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## Raman Spectroscopy of Amino Acid Crystals

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Additional information is available at the end of the chapter

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### Abstract

In this chapter, we investigate the Raman spectra of proteinogenic amino acid crystals. Amino acids are fundamental organic molecules that compose polypeptides (a linear chain of amino acids) and proteins (folded polypeptides with specific functions) found in all living beings. Surprisingly, the number of these basic molecules is not more than 22 (20 of them commonly known as the standard amino acids, plus pyrrolysine and selenocysteine). They are defined as a molecule formed by an  $\text{NH}_2$  group, a  $\text{COOH}$  group, a lateral chain (the R group), and a hydrogen atom, all of them connected to a single carbon, the  $\alpha$ -carbon. Interestingly,  $\alpha$ -amino acids show chirality, i.e., they present different distributions of group of atoms around the  $\alpha$ -carbon, being defined as L- and D-form. For amino acids and proteins found in the living beings, the L-form is the dominant form, although some exceptions have been discovered in the last decades. In this chapter, we present the Raman spectra of all standard amino acids and discuss the different kinds of vibrations found, comparing them. As complementary part of the work, we present results on vibrational properties of some amino acids using Raman spectroscopy when subjected to specific conditions, with variation in temperature or pressure. Finally, we present some perspectives as the investigation of purines, a group of molecules associated with the DNA molecule.

**Keywords:** amino acid, raman spectroscopy, vibrational property

## 1. Introduction

Amino acids constitute an impressive and mysterious class of organic molecules, impressive because they form all proteins of living beings and mysterious because of their simplicity. In fact, amino acids — in their  $\alpha$  form and in the *zwitterion* charge distribution conformation — are

constituted by one carbon, one  $\text{NH}_3^+$  group, one  $\text{CO}_2^-$  group, and a group denominated as radical. The radical ( $R$ ) characterizes the different types of amino acids; for example,  $R = \text{H}$  for glycine,  $R = \text{CH}_3$  for alanine,  $R = \text{CH}_2\text{-OH}$  for serine, etc.

As it is well established, every cell of the living being on the Earth uses a set of 20 amino acids to produce all kinds of proteins [1, 2]. The 20 standard amino acids can be classified as (i) unpolar (alanine, valine, leucine, isoleucine, methionine, proline, phenylalanine, and tryptophan) and (ii) polar (glycine, arginine, asparagine, cysteine, glutamine, lysine, aspartic acid, glutamic acid, serine, threonine, tyrosine, and histidine). Additionally, some compendia include selenocysteine and pyrrolysine as belonging to the group of proteinogenic amino acids, but this is not unanimity yet. In the last years, a series of studies have investigated the vibrational and structural properties of amino acid crystals [3–42].

One of the main techniques to investigate vibrational properties of materials, whatever it is, is Raman spectroscopy. The technique consists in the interaction of light from a laser source with the material and further scattering of the light. The scattered light carries information about the rotational, vibrational, and, eventually, electronic states of the material. Concerning the Raman scattering effect, a pivotal concept is the scattering cross-section,  $\sigma$ , which represents a likelihood of a scattering event to occur [43]. It is defined as the rate at which energy is removed from the incident photon by the scattering substance, divided by the rate at which energy in the incident photon crosses a unit area perpendicular to its direction of propagation [43], expressed as in Eq. (1):

$$\sigma = \hbar \omega_I / (2\pi \tau I_I) \quad (1)$$

In this equation,  $\hbar$  represents the Planck constant,  $\omega_I$  represents the angular frequency of the incident light,  $1/\tau$  is the transition rate of the scattering process, and  $I_I$  is the surface power density, given in units of energy/[time  $\times$  area].  $1/\tau$  is calculated through time-dependent perturbation theory from quantum mechanics. Obviously, if we are using quantum theory we need to define a Hamiltonian representing the system, as well as the states of the system. It is possible to define a Hamiltonian composed of two parts: (i) one unperturbed ( $H_0$ ), represented by the contribution of the scattering medium and by the radiation field and (ii) one perturbed ( $H_p$ ), with contributions from the electron-radiation interaction and electron-phonon interaction. An eigenstate of  $H_0$  is  $\psi_i$  that encompasses information about incident and scattered photons, phonons, and about the electrons. The process involves the excitation of the medium due to the incident photon, leading the system to an intermediate state, creating an electron-hole pair. Such pair is scattered in a different intermediate state; finally, the electron-hole pair recombines, occurring the emission of a photon. This scattered phonon will have the information about the system. Using time-dependent perturbation theory we can show that

$$1/\tau = -\text{d}/\text{d}t \sum |\Psi_f|^2 \exp[-i (H_0 + H_p) (2\pi/\hbar)] \Psi_i \quad (2)$$

with the sum performed over all possible eigenstates  $\Psi_f$ . After a crusty work using third-order perturbation theory, we can present the cross-section for the so-called one-phonon Stokes process in a relation such as:

$$\sigma = h\omega_l / (2\pi I_0) \sum (8\pi/h^2) \left| \left\{ \left[ \langle f | H_{er} | b \rangle \langle b | H_{ep} | a \rangle \langle a | H_{er} | i \rangle \right] / (E_l - E_{eh} + i\xi_1) (E_l - (E_q + E_{eh}) + i\xi_2) \right\} \right|^2 \delta(E_l - (E_s + E_q)) \quad (3)$$

In this expression, the kets  $|a\rangle$  and  $|b\rangle$  represent intermediate states with small lifetime — called virtual states —  $H_{er}$  represents the electron-radiation interaction,  $H_{ep}$  represents the electron-phonon interaction,  $E_l$  represents the energy of the incident phonon,  $E_s$  represents the energy of the scattered photon,  $E_q$  represents the phonon energy, and  $\xi_1$  and  $\xi_2$  are small parameters. Other processes are also possible such as those involving two phonons that require the use of perturbation theory in order higher than third. However, in the present text, the results are related to one-phonon processes and, consequently, at first, all quantitative analysis can be achieved with the use of Eq. (3).

In this chapter, we summarize the main studies on Raman spectroscopy applied to proteino-genic amino acid molecules and crystals and point new perspectives on the subject.

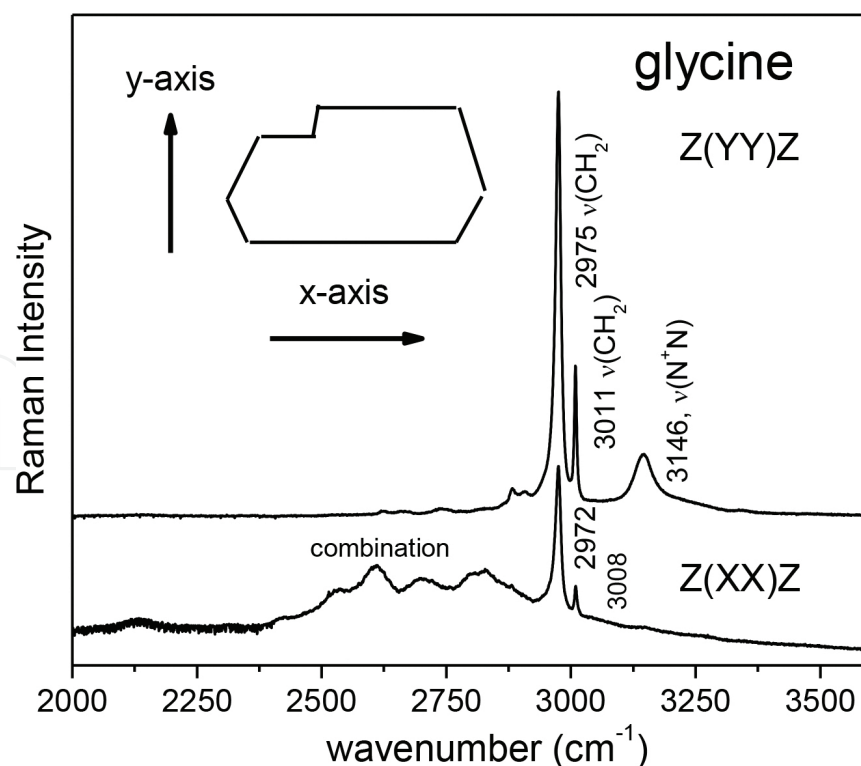
## 2. Experimental details

In the present work, we have used two experimental set-ups: an instrument using Fourier transform mechanism and a dispersive (conventional) spectrometer. On the one hand, FT-Raman spectra were recorded using a Bruker RFS100/S FTR system and a D418-T detector, with the sample excited by means of the 1064 nm line of a Nd:YAG laser. In these cases, the spectral resolution was  $4 \text{ cm}^{-1}$ . On the other hand, the conventional Raman spectra were excited with the 514.5 nm line of argon ion lasers and the scattered light was analyzed in a Jobin-Yvon T64000 spectrometer equipped with the nitrogen cooled CCD system. Typically, the spectral resolution in the conventional Raman experiments was  $2 \text{ cm}^{-1}$ . Theoretically, the bands appearing in the Raman spectrum are independent of the excitation energy of the laser, although the intensity of the scattered light is proportional to  $\lambda^{-4}$  ( $\lambda$  is the wavelength of the laser) and, consequently, long-wavenumber excitation will produce a weaker Raman spectrum. However, in some cases, the exciting photons—mainly in the visible—are sufficient to excite the system into the lowest energy electronic states, and further relaxation to the ground state will appear as a broadband fluorescence in the Raman spectrum. Regarding the samples of amino acids, we have utilized two kinds: those used to obtain FT-Raman spectra were commercial reagents, while those where polarized Raman spectra are shown, were crystals obtained through aqueous solutions at several temperatures.

### 3. Nonpolar amino acids

In the discussion, we will separate the discussion according to the polarity of the lateral groups of the amino acids. We begin with the apolar amino acids. This group is composed of the following amino acids: glycine, alanine, valine, leucine, isoleucine, phenylalanine, methionine, tryptophan, and proline.

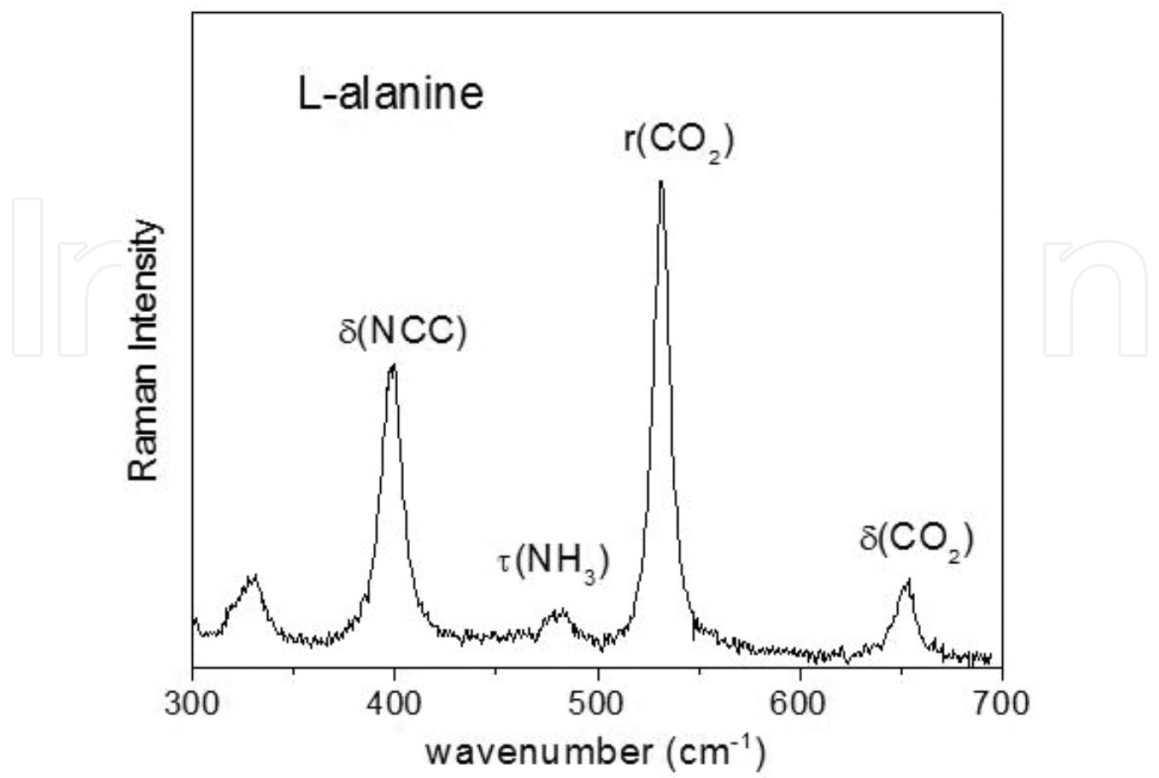
*Glycine* is the simplest amino acid, having as the radical a hydrogen atom. Although the molecular simplicity, glycine can crystallize in three different forms at atmospheric pressure and room temperature. The  $\alpha$ -form shows a  $P2_1/n$  monoclinic structure where hydrogen bonds appear in double antiparallel layers; the  $\beta$ -form shows a  $P2_1$  monoclinic structure and individual parallel layers are linked by hydrogen bonds in a three-dimensional network; the  $\gamma$ -form shows a  $P3_1$  trigonal structure with zwitterions forming helices linked in a three-dimensional network [6]. The problem is that the three forms of glycine generally crystallize simultaneously from the same solution. In order to obtain crystals of the  $\gamma$ -form, it is necessary to prepare aqueous solution containing small seeds of the  $\gamma$ -glycine; the metastable  $\beta$ -form can be obtained by a mixture of water and acetic acid having the  $\beta$ -glycine as a seed, among other possibilities [6]. The three forms were studied under high pressure or under temperature variation. From these studies different results were obtained. The  $\alpha$ -glycine under pressure is stable up to 23 GPa [7]. The  $\beta$  polymorphic form of glycine



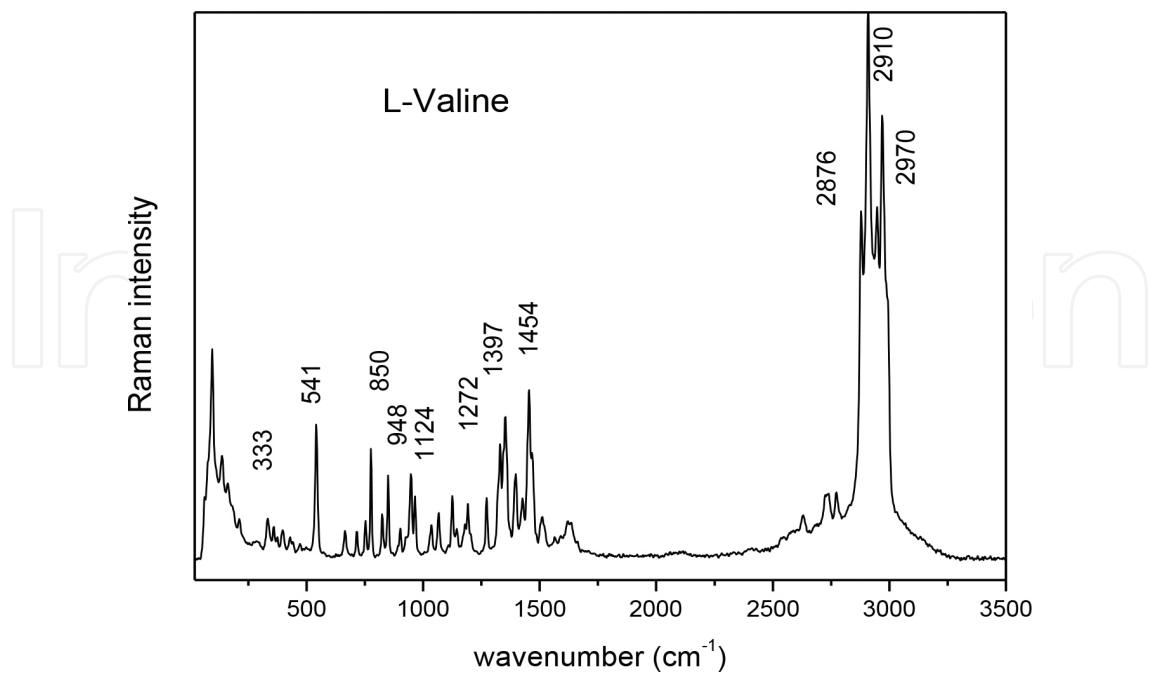
**Figure 1.** Polarized Raman spectra of glycine ( $\alpha$ -form) for two scattering geometries in the high wavenumber region of the spectrum.

presents a phase transition at 0.76 GPa [8]; the new phase,  $\beta'$  is noted by the jumps and kinks at the curve of wavenumber vs. pressure as observed in experiment of Raman spectroscopy. Although the  $\gamma$ -form is the most stable among the three atmospheric pressure possibilities, it is possible to observe at  $\sim 440$  K the  $\gamma \rightarrow \alpha$  phase transition [9]. Also, under pressure, a phase transition from the  $\gamma$ -form to a new  $\delta$ -form is verified, starting at  $\sim 2.7$  GPa [10]. Additionally, when the  $\delta$ -form is decompressed, between 0.95 and 0.2 GPa, a new polymorph is obtained, the  $\zeta$ -glycine [11]. In relation to the Raman spectroscopy, as it is expected, each of the different polymorphs exhibits different spectrum. To give an example, **Figure 1** shows the Raman spectra of the (predominantly)  $\alpha$ -form of glycine in the high wavenumber region for two scattering geometries, Z(YY)Z and Z(XX)Z. In this figure, it is possible to observe bands associated with symmetric stretching of  $\text{CH}_2$ ,  $\nu_s(\text{CH}_2)$ , at  $2971\text{ cm}^{-1}$ ; antisymmetric stretching of  $\text{CH}_2$ ,  $\nu_a(\text{CH}_2)$ , at  $3006\text{ cm}^{-1}$ , and stretching of  $\text{NH}$ ,  $\nu(\text{NH})$ , at  $3145\text{ cm}^{-1}$ . Depending on the kind of polymorph, the stretching of  $\text{NH}$ , in particular, is observed at different wavenumbers and with different intensities.

Although the chirality itself is a theme of great relevance, in the present chapter we discuss only the properties of the L-chiral sister of amino acid crystals (those present in the proteins) and do not furnish further information about the phenomenon. The simplest chiral amino acid is *alanine*. It crystallizes in an orthorhombic  $P2_12_12_1$  space group with four molecules per unit cell. The Raman spectrum of L-alanine was studied in a series of papers throughout the years [12–14]. Two studies have furnished the assignment of the normal modes through the analysis of deuterated analogs [13, 14]. Under temperature variation, L-alanine seems to be stable in the interval of 20–300 K, as indicated by several studies, including both infrared spectroscopy [15] and Raman scattering [16] studies. **Figure 2** shows the Raman spectrum of L-alanine recorded at room temperature in the interval of  $300\text{--}700\text{ cm}^{-1}$ . This spectral range presents two important vibrations, e.g., the torsion of  $\text{NH}_3^+$ ,  $\tau(\text{NH}_3)$ , and the rocking of  $\text{CO}_2^-$  unit,  $r(\text{CO}_2)$ . The study of the  $\tau(\text{NH}_3)$  mode as a function of pressure showed that its wavenumber presents  $dw/dP < 0$ , in contrast with other amino acid crystals, such as L-threonine and taurine. Such fact was interpreted as a consequence of the behavior of hydrogen bonds that due to the short dimension should move away the N, H, and O atoms (that participate of a specific hydrogen bond) from a straight line, instead of to approximate the N and O atoms [17]. The discussion about the effect of pressure on L-alanine crystal is also of relevance. A first work indicated that L-alanine crystal undergoes a phase transition at  $\sim 2.2$  GPa [18]. Additionally, the same work showed that the bands appearing at 42 and  $48\text{ cm}^{-1}$  change intensity above the pressure where the supposed phase transition occurs [18] and the variation of intensity of the two bands was also noted by Tumanov et al. [19]. However, two studies point that instead of a phase transition, in fact, at 2.2 GPa, only the inversion of the **a** and **c** crystallographic axes occurs [19, 20]. Because at  $\sim 2.2$  GPa, the two crystallographic axes had the same dimensions, technically, at this specific pressure, the structure should be tetragonal, but above the critical pressure value, the structure continues with its original orthorhombic structure. Therefore, L-alanine is an interesting example where the union of both Raman spectroscopy and X-ray diffraction furnished a wide picture about the behavior of the system.

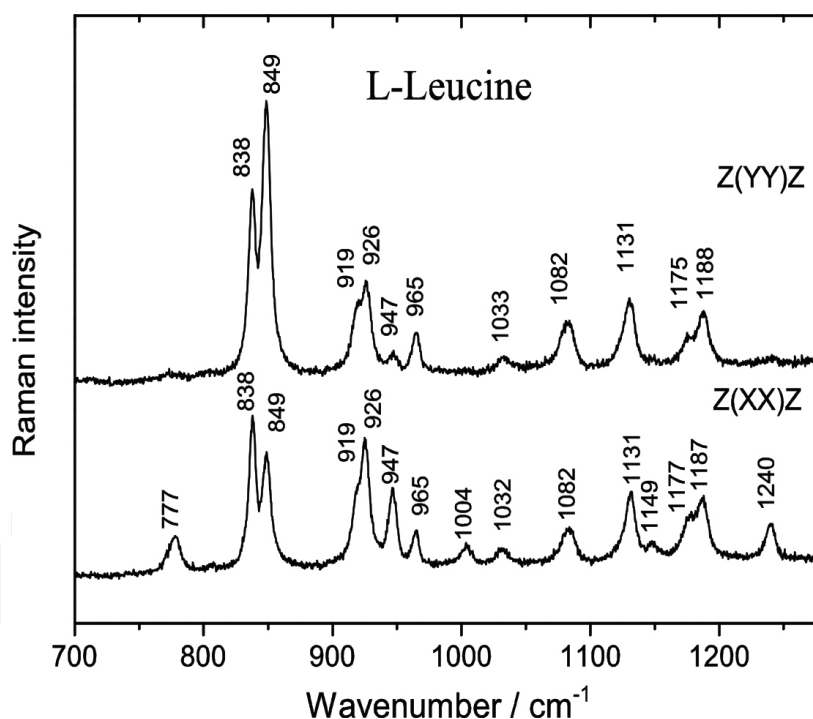


**Figure 2.** Raman spectrum of L-alanine in the spectral range from 300 to 700  $\text{cm}^{-1}$ . Some important modes related to hydrogen bonds are shown, as rocking of  $\text{CO}_2^-$ ,  $r(\text{CO}_2^-)$ , and torsion of  $\text{NH}_3^+$ ,  $\tau(\text{NH}_3^+)$ .



**Figure 3.** FT-Raman spectra of L-valine in the spectral range from 20 to 3500  $\text{cm}^{-1}$ . The most intense peaks appear in the high wavenumber region of the spectrum.

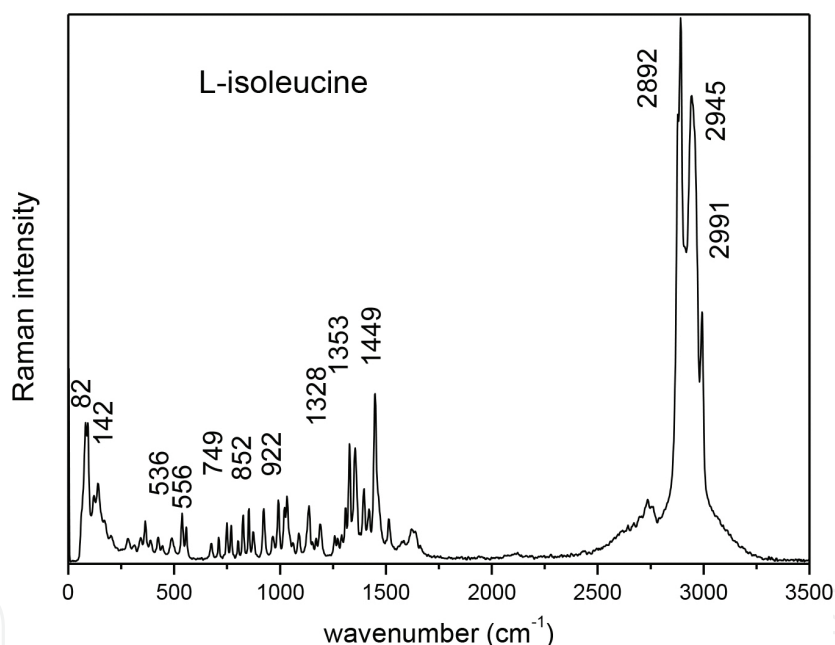
**Figure 3** shows the FT-Raman spectrum of *L-valine* in the range of 20–3500  $\text{cm}^{-1}$ . This aliphatic amino acid crystallizes in a monoclinic structure with a  $P2_1$  space group. *L-valine* is characterized by the  $(\text{CH}_3)_2\text{-(CH)}$  groups as radicals [*L-leucine* and *L-isoleucine*, which are discussed below, are characterized by  $(\text{CH}_3)_2\text{-CH}_2\text{-CH}_2$ ]. There are four molecules per unit cell of *L-valine*, two of them in the *trans* conformation and two of them in the *gauche I* conformation. As usual, the modes in the high wavenumber region are associated with the stretching of CH and  $\text{CH}_3$ ; modes related to the bending of  $\text{CH}_3$ ,  $\delta(\text{CH}_3)$ , were assigned for the bands between 1400 and 1460  $\text{cm}^{-1}$ ; modes assigned as rocking of  $\text{CH}_3$ ,  $r(\text{CH}_3)$ , were observed between 1125 and 1200  $\text{cm}^{-1}$ ; bands identified as the stretching of CC,  $\nu(\text{CC})$ , were observed between 900 and 970  $\text{cm}^{-1}$  [21]. The lattice modes were assigned as bands with wavenumber lower than 177  $\text{cm}^{-1}$  and the torsion of  $\text{CO}_2$ ,  $\tau(\text{CO}_2)$ , was identified with a band at 185  $\text{cm}^{-1}$ . A Raman spectroscopic study showed that the *L-valine* crystal seems to undergo a phase transition at  $\sim 100$  K, as indicated by changes in the lattice mode region of the spectrum [21]. In fact, unless the crystal is ferroelastic and presents domains, modification in the lattice mode spectral region means change in the symmetry of the unit cell. This behavior differs from the behavior of the *L-alanine* crystal, for example, that is stable under cryogenic conditions.



**Figure 4.** Raman spectra of *L-leucine* in the spectral range from 700 to 1280  $\text{cm}^{-1}$  for two scattering geometries.

Another amino acid with nonpolar characteristics is *L-leucine*. This amino acid crystallizes in a monoclinic structure, space group  $P2_1$ , and  $Z = 2$ . The carboxyl and the amino groups are hydrogen bonded in a double layer, in a similar fashion to *L-valine* and *L-isoleucine* [22]. **Figure 4** presents the Raman spectra of *L-leucine* for two scattering geometries in the spectral range from 700 to 1280  $\text{cm}^{-1}$ . The band observed at 777  $\text{cm}^{-1}$  is assigned as bending of  $\text{CO}_2^-$ ,  $\delta(\text{CO}_2^-)$ ;

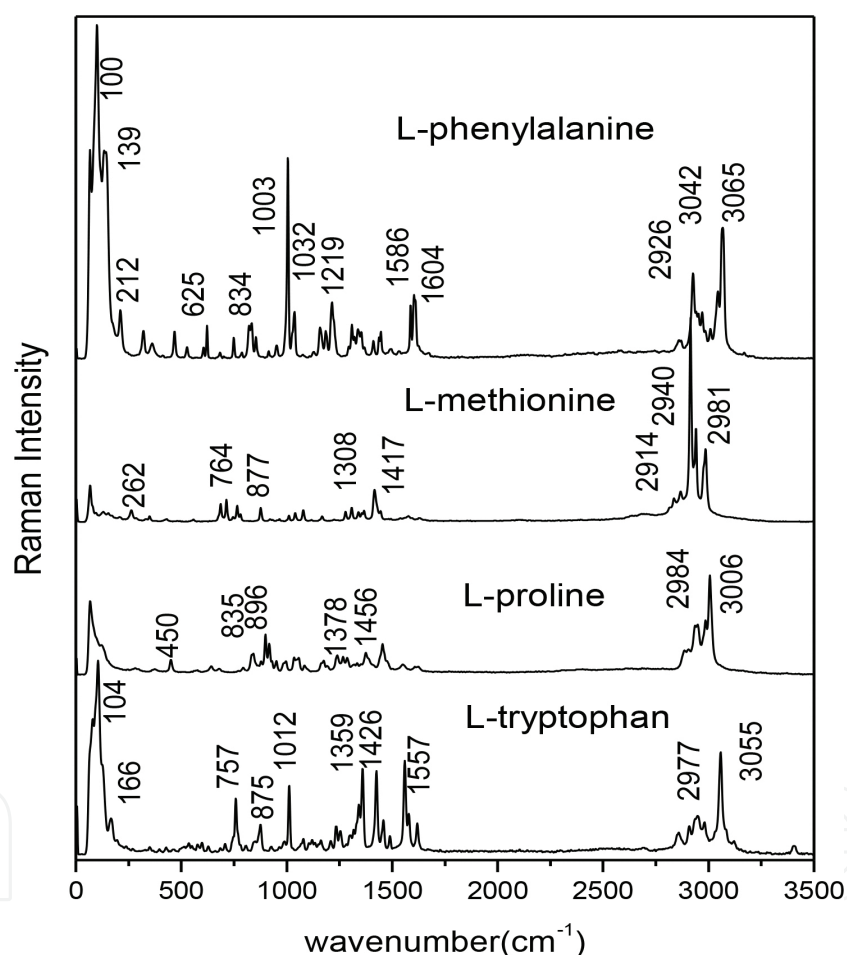
at  $838\text{ cm}^{-1}$  as out-of-plane vibration of  $\text{CO}_2$ ,  $\gamma(\text{CO}_2)$ ;  $849\text{ cm}^{-1}$  as rocking of  $\text{CH}_2$ ,  $r(\text{CH}_2)$ . The bands between  $919$  and  $1004\text{ cm}^{-1}$  are assigned as stretching vibration of  $\text{CC}$ ,  $\nu(\text{CC})$ , and the bands at  $1032$  and  $1083\text{ cm}^{-1}$  are assigned as stretching of  $\text{CN}$ ,  $\nu(\text{CN})$ , while the band at  $1131\text{ cm}^{-1}$  is assigned as rocking of  $\text{NH}_3^+$ ,  $r(\text{NH}_3^+)$  unit [22]. The Raman spectroscopic study showed a series of modifications at about  $353\text{ K}$  in both the internal and lattice modes of L-leucine, indicating a possible modification of the structure. This was interpreted as a phase transition from a  $\text{C}_2$  to a  $\text{C}_s$  structure, even with the appearance of a TO mode at high temperature. L-leucine was also investigated under high pressure with the scrutiny of Raman spectroscopy [23]. Anomalous behavior was observed in two ranges, from  $0$  to  $0.46\text{ GPa}$  and from  $0.8$  to  $1.46\text{ GPa}$ . The first anomaly was realized through the observation of the disappearance of a band in the  $\text{CH}$  and  $\text{CH}_3$  stretching region of the spectrum. The second anomaly is verified through the disappearance of lattice modes and splitting of modes in the high wavenumber region. Obviously, some of the modifications must involve molecular rearrangements due to changes of hydrogen bonds. Again, Raman spectroscopy appears as a powerful tool in order to study the phase transitions.



**Figure 5.** FT-Raman spectra of L-isoleucine in the spectral range from 20 to  $3500\text{ cm}^{-1}$ . The most intense peaks appear in the high wavenumber region of the spectrum.

**Figure 5** presents the FT-Raman spectrum of *L-isoleucine* in the spectral range from 20 to  $3500\text{ cm}^{-1}$ . We observe that the most intense bands is located in the high wavenumber region of the spectrum, corresponding to bands associated with stretching of  $\text{CH}$ ,  $\text{CH}_2$ , and  $\text{CH}_3$ ; in fact, the spectral range of  $2700\text{--}3200\text{ cm}^{-1}$  presents a very complex profile, with at least seven different bands [24]. On the other hand, the region between about  $1700$  and  $2700\text{ cm}^{-1}$  does not present bands, as occurs with most proteinogenic amino acids (exception to cysteine that presents bands associated with  $\text{SH}$  stretching vibration at about  $2500\text{ cm}^{-1}$ ). A series of bands is observed between  $500$  and  $1650\text{ cm}^{-1}$ , including vibrations associated with stretching of  $\text{CC}$ ,  $\nu(\text{CC})$ , from

872 to 1018  $\text{cm}^{-1}$ , stretching CN,  $\nu(\text{CN})$ , at 1033 and 1091  $\text{cm}^{-1}$ , rocking of  $\text{CH}_3$ , rocking of  $\text{CO}_2^-$ , at 536  $\text{cm}^{-1}$  etc. Several modes associated with bending vibrations are observed in the 300–500  $\text{cm}^{-1}$  spectral range. The vibration associated with torsion of  $\text{CO}_2^-$ ,  $\tau(\text{CO}_2^-)$ , is observed at  $\sim 177$   $\text{cm}^{-1}$ ; such vibration is common to most amino acid crystals. Finally, below 170  $\text{cm}^{-1}$  bands are observed that are generically associated with the lattice modes of the crystal. In relation to the behavior of the crystal under low temperature conditions, Raman spectroscopy showed that the L-isoleucine crystal does not present any evidence of phase transition, similarly to L-alanine and L-leucine, but differently from L-valine, which presents a modification at about 100 K. Such fact is very curious and future investigations are demanded in order to shed light in this problem.



**Figure 6.** FT-Raman spectra of L-phenylalanine, L-methionine, L-proline and L-tryptophan in the spectral range from 20 to 3500  $\text{cm}^{-1}$ .

The Raman spectra of *L-phenylalanine*, *L-methionine*, *L-proline*, and *L-tryptophan* are shown in **Figure 6**. It is very difficult to grow crystals of L-phenylalanine and L-tryptophan in their pure forms. L-proline grows mainly in a hydrated form, while it is relatively easy to grow L-methionine. As a consequence there are few studies reporting vibrational properties of L-phenylalanine [25] and L-tryptophan [26], as well L-proline [5], and a little more on

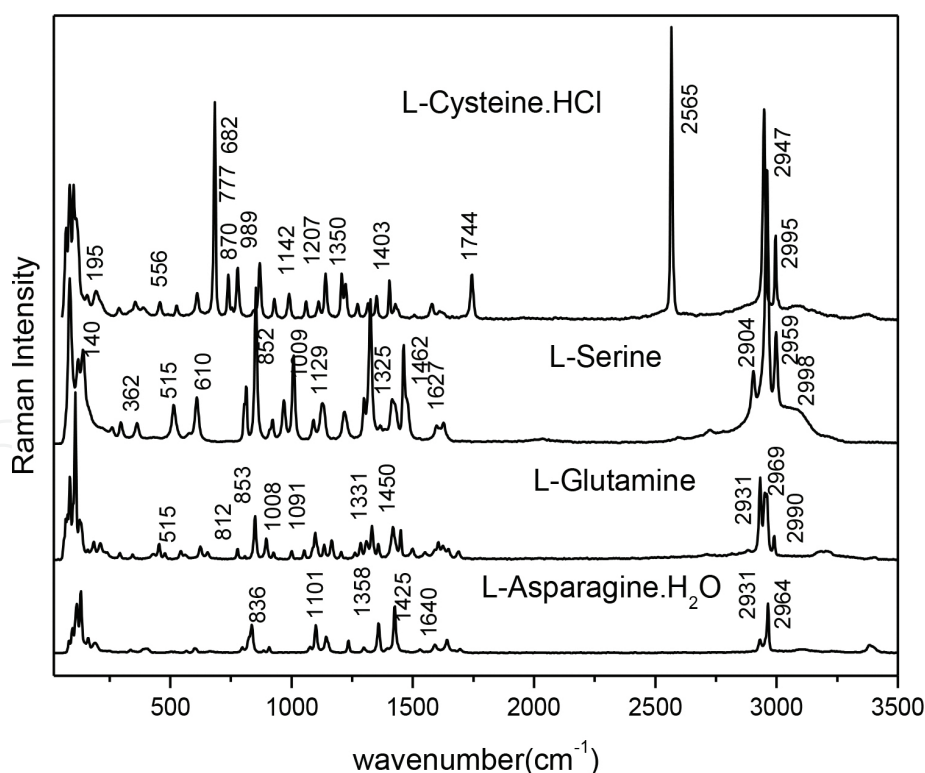
L-methionine [27]. In relation to the previous amino acid presented in this chapter, phenylalanine and tryptophan show an additional benzene ring. As a consequence, this unit presents vibrations associated with it: stretching of CH at 2979, 3030, and 3057  $\text{cm}^{-1}$ ; rocking at  $\sim 1260 \text{ cm}^{-1}$ ; deformation at about 704  $\text{cm}^{-1}$ ; ring breathing at  $\sim 1010 \text{ cm}^{-1}$  and wagging at  $\sim 744 \text{ cm}^{-1}$ , for L-tryptophan [25]. The L-phenylalanine shows that vibrations related to the benzene ring are observed at 848, 912, and 949  $\text{cm}^{-1}$  (out-of-plane bending); 1001  $\text{cm}^{-1}$  (breathing of the ring); 1025 and 1076  $\text{cm}^{-1}$  (in-plane bending); 1600  $\text{cm}^{-1}$  (stretching of CC in the ring). Some results on L-proline monohydrated were presented in reference [5], where a high pressure investigation is furnished; the work shows evidence of two phase transitions, between 0.0 and 1.1 GPa and another between 6.5 and 7.8 GPa. In relation to L-methionine, a detailed study of the Raman spectra of the crystal under high pressure was revealed in reference [27]. Although the methionine molecule presents a sulfur atom, differently from the cysteine, in the methionine there is a connection linking two carbon atoms, C–S–C; as a consequence, the very intense band observed at  $\sim 2500 \text{ cm}^{-1}$  appearing in the Raman spectrum of cysteine due to the S–H stretching is not observed in the Raman spectrum of methionine. However, a very characteristic band associated with the C–S stretching vibration is observed at 659  $\text{cm}^{-1}$  in the spectrum of L-methionine. Following this band, when the L-methionine crystal was subjected to high pressure into a diamond anvil cell, it was observed an impressive modification that was associated with a phase transition undergone by the crystal at about 2.2 GPa [27]. Also interesting is the fact that the phase transition occurs with a hysteresis of  $\sim 0.8 \text{ GPa}$ , suggesting the transition can be classified as a first-order one.

#### 4. Neutral, polar amino acids

Among the polar amino acids, serine, cysteine, asparagine, glutamine, threonine, and tyrosine are neutral. **Figure 7** shows the Raman spectra of L-cysteine.HCl, L-serine, L-glutamine, and L-asparagine.H<sub>2</sub>O as obtained through a Fourier-transform Raman spectrometer in the spectral range from 50 to 3500  $\text{cm}^{-1}$ . From 1800 to 2800  $\text{cm}^{-1}$  no mode is observed, except in the spectrum of L-cysteine.HCl, where a stretching vibration of SH appears at  $\sim 2550 \text{ cm}^{-1}$ . In fact, as mentioned in the previous paragraph, cysteine is the only proteinogenic amino acid that presents an S–H bond and, as a consequence, is the only amino acid to present a peak in this region.

It is important to remember that *L-cysteine* can be found without HCl ions in the unit cell. L-cysteine can be obtained under ambient conditions in two different crystalline polymorphs, orthorhombic, and monoclinic. The orthorhombic structure of L-cysteine crystallizes with  $Z = 4$  and space group  $P2_12_12_1$ . One of the important characteristics among the amino acid crystals is the fact that L-cysteine presents the S–H...S hydrogen bond, extending along the **b** crystallographic direction. As a consequence, L-cysteine constitutes a model to understand the important sulfur hydrogen bonds involved in some proteins of the human being. In a polarized Raman spectroscopic study, it was observed that at low temperatures, the S–H...S hydrogen bonds contribute to form an ordered crystal structure, but upon heating, the thiol-groups appear slightly disordered [28]. As pointed out in this reference, some of the S–H...S

hydrogen bonds are substituted by S-H...O bonds in such a way that at room temperature the number of the two species of hydrogen bonds is approximately the same. Interestingly enough is the fact that the change of hydrogen bonds with the substitution of sulfur by oxygen is not sharp, but occurs through a series of intermediate states [28]. We remember that L-alanine, when submitted to low temperature conditions, also presents a pathological behavior, i.e., the *c* crystallographic parameter decreases in a succession of steps and plateaus. The jumps on the *c* parameter were interpreted for L-alanine as attempts to relax some frustration [29]. Returning to the L-cysteine case, the modification in the thiol group is related to different orientations of the cysteine zwitterion, which are tuned by the strong N-H...O hydrogen bonds. Additionally, it was observed that different groups (NH<sub>3</sub>, SH, CH, and CH<sub>2</sub>) are activated in different temperature ranges, similarly with was observed for the NH<sub>3</sub> and CH<sub>3</sub> groups of L-alanine [30]. Under high pressure, the Raman spectrum of orthorhombic L-cysteine presents noticeable modifications that can be summarized as follows [31]. Above 0.1 GPa redistribution of intensities of the components of the bands associated with stretching of SH,  $\nu(\text{SH})$ , suggests a continuous decrease in the number of sulfhydryl groups participating of S-H...S hydrogen bonds. This tendency remains until the pressure arrive to 1.6–1.9 GPa, when is observed an impressive change in the Raman spectrum, associated with a phase transition. One of these changes is the downshift of the wavenumber of  $\nu(\text{SH})$  by  $\sim 40 \text{ cm}^{-1}$ , as well as the splitting of this band; such facts suggest both (i) the S-H...S hydrogen bond



**Figure 7.** FT-Raman spectra of polar (neutral) amino acids L-serine, L-glutamine and compounds L-cysteine.HCl and L-asparagine.H<sub>2</sub>O. In the spectrum of L-cysteine.HCl stands out an intense band associated with the S-H stretching,  $\nu(\text{SH})$ , similar to the monoclinic phase of L-cysteine, but different from the orthorhombic phase, where broad split bands are observed (see text).

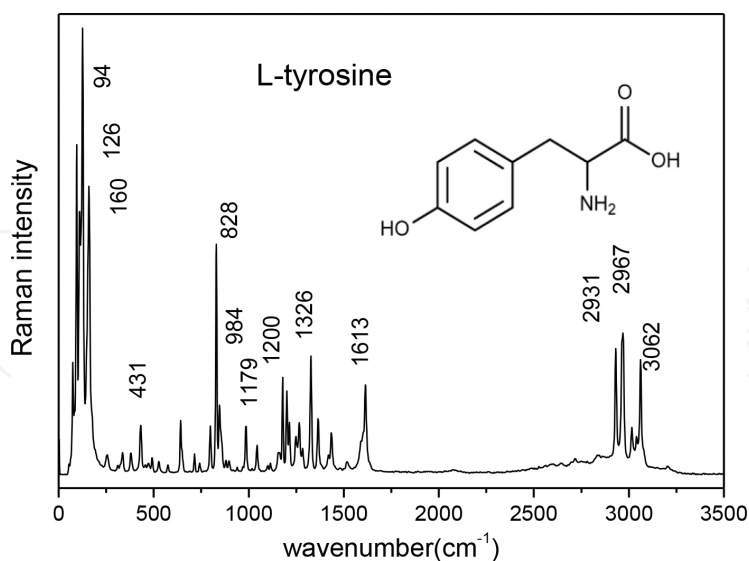
strengthen and (ii) increasing of disorder of SH group. Above 2.3 GPa, the wavenumber of  $\nu(\text{SH})$  presents a upshift of  $30\text{ cm}^{-1}$ , indicating the weakening of hydrogen bonds related to S-H groups. However, the modes are split above this pressure, and we can consider that the sulfhydryl groups are still disordered.

Obviously, if you change the sulfur atom by an oxygen atom, the hydrogen bonds involving SH groups cease to exist. As a molecule, *serine* is a copy of cysteine but with oxygen replacing the sulfur atom. L-serine crystallizes in an orthorhombic structure with space group  $P2_12_12_1$ , the same of one of the polymorphs of L-cysteine. A Raman spectroscopic study showed that L-serine presents changes at  $\sim 140\text{ K}$ . the changes were interpreted as reorientation of the side chain  $\text{CH}_2\text{OH}$  with respect to the C-C bonds of the skeleton of the molecule [32]. As a consequence, a positional disorder of the O-H...O intermolecular hydrogen bond is verified. Such a fact was realized through the analysis of the behavior of stretching of OH,  $\nu(\text{OH})$ , allowing to separate the temperature evolution of O-H...O hydrogen bond among the other formed by serine molecules in the crystal structure: N-H...O in the head-to-tail chains, N-H...O between antiparallel chains and N-H...O between **ab** layers [32]. This constitutes a very beautiful example of the power of Raman spectroscopy to play light in a so complicated theme as is hydrogen bond.

*Glutamine* is the more abundant proteinogenic amino acid in the human blood, occupying a pivotal position in the nitrogen metabolism. The radical of the amino acid is characterized by the groups  $\text{NH}_2\text{-(C=O)-CH}_2\text{-CH}_2$ . As a consequence, the high spectral region of the Raman spectrum of L-glutamine presents a rich profile. In the FT-Raman spectrum a very strong band observed at  $2933\text{ cm}^{-1}$  is associated with the symmetric stretching of  $\text{CH}_2$ ,  $\nu_s(\text{CH}_2)$ ; a doublet at  $2952$  and  $2962\text{ cm}^{-1}$  is associated, respectively, with the stretching CH,  $\nu(\text{CH})$ , and the symmetric stretching of  $\text{CH}_2$ ,  $\nu_s(\text{CH}_2)$ ; a peak observed at  $2991\text{ cm}^{-1}$  is assigned as antisymmetric stretching of  $\text{CH}_2$ ,  $\nu_a(\text{CH}_2)$ . Above  $3100\text{ cm}^{-1}$  it is possible to observe bands with low intensity associated with  $\text{NH}_2$  and  $\text{NH}_3^+$  groups: at  $3176\text{ cm}^{-1}$ , assigned as symmetric stretching of  $\text{NH}_2$ ; at  $3210\text{ cm}^{-1}$ , assigned as symmetric stretching of  $\text{NH}_3^+$  and at  $3403\text{ cm}^{-1}$ , assigned as antisymmetric stretching of  $\text{NH}_2$ . Such assignment, performed on reference [33] with the use of deuterated L-glutamine samples will be fundamental to understand the behavior of the crystal submitted to extreme conditions e.g., high pressure and low temperatures.

The radical  $\text{NH}_2\text{-(C=O)-CH}_2$  characterizes the amino acid L-*asparagine*. Although is possible to grow small crystals of the pure form, most of the studies on vibrational spectroscopy deals with the hydrate form, monohydrated L-asparagine (MLA). This crystal was studied in a series of papers [34, 35]. A Raman spectroscopic study revealed that under low temperature MLA undergoes a phase transition between  $140$  and  $150\text{ K}$ . The modification is clearly realized through the observation of splitting of a band assigned as lattice modes, at  $\sim 130\text{ cm}^{-1}$  [34]. Under high temperature, MLA also presents a phase transition, as it was shown by Raman scattering measurements [35]. At about  $363\text{ K}$ , the orthorhombic  $P2_12_12_1$  structure of MLA change drastically, as it is possible to infer from the impressive modifications of the Raman spectra above this temperature. Interesting enough, while in the low temperature phase transition the modifications are observed mainly in the low wavenumber region of the Raman spectrum, in the high temperature phase transition modifications occur in all spectral range.

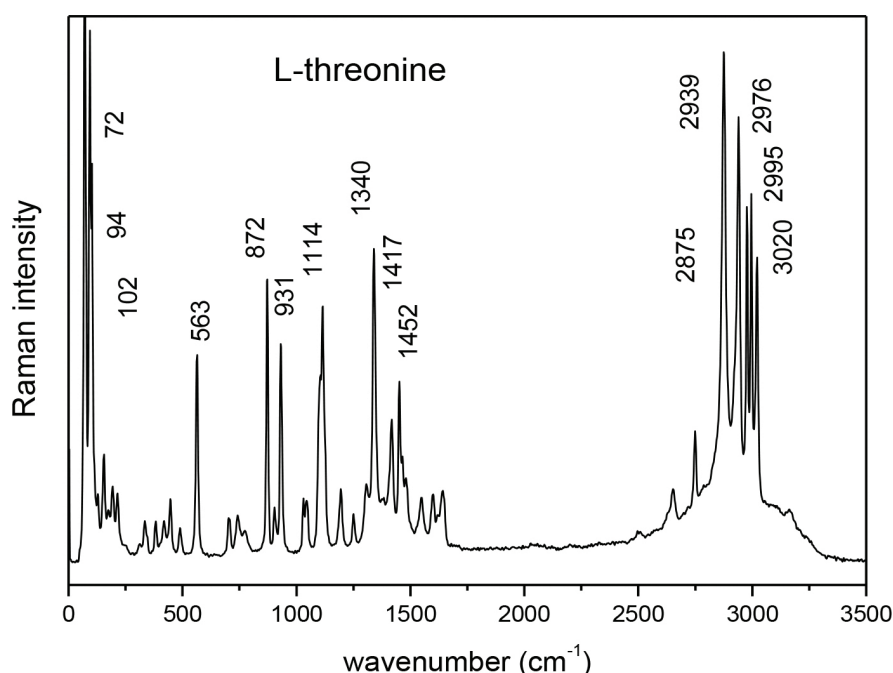
Something that also deserves attention is the behavior of MLA under high pressure. In a recent work [36] authors have investigated the material up to 30 GPa. This is the highest pressure value utilized in experiments on the vibrational properties of amino acid crystals up to now (we remember that L-alanine presents a crystal-amorphous phase transition at 15 GPa, half of the pressure value reached in the experiment with MLA). In this work, analyzing most of the Raman bands in the spectral range 30–3600  $\text{cm}^{-1}$ , it was possible to note modifications that were correlated with phase transition undergone by MLA, as well as, with conformational changes of the molecules in the unit cell of the crystal. The changes observed at approximately 10 GPa were associated with a phase transition and other modifications between 2.1 and 3.1 GPa and between 15.0 and 17.0 GPa were associated with conformational changes. In particular, the most impressive modifications occur in the high wavenumber region of the spectrum. Very suggestive is the fact that the wavenumbers of the antisymmetric stretching of  $\text{NH}_2$ ,  $\nu_{\text{A}}(\text{NH}_2)$  and symmetric stretching of  $\text{H}_2\text{O}$ ,  $\nu_{\text{S}}(\text{H}_2\text{O})$  decrease in the interval at 1 atm and 8.5 GPa, indicating that hydrogen bonds are strengthened in this pressure interval. The explanation for the anomalous behavior is because N-H...O hydrogen bond interaction is stiffened due the approximation of molecules under compression, weakening the covalent N-H interaction and, consequently, shifting the wavenumber of the two modes to lower values. During the transition at 10 GPa, the bands associated with stretching of water molecule show a positive jump, indicating a new environment for the molecules. However, between 10.6 and 15 GPa and above 17.9 GPa the symmetric stretching of water goes, respectively, to higher and to lower wavenumbers, signaling different behavior of the hydrogen bonds. As a résumé for the data on MLA, we can affirm that Raman spectroscopy furnished a precise picture about the hydrogen bonds allocated in the unit cell of the crystal.



**Figure 8.** FT-Raman spectrum of L-tyrosine. It is interesting to observe the intense peaks characterizing the low wavenumber of the spectrum. The inset presents a representation of the molecule.

The FT-Raman spectrum of *L-tyrosine* is shown in **Figure 8** (the inset shows a representation of the molecular formula). As occurs with L-phenylalanine and L-tryptophan, L-tyrosine

presents a benzene ring in its radical. Consequently, it is expected that vibrations associated with breathing of the benzene ring and CC stretching related to the atoms of the ring appear in the Raman spectrum of L-tyrosine with frequencies similar to those of L-phenylalanine and L-tryptophan. It is also interesting to observe that the low wavenumber bands are the most intense bands appearing in the Raman spectrum. Because these bands are assigned mainly as lattice modes, it will be of interest in future studies where one should search for eventual phase transitions. In the high wavenumber region of the Raman spectrum, it is possible to observe distinctly five different bands that are associated with the stretching vibrations of CH and CH<sub>2</sub>,  $\nu(\text{CH})$  and  $\nu(\text{CH}_2)$ , respectively. Up to now, there is no work published in the literature discussing the vibrational behavior of L-tyrosine subjected to neither low temperature nor high pressure conditions, among other extreme conditions.



**Figure 9.** FT-Raman spectrum of L-threonine in the spectral range 20–3500 cm<sup>-1</sup> recorded at room temperature. It is interesting the observation of a very complex profile in the high wavenumber region of the spectrum although threonine is a relatively simple molecule.

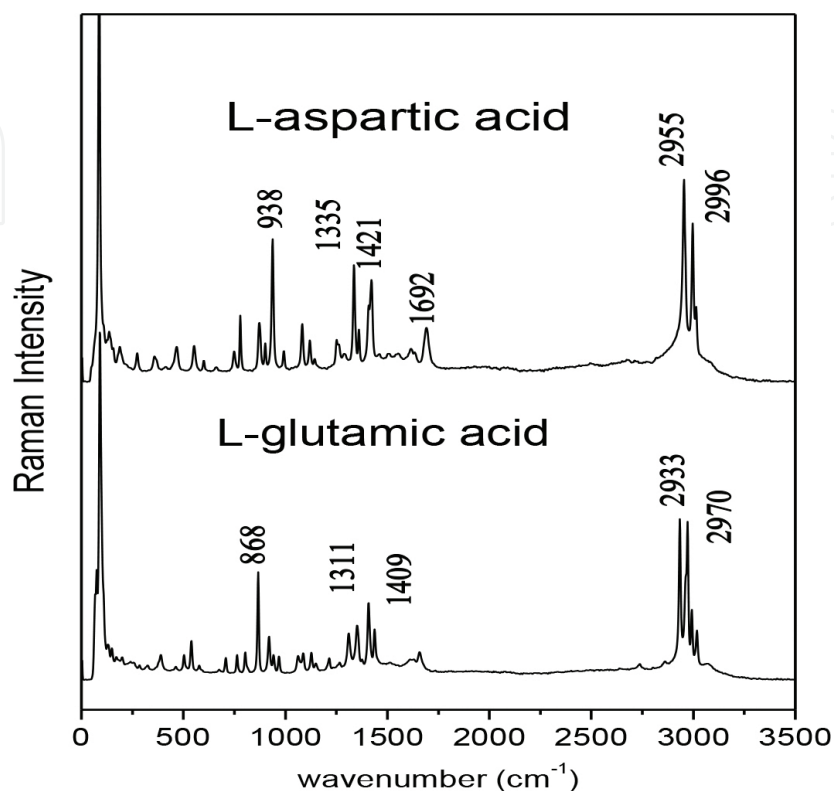
**Figure 9** shows the FT-Raman spectrum of *L-threonine* in the spectral range from 20 to 3500 cm<sup>-1</sup>. Most of the bands appearing in the low wavenumber region are associated with lattice modes and, as a consequence, their behavior can furnish information about the stability of the unit cell [37]. L-threonine crystallizes in an orthorhombic structure with space group *P*2<sub>1</sub>2<sub>1</sub>2<sub>1</sub>. Vibrations associated with CC stretching are observed as bands in the spectral range 907–940 cm<sup>-1</sup>. Rocking of CO<sub>2</sub><sup>-</sup>,  $\rho(\text{CO}_2^-)$  is observed at ~568 cm<sup>-1</sup>. It is interesting in this point remember that  $\rho(\text{CO}_2^-)$  vibration is observed at 515 cm<sup>-1</sup> in L-serine, 530 cm<sup>-1</sup> in L-histidine hydrochloride monohydrated, 553 cm<sup>-1</sup> in L-asparagine monohydrated and in L-cysteine, 545 cm<sup>-1</sup> in L-methionine and 541 cm<sup>-1</sup> in L-valine. So, this kind of vibration that appears in the Raman spectrum as a band of relatively high intensity presents a well-specific range where

it is observed. It is also relevant to point out that the torsion of  $\text{NH}_3^+$ ,  $\tau(\text{NH}_3^+)$ , is observed in L-threonine at  $497\text{ cm}^{-1}$ . A recent Raman scattering study—where a sample of L-threonine was put into a diamond anvil cell set up—showed an impressive phase transition close to 2 GPa, another between 8.2 and 9.2 GPa, and a third between 14 and 15.5 GPa. The principal indication for the occurrence of these pressure-induced phase transitions is the modification in the bands associated with external modes. Additional changes observed in bands related to  $\text{CO}_2$ ,  $\text{NH}_3$ , and  $\text{CH}_3$  units of the threonine molecule also corroborate the occurrence of the three-phase transition [38]. However, although the maximum pressure reached in the experiment was 27 GPa, no evidence of amorphization was observed. This point is being analyzed because, on the contrary, L-alanine undergoes a crystal — amorphous phase transition for pressure of only 15 GPa. Additionally, a comparative study looking for a correlation between the behavior of  $\text{NH}_3$  torsional modes of L-alanine, L-threonine, and taurine with the hydrogen bond dimensions was given in reference [17]. A possible connection between the hydrogen bond dimensions and the amorphous state of the amino acid crystal can give interesting insights about the phenomenon.

## 5. Acidic, polar amino acids

**Figure 10** shows the FT-Raman spectra of *L-aspartic acid* and *L-glutamic acid* in the spectral range from 20 to  $3500\text{ cm}^{-1}$ . In some aspects, the two spectra are similar, e.g., the lattice modes appear as very intense bands in the low wavenumber region of the spectrum and the profiles of the Raman spectra in the high wavenumber region are very similar for the two crystals. L-glutamic acid crystallizes in two possible polymorphs, called  $\alpha$  (like prisms) and  $\beta$  (as platelets). Both polymorphs crystallize in an orthorhombic structure in a  $P2_12_12_1$  space group. In **Figure 10**, the main contribution for the FT-Raman spectrum of L-glutamic acid is from the  $\beta$ -form. For this polymorph, there is no intramolecular hydrogen bond, but a strong hydrogen bond between two carboxylic groups in neighboring molecules is observed; such a bond form links along the *b*-direction. In the L-glutamic acid, the symmetric stretching of  $\text{NH}_3^+$  appears—as occurs with most of the amino acid crystals—as a weak band, observed at  $3073\text{ cm}^{-1}$ . A very strong band associated with the stretching of  $\text{CH}_2$  is observed at  $2974$  and  $2938\text{ cm}^{-1}$ . Vibrations associated with rocking of  $\text{NH}_3^+$  are observed at  $1128$  and  $1149\text{ cm}^{-1}$  and the stretching of CC is observed at  $970$  and  $1062\text{ cm}^{-1}$ . At  $804$  and  $866\text{ cm}^{-1}$  (the last one as a very strong band) bands are associated with rocking of  $\text{CH}_2$ . Vibrations associated with the skeleton of the L-glutamic acid are observed in the spectral range of  $240\text{--}398\text{ cm}^{-1}$ . Finally, the torsion of  $\text{CO}_2^-$  unit is observed at  $199\text{ cm}^{-1}$  and bands with wavenumber lower than this value are associated with the lattice vibrations of the crystal. Raman spectroscopy was used to investigate the vibrational properties of a crystal of L-glutamic acid in its  $\beta$ -form. From this study, authors have observed modifications that can be considered as evidence that the crystal undergoes some phase transitions. One modification in the Raman spectrum was observed between 0.5 and 1.3 GPa. The second modification was noted between 2.6 and 3.1 GPa; the third modification was observed for pressures between 5.4 and 6.4 GPa and the fourth change in the Raman spectra was observed for pressures between 13.9 and 15.9. Again,

it is important to inform that at these pressures there is no evidence of amorphization for the crystal of L-glutamic acid; in fact, even at the highest pressure reached in the experiments (21.5 GPa), the crystal shows all bands, given no evidence of an amorphous phase [39].



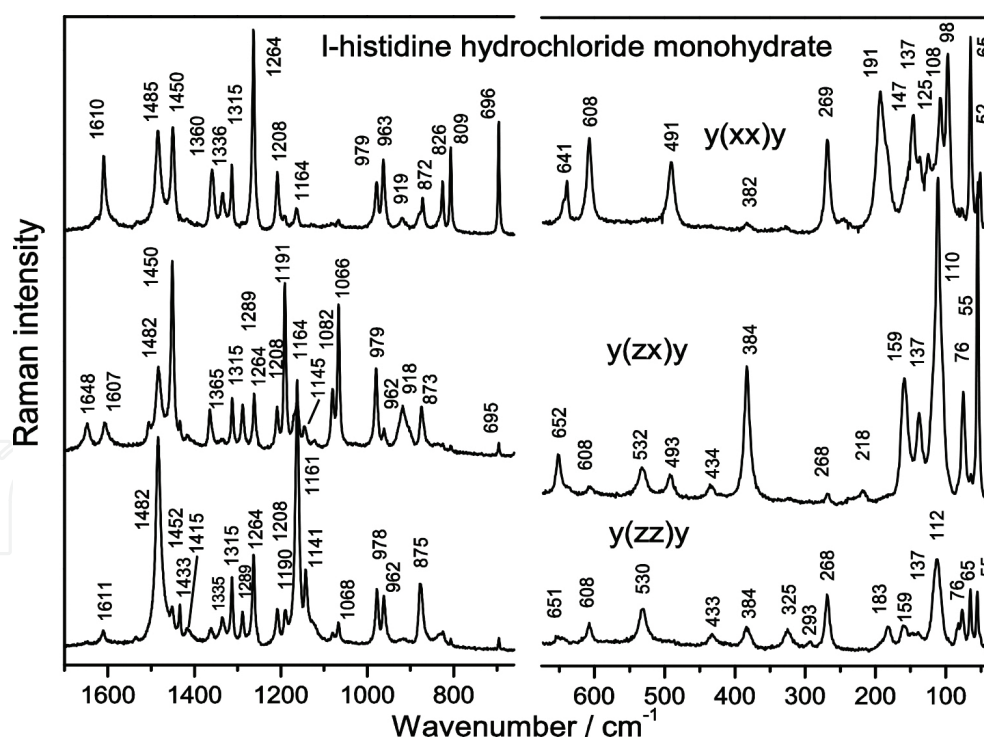
**Figure 10.** FT-Raman spectra of L-aspartic acid and L-glutamic acid in the spectral range 20–3500  $\text{cm}^{-1}$  recorded at room temperature.

## 6. Basic, polar amino acids

*L-Lysine*, *L-arginine* and *L-histidine* are the basic, polar amino acids. Lysine has as characteristic a radical composed of the following group of atoms  $\text{CH}_2\text{--CH}_2\text{--CH}_2\text{--CH}_2\text{--NH}_2$ . Almost no work on Raman spectroscopy of L-lysine was published. However, Hernández et al. [40] showed the assignment of the main modes of the Raman spectrum of L-lysine. For example, the stretching of CC is observed at 1012, 1033, 1063, and 1076  $\text{cm}^{-1}$ ; the antisymmetric rocking of  $\text{NH}_3^+$  is observed at 1143 and 1183  $\text{cm}^{-1}$ ; the antisymmetric bending of  $\text{NH}_3^+$  appears as bands at 1615 and 1650  $\text{cm}^{-1}$ ; the symmetric stretching of  $\text{CO}_2^-$  at 1415  $\text{cm}^{-1}$  and the antisymmetric stretching of  $\text{CO}_2^-$  at 1598  $\text{cm}^{-1}$ . Hernández et al. [40] also presented a tentative assignment of most bands appearing in the Raman spectrum of L-arginine. The radical characterizing L-arginine is  $\text{CH}_2\text{--CH}_2\text{--CH}_2\text{--NH--C--NH--NH}_2$ . The Raman spectrum of L-arginine shows bands at 1011 and 1035  $\text{cm}^{-1}$  which are associated with CC stretching, while a band at 970  $\text{cm}^{-1}$  is associated with CN stretching. Rocking of  $\text{NH}_3^+$  is observed at 1164  $\text{cm}^{-1}$ .

<sup>-1</sup> and antisymmetric bending of CNH<sub>2</sub> is observed at 1176 cm<sup>-1</sup>. The symmetric stretching of CO<sub>2</sub><sup>-</sup> is observed at 1581 cm<sup>-1</sup> and the antisymmetric stretching of CO<sub>2</sub><sup>-</sup> appears at 1581 cm<sup>-1</sup> [40].

L-histidine was investigated through Raman spectroscopy in a recent paper that explored the vibrational behavior of the crystal under cryogenic conditions [41]. L-histidine can crystallize in two different polymorphs with monoclinic or orthorhombic symmetry. The work of reference [41] has investigated the orthorhombic form of the crystal that presents a *P*2<sub>1</sub>2<sub>1</sub>2<sub>1</sub> space group with four molecules per unit cell. It is interesting to note that many of the amino acids crystallize in a *P*2<sub>1</sub>2<sub>1</sub>2<sub>1</sub> space group with orthorhombic structure or in a *P*2<sub>1</sub> space group with monoclinic structure; this possibly is related to the packing of molecules in the unit cell, but this point will not be explored in the present text. Returning to the case of L-histidine, the Raman scattering study in reference [41] showed a series of discontinuity in the wavenumber of bands at about 165 K. This was interpreted as consequence of a conformational phase transition through involving both CO<sub>2</sub><sup>-</sup> and NH<sub>3</sub><sup>+</sup> groups. It is interesting to add the information that L-arginine and L-histidine can also easily grow as hydrated and as chloride hydrated crystals. In **Figure 11**, the Raman spectra of L-histidine hydrochloride monohydrated crystal are shown for three different scattering geometries in order to illustrate a case of an amino acid crystallizing with water and HCl units.



**Figure 11.** Raman spectra of L-histidine hydrochloride monohydrate in three scattering geometries (adapted from reference [43]).

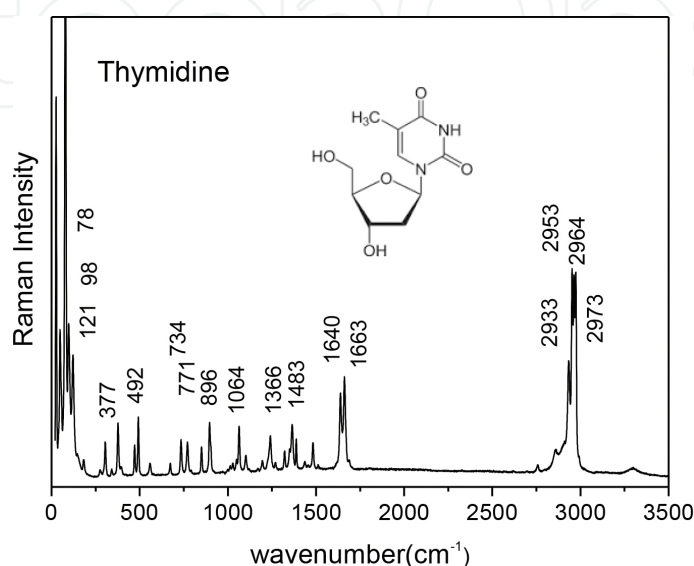
Up to now, the Raman scattering investigations have furnished an interesting picture about the vibrational aspects of diverse amino acids. Some studies have even studied the behavior

of the crystals under extreme conditions, low temperature or high pressure. However, a complete understanding involving connections, for example, between the hydrogen bonds and the physical properties of the crystal is still lacking. Obviously, some preliminary attempts are already known, such as a possible connection between the dimensions of hydrogen bonds and the behavior of torsional vibration of  $\text{NH}_3^+$  under high pressure (for L-alanine, L-threonine, and taurine [17]). A fundamental question in biochemistry is to realize why the proteins of all living beings are formed by the L-form of amino acids (the D-form is found only isolated in the plasma of certain cells). Some glimpses were given by Abdus Salam who speculates the occurrence of a phase transition explained through BCS theory, gauge field theory, and Higgs mechanism [44]. There is also suggestion that ultraviolet radiation should be able to select one of the chiral forms of the amino acid, but, in fact, all these suggestions are suppositions waiting for confirmation. This problem deserves future investigations. But, is the behavior of D-amino acid crystals the same of L-amino acids under extreme conditions? At first, the answer to this question should be positive because both L- and D-forms of the amino acids are equivalent from an energetic point of view. However, some preliminary results point to different behavior for the two forms in some special cases, but we do not have space to discuss such intriguing point in this chapter. Maybe, surprising information is waiting for us in the coming years.

## 7. Beyond amino acids

The success obtained by the investigation of amino acids has incentivized the study of other simple organic molecules of living beings. After furnishing a more or less closed picture about amino acids, the next natural step is the study of peptides, but we will not discuss them in this chapter. We prefer to analyze another natural choose, molecules involved in the DNA structure. One example we will explore in this chapter is thymidine, a nucleoside constituted of a deoxyribose and the pyrimidine base thymine. It is found in the DNA of all living organisms. The Raman spectrum presents a very intense set of bands in the low wavenumber region that are associated with the lattice modes (**Figure 12**). This is very interesting because in future analysis of the crystal under extreme conditions, the behavior of the lattice modes should be a pivotal point in order to understand eventual structural modification. A strong band observed at  $1665\text{ cm}^{-1}$  is assigned as in-plane vibration involving  $\text{C}=\text{O}$  and  $\text{C}=\text{C}$  and a band at  $1690\text{ cm}^{-1}$  is assigned as stretching  $\text{C}=\text{O}$ ,  $\nu(\text{C}=\text{O})$ . Bending of  $\text{CH}_3$ ,  $\delta(\text{CH}_3)$ , is identified as the band at  $1438$ ,  $1457$ , and  $1480\text{ cm}^{-1}$ . The band observed at  $1031\text{ cm}^{-1}$  is associated with bending of  $\text{CNH}$ ,  $\delta(\text{CNH})$ , and the band at  $1000\text{ cm}^{-1}$  is associated with bending  $\text{OCH}$ ,  $\delta(\text{OCH})$ . An out-of-plane vibration involving  $\text{CH}$  is observed at  $972\text{ cm}^{-1}$  and a pyrimidine ring breathing is observed at  $773\text{ cm}^{-1}$ . Additionally, out-of-plane vibration involving  $\text{CCH}_3$  group is observed at  $396$  and  $378\text{ cm}^{-1}$  and in plane vibration involving the same group is observed at  $276$  and  $306\text{ cm}^{-1}$ . In the high wavenumber region of the Raman spectrum is possible to observe a series of bands, among them one observed at  $3298\text{ cm}^{-1}$  that was assigned as stretching of  $\text{OH}$ ,  $\nu(\text{OH})$ . A series of bands is observed at  $2952$ ,  $2965$ ,  $2973$ , and  $2991\text{ cm}^{-1}$  and they are classified as stretching of  $\text{CH}$ ,  $\text{CH}_2$ , and  $\text{CH}_3$  units. Finally, let us single out an important point related to the study of thymidine, its behavior as a function of temperature.

In order to make the presentation of this section more complete, we have performed study of thymidine crystal under low temperature. Analysis of the Raman spectra of thymidine showed that the wavenumber of several bands presents jumps at about 160 K, suggesting the occurrence of a conformational modification due change of hydrogen bonds. A comparison with the behavior of amino acid crystals will be welcome, and we hope that in a few time we will have an overview of the subject.



**Figure 12.** Raman spectrum of thymidine; in the inset a representation of the molecule.

In résumé, in this chapter, a complete picture about the Raman spectra of the 20 proteinogenic amino acid crystals was furnished and some aspects related to the modification of these spectra under extreme conditions were also discussed. As additional information we discussed the Raman spectrum of thymidine, an organic molecule involved in the formation of DNA.

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