

PRESERVATION OF ORGANIC MATTER ON MARS: EFFECT OF HIGH RADIATION DOSES ON BIOMOLECULES

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Introduction: The strong ionizing radiation on the surface of Mars is one of the most important factors that determine the integrity of the organic matter, regardless its meteoritic or biological origin. One of the objectives of the proposed *Icebreaker* mission to Mars [1] is to find organic biosignatures in ice-rich soils of the northern plains, where transient habitable conditions might have occurred within the last 10 Myr, during high obliquity cycles [2]. Since Mars lacks a planetary magnetic field, the potential organic biomarkers generated during such recent episodes of biological activity might have suffered severe radiolytic degradation, with the subsequent total or partial loss of biological information [3]. This constrains the approaches used to search for evidence of life. As part of the IceBreaker payload, the SOLID (Signs of Life Detector) instrument [4], an antibody microarray-based biosensor, can detect large-molecular-weight compounds by Fluorescent Sandwich Immunoassay (FSI). Antibodies (Abs) recognize and bind to a small region of the antigen, called the epitope. In principle, because epitopes are comparatively small, they are more likely to be preserved to the effect of radiation than the entire organic macromolecules.

Objectives: Testing the effect of high doses of ionizing radiation on selected biochemical compounds and bacterial spores. How ionizing radiation affects the epitope recognition by different antibodies.

Methods: Representative molecular biomarkers targeted by SOLID (proteins, peptides, exopolymeric substances, bacterial spores...) were immobilized on glass slides. Slides were exposed to several 10 MeV electron radiation doses: 0, 1, 50 and 500 KGy, equivalent to 0, 0.02, 1 and 10 My radiation exposure on Mars. Direct fluorescent immunoassays were performed to test the preservation of the epitopes by the binding of the corresponding antibodies.

In addition, soil samples from the Antarctic Dry Valleys spiked with 10^7 spores/g of the Gram-positive bacterium *Bacillus subtilis*, and a nearly 1000 years old cyanobacterial mat from the McMurdo Ice Shelf (Antarctica) were subjected to the same radiation doses. After irradiation, extracts were analyzed by FSI with a microarray containing the anti-*B. subtilis* Ab and a collection of 17 anti-cyanobacterial strains Abs [5]. The corresponding fluorescent Abs were used as detectors.

Results: Fluorescent Abs can only bind to their corresponding targets if these still have intact or little modified epitopes. Bright fluorescent spots indicate

that the biomolecules are recognized by the antibodies and, consequently, they still retain intact epitopes after radiation exposure.

Effect of electron radiation on immobilized biomolecules: The fluorescent signal decreased with radiation dose, indicating damage to the epitope structure. Damage seemed to depend on epitope complexity, with smaller and simpler ones being less affected. In most cases 1 to 20% of the original fluorescence signal was retained after 500 KGy. This implies that a significant fraction of Abs could still bind to intact or partially damaged epitopes.

Effect of electron radiation on bacterial spores: Although samples were completely sterilized after 50 KGy, anti-spore Abs yielded a similar signal in samples exposed to 50 KGy as in the control sample. Even after 500 KGy, 30% of the original signal was still observable.

Effect of electron radiation on an old and dry antarctic cyanobacterial mat: The non-irradiated sample (control) showed strong reactivity with the antibodies IIC4, K14, K4 and K5 (corresponding to an antarctic permafrost extract, *Anabaena* sp., *Microcystis* sp., and *M. aeruginosa*, respectively) and less reactivity with K17 (*Plankthothrix* sp.), K15 (*Leptolyngbya* sp.), K12 (*Aphanizomenon* sp.) and K6 (*A. ovalisporum*). After 500 KGy radiation, many signals disappeared, but 20 and 100 % of the original fluorescence (depending the antibody) was retained.

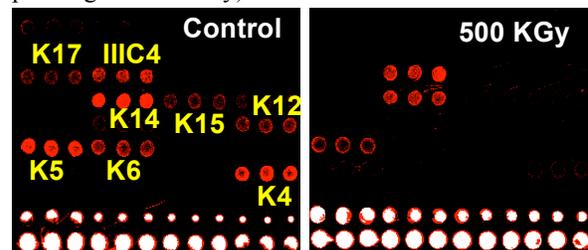


Figure 1. Microarray images showing positive reactions to cyanobacterial markers in an antarctic mat by FSI: non irradiated (control) and exposed to 500 KGy.

References: [1] McKay et al., (2013) *Astrobiology* 13, 334-353. [2] Laskar, J. et al. (2002) *Nature* 419, 375-377. [3] Dartnell, L. R., et al. (2007) *Geophys. Res. Lett.* 34.2 [4] Parro V. et al. (2011) *Astrobiology* 11, 15-27. [5] Blanco Y. et al. (2015) *Environ. Sci. Tech.* DOI: 10.1021/es5051106.

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