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DETERMINATION OF THE ABSOLUTE CONFIGURATION OF 3-AMINO-3-(TETRAHYDROFURAN) CARBOXYLIC ACID BY VIBRATIONAL CIRCULAR DICHROISM AND DFT CALCULATION

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ABSTRACT. The absolute configuration of 3-amino-3-(tetrahydrofuran) carboxylic acid was determined using vibrational circular dichroism and DFT calculation. The conformation of 3-amino-3-(tetrahydrofuran) carboxylic acid in gas phase and aqueous solution was calculated, along with the vibrational circular dichroism (VCD) and infrared (IR) spectra. In addition, experimental VCD and IR spectra were collected in aqueous solution. Each calculated IR and VCD spectra was compared to the experimental spectra. The band assignment was unambiguously determined from the DFT calculation. Based on the calculation and the experimental data it was determined that the absolute configuration of the compound was (S)-enantiomer.

Keywords: *VCD, absolute configuration, THF amino acid, DFT*

INTRODUCTION

Amino acids are essential building blocks for proteins. In addition they have been extensively used as intermediates in the synthesis of many chiral molecules.[1-9] Heterocyclic amino acids are of particular interest, as exemplified by 3-amino-3-(tetrahydrofuran) carboxylic acid (THF amino acid 1), which possesses diverse physiological activities and has been utilized as a building block for a large number of chiral drug molecules.[1,10-15] As such, the absolute configuration of amino acid is critical; yet very few methods can be applied for such determination. Single crystal X-ray is the straightforward way; however, sometimes it is very difficult, if not impossible, to get single crystals. Another method for the determination of absolute configuration is NMR; however, the technique requires chiral reagents for the formation of diastereomeric species, and the reagents are often not readily available.

Recently we have synthesized a tetrahydrofuran (THF) amino acid **1** and used it as a building block in the synthesis of a series of chiral drug candidates.[11,12] In order to pursue the synthesis in the subsequent steps it

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was important to determine the absolute configuration of the starting material. The polar nature of the compound rendered it solubile only in water. Using such solvent, the generation of a single crystal amenable to X-ray analysis was not possible.

Vibrational circular dichroism (VCD) is a spectroscopic method that was developed in the 1970s. VCD is a chiroptical technique which measures the difference in absorbance of a chiral molecule upon interacting with a left versus right circularly polarized light in the infrared part of the spectrum. As such the technique is very sensitive not only to structural changes in biomacromolecules and small molecules, but also to the mutual orientation of different chemical groups within it. VCD is also very sensitive to changes in the bond strength, bond distortion during the catalytic reaction, etc.[16] As a result, VCD provides a wealth of information about the structure and absolute configuration of chiral compounds.[17,18]

The theories of IR and VCD were first developed decades ago and significant progress has been made since then. Although the theoretical predication of VCD of large molecules such as proteins is still a difficult task, the *ab initio* quantum chemical calculations applied to small to medium size molecules have become fast and reliable even on PC computers.[19-21] Such advancements have greatly enhanced the application of VCD as a tool to elucidate the structure, and especially the absolute configuration (AC), of chiral molecules.

The calculation of VCD intensity for a chiral molecule becomes routine with the availability of commercial software such as Gaussian 98/03.[22] The density functional theory (DFT) method provides electron correlation by including an exchange correlation that is a function of the electron density, thus implicitly accounting for electron correlation. Numerous studies have shown that density functional theory (DFT) with a hybrid functional such as B3LYP and a basis set of 6-31G(d) provides the best combination of spectral accuracy and calculation expense. The commercial software Gaussian 03 computes VCD spectra along with performing the geometry optimization, which is achieved at the same level as the calculation of the VCD spectrum, generating the molecular geometry at a stationary point on the potential energy surface. Once the optimized geometry is obtained, corresponding dipole strengths, rotational strengths and vibrational frequencies are calculated based on the magnetic field perturbation (MFP) theory. [23,24]

In this paper, we present the determination of the absolute configuration and conformation of amino acid **1** by VCD, along with DFT calculation. Since amino acid **1** is only soluble in water, experimental VCD was performed in aqueous solution. The DFT calculation was first carried out on zwitterionic form, in gas phase. The comparison between the calculated and experimental results were not in agreement. We further extended the DFT calculation with the polarizable continuum model and added explicit water molecules surrounding the amino acid **1**. It is demonstrated that this model provides dramatically more accurate results, allowing for a better correlation between the experimental and calculated results and consequently for the determination of absolute configuration and solution conformation.

RESULTS AND DISCUSSION

To study the absolute configuration of amino acid **1**, we first performed the DFT calculations in gas phase with the assumption that the compound is not self-interacting and is in the neutral state. Indeed, the DFT calculation of the zwitterionic species led to a neutral structure, indicating that the stable form in gas phase is not the zwitterionic specie, but a neutral specie in which the carboxy group is a carboxyl and the amine is in the form of NH_2 (Fig. 1). The calculated VCD spectra and its comparison with the experimental results is presented in Fig. 2. All major VCD bands were labeled with numbers for clarity. The spectral region between 1700-1600 cm^{-1}, presents in the experimental spectra a (+,-,+) feature (band # 1, 1' and 2) (1674 cm^{-1}, 1662 cm^{-1} and 1627cm^{-1}, respectively).

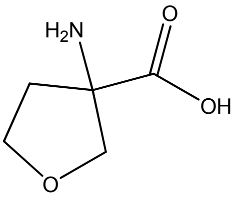
The calculation, however, showed only two negative peaks in the same spectral region (band #1 and # 2) (1674 and 1627 cm^{-1}), corresponding to C=O stretching and $-NH_2$ bending modes, respectively. In the spectral region between 1600-1400 cm^{-1}, only band #3 was observed in both the experimental and calculated spectra. This band corresponds to the methylene vibration from the THF ring. Large discrepancies between the calculated and experimental spectra were observed in the region of 1400-1200 cm^{-1}. In particular, the bands # 4' and 4 display intense negative peaks in the calculated spectra, while the experimental spectra show very weak bands in this region. These two bands correspond to the vibrations of carboxylic –OH and $-NH_2$ respectively, coupled with THF ring.

In the region between 1200-1000 cm^{-1}, calculated VCD shows three positive peaks (bands #5, #6 and #7), with relative intensities quite different from the experimental spectra. For example, band #7 (corresponding to $-NH_2$ bending coupled with ring vibration) shows intense intensity in the calculated spectra, while in the experimental spectra, only broad bands are observed. Similar disagreements were observed when comparing experimental and calculated IR spectra. Under these circumstances, the absolute configuration of amino acid **1** cannot be accurately determined due to the discrepancies between the experimental and calculated spectra.

The broadening of the bands observed in the experimental spectra can be attributed to the interaction of water with the functional groups of the amino acid. As a consequence, the DFT simulation was performed introducing molecules of water in the calculation in this case. The method involves the use of a self-consistent reaction field (SCRF). Molecules of water were placed in an environment that had the same dielectric constant as the desired solvent. There are several types of SCRF which range in complexity from a single dielectric sphere around the whole molecule, to individual spheres around each atom of the molecule, which can each be polarized independently.

Gaussian 03 provides the functions for VCD calculation using the polarizable continuum model (PCM). [25-27] We used PCM to simulate the water solvent environment. In addition, three water molecules were placed at sites in which hydrogen bonding occurred (NH_2, COOH and ether oxygen).[28,29] It is preferable to use the minimum number of water molecules that can still produce agreement with the experimental data, because calculation times increase dramatically with the number of atoms in the molecule. Thus, water molecules were placed according to Fig. 3, and the calculation was pursued. Under these parameters the calculation approximates the experimental spectrum very well, in peak frequency as well as both relative and absolute intensity (Fig. 4). This calculation also showed a reduction of the VCD intensities of bands #4, #4' and #7, previously observed without water molecules docked to the amino acid. The addition of these three molecules of water, hydrogen bonded to the carboxyl, amino and ether oxygen, accounted for a better correlation between the experimental and the calculated spectrum.

In addition to the VCD results, the model with three explicit water molecules offers a significant improvement in the calculated IR spectra. This formation of the water bridges also affected the low intensity of these groups in the experimental VCD spectrum. From the close agreement between the experimental and calculated VCD spectra, the absolute configuration of amino acid **1** can be unambiguously assigned as (***S*)-enantiomer**.



Scheme 1

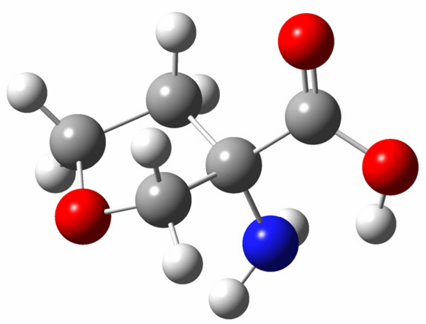


Figure 1. Structure of neutral form of amino acid 1

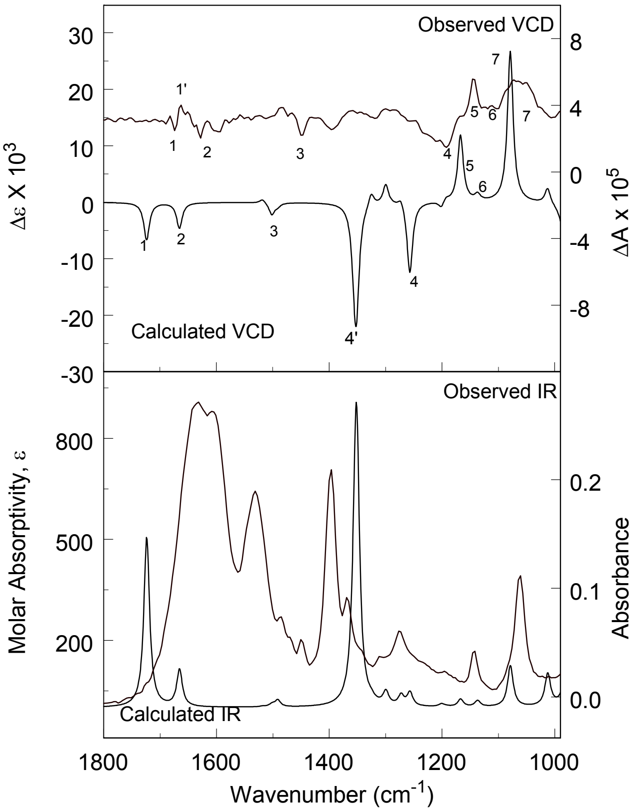


Figure 2. Comparison of calculated and experimental VCD and IR spectra of amino acid 1: calculation on neutral form.

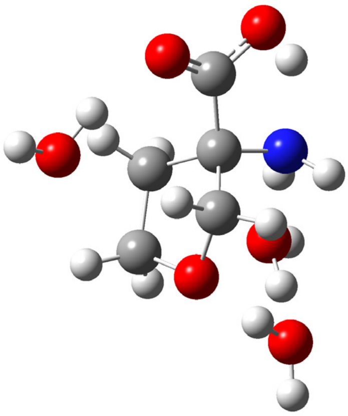


Figure 3. Structure of amino acid 1 with three explicit water molecules

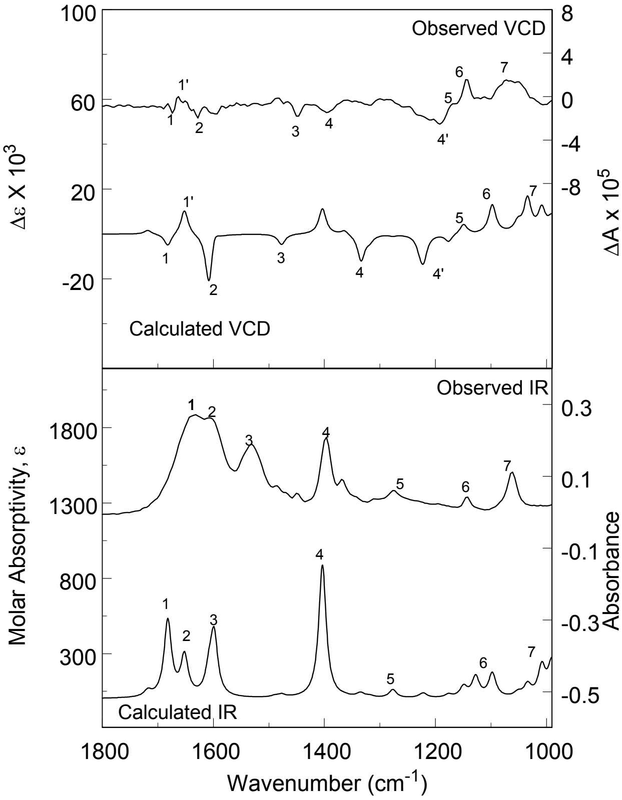


Figure 4. Comparison of calculated and experimental VCD and IR spectra of amino acid 1: calculation on PCM with three explicit water molecules surrounding amino acid 1.

CONCLUSIONS

The experimental and calculated VCD spectra of amino acid 1 are reported and the absolute configuration is determined. The PCM model with the addition of specific water molecules produces accurate VCD and IR calculated spectra for the amino acid. The model can be extended to other molecules such as peptides. This protocol presents a promising approach for the determination of the absolute configuration of amino acids in aqueous solution.

EXPERIMENTAL SECTION

The chiral amino acid was prepared from its butyl ester by hydrolysis in MeOH with NaOH, followed by purification on the column.[11,13]

To compute the structure of the amino acid, we used Hyperchem software (Hypercube, Inc., Gainesville, FL). All structural and spectroscopic calculations were performed using Gaussian 03. Structural optimizations were conducted under the polarizable continuum model (PCM) self-consistent reaction field (SCRF). Once a standard optimization was performed with PCM, the molecule was considered to be fully optimized. The VCD spectrum of the molecule was calculated in PCM, and this calculation was extended to find the VCD spectra of 1. Calculations were repeated for systems with zero or three explicit water molecules using B3LYP/6-31G(d) basis sets and functionals. Spectra were analyzed and compared. Mode assignments are based on the animation of vibrations in Gaussview 3.1.

All VCD measurements were performed on a ChiralIR spectrometer (BioTools, FL, USA). The amino acid was dissolved in H_2O solution at the concentration of 1.3 M. Spectra were collected for 4 hours in a 9 μm path length cell. The final VCD spectra were corrected by subtracting the corresponding solvent spectra (water) measured in the same conditions.

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