EPSC Abstracts
Vol. 12, EPSC2018-886, 2018
European Planetary Science Congress 2018

Effects of low and high energy proton radiation on the preservation of biomolecules as followed by their immuno-identification behavior

Yolanda Blanco[(1)], Daniel Viúdez-Moreiras[(1)], Mercedes Moreno-Paz[(1)], Aurélie Le Postollec[(2)], Sébastien Incerti[(3) (4)], Michel Dobrijevic[(2)], José Antonio Rodríguez-Manfredi[(1)] and Víctor Parro[(1)]

(1) Centro de Astrobiología (INTA-CSIC), Madrid, Spain, (2) Laboratoire d'astrophysique de Bordeaux, Université de Bordeaux, Pessac, France, (3) CNRS/IN2P3, Centre d'Etudes Nucléaires de Bordeaux-Gradignan, France, (4) Université Bordeaux, Centre d'Etudes Nucléaires de Bordeaux-Gradignan, France.
(blancoly@cab.inta-csic.es / Fax: +34915201074)

Abstract

Bio-affinity based systems, such as antigen-antibody interactions, have been proposed for searching for molecular biomarkers in planetary exploration. However, the surface of planets and moons with little or no atmosphere are generally unprotected from the high-energy ionizing radiation that may severely affect the structure and chemistry of potential biopolymers. To understand what range of energy and proton radiation doses may be more harmful to the target biomarkers, we performed fluorescent immunoassays to monitor the radiolytic effect on target epitopes (the part of the molecule to which antibodies bind) exposed to low (5.5 kGy and 7 kGy) and high doses (55 kGy and 70 kGy) of 3 MeV and 200 MeV of protons. The effect of proton radiation was monitored by measuring the loss in the immuno-identification of target molecules due to the impaired ability of the antibodies for binding their corresponding radiation-damaged epitopes.

1. Introduction

The search for signs of past or present life in planets and moons of our Solar System is one of the great challenges of the upcoming space missions. On planetary environments, potential biogenic organic molecules would be exposed to chemical and physical degradation due to environmental factors. The ionizing radiation, one of the most pervasive long-term agents of organic molecule degradation, is in the form of galactic cosmic rays (GCR) and solar energetic particles (SEP) on planetary surfaces. During the last two decades, immunosensors (bioaffinity-based biosensors using antibodies) have been proposed for life detection in planetary exploration [1] and studies on the stability of antibodies have been performed to demonstrate the possibility of their use in a mission to Mars [2, 3]. In this context, it is critical to know how a long-term exposure to ionizing radiation may affect the integrity of potential organic molecular targets.

2. Material and Methods

We selected several organic molecules and a whole microorganism that might be potential indicators of extant or extinct life on Mars. All of them were immobilized on epoxy-activated glass slides and exposed to high energy protons (3 and 200 MeV) at radiation doses equivalent to 100-500 Kyr and 1-5 Myr of exposure at 1 m of the Martian surface based on MSL data [4]. Direct immunoassays were performed to test the structural integrity and preservation of epitopes in the printed biomolecules through the recognition of their corresponding antibodies.

3. Results

Results of two-way ANOVA revealed statistical differences in the immuno-identification of the immobilized molecules as a function of the dose, the energy and the interaction of both factors, which means that the dose caused effects in a different manner as a function of the energy. Also, two-way ANOVA results showed that the energy was the most powerful factor for 56% (9/16) of the immobilized molecules and the dose in the case of *B. subtilis* spores, EPS, LPS, FtsZ, rubredoxin, AEKAC and p-azo-L-Phe (44% of the immobilized molecules). Multiple range tests were performed to test the effect of the dose for each level of energy. These tests revealed that the differences as a function of the dose were higher at the lowest energy, showing all the

immobilized molecules, except the bacterioferritin, significant effects with respect to the control at an energy of 3 MeV (Fig. 1A), whereas only 25% (4/16) of the immobilized molecules in the case of irradiation with 200 MeV (Fig. 1B).

After exposure to the lowest dose at 3 MeV, only one molecule (p-azo-L-Phe) lost a statistically significant portion of the original immuno-identification, whereas 31% (5/16) (GroEL, FtsZ, rubredoxin, AEKAC and Cys) surprisingly increased their immuno-identification signal (Fig. 1A). Under the lowest dose and 200 MeV, two molecules (AEKAC and LPS) lost and increased a significant portion of the original immuno-identification, respectively (Fig. 1B). Finally, after exposure to the highest dose, 75% (12/16) and 6% (1/16) of the immobilized molecules showed significant loss of immuno-identification at an energy of 3 and 200 MeV, respectively (Fig. 1). Three molecules (LPS, FtsZ and bacterioferritin) increased significantly their immuno-identification signal after irradiation with the highest dose at 200 MeV. However, no molecules increased its immuno-identification after irradiation with the highest dose at an energy of 3 MeV (Fig. 1A). After irradiation at the highest dose with protons of 3 MeV and 200 MeV, 25% and 94% of the tested molecules retained more 75% of their non-irradiated control immuno-identification signal, respectively (Fig. 1). On the other hand, T-tests were performed to evaluate the effect of the energy for the low and high doses. These results revealed statistically significant differences among the mean values corresponded to the two levels of energy for low doses and high doses in only 31% (5/16) of the molecules (GroEL, p-azo-L-Phe, Cys, LPS and cAMP). However, 18% (3/16) and 50% (8/16) of the molecules only showed significant differences among the two energies for the lower and the higher doses, respectively.

4. Figures

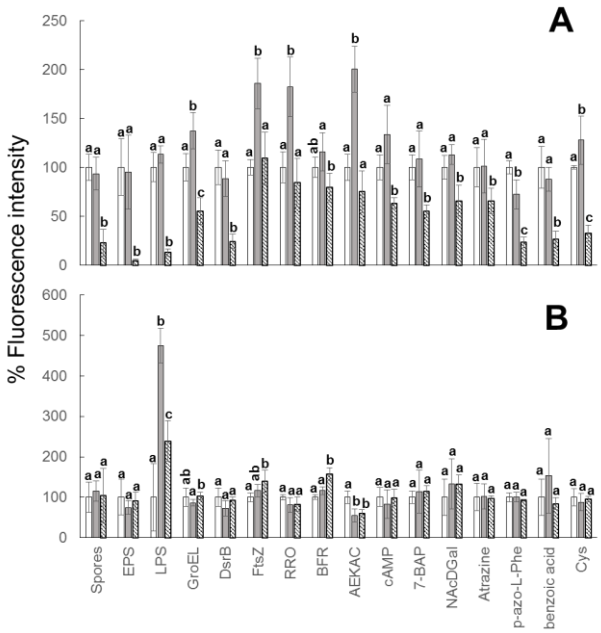

Fig. 1: Effect of proton radiation on the immobilized organic molecules. Average of the fluorescence intensity of spots containing each organic molecule after performing direct fluorescent immunoassays relativized to their respective non-irradiated control (considered as 100% of the signal). Non-irradiated samples (white bars), irradiated at low doses (5.5 kGy and 7 kGy) (grey) and irradiated at high doses (55 kGy and 70 kGy) (hatched) at energies of 3 MeV **(A)** and 200 MeV **(B)**. Letters represent statistical differences with respect to the control (0 kGy), that is, samples with the same letter are not significantly different ($p \geq 0.05$) based on Tamhane´s T2 or Bonferroni's multiple range tests.

5. Summary and conclusions

Our results revealed that the effects produced by a particular dose strongly depended on the proton energy. Also, we found a strong correlation between the radiolysis rates and the protons stopping power, related to LET (Linear Energy Transfer). We conclude that although unprotected planetary surfaces as Europa and Mars receive high energy (>100 MeV) protons, are those of lower energies (<10 MeV) the most harmful for biopolymers and on which future studies should mainly focus in.

Acknowledgements

This work has been funded by Spanish MINECO/FEDER grants Nº ESP2015-69540-R and ESP2016-79612-C3-1-R. We are very grateful to Laura Barrios (SGAI-CSIC) for her help with the statistic tests and the NCC in Gyeonggi-do (South Korea) for performing proton irradiation at 200 MeV.

References

[1] Parro V *et al*. (2011): Astrobiology 11: 15-18.

[2] Le Postollec A. *et al.* (2009): Astrobiology 9: 637-645.

[3] de Diego-Castilla G. *et al.* (2011): Astrobiology 11: 759-773.

[4] Hassler D.M. *et al.* (2014): Science 343.