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20 种 α-氨基酸的太赫兹光谱及其分子结构的相关性

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摘要: 应用太赫兹时域光谱(THz-TDS)技术, 在室温下对构成蛋白质的 20 种基本氨基酸的多晶粉末压片样品进行了光谱测试分析. 结果表明, 所有氨基酸对 THz 波反应非常灵敏, 在 0.2–3.0 THz 的有效频谱范围内, 表现出各自特征吸收峰, 故而利用 THz 光谱可以有效地区别不同种类的氨基酸. 我们以新数据验证和补充了前人的研究结果, 建立了以氨基酸分子结构及其 THz 光谱特征为基础的分类方案, 讨论并揭示了氨基酸分子的结构差异与其 THz 吸收光谱之间的相关性. 认知这些相关性将有助于鉴定氨基酸分子, 促进 THz 光谱学的理论研究以及在生物医学领域的推广应用.

关键词: 吸收光谱; 氨基酸; 太赫兹; 远红外
中图分类号: O641; O657.3

Correlations between Terahertz Spectra and Molecular Structures of 20 Standard α-Amino Acids

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Abstract: All 20 standard polycrystalline α-amino acids were examined using terahertz time-domain spectroscopy (THz-TDS) at room temperature. They are strikingly sensitive to the THz pulse and yield a complete set of THz fingerprint spectra between 0.2 and 3.0 THz. These spectra were compared to those from previous reports in terms of spectral shape and frequencies of absorption peaks. We validated the characteristic absorption peaks and provided supplementary data. For the first time, correlations between THz spectral peaks and the molecular structures of amino acids are revealed and a classification of amino acids based on both molecular structures and THz spectra was established. These correlations can help identify amino acids, trace some functional groups, and examine if the THz spectra are dominated by internal or intermolecular vibrations. These correlations can thus promote the application of THz spectroscopy in the study of biological and medicinal materials in the biomedical fields.

Key Words: Absorption spectrum; Amino acid; Terahertz; Far infrared

Human protein molecules perform very important physiological functions and they are made of total 20 types of basic structural and functional units that are called proteingenic, standard, or α-amino acids. Various spectroscopies have been used to identify and study all 20 standard α-amino acids, in biochemical and medicinal research. THz spectroscopy is a new technology, yielding highly sensitive spectra, with a high signal-to-noise ratio (SNR). Since THz pulse can penetrate through bio-specimens safely, THz spectroscopy has recently been used to examine physical and chemical properties of bio-specimens, such as pep-

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tides[1], DNA, RNA, and their fragments[2,3], amino acids (see below), proteins[4,5], and other biomolecules[6–8]. Although THz spectroscopy has been used to study amino acids only since 2003, all standard amino acids have been gradually studied with this new technology[9–27]. However, each report covered THz-spectra of only one to several standard amino acids[11] , except for one study that presented brief graphs of THz-spectra of all 20 standard amino acids. Many previous results are more or less inconsistent, possibly due to different protocols, sample preparations, system calibrations, and different THz ranges among different systems. Obviously, THz spectra of all standard amino acids should be validated with their characteristic peaks through a comprehensive study.

Large biomolecules with different molecular structures exhibit different collective rotational and vibrational transitions. These transitions carried by a passing-through THz pulse will be detected and converted into absorption spectra that consist of absorption coefficients[4,28]. Since THz-TDS can exhibit effects of the terahertz radiation on material in both amplitude and phase, it provides more information than conventional Fourier transform infrared spectroscopy (FTIR) that displays with amplitude only. On the other hand, while most major IR spectral peaks of molecules have been well assigned to specific functional groups, correlations between THz spectral peaks and specific functional groups are still unclear, because THz spectra are currently interpreted as collective modes of rotational and vibrational transitions of the whole molecules. For polycrystalline, intermolecular modes and lattice vibrations are also considered to contribute to far-infrared (FIR) or THz absorption spectra[3,29], and this makes it even harder to examine if these collective modes could also exhibit correlations between THz spectral peaks and specific functional groups. However, as a general practice in scientific research, when both control and experimental samples are tested under the same conditions, the different results would be hypothetically correlated to the different parts of the experimental samples. In serial molecules, every two neighboring molecules differ from each other with just one atom or one small functional group so that they can be used as control and experimental samples to help us to find out the correlations between different THz spectra and molecular structures when they are tested under the same conditions. All 20 standard amino acids have the same basic structure, but differ from each other with only one functional group (even just one atom) on the side chain, making them a suitable series for us to conduct a comparative study to examine if there could be some correlations between their molecular structures and their THz spectra.

We have analyzed all 20 standard α-amino acids with the same THz-TDS under the same conditions, verified their characteristic spectral peaks based on comparisons with previous data, revealed some correlations between molecular structures and spectral peaks for the first time, and established a classification of α-amino acids based on both molecular structures and THz spectra between 0.2 and 3.0 THz.

1 Experimental

All samples of amino acids were purchased from Beijing Dingguo Biotechnology Co. Ltd. and Beijing Qilin-Hongwei Company. The purities of all samples were >98%. Samples were mixed with pure polyethylene (PE) powder in different mass ratios, and then pressed with a 2000 kg force into round disks, about 13 mm in diameter and 1 mm in thickness, with smooth surfaces parallel to each other. Since samples with different mass ratios yield absorption spectra in different ranges, in this experiment, samples were prepared with various mass ratios between amino acids and PE (such as 2∶1, 1∶0, 1∶1, 1∶2, and so on) and tested to screen for the absorption spectrum with the widest spectral range. Each sample disk was scanned at least three times to select the best result.

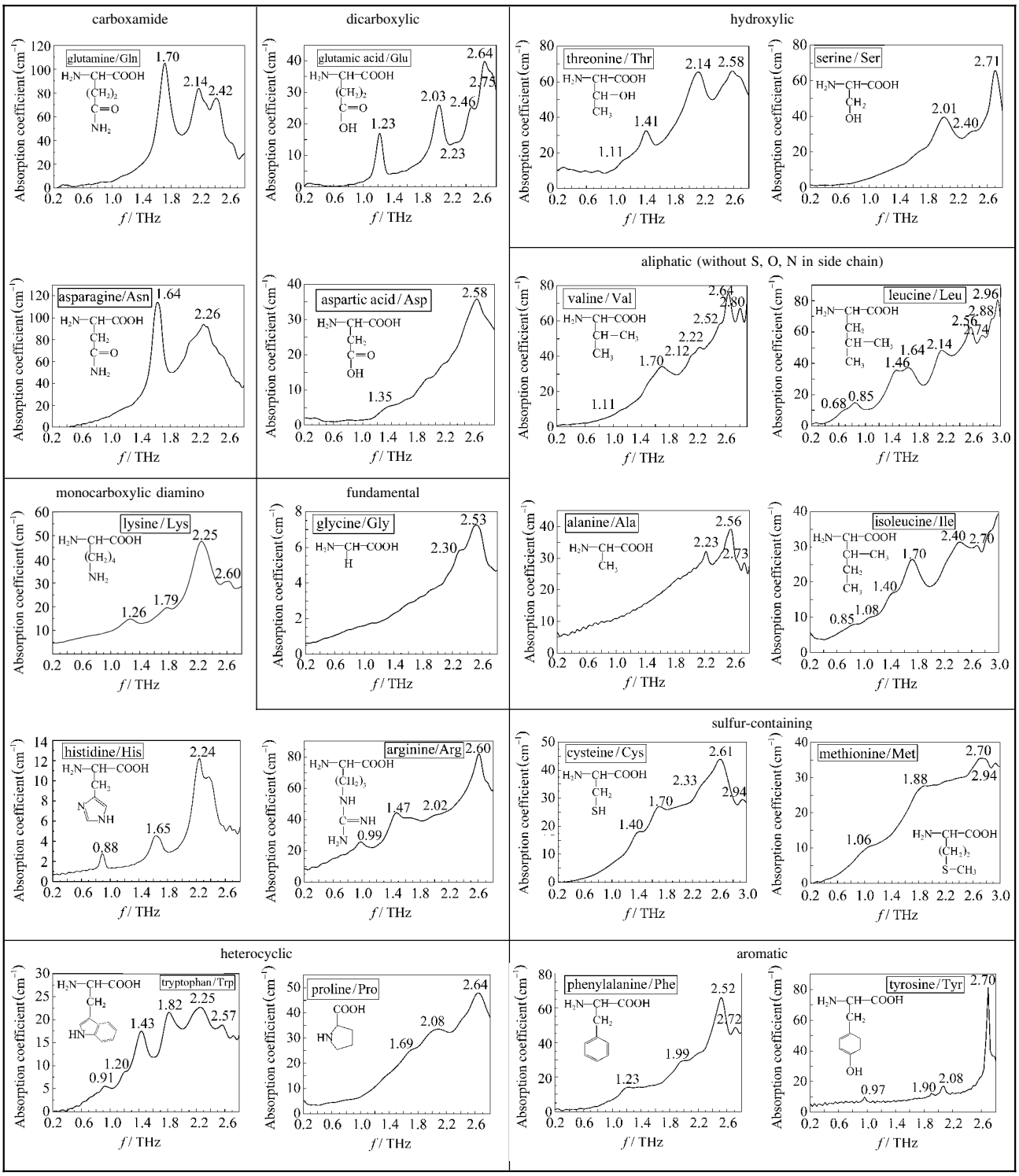
The THz-TDS system was used at room temperature (about 20 ℃), and it consists of a sample box (that is purged with dry nitrogen to keep the humidity less than 2%, to minimize the vapor effects and thus enhance the SNR and a mode-locked Mai-Tai Laser to generate laser pulse, with repetition rate 82 MHz, center wavelength 810 nm, pulse width 100 fs, and pulse power 980 mW[9]. Within the sample box, an InAs wafer with <100> orientation is used to emit THz beam and a ZnTe wafer with <110> orientation to receive signals. Sample signals were yielded when a THz beam passed through a sample between the two wafers, and reference signals were received when the THz beam passed through the box without sample. Both reference and sample signals were compared and calculated automatically to plot spectra with reflection index and absorption coefficient[9,10].

2 Results and discussion

THz absorption spectra of all 20 standard α-amino acids are compiled in Table 1. The names of amino acids, name abbreviations, and molecular structures are all noted in the spectra. Absorption peaks are marked within valid frequency ranges between 0.2 and 3.0 THz. For the convenience of numerical comparisons, our results and those from previous reports within the similar frequency ranges are presented in Table 2. Our results are generally comparable with most previous reports, but our spectra consistently exhibit more graphic and numeric details than all the previous results.

Among all standard amino acids, Gly is the most fundamental because it simply consists of only three parts, a backbone carbon between an amino group (NH_2) and a carboxyl group (COOH). These three parts also serve as a basic structure in all other standard amino acids, but the backbone carbon is attached with a functional R-group as a side chain. Based on their side chains they are classified into eight groups (Table 1) in this paper, with six groups similar to that in Devlin′s classification[30]. Table 1 is designed to show graphical patterns of both molecular structures and THz spectra of all amino acids: molecules with main absorption peaks ≤2.30 THz are placed in the left column, ≥2.70 THz in the right column, and between 2.52 and 2.64 THz in the central two columns. Graphical differences and similarities

Table 1 THz Spectra of 20 α-amino acids



among neighboring molecular spectra make it easier to reveal some associations between THz spectra and molecular structures through comparing different absorption peaks and the types, positions, and chain lengths of functional groups of the amino acids.

Fundamental amino acid Gly has a main peak at 2.53 THz that appears to be correlated with the carboxyl group (COOH) because in dicarboxylic amino acids with two carboxyl groups the main peak between 2.52 and 2.64 THz appears stronger. Gly also has a very weak peak (or a shoulder) at 2.30 THz that seems to be mainly affected by the amino group (NH_2) because in carboxamide and monocarboxylic diamino acids with two amino groups the peak between 2.24 and 2.30 THz is much stronger (see below).

Table 2 Comparison of THz spectra of 20 α-amino acids with previous data

Amino acid	Our spectral peaks	Previous data	Some of our peaks matched graphically with spectra of Ref.[11]
		fundamental	
glycine	2.30, 2.53	2.4, 2.7[16]	2.53
		aliphatic	
alanine	2.23, 2.56, 2.73	2.23, 2.57[17]	2.23, 2.56
valine	1.11, 1.70, 2.12, 2.22, 2.52, 2.64, 2.80		1.70, 2.22, 2.80
isoleucine	0.85, 1.08, 1.40, 1.70 2.40, 2.70		0.85, 1.08, 1.40, 1.70, 2.40
leucine	0.68, 0.85, 1.46, 1.64, 2.14, 2.56, 2.74, 2.88, 2.96		0.68, 0.85, 1.46, 1.64, 2.14
		hydroxylic	
serine	2.01, 2.40, 2.71	2.01, 2.22, 2.55, 2.70, 2.94[14]*(FTIR data)	2.01, 2.71
threonine	1.11, 1.41, 2.14, 2.58		1.41, 2.14, 2.58
		sulfur-containing	
cysteine	1.40, 1.70, 2.33, 2.61, 2.94	1.38, 1.68, 2.13, 2.40, 2.91[14]*(FTIR data)	1.40, 1.70
methionine	1.06, 1.88, 2.70, 2.94		1.88
		dicarboxylic (acidic)	
aspartic acid	1.35, 2.58	2.6 (wide)[18]	2.58
glutamic acid	1.23, 2.03, 2.23, 2.46, 2.64, 2.75	1.2[24]; 1.21, 2.05[25];1.24[26]; 1.74, 2.24, 2.46[10]	1.23, 2.03, 2.23, 2.46, 2.64, 2.75
		carboxamide	
asparagine	1.64, 2.26	1.642–1.758, 2.266[12]	not matched
glutamine	1.70, 2.14, 2.42		1.70, 2.14–2.42
		diamino (basic)	
lysine	1.26, 1.79, 2.25, 2.64		1.79, 2.25, 2.64
arginine	0.99, 1.47, 2.02, 2.60		0.99, 1.47, 2.60
histidine	0.88, 1.65, 2.24, 2.38		2.24
		heterocyclic	
proline	1.69, 2.08, 2.64		1.69, 2.08
tryptophan	0.91, 1.20, 1.43, 1.82, 2.26, 2.57	1.20, 1.435, 1.842[21]; 1.43, 1.8[23]	0.91, 1.20, 1.43, 1.82, 2.26
		aromatic	
phenylalanine	1.23, 1.99, 2.52, 2.72	1.23, 1.99[20]; 2.72[18]	1.23, 1.99, 2.72
tyrosine	0.97, 1.90, 2.08, 2.70	0.96, 1.91, 2.08, 2.70[18]; 0.96, 1.92[23]	0.97, 1.90, 2.08, 2.70

*converted from wavenumbers of FTIR data

Aliphatic group has four amino acids that lack of S, O, and N, but have CH_2 and CH_3 only, on the side chains. The Ala molecule can be considered as a Gly molecule joined with a methyl group (CH_3). Associated with this addition, the main peak is shifted to 2.56 THz and the minor peak is strengthened and shifted to 2.23 THz, but more importantly a new peak occurred at 2.73 THz on the higher frequency side. Comparing Ala and Val spectra, the above correlation is further evidenced: as the side chain is elongated and added with a lateral methyl group onto the side chain, Val spectrum has several more peaks occurred on the lower frequency side, the main peak is shifted higher to 2.64 THz, and the peak at 2.73 THz shifted to 2.80 THz. The above correlation can be observed again when comparing Leu with Val, and then the peak at 1.70 THz is split into two peaks at 1.46 and 1.64 THz.

However, as the side chain of Ile is elongated (comparing to that of Val), the THz spectrum does not have more absorption peaks, although the peaks on the lower frequency side (especially the peak at 1.70 THz) are strengthened. This is possibly because the lateral methyl group remains at the original position. Between the isomers, Leu and Ile, the position of the lateral methyl group is the only difference, and its distal position in Leu spectrum appears to be responsible for the occurrence of more absorption peaks. So, it appears that when a methyl group is in a terminal position on the side chain, the molecule will be less stable or more active and thus more absorption peaks would occur.

Dicarboxylic group includes two acidic amino acids, Asp and Glu. Each molecule has a second carboxyl group (COOH) on the side chain. Between Gly and Asp, the addition of the second carboxyl group did not affect the main peak but shifted it to 2.58 THz, suggesting both carboxyl groups possibly share the same, overlapped vibrational rotational modes. This is further supported with Glu spectrum that its main peak shifted to 2.64 THz and had obvious peaks at 1.23 and 2.03 THz on the lower frequency side, and these changes are associated with the insertion of a CH_2 and that is the only difference between Asp and Glu.

Hydroxylic amino acid group includes Thr and Ser, both are characterized with a hydroxyl group (OH) on the side chain. Comparing with Val, Thr has the OH group replaced the lateral CH_3 on the side chain, and this is associated with a decrease of

absorption peaks. Between Thr and Ser spectra, more peaks disappeared without the terminal CH_3. These suggest that the hydroxyl group (OH) on the side chain is much less dynamic than a methyl group, and its appearance is associated with the decrease of absorption peaks.

Monocarboxylic diamino acids include Lys, His, and Arg. Each has a second amino group (NH_2) on the side chain or the side ring. Between Gly and Lys, the side chain is elongated with several CH_2 groups and ended with the second amino group. Similar to Glu, the addition of CH_2 groups is associated with the occurrence of two peaks at 1.26 and 1.79 THz, but it appears having nothing to do with the main peak at 2.25 THz that should be solely related to the addition of the second amino group. It is the second amino group that appears to strengthen the peak at 2.25 THz to overtop the peak at 2.60 THz. In other words, amino group is associated with the peak between 2.23 and 2.30 THz. Similarly, the main peak at 2.24 THz of the His spectrum also seems to be related to the second amino group, but other peaks appear to be related to the ring structure.

Comparing with Lys, Arg has NH replaced the fourth CH_2 and the side chain is inserted with a C═NH group before the terminal H_2N. These changes are associated with the diminishing of the absorption peak between 2.23 and 2.30 THz, but the peak at 2.60 THz is strengthened.

Carboxamide group includes Asn and Gln, both have a carboxamide group (CO—NH_2) that consists of a carbonyl group (C═O) and an amino group (NH_2). Similar to that in Lys, the second amino acid in Asn appears to be associated with the peak at 2.26 THz, but the main peak at 1.64 THz is associated with the carbonyl group that is much more active. Similarly, Gln spectrum has the main peak at 1.70 THz and two peaks at 2.14 and 2.42 THz. It is the elongation of the side chain that appears to be related to the peak splitting in Gln spectrum.

Sulfur-containing amino acids include Cys and Met. Their spectra have two common features: a peak at 2.94 THz in the same shape, and a relative flat platform (that is not seen in spectra of other amino acids). The relative flat platform and the peak at 2.94 in both Met and Cys could be related to the sulfur, but this needs to be further studied.

Aromatic group includes Phe and Tyr, both are structurally very similar to each other in having an aromatic ring on the side chain, but the latter has a terminal hydroxyl group (OH) that appears to be correlated with the shifting of the main peak to 2.70 THz. Moreover, all the absorption peaks of Tyr are highly symmetric and it appears to be associated with the symmetry of the side chain.

Heterocyclic amino acids include Pro and Trp, both are structurally very different from each other. The spectrum of imino acid Pro is slightly similar to that of Phe, but with a wider peak band centered at 2.64 THz, possibly due to the existence of the NH in the ring. Trp spectrum is very different from other amino acids and this is possibly because the molecule has two different rings.

3 Conclusions

With the above results, the carboxyl group appears to be associated with an absorption peak between 2.52 and 2.64 THz, and the amino group associated with a peak between 2.23 and 2.30 THz. The two peaks should appear in spectra of all amino acids unless they are masked or offset by the effects of other functional groups. Among aliphatic amino acids, the terminal position of methyl group(s) on the longer side chain appears to make the molecule more active, resulting in more vibrational and rotational transitions, i.e., more absorption peaks. However, a terminal hydroxyl group on the side chain seems to reverse the effects of methyl groups and thus decrease number of absorption peaks. Between isomer Leu and Ile, different position of the methyl group appears to be correlated with different THz spectral peaks. On the other hand, although elongation of the side chain is commonly associated with the occurrence of more absorption peaks, the peaks may not increase when the side chain is symmetric (e.g., in Phe and Tyr), or the side chain has the same functional group as that in the basic structure of the amino acid (e.g., in Asp) because the same vibrational and transitional modes of two identical functional groups could overlap each other. Obviously, recognizing these correlations could help us to identify and trace some functional groups in different molecules through analyzing their characteristic peaks.

Although we have found some correlations between THz spectral peaks and molecular structures, the physical mechanisms of these correlations remain unclear. Currently, it is generally believed that mid-infrared spectroscopy can help to study the internal vibration of molecules, and THz spectroscopy can help to observe the intermolecular vibration. THz spectral peaks of some amino acids are considered contributed by the intermolecular vibrational modes mediated by hydrogen bonds, and THz absorption bands are also reported to be sensitive to crystal structures of amino acids. However, whether the THz absorption bands are dominated by intermolecular or intramolecular vibration may also be affected by other factors, such as temperature. It also could be determined by molecular size, because large molecules with more different functional groups were more active and could cause more absorption peaks. However, whether the THz absorption peaks are dominated by internal or intermolecular vibration in small molecules need to be further studied. Nevertheless, recognizing the correlations between molecular structures and THz spectra can help us to reveal the physical mechanisms of formation of absorption peaks.

In short, we have presented a whole set of spectra between 0.2 and 3.0 THz of all standard amino acids with more graphic and numeric details than the previous reports, so the set of THz spectra has been validated and may be used as fingerprints in identification of amino acids. Through a comparative study of serial molecules, we have found some correlations between molecular structures and their THz spectra and established a new classification of 20 standard α-amino acids, reflecting the correlations. Although the physical mechanisms of these correlations and the

formation of the absorption peaks still need to be further studied, these correlations can help to identify and trace some functional groups in different molecules, and thus promote the application of THz spectroscopy in study of biological and medicinal materials in biomedical fields.

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