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Chiral Analysis of Re-crystallized Mixtures of D-, L-amino Acid Using Terahertz Spectroscopy

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Abstract: Distinguishability by terahertz absorption between D- and L-amino acids and a change in terahertz absorption based on the re-crystallization condition was examined. Terahertz spectra which were obtained from the re-crystallized each enantiomer or mixtures of D- and L-leucine or alanine were compared with those of the purchased reagents. The peak tops of the re-crystallized L- and D-leucine mixtures were shifted toward low frequency side and the half width of the peak at 3.7 THz became narrow to approximately half of that of the reagent. Difference of spectral features from 2.1 THz to 2.8 THz in the THz spectra between D- and L-alanines was observed. According to the result of peak separation against these spectra, distinction between the purchased D- and L-alanine was possible to compare the intensities of the sub-peaks. Moreover, changes of the integrated values of the peaks obtained from L- or D-form-rich mixture of leucine were observed. These results suggested that feasibility of chiral analysis of enantiomers using terahertz spectroscopy would be shown.

Keywords: Terahertz spectroscopy, polymorphs, enantiomers, crystals

Introduction

Terahertz (THz) electro-magnetic region (0.1 THz to 10 THz, 3.3 cm^{-1} to 333 cm^{-1}) detects a weak inter-molecular energy such as a hydrogen bond, and a crystal lattice vibration. In the pharmaceutical and the chemical industries, detection of polymorphs (1-8) and unique THz spectra obtained from active pharmaceutical ingredients (APIs), illegal drugs and explosives have been reported (1, 3, 6). The hydrogen bond and van der Waals force, which contribute to form the function-able structure of protein and amino acids, would be sensitively detected in THz region. Thus, absorption on THz electro-magnetic region would be expected to provide useful information for investigation of the function and the dynamics of these compounds. Amino acids which are components of protein are used as not only a supplement for keeping health but also a medication for certain diseases. Amino acids have hydrogen bond network in their crystals. Thus, an investigation concerning property of hydrogen bonds and intermolecular interactions would be useful to understand the functions of these compounds. Moreover, amino acids exist as L-form in nature, and their biological function is different by stereo- configuration. In study about an optical isomerism by THz spectroscopy, distinction between racemate and enantiomer by THz peaks has been reported (9, 10). However, distinguishability between both enantiomers has not been established yet. The authors examined the THz spectral change of the re-crystallized amino acids, leucine and alanine using THz electro-magnetic wave.

Materials and Methods

L-, D- and DL-leucine (racemate), and L-, D-, and DL-alanine (racemate) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Polyethylene powder for making pellets was purchased from THz Laboratory Co., Ltd. (Akita, Japan).

Re-crystallization of these compounds was performed with water. Sample powder was dissolved in water at around 90 ºC and cooled down to room temperature. Then, this solution was put into a refrigerator at 4 ºC. Re-crystallized sample was filtered and washed with iced water, and dried in a desiccator under reduced pressure. The mixture of L- and D-amino acids which contains 25 % or 75 % of L-enantiomer was re-crystallized to make D- or L-form-rich mixed sample. The re-crystallized sample was diluted to concentration of 0.25 mol/l with polyethylene powder and then the mixture was compressed at 9.8 kN for 2 min to make pellets for transmittance measurements.

Measurements of THz spectra were performed by the Gallium-Phosphorus (GaP) THz signal generator system equipped with a pyroelectric DTGS detector. This THz generator system has been developed and constructed by Nishizawa et al (11-19). The optical system of this instrument is shown in Fig. 1. The spectra were measured from 1 THz (33 cm^{-1}) to 5 THz (167 cm^{-1}) at 15 GHz measurement steps. Measurements of optical rotations of the re-crystallized D-, L-amino acids and their mixtures were performed using DIP-360 polarimeter (JASCO, Tokyo, Japan).

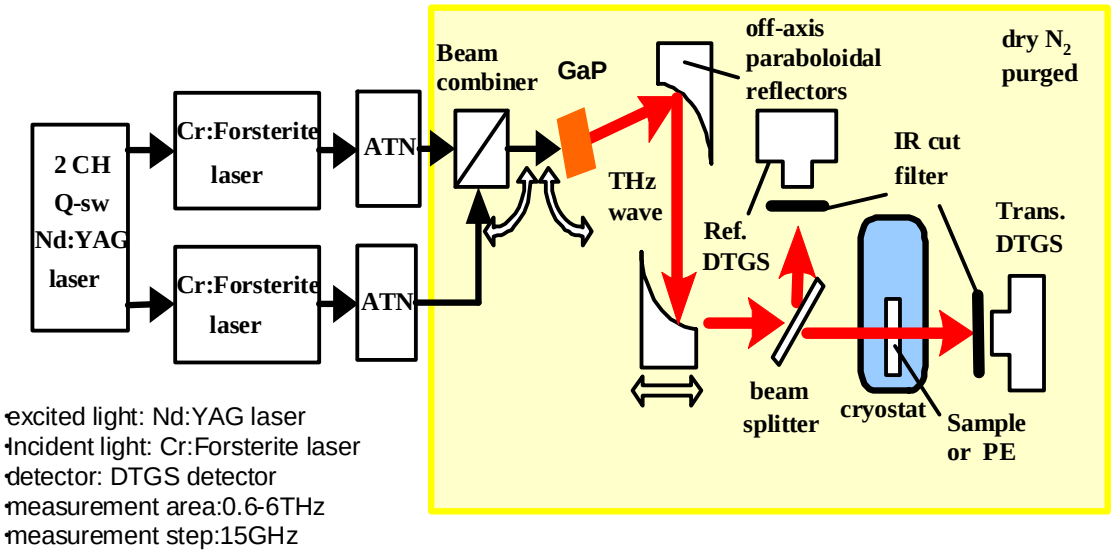


Figure 1: Schematic diagram of GaP-THz spectrometer via differential frequency generation

Results and Discussion

The baseline corrected THz spectra obtained from the purchased D-, L-, and leucine racemate are shown in Fig. 2(A). The peak at 3.7 THz was observed in both enantiomers. However, this single peak was shifted to 3.6 THz in racemate and the half width of the peak height of the enantiomers (0.7 THz) was narrowed to 0.4 THz. The baseline corrected THz spectra obtained from the re-crystallized mixtures are shown in Fig. 2(B). The intensity (a relative absorption coefficient) of D-leucine was approximately 20 % higher than that of DL-leucine. On the other hand, L-leucine showed 20 % lower intensity than that of racemate. No significant difference was observed about the waveforms, the peak position, and the half width of peak height at 3.7 THz between D- and L-leucine. In case of the mixtures, the peak positions were shifted to lower side of frequency, and the half width of peak height at 3.7 THz also became narrow as well as the purchased leucines. In order to investigate the THz peaks, the peak separation by Gaussian function was carried out. The single peak at 3.7 THz was separated to one main peak and 2 sub-peaks. Figure 3 shows the correlations between the spiked amount of L-form and the peak positions or the integrated values of peaks. Although the peak positions of the main peak were same in all mixtures, the peak positions of the sub-peaks of L- or D-form-rich mixture came up to that of the racemate. These results suggest that the existence of the other enantiomer would affect the phonon of crystallized leucine molecules. On the other hand, the correlation between the integrated values and the spiked amount of L-leucine shows the integrated values of the main peak were smallest on the D- or L-form-rich mixture resulting in a W-like pattern. The highest integrated value of the low sub-peak was observed in the D- or L-form-rich mixture, and shows an inverted W-like pattern. Moreover, the integrated value of L-leucine was comparatively higher than that of D-leucine. The peak integrated value of D-form-rich mixture of the high sub-peak was small, but it became bigger as the D-form content was increased. Furthermore, the integrated value in L-enantiomer remarkably increased and the value doubled compared with that of D-enantiomer. These results suggest that those values of D- or L-leucine were influenced by the existence of the other enantiomer, and the value of the high sub-peak at 4.1THz had correlated with the spiked content of D- or L-leucine in the re-crystallized mixture. The baseline corrected THz spectra obtained from the purchased D-, L-, and DL-alanine (Racemate) are shown in Fig. 4(A). Each optical isomer shows 2 peaks at 3.2 THz and 3.4 THz, racemate has the single peak at 3.1 THz. After re-crystallization, the two peaks on the THz waveform on D- or L-enantiomer were shifted to 4.2 THz and 4.4 THz. Moreover, the single peak of racemate was shifted to 4.1 THz on the THz wave obtained from L-form-rich or D-form-rich mixture (Fig. 4(B)). These results suggest that crystalline forms were changed from those of the purchased alanines because the distributor of these reagents would have done a different purification process from the re-crystallization method which was used in this study. In the THz waveform of the re-crystallized racemate, an increase of the relative molar absorbance was observed as the spiked content of D-enantiomer increased. In the THz waveform obtained from the purchased D- and L-alanine, small differences were observed at the frequency range from 2.1 THz to 2.8 THz.

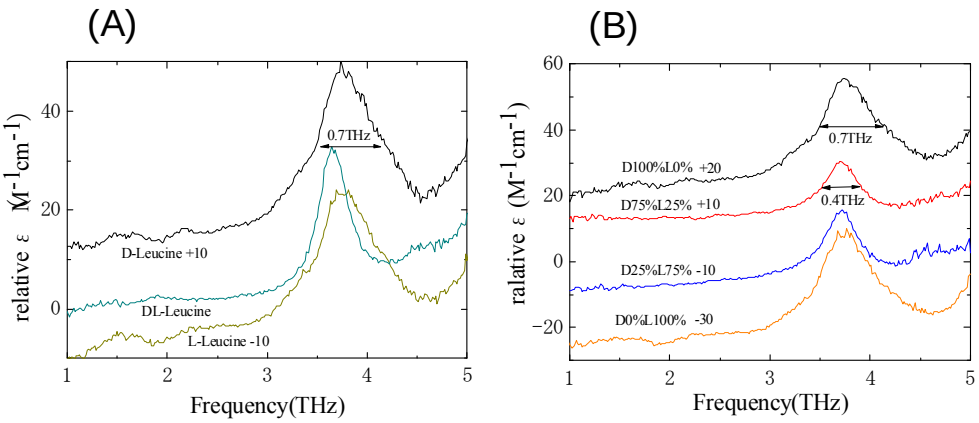


Figure 2: THz spectra of leucines (A: D-, L-, and DL-leucine (racemate) as purchased B: four kinds of re-crystallized D- and L-mixture)

In order to characterize the small differences, the curve separation was employed. Figure 5 shows the calculated D- and L-alanine waveforms by the Gaussian function. The three main peaks at 2.25 THz (No.1), 2.45 THz (No.2) and 2.6 THz (No.3) and the sub-peak at 2.7 THz (C) were calculated from the waveform of D-enantiomer. In case of L-enantiomer, the intensity of the peak No.2 became smaller compared with that of D-enantiomer. Moreover the two sub-peaks at 2.2 THz and 2.35 THz appeared and then the sub-peak C which was observed in D-enantiomer disappeared. While no significant differences between both enantiomers about the peak positions and the integrated values of peaks were observed, the peak intensities at the peak No.2 and No.3 of L-enantiomer were smaller than D-enantiomer. Although further study is necessary to explain the detail of these observations, the purchased D- and L-alanine may be distinguishable using THz spectroscopy.

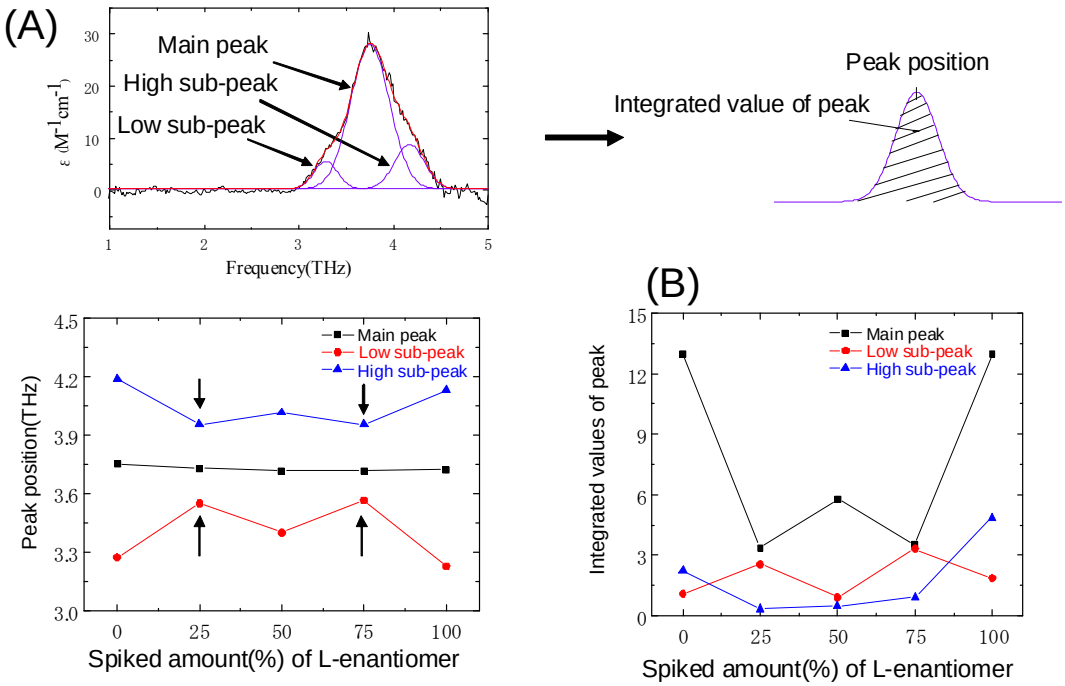


Figure 3: Correlations between re-crystallized mixtures and THz peaks (A: Peak positions (THz), B: Integrated values of peaks)

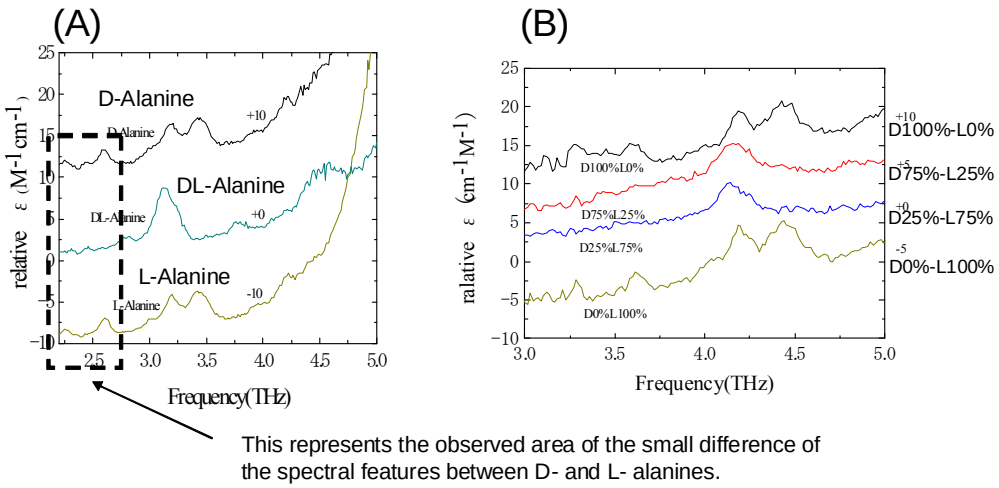
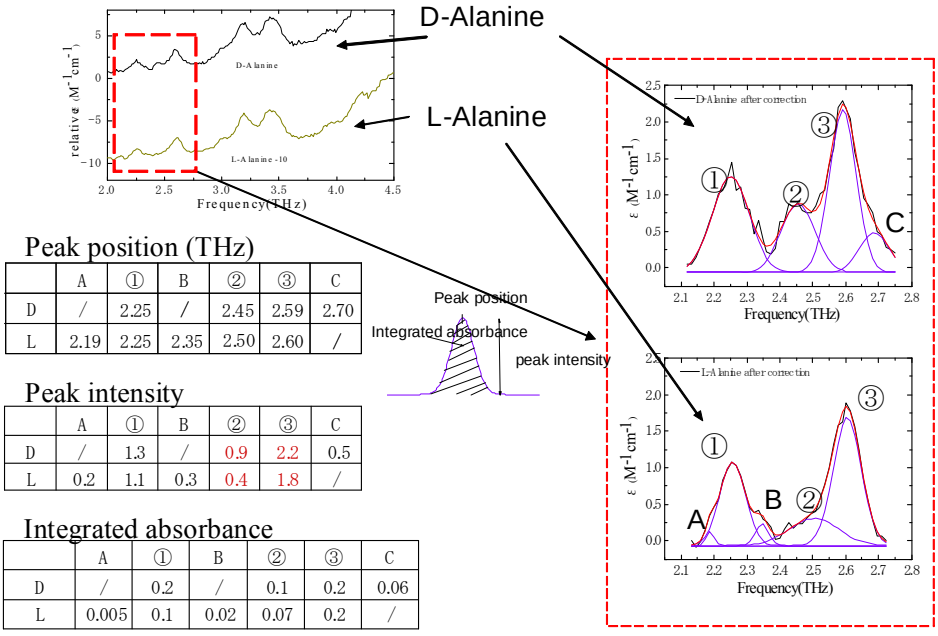


Figure 4: THz spectra of alanines (A: D-, L-, and DL-alanine (racemate) as purchased B: four kinds of re-crystallized D- and L-mixture)



Peak position (THz)

	A	①	B	②	③	C
D	/	2.25	/	2.45	2.59	2.70
L	2.19	2.25	2.35	2.50	2.60	/

Peak intensity

	A	①	B	②	③	C
D	/	1.3	/	0.9	2.2	0.5
L	0.2	1.1	0.3	0.4	1.8	/

Integrated absorbance

	A	①	B	②	③	C
D	/	0.2	/	0.1	0.2	0.06
L	0.005	0.1	0.02	0.07	0.2	/

Figure 5: Fitted THz peaks of alanine and calculation results of three evaluation parameters

While comparative large two peaks (from 4.1 THz to 4.6 THz) were observed in D- or L-leucine, only single peak was detected in racemate and the re-crystallized mixtures. The authors are thinking that a hydrogen bond network of racemate which has a crystal structure formed by distributing of D- and L-enantiomer alternately is different from a hydrogen bond network in a crystal structure of each enantiomer. For example, a hydrogen network in three-dimensional structure of D- or L-enantiomer can be illustrated as Fig. 6 based on the crystalline structure of L-enantiomer (20).

On the other hand, a hydrogen bond network of racemate is formed by distributing both enantiomers alternately. Then a single molecule in crystal formed from D- or L-form molecule may construct a hydrogen bond network ([-O --- H-N-]) between adjacent two molecules. In case of racemate, D- or L-form molecule may construct two hydrogen networks between the other L- and D-form molecules (Fig. 7). The authors predict that inter-molecular vibration modes detected in THz spectra are correlated with an infra-red activity mode. Symmetry of crystal structure between enantiomer and racemate is different. Then, an infra-red active inter-molecular mode in enantiomer might change into an inactive mode in racemate. And

the opposite changing may be also observed. Of course further investigation is necessary to test this hypothesis; a difference of configuration to take high-density and/or stable crystal structure may affect THz spectral features.

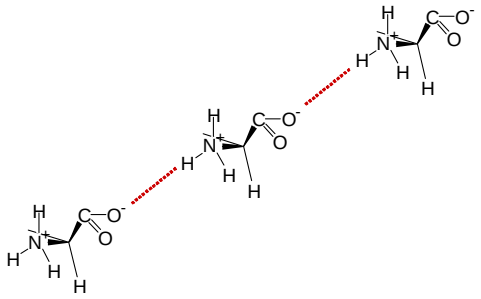


Figure 6: The image of a hydrogen bond between L-leucine and an adjacent one (Only one hydrogen bond is shown here.)

The different spectral feature between D- and L-alanine was observed in the range from 2.1 THz to 2.8 THz. In the calculated three peaks, the intensity of the peak No.2 was significantly decreased. Moreover, different calculated sub-peaks pattern between D- and L-enantiomer was observed as described in the paragraph 3.2. D- and L-enantiomer has the identical physical properties except an optical rotation. Although there is a possibility that an impurity derived from a different purification process between D- and L-leucine may cause spectral differences, it also has possibility that THz spectroscopy can sensitively detect small differences concerning a hydrogen bond network or a crystal lattice vibration.

A difference of spectral features between D- and L-leucine was not observed, but the differences of the integrated values of the peaks (shown in Fig. 5) obtained from the two sub-peaks between racemate and L- or D-form-rich mixture were observed. The optical rotation of re-crystallized mixture, L0%-D100%, L25%-D75%, L50%-D50%, L75%-D25% and L100%-D0% were -15.74 ± 0.02°, -0.06 ± 0.01°, 0.10 ± 0.02°, 0.12 ± 0.01° and 15.58 ± 0.01°, respectively. An isomerization in the aqueous solution and a thermo-isomerization of enantiomer was not observed. According to these results, According to these results, these re-crystallized mixtures did not include whole spiked amounts of L- or D-enantiomer. Most of these mixtures were formed by racemate, and a portion of excess L- or D-enantiomer was distributed in these crystals. Furthermore, no significant difference of the optical rotation between L50% and L75% mixtures was calculated. Thus, it is presumed that a racemization of D- and L-leucines in the solution was first progressed and then a portion of excess D- or L-enantiomer was taken in the racemate crystals. Although investigation of crystal structure of these mixtures is necessary to explain this observation, each D- or L-form-rich mixture showed the characteristic THz spectral features.

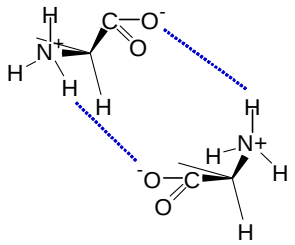


Figure 7: The image of assumed hydrogen bonds between two adjacent molecules in racemate

Conclusion

In this study, distinguishability of chiral compounds by THz spectroscopy was examined. Characteristic spectral differences between enantiomers and mixtures were observed by detailed analysis of spectral features. Because THz electro-magnetic wave can detect small but meaningful differences of crystal structure, more applications of THz electro-magnetic wave to analyze and to evaluate crystalline state and physical properties will be expected in the future.

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