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Vibrational and structural properties of L-Alanyl-L-Phenylalanine dipeptide by Raman spectroscopy, infrared and DFT calculations

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ABSTRACT

A study of vibrational and structural properties of L-Alanyl-L-Phenylalanine hydrophobic dipeptide (molecular formula $C_{12}H_{16}N_2O_3$) is reported. The L-alanyl-L-phenylalanine is formed by two hydrophobic amino acids, L-alanine and L-phenylalanine, through a peptide bond. Individually these compounds have many important properties, including increasing immunity and providing energy to muscle tissue, brain and central nervous system (L-alanine). We have performed measurements of Raman and Fourier transform infrared spectroscopy, between 3500-50 cm^{-1} and 4000-130 cm^{-1}, respectively, at ambient conditions. To support the experimental results calculations using the density functional theory (DFT) with B3LYP functional, 6–31 G + + (d, p) basis sets and the polarizable continuum model of solvatation in an isolated molecule in the zwitterionic form were done. The assignment of the normal modes was described by the potential energy distribution analysis. In this way, it was possible to analyze the vibrational and structural properties of L-alanyl-L-phenylalanine, allowing the assignment of each vibrational normal mode of the molecular structure, associating them with those results obtained experimentally.

1. Introduction

Short peptides have arisen the interest of the scientific community around the world. Although the peptides have less complex structures than the proteins, they exhibit important biological activities. Hydrogels formed by self-assembly of peptides have been used in 3D cell culture as constituents of compounds with regenerative properties [1,2], which act in the regeneration of several tissues with low regenerative capacity, such as bone tissue, dental tissue, skeletal tissue and cartilage [3–6]. Some peptides have antioxidant properties and act as pH regulator in muscle cells [7], avoiding the oxidation of organic molecules and metals, helping in glycemic control [8] and the treatment of type 2 diabetes [9], besides being used in the diet of slimming [10].

The L-alanyl-L-phenylalanine (L-Ala-L-Phe) is a dipeptide formed by two hydrophobic amino acid residues. The hydrophobic dipeptides constitute a very diversified group of crystalline structure and have been a source of stable microporous materials [11,12], which are originated from molecular self-assembling dictated by the formation of hydrogen bonds and by the aggregation of hydrophobic entities in the side chains [13].

A series of works, using the most varied solvents, shows these dipeptides tend to incorporate molecules of organic solvents into their structure, which then act as acceptors for one of the three H atoms of the N-terminal amino group NH_3^+ [14–18]. The change of solvent can result in the formation of different structures, for example, nanotubes, nanofilms, nanospheres, nanofibers, and materials with large channels filled with solvent [19–21]. These structures have been used in different areas, such as in the construction of biosensors [21], nanocarriers for drug and gene delivery [22] and natural encapsulates [23,24]. In addition, the use of dipeptides has been tested in the fight against diseases such as zyka virus [25], cancer [26], diabetic osteoporosis [27], HIV [28], malaria [29], anti-tumor [30,31], and antibacterial and antifungal agents [32].

In previously published works, crystalline structures with L-Ala-L-Phe dipeptide was obtained with 2-propanol solvent [33], ammonium [34], hydrochloride dehydrate [35], as well as structures interacting with copper [26] and gold [36], although the crystalline structure of pure L-Ala-L-Phe dipeptide is still unknown. Similar structures containing the phenylalanine showed to be an effective hydrogelator, when used with the specific solvent. In this manner the L-Ala-L-Phe is a good candidate to gel-forming [37–40].

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Structural analysis and vibrational properties of dipeptides using the density functional theory (DFT) [41,42] have been reported in the literature, being important to understanding the behavior of aggregation of amino acid in complex structures as peptides and proteins [43–49]. The present work reports a detailed study about the structural and vibrational properties of L-Ala-L-Phe dipeptide (molecular formula $C_{12}H_{16}N_2O_3$) including data about important torsion angles of the molecular structure and the assignment of vibrational modes.

2. Experimental

L-Alanyl-L-Phenylalanine (L-Ala-L-Phe) dipeptide, which consists of a white polycrystalline powder, was used as provided by Sigma-Aldrich. The Raman spectra of L-Ala-L-Phe were performed using a triple spectrometer (Jobin-Yvon T64000) equipped with an N_2-cooled charge-coupled device detector (CCD). The 532 nm line of a semiconductor laser was used as the exciting source, with an output power of 70 mW. The spectral resolution was ~ 2 cm^{-1}. An Olympus microscope lens with focal distance 20.5 mm (N.A. = 0.35) was used to focus the laser beam on the sample at room temperature. The spectral region analyzed covered the region from 3500 to 40 cm^{-1}.

The Fourier Transform infrared (FT-IR) measurement was obtained through a Bruker Vertex 70v spectrometer. The infrared spectrum was recorded from 4000 to 130 cm^{-1}, using the Attenuated Total Reflectance (ATR) technique with vacuum by absorbance of radiation to eliminate CO_2 residues and humidity with a spectral resolution of 2 cm^{-1} and accumulating 128 scans per spectrum.

3. Calculations

Calculation and normal mode assignment of IR and Raman spectra is widely utilized to understand organic materials like amino acids, peptides and proteins [50–59]. The vibrational modes of L-Ala-L-Phe dipeptide were calculated using density functional theory (DFT). For this purpose, we used the Gaussian 09 package [60], with the B3LYP functional [61] and 6-31 + + G(d,p) basis set. Solvatation was also incorporated for assignment by using the polarizable continuum model (PCM). Our calculations were established for an isolated molecule in the zwitterionic form. In general, zwitterionic states are predominantly observed in the crystalline medium for a variety of amino acids and dipeptides [62].

The initial molecular structure of L-Ala-L-Phe dipeptide used was that reported by Görbitz [33], and then optimized for a minimum of energy. The optimized molecular structure was subjected to investigate the conformational structure by calculation of energy surface potential (scan) of torsion dipeptide angle and, finally, to calculate the frequency of vibrational modes for this molecule (3N-6).

Using VEDA program [63] for molecular visualization and potential energy distribution (PED) calculations, the vibrational assignments of the normal modes were obtained. Here we consider only values with contributions of at least 10%. For a better correspondence between the theoretical and experimental spectra, we made use of the scale factors suggested by the reference [64] of 0.977 for vibrations modes under 1800 cm^{-1}, and 0.955 for vibrational modes above 1800 cm^{-1}.

The calculations were made in the conformer of the lowest energy. Although the calculations had been performed in a molecule, the vibrations of both single molecule and molecules in the crystal are similar, exceptions occur with vibrations related to part of the molecule involved in hydrogen bond, for example, and vibrations related to the lattice modes that obviously do not appear in the calculation of the molecule. It is important mentioning that the possible coincidence of the molecular conformation in the solid state with the molecular conformation in the gas phase or in solution has supporters and opponents; this question remains unresolved. As can be seen in Table 2, there is a good agreement between the calculated wavenumbers and the experimental wavenumbers of the Raman and infrared bands to the internal modes of the Ala-Phe molecule.

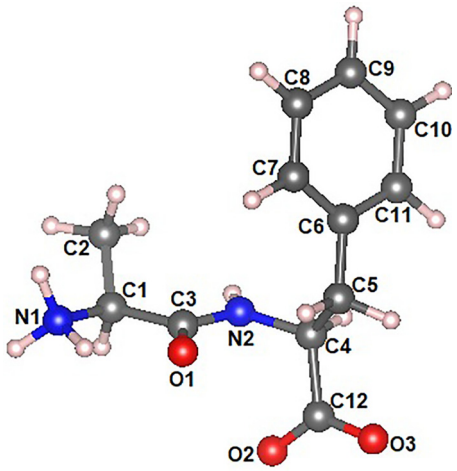

Fig. 1. Representation of molecular structure of L-Ala-L-Phe in zwitterionic form.

4. Results and discussions

The molecular structure of L-Ala-L-Phe (illustrated in Fig. 1) has 33 atoms, molecular weight of 236.27 g/mol and molecular area of approximately 95.5 $Å^2$. The data of positions of atomic coordinates and the geometric parameters (bond lengths, bond angles and dihedral angles) of the optimized molecular structure of L-Ala-L-Phe dipeptide are listed in Tables S1 and S2 of Supplementary Material, respectively.

4.1. Potential energy surface (PES)

An important torsion angle in dipeptides deserves attention, due to it considerably influences the conformation of peptides and proteins. This torsion angle is formed by the alpha carbon ($_\alpha$C) and the beta carbon ($_\beta$C) of the first amino acid residue with the alpha carbon and the beta carbon of the second amino acid, interchanged by amide plane (OCNH), $\theta = {}_\beta C_1\text{-}{}_\alpha C_1 \cdots {}_\alpha C_2\text{-}{}_\beta C_2$, which defines the relative positions of the two sides chain in the structure of dipeptide [19].

This dihedral angle is the simplest way to describe the conformational configuration of the molecule, characterizing the influence of the inter- and intra-molecular interactions in the crystalline medium. In order to investigate the energetic stability of different conformational configurations of L-Ala-L-Phe, the potential energy surface (PES) calculation was carried out, varying the dihedral angle θ from -180° to +180° degrees in steps of 10°. The relative energy with respect to the global minimum (ΔE) was plotted against the dihedral angle, as shown in Fig. 2. In this figure, we can see a global minimum at -110° and local minima around -150°, +20° and +150°. At room temperature the thermal energy (kT) is approximately 2.45 kJ/mol, which corresponds to the dashed red line in the plot of Fig. 2. We can observe two regions above the thermal energy at room temperature, the first region between -85° to -115° and the second region between -177° to -130° separated by a barrier of 1.96 kJ/mol.

In the polypeptide chain the definition of protein folding depends on the torsion angles prior to alpha carbon, called phi angle (ϕ), and on the torsion angle after the alpha carbon, called the psi angle (ψ) [65]. The analysis of rotation of these angles led to the identification of permitted regions where there is no clash between atoms, and non-permitted regions, where there is clash between the atoms. In Fig. 2 we also show the correspondence between each value of ϕ and ψ with dihedral angle θ. In this case, while the θ angle vary from −180° to + 180°, the ϕ angle vary between −40° and 25° and the ψ angle vary between 80° and 110°, respectively.