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Photo induced dissociation of amino acids free from thermal degradation effects: A case study applied to DL-Valine



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ABSTRACT

We present a careful study of the thermal degradation effects in the mass spectrum of DL-Valine using a quadrupole mass spectrometer and a time of flight – mass spectrometer. This allows setting the temperature of $95 \pm 10^\circ\text{C}$ as threshold for the sublimation of our solid sample. Based on the assignments for each ionic fragment detected, it is possible to separate the mass peaks in groups, explaining what are the principal bond breaks involved in the specific ionic yield, whose procedure can be extended to other amino acids.

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1. Introduction

Amino acids are the building blocks of proteins. Their spectroscopic study is of importance to understand the properties of biological systems such as living cells. The demand for gas phase information of amino acids arises from the anticipation that many biological phenomena can be traced to the fundamental properties of molecular constituents. Despite the fact that the amino acids are not neutral in its crystalline and solution form, which may be in the anionic, cationic or zwitterionic form in the case of solutions (pH-dependent) and in the zwitterionic form when they are in its crystalline form, intrinsic properties of biomolecules, difficult to rationalize in the complex medium of biological systems, can be understood in isolated environments as in gas phase [1]. As an example, we have the photo dissociation of amino acids when irradiated by vacuum ultraviolet (VUV) photons or electron impact, which was widely studied by mass spectrometry [2–10]. The study of such molecular systems in the absence of solvents provides an understanding of their geometrical and electronic structure allowing the distinction between intrinsic properties and those due to the interaction with the environment.

All amino acids, in standard conditions (25°C and 100 kPa), exist in solid state. The low volatility of amino acids provides a significant experimental challenge to transport these fragile biomolecules into gas phase. Some of the proposed methods to solve this limitation,

however, produce biomolecules as multicharged ions, which are incompatible with spectroscopy studies of neutral species [9]. A technique, based on aerosol thermal desorption, provides neutral species of very fragile biomolecules [10]. A more conventional approach may be possible for less critically fragile biomolecules. In order to produce a molecular beam with a desirable density, an oven-like device is used to heat the solid sample. Consequently, great care is required to be taken in order to guarantee no spurious effect due to thermal decomposition and degradation [8].

An improvement in the ability to monitor thermal degradation in amino acids is obtained by recording mass spectrum as a function of the temperature. In this way, the temperature threshold where degradation starts to take place can be obtained. The mass spectrum is a fingerprint of a given molecule for certain excitation energy. This permits to monitor all the heating process and detect when changes start to appear in the mass spectrum. In this way, a safe upper limit to the heating temperature can be obtained.

Although the strongest evidence of thermal degradation is the presence of additional spurious peaks in the mass spectrum, a much more sensitive procedure is to observe the changes in the relative intensity of each peak as a function of the temperature. For so, a quadrupole mass spectrometer is suitable to this evaluation.

Valine ($\text{NH}_2(\text{CH}_3)_2(\text{CH})_2\text{COOH}$) is an amino acid found in most proteins and is essential in human diet. The molecular structure of DL-Valine is shown in Fig. 1. Valine together with alanine, isoleucine and isoValine, although not interacting well with water, interact with each other and form a structure where the DNA molecule is assembled [11]. There are some publications in which the gaseous phase of Valine is analyzed by different spectroscopic methods

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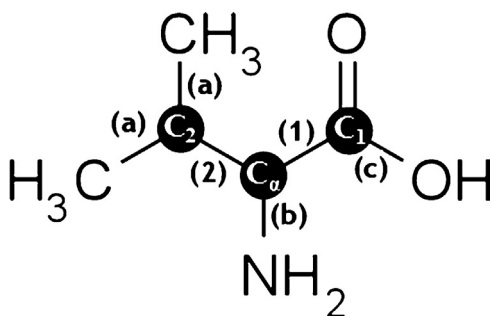


Fig. 1. Schematic representation of DL-Valine. The chemical bonds and the structural carbons are labeled by numbers and letters to help the reader: (1) $C_{\alpha}-C_1$ bond, (2) $C_{\alpha}-C_2$ bond, (a) C_2-CH_3 bond, (b) $C_{\alpha}-NH_2$ bond, (c) C_1-OH bond. The $C_1=O$ bond (double bond) was not labeled. The fragmentation pathways groups, disposed in the text, are labeled based on which chemical bonds are broken.

[2–5,7]. In these studies, several temperatures are employed to volatilize the amino acid. In order to study the thermal degradation of amino acids, we use the DL-Valine and compare with previous results.

2. Experiment

The study of thermal degradation using a Pfeiffer Vacuum Quadrupole Mass Spectrometer (QMS), model IO 220 D-35614 Asslar, was performed keeping a constant pressure of approximately 1×10^{-6} mbar in the main chamber. The QMS used 70 eV electron impact for the sample ionization. The DL-Valine samples, used without any further purification, are made of crystalline powder purchased from Aldrich® with a stated purity higher than ninety seven per cent. The sample was evaporated by heating the pure Valine in a resistively heated oven that is directly connected to the main chamber by a needle. The needle itself is heated to prevent condensation of the molecules in the walls. The temperature is measured by a thermocouple inserted in the external wall of the oven. The oven is inside a stainless steel cylinder. Therefore, the temperature is kept uniform as uniform as possible over the whole oven. The DL-Valine samples were slowly heated, approximately 10°C/h , to ensure the thermal equilibrium between the sample and the oven. More details in Ref. [4].

The coincidence mass spectrum has been recorded at the Toroidal Grating Monochromator beamline (D05-TGM) in the Brazilian Synchrotron Light Laboratory (LNLS), at Campinas, using a Time of Flight Mass Spectrometer (TOF-MS), which allows Photoelectron-Photoion Coincidence (PEPICO) spectra to be recorded [12]. The apparatus is mounted in a chamber that can rotate with respect to the polarization vector of the light beam, and was kept at the angle of 54.7° . The spectrometer (TOF-MS) has been described in details in Ref. [12–15]. The TGM beamline was operated using a Neon gas filter [15]. The filter parameters used in the present experiment guarantee five orders of magnitude high harmonics energy suppression, thus these effects are totally negligible in our case.

3. Thermal degradation analysis

A careful analysis of the thermal degradation effects upon heating DL-Valine was obtained by monitoring changes in the relative intensities of peaks as well as possible growth of spurious peaks in the mass spectrum of two different mass analyzers. Each study is described in detail below.

3.1. Relative intensities using the quadrupole mass spectrometer

The quadrupole mass spectrum (QMS) of DL-Valine was taken as a function of the oven temperature. Table 1 shows the evolution of relative intensities (RI) for all DL-Valine fragments detected. As expected, the amount of evaporated molecules increases with temperature. If no degradation is present, the ratio of any peak in the mass spectrum with respect to another shall be constant. A different situation occurs when thermal degradation starts to take place. In this case, the neutral molecule will break into neutral fragments and ionization of those produces a mass spectrum corresponding to the ions from neutral fragment instead of the entire molecule. A common result of thermal degradation is the appearance of smaller ion fragments in the mass spectrum. These new ionic fragments might already be present in the QMS compounding the intensity of ions from the molecule or also new ionic species may appear. The increase of ions with relatively larger mass is unlikely. Normalizing the peaks in the QMS with respect to a larger mass ion peak facilitates the task of spotting the presence of new peaks and the increase of the relative intensity of smaller mass peaks. The RI of a given peak is defined as the ratio between the area of this peak and the area of the peak $m/q = 72$ ($NH_2(CH_3)_2(CH)_2^+$). Peak $m/q = 72$ is the most intense peak in the mass spectrum, corresponding to the loss of the COOH neutral radical from DL-Valine (breaking of $C_{\alpha}-C_1$ bond, Fig. 1).

As can be seen in Table 1, the ions of m/q smaller than 45 have their RI increased to temperatures greater than 95°C . And around $T = 120^\circ\text{C}$ the RI of singly ionized water $m/q = 18-H_2O^+$ changes drastically, jumping from 20% to 80% in the range of ($120-144^\circ\text{C}$). The presence of water contamination in our sample could be claimed as originating the behavior. However, our sample prior to its utilization was warmed during 24 h at 50°C in the oven in vacuum. We therefore put forward the interpretation that H_2O^+ is a reaction product of photoionization of DL-Valine, and the rapid growth observed suggests that thermal degradation is occurring above 120°C . We observe other signs of thermal degradation, which would not depend on water free sample condition: a strong thermal degradation sign is observed at temperature above 120°C . Above this temperature two new spurious peaks, in the mass spectrum, appears with $m/q = 50$ and $m/q = 81$.

In Table 1 we can observe that fragments $m/q = 41, 42, 43$ and 44 , all hydrocarbons assigned as $C_3H_x^+$, $x = 5.8$ respectively, present significant relative growth given by 15–19%, 6–11%, 10–14%, and 6–9% respectively, when the temperature is raised from 95 to 110°C . Furthermore no change is observed when the temperature is raised from 83 to 95°C . Moreover, fragments 27 and 28 increased from 20% to 23% and 64% to 73% when the temperature is raised from 95 to 110°C . Perhaps less sensitive to thermal degradation below 120°C are fragments 29 and 30. They increased from 24% to 29% and 36% to 42% respectively when the temperature raised from 110 to 120°C . These results show that thermal degradation takes place for Valine molecule for temperatures above $95 \pm 10^\circ\text{C}$. In conclusion, fragments $m/q = 41-44$ and $m/q = 27-28$ seem to be good candidates as indicators of thermal degradation effects. Indeed, they seem to be more sensitive to thermal degradation effects while the difficult requirement of a water free sample does not need to be fulfilled. From the analysis of the Table 1 the oven temperature was set at 95°C where thermal degradation does not occurs. In this temperature the chamber pressure was around 10^{-6} mbar, which is the same working pressure reported by other researches [2–5]. We notice, however, that other studies reported a substantially higher oven/sample operating temperature: undetermined [2], 200°C [3], $115-165^\circ\text{C}$ [4], $150-200^\circ\text{C}$ (chamber temperature) [5]. Therefore, the present study raises questions like the possibility of significant contributions of degraded sample in the experimental results of aminoacids, and to what extent this affects the spectrum

Table 1

Relative intensities (RI) of cations formed upon electron impact ionization of Valine in the gas phase in function of the temperature.

<i>m/q</i>	Ion	RI photon impact present results <i>T</i> = 95 °C	RI electron impact, Junk and Svec [2] **	RI photon impact Jochims et al. [5] *	RI electron impact, Denifl et al. [7] <i>T</i> = 120 °C
118	ValH ⁺	–	–	–	0.5
117	Val ⁺	–	0.1	1	0.1
75	NH ₂ CH ₂ COOH ⁺	18	16	12	11
74	NH ₂ CHCOOH ⁺	37	34	45	30
73	C ₂ H ₃ NO ₂ ⁺	6	5	–	^b
72	NH ₂ (CH ₃) ₂ (CH) ₂ ⁺	100	100	100	100
70	C ₄ H ₈ N ⁺	3	1	–	^b
58	C ₃ H ₈ N ⁺	16	4	–	^b
57	C ₃ H ₇ N ⁺	35	29	60	23
56	(CH ₃) ₂ (CH) ₂ ⁺	22	11	Present but unresolved	15
55	C ₄ H ₇ ⁺	26	30	64	38
53	C ₃ H ₃ N ⁺	1	–	–	^b
46	NH ₂ CHOH ⁺	11	5	13	10
45	HOCO ⁺	1	3	6	3
44	C ₃ H ₈ ⁺	6	2	–	^b
43	(CH ₃) ₂ CH ⁺	8	7	13	8
42	C ₃ H ₆ ⁺	2	4	Unresolved	4
41	C ₃ H ₅ ⁺	6	8	9	10
39	C ₃ H ₃ ⁺	1	8	3	9
30	NH ₂ CH ₂ ⁺	16	12	19	15
29	NH ₂ CH ⁺	24	21	36	21
28	HCNH ⁺	29	31	36	33
27	C ₂ H ₃ ⁺ , HCN ⁺	4	9	6	10
19	H ₃ O ⁺	1	1	–	^b
18	H ₂ O ⁺ , NH ₄ ⁺	6	4	^a	3
17	OH ⁺	–	–	–	–

^a High water impurity^b Only the most intense cations are displayed at the table.

* The specific pick up cell temperature is not displayed for valine, but the chamber was heated to temperatures, typically 150–200 °C.

** The specific valine sublimation temperature is not mentioned.

obtained at high temperatures. In the next section we will compare our results using the TOF spectrometer with other studies already published with different temperatures.

3.2. Relative intensities using TOF spectrometer

We hypothesized that the quadrupole mass spectrometer (QMS) may not be the most accurate device to determine the thermal degradation temperature (TDT). The QMs takes into account

gas contribution from the whole chamber, as an average. In this way, fragments produced at different temperatures may contribute, leading to an incorrect measure of an upper safe operational temperature limit. In fact, if molecules produced at lower temperature do contribute strongly to the average seen by the QMs, artificially higher thermal degradation temperature will be obtained. Hence, to investigate this hypothesis, we proceed with the same study based upon relative intensities using TOF spectroscopy.

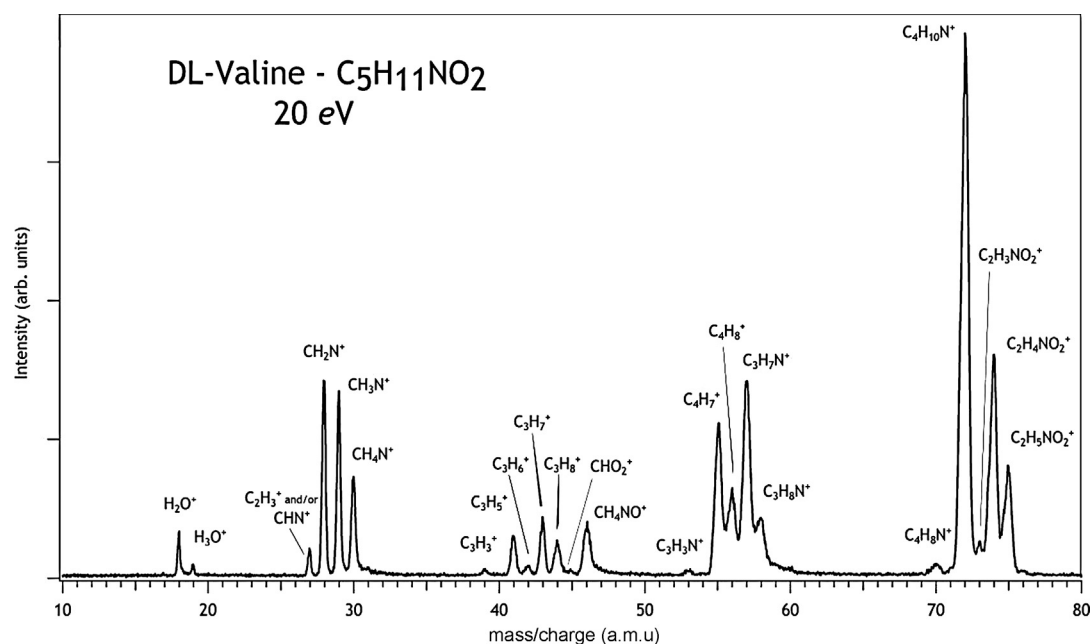


Fig. 2. DL-Valine coincidence mass spectrum: molecule irradiated by photons of 20 eV at 95 °C. The assignment of peaks are indicated.

This method is far more sensitive because the detected ions in a TOF spectrum come from ionized molecules just after they leave the oven needle tip. This is ensured because the ions are created, in practice, only at the crossing of the molecular beam and the photon beam which is placed few millimeters below the needle tip. Simulations, including molecular beam density variation, and focusing conditions of the TOF lens elements, contained in Ref. [12], guaranteed the averaging problems affecting QMs are not present at TOF data.

Surprisingly, TOF and quadrupole data lead us to obtain the same TDT. Probably this came from the fact that we waited at least 1 h after temperature stabilization in the oven to test sample integrity with the QMS. From the above findings we conclude that the substantially simpler methods based on QMs analysis may be sufficient for TDT, however, this comes at price of a longer waiting time for stabilization.

In Fig. 2, we present a coincidence mass spectrum of DL-Valine at 95 °C, obtained by photon ionization at 20 eV. The assignments of peaks are indicated in the figure. Fragmentation patterns of the Valine are widely discussed by Lago et al. [4] and Jochims et al. [5]. Although we do not explicitly describe each of the fragmentation pathways, we can separate the peaks in groups explaining which are the bond breaks, based on pathways previously published [2,5] and in our present result. Fig. 1 is labeled to help in this analysis. The photodissociation of DL-Valine takes the following pathways: a single backbone break in **groups (1) and (2)**; a double backbone breaks in **groups (1)+(2), (1)+(a), (1)+(b) and 1+(c)**. Belonging to **group (1)** we group peaks $m/q = 45, 70$ and 72 which are formed by the rupture in the $C_{\alpha}-C_1$ bond, see Fig. 1. Fragment $m/q = 46$ (NH_2CHOH^+) could also be assigned to fragment $HCOOH^+$ but his fragmentation path is not supported by experiments with α -Valine- d_3 [5], and in that case it is not placed at the **group (1)**. The fragments that we classified belonging to **group (2)** are $m/q = 39, 41, 42, 43, 44, 73, 74$ and 75 formed by the rupture in the chemical bonding between the α -carbon (C_{α}) and the $CH(CH_3)_2$ radical, which characterizes Valine. The **group (1)+(2)** represents fragments which result from two bond cleavages in the molecular main backbone. They are $m/q = 28, 29$ and 30 . Note that radiation causing two bond cleavages in biological systems leads to damage much harder to be repaired than single back-bond break and can be easier to spot using our proposed classification. The last three groups – **(1)+(a)**, **(1)+(b)** and **(1)+(c)** – are made of rupture in **(1)** and a methyl **(a)**, amine **(b)** or hydroxyl radical **(c)** loss. In the specific case of hydroxyl radical loss **(1)+(c)** group, it is perfectly possible that the double bond $C=O$ is broken, so this group describes any rupture between $C-O$ bonds forming H_xO^+ ions ($x = 1, 2$). Classification of fragments 46 and 27 are given further down in the text. Listing the groups composed by double fragmentations: **group (1)+(a)** – $m/q = 53, 57$ and 58 –, **group (1)+(b)** – $m/q = 55$ and 56 – and finally **group (1)+(c)** – $m/q = 18$ and 19 .

The Jochims et al. spectrum [5] shows the peaks $m/q = 57$ overlapped with the peak $m/q = 58$, whose behavior could be interpreted as a metastability of the peak $m/q = 57$, but we observe a clear separation of the peaks $m/q = 57$ and 58 as confirmed by the high resolution mass spectra of Denifl et al. [7]. These fragments $m/q = 57$ and $m/q = 58$ are probably $C_3H_7N^+$ and $C_3H_8N^+$, respectively. Furthermore, fragment $m/q = 27$ is assigned to $C_2H_3^+$, fragments $m/q = 45$ is assigned to $COOH^+$ and the fragment $m/q = 46$ is assigned to NH_2CHOH^+ , all of them is confirmed in the α -valine- d_3 mass spectrum [5].

We did not observe the parent peak $m/q = 117$ ($NH_2(CH_3)_2(CH_2)_2COOH^+$) corresponding to the singly ionized Valine molecule, suggesting instability of these species in the time scale of the experiments and an efficient molecular bond rupture upon ionization [4]. This result is consistent with previous studies [4–7] that find no peak or very small, less than 1% RI for

Table 2

Relative intensities of cations formed upon electron impact ionization of Valine in the gas phase. The present values are compared with a previous electron impact study [2,5] and photoionization studies [7].

m/q	Ion	Relative intensities					
		83 °C	95 °C	110 °C	120 °C	134 °C	144 °C
118	ValH ⁺	*	*	*	*	*	*
81		0%	0%	0%	0%	1%	10%
75	NH ₂ CH ₂ COOH ⁺	13%	13%	14%	13%	12%	12%
74	NH ₂ CHCOOH ⁺	32%	32%	32%	31%	30%	30%
73	C ₂ H ₃ NO ₂ ⁺	6%	6%	6%	6%	7%	6%
72	NH ₂ (CH ₃) ₂ (CH) ₂ ⁺	100%	100%	100%	100%	100%	100%
70	C ₄ H ₈ N ⁺	2%	2%	4%	4%	4%	5%
58	C ₃ H ₈ N ⁺	7%	6%	6%	7%	7%	7%
57	C ₃ H ₇ N ⁺	38%	38%	38%	39%	41%	41%
56	(CH ₃) ₂ (CH) ₂ ⁺	18%	18%	20%	22%	21%	21%
55	C ₄ H ₇ ⁺	45%	45%	45%	47%	51%	51%
54		4%	4%	5%	5%	5%	6%
53	C ₃ H ₃ N ⁺	2%	2%	3%	3%	3%	4%
50		0%	0%	1%	1%	1%	5%
46	NH ₂ CHOH ⁺	11%	11%	10%	12%	12%	11%
45	HOCO ⁺	6%	6%	7%	7%	7%	7%
44	C ₃ H ₈ ⁺	6%	6%	9%	10%	20%	22%
43	(CH ₃) ₂ CH ⁺	10%	10%	14%	16%	16%	18%
42	C ₃ H ₆ ⁺	6%	6%	11%	12%	12%	16%
41	C ₃ H ₅ ⁺	15%	15%	19%	22%	24%	26%
40		2%	2%	3%	3%	3%	4%
39	C ₃ H ₃ ⁺	15%	15%	18%	20%	20%	22%
31		1%	1%	1%	1%	3%	17%
30	NH ₂ CH ₂ ⁺	25%	24%	24%	29%	41%	38%
29	NH ₂ CH ⁺	36%	36%	36%	42%	43%	43%
28	HCNH ⁺	65%	64%	73%	84%	81%	83%
27	C ₂ H ₃ ⁺ , HCN ⁺	20%	20%	23%	28%	28%	30%
26		3%	3%	4%	5%	5%	5%
18	H ₂ O ⁺ , NH ₄ ⁺	16%	17%	21%	27%	76%	88%
17		3%	3%	5%	7%	21%	24%
16		1%	1%	3%	4%	8%	8%
15	CH ₃ ⁺ , NH ⁺	6%	7%	7%	9%	10%	11%

* Indicates that we do not find the corresponding fragment (parent ion) in our measurements.

the parent ion, see Table 2. Table 2 presents the RI of the most intense ions observed in the TOF spectrum. Also included in the table for comparison are data for previous studies obtained from photoionization by Lago et al. [4] and Jochims et al. [5] and from electron impact by Junk and Svec [2] and Denifl et al. [7]. The most abundant cation is the $NH_2(CH_3)_2(CH)_2^+$, $m/q = 72$ a.m.u., and is equal in all studies. This ion is formed by the lost of the carboxyl group $COOH$. It is interesting to note that, the complementary ion $COOH^+$ is hardly observed.

We did not observed the protonated Valine (ValH⁺), which is in agreement with previous works. The only exception is the Denifl et al. [7] study. The major differences found in Table 2 where in RI for $m/q = 57$ and 58 and for $m/q = 55$ and 56 . These peaks in our TOF-MS are well resolved and the areas can be calculated which better precision.

4. Conclusions

The study of thermal degradation in the DL-Valine allowed establishing a limit for the oven temperature used for sublimation of the samples at 95 ± 10 °C. Fragments ($m/q = 41–44$ and $m/q = 27, 28$) are good candidates as thermal degradation indicators. Their relative intensities increase above 95 °C thus revealing that thermal degradation occurs above this temperature. The same methods can be applied to other biomolecules that need to be vaporized directly from its solid form.

Although we did not explicitly describe each of the fragmentation pathways, we can separate the peaks in groups explaining which are the principal bond breaks involved in the fragmentation paths. Particularly, the higher resolution of our spectrometer allows

identification of a new peak $m/q = 58$ in the valine mass spectrum. Now, the peak $m/q = 58$ is assigned to $C_3H_8N^+$ and peak $m/q = 57$, assigned to $C_3H_7N^+$. They were not well resolved in previous works [6,8], peak $m/q = 58$ being confused with the metastability of the peak $m/q = 57$.

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