried out to assist the analysis of the vibrational frequencies.

Figure 1 Chemical structures of the investigated HCA derivatives.

2 Materials and Methods

Caffeic acid (Acros), sinapic acid (Acros), ferulic acid (Sigma-Aldrich) and chlorogenic acid (Fluka) were used without further purification. Samples were made into pellets with thickness between 1.0 and 1.2 mm by applying an approximate pressure of 22 MPa with an oil press. X-ray diffraction measurements revealed that all samples were in poly-crystalline form (Rigaku, X-ray Diffractometer D/max-2550VB/PC). All crystals were crushed well before making the pellets in order to ensure that the particles were of sub-micron size, and that the observed features were not a result of Rayleigh and/or Mie scattering. The grain sizes are about 10 μm which were measured by a laser diffraction particle size analyzer (Oasis Dry, Sympatec GmbH, Germany).

The illustration of transmittance terahertz time-domain spectroscopy we used is shown in Figure 2 and has been discussed in detail elsewhere [7, 16]. In brief, a mode-locked Ti: sapphire laser provides pulses of 100 fs duration at a center wavelength of 805 nm with average power of 600 mW and a repetition rate of 80 MHz. The output is split into two parts: a pump beam is focused onto the surface of a biased GaAs photoconductive emitter for THz generation, a beam serves as the probe beam for electro-optic detection using a 2 mm thick ZnTe crystal. The variable delay stage that provides the time delay between the THz pulse and the probe pulse is scanned over a distance of 4 mm, providing a spectral resolution of 40 GHz. The useful bandwidth of the spectrometer is 0.3–2.0 THz. The terahertz beam path was placed in a closed box and purged with dry nitrogen in order to minimize absorption by residual water vapor. The temperature near the sample is $21 \pm 0.5^\circ\text{C}$ and the humidity is controlled below 4% during data collection.

Quantum chemical calculations with full geometry optimizations and frequency analysis of the optimized structures were performed using the Gaussian 03 [17] program package.

Figure 2 Experimental setup of THz time-domain spectroscopy.

Ab initio DFT calculations on the isolated molecule and dimer were all carried out with the 6–31G basis set and B3LYP functional. The B3LYP functional was used, together with the DNP basis set, which includes polarization d-functions on all non-hydrogen atoms and polarization p-functions on all hydrogen atoms to include hydrogen bonding effects. Minimum energy structures were found, which were confirmed by vibrational analysis. The atom labeling and visualization of the normal modes can be found using Gaussview molecular modeling software [18].

3 Results and Discussion

The electric field of a THz pulse transmitted through a sample is modified by dispersion and absorption of the specimen. The ratio of the electric field strengths before (E_r) and after (E_s) transmission is given by

$$E_s/E_r = T(n) \times \exp\{-\alpha d + i\omega d/c\}$$

where d is the thickness of the sample, ω is the frequency of the radiation, c is the speed of light in vacuum and $T(n)$ is the reflection loss at the sample surface [15]. The power absorption coefficient α and the refractive index n of the sample can be accurately determined from the ratio of the measured THz fields. Furthermore, the frequency-dependent complex dielectric response ϵ can be described by the general relationship $\epsilon = \epsilon_1 + i\epsilon_2 = (n_r + in_i)^2$, where n_r and n_i are the real and imaginary index of refraction, respectively. The imaginary refractive index is determined by measuring the power absorption coefficient $\alpha = 4\pi n_i/\lambda$.

The measured THz power absorption coefficients α and the refractive indices n of the four HCA derivatives are shown in Figure 3. The data below 0.3 THz are not considered due to multiple THz reflections inside the sample pellets and the detection crystal. All spectra show distinctive absorption peaks and continuous absorption increasing with frequency. It can be seen obviously that caffeic acid has a sharp absorption peak at 1.36 THz and a wide band centered at about 1.83 THz, ferulic acid displays four separate bands at 0.94, 1.16, 1.47 and 1.88 THz, sinapic acid has three absorption peaks at 1.34, 1.64 and 1.73 THz, while chlorogenic acid has a peak at 1.33 THz and a series of shoulder band at 0.70, 0.97 and 1.14 THz. Another wide band of chlorogenic acid is near 2 THz, which does not display its full profile within the experimental range but does show a weak band at 1.73 THz. All absorption peaks were further confirmed by the corresponding characteristic change of the refractive index. Furthermore, the real part and imaginary part of ϵ of the four HCA derivatives were also obtained from experimentally-determined α and n with the relationship $\epsilon = \epsilon_1 + i\epsilon_2 = (n_r + in_i)^2$. The real part of the dielectric function indicates a resonance process related with a similar mechanism as the optical absorption. Meanwhile, the imaginary part describes the dielectric loss of such a resonance process. Since the real part and the imaginary part of the dielectric function showed distinctive bands which displayed the same trend as they showed in the power absorption and refractive index spectrum respectively, the plots are not given in this letter. The marked differences in the THz spectra provide a convenient, rapid and accurate method to distinguish specimens with similar structure. It also indicates the tremendous possibilities of the THz-TDS technique in the pharmaceutical industry and quality control. It can be noticed that all the samples show a similar absorption band around 1.36 THz. Based on the

Figure 3 a-d The measured power absorption coefficient α and index of refraction n of caffeic acid, ferulic acid, sinapic acid and chlorogenic acid. Solid lines: absorption coefficient α ; the dotted lines: index of refraction n .

previous research, it is known that the vibrational modes in THz range were mostly related to the structural characteristics of the specimens. The four samples have the same structural domain as of 4-hydroxycinnamic acid. We infer that the band at about 1.36 THz may originate from the vibration of the same structural unit.

Though all samples have a similar structural domain, the HCA derivatives still show distinctive differences in their THz spectrum. This illustrates again that slight variations in the crystal structure can considerably influence the low-frequency vibration modes. To understand THz spectroscopy more thoroughly, the FT-IR spectra of the samples were measured at room temperature with a Nicolet Avater-360 spectrometer (Figure 5). In the FT-IR spectra, all substances show wide absorption bands of ν_{OH} between $3,448\text{ cm}^{-1}$ and $2,880\text{ cm}^{-1}$ and characteristic bands of $\nu_{\text{C=O}}$ at $1,700\text{--}1,640\text{ cm}^{-1}$. Comparing with the OH stretch fundamental, there is a frequency shift and a corresponding broadening. The formation of hydrogen bonds and the π -conjugated system with the C=C bond and the phenyl ring makes the C=O stretch fundamental shift significantly to lower frequencies. The hydride stretch region of the infrared spectra has long been used to diagnose the presence and strength of hydrogen bonds involving the XH group. When an XH group forms a hydrogen bond, the XH stretch fundamental undergoes a large frequency shift, the magnitude of which reflects the strength of the hydrogen bond formed [19]. The frequency shift of the OH stretch fundamental and the characteristic band of C=O implied the existence of dimer held together by two cooperatively strengthened $\text{OH}\cdots\text{O}=\text{C}$ hydrogen bonds.

Based on this result, theoretical calculations with density function theory (DFT) were performed to give a more reasonable interpretation. *Ab initio* DFT calculations on the isolated molecule and dimer (except the dimer of chlorogenic acid) were all carried out using Gaussian 03 with the B3LYP functional and the 6-31G (d, p) basis set. The structure of chlorogenic acid does not form dimers easily because the hexahydric ring produces huge resistance to the formation. Figure 4 shows the optimized structure of caffeic acid dimer together with the calculated structural parameters. The calculated bond lengths are nearly all longer than the crystallographic data from [20], except for the bond between 4-hydroxyl group and the phenolic ring. This is due to the packing forces of the crystal which modify the geometry in the solid state. In contrast, the shorter bond (C5–C6) is caused by the additional hydrogen bonds formed between screw-axis-related 4-hydrogen groups in the crystal. This fact is confirmed in our IR data, which show a characteristic frequency corresponding to the hydrogen-bonded ($3,214\text{ cm}^{-1}$) hydroxyl groups. The phenolic ring and the carboxylic group are almost in the same plane which also differs from the crystallographic data (4.338°). The longer bond lengths and the small dihedral angle all illustrate the different environments experienced by the molecule in solid state and in isolation.

Figure 5 presents the predicted frequencies together with the FT-IR experimental data. It can be noted that the theoretical results for the dimer agree well with the experimental data

Figure 4 View of a calculated dimer of caffeic acid and the associated structural parameters.

Figure 5 Calculated frequencies and FT-IR experimental results of HCA derivatives.

and were obviously more accurate than those on isolated molecule. This agreement confirmed the existence of hydrogen bonds. However, it can be seen that there are errors in the calculated data compared with the experimental results. We took the stretching band of $C=O$ as an example. The experimental $\nu_{C=O}$ of caffeic acid is at about $1,644\text{ cm}^{-1}$ while the calculated data of the isolated molecule and the dimer were at $1,766\text{ cm}^{-1}$ and $1,759\text{ cm}^{-1}$ respectively. For the $\nu_{C=O}$ of chlorogenic acid, the experimental data is at $1,687\text{ cm}^{-1}$ while the calculated data of the isolated molecule is at $1,766\text{ cm}^{-1}$. The deviations were caused by the real and complicated situations. The measurement was affected by many factors such as the crystalline state, the process of the sample's preparation, temperature and ambient humidity etc. Table 1 shows the detailed assignments of low-energy vibrational modes. It can be seen that the terahertz responses can be concluded to result from the collective modes mediated by the cooperatively strengthened $OH\cdots O=C$

Figure 5 (Continued).

hydrogen bonds. Though the vibrational modes had been assigned based on theoretical calculations, it has to be mentioned that vibrational motions in the THz region must be considered in a global sense where all of the atoms in the entire molecule are participating in the intramolecular or intermolecular motion. Simulations on dimers may not be adequate to model THz spectra for this multi-hydrogen bonding system. According to the crystal structure of caffeic acid, pairs of acid molecules were associated through their carboxylic groups by hydrogen bonds. Additional hydrogen bonding occurs between 4-hydrogen groups [20]. The vibrations of dimer molecules will become sluggish due to the extra hydrogen bonding. The whole THz spectra will therefore be rendered by this anchoring between phenolic groups. For ferulic acid, the existence of $\text{C-H}\cdots\text{O}$ will also influence the predicted THz spectrum [25]. The application of DFT in THz range is still controversial though some researches have simulated THz absorption successfully with DFT calculations [21–24]. Solid-state modeling on crystal unit cell may produce more accurate results in this

Table 1 Assignment of observed vibrational frequencies of HCA derivatives

	Calculated frequency (THz)		Experimental frequency (THz)	Assignments
	Single	Dimer		
Caffeic acid	0.58	0.67		
	2.0	1.58	1.36	Scissoring
		1.78	1.83	Twist around the conjugated plane
Ferulic acid	0.96	0.69	0.94	Twist of the domain of H-bonds and double bond
	1.97	1.23	1.16	Tilting
		1.39	1.47	Stretching of whole dimer linked by H-bonds
Sinapic acid		1.99	1.88	Twist around the conjugated plane
	0.73	0.68		Twist of the domain of H-bonds and double bond
	1.70	1.17	1.34	Tilting
	1.86	1.62	1.64	Twist of four methyl groups
Chlorogenic acid		1.98	1.73	Twist around the conjugated plane
	0.41			
	0.53		0.70	Twist of the domain of H-bonds and double bond
	0.94		0.97	Scissoring
	1.26		1.14	Stretching of whole dimer linked by H-bonds
	1.86		1.33	Tilting of two phenyl rings

range [26]. Nevertheless, the calculated frequencies can at least provide a primary profile of the origination of the vibrational modes.

4 Conclusions

In this paper the FIR absorption spectra of the four HCA derivatives including caffeic acid, ferulic acid, sinapic acid and chlorogenic acid were measured by THz-TDS at room temperature. The distinct absorption features in frequency range between 0.3 and 2.0 THz were attributed to collective vibration modes based on theoretical calculations on B3LYP/6–31G (d, p). These results provide basic optical data for further investigation on the charge/electron transfer interaction process occurring between HCA derivatives and DNA or proteins with time-resolved THz spectroscopy. The experiments also illustrate that THz spectroscopy will offer tremendous potential for chemical, biological and pharmaceutical applications.

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