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Radiolysis and Thermolysis of Cytosine: Importance in Chemical Evolution

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Abstract An important aspect of chemical evolution is the study of the stability of organic molecules with biological significance in primitive conditions, especially in the presence of constant energy sources. An example of sets of biologically important organic compounds is nitrogenous bases. The presence of these compounds in prebiotic environments is very important in forming more complex systems, such as nucleic acids, in which nitrogenous bases are an essential component. The aim of the present work is to study the stability of cytosine, a pyrimidine base, in high-radiation fields or at high temperature and to evaluate its recovery. Our results show that the cytosine ($1x10^{-4}$ M aqueous solution, oxygen-free) decomposed completely at a dose of 22 kGy, and 25% recovery was obtained with a dose of 7.4 kGy. The analysis of irradiated samples was followed by HPLC, HPLC-mass spectrometry and UV-VIS spectroscopy. The main product in both thermolysis and radiolysis was uracil, formed via a deamination reaction. Uracil is another nitrogenous base with biological significance.

Keywords: gamma radiation, thermolysis, nitrogenous base, chemical evolution, cytosine.

1. INTRODUCTION

The study of the origin of life has been divided into three main stages: chemical evolution, pre-biological evolution and biological evolution [3]. Chemical evolution is defined as the series of physical and chemical processes that led

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to the abiotic formation of organic compounds of biological importance. To gain insight into these processes, laboratory simulations are carried out under conditions that likely existed on early Earth. The objective of these kinds of experiments is to find possible mechanisms by which organic molecules were formed and increased their complexity [4, 8, 10]. In addition, the stability of the products synthesized in the prevailing environment [6] is also a relevant topic in chemical evolution.

An important aspect of chemical evolution is energy sources, since energy is responsible for starting, promoting and directing all physicochemical processes. Several energy sources could plausibly have contributed to chemical evolution [5], including radiation and thermal energy [2, 7, 11-12].

Particularly for nitrogenous bases, cytosine is an important molecule in biological systems. It is part of nucleic acid molecules, which are responsible for storing and transmitting genetic information in living organisms, as well as part of energetic molecules, such as CTP and CDP. However, despite its importance in prebiotic environments, few articles deal with the stability of this compound under possible prebiotic conditions, particularly with high radiation fields or at high temperatures. Therefore, it is necessary to have a balance between the formation and destruction of these molecules to have them available for further use [6].

This work focuses on the stability of cytosine in aqueous solution under high temperatures and gamma irradiation fields. The present study contributes to a better understanding the behavior and stability of organic compounds of biological importance by simulating chemical reactions in a primitive environment.

2. EXPERIMENTAL

2.2 Chemicals and materials

All of the chemicals were purchased from Sigma Aldrich Co., USA and were of the highest purity (cytosine, uracil, ammonium acetate and formic acid). The HPLC-grade solvents (water and methanol) were purchased from Honeywell Burdick & Jacson (NJ, USA). The glassware was treated with a warm mixture of HNO_3 and H_2SO_4 for 20 minutes, followed by a wash with distilled water and heating in an oven at 300 °C overnight. All of the chemical and glassware were handled to minimize contamination [9].

2.2 Preparation of samples

A standard stock solution of cytosine $1x10^{-4}$ M was prepared using triple distilled [1, 9] and deionized, oxygen-free water by bubbling Ar for 20

minutes. Then, 10 mL of the solution was placed in culture tubes and sealed for the irradiation experiments, and 1 mL was placed in glass vials and sealed, for experiments with thermal energy. All of the solutions were stored in a refrigerator at 4 °C when not in use.

2.3 Irradiation of Samples

The samples were irradiated at room temperature by a high-intensity ^{60}Co gamma source (Gammabeam 651 PT) at ICN-UNAM at different doses (0 to 22 kGy) and a dose rate of 248 Gy/min. The dose was evaluated using a ferrous sulfate–copper sulfate dosimeter [13]. The samples were analyzed immediately after irradiation.

2.4 Thermolysis experiments

The aqueous solutions of cytosine were heated in a Parr Reactor 4838 in glass vials at 92 °C at different times (0 to 1,026 hours) and at 1 bar pressure. Afterward, the samples were cooled at room temperature and immediately analyzed by a liquid chromatographic/mass spectrometry system.

2.5 Analysis of samples

2.5.1 *LC-ESI-MS analysis*

Liquid chromatographic analysis was performed on an HPLC system (515-pump from Waters Corp.) coupled with a Single Quadrupole Mass Detection system (SQ-2 manufactured by Waters Corp.), with an electrospray ionization positive mode (ESI+) source. Analysis was done within 5.0 min in Symmetry C18 column (4.6 x 75 mm, 3.5 µm spherical particle size, by Waters Corp.) under an isocratic elution of mobile phase (0.2 M ammonium acetate solution; 80% methanol, 20% water at pH=4) at a constant flow of 0.4 mL/min. A definite sample volume (20 µL) was injected using a loop.

2.5.2 UV spectrophotometry analysis

UV analysis was performance at 258 nm for uracil and 267 nm for cytosine in a Varian Cary 100 Scan Spectrophotometer using a using a 1-cm quartz cell at room temperature.

3. RESULTS

The principal reaction of the thermolysis and irradiation experiments with cytosine in aqueous solution ($1x10^{-4}$ M) was the deamination of the pyrimidine base to generate another pyrimidine base: uracil (Figure. 1).

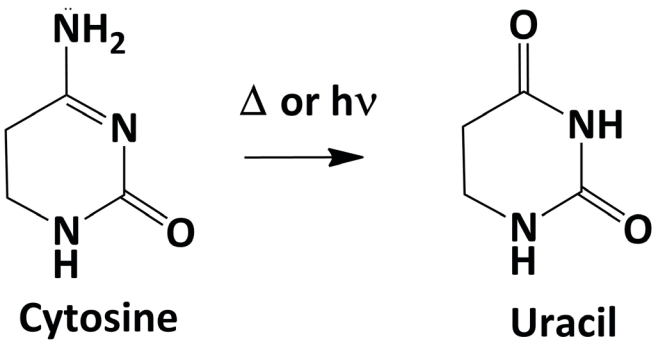

Figure 1: Deamination reaction of cytosine.

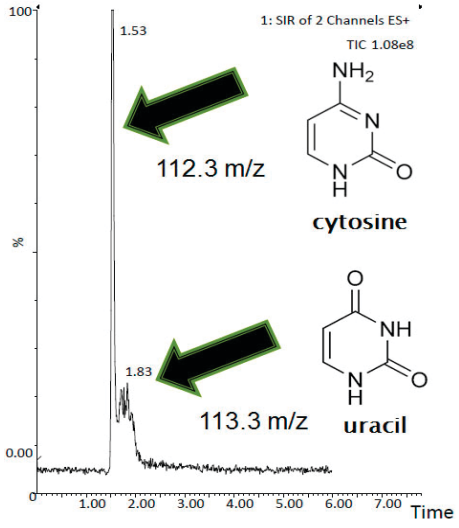

Figure 2: HPLC-MS analysis of cytosine.

As the principal method of decomposition identified by HPLC and MS fragmentation spectra (Figure 2), cytosine decomposition was quantified by UV spectrophotometry at 267 nm(Figure 3)., with experimental ε_{267} =855.84 mol^{-1} L cm^{-1} (Figure 4).

Uracil formation was quantified by UV spectrophotometry at 258 nm. Uracil formation is dose dependent, since increases in the dose showed formation of the pyrimidine base (Figure 5).

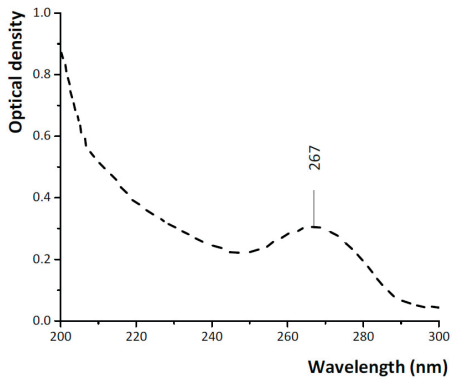

Figure 3: UV analysis of cytosine at 267 nm**.**

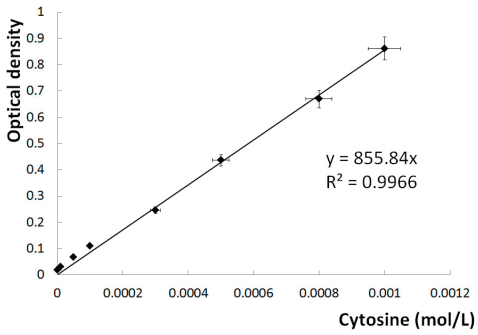

Figure 4: Cytosine decomposition quantified by UV.

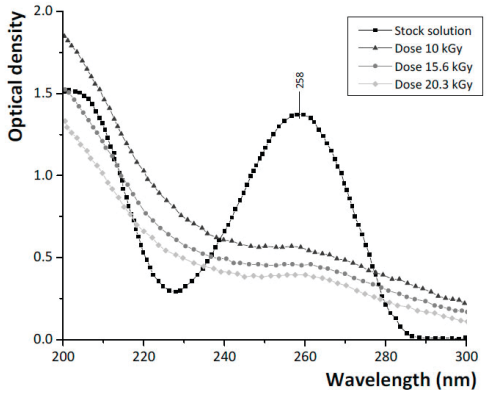

Figure 5: UV analysis of uracil at 258 nm.

3.1 Gamma irradiation experiments

Cytosine decomposition was dose dependent, since with increases in the dose showed high decomposition of the pyrimidine base (Figure 6). The radiolysis showed that the cytosine ($1x10^{-4}$ M aqueous solution, oxygen free) decomposed completely at a dose of 22 kGy, while 75% decomposed with a dose of 7.4 kGy.

Thermolysis experiments

The main thermolysis product of cytosine in aqueous solution ($1x10^{-4}$ M) at 92 °C was uracil. The thermolysis reaction is time dependent; after 1,026 hours of heating, the recovery was 72%. Figure 7 shows the evolution of cytosine decomposition as a function of time. The possible reaction mechanism for the thermolysis is showed in figure 8.

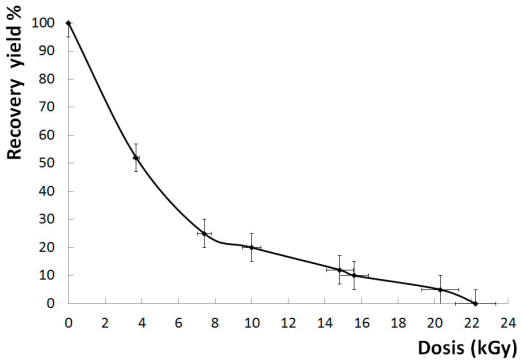

Figure 6: Decomposition of cytosine as a function of absorbed dose.

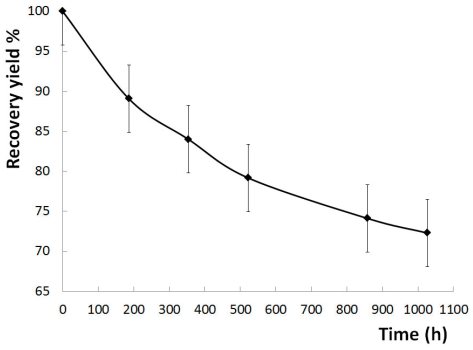

Figure 7: Dependence of cytosine deamination on time.

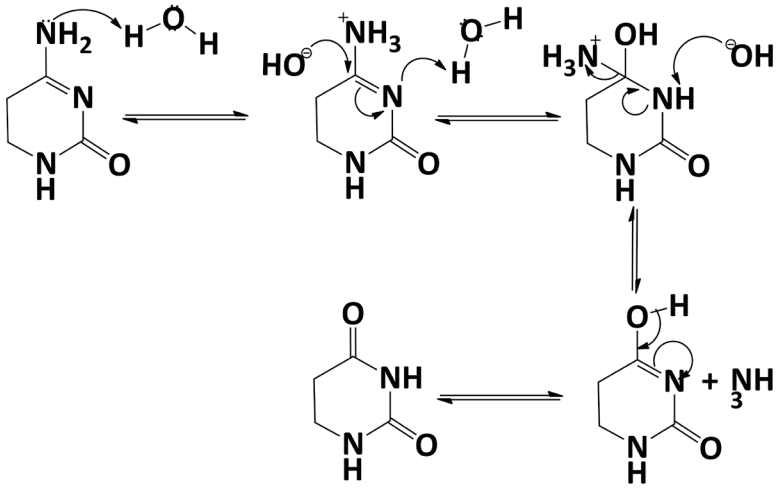

Figure 8: Suggested mechanism for the formation of uracil from cytosine thermolysis.

4. REMARKS

Despite the importance of nucleic acid bases in the prebiotic environment, there have been few reports on the stability of these types of compounds. Considering that the dose rate of high-radiation energy sources in the primitive Earth was low, the results presented in this paper show that cytosine in an aqueous medium was relatively stable under gamma irradiation or high temperatures, with a good yield of recovery, and its decomposition produced was another pyrimidine base. This behavior is a distinctive advantage of these types of molecules because they needed to survive to form more complex ones. The role of energy in the early Earth during the period of chemical evolution must have been important for the reactions, inasmuch as the energy was responsible for starting, promoting and directing all physicochemical processes. Many authors have proposed different energy sources on the early Earth. However, it cannot be said that one of them was the principal source; rather, only the participation of all possible sources may have contributed to the synthesis of the majority of organic compounds with biological significance.

5. ACKNOWLEDGMENTS

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