Gamma Irradiation of Aqueos Solution of L-Aspartic Acid, L-Aspartic Acid in Solid State, and L-Aspartic Acid Adsorbed into Na-Montmorillonite: Its Relevance in Chemistry Prebiotic

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ARTICLE INFORMATION

ABSTRACT

Aspartic acid is an amino acid present in the modern proteins, however, is considered a primitive amino acid hence its importance in prebiotic chemistry experiments studies. In some works of prebiotic chemistry have been studied the synthesis and the stability of organic matter under high energy sources, and the role of clays has been highlighted due to clays that can affect the reaction mechanisms in the radiolytic processes. The present work is focused on the study of the role of Na-montmorillonite in the gamma radiolysis processes of L-aspartic acid. Gamma radiolysis processes were carried out in three different systems a) L-aspartic acid in aqueous solution; b) L-aspartic acid in solid-state; and c) L-aspartic acid adsorbed into Na-montmorillonite. L-aspartic acid was analyzed by high-performance liquid chromatography−electrospray ionization− mass spectrometry (HPLC-ESI-MS). The results showed that the decomposition of L-aspartic acid considerably decreased in the presence of clay thus highlighting the protector role of clays and favors the stability of organic matter even under the possible high energy conditions of primitive environments. The principal product of gamma radiolysis of L-aspartic acid was succinic acid produced by deamination reaction. On the other hand, when aspartic acid was irradiated in solid-state the main product was the L-aspartic acid dimer. Both radiolysis products are important for chemical evolution processes for L-aspartic acid in primitive environments.

1. Introduction

Amino acids are the components of proteins, which are fundamental pieces for live organisms. Aspartic acid $(C_4H_7NO_4)$ is an amino acid, is metabolized from L-glutamic acid. Its biosynthesis occurs by transamination of oxaloacetic acid, an intermediate metabolite of the Krebs cycle, this cycle being part of current metabolism. These reactions can be grouped into metabolic pathways that contain a sequence of chemical reactions [1]. However, the synthesis of this amino acid can not only be the result of chemical reactions that occur in systems as organized like that of a cell, but it has also been proven that the synthesis of this and other amino acids occurs through abiotic processes. An example of this is given when irradiating with high-energy particles, CO and N_2 gases, proposing that cosmic rays can be an efficient energy source for the abiotic formation of the matter of biological importance [2]. This energy is not the only one used in these experiments; it has also been suggested that UV radiation could have a fundamental role among others [3]. Some authors have used UV and IR energy, gamma radiation, etc. [4–6]. The energy sources available for primigenial environments are ionizing radiation, coming from the radioactive decay of ^{40}K, ^{232}Th, ^{235}U, ^{244}Pu, among others [7]. The main characteristics of ionizing radiation are its specificity to deposit energy regardless of the concentration of the reactants, pH, state of aggregation, and optical properties, resulting in two different events: ionization and excitation. These events are important for prebiotic chemistry in the synthesis of organic molecules in primigenial environments. However, the high radiation field could have promoted the disintegration of organic matter, which could have been a disadvantage. Therefore, a possible protection mechanism is studied to understand how the organic matter was stable under high radiation fields.

Several works have demonstrated that clays might protect organic molecules from high-energy radiation, permitting organic molecules' stability under high radiation fields [8, 9]. This study aims to extend our knowledge of the possible role that some minerals played as a protector of some organic matter in primitive environments.

2. Material and Methods

Milli Q water and high purity reagents (Sigma, Co., USA) were employed in all the experiments. Na-Montmorillonite (SWy-2) from Clay Mineral Repository of Crook Country, Wyoming, USA., was employed as an adsorbent surface.

2.1. Preparation of Samples

2.1.1. L-Aspartic Acid in Aqueous Solution

Aqueous solution of L-aspartic acid 1×10^{-4} mol L^{-1}, was placed in a glass tube in the presence of oxygen. After gamma irradiation processes the samples were analyzed by HPLC-ESI-MS.

2.1.2. L-Aspartic Acid in Solid-State

100 mg (as a powder) was placed in a glass tube in the presence of oxygen. After gamma irradiation processes, the samples were analyzed by HPLC-ESI-MS after prepared an aqueous solution.

2.1.3. L-Aspartic Adsorbed into Na-Montmorillonite. Adsorption-desorption Experiments

For adsorption processes, 0.1 g of clay was mixed with 3 mL of the L-aspartic acid solution (1×10^{-4} mol L^{-1}) in continuous agitation on a 150-rpm plate at 30 min. To evaluate the effect of pH in the system, three sets of experiments were made: at natural pH solutions (pH 4.5), at pH 1.5 (adding formic acid), and pH 9 (adding ammonium hydroxide). After agitation, all samples were centrifuged at 15,000 rpm for 30 min. The percentage of adsorption was determined by comparing the response by high-performance liquid chromatography coupled to mass spectrometry (HPLC-ESI-MS) the supernatant with a standard solution (1×10^{-4} mol L^{-1}).

After the irradiation procedure, desorption experiments were carried out by changing the pH and adding potassium hydroxide (KOH, 0.1 mol L^{-1}) to the solid previously separated by centrifugation. The recovery of the L-aspartic acid was made after three cycles of treatment with KOH. It was possible to desorb all L-aspartic acid from the clays to analyze by HPLC-ESI-MS.

2.2. Irradiation Procedure

The aqueous solution of L-aspartic acid, amino acid powder, and L-aspartic acid adsorbed into Na-montmorillonite at pH 1.5 were exposed to gamma irradiation cobalt-60 Gamma beam 651PT facility at Instituto de Ciencias Nucleares, UNAM. The absorbed doses were between 1.5 and 200 kGy at a fixed position with a 161 Gy/min dose rate. The samples were irradiated at room temperature (298 K). The dose rate was determined using the ferrous ammonium sulfate-cupric sulfate dosimeter [10].

2.3. HPLC-ESI-MS Analyzes

The analyses were performed by the HPLC system (Waters Corp®. 515 pump), along with a simple quadrupole mass detection system (SQ-2 manufactured by Waters Corp®) and an EDM instrument in negative mode (ESI-). Working conditions were adjusted for 1.58 kV capillary, 19 V cone, at a temperature of 350°C, and a desolvation gas flow of 650 Lh^{-1}, using a Symmetry C18 column (4.6 x 75 mm, size of 3.5 μm spherical particle, by Waters Corp®.) under an isocratic elution with a mobile phase (98% methanol and 2% formic acid), and with a flow rate of 0.4 mLmin^{-1}. A fix volume of sample (20 μL) was injected using a loop. The retention time of the analyte was 1.4 min. A calibration curve was made, aqueous solutions of L-aspartic acid were analyzed 1×10^{-5}, 2.5×10^{-5}, 5×10^{-5}, 6×10^{-5}, 9×10^{-5} and 1×10^{-4} mol L^{-1}. After L-aspartic acid adsorption and desorption experiments on sodium montmorillonite, the supernatants were analyzed by this technique.

3. Results and Discussion

3.1. L-Aspartic Acid Analyzes by HPLC-ESI-MS

HPLC-ESI-MS analyses for L-aspartic acid show a linear relationship ($R^2=0.9929$) between concentration (mol·L^{-1}), and absorbance was determined for the systems (Figure 1).
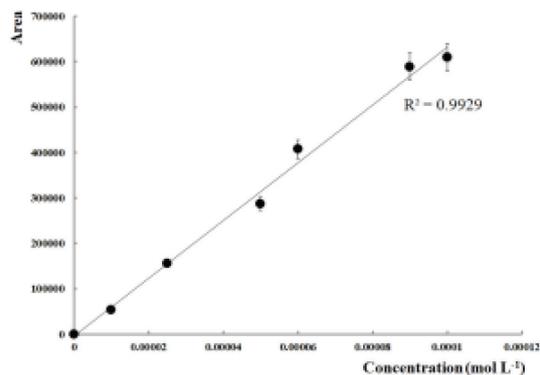

Figure 1: Linear relationship for L-aspartic acid analysis by HPLC-ESI-MS.

3.2. Adsorption-Desorption Experiments

The mechanism of adsorption and the affinity between L-aspartic acid and Na-montmorillonite can be obtained by studying the dependence of L-aspartic acid adsorbed as a pH function. The adsorption of L-aspartic acid onto montmorillonite was strongly pH-dependent (Figure 2).

After a cycle of treatment with KOH to 60 minutes for the L-aspartic acid-Na-montmorillonite system, 100% of L-aspartic acid was desorbed.
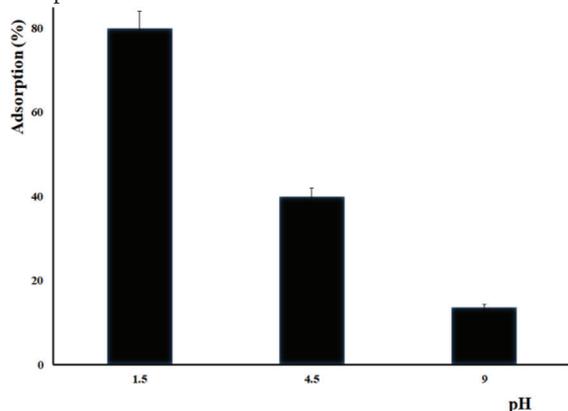

Figure 2: Adsorption of L-aspartic acid at different pH onto Na-montmorillonite.

3.3. Gamma Irradiation of Different Systems

After gamma irradiation processes of L-aspartic acid in three different systems, the results show that as the dose increased, the decomposition of L-aspartic acid also increased in the three systems; the remnants were:

1. 5% of L-aspartic acid in aqueous solution irradiated from 1.5 to 20 kGy.
2. 90% remnants of L-aspartic acid adsorbed into the clay at pH 1.5 irradiated from 1.5 to 200 kGy.
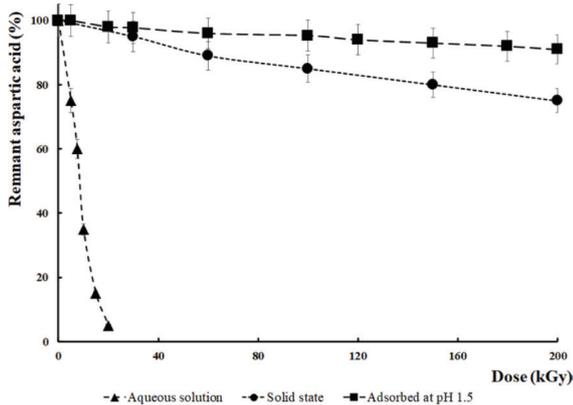3. 75% remnants of aspartic powder from 1.5 to 200 kGy (Figure 3).

Figure 3: Decomposition of L-aspartic acid in different system as a function of the irradiation dose.

An HPLC-ESI-MS analysis shows that succinic acid (118 gmol[-1]) is the main product of the radiolysis for L-aspartic acid in aqueous solution, and aspartic-acid-Na-montmorillonite systems. Succinic acid is obtained after a deamination reaction. The reactions involved are through free radicals, where the products of water's radiolysis (equation 1) play a fundamental role. Succinic acid is a dicarboxylic acid that ionizes in aqueous solution, producing succinate ions involved in the Krebs Cycle.

$$H_2O -w \rightarrow H^\bullet, OH^\bullet, H_2, H_3O^+, H_2O_2, e_{aq} \qquad (1)$$

Krebs Cycle is a metabolic pathway, an integral part of aerobic cellular respiration, hence the importance of studying radiation processes of amino acids in prebiotic chemistry. According to the L-aspartic acid radiolysis result, a dicarboxylic acid is produced, succinic acid (Figure 4), a very stable reaction product in aqueous solution.
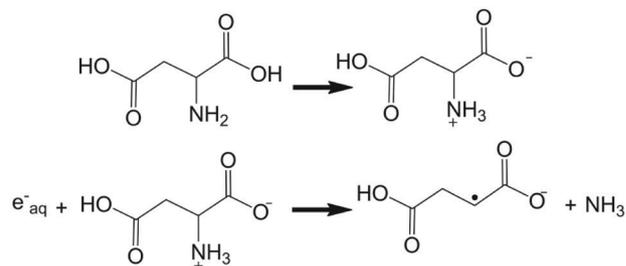

Figure 4: The proposed reaction mechanism for the production of succinic acid (118 g mol^{-1}).

On the other hand, the main product for L-aspartic acid irradiated in solid-state was the L-aspartic acid dimer (266 gmol[-1]) identified by HPLC-ESI-MS, highlighting its importance the reaction medium in chemical evolution processes.

Final Remarks

L-aspartic acid is readily adsorbed onto the clay at acid pH and is desorbed after KOH treatment. The presence of the Na-montmorillonite modifies the decomposition of L-aspartic acid by gamma radiation. The decomposition considerably decreased in the presence of clay. These results show that clays may have played an essential role as protective agents toward the degradation of some type of molecules exposed to a high radiation field when organic molecules are adsorbed into them. Clays' protective character is a clear advantage in primitive environments since the molecules produced by ultraviolet radiation, ionizing radiation, or electric discharges had to be stable to interact with each other and form more complex molecules. This stability could be provided through the clays.

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