Polarized Raman Spectra and Infrared Analysis of Vibrational Modes in L-Threonine Crystals

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Polarized Raman and infrared spectra of l-threonine crystals have been obtained at room temperature and an assignment of normal modes is proposed based on group theory analysis. The usefulness of these assignments comes from the fact that some modes are related to important vibrations that can show changes under different pressure and temperature conditions.

I. Introduction

Studies of temperature vibrational spectra of amino acids by Raman and infrared spectroscopies are useful in obtaining information regarding the behavior of normal modes and stability of the structure under changes of external conditions, molecular conformation, the effects of various types of intermolecular forces on vibrational frequencies and, sometimes, the nature of hydrogen bonding in these biologically fundamental substances. Also, optically active amino acids contain many highly efficient optical second-harmonic generators and are promising candidates for a great number of applications [1-3].

L-threonine is an important amino acid found in several proteins of human being such as γ-globulin, β-lactoglobulin, hemoglobin, insulin, silk fibroin, among others [2]. By the physical point of view the l- threonine investigation is relevant both owing to the possibility to observe the behavior of a system where the hydrogen bond plays a fundamental role [3- 5] and the technological importance of a material which shows a second-harmonic conversion efficiency greater than 1 relative to potassium dihydrogen phosphate [1].

In this paper we present Infrared (I.R.) absorption measurements and polarized Raman scattering results for l-threonine at room temperature. Assignments of some internal modes are also given.

II. Experimental

The experiments were performed on monocrystalline samples grown at controlled temperature (40°C) from acqueous solutions of l- threonine powder. Infrared spectrum between 400 and 4000 cm^{-1} of l- threonine powder was obtained by mixing l-threonine powder with KBr using a Hartmann & Braun MB100 spectrometer. The excitation source in the Raman experiments was a 514.5 nm radiation from an Argon ion laser and the scattered light was analyzed using a Spex

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1402 double monochromator and a Model C 31034-RF photomultiplier from RCA. The samples were placed into an helium closed-cycle refrigeration system where the temperature could be maintained constant to ± 0.5 K and temperature measurements were performed with a copper-constantan termocouple. Geometries for the spectra listed in the text and figures follow the usual Porto notation, A(BC)D.

III. Crystal structure

The crystal structure and the information about the hydrogen bonds were accurately determined by Shoemaker *et al.* in an X-ray diffraction study [2]. It was found that the crystal is orthorhombic with four molecules per unit cell, space group D_2^4 ($P2_12_12_1$). L-threonine has a zwitterion configuration and in the crystal the molecules are held by a three-dimensional network of hydrogen bonds with the amino group forming two hydrogen bonds of lengths 0.290 and 0.280 nm and the hydroxyl group forming a hydrogen bond of length 0.266 nm. These hydrogen bonds are linked to the carboxyl oxygen atoms: one of the carboxyl hydrogen atoms is hydrogen bonded to a hydroxyl group and the other to two amino groups [2].

In the primitive cell of l-threonine crystal, each ion has a C_1 local site symmetry. The 204 vibrations can be decomposed into the irreducible representations of the factor group D_2 as

$$, = 51(A + B_1 + B_2 + B_3)\ . \qquad (1)$$

Among these modes, 1 B_1 , 1 B_2 and 1 B_3 belong to the acoustic branch and the others are optical modes. All representations are Raman active and the $B_1 + B_2 + B_3$ are I.R. active.

IV. Assignment

The infrared spectrum was taken between 400 and 4000 cm^{-1} and is shown in Fig. 1. In Table 1 we present the tentative assignment of these modes. In order to do the assignment we used the knowledge of some group wavenumbers and some vibrations in amino acid crystals with similar structures as we will discuss below. In Fig. 2 we show the Raman spectra of l-threonine crystals at 300 K for four scattering geometries which display the modes belonging to the four irreducible representations of the factor group D_2: Y(ZZ)X - A, Y(XY)Z - B_1, Y(ZX)Z - B_2 and Y(ZY)X - B_3. The wavenumber range comprises mainly two regions, one from 0 to 1700 cm^{-1} and another between 2700 and 3300 cm^{-1}. In the first region the spectra were taken in three different scanning steps and this explain the two discontinuities in the background lines.

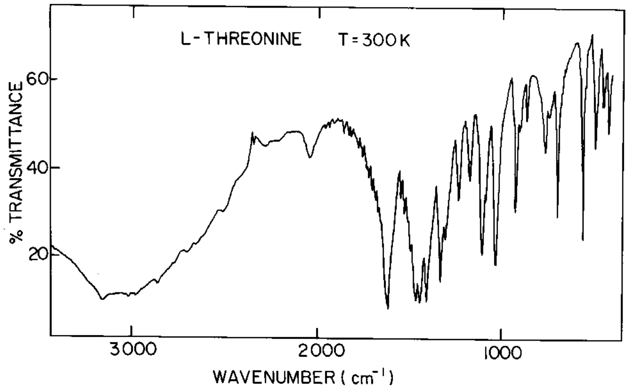

Figure 1. Infrared spectrum of l-threonine crystal at room temperature.

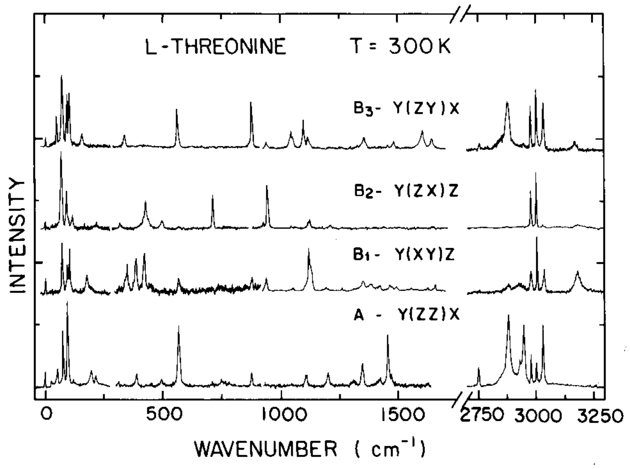

Figure 2. Polarized Raman spectra of l-threonine crystals in the four irreducible representations of D2 factor group at room temperature.

The following discussion refers to Figs. 1 and 2. The modes with low wavenumbers are related to external, librational and rotational modes and certainly are compressed torsion of CC structure, bending of CCC structure, among others [6]. We made a generic assignment - lat. (from lattice) - because it is very difficult to especify exactly any mode in this spectral region; however, it is clear that librational and rotational modes are also possible to be found in this spectral region.

TABLE I:
Assignment of the normal modes of l-threonine crystal.

IR	A Y(ZZ)X	B_1 Y(XY)Z	B_2 Y(ZX)Y	B_3 Y(ZY)X	Assignment
	52				
	77	74	68	71	
			91	93	
	96	96	98		
		104		102	
	120		114		
		175		156	
	194	192			
	215		216		
	312	315	317		
		347		338	
	388	385			
418		421	426	424	
447	450	448	448		
491	493		497		$\tau (NH_3)$
560	570	570	568	564	$r(CO_2^-)$
701	709		714		$w(CO_2^-)$
747	750		761		$\tau (COH)$
769	776		773		$\delta (CO_2^-)$
871	878	879		878	$\nu (CCN)$
907	910				$\nu (CC)$
932	930		925		$\nu (CC)$
		940	946	940	$\nu (CC)$
1040	1032				$\nu (CN)$
	1047	1051	1044	1047	$\nu (CN)$
			1063		
1093	1108			1101	$\nu_a(CCN)$
1111		1124	1120	1117 - 1124	$r(NH_3)$
1185	1202	1197			$r(NH_3)$
			1213		
1246			1263	1259	$\delta (CH)$
1318	1310				$\delta (CH)$
1347	1349	1350		1355	$\delta (CH)+$
					$\delta_s(CH_3)$
1383		1385			$\delta_s(CH_3)$
1418	1421	1423			$\nu_s(CO_2^-)$
1457	1460		1443		$\delta_a(CH_3)$
	1471	1471			$\delta_s(NH_3^+)$
1480		1491		1483	$\delta_s(NH_3^+)$
		1558			
				1606	$\nu_a (CO_2^-)$
1626	1627	1629			$\delta_a(NH_3^+)$
1651		1658	1651	1641- 1647	$\delta_a(NH_3^+)$
	2753			2757	
	2790				
2873	2882	2880	2886	2879	$\nu (CH)$
	2932	2930			$\nu (CH)$
	2947		2948	2950	$\nu_s(NH_3)$
2978	2982	2981	2981	2979	
2998	3003	3007	3004	305	
3026	3035	3034	3034	3035	$\nu_a(NH_3)$
3169		3184	3182	3170	$\nu_a(NH_3)$

Now we begin the tentative assignment of the bands observed with wavenumbers greater than 400 cm^{-1}. To throw light on these assignments knowledge of the I.R. and Raman vibrations in some similar complex molecules is of great importance [7, 8]. In Fig. 1 the band observed at 491 cm^{-1} was assigned as a torsional mode of NH_3, $\tau(NH_3)$. Such a band was observed with wavenumber of 493 and 497 cm^{-1} in the A and B_2 representations by Raman scattering as is shown in Fig. 2. It is interesting to note that in other materials with amino groups the $\tau(NH_3)$ is observed near this wavenumber: in a taurine crystal the band was observed around 470 - 480 cm^{-1} [9]; in alanine crystal the $\tau(NH_3)$ was observed at 478 cm^{-1} [10]; in a monohydrated l-asparagine crystal, the band was observed at 429 cm^{-1} [11] and in a l-asparagine powder the band was observed at 409 cm^{-1} [12]. Another band associated with torsion of the structure is that observed with a wavenumber of 418 cm^{-1} which is related to a torsion of the CC structure, as was observed in l- asparagine [12].

The band observed with wavenumbers of 560 and 570 cm^{-1}, respectively, in the I.R. and in the Raman spectra, are tentatively assigned as a rocking of CO_2^-, r (CO_2^-). This is because in l-alanine the r (CO_2^-) is observed at 532 cm^{-1} [6] and has an intensity greater than the intensity of the $\tau(NH_3)$, exactly as occurs with the l-threonine crystal. Also based on the work of Wang and Storms [6], we believe that the band with wavenumber of 709 cm^{-1} in the I.R. and 714 cm^{-1} in the Raman spectrum is due a wagging vibration of the CO_2^- structure, w(CO_2^-).

A torsion of COH structure, τ(COH), as observed in serine at 752 cm^{-1} [13], was observed by us in the l-treonine around 750 - 761 cm^{-1} in Raman spectra and with wavenumber of 747 cm^{-1} in the I.R. spectrum. The bending of CO_2^-, $\delta(CO_2^-)$, was observed by us with wavenumber of 769 cm^{-1} and 776 cm^{-1}, in the I.R. and in the A representation of Raman spectra, respectively. The same band was observed in l-alanine [6] in both I.R. and Raman at 769 $\text{cm}^{-1}$and 771 cm^{-1}, respectively.

In the Y(ZY)X spectrum of Fig. 2 we observed a very intense band at 878 cm^{-1}, which is observed in the I.R. spectrum at 871 cm^{-1}. This band is related to a stretching band of CCN structure, ν(CCN); this assignment is based on the assignment of similar structure in the l-serine and l-cysteine amino acids in water solution, as observed in Ref. [14]. Bands with wavenumbers of 910, 925 cm^{-1} in the A symmetry and with 940 cm^{-1} in the B_1 and B_3 symmetries are associated to CC stretching vibrations, ν(CC). These assignments are based in the ν(CC) vibrations of dl-alanine [12]. Bands appearing in the A symmetry with wavenumbers of 1036 cm^{-1} and 1047 cm^{-1} are possibly associated to ν(CN), a stretching vibration involving a carbon of the structure and the nitrogen of the amino group. The band observed with wavenumber of 1093 cm^{-1} in the I.R. spectrum is due to CCN asymmetric stretching vibration as suggested by Diem et al. in a work performed in l- alanine [15]. The rocking of NH_3 structure, r(NH_3), was observed in l- threonine with wavenumbers 1111 $\text{cm}^{-1}$and 1185 cm^{-1} in the I.R. spectrum, showing similar wavenumbers to those observed in l-alanine as studied in ref. [15]. In the Raman spectrum of the B_3 - Y(ZY)X scattering geometry we observed bands at 1117 cm^{-1} and 1124 cm^{-1}. All bands observed above are related to the α'_{zy} tensor element and are due to polar modes. The band with two peaks at 1117 cm^{-1} and 1124 cm^{-1} is possibly due to the additional scattering mechanism [16] which exists as a result of the interaction between polar modes. Since the splitting of the closely spaced components is small, the effect of the long-range interaction of the internal modes is small. Similar results were observed in the Raman scattering both from γ- glycine [17] and from l-alanine [6]. In the latter case four pairs of Raman lines were interpreted as the secondary mechanism of scattering involving the polar modes.

Bending vibrations of CH group, δ(CH), were found in l- threonine with wavenumbers 1246 cm^{-1}, 1318 cm^{-1} and 1347 cm^{-1} in the I.R. spectrum. We point out that the band corresponding to the I.R. peak at 1246 cm^{-1} has low intensity in the Raman spectrum observed in B_2 (1263 cm^{-1}) and B_3 (1259 cm^{-1}), as can be seen in the Fig. 2. Our assignment is consistent to that carried out in l-threonine in water solution [14] and in l-alanine [15]. Bending vibrations of the CH_3 group, $\delta(CH_3)$, were found in l-threonine crystal with wavenumbers 1383 cm^{-1} and 1457 cm^{-1} in the I.R. spectrum and with similar values in the Raman spectra; once more, assignment is based on the work of Refs. [14 - 15].

The symmetric stretching of CO_2^- structure, $\nu_s(CO_2^-)$, was found at 1421 cm^{-1} and 1423 cm^{-1} in the Raman spectra of A and B_1 symmetries. Our assignment is based on the work of both Ref. [14] where such a mode was observed with wavenumber 1409 cm^{-1} in l-threonine water solution and Ref. [15] where the $\nu_s(CO_2^-)$ was observed with wavenumber of 1410 cm^{-1} in l-alanine.

The symmetric bending of the NH_3, δ_a (NH_3^+), was observed in l-threonine crystals at the wavenumber 1471 cm^{-1} and 1491 cm^{-1} in the B1 symmetry by Raman scattering. This assignment is consistent with the work by Diem *et al.* [15], who observed that mode with wavenumber 1498 cm^{-1} in l-alanine crystal. The mode observed in the B_3 - Y(ZY)X scattering geometry with wavenumber 1606 cm^{-1} was assigned as the asymmetric stretching of CO_2^-, $\nu_a(CO_2^-)$, and the modes observed with wavenumbers 1641 cm^{-1} - 1647 cm^{-1} were assigned as the asymmetric bending of the NH_3^+ , $\delta_a(NH_3^+)$. We point out that in l-alanine crystals the $\nu_a(CO_2^-)$ and $\delta_a(NH_3^+)$ modes were observed at wavenumbers 1595 cm^{-1} and 1647 cm^{-1}, respectively [6]. Again, we observed a pair of Raman lines (1641 cm^{-1} - 1647 cm^{-1}) originating from only one vibrational mode as consequence of the interaction between polar modes producing a secondary scattering mechanism.

Finally, we discuss the modes with high wavenumbers. These modes are due to the stretching vibrations in both the NH_3 and CH structures. The CH stretching vibrations, ν(CH), were found with wavenumbers 2882 cm^{-1} and 2932 cm^{-1} in the A symmetry of the Raman spectrum, while in the I.R. spectrum the above specified vibration was found with wavenumber 2873 cm^{-1}. This assignment is based mainly on the work of Ref [15] where a meticulous study in l-alanine showed such vibration with wavenumber of 2962 cm^{-1}.

The NH_3 stretching vibrations, $\nu_s(NH_3)$, are present in all amino acids and also in other amino substances such as taurine [9]. Among the free NH_3 molecule vibrations we known that the symmetric stretching has no degeneracy, but the asymmetric stretching, $\nu_a(NH_3)$, is doubly degenerate. The NH_3 molecule symmetry is C_{3v} while the symmetry of the amino group in the crystal environment of the l-threonine is C_1. The C_1 local site of NH_3 is the same in other amino acid crystals. Because of this lowering in symmetry, the degeneracy of the asymmetric stretching mode is raised and we can observe two bands corresponding to the $\nu_a(NH_3)$ vibration. For example in the work of Diem *et al.* [15] the two modes were observed at wavenumbers 3080 cm^{-1} and 3060 cm^{-1} ; in Ref. [18] the two modes are also observed. In a Raman polarized study performed in a monohydrated l-asparagine crystal [11] were observed the two $\nu_a(NH_3)$ modes with wavenumbers 3099 cm^{-1} and 3115 cm^{-1}; in a Raman study performed in l-asparagine powder it was observed a large band assigned as the asymmetric stretching of NH_3 and possibly in such a band the two modes can be found. In the material investigated in the present study, l-threonine crystal, we observed the $\nu_s(NH_3)$ with wavenumber 3003 cm^{-1} for the A symmetry and 2998 cm^{-1} in the I.R. absorption spectrum. Such a mode was observed with a low intensity in the A symmetry but in the spectrum of the B_3 symmetry it is observed as the most intense band in the spectrum. One of the NH_3 asymmetric stretching vibration was observed in l-threonine crystals by Raman scattering with wavenumber 3034 cm^{-1} in the B_1 and B_2 and wavenumber 3035 cm^{-1} in the B_3 and A representations. The other NH_3 asymmetric stretching vibration was observed by Raman scattering with wavenumbers between 3170 cm^{-1} and 3184 cm^{-1} in the Bs representations, while it was not observed in the A representation. Finally, by I.R. measurements, the asymmetric stretching of the NH3 was observed with wavenumbers 3026 cm^{-1} and 3169 cm^{-1}.

As a conclusion, polarized Raman spectra in the four irreducible representations of the D_2 factor group of the l-threonine crystal were analyzed. As a future work we will investigate amino acid crystals under high hydrostatic pressure and temperature changes in order to investigate eventual phase transitions driven by changes in hydrogen bonds. Structures bonded by ionic bonds are harder than structures bonded by hydrogen bonds. This explain, for example, why the fusion of hydrogen bonded materials occurs at temperatures lower than the temperatures where it is observed the fusion of ionic materials. This suggests that the amino acid crystals can show a great number of phase transitions. Hydrostatic pressure is a powerful technique to induce phase transitions in crystals and we believe that with relatively low pressure we can change the distance between the atoms that participate in the hydrogen bond in the amino acid crystals, and consequently, we could induce phase transitions, as was already observed in the monohydrated l-asparagine crystal [19] and in the l-threonine crystal[20].

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