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Research Article

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Terahertz absorption spectra of short-chain peptides

Li Cheng[1], Yuichi Ogawa[2] and Shin'ichiro Hayashi[3]

[1]*College of Life Sciences, Heilongjiang University, Harbin, China*
[2]*Graduate School of Agriculture, Kyoto University, Kyoto, Japan*
[3]*RIKEN Sendai, Sendai, Japan*

ABSTRACT

The Terahertz (THz) absorption spectra of some short-chain peptides have been measured using a THz-FTIR at room temperature. Compared with the spectra in the mid-infrared region the THz absorption features were remarkable sensitivity to the chain-length dependence and sequence dependence. With the length of the peptide increasing the sharp spectra features became broadening. And the peptide and its reverse sequence produced a different and identifiable spectrum. These results demonstrate that the THz-FTIR is a promising method for studies on the low-frequency spectra of peptides.

Key words: Terahertz, absorption spectra, short-chain peptides

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INTRODUCTION

Terahertz (THz) vibration spectroscopy of bio-molecular systems, which can be used to characterize far-infrared vibration modes (*i.e.*, rotation, torsion, phonon, and inter- and intra-molecular modes), is widely recognized for its sensitivity to force constants influencing the global nuclear motions that extend over a large fraction of the bio-molecular framework. Previous studies of sugars [1, 2], amino acids [3, 4], proteins [5-8], DNA [9, 10], and other bio-molecules [11-13] have examined the dependence of THz absorption on factors such as the degree of hydration and amorphous versus crystalline forms. From these studies, THz spectroscopy has emerged as an important tool for examining new aspects of bio-molecular structure and dynamics, providing a new method for analyzing the conformation of bio-molecules. Many studies have shown that THz spectroscopy allows the selective detection of weak inter- and intra-molecular vibration modes, permitting the identification or analysis of molecular structures by using the unique information obtained from the THz spectra.

Peptides are short chains of amino acid monomers linked by peptide bonds. Their properties are determined by their conformation and structure [14, 15]. A single difference in the combination of amino acids, such as the length of the chain or the sequence of the amino acid, often makes for a completely different physiological function. So the complexity and structural information become more important for researchers. On the other hand, in recent years, with the existence of the collective rotation modes and vibration modes in the THz region, the terahertz (THz) spectroscopy are widely used as a powerful technique to investigate the conformation and structure of various molecule.

In this paper we present the THz absorption spectra of the peptide $(Gly)_2$-$(Gly)_6$ which length and masses increases gradually with a standard FTIR spectrometer have been performed at wave number between 20cm^{-1}-650cm^{-1} at room temperature. And we use the best-fit simulated THz spectra for calibration, linearization and manipulation of the experimental data. Also we present the THz absorption spectra of some di-peptide and their reverses.

EXPERIMENT SECTION

Fig. 1 shows the experiment setup. It is the THz-Fourier Transform Infrared spectrometer (THz-FTIR) which is reconstructed by JASCO Co., Led. **Fig. 2** shows the schematic of the light path of the THz-FTIR. It is composed by light source, interferometer, and sample compartment and detector unit. The THz wave source is a high pressure mercury lamp which generates a continuous wave. The THz beam is collimated by an off-axis parabolic mirror, and then split into two parts by the beam splitter. The two parts of the beam will recombine at the beam splitter where interference between the beams takes place. Interfacial waves were focused on the sample by another off-axis parabolic mirror. The transmitted intensity of the THz waves is detected by a pyroelectric detector (DLATGS: Deuterated L-Alanine Triglycine Sulphate) or a liquid cooled indium antimonide hot electron bolometer.

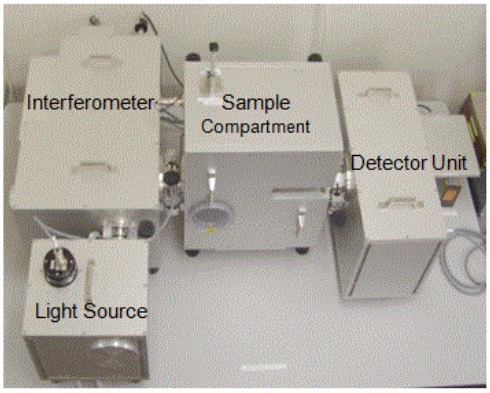


Fig. 1 Picture of the THz-FTIR

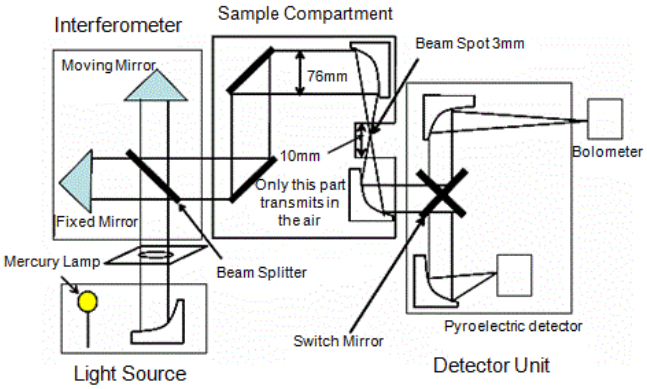


Fig. 2 Schematic of the light path of the THz-FTIR

Solid lyophilized short-chain peptide were purchased from Sigma-Aldrich Co. and used without further purification. Powders requiring low temperature storage were maintained at 227K until use to prevent decomposition or exposure to atmosphere. Samples for THz absorption measurements were prepared by first weighing 10 mg of solid and homogenizing the material in a mortar and pestle to reduce the solid particle size distribution. This procedure ensures particle sizes sufficiently smaller than THz wavelengths to reduce baseline offset at higher frequencies arising from non-resonant light scattering. Each sample was rapidly prepared by mixing with the dry spectra photometric grade high density polyethylene powder (Sharmrock Co., Inc. with < 150 μm particle size) by shaking in a container at concentrations of approximately 1%. A vacuum die (Shimadzu Co.; Vacuum pump of exhausting speed 10 ~30 *l* / min, Shimadzu Gaede Rotary Vacuum Pump Model SA18-3M) was used to press mixture sample at the lowest possible pressure (1500kg/cm^2) to minimize decomposition from transient heating. And for this conditions used to pulverize the crystalline samples and press pellets, we have found no evidence frequency shift for pressure dependence. Typical, pellet disks were 1.8mm thick and 13mm in diameter. Pure polyethylene disks were similarly pressed for background scan. The absorption spectra in the 20cm^{-1}-650cm^{-1} at 2cm^{-1} resolution were obtained with a Terahertz Fourier Transform Infrared Spectrometer (THz-FTIR) using a 3mm thick silicon beam-splitter and room temperature DTGS detector fitted with a high density polyethylene window.

The Infrared (IR) spectra of the pure crystallized samples were taken from 400cm^{-1}-4000cm^{-1} at room temperature with instrument resolution set at 2cm^{-1} by Fourier Transform Infrared-Attenuated Total Internal Reflector (FTIR-ATR) Spectrometer (α-FTIR-ATR: Bruker Optics Inc). The instrument is equipped with standard mid-IR beam splitter (T303) measured with standard mid-IR source (low voltage / air cooled) and DTGS (KBr/Dla TGSD301) detector which S/N> 4×10^8 cm Hz$^{1/2}$ W^{-1}. And no effort was made to completely remove the residual water and CO_2 lines near 2350cm^{-1}.

An X-ray diffraction (XDR) pattern is obtained by an X-ray diffractometer.

RESULTS AND DISCUSSION

THz absorption spectra of the $(Gly)_n$ n=1-6

Some papers have appeared concerning the influence of the peptide length on their stability [16] and activity [17, 18]. Peptide length is an important factor for study on relationship between the structure and the function. The peptide length dependence of the THz spectra was investigated. The IR spectra of peptides that is the homologous series of glycine, $(Gly)_n$ n=1-6, being composed by the simplest amino acid, were obtained at room temperature and shown in the **Fig.3**. While overall IR spectra are quite similar, especially $(Gly)_3$-$(Gly)_6$, and it's difficult to distinguish these samples from their IR spectra features. The same features are seen to exist in all spectra, although

the relative intensities over broader spectral regions near 800 cm^{-1}- 3600 cm^{-1} are some different. The THz spectra for the $(Gly)_n$ n=1-6 are presented in **Fig.4**. Compared with the IR spectra, each species yields characteristic and highly structured spectra in this region. Distinctly new absorption features become visible as the chain length increases. Each spectrum has a uniquely identified characteristic and equally complex spectral structure. Weak absorption features below 100 cm^{-1} are observed only for $(Gly)_2$ and disappear for the peptide $(Gly)_3$-$(Gly)_6$. Higher frequency absorptions seem to shift to lower frequency with the chain-length increases and the density of the features increases, because the addition of another amino acid to a peptide backbone chain brings additional absorption features arising from increased mode and spectral density. From **Fig.4**, it was also obtained that there was an underlying very broad absorption between 120 cm^{-1} to 320 cm^{-1} which may be caused by intermolecular hydrogen bonding between neighboring constituents, crystallite surface absorbed water, or water incorporated within the samples which is difficult to remove from these biomaterials.

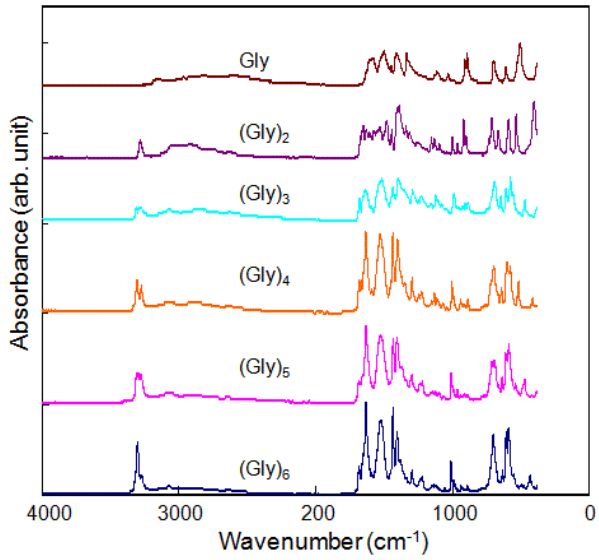


Fig. 3 IR absorption spectra of the $(Gly)_n$ n=1-6 (the absorption spectra of $(Gly)_n$ n=1-5 are offset along the vertical axis)

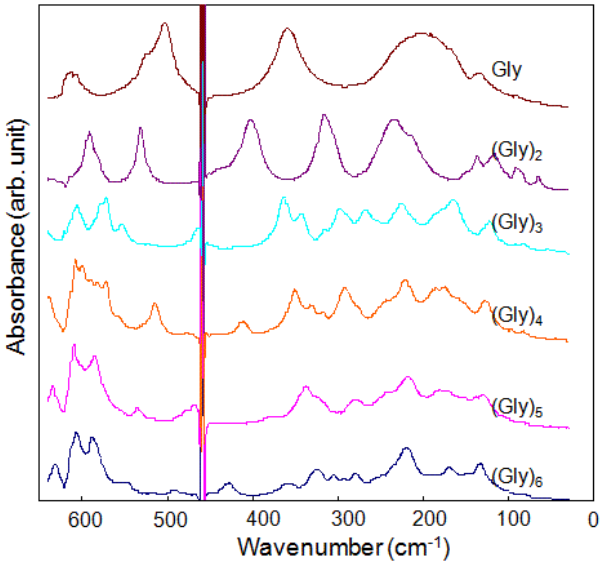


Fig. 4 THz absorption spectra of the $(Gly)_n$ n=1-6 (the absorption spectra of $(Gly)_n$ n=1-5 are offset along the vertical axis)

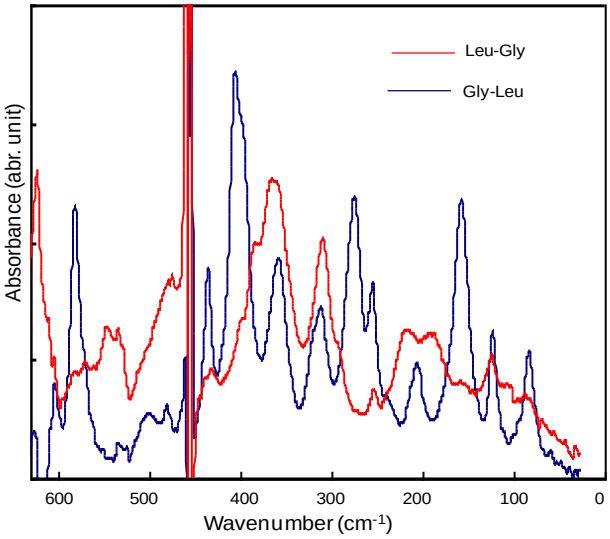


Fig. 5 THz absorption spectra of the Leu-Gly and its reverse Gly-Leu

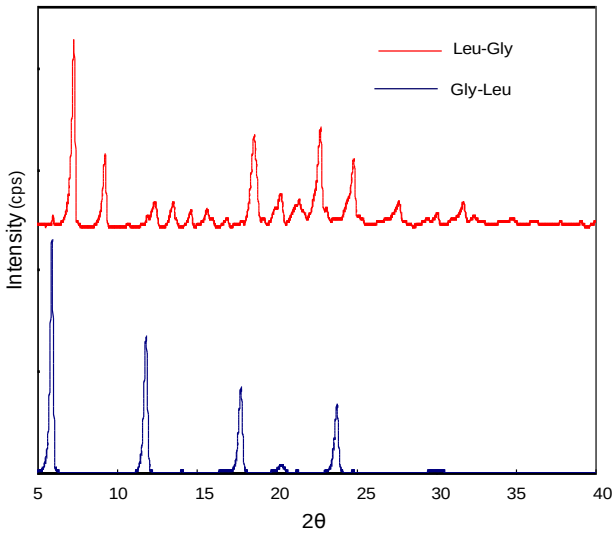
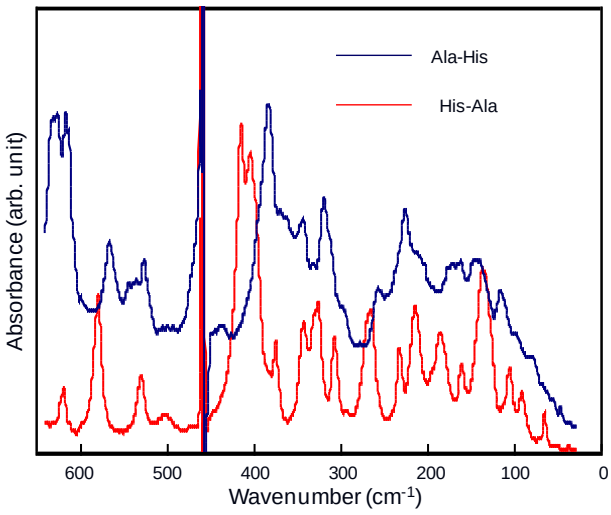
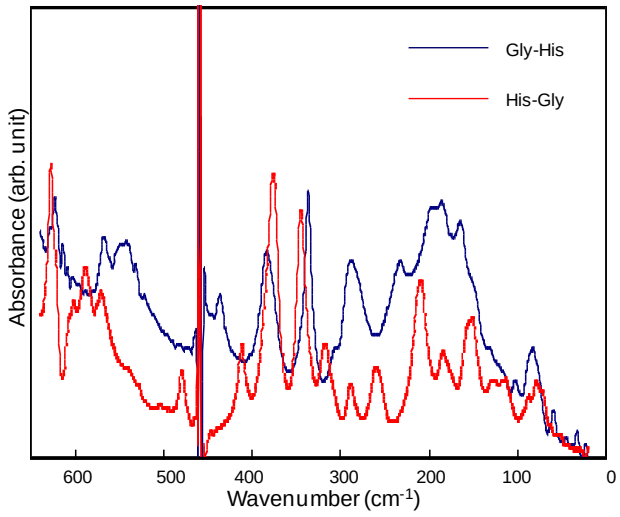


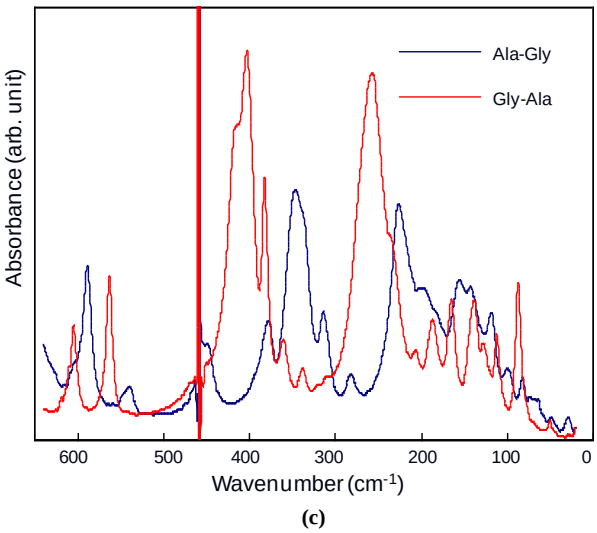
Fig. 6 X-ray diffraction patterns of the Leu-Gly and its reverse Gly-Leu (the Leu-Gly is offset a long the vertical axis)



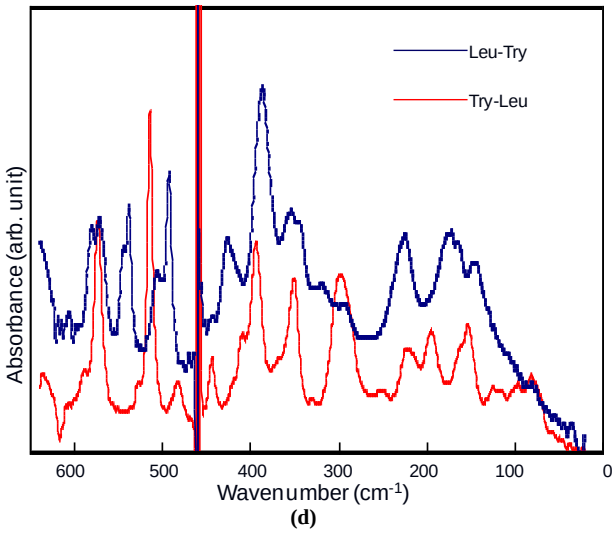
(a)



(b)



(c)



(d)

Fig.7 THz absorption spectra of di-peptides and their reverses
(a)His-Gly. His-Gly (b)Ala-Gly and Gly-Al (c) Leu-Tyr and Tyr-Leu (d) Ala-His and His-Ala

THz absorption spectra of some di-peptides and their reverses

Because the peptides have C-terminations and N-terminations, they are sequence specific. And the difference in the sequence will cause the changes in the biological activity and stability. Investigations show the effect of the molecular structure (N- and C-termini) of di-peptides on their half-life and hydrolysis in plasma, their recovery from tissues and urine, and their enrichment of amino acid pools in plasma and tissues [19]. For example the Leu-Gly and the Gly-Leu, the plasma half-life and hydrolysis of the Leu-Gly is lower than Gly-Leu; the speed of the transport of the Leu-Gly in the small intestine is higher than the Gly-Leu. So, we chose some kinds of these peptides and investigated the sequence dependence of the peptide in THz spectra. **Fig.5** shows the results of the THz absorption spectra of the Leu-Gly / Gly-Leu. The obviously spectral difference of the di-peptide Leu-Gly and its reverse sequence Gly-Leu can be observed. These indicate that the THz spectra are potentially sensitive to the sequence dependence (dependent on N-terminus versus C-terminus ordering). These also reflect the differences in the force fields associated with the vastly different network of hydrogen bonds that characterize the structures. In **Fig.6** the X-ray diffraction patterns of these two peptides are presented. From the difference of the patterns we can see the crystal structure of the two peptides is completely different. The different crystal structure causes the different absorption features in the spectra. It is certified that the THz spectra are very powerful means for the peptide structure analysis and very sensitivity to the crystal structure. We also analyzed some other di-peptides and their reverse sequence peptides, such as **Fig.7 (a)** Gly-His / His-Gly, **(b)** Ala-Gly / Gly-Ala, **(c)** Leu-Tyr / Tyr-Leu, **(d)** Ala-His / His-Ala, and the same results could be obtained.

CONCLUSIONS

In this paper, the THz spectra were successfully used in peptide analysis. Compared with IR, XRD, the THz absorption spectra showed intermolecular interaction and give distinct information on peptide structure. Because fine changes in peptide structure were reflected in THz spectra, these techniques showed obvious advantages for structural analysis. THz spectra were able to detect differences in peptide length, sequence, C-terminal composition. Increasing peptide length increased spectral density and the complexity of the intermolecular hydrogen bond. Sequence changes alter the network of hydrogen bonds. Difference in the C-terminal composition altered interactions between molecules. Finally, unique vibration signatures could be used to identify these structures. Therefore, THz absorption spectra were good means of analyzing peptide structure.

Acknowledgements

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