

Radiolysis of Nucleosides: Study of Sedimentary Microenvironment Models for the Protection of Bio-Organic Molecules on Early Earth

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Published online: August 07, 2017

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Abstract Nucleic acid bases and their derivatives are important compounds in biological systems. Many efforts have been made to demonstrate the possible prebiotic origin of these molecules, but the abiotic synthesis of these compounds has proved to be very difficult in that conditions. So, if their synthesis actually took place, a study of their stability in prebiotic conditions is quite relevant in chemical evolution studies. In this work, it has been examined and compared the influence of Sodium Montmorillonite on the chemical transformations undergone by two nucleosides (guanosine – purinic– and uridine, –pyrimidinic–) when subjected to conditions simulating the primitive Earth during the period of chemical evolution. The experiments prove the concentration capacity and protective role against external sources of ionizing radiation (specifically γ -ray) that clays can provide to these specific compounds adsorbed on them. By using X-ray diffraction, UV-vis spectrophotometry and HPLC for the analysis, it was found that purinic nucleosides (more than pyrimidinic) are quickly adsorbed on clay at low pH values, and the temperature of mineral desiccation applied after adsorption promotes their decomposition into their corresponding nitrogenous bases. In both, purinic and pyrimidinic, desorption occurs in neutral or slightly basic aqueous solutions, and both are protected by clay. Pyrimidinic nucleosides show more resistance to heat, but less resistance towards ionizing radiation, even when adsorbed in clay.

Keywords: Chemical Evolution, Clays, Nucleosides, Ionizing Radiation.

Journal of Nuclear
Physics, Material
Sciences, Radiation and
Applications
Vol-5 No-1
August 2017
pp. 103–111

1. INTRODUCTION

The Oparin-Haldane hypothesis indicates that life originated through the abiotic synthesis and the consequent accumulation of organic compounds, through a series of physical and chemical processes that took place in the space and/or in an early Earth [4]. Environmental conditions in those scenarios represented a challenge for the survival of simple organic molecules and their evolution into more complex ones. Organic molecules diluted into vast oceans and the presence of high radiation fields (of ionizing radiation sources, like radioisotopes and cosmic rays, with high penetration capacity) might have made specially complicated for organic molecules to react with each other to evolve. That is why a mechanism for concentration and protection of these first molecules should be necessary. One possibility is the adsorption of the organic compounds onto mineral surfaces, like proposed by Bernal in 1951 [1].

Nucleic acid bases and their derivatives are important compounds in biological systems, because are the building blocks of RNA and DNA. Their synthesis and stability in environmental conditions is of paramount importance in chemical evolution [9-10]. Nucleosides consist of a nucleobase that is bound to a ribose. Depending on structure, they can be purinic (e.g., adenosine, guanosine) or pyrimidinic (e.g., cytidine, uridine). Up to now, the abiotic synthesis of these compounds has not been fully understood [9], and their synthesis, especially if the base is a pyrimidine, is very difficult in prebiotic chemistry [5,9]. Studies of their stability in primordial environments are scarce, especially those related to the stability in an aqueous medium at high temperatures or in the presence of high radiation fields. Some studies of the stability of nucleic acid components in aqueous solutions have revealed that these types of compounds are decomposed, although the pyrimidine ring is quite stable, but the nucleosides have different half-lives [5].

To endure the primitive Earth conditions or interstellar space these molecules may have had a continuous synthesis to reach steady state concentration, or have had a long half-life in that conditions, or have had a mechanism of protection if they were unstable. Clays may have been that mechanism. They were proposed due to their ancient origin (geological evidence indicates their presence during the Archean [2]), wide distribution (they have even been detected on Mars and in a comet [2]), and especially because of their physical-chemical affinity for organic compounds [6].

Studies in radiation chemistry can provide a deeper insight into chemical processes that may be important for chemical evolution studies on the origin of life. To this end, we investigated the survival of nucleosides exposed to a high radiation field in an aqueous solution and adsorbed on a clay mineral.

2. METHODOLOGY

The experimental work was divided into groups of experiments: (a) adsorption/desorption tests (b) desiccation tests, and (c) irradiation tests.

The adsorption and desorption tests (a), were performed by changes in the pH of nucleoside solutions in the presence and absence of clay, verifying the adsorption and desorption using high-performance liquid chromatography (HPLC) coupled with a UV detector. Irradiation tests (c) were carried out in solution in the absence of oxygen by exposing the sample to different doses of gamma radiation from a cobalt-60 gamma-ray source (Gammabeam 651 PT) at an average fixed dose rate (175 Gy/min). The dose interval was from 0 to 52 kGy. All glassware used was previously treated with nitrating mixture and triple distilled water, and finally dried in a furnace at 300 °C, according to Draganič and Draganič protocol for removing organic matter [3].

In this study, guanosine (99%) and uridine (99%) were sampled from Aldrich Company, USA, and used without further purification. Namontmorillonite from Crook County, Wyoming, USA, clay mineral standard batch SWY-1, was used for the experiments. The nucleoside solutions used in these studies were 1×10^{-3} mol/L in triple distilled water prepared according to the techniques used in radiation chemistry [7]. The oxygen was removed, saturating the solutions with argon.

The fraction of organic molecules adsorbed on the clay surface was calculated from extinction coefficients (ϵ), determined by measuring the wavelength in the ultraviolet region for maximum absorbance at pH 2, 7, and 10, using a Carry 100 spectrometer, in the range of 200–350 nm. Maximum absorption was recorded for all samples, and pH was measured with an LSX Sargent-Welch pH meter. A Beckman Allegra XL-90 centrifuge was used to separate the fine-particle solids from the liquid phases. The nucleoside left in the solution were analyzed by HPLC/UV using a Varian chromatograph model 8055 with a column Ultra IBD (150 mm), coupled to a Varian ultraviolet detector at 260 nm. The mobile phase was a mixture of two solutions. Solution A was a methanol–water mixture (80:10 v/v), and solution B was a pH 2-buffer solution of sodium acetate 0.01 mol/L. The gradient used was from 94% of solution A to 60% of solution B in 12 min.

The adsorption of organic molecules by montmorillonite was followed by X-ray diffraction of powders spectroscopy, using a D5000 Siemens diffractometer, with filtered $\text{CuK}\alpha$ radiation at 2θ angles from 15 to 2° , at 30 mA during 58 min and 35 kV. The spectrometer was calibrated using Si, quartz, and kaolinite reference materials.

3. RESULTS AND CONCLUSIONS

3.1 Adsorption/Desorption Tests

The adsorption mechanism of organic molecules with clays is a complex phenomenon in which different processes take place: H-bonding, ion–dipole, and interactions of the van der Waals type. Organic compounds may also form complexes with the counter ions of the clay, or they may undergo ionic exchange [6]. The Na-montmorillonite clay used in this study has two binding sites: the interlaminar channel and the edges of the crystal. The interlaminar channel has a negative charge produced by the substitution of atoms in the layers that is compensated by Na⁺ cations. In addition, these interlayer spaces can accept water molecules. The edges present a positive charge.

The cation exchange capacity of the sodium montmorillonite was 101 meq/100 g of clay. For the adsorption/desorption experiments an amount below that level of nucleosides was used (100 mg of clay was mixed with 3 mL of nucleoside dissolution 1×10^{-3} mol/L) in an oxygen-free system. The samples were adjusted to pH 2 and 6 with HCl to test the effect of pH in the adsorption process. After shaking them for different increasing periods of time and centrifuging them, the supernatant (that contained the nucleoside that was not adsorbed onto the clay) was analyzed by UV–Vis spectroscopy and HPLC. The solid was analyzed by X-ray spectroscopy. After centrifuging, the clay was extracted with solutions at pH 8 by shaking the suspensions for 60 min. The supernatant was then examined by HPLC and the clay was analyzed by X-ray spectroscopy.

It was observed for both nucleosides that maximum adsorption occurs after around 10 minutes of contact between solutions and mineral, and after that, the maximum rate stayed steady (Fig. 1). Guanosine had a maximum adsorption percentage of 82 at pH 2, and practically no adsorption at pH 6. Uridine had very low adsorption at pH 2 (2.6%) and, like guanosine, no adsorption at pH 6. In both cases, the adsorption at low pH values indicates that the principal adsorption mechanism is ionic exchange, because in that circumstance these kinds of molecules are protonated and can get adsorbed inside the interlaminar channel. The difference between guanosine and uridine can be explained by the different pK₁ values of the molecules. While guanosine has a pK₁ of 4 (which means that at pH 2 all molecules are completely protonated), uridine has a pK₁ lower than 0.5 (so the uridine molecules are not completely in their cationic form). At pH 6, the molecules are both close, but not completely neutral (pK₂ of this nucleosides is between 9.25 and 9.3), so, molecules cannot get inside the interlaminar channel nor get adsorbed outside it.

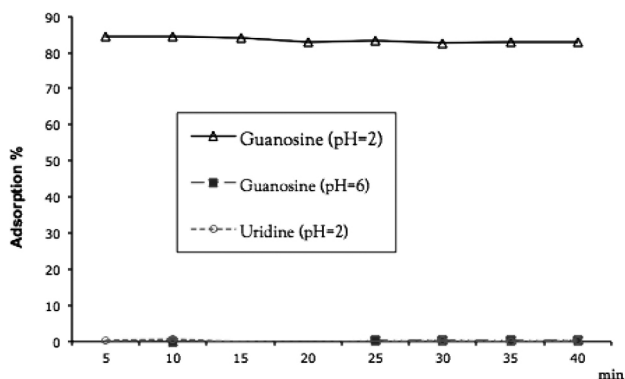


Figure 1: Adsorption curves by analysis with HPLC/UV for guanosine and uridine at pH 2 and 6 onto sodium montmorillonite.

Analysis by X-ray diffraction of powder of clay before and after adsorption process corroborates the assumption made related to the absorption sites on sodium montmorillonite (Fig. 2). In the patterns is possible to observe the widening of the interlamellar channel from 11.91 Å to 12.33 Å and to 13.60 Å for adsorption of uridine and guanosine, respectively.

Desorption experiments were carried in the same way as the adsorption test, changing the pH value of solutions to extract nucleosides from clay where

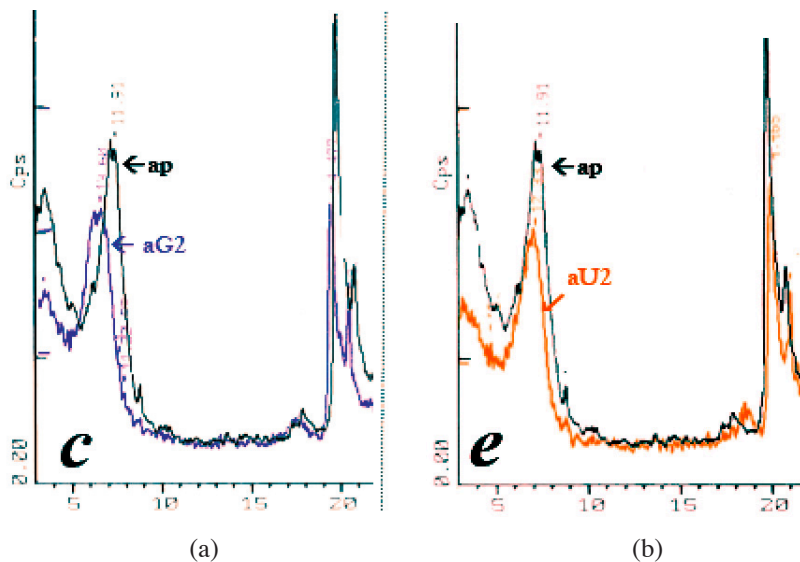


Figure 2: X-ray patterns of (a) guanosine and (b) uridine adsorbed on clay at pH 2 compared to sodium montmorillonite’s X-ray pattern (blank “ap”).

Aguilar-Ovando, E.Y. adsorption previously occurred. By using HPLC/UV and considering the
 Negrón-Mendoza, A. areas under the graphics, nucleosides desorption percentages were calculated. Desorption is possible in variable percentages at pH 5 and 8 (this last one gives the best desorption) in guanosine, and only at pH 8 in uridine, but for both generally produces partial decomposition of nucleosides into their corresponding nitrogenous bases.

3.2 Desiccation Tests

To evaluate the nucleosides stability to changes in temperature once adsorbed by the clay, desiccation experiments were carried out at 3 different temperatures: 22, 30 and 60 °C. After desiccation process, products of extraction (desorption at pH 8) were analyzed by HPLC/UV (Table 1).

Table 1: Desiccation experiments at 22, 30 and 60 °C.

Sample		Drying Temperature		
		22 °C	30 °C	60 °C
Guanosine	Nucleoside %	87.1	77.2	46.3
	Nucleobase %	9.1	17.4	41.9
Uridine	Nucleoside %	78.3	76.9	79.5
	Nucleobase %	0	0	0

It was observed that the increase in drying temperature leads to hydrolysis of only purinic nucleosides (pyrimidinic remain mostly the same).

3.3 Irradiation Tests

For the radiolysis experiments, 6 mL of oxygen-free aqueous solutions of nucleosides at pH 2 were irradiated with different doses of ionizing radiation from a ⁶⁰Co gamma-ray source. After irradiation, the samples were centrifuged. The supernatant was removed and analyzed by HPLC/UV. Desorption from the clay was done at pH 8 and the supernatant again analyzed by HPLC/UV.

More than 50% destruction of the nucleosides was observed after a dose of 52 kGy (Fig. 3). Uridine decomposes faster with gamma radiation than guanosine in aqueous solutions. Differences may be explained due the particular structure of purinic and pyrimidinic nucleosides, being the first ones the ones that have a larger structure with more π orbitals able to delocalize energy and radicals (that cause breaking of the molecule) during a longer period.

It was observed that the uridine curve of decomposition by radiolysis tends to increase a little after a dose of 6 kGy. This might be due to a recombination

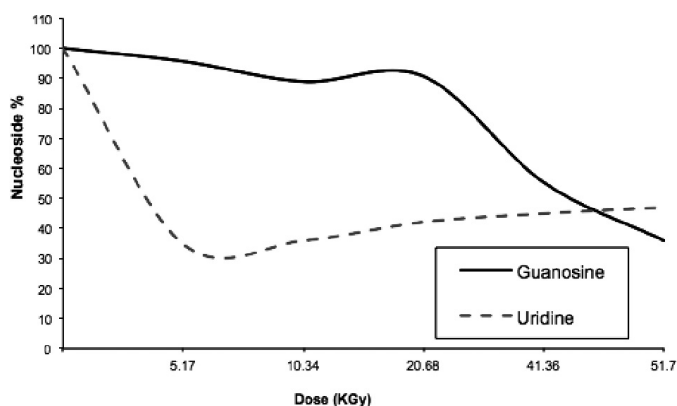


Figure 3: Effect of irradiation dose in the survival of guanosine and uridine in aqueous solutions.

process of radicals in the sample during irradiation, which leads to a partial regeneration of the molecule.

In the heterogeneous radiolysis of the water-nucleoside-clay system, 3 mL of the clay suspension (100 mg of clay) were irradiated until no nucleoside was left in aqueous solution without clay at a fixed dose rate of 186 Gy/min, evaluated by copper-ferrous sulfate dosimeter [7]. Nucleosides irradiation in the presence of clay, demonstrated that the presence of mineral decreased the damage suffered by the guanosine as a result of the action of gamma radiation (Table 2). The mechanism for this protection is not well established, but probably has partial explanation in the capacity of mineral structure to delocalize energy and restrain the movement of radicals inside the structure, so less energy and radicals attack the organic molecules adsorbed on clay. Probably due to the fact that uridine is poorly adsorbed under the experimental conditions, it was impossible to recover it after irradiation in presence of clay. More experimentation with pyrimidinic nucleosides considering different environmental conditions may lead to more conclusions related to their probable prebiotic chemistry.

Table 2: Remaining percentage guanosine and uridine after irradiation with a 60-cobalt gamma ray source, at pH 2, in presence and absence of clay.

Sample	Adsorbed %	In presence of clay		In absence of clay
		% Desorbed without irradiation	% Desorbed after irradiation	% Remnant after irradiation
Guanosine	88.5	73.0	25.8	0
Uridine	4.4	50.3	0	0

Aguilar-Ovando, E.Y. **REMARKS**
Negrón-Mendoza, A.

These results confirm the capacity of concentration and protection against ionizing radiation that minerals like clay may provide to nucleosides. This kind of research gives clues to a better comprehension of the chemical evolution that originated life on our planet. Adsorption of nucleic acid bases and their derivatives on clay minerals may have played an important role in primordial organic chemistry as first proposed by Bernal in 1951, especially considering the difficulties of synthesis of this particular kind of molecules. If their synthesis happened on early Earth, the presence of this kind of minerals may have played a special role in their survival and evolution. In this study, it was established that maximum adsorption of nucleosides occurred at low pH values, depending on the particular structure of the organic molecule, and then decreased steadily as pH increased. The purinic nucleoside is adsorbed in larger quantities than the pyrimidic one. The major mechanism for adsorption is cation exchange, but other mechanisms like ion-dipole coordination interactions and hydrogen bonding also might take place in the process. The dependence of these mechanisms is due mainly to the iso-electric point, molecular size, shape of the molecule, and pH of the solution. The effect of environmental temperature around the nucleoside adsorbed on clay was examined too, showing special relevance to purinic nucleosides. Pyrimidinic nucleosides show more resistance to heat, but less resistance against ionizing radiation, even when adsorbed in clay. Finally, survival of the nucleosides under study was greater when the nucleosides were irradiated while adsorbed on a clay mineral, compared with their survival when irradiated in solution, so the protection role of the clays against ionizing radiation was observed.

ACKNOWLEDGMENTS

This work was supported by DGAPA grant IN111116. One of us (E.A.O.) was supported by CONACYT fellowship. We thank to the Posgrado en Ciencias Químicas-UNAM. The technical support from C. Camargo, B. Leal, and F. García-Flores is acknowledged.

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