REACTIVITY OF SELECTED PRIMITIVE AMINO ACIDS INDUCED BY GAMMA IRRADIATION IN ASTROCHEMICAL CONTEXT*

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Abstract. *Amino acids in meteorites were preserved from the action of high energy sources (cosmic rays and ultraviolet protons) by their collocation, at a depth of 20 m. At the same time, the presence of radioactive elements was the cause of amino acids' degradation. The radioactive elements produced a total radiation dose of 14 MGy during the life of the Solar System (4.6x10^9 years). Aside from the amino acids' degradation, radiations promoted a radioracemization process that was able to reduce the L-enantiomeric excess of amino acids. Our studies are aimed at identifying the radiation products formed in a solid state radiolysis using mass spectrometric techniques. Moreover, we are analyzing the radioracemization process at different irradiation conditions for proteinogenic and non proteinogenic amino acids. The amino acids show a relevant radiation and radioracemization resistance, especially the proteinaceous ones, such as leucine, valine and isoleucine. Some identified degradation pathways are significant due to their final products which can be considered precursors of more complex intermediates.*

Key words: *Amino acids, gamma irradiation, meteoritic context*

DOI: 10.21175/RadJ.2016.02.025

1. INTRODUCTION

Amino acids found in meteorites are the result of reactions between small organic molecules, such as CO_2, CO, CH_3OH, NH_3 and H_2O, the components of ice layers in solid dust particles. Once formed, they were incorporated into silicate structures which are the base of Solar System bodies [1]. Calculations show that amino acids and other organics are buried at a depth >20 m in asteroids, comets or other primitive bodies. On one hand this preserves them from the action different energy sources, mainly ultraviolet photons and cosmic rays. On the other hand, the presence of radioactive elements in meteoritic body, principally ^{40}K, ^{232}Th, ^{235}U, ^{238}U and ^{26}Al, can play a role against the preservation of amino acids [2, 3]. In fact, the radionuclide decay was the main cause of the amino acids degradation in astrochemical context. According to Urey's works [4, 5], radioactive elements produced a total radiation dose of ≈ 14 MGy during the life of Solar System (4.6x10^9 years). Beside the fact that they can destruct the amino acids [6], radiations can also cause radioracemization, a transformation process leading to a reduction in optical activity as a consequence of two main events: firstly, the radiolysis of chiral molecules and their degradation into products without asymmetric centre; secondly, the inversion of chiral centre due to the high energy involved during irradiation [7]. In order to evaluate the radiolysis resistance of amino acids and their preservation of chirality to high energy radiations, we have started a systematic study on the radiolysis of proteinogenic and non proteinogenic amino acids, the latter ones predominant in meteorites.

The used radiation dose was 3.2 MGy, which corresponds to 22.8% of the total dose they have received inside asteroids, comet and other bodies of the Solar System [8-11]. Here our attention is particularly focused on three primitive amino acids (valine, isoleucine and leucine), in other word amino acids formed at the first stages of Solar System life. Valine, isoleucine and leucine are essential amino acids and are some of the most commonly found proteinogenic amino acids found in meteorites [12]. Using mass spectrometric techniques, we were able to identify the radiation products, investigate the possibility for amino acids to retain their initial chiral signature and examine how the lack of oxygen can influence the amino acids radioracemization.

2. EXPERIMENTAL

The amino acids L-valine, L-leucine and L-isoleucine (Fig. 1) were obtained from Sigma-Aldrich (Milan, Italy) and used as received (reagent grade min 98% TLC).

2.1. Irradiation experiments

The irradiation with γ rays was conducted in solid phase in a ^{60}Co Gammacell 220 from Atomic Energy of Canada. The dose rate was 0.8 kGy/h for a total dose of 3.2 MGy. Each amino acid (300 mg for each) was irradiated separately from one another and in absence of air.

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* The paper was presented at the Third International Conference on Radiation and Applications in Various Fields of Research (RAD 2015), Budva, Montenegro, 2015.

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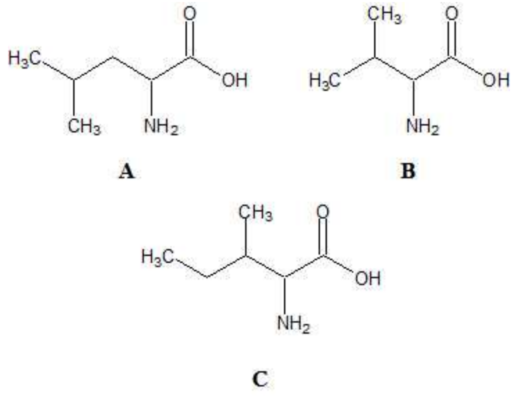

Figure 1. Structure of analyzed amino acids: Leucine (A), Valine (B) and Isoleucine (C)

2.2. Mass spectrometric analysis

The amino acids were analyzed by a mass spectrometer Finnigan LXQ, a linear ion trap system equipped with an ESI ion source. The mass spectra were obtained by a direct injection of amino acids solutions in the mass spectrometer. L-Val, L-Leu and L-Ile were dissolved in 1 mL methanol and 1 mL of ammonium acetate solution 45 mM. The concentration for the three amino acids were, respectively, 1.0x10^{-2} M, 7.8x10^{-3} M and 6.7x10^{-3} M and they were the same for irradiated and pristine samples, in order to have a more direct comparison for every amino acid. The flow injection was 10 μL/min and the analysis were conducted both in positive and in negative ion polarity mode, so as to identify products which retain the basic group (positive ion mode) and products which maintain the carboxylic group (negative ion mode). New products originated during irradiation were discovered comparing the mass spectra of pristine, non radiolyzed amino acids and the spectra of samples irradiated in absence of air. Whenever a new ion was detected in the spectrum, it was isolated in the ion trap and fragmented by collision induced dissociation (CID), operating a MS^n analysis, with n up to 5. The possibility to isolate and fragment the ions derived from a first CID analysis enabled us to determine the chemical structure of irradiation products, drawing a fragmentation pathway for every new ion .

Coupling the ESI-MS with a HPLC system equipped with a teicoplanine based chiral column (stainless steel column, Astec CHIROBIOTIC™ T, 5 μm particle size, 150 mm x 4.6 mm) allowed us to measure the amount of D-enantiomer formed by irradiation process. The HPLC analysis was made using a Shimadzu liquid chromatograph LC-10AD VP. The mobile phase was a mixture of methanol, water and formic acid (70 MeOH – 30 H_2O – 0.02 CH_2O_2) and the flow rate was 0.8 mL/min, split with a ratio 60/40 (waste/mass spectrometer ESI source). The purpose of the splitting was to increase sensitivity of mass spectrometer without losing resolution from the chiral column. To determine the quantity of D-enantiomer formed after irradiation, a calibration curve was constructed from standard solutions of L- and D-enantiomer. The Xcalibur program was used to calculate the relative percentage of D-enantiomer formation.

3. DISCUSSION

The mass spectra of L-Leu, L-Ile and L-Val irradiated in vacuum condition are reported in Fig. 2, where it can be seen spectra recorded both in positive (Fig. 2a) and in negative ion mode (Fig. 2b). In every spectra the ions enlightened by an arrow represent products of irradiation. The structures can be identify operating an MS^n analysis.

It is possible to observe that each amino acid presents two types of radiation products: deamination and decarboxylation products. These two processes are well known mechanism of amino acid reaction towards radiations [6, 13, 14]. Due to the high energy involved in the radiation process, a series of reactions can occur in the vial, increased by the long time of irradiation. In this lapse of time the radicals can react with each other, react with neutral molecules or rearrange. The interaction with another radical or a neutral molecule leads to the formation of products with a higher molecular mass than the amino acid itself.

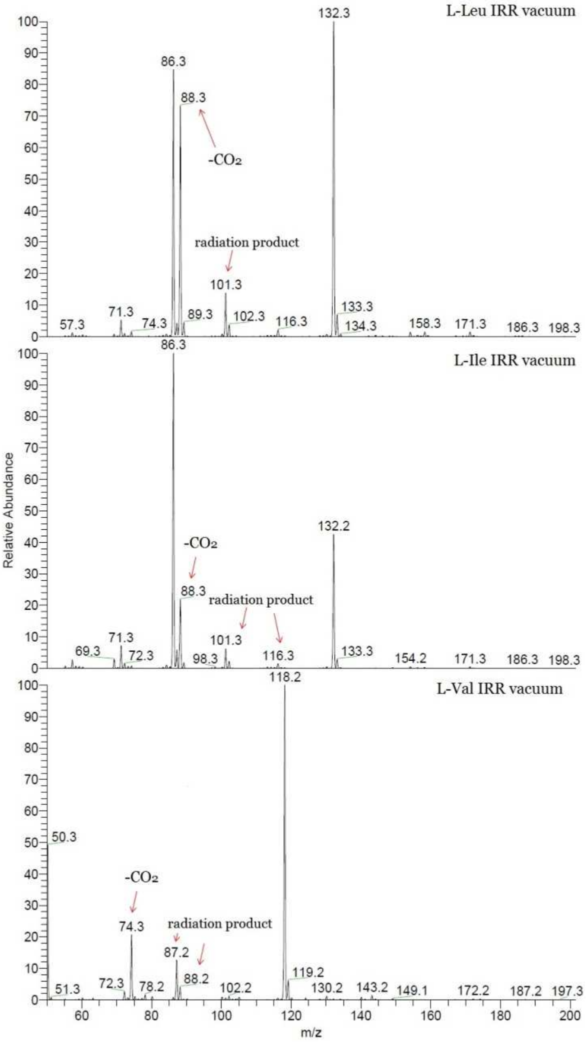

Figure 2a. Mass spectra of L-Leu, L-Ile and L-Val irradiated in vacuum condition, recorded in positive ion mode with a mass range m/z 50-200. The amino acids were dissolved into a solution 1:1 MeOH-AcNH$_4$ 45 mM

Some interesting products are the ones in which a radical was able to react with the ammonia group of the amino acid. In this case the N-substitution gives birth to molecules considered to be precursors of more complex intermediates, such as pyrimidines and purines. In Fig. 3 there are some products identified for L-Val, while in Fig. 4 there are some others identified for L-Ile and L-Leu.

Although isomers, leucine and isoleucine present some different irradiation products. For example, in isoleucine case, it is possible to observe the formation of glycine, absent in leucine spectra, where, instead, there are detected products derived from the reactions of side chain radicals. Contrary to what happens when the irradiation experiment were conducted in presence of air, in vacuum condition it was not possible to observe oxidation products, because the lack of both oxygen and humidity.

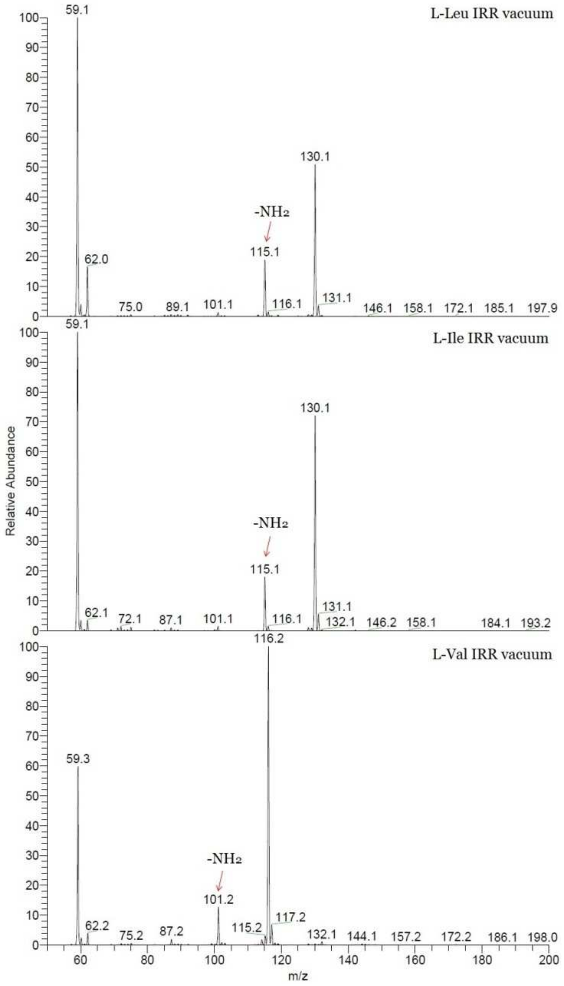

Figure 2b. Mass spectra of L-Leu, L-Ile and L-Val irradiated in vacuum condition, recorded in negative ion mode with a mass range m/z 50-200. The amino acids were dissolved into a solution 1:1 MeOH-AcNH$_4$ 45 mM

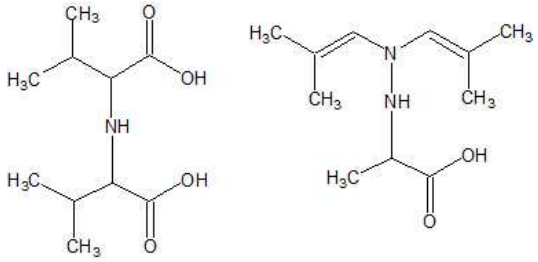

Figure 3. Some radiation products identified for valine irradiated in vacuum condition

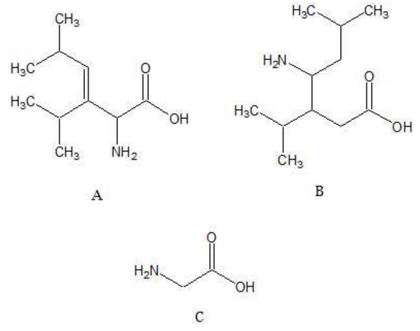

Figure 4. Radiation products identified for leucine irradiated in vacuum condition (A and B) and glycine (C) identified in the spectrum of isoleucine irradiated in absence of air

Basically we can identify four main categories of products. The loss of small radicals from the amino acid molecules is the first category. It is represented by all the products in which there is the loss of a small group, such as CO$_2$, NH$_3$, CH$_4$. Decarboxylation and deamination are the most important irradiation processes and they are considered to be the starting point of other radiation pathways, due to the fact that the formed primary radiation products were able to further react (Fig. 5).

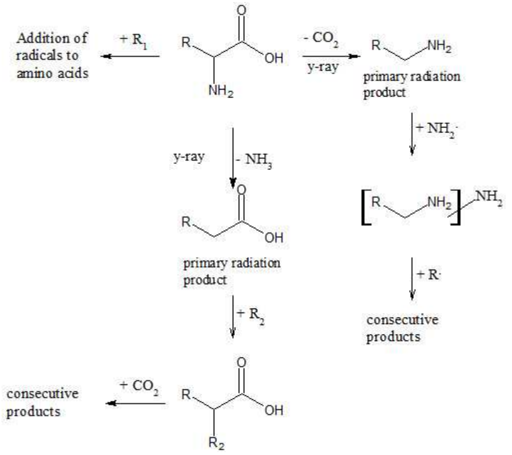

Figure 5. Processes induced by radiation, starting from decarboxylation and deamination of amino acid neutral molecules

A second category is the one in which radicals were able to react with amino acid molecules. These radicals could be either small (CO_2, NH_3, CH_4) or large radicals, the latter derived from the amino acid side chain. The last two categories refer to the products of small and large radicals addition to those primary radiation products already mentioned. They were the most abundant types of radiation products because of their mechanisms of formation. In fact, the primary radiation products react easily with radicals. It is thought that these radicals remained in close proximity to the primary radiation products due to the fact that both of them were directly produced, at the same time, by irradiation processes.

What it is important considering the radiations in meteorites is the role they played in the survival of L-enantiomer [15]. The lack of oxygen during irradiation experiments highlight how it can be important for the radioracemization. Through the construction of a calibration curve with standard solution of L- and D-enantiomers at different concentration (2, 5, 10, 15 and 20 percentage of D-enantiomer solution), we can estimate the amount of D-enantiomer formed. From the area data provided by the Xcalibur program of mass spectrometer, it was possible to calculate the amount of D-enantiomer (Table 1). Due to the presence of a β chiral carbon, there is the formation of D-alloisoleucine rather than of D-isoleucine [16].

The presence of oxygen seems to inhibit, at some extent, the formation of D-enantiomer. For L-Val and L-Ile the presence of D-enantiomer is halved by the oxygen action, while in the case of L-Leu there is no evidence of radioracemization when the irradiation occurred in atmospheric condition.

Table 1. Percentage of D-enantiomer formation in presence or absence of air

Amino acid	% of D-enantiomer formation in presence of air	% of D-enantiomer formation in vacuum condition
Valine	0.6	1.0
Isoleucine	0.3*	0.6*
Leucine	n.d.	1.8

* D-alloisoleucine

We are actually carrying out other irradiation experiments in which eleven selected amino acids are irradiated in presence of olivine, a mineral present in meteorites. The choice of amino acid was made following the quantity of amino acids found in meteorites [12], so that only the most abundant (proteinogenic and non proteinogenic) are irradiated. In order to study how the presence of olivine can influence radiation processes, four different ratios amino acids: olivine where chosen (1:10^4, 1:100, 1:10 and 1:2). In addition to the liquid mass spectrometry, the use of a gas chromatograph coupled with a mass spectrometer allows us to study the formation of volatile products, so to obtain a more precise analysis of radiation products. Moreover, we want to investigate if the presence of a mineral can further influence the radioracemization, either protecting the enantiomers or promoting radioracemization.

4. CONCLUSION

The mass spectrometric study of irradiated amino acids in solid phase can give us some answers to the question of "how" and "how much" the amino acids were able to survive to radiations emitted by radioactive elements present in meteorites. The identification of radiation products can be achieved thanks to MS^n analysis, through isolation and fragmentation processes and CID technique. At the same time, a quantitative study is conducted to estimate the percentage of amino acids unmodified after irradiation. It seems that amino acids are relatively stable to radiations: despite the high radiation dose, all of the three amino acids survived at a percentage major to 70. This result is in agreement to what is found in meteorites, where leucine, isoleucine and valine are present in relative great amount [12]. All the three amino acids, as well as other amino acids, are present in enantiomeric excess, with the L-enantiomer prevalent. It is thought that this excess was more relevant at initial phase of Solar System life and it had decreased due the process of racemization induced by radiation.

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