MODELLING AMINO ACID THREONINE UNDER IONIZING RADIATION: MECHANISM OF FRAGMENTATION

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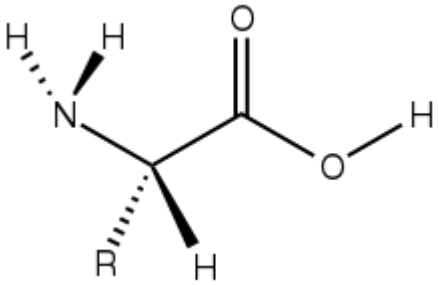
Abstract. *This work grew from our observations, mainly of the second co-author, over the years on the chemical reactions of amino acids under ionization that have been often debated, interpreted differently and not always clearly. Usually chemical reactions produce messy mixtures of products. The reaction of the interaction of amino acids with ionization radiation - or, simply, ionization - is no exception. The intricacies of its description has attracted the attention of researchers over the past few decades. On the contrary, we set ourselves the goal of elucidating the molecular orbital mechanism of the fragmentation process, which underlies the aforementioned reaction and prevails in the latter, using the amino acids threonine whose interaction with radiation is considered in this work quantum mechanically, within the density functional theory. And thus, we came to the conclusion that along with fragmentation, there is in parallel some alternative pathway that fuses fragmentation products via a novel "fragmentation bonding". The latter is studied in detail on the example of threonine ionization, extended to the 2nd order of the Möller-Plesset perturbation theory.*

1. Introduction

This topic is not new. In the 21st century, the century of gadgets and of the second technological revolution, only the lazy does not discuss interactions of life organisms, containing, as a human body, hundreds of trillions of cells, with ionizing radiation[1] in many of possible manifestations: Chernobyl and Fukushima (high-energy radiation), cosmic rays, rocks' nucleotides, different medical treatments, and the other sources of rather low-energy radiation (see Refs. [5-10] and references therein).

At the cell or biomolecular level, these interactions are a matter of life and death since the resulted low-energy electrons produce the long-term effects that cause a variety of biological damages, including an increased risk of cancer in particular. For instance, the life of the next generation inherits, throughout a death, various traits - genetic information - from the previous-life one. This genetic information is transmitted via some architect-like rules which altogether are named as the genetic code. The latter translates the genetic information that is stored in the DNA into proteins by means of 20 amino acids, the group of organic molecules containing both acidic carboxyl group (-COOH) and a basic amino group ($-NH_2$) and the building blocks of proteins in living cells, that codes each specific codon(s).

[1] In conventional wisdom, "radiation is the emission of energy in the form of waves or particles, such as electrons for example, through space or ... a material medium" ([1,2]; see also Wikipedia and Ref. [3]). "Ionizing radiation is a term used for any radiation that is capable of ionizing (i.e. removing electrons from) atoms or molecules of the medium being traversed. Ionizing radiations are usually classified as either electromagnetic or particulate" [4]. Note that the concept of ionization is closely related to the formation of the Universe: A few minutes after the Big Bang, the entire Universe was ionized, i.e. the nuclei of hydrogen and helium had no electrons bound to them (S.E.I. Bosman et al. Hydrogen reionisation ends by z = 5.3: Lyman-α optical depth measured by the XQR-30 sample. ArXiv: 2108.03699 (2022); P. Plait, What reionized the Universe, and when? https://www.yahoo.com/entertainment/reionized-universe-130013955.html). .

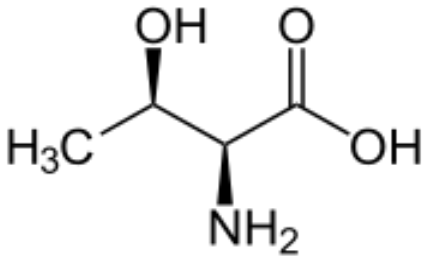


Scheme 1. **A general neutral structure of L-amino acid (from Wiki: https://en.wikipedia.org/wiki/Amino_acid). α-amino acid is the amino acid whose amino nitrogen atom is bonded to the α-carbon which is bonded to the carboxylate group which is now is in *cis*-position.**

Under ionizing radiation the amino acid exposes to damage, in particular undergo a fragmentation along the [H_2OOC-NH_2-H]*C2*-*C7*-OH-CH_3 bond that has already been studied experimentally [3,11-19]: for L-threonine, for instance, the fragmentation channel is the following [16]:

$$C_4H_9NO_3 \rightarrow C_2H_5NO_2 + C_2H_4O\,. \qquad (1)$$

These patterns may then spread throughout the genetic code and cause its damage that, in turn, produces damaged cells. The deleterious effects of the latter on living organism can be so varied that it is even hard to imagine (e.g. [7,8]).



Scheme 2. **L(left-handed)-amino acid threonine (Thr: R = CH_3-) $C_4H_9NO_3$ [1].**

The present work aims to computationally unveil the physics of events that occur under bombarding the amino acid Thr by low-energy electrons with different radiation doses and that precede the fragmentation, and thus, constitute the pre-fragmentation stage. In particular, we will demonstrate that these events involve novel fragmentation patterns which mechanism of formation will be studied as well. To model amino acids participating in the above experiments, we consider threonine ([20]; see also [21]) shown in Scheme 2.

Speaking generally, the problem of fragmentation of amino acids and peptides they compose under ionization has been an issue that has lingered among chemists and biophysicists for decades [22-24]. Thus, the main motivation of the present paper was to computationally resolve a longstanding controversy in the science community about the mechanism of fragmentation, which we propose on the basis of molecular-orbital theory that includes the concept of a so called Dyson orbitals [25-27]. For this purpose, we have chosen the following layout. The second Section describes the computational methodology and the third one step by step reveals some backstage simulating the fragmentation of Thr. Section 4 concludes this work

2. Computational Methodology

The computational methodology is based on using the GAUSSIAN package of quantum-chemical programs [28] and on the B3LYP density functional theory (DFT) in junction with

[1] Threonine is encoded by the codons starting with AC (ACU, ACC, ACA, and ACG), respectively.

the Pople's 6-311++G(d,p) basis set which has already been employed for such class of tasks (see, e.g. [14,29] that reports six and seven conformers of L-threonine in the gas phase, respectively).

All reported energies, except those in Table 1, were calculated by taking the zero-point energies (ZPE) into account.

3. Backstage of Fragmentation of L-threonine

Since the beginning of our narrative was referred to L-threonine, the latter starts this Section.

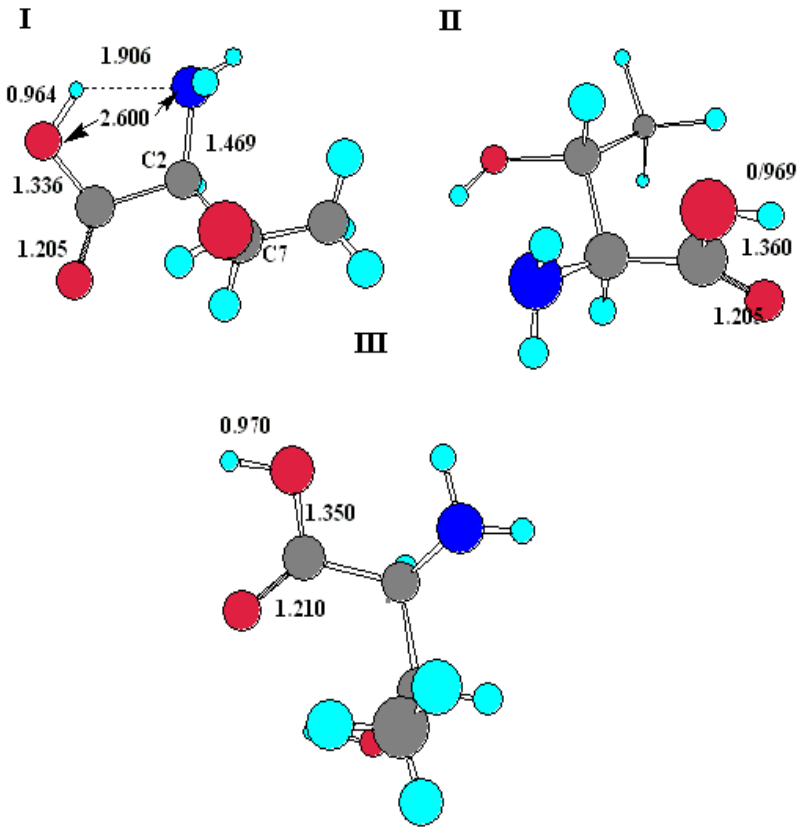


Fig. 1. **Low-energy conformers I - III of L-threonine studied in the present work. Bondlengths are given in Å, and bond angles in °. Their energies and ZPEs are listed in Table 1. Conformer I is drawn on the cover page of [21]**

On the lower-energy portion of the ground-state potential energy surface (PES) of L-threonine, we determine three noteworthy structures I - III which are displayed in Fig. 1. Some their properties are listed in Table 1.

According to Scheme 3, conformers I - III lie within ~3 kcal·mol^{-1}. The lowest one is I, which highest stability is perhaps due to the hydrogen bonding C-O-H···NH_2. To verify this suggestion, let consider the rotational isomer of I which is *cis*-I (Fig.1) - denote it as *trans*-I. The energy difference between them amounts to 2.6 kcal·mol^{-1}. Neglecting a possible and rather weak H-bonding between *trans*-O-H group and C=O in COOH, one can characterize the above H-bond C-O-H···NH_2 in I by the frequency shift $\Delta\nu$ = -286 cm^{-1} (red shift) and Δr(O-H) = -0.006 Å (bond contraction) with the H-bondlength R = 1.906 Å.

This Table 1 shows that conformer I resembles the conformer IIb of [29] which lies only 34 cm^{-1} above the ground-state one (Fig.5, [29]), II - III'$_\alpha$a (lies 284 cm^{-1} above the ground-state), and III is apparently new. According to the present calculations, I is at the bottom of our PES, II and III are 1.08 and 3.31 kcal·mol^{-1}, correspondingly, above it.

Table 1

Rotational constants, A, B, and C (in MHz), electric dipole moment components (in D), energy (hartree), and ZPE (kcal·mol^{-1}), vertical (VIE) and adiabatic ionization (AIE) energies (in eV) of the studied low-energy conformers I-III of L-threonine and their cations (in brackets; energy and ZPE)

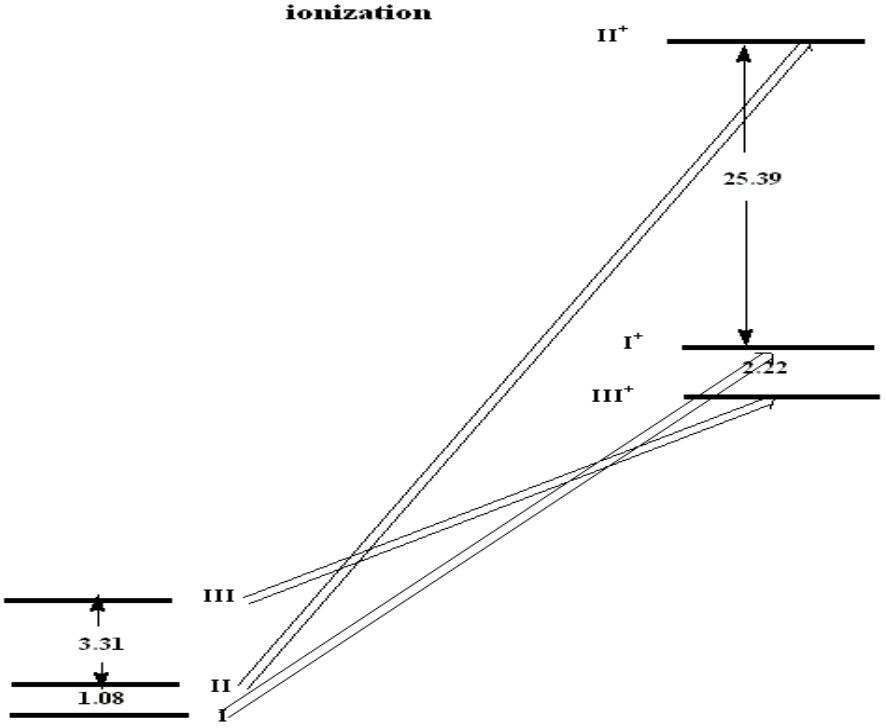
Structure	I= IIb	II= III'$_{\alpha}$a	III
A	3204	2880	2731
B	1500	1549	1719
C	1256	1223	1340
$\|\mu_x\|$	3.07	2.08	2.48
$\|\mu_y\|$	2.98	1.57	1.66
$\|\mu_z\|$	0.90	1.28	0.10
$\|\mu_{total}\|$	4.37	2.90	2.99
Energy +438, hartree	-0.426916 (-0.152258)	-0.424871 (-0.114167)	-0.421117 (-0.157004)
ZPE, kcal·mol^{-1}	88.73 (85.00)	88.53 (86.49)	88.40 (85.75)
VIE, AIE, eV	9.602 7.312	9.372 8.366	9.404 7.072

Let us move on to the topic of this paper. To ionization of the studied conformers. Their scenarios of ionization - or ionization paths - are demonstrated in Figs. 2-4 that end at the correspon-ding cations. The first step on these paths are resulted in the conformers whose structures are identical to those of neutrals and characterized by the VIEs which are tabulated in Table 1. The highest VIEs correspond to the ionization paths of I and III which resemble the fragmentation (1), though each in its own manner, and resemblance has the meaning to some extent, rigorously speaking.

Let explain. The present calculations demonstrate that

(**i**) the ionization path of I ends at (Fig. 2)

$$[C_4H_9NO_3]^+ \rightarrow [C_2H_4NO_2]^{+0.93} @ [C_2H_5O]^{+0.07}\ ; \qquad (2)$$



Scheme 3. **Energetic ordering of conformers I - III (on the left side) and their cations (on the right)**

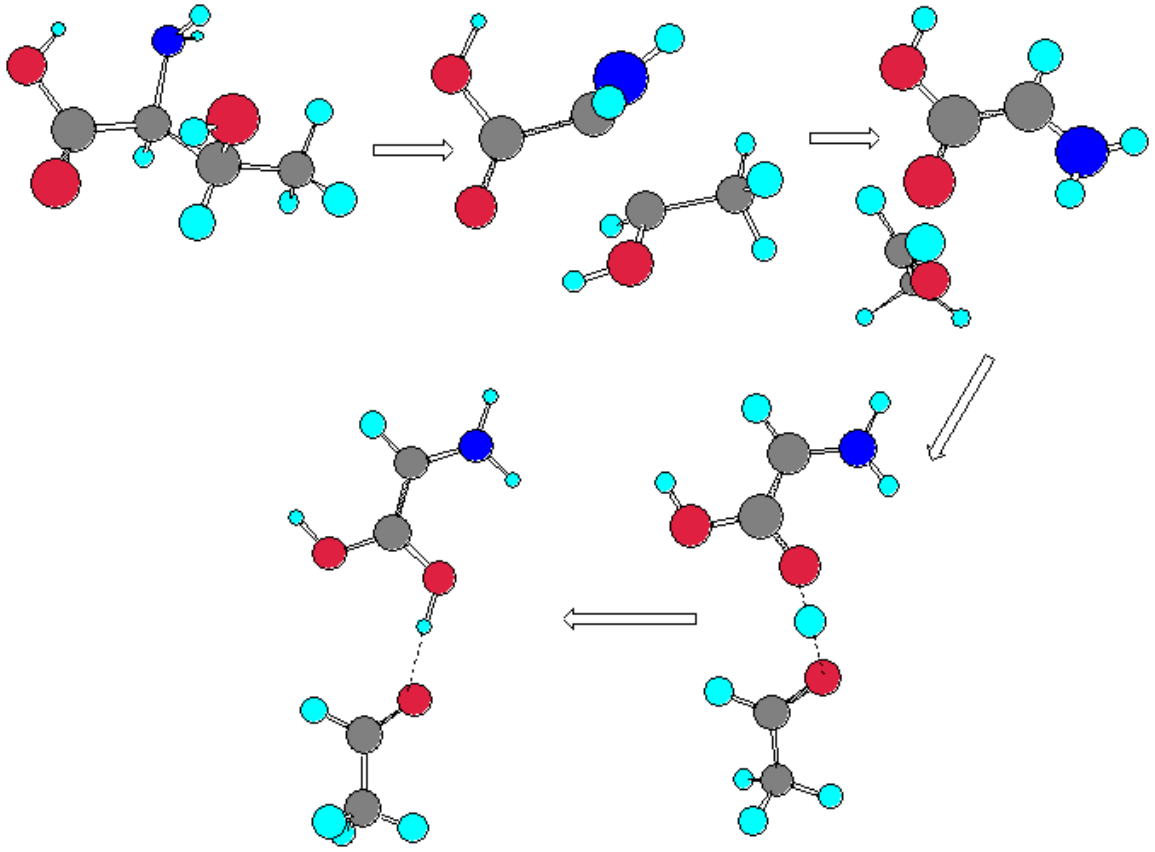


Fig. 2. **Scenario of ionization of the conformer I of threonine.**

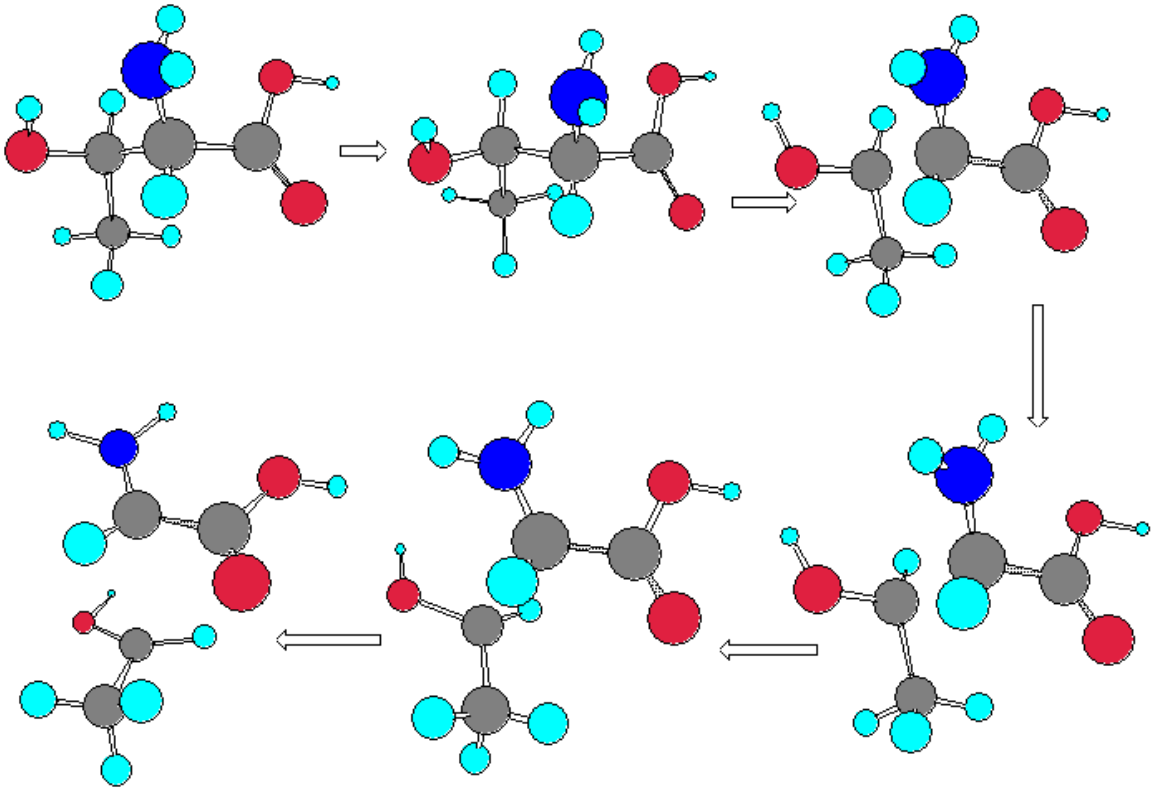


Fig. 3. **The ionization path of the conformer II of threonine.**

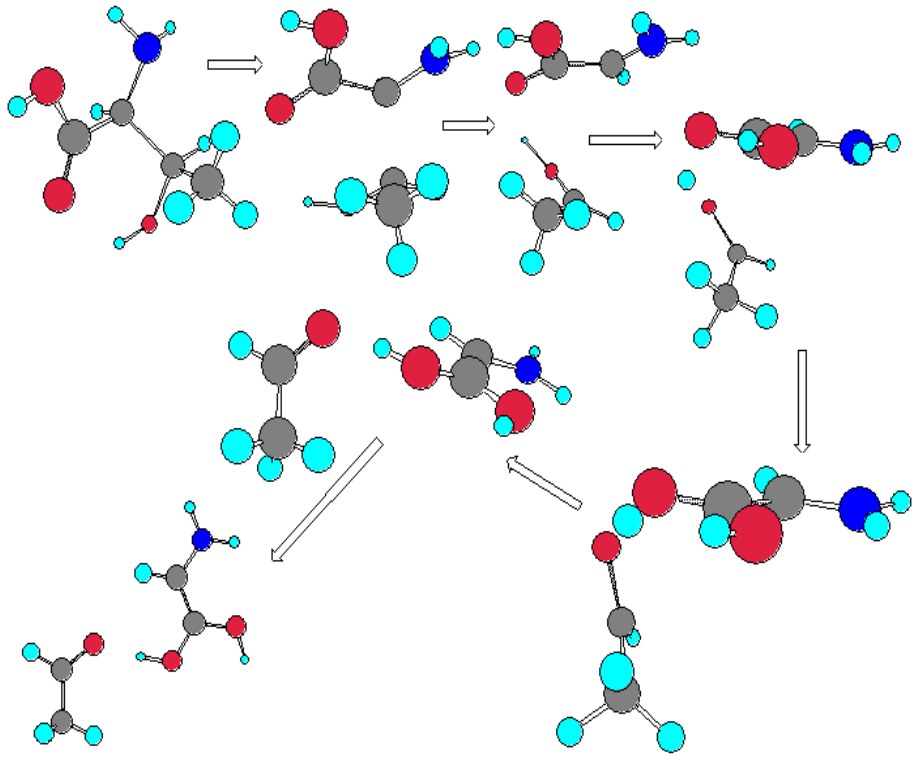


Fig. 4. **Scenario of ionization of the conformer III of threonine.**

(**ii**) the ionization path of III ends at (Fig. 4)

$$[C_4H_9NO_3]^+ \rightarrow [C_2H_4NO_2]^{+0.92} @ [C_2H_5O]^{+0.08} ; \quad (3)$$

where the superscript charges are derived from the corresponding Mulliken charges, and where the sign @ is introduced in (2) and (3), first, to indicate a hydrogen bond O-H···O that holds together sub-fragment cationic species therein, and second, to emphasize its difference from the sign '+' that in (1) means the fragmentation[1]. Therefore, we conclude that the type of reactions (2) and (3) do not refer to fragmentation[2]. We bestow a word 'fragmentation' two meanings: one is a real fragmentation when a system under consideration is partitioned into two or more subsystems separated from each other by an infinity distance, and the other is a so called 'fragmentation bonding' when a molecule under study is partially dissociated and the product species are formed. Note that the ionization path of III is by ~2.2 kcal·mol^{-1} more stable that of I. The path of II is placed ca. 25.4 kcal·mol^{-1} above the path of I. The ionization path of II that is shown in Fig.3 is different from (2) and (3) and ends actually at

$$[C_4H_9NO_3]^+ \rightarrow [C_2H_4NO_2]^{+0.40} @ [C_2H_5O]^{+0.60} . \quad (4)$$

meaning a non-covalent complex of sub-fragment cations on the r.h.s.

[1] According to the IUPAC Gold Book, Compendium of Chemical Terminology (2005-2022; Version 3.0.1: https://goldbook.iupac.org/), fragmentation is defined either as "1. The heterolytic cleavage of a molecule according to the general reaction: a–b–c–d–X → (a–b)$^+$ + c=d + X$^-$; 2. The breakdown of a radical into a diamagneticmolecule or ion and a smaller radical ; or 3. The breakdown of a radical ion in a mass spectrometer or in solution, forming an ion of lower molar mass and a radical." And further: "A fragmentation reaction may be written $M_1^+ \rightarrow M_2^+ + M_3$."

[2] The fact that, on the one hand, the word 'fragmentation' appears in the title of the work can lead to misunderstanding, and, on the other hand, the reaction under consideration, although similar to it to some extent, is fundamentally not one. This is just a tribute to fashion or this topic.

Figure 5 shows the final products of the fragmentation of cations of conformers I - III according to reactions (2)-(4). Regarding the cations of the conformers I and III, the corresponding angles and distances are indicated for the O-H-O bond. In the case of II, the distance between C2 and C4 atoms is shown as well.

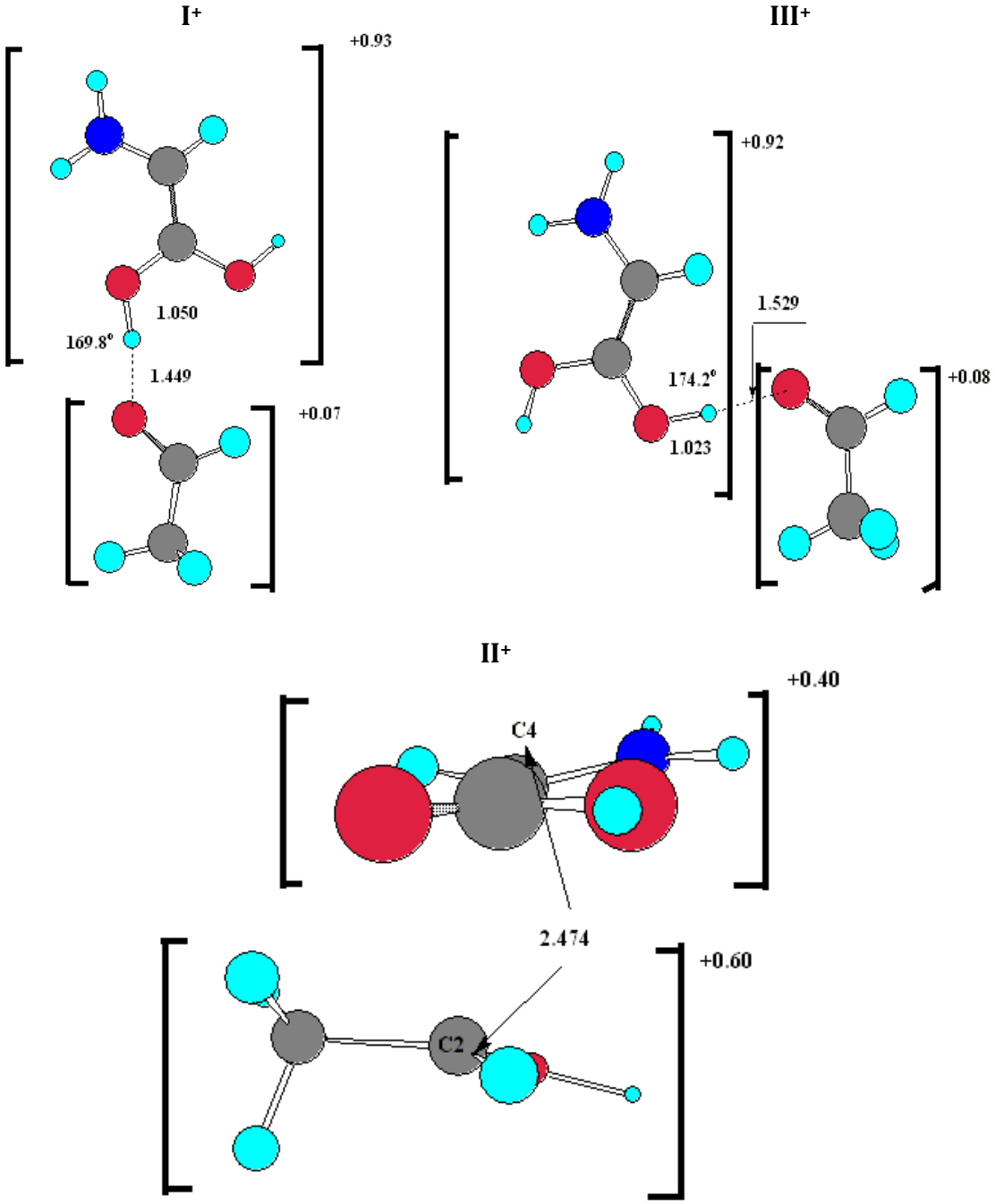


Fig. 5. **Threonine cations I+, II+, and III+.**

4. Afterword: Conclusions

Summarizing, physics behind ionization of threonine looks like this. Previously, it was believed that ionization and subsequent dissociative ionization of the aliphatic amino acids occurs due to removal of one of the electrons from the unbounded pair on the nitrogen ([22], p. 839) via a very simple mechanism.

The present computer simulation demonstrates that the ionizing electrons interact with the outer electrons of threonine that fill its least energetically bound orbital - HOMO. The HOMO of the studied threonine is depicted in Fig. 6. This leads to appearance of their fragmentation (see, e.g. [30]). A free electron(s) deposit energy into it, which can be sufficient either for its complete or partial excitation. This constitutes the threonine ionization process. Since part of the HOMO is located on the *C2-C7* bond, this ionization destroys it and opens a new reaction pathway that leads to the formation of the so-called "fragmentary bond" between threonine ionization products, somewhat differently (non-integer), ionized. This generates a new, intermediate cationic threonine complex which is energetically located below the fragmentation limit, composed of integer-ionized decay products.

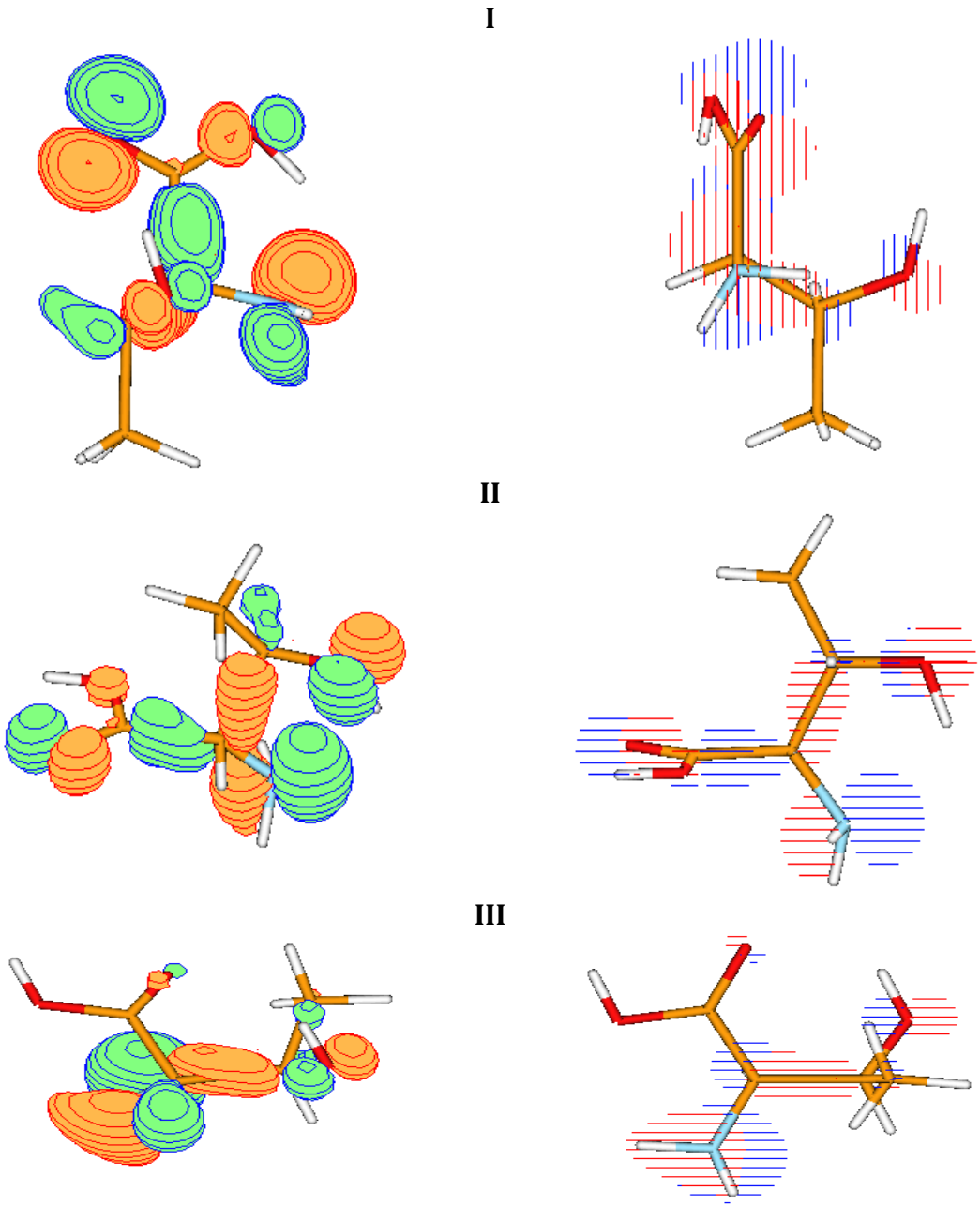


Fig. 6. **The HOMOs of the studied conformers I-III of threonine: left-orbital mode; right-molecular mode**

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