Solid adenine and seawater salts exposed to gamma radiation: An FT-IR and EPR spectroscopy analysis for prebiotic chemistry

João Paulo T. Baú a, Daniel F. Valezi b, Sául A. Villafañe-Barajas c, María Colín-García c, **, Alicia Negrón-Mendoza d, Eduardo Di Mauro b, Dimas A.M. Zaia a, a, 

a Laboratorio de Química Prebiótica, Departamento de Química-CCE, Universidad Estadual de Londrina, 86051-990, Londrina, PR, Brasil 
b Laboratorio de Resonancia Paramagnética Electrónica (LARPE), Departamento de Física-CCE, Universidad Estadual de Londrina, 86051-990, Londrina, PR, Brasil 
c Instituto de Geología, Universidad Nacional Autónoma de México, Ciudad Universitaria, 04510, C. Mx., Mexico 
d Instituto de Ciencias Nucleares, Universidad Nacional Autónoma de México, Ciudad Universitaria, 04510, C. Mx, Mexico

A R T I C L E   I N F O

Keywords:
Physical chemistry

A B S T R A C T

Solids of adenine obtained from distilled water and seawater lyophilized solutions were gamma irradiated at a 94.52 kGy dose. Results indicate that pure solid adenine had a low degradation rate, likewise the solid containing seawater salts. However, EPR spectroscopy analysis suggests a high interaction of the radiation with seawater salts. These outcomes are of interest for prebiotic chemistry, since ions could have played important roles in chemical evolution. In addition, Martian soil is rich in sulphate salts, thus these salts could protected organic molecules being degraded by γ-radiation.

1. Introduction

Prebiotic Chemistry is a field in Astrobiology, which investigate the origins of life on Earth, from chemical (geochemical) to biological systems. The reactivity and decomposition of biomolecules such as amino acids, nucleic acid bases and sugars (building blocks of life) are one of the main aims on Prebiotic Chemistry [1]. The emergence of life probably occurred with the participation of these biomolecules. Thus, experiments focusing on these molecules should be carried out in accordance with Primitive Earth conditions [2].

It is known that at the early Earth, environmental ionizing radiation was more intense than nowadays [3]. This source of energy could initiate a variety of chemical reactions, including decomposition and polymerization. Zaia [2] suggested an artificial seawater to be used in Prebiotic Chemistry experiments that better resembles saline concentration of the primitive seas at 4.0 billion years ago. It should be noted that most radiolysis experiments were performed in distilled water or sodium chloride solutions or salt solutions. The artificial seawater suggested by Zaia [2] has high Mg²⁺ and SO₄²⁻ concentrations unlike the current seawater that has a higher Na⁺ and Cl⁻ concentrations [4]. In addition, the interaction between salts of artificial seawater [2] and nucleic acid bases change their reactivity [5, 6]. The effects of ionizing radiation on seawater decomposition, and the effect of ions on the radiolysis products of water has been reported recently [7, 8, 9]. These reports demonstrated that ionizing radiation can interact with different ions, changing the yields of water radiolysis products. The γ-irradiation of seawater promoted the formation of H₂, O₂ and H₂O₂ [7, 8, 9]. The Br⁻ and Cl⁻ anions enhanced the formation of H₂ because, they inhibit the reaction of hydrogen with hydroxyl radical [7, 8, 9].

This work aims to understand the interactions promoted by gamma radiation on solid samples of adenine obtained from both distilled water and seawater solution, resembling conditions from 4.0 billion years ago. The samples were analyzed with infrared (FT-IR) and electron paramagnetic resonance (EPR) spectroscopy.

2. Experimental

2.1. Samples preparation and cleaning of the glassware

Solid dry samples were obtained from solutions of adenine at 500 µg.ml⁻¹ (in distilled water and seawater solution). The solutions were frozen in a refrigerator and further place to a pump lyophilizer (high vacuum, 50 mPa, −50 °C). The 4.0 Ga seawater solution was prepared according to Zaia [2], by the dissolution in 1.0 L of purified water...
(MilliQ) the following salts: Na$_2$SO$_4$ (0.271 g), MgCl$_2$.6H$_2$O (0.500 g), CaCl$_2$.2H$_2$O (2.50 g), KBr (0.050 g), K$_2$SO$_4$ (0.400 g), MgSO$_4$ (15.00 g).

All the glassware was cleaned according to the chemical radiation procedures. It was immersed in a hot mixture of HNO$_3$ and H$_2$SO$_4$ for four hours, followed by rinsing of with bidistilled water and MilliQ purified water in this order. The glassware was evolved in aluminum foil and heated at 300 °C overnight, ensuring the elimination of all organic matter.

2.2. Radiolysis

The samples were irradiated in a gamma ray source (Gammabean 651-PT, located at Institute of Nuclear Sciences, UNAM) at room temperature (298 K). The irradiation dose was determined using a ferrous sulfate-copper sulfate dosimeter. The dose rate was 197 Gy min$^{-1}$. Irradiation dose was 94.52 kGy.

2.3. Infrared spectroscopy (FT-IR)

The FT-IR spectra were obtained with a reflectance accessory (PerkinElmer spectrum 400, USA) in a spectrometer (PerkinElmer spectrum 400, USA). The spectra were recorded at transmittance mode from 4000 cm$^{-1}$-650 cm$^{-1}$ and a resolution of 4 cm$^{-1}$ over 10 scans.

2.4. Electron paramagnetic resonance spectroscopy (EPR)

The samples were submitted to EPR spectrometer (Jeol JES-TE300) from Chemistryl Institute, UNAM, operating at X-band, with a 100 kHz modulation frequency and a cylindrical cavity in the mode TE011. The magnetic field received external calibration with a precision gaussmeter, JeolES-FC5. The samples were placed in a flat-type quartz cell and measured at room temperature.

![FT-IR spectra](image-url)

Fig. 1. FT-IR spectra of the solid of adenine obtained from the lyophilization of the adenine solution (500 μg.mL$^{-1}$) in: a) distilled water and; b) seawater solution (4.0 Ga). Control sample (black line) and irradiated sample at 94.52 kGy (red line).
3. Results

Solid powder samples were obtained from the lyophilization of adenine solutions at 500 μg mL⁻¹ (distilled water and seawater solution). These solids were gamma irradiated at a dose of 94.52 kGy. No changes (shift nor new bands formation) were observed in the FT-IR spectra of the solids obtained from distilled water solution (Fig. 1-a), which suggest a low or none decomposition of adenine. The same irradiation procedure was performed on the solid obtained from seawater solution. In this last case, no bands of adenine were observed, due to the minor concentration of adenine, compared to the salts of seawater (Fig. 1-b). In the case of the sample containing adenine and seawater salts, the FT-IR spectra show three bands at 667, 1104 and 1636 cm⁻¹. The high intense band at 1104 cm⁻¹ is due to the vibrational modes v₁ of the sulfate (SO₄²⁻) species [10]. The third band at 1636 cm⁻¹ is attributed to the bending β(H-O-H), from the hydration of the salts [11]. The control sample (without irradiation) shows a band at 667 cm⁻¹, which could be related to the sulfate bending β(SO₄²⁻), due to formation of calcium sulfate (CaSO₄). The gypsum mineral has the same vibration frequency at the FT-IR spectra [12], as sulfate calcium salt does [13]. Nevertheless, after gamma-irradiation, this frequency was observed to decrease in intensity. These results suggest an interaction of the gamma radiation with the salts present in the seawater, and not merely the interaction with adenine molecule.

Moreover, the irradiation of sulfate promotes the formation of other

Fig. 2. EPR spectra of the solid obtained from lyophilization of the solution of: a) adenine (500 μg mL⁻¹) in: distilled water solution and irradiated at 94.52 kGy and; b) seawater solution and irradiated at 94.52 kGy.
species like $\text{SO}_4^{3-}$, $\text{SO}_2$, $\text{O}^-$ and $\text{O}_3^-$ by the formation of electron-hole trapping centers [14], being $\text{SO}_2$ the primary defect produced (Eq. (1)), which may be monitored by EPR spectroscopy [15, 16].

$$\text{SO}_4^{3-} \rightarrow \text{SO}_3^- + e^- \tag{1}$$

Solid samples obtained from the lyophilization of adenine in distilled water and seawater solution were both analyzed by EPR spectroscopy before and after gamma irradiation. Samples before irradiation showed no EPR signals. Whereas, irradiated samples showed EPR lines which represent the formation of free radicals induced by the gamma-radiation (Fig. 2).

The EPR of the solid obtained from distilled water solution showed a resonance with $g = 2.004$, which is characteristic of free radical. This radical is very likely prominent of degradation of adenine [17]. Zehner et al. [18], describes the formation of radicals in adenine derivatives through X-rays irradiation, by the addition of a proton at carbon C2 or C8. The EPR of the solid obtained from seawater solution shows a much more intricate spectrum. There are several resonance lines in the spectrum and some of them are superimposed, which makes their identification difficult. This occurs because of the mixture of salts (cations and anions) in the sample.

The amount of each species at the sample of adenine plus seawater solution is described at Table 1. It should be noticed that the salt with minor concentration is the potassium bromide (KBr). Thus, the amount of the ions and adenine is related to one species (or mol) of a bromide ion ($\text{Br}^-$). The main species at this sample are the ions $\text{SO}_4^{2-}$, $\text{Cl}^-$, $\text{Mg}^{2+}$ and $\text{Ca}^{2+}$.

After irradiation, sulfates (both the $\text{Na}_2\text{SO}_4$ and $\text{K}_2\text{SO}_4$) could generate free radicals, which are detected by EPR. Some species, such as $\text{SO}_3$ in different environments could show different $g$ values, as described in literature [19], this species generates even more probability of superimposition lines. The formation of this type of radical is well documented in the literature by EPR, including in other compounds like $\text{BaSO}_4$ [20, 21].

4. Discussion

In the spectrum of adenine plus seawater solution, the resonance at $g = 2.004$ is very likely due to $\text{SO}_3^-$. Among the radicals that could be formed after irradiation, $\text{SO}_3$ appears in literature as the more intense, showing an isotropic spectroscopic factor of $g = 2.0037$ [22] and $g = 2.0033$ [15] for $\text{K}_2\text{SO}_4$ and $g = 2.004$ for $\text{Na}_2\text{SO}_4$ [23]. Although this radical shows the same $g$ value as the radical of the solid obtained from distilled water solution, this does not necessarily means that it is the same radical. The difference in $g$ would appear in a measure with greater precision.

Another species probably present is the $\text{SO}_4$ radical. However, this radical was very difficult to identify in our spectrum since it generates several resonance lines, and its intensity is much weaker than the one from $\text{SO}_3^-$. Unlike $\text{SO}_3^-$, the $\text{SO}_4$ radical is anisotropic, showing three $g$ values [22, 23]. The $\text{SO}_4$ can occupy two non-equivalent sites when present in $\text{K}_2\text{SO}_4$ structure, [22]. In this case, instead three $g$ values, we would recommend six. Another consideration, for $\text{Na}_2\text{SO}_4$, is the possibility of hyperfine interaction with 3/2 nucleus [23]. All these resonance lines are probably superimposed in our spectrum.

The $\text{SO}_3^-$ radical, as in the case of $\text{SO}_2^-$, shows an anisotropic $g$ both in $\text{K}_2\text{SO}_4$ and in $\text{CaSO}_4$ [22, 23]. However, it was very difficult the identification, because of the salt mixture.

Lastly, although the mixture of salts and the several resonance lines, it is possible to identify the $g = 2.011$ as probably due to $\text{O}_3^-$. This radical appears in literature as an isotropic $g$ value resonance, showing $g = 2.0116$ for $\text{K}_2\text{SO}_4$ structure [22] and 2.0100 for $\text{Na}_2\text{SO}_4$ [23]. It should be noticed that some lines in the spectrum could be due to hyperfine interaction from others nucleus spin configuration. Nevertheless, FT-IR points out to modifications on the sulfate species.

In terms of prebiotic chemistry, the interactions of biomolecules with ions from seawater salts could give a clue on Chemical Evolution processes. Cations from seawater can decrease the free energy of nucleic acid bases, such as adenine [5]. The results here discussed points out some remarks. Firstly, in solid state, some reactivity of the gamma-irradiated adenine is observed. Second, in seawater solution due to the salts, it is possible that a minor amount of adenine molecules interacts with the gamma radiation; this effect could have diminishing the decomposition of organic molecules.

It should be pointed out that Mars’s surface has a high concentration of sulfate salts [24, 25, 26]. Thus, organic matter might be preserved by the mechanism here described. These sulfates present in Martian soils would interact with ionizing radiation that reaches surface of Mars.

5. Conclusion

At solid state, the gamma-irradiation of pure adenine promotes the formation of free radicals, detected by EPR spectroscopy, followed by small decomposition. Nevertheless, for the mixture of solid adenine and several salts from seawater, a minor interaction of gamma radiation with adenine is observed, due to the effects with seawater ions detected. In order to a fully comprehension of the phenomena, some experiments including one or two sulfates species could be helpful.

The results hereby presented highlight the relevance of one scenario in prebiotic chemistry: the environments with wet/dry cycles, like tidal ponds. At the dry period, none or a very small amount of water is present. Thus, the presence of small organic species should be favored in a saline medium, since salts (ions) may have decreased the interaction with radiation effects.

Table 1

Amount of each species at the sample containing adenine plus seawater salts.

| Specie   | n   |
|----------|-----|
| $\text{Na}^+$ | 9.1 |
| $\text{K}^+$  | 11.9|
| $\text{Mg}^{2+}$ | 302.5|
| $\text{Ca}^{2+}$ | 40.5|
| $\text{SO}_3^-$ | 306.6|
| $\text{Cl}^-$  | 92.7|
| $\text{Br}^-$   | 1.0 |
| Adenine        | 8.8 |

The amount of $\text{Br}^-$ in mol was taken as 1.0.

Declarations

Author contribution statement

João Paulo T. Baú: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Daniel F. Valezi, Dimas A. M. Zaia, Eduardo D. Mauro: Analyzed and interpreted the data; Wrote the paper.
Sául A. Villañá-Barajas: Performed the experiments.
María Colín-García, Alicia Negrín-Mendoza: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Funding statement

João Paulo T. Baú was supported by CAPES for the funding. This work was supported by DGAPA-PAPIIT (IA203217). This work was supported by a grant from CNPq/Fundação Araucária (Programa de Apoio a Núcleos de Excelência – PRONEX, protocolo 24732).
Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

The authors thank the Laboratory of Chemical Evolution at the Institute of Nuclear Sciences and the Institute of Geology at the National Autonomous University of Mexico, Mexico City, for kind assistance in the lab work. In addition, the authors thank the Laboratory of EPR from Institute of Chemistry at the National Autonomous University of Mexico, for the EPR analysis.

References

[1] N. Kitadai, S. Maruyama, Origins of building blocks of life: a review, Geosci. Front. 9 (2018) 1117–1153.
[2] D.A.M. Zaia, Adsorption of amino acids and nucleic acid bases onto minerals: a few suggestions for prebiotic chemistry experiments, Int. J. Astrobiol. 11 (2012) 229–234.
[3] A. Negron-Mendoza, S. Ramos-Bernal, M. Colín-García, A. Heredia, Chemical evolution: an approach from radiation chemistry, Rad. Appl. 1 (3) (2016) 159–164.
[4] G. Beamam, in: E. Brown, A. Colling, D. Park, J. Phillips, D. Rothery, J. Wright (Eds.), Seawater: its Composition, Properties and Behavior, The Open University, Oxford, 2004.
[5] P.R. Anizelli, J.P.T. Baú, H.S. Nabeshima, M.F. da Costa, D.A.M. Zaia, An experimental and theoretical vibrational study of interaction of adenine and thymine with artificial seawaters: a prebiotic experiment, Spectrochim. Acta Mol. Biomol. Spectrosc. 78 (3) (2011) 1023–1026.
[6] J.P. Bibring, Y. Langevin, A. Gendrin, B. Gondet, F. Poulet, M. Berthieu, A. Soufflot, R. Avidson, N. Mandgol, J. Mustard, P. Drossart, Mars surface diversity as revealed by the OMEGA/Mars Express observations, Science 307 (5715) (2005) 1576–1581.
[7] J.P. Bibring, Y. Langevin, J.F. Mustard, F. Poulet, R. Avidson, A. Gendrin, B. Gondet, N. Mandgol, P. Pinet, F. Forget, OMEGA Team, Global mineralogical and aqueous Mars history derived from OMEGA/Mars Express data, Science 312 (5772) (2006) 400–404.
[8] A. Wang, L.A. Hackin, S.W. Squyes, R.L. Jolliff, L. Crumpler, R. Gellert, C. Schröder, K. Herkenhoff, J. Hurowitz, N.J. Tosca, W.H. Farrand, R. Anderson, A.T. Knudson, Sulfate deposition in subsurface regolith in Gusev crater, Mars, J. Geophys. Res. Plan 111 (E2) (2006).

K. Hata, H. Inoue, T. Kojima, A. Iwase, S. Kashara, S. Hanawa, F. Ueno, T. Tsukada, Hydrogen peroxide production by gamma radiolysis of sodium chloride solutions containing a small amount of bromide ion, Nucl. Technol. 193 (3) (2016) 434–443.

N.B. Colthup, Lawrence H. Daly, Stephen E. Wiberley, in: N.B. Colthup, L.H. Daly (Eds.), Introduction to Infrared and Raman Spectroscopy, third ed., Academic Press, Inc., San Diego, 1990.

B.S. Ault, Infrared spectra of argon matrix-isolated alkali halide salt/water complexes, J. Am. Chem. Soc. 100 (8) (1978) 2426–2433.

G. Anbalagan, S. Mukundakumari, K.S. Murugesan, S. Gunasekaran, Infrared, optical absorption, and EPR spectroscopic studies on natural gypsum, Vib. Spectrosc. 50 (2009) 226–230.

P.A. Miller, C.H. Wilkins, Infrared spectra and characteristic frequencies of inorganic ions, Anal. Chem. 24 (8) (1952) 1253–1294.

T.N. Nurakhmetov, K.A. Kuterbekov, D.H. Daurenbekov, Z.M. Salikhodzha, A.K. Kainarbay, A.M. Zhunusbekov, K. Bekmyrza, Radiation defects in alkali metal sulfates, Radiat. Phys. Chem. 119 (2016) 218–222.

V.V. Gromov, J.R. Morton, Paramagnetic centers in irradiated potassium sulfate, Can. J. Chem. 44 (4) (1966) 527–528.

J.R. Byberg, O− detected by ESR as a primary electron-excess defect in X-irradiated K2SO4, J. Chem. Phys. 84 (11) (1986) 6083–6085.

K. Hata, H. Inoue, T. Kojima, A. Iwase, S. Kashara, S. Hanawa, F. Ueno, T. Tsukada, Hydrogen peroxide production by gamma radiolysis of sodium chloride solutions containing a small amount of bromide ion, Nucl. Technol. 193 (3) (2016) 434–443.