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

Á. Miranda da Silva[a], A. Mocellin[a], H. Farrokhpour[b], M.S.P. Mundim[a], A. Naves de Brito[c,*]

[a] Instituto, Universidade de Brasília, Box 04455, 70919-970 Brasília-DF, Brazil
[b] Chemistry Department, Isfahan University of Technology, 84156-83111 Isfahan, Iran
[c] Instituto de Física "Gleb Wataghin", Universidade Estadual de Campinas, 13083-859 Campinas, SP, Brazil

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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 ± 10 °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.

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 ($NH_2(CH3)_2(CH)_2COOH$) 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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* Corresponding author. Tel.: +55 19 3521 5376.
E-mail addresses: arnaldo.naves@gmail.com, arnaldo@ifi.unicamp.br (A.N.d. Brito).

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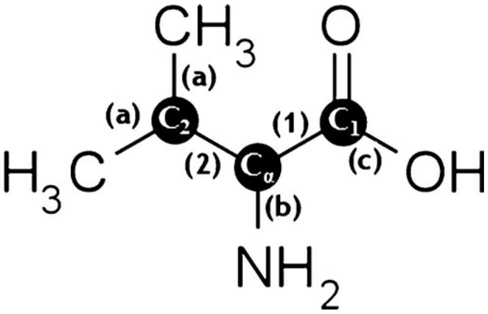

Fig. 1. Schematic representation of DL-Valine. The chemical bonds and the structural carbons are labeled by numbers and letters to help the reader: (1) C_α—C_1 bond, (2) C_α—C_2 bond, (a) C_2—CH_3 bond, (b) C_α—NH_2 bond, (c) C_1—OH bond. The C_1=O bond (double bond) was not labeled. The fragmentation 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 Pfeffeir Vaccum 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_α—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 $T = 120$ °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 °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. Further more 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 termal 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 ± 10 °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 °C [4], 150–200 °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

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