Conformational landscape of L-threonine in neutral, acid and basic solutions from vibrational circular dichroism spectroscopy and quantum chemical calculations

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A B S T R A C T

The biological relevance of amino acids is well known. They can be used as zwitterionic, cationic or anionic forms according to the pH of the medium where they are. Thus, our aim herein was to study the conformational preference of the polar amino acid l-threonine [C4H9NO3 (2S,3R)-2-amino-3-hydroxybutyric acid] under different pH conditions. A conformational study in an aqueous solution of the dissociation equilibrium of the amino acid l-threonine was carried out for this purpose. We recorded, at room temperature, the Mid-IR, Far-IR, Raman and VCD spectra of l-threonine from the aqueous solutions at pH values 5.70 (zwitterionic species), 1.00 (protonated species) and 13.00 (deprotonated species). The number of conformers found with the conformational search was 9 zwitterions, 27 anions and 52 cations. Both the study of the conformational landscape and the theoretical analysis of the vibrational features were accomplished by using DFT and ab initio calculations, that is, B3LYP/6-311++G(d,p) level of theory for all the conformers obtained from the conformational search, M062X/6-311++G(d,p) and MP2/6-311++G(d,p) levels of theory for the most stable conformers. The presence of water was included with the IEF-PCM implicit hydration model. With regard to the zwitterion, the importance of the analysis of the low frequency region (700–30 cm−1) in the Far-IR spectra should be noted, because it provides relevant information that can be used to determine the presence of the most stable structures.

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1. Introduction

Amino acids not only play a key role in protein folding but also take part in lyophobic interactions and influence secondary structure properties. They are bifunctional compounds, which contain an amine and a carboxylic acid group and have a side chain that varies among the different amino acids.1,2 Furthermore, understanding the structures and conformational preferences of the zwitterionic forms is important to gain insight into protein folding, which is governed by hydrophobic and hydrophobic interactions which determine the H-bonding scheme.3–7 Studying their behaviour in water is a major interest because it is the natural medium for biological molecules.7,8 Naturally occurring amino acids have a large conformational flexibility, and computational studies should correctly determine conformers and statistical populations found from experimental data.8,10 The dissociation equilibrium depends on the pH of the medium in which the amino acid is placed. The experimental dissociation constants of threonine, pKα, corresponding to the dissociation of the carboxyl and protonated amino groups are 9.10 and 2.09, respectively. Thus, we can deduce that the presence of anions will be found at pH 13.00, cations at pH 1.00 and zwitterions at pH 5.70.11

Polar amino acids, such as l-serine (l-Ser or S) and l-threonine (l-Threo, l-Thr or T), with a hydroxyl group on their side chains, take part in pivotal biological processes, such as biosynthesis and phosphorylation.12–16 Threonine13 is a polar α-amino acid with the chemical formula HO2CCH(NH2)CH(OH)CH3. Together with serine, threonine is one of two proteinogenic amino acids that have an alcohol group (tyrosine is not an alcohol but a phenol, since its hydroxyl group is bonded directly to an aromatic ring, giving it different acid/base and oxidative properties). It is also one of two common amino acids that bear a chiral side chain, along with isoleucine. Moreover, the threonine residue is susceptible to numerous posttranslational modifications. The hydroxy side-chain can undergo O-linked glycosylation. In addition, threonine residues undergo phosphorylation through the action of a threonine kinase.
It can be obtained in its phosphorylated form as phosphothreonine.\(^{17}\) It is also one of two amino acids out of the 20 with two stereo- genetic centers. It can thus exist as four possible stereoisomers with the following configurations: (2S,3R), (2R,3S), (2S,3S) and (2R,3R). However, the name l-threonine is used for one single dia- stereomer, (2S,3R)-2-amino-3-hydroxybutanoic acid. The second stereoisomer (2S,3S), which is rarely present in Nature, is called l-allo-threonine.

Hernández et al.\(^{23}\) reported an analysis of the IR–Raman spectra of l-Ser and l-Threo in H\(_2\)O and D\(_2\)O solutions in the 2000–800 cm\(^{-1}\) and 2000–500 cm\(^{-1}\) regions, respectively. Additionally Xu and Lin carried out a detailed theoretical study with ab initio calculations of l-Threo and l-allo-Threo in the gas phase.\(^{18}\)

Herein, an experimental and theoretical study of Mid-IR, FarIR, Raman and VCD spectroscopic features of l-threonine in aqueous solutions under three representative pH conditions is reported (see Fig. 1 for the atom numbering). The pH values were: 5.70 (zwitterionic species, ThreoZW), 1.00 (protonated species, ThreoCAT) and 13.00 (deprotonated species, ThreoAN).

Based on the vibrational analysis of the zwitterions, determining the number of possible conformers present in solution is a complicated task, given the similar IR and Raman signature of the most stable conformers.\(^{19–27}\) Thus, in order to obtain additional information, we also analysed the FarIR spectrum in the solid phase of l-threonine. Furthermore, VCD measures the differential absorption of left versus right circularly polarized IR incident light in the molecular vibrational transition by a chiral molecule.\(^{28}\) The signs of the rotatory strength could be helpful in determining the absolute configuration of the l-threonine. As vibrational tech- niques, IR and Raman, VCD technique is also sensitive to the presence of different conformers.\(^{28–30}\) The vibrational studies of the cations, anions and zwitterions of this amino acid performed here- in are more complete than those in the literature,\(^ {31}\) not only because of the study by VCD, but also because the region between 700 and 30 cm\(^{-1}\) is analysed by means of IR spectroscopy in the case of the zwitterions.

In many works, biomolecules have been studied by using chir- optical techniques such as vibrational circular dichroism (VCD) or Raman optical activity (ROA); an explicit solvation model has been considered to account for the solvent effects.\(^ {31–43}\)

Our strategy was to test the influence of two different DFT func- tionals, B3LYP and M062X, in the prediction of the most stable con- formers at different pH values. We concluded that a good election in the functional is also important in order to get a reasonable set of the most stable conformers. In order to stabilize the zwitterionic, anionic and cationic structures of l-Threo, solvent (water) effects were taken into account by using IEF-PCM formalism as imple- mented in Gaussian09. The M062X functional is also a high nonlocal- ity functional with double the amount of nonlocal exchange (2\(\times\)). It is parametrized for non-metals and contains a treatment for non- covalent interactions. The B3LYP functional does not contain a noncovalent interaction treatment. Due to the system under study, l-Threo is a polar flexible molecule which presents noncovalent interactions, it would be reasonable to assume that the result of the calculation of the relative energies for it with the M062X functional was better than those obtained with the B3LYP functional. Similar results have been obtained in the study of the zwitterionic structures of l-Phe and l-Tyr.\(^ {27}\)

In the literature,\(^ {44}\) a thorough study on the molecular properties of H-bonded complexes using a large set of functionals has been re- ported. In these complexes, the charge transfer interactions play an important role in successfully describing the structure and

![Figure 1. Molecular structure and atom numbering adopted herein for the l-Threo in the zwitterionic, protonated and deprotonated forms.](image-url)
optimized at the B3LYP, M062X and MP2 levels of theory. Calculated molecular populations and relative energies (with ZPE correction) for the zwitterionic (ZW), protonated (cation) and deprotonated (anion) conformers of 1-Threo optimized at the B3LYP, M062X and MP2 levels of theory.

2. Experimental procedure

A commercial 1-threonine sample (99%) was purchased from Sigma-Aldrich. Mid-IR, Raman and Far-IR spectra of 1-threonine were recorded for the solid samples, without performing any previous purification. Likewise, the IR and Raman spectra were recorded in aqueous solutions at room temperature and different pH conditions: 5.70 (neutral media), 1.00 (acid media) and 13.00 (basic media). Zwitterionic, protonated (cation) and deprotonated (anion) species are present at these three representative pH values. We also recorded the thin film spectra of these three solutions by means of a Bruker Vertex 70 in the 700–30 cm⁻¹ range, with a resolution of 1 cm⁻¹ and 200 scans, using a platinum ATR (single reflection diamond ATR accessory) and the silicon beamsplitter for the Far-IR region.

The VCD spectra of 1-threonine in thin film were recorded using a JASCO FVS-400 FTIR spectrometer, equipped with MCTV (2000–800 cm⁻¹) detector. In order to obtain very thin films of the sample, we prepared very low concentration solutions of the sample at different pH values and we evaporated the solvent on KCl ‘Real Crystal IR Sample Cards’. These films were measured in several positions by rotating the sample around both the beam propagation axes (90° and 180°) and that perpendicular to it (180°) in order to minimize the presence of artifacts in the VCD spectra. All spectra were recorded using a KCl support, with a resolution of 4–8 cm⁻¹ and 4000–8000 scans by blocks of 2000 scans. With regard to the baseline correction, we subtracted the KCl support signals for the film spectra.

Table 1: Calculated molecular populations and relative energies (with ZPE correction) for the zwitterionic (ZW), protonated (cation) and deprotonated (anion) conformers of 1-Threo optimized at the B3LYP, M062X and MP2 levels of theory.

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a Boltzmann population from ΔE₀ taking T = 298.16 K. ΔE₀: zero point corrected relative energy; ΔEₑ: equilibrium relative energy.
b ThreeAN2 conformer only found with the M062X functional.
3. Theoretical methodology

The vibrational study of L-Threo required an extensive conformational search, which was performed for the ThreeZW, ThreeCAT and ThreeAN forms by means of molecular mechanics with different force fields (MMFF and SYBYL)\(^{50,51}\) and using a SPARTAN program.\(^{52}\) When a fulfilled conformational energy was reached (20 kJ/mol) giving 9, 27 and 52 conformers for the zwitterionic, anionic and cationic structures of L-Threo, all of these conformers were optimized using B3LYP\(^{33-55}\) functional and the 6-311++G(d,p) basis set. Moreover, the lowest non-redundant conformers were selected for further optimization of the structures and the calculation of their relative energies (with ZPE corrections) using another density functional approach (M062X) and the 6-311++G(d,p) basis set. The M062X functional is a high nonlocality approach with double the amount of nonlocal exchange (\(2x\)); it is parametrized only for non-metals and contains a treatment of noncovalent interactions.

To compare the results obtained with different functionals, the B3LYP and M062X approaches with those obtained using the B3LYP functional. Finally, a second-order perturbation theory (MP2) calculation\(^{56}\) with the 6-311++G(d,p) basis set was accomplished in order to compare the MP2 relative energies with the DFT results. These two different DFT methods were compared to surmise that the result of the calculation of their relative energies (zero point corrected, \(\Delta E_0\) and equilibrium, \(\Delta E_e\) and Boltzmann populations (ZPop) obtained for the L-Threo at three different theoretical levels: B3LYP, M062X and MP2 with the same 6-311+G(d,p) basis set. Table 2 shows some relevant molecular structural parameters, in particular the dihedral angles, of this amino acid obtained at the same levels of theory specified above. Meanwhile, Fig. 2 shows the structures, relative energies and intra-molecular H-bond distances of the most stable zwitterions, protonated (cation) and deprotonated (anion) forms of L-Threo optimized at these three theoretical levels.

The conformational landscape derived from the DFT and MP2 results corroborates that the most populated structures are: (i) four conformers for ThreeZW (92% of population); (ii) three conformers for ThreeCAT (85% of population); and (iii) three conformers for ThreeAN (89% of population).

The conformational distribution changes for ThreeZW, ThreeCAT and ThreeAN when DFT and MP2 methods are used, with the same

### Table 2

Some relevant molecular structural parameters of L-Threo (zwitterionic, protonated and deprotonated) performed at the B3LYP, M062X and MP2 levels of theory. See Fig. 1 for for atom numbering

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<tr>
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* B3LYP: 6-311+G(d,p) basis set.
* M062X: 6-311+G(d,p) basis set.
* MP2: 6-311+G(d,p) basis set.

4. Results and discussion

4.1. Theoretical conformational stability

The conformational search with the MMFF and SYBYL force fields found respective totals of 9, 27 and 52 conformers for the zwitterionic, anionic and cationic structures of L-Threo. All of these structures were optimized at the B3LYP/6-311+G(d,p) level of theory. The number of non-redundant conformers was 7 zwitterions, 19 anions and 27 cations. The most stable conformers were also optimized at the M062X/6-311+G(d,p) level and the relative energies of some of these conformers were calculated with the MP2/6-311+G(d,p) method. Table 1 gives the relative energies (zero point corrected, \(\Delta E_0\) and equilibrium, \(\Delta E_e\) and Boltzmann populations (ZPop) obtained for the L-Threo at three different theoretical levels: B3LYP, M062X and MP2 with the same 6-311+G(d,p) basis set. Table 2 shows some relevant molecular structural parameters, in particular the dihedral angles, of this amino acid obtained at the same levels of theory specified above. Meanwhile, Fig. 2 shows the structures, relative energies and intra-molecular H-bond distances of the most stable zwitterions, protonated (cation) and deprotonated (anion) forms of L-Threo optimized at these three theoretical levels.
basis set, are other notable points that require comment. For instance, the B3LYP, M062X and MP2 methods predict that Threo\textit{ZW1} conformer is the most stable, with a population around 40%. The Threo\textit{ZW2} conformer is predicted to be the second in terms of energy with the use of B3LYP (34%) and M062X (25%) approaches, but the third with the MP2 method (20%). Furthermore, the B3LYP/6-311++G(d,p) level of theory predicts that Threo\textit{ZW3}, Threo\textit{ZW4} and Threo\textit{ZW5} conformers are fifth, third and the fourth in energy, with respective populations at approximately 8%, 11% and 10%. If we calculate the relative energies for these three conformers with the M062X/6-311++G(d,p) or the MP2/6-311++G(d,p) levels, the results change drastically because Threo\textit{ZW4} and Threo\textit{ZW5} are predicted to be the fourth and fifth in energy, with populations at approximately 10 and 8%, respectively. Threo\textit{ZW3} is predicted to be the third (14%) with M062X and the second (21%) with MP2 level.

In the cases of Threo\textit{AN} and Threo\textit{CAT}, the similarity among relative energies obtained with B3LYP and MP2 levels of theory should be noted. Threo\textit{AN2} is only found with the M062X functional and, as discussed below, it does not seem to be present experimentally. Table 1 offers further details on the conformational landscapes of Threo\textit{CAT} and Threo\textit{AN}.

These results are important because the calculated IR, Raman and VCD spectra are different depending on the predicted relative energies and, consequently, populations. The conformational preference changes according to the theoretical model used. Since the

<table>
<thead>
<tr>
<th>Cation(protonated)</th>
<th>ZW</th>
<th>Anion (deprotonated)</th>
</tr>
</thead>
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<tr>
<td>Threo\textit{CAT1} (0.0, 0.0, 0.0)</td>
<td>Threo\textit{ZW1} (0.0, 0.0, 0.0)</td>
<td>Threo\textit{AN1} (0.0, 0.0, 0.0)</td>
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<tr>
<td>Threo\textit{CAT2} (3.5, 3.0, 1.4)</td>
<td>Threo\textit{ZW2} (1.2, 1.8, 0.3)</td>
<td>Threo\textit{AN2} (0.8, *, *)</td>
</tr>
<tr>
<td>Threo\textit{CAT3} (4.7, 1.5, 0.1)</td>
<td>Threo\textit{ZW3} (2.7, 1.6, 3.8)</td>
<td>Threo\textit{AN3} (1.7, 1.2, 2.7)</td>
</tr>
<tr>
<td>Threo\textit{CAT4} (5.3, 2.1, 0.2)</td>
<td>Threo\textit{ZW4} (3.2, 3.5, 3.0)</td>
<td>Threo\textit{AN4} (6.2, 6.5, 4.2)</td>
</tr>
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</table>

*The minimum was neither found with B3LYP nor with MP2

Figure 2. Structures of the most stable zwitterions, protonated (cation) and deprotonated (anion) structures of \textit{i}-Threo optimized at the M062X(6-311++G(d,p)) level of theory. Relative energies (zero point corrected) are given in kJ/mol in parenthesis at the M062X(6-311++G(d,p)), MP2(6-311++G(d,p)) and B3LYP(6-311++G(d,p)) levels of theory, respectively. H-bond values are shown in pm.
basis set is the same in all three cases, the relative energy differences found using DFT methods are due to the use of different treatments for electron density, that is, B3LYP and M062X functionals. A good prediction of the most stable structures will contribute much more to our understanding of the experimental features.

Table 2 shows some relevant molecular structural parameters of L-Threo (ZW, CAT and AN), with particular attention paid to some characteristic dihedral angles, calculated at the DFT (B3LYP and M062X functionals) and MP2 calculations (see Fig. 1 for atom numbering). The variations in the compared dihedral angles are smaller than 17°, when we compare the results obtained with B3LYP, M062X and MP2 calculations with the 6-311++G(d,p) basis set. This fact confirms that the optimized structures are the same in all three cases. The best results correspond to the MP2 calculation, but this method is also the most demanding in terms of computational cost. It seems that the best choice in terms of accuracy (structure, energy, and harmonic spectra) and computational cost could be the M062X functional for ThreeZW conformers. This functional is more demanding in computational cost than the B3LYP one but much less than the second-order perturbative (MP2) calculations. Nevertheless, B3LYP and M062X functionals seem to work well for ThreeAN and ThreeCAT structures.

With regard to the ThreeZW1 and ThreeZW2 conformers of L-Threo, the torsion angle θ6 (H13–O10–C6–C2) was found to have respective values of 73° and −174°. In the ThreeZW1 conformer, a weak intramolecular hydrogen bond was formed between O10–

H17 and O6–C6, with a length of 239 pm. However, another different intramolecular hydrogen bond is formed between H13–O10 and H12–N1 in the ThreeZW2 conformer, with a length of 216 pm. The only difference between these conformers is a turn of the HO–(hydroxyl) group, which changes the torsion angle value from 73° to −174°, giving a different and stronger H-bond in ThreeZW2. For ThreeCAT1 and ThreeCAT2, all angles were seen to have similar values except for the torsion angle θ6 (H13–O10–C6–C2); with values of −175° and 95°, respectively. These values are different because the hydroxyl group turns again. There are two H-bonds that stabilize both conformers: one between H2–O10 and H7–N1, and the other between N1–H17 and O6–C6.

For ThreeAN, dihedral angles of ThreeAN1, ThreeAN3 and ThreeAN4 have different values. In ThreeAN1, an intramolecular hydrogen bond is formed between N1–H3 and O6–C6, with a length of 215 pm. However, the amino group turns in ThreeAN3 giving a different hydrogen bond between N1–H3 and O6–C6, with a length of 225 pm. In ThreeAN4 it not only turns the amino but also the –CH(OH)–CH3 group, causing one different hydrogen bond between H13–O10 and H12–N1, with a length of 214 pm.

Finally, it should be noted that the structures (M062X and MP2) obtained herein are in good agreement with the most stable structures obtained in the literature, using the explicit solvation model.

In the case of L-Threo zwitterions: our most stable structure ThreeZW1 corresponds with the first and second conformers obtained in the literature (g–g and +g+g conformers), that is, the orientation of the OH, COO− and NH3+ groups of threonine moiety is similar. Additionally, our second conformer, ThreeZW2, is found as the third structure in the literature, that is, tg−. This way, we can say that our conformational research with an MM method and the later optimization of the structures give reasonable results. The similarity between the most stable conformers obtained by us and the literature confirms the good agreement between our experimental data and theoretical data.

4.2. Experimental analysis and spectroscopic features

The experimental IR (including the FarIR region for the ThreeZW) and Raman and VCD (Figs. 3–6) spectra confirm the presence of the most stable conformers of the ZW (Figs. 3 and 6), CAT (Figs. 4 and 6) and AN (Figs. 5 and 6) of L-Threo in an aqueous solution and the solid phase (only for the zwitterionic structures), and is in good agreement with the theoretical calculations, especially M062X for the ThreeZW form and B3LYP-M062X for the ThreeCAT and ThreeAN structures. The single scaling factors from the NIST database61 for the B3LYP/6-311++G(d,p) (0.967) and M062X/6-311++G(d,p) (0.950) levels were used to carry out the vibrational analysis of the species studied. All of the percentages of the normal modes contributions were taken from the P.E.D. matrix obtained with the VEDA program.62,63

ThreeAN, ThreeZW and ThreeCAT forms have, respectively, 42, 45 and 48 vibrational normal modes, belonging to the unique irreducible representation (A) of its common symmetry point group, C1. The recorded IR, Raman and VCD spectra of L-Threo at different pH in aqueous solution, thin film and powder sample (only for zwitterions) are displayed in Figures 3–6.

For vibrational analysis, we took into account the IR–Raman–VCD data of L-Threo in the solid phase and in solution at different pH conditions. As can be seen, the IR, Raman and VCD spectra (see Figs. 3–6) are complex and in order to accomplish a reasonable interpretation of them, some remarks about the most helpful and relevant spectroscopic features are needed.

4.2.1. IR and Raman spectra

4.2.1.1. Neutral pH. Figure 3 shows the recorded IR, Raman and FarIR spectra of L-Threo in aqueous solution at pH = 5.70, as well as in thin film and powder sample. Fig. 3 also shows the theoretically calculated spectra of the four most stable zwitterionic forms at the B3LYP/6-311++G(d,p) and M062X/6-311++G(d,p) levels of theory. As it can be seen, this last level reasonably reproduces the experimental spectra. With regard to the IR data (Fig. 3a), we could assign some bands to the contribution of different conformers of ThreeZW, for instance: (a) The experimental band observed at 1600 cm−1 (IR, 1632 cm−1 in Raman) could be assigned to a normal mode with a contribution from the P.E.D. matrix of 86% of asym. COO− str. motion of the four most stable zwitterionic structures; (b) The band at 1506 cm−1 (IR, 1493 cm−1 in Raman) could be assigned to a normal mode with contributions of 72% asym. NH3+ bend. and 16% HNCC torsion motions of the four most stable ThreeZW conformers. (c) The experimental band at 1403 cm−1 (IR and Raman) could be assigned to two normal modes of the four most stable zwitterions of L-Threo. The first one has a contribution of 70% sym. NH3+ bend. and the second one has a contribution of 56% asym. CH3 bend. and 14% HCCC torsion. A contribution of 25% of HOC bend. is also found for ThreeZW3 in the last normal mode. For ThreeZW2, ThreeZW3 and ThreeZW4 conformers, the first one also has an additional contribution of 14% sym. COO− str. (d) The band observed at 1041 cm−1 (IR and Raman) could be also assigned to two normal modes mainly due to the presence of ThreeZW1 and ThreeZW2. The first one with a contribution of 12% asym. NH3+ bend., 26% CH wagg. and 34% HNCC torsion motions for both conformers, and the second one with a contribution of 40% CC str. and 22% HOC bend. motions for ThreeZW1 conformer and 14% CC str., 28% HOC bend., 11% HNCC torsion and 10% HCCC torsion for ThreeZW2 conformer.

Figure 3b shows the FarIR spectrum of the solid L-Threo. This region shows features that correspond to normal modes with contributions from waggings, rockings and torsions. We highlight one of the most strong and relevant bands, which is assigned to the OHCC torsion normal mode. This appears at different wavenumbers depending on the conformer: at 560 cm−1 (FarIR and Raman of the solid) for ThreeZW3 with a theoretical contribution of 73%, 487 cm−1 (FarIR) for ThreeZW1 with a theoretical contribution of 93%, 310 cm−1 (FarIR) for ThreeZW2 with a contribution of 81% and 9% of HCCC torsion and at 222 cm−1 with 90% (FarIR) for
Furthermore, the bands assigned to the –NH$_3^+$ torsion normal mode of the four most stable conformers of L-Threo are also calculated at different wavenumbers, but they are predicted to be so weak that they cannot be identified in the experimental spectrum. Finally, the experimental band observed at 92 cm$^{-1}$ (FarIR) could be assigned, mainly, to the torsion normal modes of the most stable conformers of zwitterions. The major contributions are 83% of CCCN torsion motion for ThreoZW1 and ThreoZW2 conformers and 82% of CNCC torsion motion for ThreoZW4 conformer. In the case of the ThreoZW2 conformer, a contribution of 9% CNCC torsion motion is also present. Thus, it has been shown that the low IR frequency region is suitable for the detection of contributions of different conformers.

Figures 7b and 8b from the literature\textsuperscript{9} show the Raman and IR spectra obtained for the zwitterionic forms of L-Threo (neutral conditions). The best theoretical/experimental agreement obtained
was with the explicit solvation model, but it is evident that their reproduction of the experimental IR–Raman data is not better than our own theoretical/experimental agreement obtained with the implicit solvation model and the M062X functional. This fact illustrates the great difficulty of this topic. Moreover, our data include both IR and Raman spectra in aqueous solutions and a comparison with the solid ones (less interesting in terms of biological importance). In Figure 3, our experimental/theoretical comparison is shown, where the Raman data reveal a lot of information and we can see how the bands at 1400 cm\(^{-1}\), 1300 cm\(^{-1}\), and 1100 cm\(^{-1}\) are well reproduced by the weighted Raman spectrum. In our opinion, this fact is due to the similar most stable conformational set obtained with the M062X and MP2 methods, which is comparable to that given in the literature.\(^9\)
4.2.1.2. Acid pH. The recorded IR (4a) and Raman (4b) spectra of L-Threo in acid solution are shown in Figure 4. Some bands in these spectra could be assigned to the presence of the two most stable cationic structures of this species, for example: (a) The band observed at 1728 cm\(^{-1}\) (IR and Raman) could be assigned to a normal mode with a contribution from the P.E.D. matrix of 87% of C=O str. motion of the two most stable cationic forms. This indicates that, as expected, the protonation of the carboxylate group takes place at a highly acid pH. (b) The experimental band at 1621 cm\(^{-1}\) (IR, broad band at 1637 cm\(^{-1}\) in Raman) could be assigned to a normal mode with contributions of 72% asym. NH\(_3^+\) bend. and 21% HNCC torsion motions owing, mainly, to the presence of ThreeoCAT1 and ThreeoCAT2 conformers. (c) The band at 1527 cm\(^{-1}\) (IR) could be assigned to a normal mode, which is a combination of 81% of sym. NH\(_3^+\) bend. and 8% asym. CH\(_3\) bend. of the two most stable ThreeoCAT conformers. (d) The band observed at 1455 cm\(^{-1}\) (IR and Raman) could be assigned to two normal modes of the two most stable ThreeoCAT conformers. The first one has a contribution of 65% asym. CH\(_3\) bend. and 17% HCCC torsion. An additional contribution of 11% sym. NH\(_3^+\) bend. is only present in the case of ThreeoCAT2 conformer. The second one has a contribution of 34% CC str. and 14% CH wag. for ThreeoCAT1 and, likewise, a contribution of 7% CC str., 18% C=O str. and 13% CH wag. for ThreeoCAT2. (e) The experimental band at 1124 cm\(^{-1}\) (IR and Raman) could be assigned once again to the combination of the two normal modes of the two most stable cationic forms. With regard to the ThreeoCAT1 conformer, the first one has a contribution of 18% CC str. and 59% HOC bend. (COOH group). However, the contribution of 24% C=O str. and 47% HOC bend. (COOH group) was found for the ThreeoCAT2 conformer. The second one has a contribution of 26% CC str. and 11% HCCC torsion for both conformers. However, there are additional contributions, 11% HNCC torsion in ThreeoCAT1 and 9% C=O str. in ThreeoCAT2. (f) The band at 1052 cm\(^{-1}\) (IR and Raman) could be assigned to two normal modes with a contribution of 10% asym. NH\(_3^+\) bend., 19% CH wag. and 35% HNCC torsion for both conformers from the first one. In the second one, the main contributions are 24% HOC bend. (–CH\(_2\)-OH group), 22% CC str., 12% NC str., 12% HCCC torsion and 8% HNCC torsion for ThreeoCAT1 and 24% HOC bend. (–CH\(_2\)-OH group), 7% CC str., 9% C=O str. and 21% HCCC torsion for ThreeoCAT2. (g) The band observed at 935 cm\(^{-1}\) (IR, 934 cm\(^{-1}\) in Raman) could be also assigned to two normal modes. The first one has the main contributions of 41% NC str. and 26% HCCC torsion due to the presence of the two most stable cationic forms. For ThreeoCAT1, a small contribution of 11% asym. CH\(_3\) bend. is also present. The second one has a contribution of 46% CC str. and 18% HCCC torsion for the two conformers. In this case the small contribution of asym. CH\(_3\) bend. is due to ThreeoCAT2 conformer. (h) The experimental band observed at 748 cm\(^{-1}\) (Raman) could be assigned to different normal modes depending on the conformer. For ThreeoCAT1, the normal mode has contributions of 23% CC str., 20% OCOC torsion, 15% OCO bend. and 12% NC str. For ThreeoCAT2, a 7% C=O str., 17% OCOC torsion, 16% OCO bend. and 30% NC str. contribute to the corresponding normal mode.

4.2.1.3. Basic pH. Fig. 5 shows the recorded IR (5a) and Raman (5b) spectra of L-Threo in basic solution. Some bands in the IR and Raman spectra could be assigned to the presence of the three most stable anionic structures if we carried out the analysis of the P.E.D. matrix obtained with VEDA program. For instance: (a) The band at 1655 cm\(^{-1}\) (IR, broad band at 1635 cm\(^{-1}\) in Raman) could be assigned to a normal mode with contributions of 71% asym. COO\(^{-}\) str. and 10% NH\(_3^+\) scissor motions of the three most stable conformers of ThreeoAN; (b) However, the band at 1560 cm\(^{-1}\) (IR) could be assigned to a normal mode with contributions of
15% asym. CO\textsuperscript{−} str., 62% NH\textsubscript{2} scissor, and 18% HNCC torsion motions of the Threo\textsuperscript{AN1} and Threo\textsuperscript{AN3} conformers. These bands are not present in Threo\textsuperscript{ZW} and Threo\textsuperscript{CAT} spectra, because the NH\textsubscript{2} group is only found at basic pH. (c) The experimental band observed at 1265 cm\textsuperscript{−1} (IR, 1269 cm\textsuperscript{−1} in Raman) could be assigned to a normal mode with contributions of 28% sym. CO\textsuperscript{−} str., 21% CH wagg., 15% HCCO torsion and 15% NH\textsubscript{2} OH group), 10% CC str. and 10% HCCC torsion motions of the three most stable conformers, but with a major contribution of Threo\textsuperscript{AN1}; (e) The band observed at 984 cm\textsuperscript{−1} (IR and Raman) could be assigned to a normal mode with contributions of 26% HCCC torsion, 11% CC str. and 10% CCH bending motions of the three most stable conformers of Threo\textsuperscript{AN}; (f) The experimental band at 841 cm\textsuperscript{−1} (Raman) could be assigned to a normal mode with contributions of 21% CC str., 21% HNCC torsion and 13% NH\textsubscript{2} CH wagg. motions of the three most stable conformers.

4.2.2. VCD spectrum

We examined the chiroropical properties of l-threonine in relation to its structure by means of the combined use of vibrational circular dichroism (VCD) and computational calculations. The interpretation of the VCD spectroscopic features of medium size chiral compounds is a difficult task.\textsuperscript{20,28} VCD spectroscopy is a useful technique in determining the absolute configuration of the structures at different pH values of l-threonine. A good agreement was obtained between the experimental and theoretical VCD spectra, confirming our previous vibrational analysis. Figure 6 displays the recorded and theoretical VCD spectra of zwitterions (a), cations (b) and anions (c) in the 2000–900 cm\textsuperscript{−1} spectral region. In this figure, the bottom and top panels show the predicted scaled VCD spectra for the most stable conformers and the middle panels show experimental VCD spectra. The DFT and MP2 calculations are performed in the harmonic approximation. A few experimental VCD bands evidence the presence of the Threo\textsuperscript{ZW} (Fig. 6a), Threo\textsuperscript{CAT} (Fig. 6b) or Threo\textsuperscript{AN} (Fig. 6c) chiral structures, taking into account the most stable conformers. We have commented on some of the relevant bands below, which were observed in IR and/or Raman and previously mentioned in Section 4.2.1:

(a) In the case of the zwitterion, the three (−, +, +) bands at 1627 cm\textsuperscript{−1} (VCD and film IR), 1560 cm\textsuperscript{−1} (VCD and film IR) and at 1405 cm\textsuperscript{−1} (VCD and film IR) could be due to the presence of the four most stable Threo\textsuperscript{ZW} conformers. In addition, another interesting (−) band at 1038 cm\textsuperscript{−1} (VCD, 1041 cm\textsuperscript{−1} in film IR) could be due to the presence of Threo\textsuperscript{ZW1} and Threo\textsuperscript{ZW2}. These match well with those predicted by theoretical spectra.

(b) With regard to the cation, once again we could assign some relevant bands to the most stable conformers: the (−, +) bands observed at 1744 cm\textsuperscript{−1} and 1738 cm\textsuperscript{−1} (VCD, 1738 cm\textsuperscript{−1} in film IR) and the (−) band at 1595 cm\textsuperscript{−1} (VCD, 1600 cm\textsuperscript{−1} in film IR) could be due to the presence of the two most stable Threo\textsuperscript{CAT} conformers. The three (+, +, −) bands at 1414 cm\textsuperscript{−1} (VCD, 1422 cm\textsuperscript{−1} in film IR), 1115 cm\textsuperscript{−1} (VCD, 1130 cm\textsuperscript{−1} in film IR) and at 1034 cm\textsuperscript{−1} (VCD and film IR) could be due to the presence of the two most stable conformers of the cation. These match well with the experimental and theoretical results.

(c) Concerning the anion, the following (−, −) band: at 1583 cm\textsuperscript{−1} (VCD, 1580 cm\textsuperscript{−1} in film IR) and at 1290 cm\textsuperscript{−1} (VCD and film IR), could be due to the presence of the three most stable Threo\textsuperscript{AN} conformers. These are also in good agreement with theoretical data.

5. Conclusion

The conformational preference of l-Threo at different protonation states has been studied from theoretical and experimental point of views. We have measured the Far-IR, IR, VCD and Raman spectra of l-threonine in solution at different pH values as well as in the solid-state, and all of the bands were assigned based on scaled MO62X/6-311+G(d,p) calculations. The experimental spectra have been compared with a set of quantum chemical calculations, that is, DFT (B3LYP and MO62X functionals) and MP2 methods, with the same 6-311+G(d,p) basis set. All of the experimental spectra were in good agreement with the theoretical spectra. It can be clearly seen that different structures of l-threonine are present according to the pH of the medium.

The analysis of the film VCD spectra threw light on this matter, especially with regard to the determination of the most stable conformers depending on the pH, that is, four zwitterions at pH 5.70, two cations at pH 1.00 and three anions at pH 13.00.

The analysis of the low frequency region (700–30 cm\textsuperscript{−1}) in the Far-IR spectra reveals important information in order to identify the most stable conformers of Threo\textsuperscript{ZW} in the solid phase, which confirms the presence of four zwitterion structures, which is in agreement with the aqueous solution data.

The theoretical implicit model IEF-PCM used for the simulation of the water seems to reproduce in a suitable way the experimental data when the MO62X functional is used for the zwitterions of this molecule and when the B3LYP functional is used for cations and anions, even without the explicit treatment of water. Moreover, some conclusions can be addressed in the case of zwitterions of l-threonine: (i) MP2 and MO62X approaches give the same results in terms of relative energy; (ii) even if the B3LYP functional calculates the same structures as the MP2 and MO62X approaches, the calculated relative energy seems to be very different; (iii) the experimental data agree better with the MO62X and MP2 results than with B3LYP results. Yet, in the case of cations and anions, the B3LYP functional agrees better with the MP2 method in relation to relative energies and similarity with experimental spectra.

In conclusion we have shown that IR, Raman and, in particular, Far-IR and VCD techniques, are complementary techniques in the study of biologically interesting species. The use of them combined with quantum chemical calculations may help clarify the conformational landscape of flexible biological species such as the ones described herein.

Acknowledgments

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References
