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Terahertz Time Domain Spectroscopy of Crystalline α-Lactose Monohydrate

Euna Jung[1], Jeonghoi Kim[1], Younho Han[1], Kiwon Moon[1], Meehyun Lim[1] & Haewook Han[1]

[1]National Research Laboratory for Nano-THz Photonics, Department of Electrical and Computer Engineering, Pohang University of Science and Technology, Pohang 790-784, Korea
Correspondence and requests for materials should be addressed to H. Han (hhan@postech.ac.kr)

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Abstract

Fundamental biological processes require both small- and large-scale structural changes within molecules, including low frequency collective vibrational motions. Many theoretical calculations have predicted that the collective vibrational motion can be characterized as the large-scale intermolecular motions in the terahertz (THz) frequency range. A new measurement technique based on THz time domain spectroscopy (TDS) can probe directly the collective vibrational motions of biomolecules which involve structural changes and specific biological functions. We report the THz TDS of solid state α-lactose monohydrate to characterize the collective vibrational motions. Our measurements show that the THz transmission spectrum of α-lactose monohydrate has strong absorption peaks centered at 0.531, 1.195, 1.38, and 1.81 THz, which result from intermolecular interactions.

Keywords: Terahertz time domain spectroscopy, Low frequency vibrational modes, α-Lactose monohydrate, Intermolecular interactions, Biosensing

Introduction

Terahertz (THz) radiation is an electromagnetic wave in the frequency range of 0.1 to 10 THz between microwave and infrared bands[1,2]. Due to its non-ionizing and non-invasive characteristics, there has been much attention for various applications of THz radiation, including semiconductor characterization[3], medical diagnostics[4], label-free genetic analysis[5], and chemical and biological sensing[6].

The low frequency vibrational and torsional motions of biomolecular systems in condensed phase depend strongly on intermolecular interactions dominated by hydrogen bonding networks[7]. The theoretical predictions based on simulations of molecular dynamics suggest that the low frequency collective motions of biomolecules occur in THz frequency range[8-10]. Such motions involve the large-scale "breathing" of entire subunits of the macromolecules, which affect the structural changes and functions of biomolecules. The low frequency collective modes are critical for conformational changes in a variety of biological processes, ranging from primary photoisomerization of rohodopsin to enzyme reactions[11]. The identification of these collective motions can offer the structural and functional information of molecular systems in living cells[12].

THz time domain spectroscopy (TDS) is a new technique to investigate the low frequency molecular dynamics of biomolecular structures in both solid and liquid phases. Many studies have already shown that THz TDS can provide spectroscopic information for a variety of biomolecules such as DNA/RNA[13,14], polysaccharides[15], amino acids[16], and proteins[17]. In recent years, several research groups have investigated saccharide related molecules at THz frequencies because they are simple enough to study in detail and also of great biological significance[15,18]. Specifically, α-lactose monohydrate, as shown in Figure 1, is one of the most common disaccharides. In this work, we present our preliminary results on THz TDS of solid state α-lactose monohydrate at 0.2-2.0 THz.

Results and Discussion

The THz pulse signal transmitted through a lactose pellet is shown in Figure 2. The incident THz pulses are delayed due to the refractive index of the samples

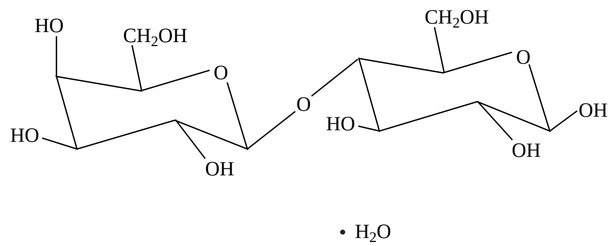

Figure 1. Molecular structure of α-lactose monohydrate.

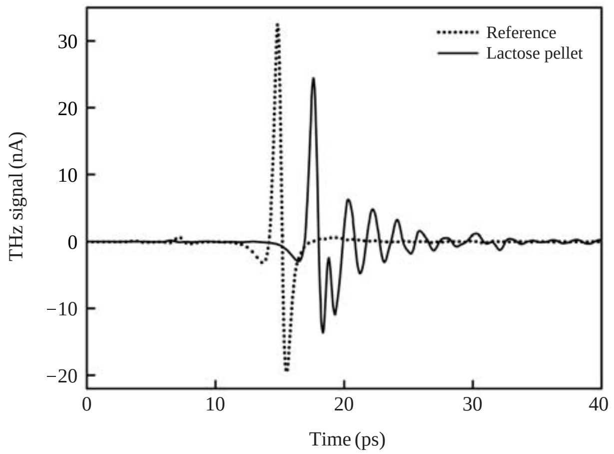

Figure 2. THz signal transmitted through the 1-mm thick lactose pellet. The reference signal was measured without the pellet.

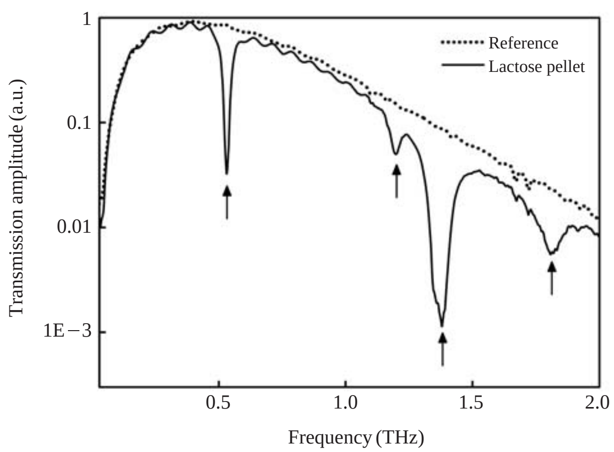

Figure 3. THz Transmission spectrum of α-lactose monohydrate at room temperature.

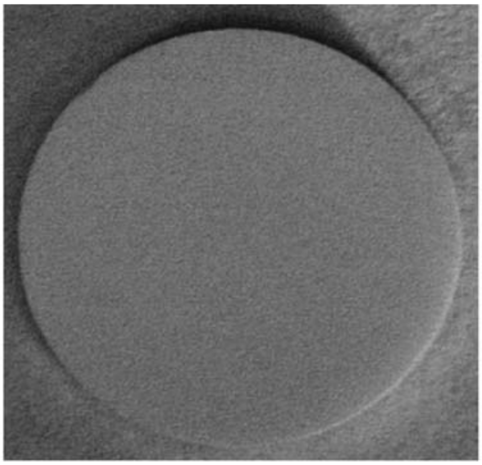

Figure 4. Photograph of α-lactose monohydrate pellet.

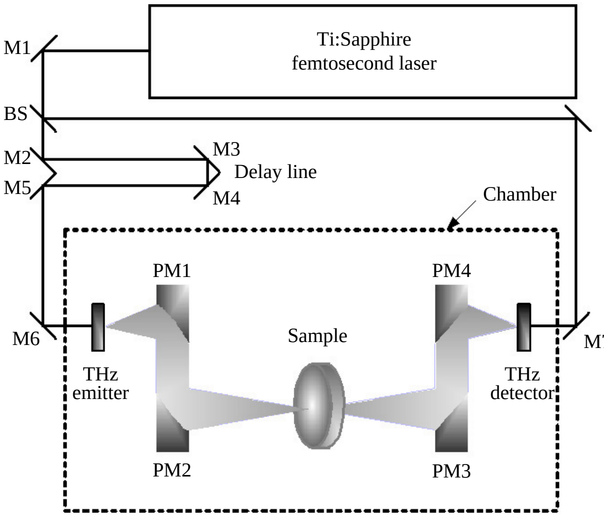

Figure 5. Schematic of THz TDS setup with transmission geometry. M: mirror, BS: beam splitter, PM: off-axis parabolic mirror.

and attenuated due to absorption and Fresnel loss. The additional small peaks results from the multiple reflections at the two sample-air interfaces and also the dispersion effect due to the complex permittivity of α-lactose monohydrate.

The transmission spectrum of solid state α-lactose monohydrate at room temperature is shown in Figure 3 where the absorption peaks are indicated by arrows. The THz spectrum indicates that α-lactose monohydrate has absorption peaks centered at 0.531, 1.195, 1.38, and 1.81 THz. As reported in a previous work, this strong absorption results from intermolecular vibrations[19].

α-lactose consists of two monosaccharides linked by a glycosidic bond. Density functional theory (B3LYP functional) of isolated α-lactose monohydrate predicts intramolecular twisting motion at 0.858 THz[20]. However, there was no absorption peak around 0.858 THz in our measured spectra, indicating that THz absorption signatures cannot be explained by the intramolecular interactions in an isolated molecule. In addition, a recent study suggests that the lowest frequency THz absorption at 0.531 THz is due to the externally hindered rotational mode with no internal mode contribution[20]. Our measurements confirm that the low frequency collective motions are mainly affected by intermolecular rather than intramolecular interactions in nature. These collective motions are associated with interactions between molecules in the hydrogen bonded networks of molecular crystals.

Conclusion

THz absorption spectra of crystalline α-lactose monohydrate with a glycosidic linkage between 0.1 and 2.0 THz were measured by THz TDS. The THz transmission spectra have strong absorption peaks centered at 0.531, 1.195, 1.38, and 1.81 THz. We interpret these THz absorption peaks as the signatures of intermolecular interaction motions of the hydrogen bonded crystalline structure of α-lactose monohydrate. We expect that in near future the THz TDS technique can be applied for biosensors and biochips to identify the spectroscopic fingerprints of specific biomolecules.

Materials and Methods

Sample Preparation

Crystalline spectrophotometric-grade α-Lactose monohydrate powder (Sigma-Aldrich, USA) was used without further purification. To identify the intrinsic spectral features of the biomolecules, the crystalline powder was pressed into 1-mm thick pellets, following standard practice. Figure 4 shows the lactose pellet with 13-mm diameter.

Experimental System

We used a conventional THz TDS system with transmission geometry[21], shown in Figure 5. The THz pulse was generated by a InAs wafer pumped by a Ti:sapphire laser (Tsunami, Spectra Physics, USA) with a center wavelength of 790 nm, a pulse width of 100 fs, and a repetition rate of 80 MHz. The generated THz pulse was collimated and focused by off-axis parabolic mirrors. The sample was placed at the THz beam waist between two off-axis parabolic mirrors. The transmitted THz signal was detected by a photoconductive antenna fabricated on a low-temperature grown GaAs using the standard optical gating and phase-sensitive detection techniques[22]. To avoid the absorption due to atmospheric water vapor, the entire THz TDS system was enclosed in a chamber and continuously purged with dry air during the measurements.

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