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Research Article

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On the influence of water on fragmentation of the amino acid L-threonine

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Abstract: The present study describes the fragmentation of the amino acid L-threonine ($C_4H_9NO_3$) with and without the inclusion of water influence. The fragmentation of the L-threonine molecule was theoretically studied using the Becke's three-parameter hybrid functional method by applying the non-local correlation provided by Lee, Yang and Parr (B3LYP) with the correlation consistent triple zeta basis (cc-pVTZ). The polarizable continuum model (PCM) was used to evaluate the influence of water. Fragments were selected based on data from mass spectrometry experiments. The chemical compositions of fragments were identified, and the appearance energy was calculated. Based on the obtained results, we can conclude that water affects the appearance energy, the fragment structure and the fragment formation processes.

Keywords: L-threonine, fragmentation, water influence

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1 Introduction

Amino acids (AAs) are defined as organic compounds that contain both acid and amino groups. Except for glycine, all AAs have L- and D-isomers. All proteinogenic AAs located in humans and animals are the L-form, while several D-form AAs are found in some bacteria [1]. Several L-AAs regulate key metabolic reactions that are necessary for growth and immunity. Physiological concentrations of AAs are required for the homeostasis and functions of the human body [2]. Furthermore, AAs are essential substrates for the synthesis of many biomolecules with a low molecular weight (*e.g.*, melanin, carnosine) [3].

As an essential amino acid, L-threonine is not synthesized in humans. Therefore, we must obtain L-threonine from food in the form of L-threonine-containing proteins. This AA is the precursor of another amino acid: L-glycine [4]. Moreover, L-threonine is necessary to synthesize the protein mucin, which is required to maintain intestinal integrity and function, as well as synthesis [5]. L-threonine is also necessary for the stimulation of antibody production, lymphocyte proliferation, and apoptosis inhibition [6].

All living organisms are constantly affected by ionizing radiation emitted by natural sources such as cosmic rays and radionuclides contained in rocks from the Earth, air, food, and water. Furthermore, radiation exposure also occurs during medical procedures. For example, X-rays are used to evaluate the condition of teeth, broken bones, tumours and foreign bodies, whereas gamma rays are used to treat cancer. Low-energy (secondary) electrons are produced due to the interaction of X-rays and gamma rays with biomolecules. These low-energy electrons cause chemical and biological damage to bioorganic molecules, including AAs, *e.g.*, threonine [7].

The ionization of L-threonine due to electrons in vacuum has been theoretically and experimentally researched by Farajmand and Bahrami [8]. The researchers calculated the enthalpies of L-threonine, its isomers and the products of their fragmentation. The dominant peak in the mass spectrum of L-threonine isomer fragments that they measured is at the mass of m/z=57. The above-mentioned authors published all fragments that had the same mass (*e.g.*, m/z=57) but different chemical compositions (*e.g.*, C_3H_7N, C_2H_3NO) without considering which fragment is most likely to form [8]. Serafin *et al.* researched the formation of fragment with m=18 a.m.u. (water) in the gaseous phase due to the collision activated dissociation (CAD) of protonated threonine [9]. The results of their research showed differences in the abundances of the identical ion fragments. These differences depend on the structures of diastereomers. The formation patterns of the fragments depend on the collision energy. Hence, an analysis by Serafin *et al.* revealed that the appearance energy

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of fragments depends on the conformation of L-threonine. Previously, far too little attention has been paid to evaluating the most likely fragments in the L-threonine process. Therefore, we decide to investigate which fragments with the same mass but different compositions and which formation processes of these fragments are the most likely.

Water molecules are the most prevalent chemical component in cells, accounting for approximately 70% of weight of cell's weight. Most biochemical reactions and biophysical processes take place in water [10]. The solvent (water) strongly affects the structure of AAs [11]. In the gaseous phase, AAs exist in the canonical form, whereas zwitterionic conformers are predominant in water [12]. The zwitterionic forms of AAs have both a positively charged protonated amine group (-NH_3^+) and a negatively charged deprotonated carboxyl group (-COO^-) [11].

Ren and Kresin [13] researched diglycine and polyglycine fragmentation in the gaseous phase and inside He nanodroplets with and without embedding with water. The authors determined that the fragmentation of diglycine and polyglycine molecules is greater and occurs with the same process as in the gaseous phase if water is not embedded in the He nanodroplets. The abovementioned authors noted that fragmentation of larger (tetraglycine) peptides in water greater than that of smaller (diglycine) peptides [13]. Overall, water affects the fragmentation of peptides. On the other hand, to our knowledge, no previous studies have investigated the influence of water on free amino acid fragmentation. Due to this reason, the aim of this research is to determine whether the appearance energies, as well as the structure and fragment which mass are m=27 a.m.u., 30 a.m.u., 45 a.m.u., 56 a.m.u., 57 a.m.u., 74 a.m.u. and 75 a.m.u. formation processes, differ between conditions with and without the influence of water included.

We choose these mass fragments because the strongest peaks in the mass spectrum of L-threonine correspond to these mass [8, 14].

It should be emphasized that the purpose of this research was to study L-threonine fragment production with and without the influence of water only after ionization by radiation exposure and not to study the other processes (*e.g.*, electron interaction with medium) that could occur.

The paper is organized as follows. In Section 2 we explain the method of computation. The results of the research are given in Section 3. Finally, the conclusions of our study are presented in Section 4.

2 Methods

Several structures and fragments of the L-threonine molecule were studied by the Becke's three-parameter hybrid functional applying the non-local correlation provided by the Lee, Yang and Parr (B3LYP) [15] method, which is a representative standard density functional theory (DFT), along with the correlation consistent triple zeta basis (cc-pVTZ) [16]. We have used the abovementioned theoretical methods while studying the fragmentation of other AAs, such as proline, tryptophan and asparagine, induced by low-energy electrons [17–19]. The results of theoretical research correspond to the experiment results, indicating that the abovementioned methods are appropriate for studying the fragmentation of AAs.

It is well known that the absolute configuration of AAs (L- or D-isomers) is defined with reference to glyceraldehyde. In this study, we investigated only proteinogenic L-threonine. Thus, the orientation of the -NH_2 group was chosen first. Second, the orientations of the R =–CH(OH)–CH_3 side chain, C_α-COOH and C_β–OH bonds were examined. The abovementioned side chain and the -COOH and -OH groups were rotated clockwise every 20 degrees, and twenty-nine different conformers were designed. To predict the most stable structures, we applied the DFT B3LYP method with the cc-pVTZ basis to obtain the total energy of each designed conformer. The geometry of the conformers was optimized without any symmetry restrictions until the equilibrium point was reached. The vibration frequencies of each conformer were investigated to ensure that the real equilibrium point was obtained. The total energies of the conformers at their equilibrium point were compared, and the three conformers possessing the lowest total energy were named the most stable conformers and chosen for further investigation of fragmentation by low-energy electrons.

The bond lengths and bond order of the L-threonine molecule conformers were analysed to identify the weakest bonds, which are most likely to be cleaved. 'Weakest bond' means that after ionization of the molecule, the bond length increases, while the bond order decreases [20]. The ionization energy (IE) was also researched. IE was calculated as the difference in the energy between the positively charged L-threonine molecule and a neutral one [21]. We also determined the chemical composition of fragments. The appearance energy of the positively charged fragments, E_{ap}, was calculated as follows:

$$E_{ap} = |E_{Thr}| - |\Sigma(E_i)|, \qquad (1)$$

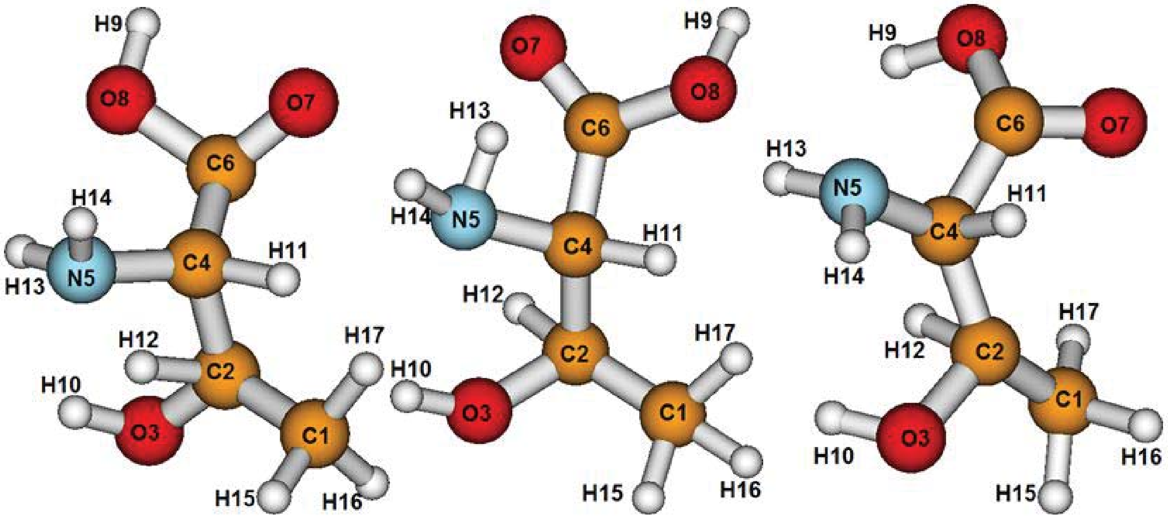

Figure 1: Views of the neutral L-threonine canonical conformers. IC is on the left, IIC is in the centre, and IIIC is on the right.

where E_{Thr} is the total energy of the neutral L-threonine molecule and E_i is the total energy of all fragments that are produced. It is predicted that fragments for which the appearance energy is the lowest are the most likely to form. In some cases, the appearance energy of fragments with the same mass but different chemical compositions is very similar. To determine which fragment product is more probable, we calculated the binding energy per atom. The binding energy per atom was calculated as follows:

$$E_{bind.} = (E_{fragm.} - |\Sigma E_i|)/n_{all}, \qquad (2)$$

where $E_{fragm.}$ – fragment energy, E_i – energy of the atom of the fragment's chemical element, and n_{all} – number of atoms in the fragment. The more stable the fragment is, the higher the binding energy per atom is present. Detection of a more stable fragment in experiments is more probable.

The amount of energy required to form positively charged fragments when the other fragment formed during the same reaction was positive or negative, or neutral, were calculated, *i.e.*, the reactions of a dissociative ionization, dipolar dissociation, dissociative electron attachment, etc. were studied to determine the reaction pathways that were energetically most likely. The most likely fragmentation reactions of L-threonine were determined according to the lowest appearance energy of the fragments of the above processes. It is predicted, that the most likely reactions are those that require the least amount of energy to produce fragments. Zero point corrections were taken into account.

As mentioned above, the strongest peaks in the mass spectrum of L-threonine correspond to m=27 a.m.u., 30 a.m.u., 45 a.m.u., 56 a.m.u., 57 a.m.u., 74 a.m.u. and 75 a.m.u. [8] and [14]. In some cases, the theoretically calculated energy required for the appearance of fragments was lower/higher than expected after analysing peaks intensity in experimentally measured mass spectra. Therefore, we calculated the Gibbs free energy of the reactions during which the fragments formed [22].

The polarizable continuum model (PCM) method was used to evaluate the influence of a solvent (water) [23].

Mass spectrometry data on the positively charged L-threonine fragments available in the National Institute of Standards and Technology (NIST) (without the inclusion of water effects) database [14] and the article by Farajmand and Bahrami [8] were used during this study to select the most probable fragments produced. We emphasize that NIST database does not provide information on the chemical composition of fragments.

In the research, the Gaussian 03 Rev D.01 [24], Gaussian 09 Rev D.01 [25] and Molden [26] programs were used. The GIMP program [27] was used to create the pictures.

3 Results

The views of the most stable L-threonine canonical conformers and the numbers of atoms used in this paper are presented in Figure 1. Further, we use the 'C' index after the number of a conformer (*i.e.*, IC, IIC and IIIC) to indicate canonical conformers.

It is necessary to mention that the structure of the most stable threonine conformers was identified previously. Conformers in which the orientation of the side chain R = $-CH(OH)-CH_3$ and the bonds $C_\alpha-NH_2$, C_α-COOH and $C_\beta-OH$ are similar represent the same conformers.

Zhang and Lin determined 71 different threonine conformers. The eight most stable of these conformers were published in [28]. Some conformations of L-threonine identified by Zhang and Lin [28] are similar to the most energetically favourable conformers, IC, IIC and IIIC, that we have identified. Farajmand and Bahrami found six lowest energy conformers of L-threonine [8]. Regardless of the fact that we used the DFT B3LYP method with the cc-pVTZ basis, whereas Farajmand and Bahrami applied the second-order Møller–Plesset (MP2) perturbation theory/6-311G basis set with the inclusion of the (d,p) polarization and diffusion functions (6-311++G(d,p)) method, the most stable L-threonine conformers identified in this paper (IC, IIC and IIIC) are similar to conformers identified by Farajmand and Bahrami [8]. Alonso *et al.* determined the ten L-threonine conformers with the lowest energy [29]. The authors used the MP2/6-311++G(d,p) method, which is the method that was used by Farajmand and Bahrami. Seven structures were identified by Alonso *et al.* experimentally using rotational spectroscopy in combination with molecular beam and laser ablation (laser-ablation molecular-beam Fourier transform microwave (LA-MB-FTMW) spectroscopy). The most stable L-threonine conformers, IC, IIC and IIIC, are similar to the conformers identified by Alonso *et al.* [29]. Therefore, the most stable conformers of L-threonine that we have identified match those identified by the other authors despite the small number of conformers that we have investigated.

If the water influence is included, the main structural differences in L-threonine (positions of hydrogens of the carboxyl and amine groups) disappeared. However, six zwitterionic conformers of L-threonine were designed and investigated. The comparison of total energy obtained with the influence of water allows us to choose the most stable zwitterionic conformers of L-threonine. The view of the most stable L-threonine zwitterionic conformer and the numbers of atoms used in this paper are presented in Figure 2. Further, we use the ’Z’ index after the number of a conformer (*i.e.*, IIZ) to indicate that the zwitterionic conformer was investigated.

The total energy and the IE of the IC, IIC, IIIC and IIZ L-threonine conformers are presented in the Table 1.

The total energy and the IE of the IC, IIC, IIIC and IIZ L-threonine conformers in the presence of water are presented in the Table 2.

According to our results for total energy (Table 1), the IIC conformer is predominant in vacuum. The zwitterionic L-threonine conformer is predominant when the influence of water is included, as indicated by the comparison of the total energies of the investigated compounds. The total energy of the IIZ conformer is lower than that of IC, IIC and IIIC in investigations performed with the influence of water included. As mentioned above, the ionization energy (IE) was also investigated. The obtained results can be summarized as follows: the IE of the IC-IIIC conformers varies from 9.77 eV to 9.85 eV, whereas that of the IIZ conformer

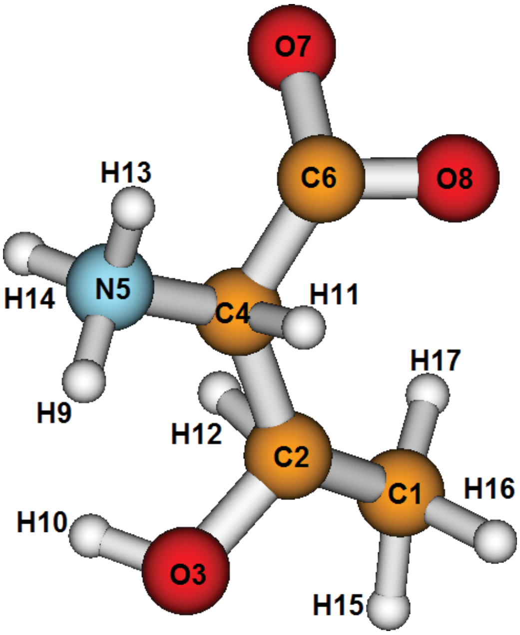

Figure 2: View of the neutral L-threonine molecule zwitterionic IIZ conformer obtained with the influence of water included

Table 1: Total energy and ionization energy of the canonical conformers (IC, IIC and IIIC) and zwitterionic conformer (IIZ) of L-threonine

Conformer	Total energy, a.u.	Ionization energy, eV
IC	−437.728	9.81
IIC	−437.730	9.85
IIIC	−437.726	9.77
IIZ	−437.649	9.69

Table 2: Total energy and ionization energy of the canonical conformers (IC, IIC and IIIC) and zwitterionic conformer (IIZ) of L-threonine obtained with the influence of water included

Conformer	Total energy, a.u.	Ionization energy, eV
IC	−437.789	10.73
IIC	−437.792	10.75
IIIC	−437.785	10.71
IIZ	−437.874	10.82

is 9.69 eV (Table 1). The IE calculated with the influence of water included varies from 10.71 eV to 10.75 eV for the IC-IIIC conformers and is equal to 10.82 eV for the IIZ conformer (Table 2). We compared the IE calculated for canonical conformers in vacuum with the experimentally measured ionization energy of 10.2 eV, which was taken from the NIST database [14]. Our calculated IE values also compared with the values of IE calculated by Sarkar and Kronik (~9.47 eV when using the B3LYP method and ~10.05 eV when using the BHLYP method) [30]. Our calculated IEs match with those Sarkar and Kronik calculated and experimentally measured IEs from NIST. According to our research results (Tables 1, 2), the ionization energy of the L-threonine conformers is lower in vacuum, *i.e.*, the fragment appearance energy in water could be higher.

The bond lengths and bond orders of the neutral and ionized canonical conformers of L-threonine are listed in Supplementary materials (Tables S1 and S2). The weakest bond is C2-C4. After ionization of the molecule, the length of this bond increases while the bond order decreases.

Other bonds of conformers IC, IIC and IIIC that tend to break are as follows: C4-N5 and C6-O8. It important to note that some fragments are formed when several bonds are broken, which could be a consequence of the cleavage of the abovementioned bonds.

3.1 L-threonine fragmentation in vacuum

The strongest peaks in the mass spectrum of L-threonine mostly correspond to m=27 a.m.u., 30 a.m.u., 45 a.m.u., 56 a.m.u., 57 a.m.u., 74 a.m.u. and 75 a.m.u. [8] and [14]. Fourteen chemical compositions of the abovementioned fragments are found. Below, we present our calculated appearance energies (in eV) required for the formation of fragments from the IC, IIC and IIIC conformers of L-threonine in vacuum and from the IIZ conformer of L-threonine (with the influence of water included) (Table 3). The fragmentation reactions (1—14 reactions) of the most stable IIC conformer are listed below. We also present a comparison of the chemical compositions of the positive ions that we have identified with the chemical compositions of the cations (45 a.m.u., 56 a.m.u., 57 a.m.u., 74 a.m.u. and 75 a.m.u.) identified by Farajmand and Bahrami [8]. The chemical compositions of the fragments with the mass m=27 a.m.u. and m=30 a.m.u. were not published in the paper of Farajmand and Bahrami.

It was determined that the cation with m=27 a.m.u. is $C_2H_3^+$. This fragment (see Table 3) is produced when the molecule of L-threonine is split into a pair of ions according to the following reaction:

$$\begin{aligned} C_4H_9NO_3 + e &\rightarrow C_2H_3^+ + (C_2H_4NO_2 + OH + H)^- + e \\ &\rightarrow C_2H_3^+ + C_2H_6NO_3^- + e. \end{aligned} \tag{1}$$

Based on our results presented in Table 3, the m=30 a.m.u. cation is CH_4N^+. Notably, the formation of the CH_4N^+ fragment involves not only bond cleavage but also the migration of one hydrogen atom (see reaction (2)).

$$\begin{aligned} C_4H_9NO_3 + e &\rightarrow (CH_3N + H)^+ + (CO_2H + C_2H_4O)^- + e \\ &\rightarrow CH_4N^+ + C_3H_5O_3^- + e. \end{aligned} \tag{2}$$

Farajmand and Bahrami determined that fragment of m=45 a.m.u. could be $C_2H_5O^+$ and CHO_2^+ [8]. According to our calculations, the $C_2H_5O^+$ ion forms as a result of the rupture of the C2-C4 bond according to reaction (3).

$$C_4H_9NO_3 + e \rightarrow C_2H_5O^+ + C_2H_4NO_2^0 + 2e. \tag{3}$$

A carboxyl group (CHO_2^+ fragment) may form after cleavage of the C4-C6 bond of L-threonine (reaction (4)).

$$C_4H_9NO_3 + e \rightarrow CHO_2^+ + C_3H_8NO^- + e. \tag{4}$$

We determined that the appearance energy required for the formation of the m=45 a.m.u. fragment ($C_2H_5O^+$) is on average only ~0.18 eV (for the IC-IIIC conformers) smaller than that for the fragment CHO_2^+, which has the same mass but a different composition and forms when the weakest bond, C2-C4 ruptures (Tables S1 and S2 in Supplementary materials). This result is insufficient to predict which fragment will predominate; therefore, we compared the stability of the $C_2H_5O^+$ and CHO_2^+ fragments, *i.e.*, the binding energy per atom was calculated. The binding energy per atom of $C_2H_5O^+$ is equal to 2.95 eV and that of CHO_2^+ is 1.5 eV. Thus, $C_2H_5O^+$ fragment is more stable. This way, according to our results– the appearance energy of the $C_2H_5O^+$ and CHO_2^+ fragments and the binding energy per atom – the $C_2H_5O^+$ fragment is more likely to form than the CHO_2^+ fragment.

Farajmand and Bahrami determined two different chemical compositions for the fragment of m=56 a.m.u.: $C_2H_2NO^+$ and $C_3H_6N^+$ [8]. On the other hand, the mass of $C_3H_4O^+$ is also 56 a.m.u., and this fragment could form as well. Therefore, we examined the possible ways for these fragments to form. The $C_2H_2NO^+$ cation forms when L-threonine splits into a pair of ions according to the following reaction:

$$\begin{aligned} C_4H_9NO_3 + e &\rightarrow C_2H_2NO^+ + (C_2H_5O + OH + H)^- + e \\ &\rightarrow C_2H_2NO^+ + C_2H_7O_2^- + e. \end{aligned} \tag{5}$$

Table 3: Calculated appearance energies (in eV) of fragments formed from the investigated L-threonine IC, IIC and IIIC conformers in vacuum and the L-threonine IIZ conformer with the influence of water included

Fragment		Conformer IC	Conformer IIC	Conformer IIIC	Conformer IIZ
		Appearance energy, eV			
$C_2H_3^+$ (m=27 a.m.u.)	$C_2H_6NO_3^-$ (m=92 a.m.u.)	15.82	15.65	15.92	16.74
CH_4N^+ (m=30 a.m.u.)	$C_3H_5O_3^-$ (m=89 a.m.u.)	13.74	13.76	13.84	14.60
$C_2H_5O^+$ (m=45 a.m.u.)	$C_2H_4NO_2^0$ (m=74 a.m.u.)	10.56	10.52	10.64	11.54
CHO_2^+ (m=45 a.m.u.)	$C_3H_8NO^-$ (m=74 a.m.u.)	10.84	10.72	10.73	11.95
$C_2H_2NO^+$ (m=56 a.m.u.)	$C_2H_7O_2^-$ (m=63 a.m.u.)	14.81	14.87	15.01	16.03
$C_3H_4O^+$ (m=56 a.m.u.)	$CH_5NO_2^-$ (m=63 a.m.u.)	15.77	15.80	15.95	16.88
$C_3H_6N^+$ (m=56 a.m.u.)	$CH_3O_3^-$ (m=63 a.m.u.)	16.78	16.89	16.92	17.83
$C_2H_3NO^+$ (m=57 a.m.u.)	$C_2H_6O_2^0$ (m=62 a.m.u.)	9.98	9.98	9.99	10.87
$C_2HO_2^+$ (m=57 a.m.u.)	$C_2H_8NO^0$ (m=62 a.m.u.)	10.53	10.51	10.55	11.46
$C_3H_7N^+$ (m=57 a.m.u.)	$CH_2O_3^0$ (m=62 a.m.u.)	11.75	11.86	11.83	12.11
$C_2H_4NO_2^+$ (m=74 a.m.u.)	$C_2H_5O^0$ (m=45 a.m.u.)	10.48	10.49	10.51	11.44
$C_3H_8NO^+$ (m=74 a.m.u.)	CHO_2^- (m=45 a.m.u.)	12.42	12.41	12.48	13.42
$C_2H_5NO_2^+$ (m=75 a.m.u.)	$C_2H_4O^-$ (m=44 a.m.u.)	11.10	11.14	11.18	12.09
$C_3H_9NO^+$ (m=75 a.m.u.)	CO_2^- (m=44 a.m.u.)	11.78	11.77	11.81	12.75

Thus, the formation mechanism of the $C_2H_2NO^+$ ion includes detachment of the OH group of a carboxyl group and a dissociation of the H11 atom from the initial molecule, along with rupture of the C2-C4 bond.

We determined that the $C_3H_6N^+$ ion forms due to detachment of the H11 atom and the OH and COOH groups (reaction (6)).

$$C_4H_9NO_3 + e \rightarrow C_3H_6N^+ + (CHO_2 + OH + H)^- + e \rightarrow C_3H_6N^+ + CH_3O_3^- + e. \qquad (6)$$

The fragment of 56 a.m.u. ($C_3H_4O^+$) can form as a result of reaction (7).

$$C_4H_9NO_3 + e \rightarrow C_3H_4O^+ + (NH_2 + CHO_2 + 2H)^- + e \rightarrow C_3H_4O^+ + CH_5NO_2^- + e. \qquad (7)$$

Comparing the appearance energy of fragments of m=56 a.m.u. (Table 3) shows that the formation of the $C_2H_2NO^+$ fragment is more probable than formation of $C_3H_4O^+$ and $C_3H_6N^+$ and that formation of the $C_3H_4O^+$ cation is more probable than formation of $C_3H_6N^+$. Hence, the $C_2H_2NO^+$ fragment is predominant.

The strongest peak in the experimentally measured mass spectrum of positively charged L-threonine fragments from the NIST database [14] and article [8] correspond to mass m=57 a.m.u. Farajmand and Bahrami determined that the ion with m=57 a.m.u. could be $C_2H_3NO^+$ or $C_3H_7N^+$ (8). We found that the cation $C_2H_3NO^+$ may be produced due to cleavage of C2-C4 (dissociation of the L-threonine side chain -CH(CH_3)–OH) and the bond C6-O8 (dissociation of the -COOH hydroxy group) as a result of reaction (8).

$$C_4H_9NO_3 + e \rightarrow C_2H_3NO^+ + (OH + C_2H_5O)^0 + 2e \quad (8)$$
$$\rightarrow C_2H_3NO^+ + C_2H_6O_2^0 + 2e.$$

We noticed that the $C_3H_7N^+$ cation can form as a result of the following reaction:

$$C_4H_9NO_3 + e \rightarrow C_3H_7N^+ + (OH + CHO_2)^0 + 2e \quad (9)$$
$$\rightarrow C_3H_7N^+ + CH_2O_3^0 + 2e.$$

Additionally, we determined that the fragment of m=57 a.m.u. may also be $C_2HO_2^+$. It is most likely that the $C_2HO_2^+$ fragment will form during reaction (10) when the weakest C2-C4 and C4-N5, C4-H11 bonds rupture.

$$C_4H_9NO_3 + e \rightarrow C_2HO_2^+ + (C_2H_5O + H + NH_2)^0 + 2e \quad (10)$$
$$\rightarrow C_2HO_2^+ + C_2H_8NO^0 + 2e.$$

Based on the appearance energies required for the formation of these fragments (Table 3), formation of the $C_2H_3NO^+$ cation is more likely than formation of $C_2HO_2^+$ and $C_3H_7N^+$, *i.e.*, process (8), is more probable than the other processes.

Another strong peak in the mass spectrum of L-threonine positive fragments [8, 14] is at m=74 a.m.u. According to the authors [8], this peak can be identified as belonging to $C_2H_4NO_2^+$ or $C_3H_8NO^+$. We determined that formation of $C_2H_4NO_2^+$ is more probable and that the following reaction is the most energetically favourable reaction for the formation of this fragment:

$$C_4H_9NO_3 + e \rightarrow C_2H_4NO_2^+ + C_2H_5O^0 + 2e. \quad (11)$$

The fragment of m=74 a.m.u. ($C_3H_8NO^+$) could form during the reaction due to the rupture of the C4-C6 bond of L-threonine (reaction (12)).

$$C_4H_9NO_3 + e \rightarrow C_3H_8NO^+ + CHO_2^- + e. \quad (12)$$

Farajmand and Bahrami found that the cation of m=75 a.m.u. could be $C_2H_5NO_2^+$ or $C_3H_9NO^+$ [8]. The chemical compositions of the fragment of m=75 a.m.u. that we identified match those that were identified by the abovementioned authors. We noticed that formation of the fragment of m=75 a.m.u. ($C_2H_5NO_2^+$) is related to the migration of the atom H12 to the atom C4 (see reaction (13)).

$$C_4H_9NO_3 + e \rightarrow (C_2H_4NO_2 + H)^+ + C_2H_4O^- + e \quad (13)$$
$$\rightarrow C_2H_5NO_2^+ + C_2H_4O^- + e.$$

We determined that formation of the fragment m=75 a.m.u. ($C_3H_9NO^+$) is possible during the following reaction:

$$C_4H_9NO_3 + e \rightarrow (C_3H_8NO + H)^+ + CO_2^- + e \quad (14)$$
$$\rightarrow C_3H_9NO^+ + CO_2^- + e.$$

Table 4: Gibbs free energy of the reactions in vacuum and in water

Reaction	Gibbs free energy, kcal/mol
in vacuum	
$C_2H_4NO_2^+ + H^0 \rightarrow C_2H_5NO_2^+$	−4.018
$C_3H_8NO^+ + H^0 \rightarrow C_3H_9NO^+$	−3.782
in water	
$C_2H_4NO_2^+ + H^0 \rightarrow C_2H_5NO_2^+$	−2.876
$C_3H_8NO^+ + H^0 \rightarrow C_3H_9NO^+$	−1.019

According to our results (Table 3), formation of the cation of m=75 a.m.u. ($C_2H_5NO_2^+$) is more probable than formation of the m=75 a.m.u. cation ($C_3H_9NO^+$), *i.e.*, the appearance energy of $C_2H_5NO_2^+$ is smaller.

Based on our comparison of the appearance energy (Table 3), formation of $C_2H_4NO_2^+$ (m=74 a.m.u.) is more probable than formation of $C_2H_5NO_2^+$ (m=75 a.m.u.). This conclusion allows us to predict that the peak at the mass of m=74 a.m.u. would be stronger than the peak at the mass of m=75 a.m.u.; this prediction contradicts the experimental measurement of a stronger peak at the mass of m=75 a.m.u. [8, 14] than at the mass of m=74 a.m.u. Thus, the assumption was made that the m=75 a.m.u. fragment can form from the m=74 a.m.u. fragment.

Thus, we calculated the Gibbs free energy (Table 4) of reactions (15) and (16).

$$C_2H_4NO_2^+ + H^0 \rightarrow C_2H_5NO_2^+. \quad (15)$$

$$C_3H_8NO^+ + H^0 \rightarrow C_3H_9NO^+. \quad (16)$$

According to our results (Table 4), reactions (15) and (16) are exergonic, *i.e.*, progress spontaneously in the forward direction. Comparison of the appearance energy (Table 3) and Gibbs free energy (Table 4) data revealed that the $C_2H_5NO_2^+$ and $C_3H_9NO^+$ (m=75 a.m.u.) cations form not only during direct fragmentation of L-threonine but also when $C_2H_4NO_2^+$ and $C_3H_8NO^+$ (m=74 a.m.u.), respectively, attach the H^0 atom.

Our calculations allow us to predict the most energetically favourable fragmentation reactions (1-14) for the L-threonine molecule and formation of the fragments of m=75 a.m.u. from fragments of m=74 a.m.u. (reactions 15, 16).

3.2 L-threonine fragmentation under the influence of water

As mentioned above, experimental data [31] show that the canonical conformers of L-threonine transform into the

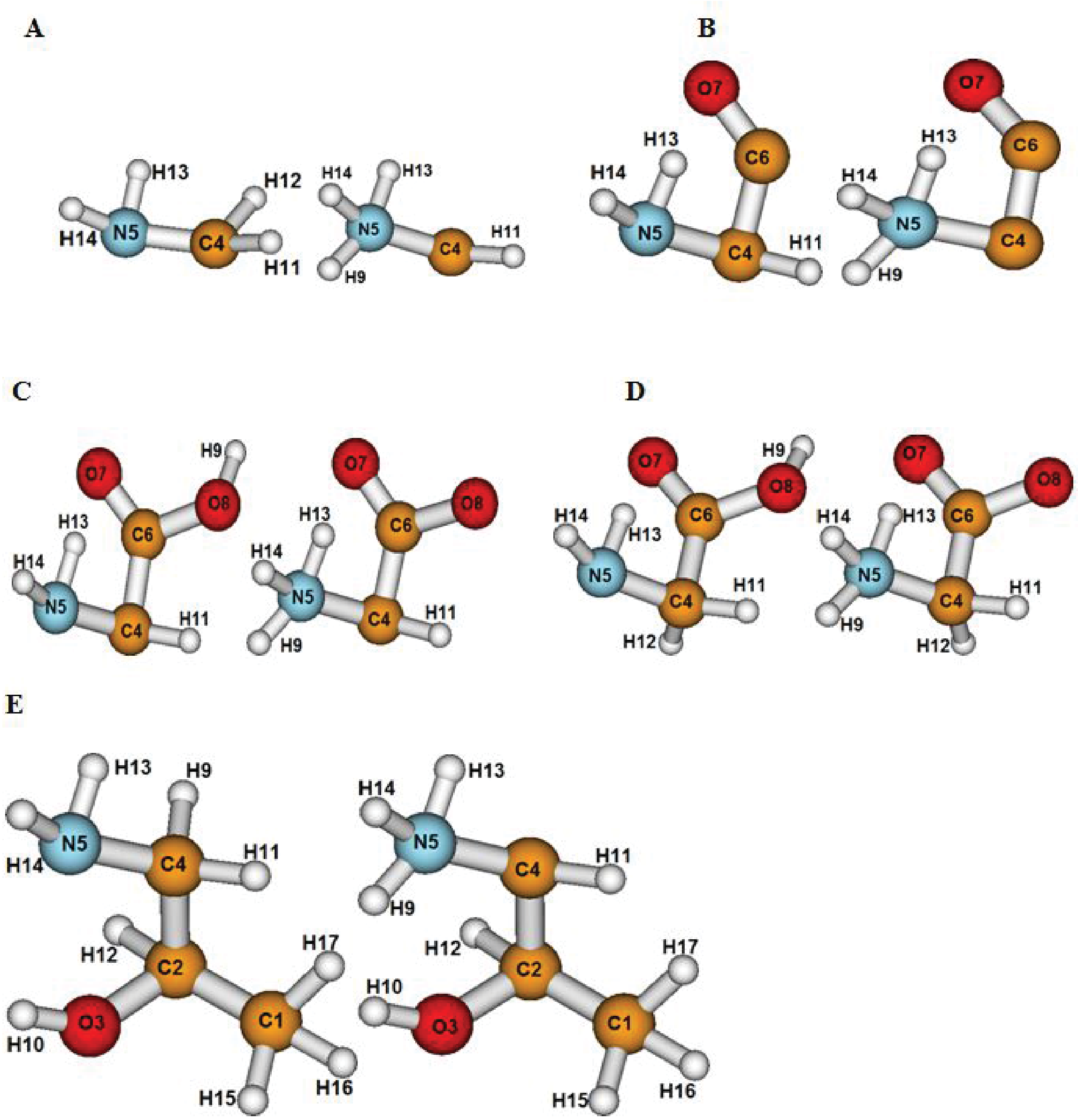

Figure 3: Views of the L-threonine fragments of the same mass and chemical composition in vacuum and in water. A — CH_4N^+ (m=30 a.m.u.) structures, B — $C_2H_3NO^+$ (m=57 a.m.u.) structures, C — $C_2H_4NO_2^+$ (m=74 a.m.u.) structures, D — $C_2H_5NO_2^+$ (m=75 a.m.u.) structures and E — $C_3H_9NO^+$ (m=75 a.m.u.) structures.

zwitterionic forms, *i.e.*, the amine group (-NH_2) is protonated, while the carboxyl group (-COOH) is deprotonated when water influence is included. Our results are in line with those of previous studies.

The view of the most stable neutral zwitterionic IIZ conformer of L-threonine (Figure 2) and the bond lengths and orders of the neutral and ionized IIZ conformer (Table S3 in Supplementary materials) are presented.

Furthermore, we calculated the Gibbs free energy for reactions (15-16) in water (Table 4). We also present the main differences between the fragmentation of L-threonine with or without the influence of water. Section 3.1 describes which fragment of each mass (m=27 a.m.u., 30 a.m.u., 45 a.m.u., 56 a.m.u., 57 a.m.u., 74 a.m.u. and 75 a.m.u.) is the most probable in vacuum. Therefore, in this section, only differences in the most likely cation structures and the fragmentation reactions during which these structures are formed in vacuum and water are presented. Moreover, the differences in the fragment $C_3H_9NO^+$ (m=75 a.m.u.), which forms when $C_3H_8NO^+$ (m=74 a.m.u.) attaches a H^0 atom, are described in vacuum and in water.

Table 5: Total energy of canonical and zwitterionic fragments obtained in the vacuum and calculated with the inclusion in water

Fragments	Canonical fragment total energy, a.u. (in vacuum)	Zwitterionic fragment total energy, a.u. (in vacuum)	Canonical fragment total energy, a.u. (in water)	Zwitterionic fragment total energy, a.u. (in water)
CH_4N^+ (m=30 a.m.u.)	−94.664	−94.637	−94.887	−94.920
$C_2H_3NO^+$ (m=57 a.m.u.)	−207.394	−207.337	−207.530	−207.601
$C_2H_4NO_2^+$ (m=74 a.m.u.)	−283.258	−283.220	−283.260	−283.299
$C_2H_5NO_2^+$ (m=75 a.m.u.)	−283.809	−283.783	−284.014	−284.045
$C_3H_9NO^+$ (m=75 a.m.u.)	−248.424	−248.405	−249.426	−249.457

According to our research results (Tables S1, S2 and S3 in Supplementary materials), the O8-H9 bond is specific to canonical conformers, while the N5-H9 bond is specific to the IIZ conformer. The weakest bond of the IIZ conformer of L-threonine is C2-C4. Other weak IIZ bonds are C4-N5 and C6-O8. As mentioned above, the same bonds (C2-C4 and C4-N5, C6-O8) are also the weakest bonds in the IC, IIC and IIIC conformers of L-threonine. The fact that the same bonds are the weakest in both the canonical and zwitterionic structures of L-threonine explains why fragments with the same chemical composition are the most likely in vacuum and water.

The Gibbs free energy that we calculated for reactions (15) and (16) is presented. Based on our results (Table 4), in water, reactions (15) and (16) are exergonic. Thus, the $C_2H_5NO_2^+$ and $C_3H_9NO^+$ (m=75 a.m.u.) positively charged fragments can form, when $C_2H_4NO_2^+$ and $C_3H_8NO^+$ (m=74 a.m.u.), respectively, attach the H^0 atom.

The comparison of the fragment production in vacuum and in water revealed some interesting differences. First, our results indicate that the appearance energy required for the formation of fragments with the same mass and chemical composition (*e.g.*, the most possible m=57 a.m.u. ($C_2H_3NO^+$)) is lower for the decomposition of the IC, IIC and IIIC L-threonine conformers in vacuum than the value required for the fragmentation of the IIZ L-threonine conformer in water.

Another observation is that some ion structures, that is, m=30 a.m.u. (CH_4N^+), m=57 a.m.u. ($C_2H_3NO^+$), m=74 a.m.u. ($C_2H_4NO_2^+$) and m=75 a.m.u. ($C_2H_5NO_2^+$ and $C_3H_9NO^+$), are different (Figure 3). Figure 3 shows fragment structures that form due to fragmentation of conformers IIC and IIZ. The main differences that are observed are caused by the amine group (-NH_2)/ protonated amine group (-NH_3^+) and carboxyl group (-COOH)/deprotonated carboxyl group (-COO^-).Thus, the medium can affect not only the appearance energy but also to the geometry of L-threonine fragments.

We further use the term 'canonical fragment' to indicate that this fragment can form as a result of the fragmentation of a canonical conformer of L-threonine and use the term 'zwitterionic fragment' for a fragment that can form due to the fragmentation of a zwitterionic conformer. We have investigated the possibility of transforming the canonical fragments into zwitterionic fragments and vice versa in vacuum and in water. Our research results are presented below.

Based on these results (Tables 5), the canonical fragments CH_4N^+, $C_2H_3NO^+$, $C_2H_4NO_2^+$, $C_2H_5NO_2^+$ and $C_3H_9NO^+$ are predominant in vacuum, while their zwitterionic forms are more probable in water.

In this paper, the formation differences between L-threonine fragments in vacuum and in water are classified into types I, II and III, depending on the migration of the hydrogen atom. In the first case, migration of the H atom occurs only in vacuum (type I). In the second case, only chemical bonds rupture without migration of the H atom in vacuum an in water (type II). In the third case, migration of the H atom occurs both in vacuum and in water (type III). Formation of the CH_4N^+ and $C_3H_9NO^+$ cations is classified as type I; $C_2H_3NO^+$ and $C_2H_4NO_2^+$, type II; and $C_2H_5NO_2^+$, type III.

The results of our research show that in some cases, the same fragments are produced during different fragmentation reactions in vacuum and in water. Below, we present the different ways to produce the same mass fragments via IIC conformer fragmentation in vacuum and via IIZ conformer fragmentation in water. We observed that the m=30 a.m.u. (CH_4N^+) fragment forms due to the cleavage of the C2-C4, C4-C6 and C2-H12 bonds of L-threonine and migration of the H12 atom to the C4 atom (see reaction (2)). This cation can form due to rupture of the same chemical bonds, without detachment of H12 when IIZ decomposes. We also noticed that the m=56 a.m.u. ($C_2H_2NO^+$) positive ion is produced when the weakest C2-C4 bond and the C4-H11 and C6-O8 bonds of L-threonine rupture (see reaction (5)). On the other hand, cleavage of the bond N5-H9 has to occur as well in order for this fragment to form during the decomposition of IIZ. Them=57 a.m.u. ($C_2H_3NO^+$) fragment is most likely produced when the weakest bonds C2-C4 bond and C6-O8 cleave (according to reaction (8)), while the chemical bond C4-H11 also cleaves when the fragment forms during decomposition of the IIZ conformer of L-threonine. We also observed that m=75 a.m.u. ($C_3H_9NO^+$) is produced in $C_3H_8NO^+$ (m=74 a.m.u.) when the H9 atom attaches to C4 of L-threonine IIC and when the H11 atom attaches to the C4 atom of the IIZ conformer.

4 Conclusions

The fragmentation of L-threonine conformers in vacuum has been theoretically studied with the use of the DFT B3LYP method with the cc-pVTZ basis. The PCM method has been used to include the influence of water. Cations were selected based on the experimental mass spectrometry data in the NIST database [14] and the article by Farajmand and Bahrami [8].

We determined that the fragments $C_2H_5NO_2^+$ and $C_3H_9NO^+$ (m=75 a.m.u.) can be produced not only during direct fragmentation of L-threonine but also when $C_2H_4NO_2^+$ and $C_3H_8NO^+$ (m=74 a.m.u.), respectively, bind to a H^0 atom.

The results of our research with and without the influence of water indicate that the energy required to form fragments with the same molecular mass and chemical composition is different. More energy is needed for the formation of identical fragments in water.

Moreover, the results of our theoretical research show that the structures of some cations should be different in vacuum and in water.

Furthermore, the medium where fragmentation occurs can affect the reactions underlying the formation of fragments with an identical mass and identical chemical composition: in different mediums, the fragment formation processes can be different.

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References

[1] Cava F., Lam H., De Pedro M.A., Waldor M.K., Emerging knowledge of regulatory roles of D-amino acids in bacteria, Cell Mol. Life Sci., 2011, 68, 817-831.

[2] Wu G., Amino acids: metabolism, functions, and nutrition, Amino acids, 2009, 37,1-17.

[3] Wu G., Functional amino acids in nutrition and health, Amino acids, 2013, 45, 407-411.

[4] Wang W., Wu Z., Dai Z.,Yang Y., Wang J., Wu G., Glycine metabolism in animals and humans: implications for nutrition and health, Amino acids, 2013, 45, 463-47.

[5] Mao X., Zeng X., Qiao S., Wu G., Li D., Specific roles of threonine in intestinal mucosal integrity and barrier function, Front Biosci. (Elite Ed), 2011, 3,1192-1200.

[6] Li P., Yin Y. L., Li D., Kim S. W., Wu G., Amino acids and immune function, Br. J. Nutr., 2007, 98, 237-252.

[7] Hall E. J., Graccia, A. J., Radiobiology for the radiologist, 6th. Lippincott Williams & Wilkins, USA, 2006.

[8] Farajmand B., Bahrami H., Electron Ionization of Serine and Threonine: a Discussion about Peak Intensities, Phys. Chem. Res., 2016, 4, 539-551.

[9] Serafin S.V., Zhang K., Aurelio L., Hughes A.B., Morton T.H., Decomposition of protonated threonine, its stereoisomers, and its homologues in the gas phase: evidence for internal backside displacement, Org. Lett., 2004,10,1561-1564.

[10] Alberts B., Bray D., Hopkin K., Johnson A., Lewis J., Raff M., Roberts K., Walter P., Essential Cell Biology, 4th edn. Garland Science, Taylor & Francis Group, USA, 2014.

[11] Seager S.L., Slabaugh M.R., Chemistry for Today, 8th edn. Cengage learning, USA, 2014.

[12] Bettelheim F.A., Brown W.H., Campbell M.K., Farrell S.O., Introduction to General, Organic and Biochemistry, 9th edn. Cengage learning, USA, 2009.

[13] Ren Y., Kresin V.V., Suppressing the fragmentation of fragile molecules in helium nanodroplets by coembedding with water: Possible role of the electric dipole moment, J. Chem. Phys., 2008,128, 074303.

[14] National Institute of Standards, Standard Reference Database: Chemistry Webbook. 01 October 2015. http://webbook.nist.gov/chemistry/.

[15] Becke A.D., A new mixing of Hartree–Fock and local density-functional theories. J. Chem. Phys., 1993, 98,1372-1377.

[16] Kendall R.A., Dunning Jr T.H., Harrison R.J., Electron affinities of the first-row atoms revisited. Systematic basis sets and wave functions, Chem. Phys., 1992, 96, 6796-6806.

[17] Tamuliene J., Romanova L., Vukstich V., Papp A., Shkurin S., Baliulyte L., Snegursky A., On the influence of low-energy ionizing radiation on the amino acid molecule: proline, EPJ D, 2016, 70,1-10.

[18] Tamuliene J., Romanova L.G., Vukstich V.S., Papp A.V., Snegursky A.V., Electron-impact-induced tryptophan molecule fragmenta-

tion, EPJ D, 2015, 69,1-10.
[19] Tamuliene J., Romanova L.G., Vukstich V.S., Papp A.V., Snegursky A.V., Electron-impact-induced asparagine molecule fragmentation, EPJ D, 2014, 68,1-17.
[20] Mulliken R.S., Electronic population analysis on LCAO–MO molecular wave functions. II. Overlap populations, bond orders, and covalent bond energies, J. Chem. Phys., 1955, 23, 1841-1846.
[21] Smalo H.S., Astrand P.O., Ingebrigtsen S., Calculation of ionization potentials and electron affinities for molecules relevant for streamer initiation and propagation, IEEE Trans. Dielectr. Electr. Insul., 2010, 17.
[22] Ochterski J.W., Thermochemistry in gaussian., Gaussian Inc., 2000, 1-19.
[23] Skyner R.E., McDonagh J.L., Groom C.R., van Mourik T., Mitchell J.B.O., A review of methods for the calculation of solution free energies and the modelling of systems in solution. Phys. Chem. Chem. Phys., 2015, 17, 6174-6191.
[24] Gaussian 03, Revision C.02, Frisch M.J., Trucks G.W., Schlegel H.B., et al., Gaussian, Inc. Wallingford C.T., 2004, http://gaussian.com/g03citation/. 02 October 2015.
[25] Gaussian 09, Revision A.02, Frisch M.J., Trucks G.W., Schlegel H.B., et al., Gaussian, Inc. Wallingford C.T., 2016, http://gaussian.com/g09citation/. 01 September 2018.
[26] Schaftenaar G., Jan H.N., Molden: a pre-and post-processing program for molecular and electronic structures, J. Comput. Aided Mol. Des., 2000, 14, 123-134.
[27] Lecarme O., Delvare K., The Book of GIMP–A Complete Guide to Nearly Everything, No starch press, San Francisco, 2013.
[28] Zhang M., Lin Z., Ab initio studies of the conformers and conformational distribution of the gaseous hydroxyamino acid threonine, J. Mol. Struct., 2006, 760, 159-166.
[29] Alonso J.L., Pérez C., Sanz M.E., López J.C., Blanco S., Seven conformers of l-threonine in the gas phase: a LA-MB-FTMW study, Phys. Chem. Chem. Phys., 2009, 11, 617-627.
[30] Sarkar S., and Kronik L., Ionisation and (de-)protonation energies of gas-phase amino acids from an optimally tuned range-separated hybrid functional, Mol. Phys., 2016, 114,1218–1224.
[31] Quesada-Moreno M.M., Márquez-García A.Á., Avilés-Moreno J.R., and López-González J.J., Conformational landscape of L-threonine in neutral, acid and basic solutions from vibrational circular dichroism spectroscopy and quantum chemical calculations, Tetrahedron Asymmetry, 2013, 24, 1537–1547.

Supplementary Materials

Table S1: Bond lengths of the neutral and ionized canonical conformers (IC, IIC and IIIC) of the investigated L-threonine molecule

Bond	Conformer IC		Conformer IIC		Conformer IIIC	
	Bond length, Å neutral	Bond length, Å ionized	Bond length, Å neutral	Bond length, Å ionized	Bond length, Å neutral	Bond length, Å ionized
C1-C2	1.516	1.471	1.450	1.408	1.524	1.520
C1-H15	1.089	1.098	1.089	1.100	1.091	1.098
C1-H16	1.089	1.091	1.089	1.192	1.087	1.091
C1-H17	1.090	1.087	1.089	1.081	1.091	1.083
C2-O3	1.421	1.311	1.400	1.348	1.422	1.314
C2-C4	1.556	1.887	1.450	1.819	1.557	1.883
C2-H12	1.096	1.085	1.089	1.079	1.096	1.083
O3-H10	0.964	0.968	2.796	2.898	0.964	0.969
C4-N5	1.329	1.465	1.440	1.595	1.465	1.499
C4-C6	1.523	1.468	1.450	1.440	1.523	1.478
C4-H11	1.079	1.090	1.089	1.179	1.089	1.098
N5-H13	1.012	1.011	1.008	0.905	1.013	1.011
N5-H14	1.011	1.009	1.008	1.002	1.013	1.009
C6-O7	1.204	1.202	1.220	1.202	1.204	1.203
C6-O8	1.541	1.688	1.400	1.635	1.354	1.479
O8-H9	0.969	0.970	0.950	0.973	0.969	0.969

Table S2: Bond orders of the neutral and ionized canonical conformers (IC, IIC and IIIC) of the investigated L-threonine molecule

Bond	Conformer IC		Conformer IIC		Conformer IIIC	
	Bond order, neutral	Bond order, ionized	Bond order, neutral	Bond order, ionized	Bond order, neutral	Bond order, ionized
C1-C2	0.331	0.312	0.281	0.273	0.335	0.312
C1-H15	0.393	0.377	0.327	0.314	0.394	0.377
C1-H16	0.384	0.395	0.313	0.396	0.384	0.395
C1-H17	0.396	0.392	0.356	0.344	0.396	0.392
C2-O3	0.350	0.390	0.306	0.372	0.350	0.390
C2-C4	0.234	0.135	0.171	0.101	0.234	0.135
C2-H12	0.401	0.405	0.340	0.404	0.401	0.404
O3-H10	0.337	0.329	0.211	0.205	0.369	0.329
C4-N5	0.326	0.302	0.401	0.337	0.302	0.246
C4-C6	0.292	0.289	0.258	0.220	0.293	0.253
C4-H11	0.421	0.401	0.200	0.193	0.409	0.361
N5-H13	0.385	0.382	0.370	0.322	0.385	0.383
N5-H14	0.379	0.379	0.307	0.379	0.379	0.379
C6-O7	0.760	0.753	0.652	0.753	0.760	0.752
C6-O8	0.423	0.434	0.360	0.310	0.423	0.434
O8-H9	0.312	0.332	0.330	0.352	0.312	0.333

Table S3: Bond lengths and orders of the neutral and ionized IIZ conformers of the investigated L-threonine molecule with the influence of water included

Bond	Conformer IIZ			
	Bond length, Å neutral	Bond length, Å ionized	Bond order, neutral	Bond order, ionized
C1-C2	1.450	1.409	0.316	0.302
C1-H15	1.089	1.099	0.394	0.373
C1-H16	1.089	1.099	0.350	0.396
C1-H17	1.089	1.078	0.415	0.393
C2-O3	1.400	1.340	0.332	0.391
C2-C4	1.450	1.811	0.207	0.090
C2-H12	1.089	1.078	0.253	0.404
O3-H10	0.950	0.969	0.362	0.327
C4-N5	1.440	1.663	0.147	0.091
C4-C6	1.450	1.429	0.431	0.392
C4-H11	1.089	1.179	0.246	0.231
N5-H9	1.008	0.973	0.368	0.385
N5-H13	1.008	0.911	0.340	0.315
N5-H14	1.008	1.002	0.337	0.553
C6-O7	1.400	1.202	0.664	0.735
C6-O8	1.220	1.448	0.718	0.652