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The AMINO experiment: RNA stability under solar radiation studied on the EXPOSE-R facility of the International Space Station

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Abstract: Careful examination of the present metabolism and *in vitro* selection of various catalytic RNAs strongly support the RNA world hypothesis as a crucial step of the origins and early life evolution. Small functional RNAs were exposed from 10 March 2009 to 21 January 2011 to space conditions on board the International Space Station in the EXPOSE-R mission. The aim of this study was to investigate the preservation or modification properties such as integrity of RNAs after space exposition. The exposition to the solar radiation has a strong degradation effect on the size distribution of RNA. Moreover, the comparison between the in-flight samples, exposed to the Sun and not exposed, indicates that the solar radiation degrades RNA bases.

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Key words: EXPOSE-R mission, ISS, RNA, space conditions, solar radiation.

Introduction

Prebiotic chemistry experiments search to explain chemical reactions that have been involved in the early life (Eschenmoser & Loewenthal 1992; Sutherland & Whitfield 1997; Maurel & Décout 1999; Powner *et al.* 2009). Water, carbon monoxide, sulphur dioxide, methane and dinitrogen molecules present in the atmosphere of the primitive Earth might have reacted to form the elementary building blocks of life such as ammonia, hydrogen cyanide, formaldehyde, acetonitrile, cyanogen and cyanoacetylene. Cosmic rays, ionizing reactions, electric discharges, mechanical and radioactive processes could induce ion–molecule and radical reactions. From these molecules, formation of amino acids and nucleic acid bases could be explained (Miller 1953; Oro 1961; Kobayashi *et al.* 1998; Miyakawa *et al.* 1999; Bossa *et al.* 2009; Meinert *et al.* 2012). Many prebiotic molecules could have been present in comets. Oro *et al.* (1992) suggested that comets may have delivered life's early building blocks on Earth. Delsemme (2000) emphasized that comet bombardments have brought to Earth all the carbon compounds present in organic molecules used by life. Furthermore, nucleobases have a remarkable photo-stability in spite of their high absorption in the ultraviolet (UV) radiation (Saiagh *et al.* 2014); huge amounts have been found in meteorites (Callahan *et al.* 2011).

The discovery of catalytic RNAs supported the RNA world hypothesis in which the earliest forms of life may have relied on RNA to store genetic information and to catalyse chemical reactions (Guerrier-Tanaka *et al.* 1983; Gilbert 1986; Cech 1987; Maurel 1992; Gesteland & Atkins 1993; Maurel & Haenni 2005). The Systematic Evolution of Ligands by EXponential (SELEX) enrichment method has been developed to generate and screen large libraries of nucleic acid molecules differing in their sequences and their folding properties. The success of this method to select ribozymes, RNA-possessing catalytic properties and aptamers, RNAs that specifically bind molecules such as amino acids, ATP or enzymatic cofactors, lend experimental support to the RNA world hypothesis. The use of small exogeneous cofactors by ribozymes could have been an easy way of expanding their catalytic and metabolic properties especially in a putative 'soup' of small oligoribonucleotides binding with small organics and metals. Adenine is a highly plausible prebiotic organic compound (Oro 1961; Maurel & Ninio 1987; Vergne *et al.* 2000), and therefore adenine-binding aptamers (Meli *et al.* 2002) could mimic structures showing primitive functionalities. We discovered the adenine-dependent hairpin ribozyme (ADHR) which requires adenine as catalytic cofactor (Meli *et al.* 2003) and proposed that this assembly is a good candidate for early 'ribo-organisms'. We showed that

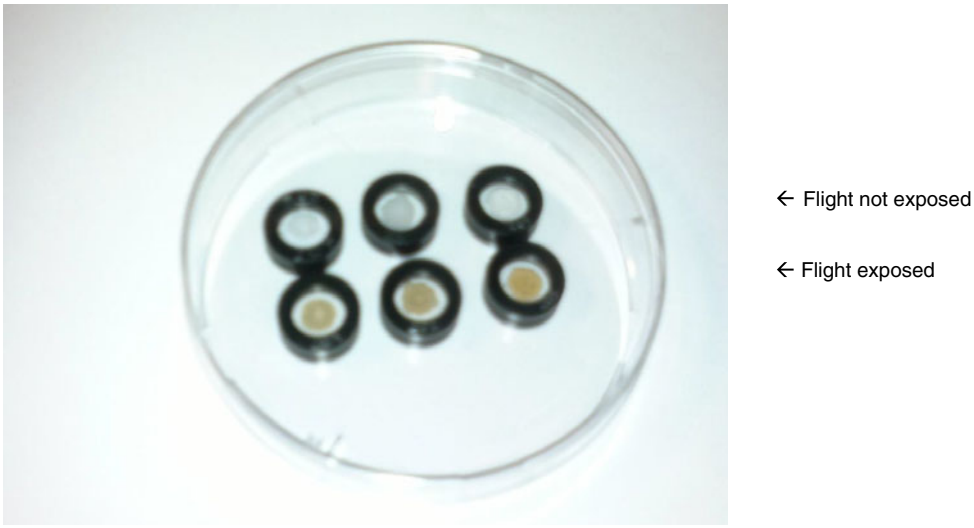


Fig. 1. Windows recovered with samples exposed to the Sun (yellow) and non-exposed samples (white) onboard ISS.

ADHR can react with a wide range of adenine analogues and acts with different catalytic strategies compared with the wild-type ribozyme. Finally, the structural properties of plausible RNA vestiges of early life were recently studied according to external perturbations (Delan-Forino *et al.* 2014; Hui-Bon-Hoa *et al.* 2014).

In the present study, we have investigated effect of UV solar radiation on ADHR molecules on-board the International Spatial Station (ISS) in the EXPOSE-R mission. The goal of the research reported here is to unravel the stability of these components in space conditions. The study of the effects of extreme conditions on RNA molecules and the protective effect of trapping in NaCl crystals is of crucial interest for looking for life elsewhere. Indeed, there is evidence that primitive oceans on Earth were highly saline (Knauth 1998) and early life might be considered in hypersaline conditions (Dundas 1998). Salt is also abundant on planets such as Mars and Europa, a satellite of Jupiter (Kargel 1998). Moreover, salt crystals have been shown to play a protective role, stabilizing RNA molecules against thermal degradation (Maurel & Zaccai 2001; Tehei *et al.* 2002; Cornée *et al.* 2004; Vergne *et al.* 2006; El-Murr *et al.* 2012). These arguments give interest to study the effect of solar radiation on RNA in the presence of NaCl. At last, the study of the stability of RNAs in space allows us to simulate the primitive conditions of the RNA world when there was not the protective effect of Earth's atmosphere against irradiations.

Materials and methods

RNA

Starting from the ADHR obtained in the experiment of Meli *et al.* (2003), the ADHR1 variant (85 nucleotides) was produced by *in vitro* transcription of its DNA coding sequence, by the T7 RNA polymerase in presence of the four ribonucleotides and buffer (Fermentas) followed by DNase treatment and deproteinization. After ethanol precipitation, RNA was purified by electrophoresis on 10% denaturing polyacrylamide gel.

Samples of 30 µl solution containing 0.1 mg RNA, either alone or with NaCl (0.2–2 mg) were loaded on MgF_2 windows and vacuum-dried for exposed and not exposed samples to solar radiation outside the ISS. Other samples were also prepared for ground variable $T°$, ground constant $T°$ and ground −20 °C controls.

Characteristics of EXPOSE-R mission

The samples were taken aboard ISS from 10 March 2009 to 21 January 2011, that is, 683 days (Rabbow *et al.* 2014). A set of samples was exposed to the Sun (6) while a replicate of the sample was stored right below the exposed layer. The EXPOSE-R top surface was irradiated uniformly and received a total dose of 13.2×10^9 J m^{-2} with a wavelength range between 100 and 1 mm including a UV dose (100–400 nm) of 1.02×10^3 MJ m^{-2} (Beuselinck & Bavinchove 2011; Cottin *et al.* 2014, personal communication). About 77% of the dose reached to the samples below the MgF_2 windows.

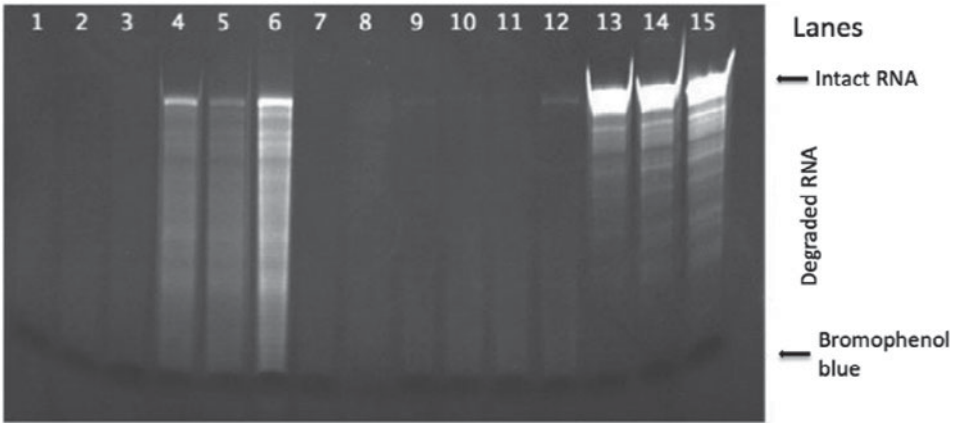
On the ground, controls were made with six samples kept at a constant temperature of 5 °C, a sample kept at −20 °C under atmospheric pressure was used as a 'stable reference' and six samples were submitted to temperature variations reproducing the cycles of the temperature during the flight. These controls were maintained under vacuum in the dark. The average temperature tray close to the sample temperature is of 19 °C, knowing that the temperature could reach two extremes equal to −27 and 46 °C. Although temperatures were higher than 0 °C for most of the EXPOSE-R mission, 285 freeze–thaw cycles were identified, including 11 freeze periods with a duration longer than the 90 min orbital period.

Recovery of samples

Samples were recovered in 100 µl H_2O; some insoluble aggregates were present in the exposed samples. Recovery of

Table 1. *Absorbance ratios,* A_{260}/A_{280} *measured with a NanoVue spectrometer*

Flight exposed	Flight not exposed	Ground variable (*T*°)	Ground constant (*T*°)	Ground −20 °C
NaCl ('mg/window')				
0 0.2 2	0 0.2 2	0 0.2 2	0 0.2 2	0 0.2 2
Absorbance ratio, 260/280				
1.89 1.93 1.90	2.05 2.04 2.06	2.08 2.07 2.11	2.06 2.08 2.10	2.03 2.03 2.05



Flight Exposed /Flight Not Exposed /Ground variable T°/Ground constant T°/Ground -20 °C

NaCl ("mg / window")

0 0.2 2 / 0 0.2 2 / 0 0.2 2 / 0 0.2 2 / 0 0.2 2

Fig. 2. Gel pattern of the 15 samples analysed by electrophoresis on denaturing polyacrylamide gel. Lanes 1–3: in-flight samples exposed to the Sun with 0, 0.2 and 2 mg/window of NaCl, respectively; Lanes 4–6: in-flight samples not exposed to the Sun with 0, 0.2 and 2 mg/window of NaCl, respectively; Lanes 7–9: samples on ground exposed to the same cycle of temperature as the in-flight sample with 0, 0.2 and 2 mg/window of NaCl, respectively; Lanes 10–12: samples on ground maintained at 5 °C with 0, 0.2 and 2 mg/window of NaCl, respectively; Lanes 13–15: reference samples on ground maintained at −20 °C with 0, 0.2 and 2 mg/window of NaCl, respectively.

each sample was quantified by absorbance measurement at 260 nm absorbance units (A_{260}) on a NanoVue spectrometer.

Integrity analysis of the samples by electrophoresis

RNA amounts of 0.07 A_{260} for each sample were preincubated at 45 °C in 50% formamide and loaded onto a denaturing 10% polyacrylamide gel for electrophoresis, pre-run for 1 h, and run at 450 V, 40 mA, 18 W, until the migration dye, bromophenol blue, reached the bottom of the gel. The running buffer was TBE (buffer: Tris base, boric acid and EDTA). RNAs were stained with ethidium bromide.

Results and discussion

The recovered windows with the samples exposed to the Sun aboard ISS were yellow coloured, whereas those not exposed remained white (Fig. 1). This colour was most probably due to a photosensitive volatile contaminant inside the tray. This contamination issue is further discussed in Demets *et al.* (2014).

The samples were characterized by the ratio of the absorbance at 260 over the absorbance at 280 nm (Table 1). At both wavelengths the absorption of the RNA only arised from the electronic transitions of the bases. The absorbance ratios of the samples exposed to the Sun slightly decreased. We cannot ascertain that it was only due to the presence of a contaminant. The absorption of the bases probably was slightly modified. For the in-flight samples not exposed to the Sun, for the sample on ground exposed to the same cycle of temperature as the in-flight samples and for the samples maintained at 5 °C, the fairly constant absorption ratio indicates that the bases still absorb UV radiation as in the reference samples maintained at −20 °C (Table 1). Thus, for the in-flight samples not exposed to the Sun and those on ground in the dark, under vacuum, the absorption of the bases did not show any sign of degradation as opposed to the samples exposed to the Sun during the flight. The fairly constant absorption ratio of all the samples, except the sample exposed to the Sun during the flight, shows that the bases were not affected during the experiment. The comparison between the in-flight samples, exposed to the Sun and not exposed, probably shows that the solar radiation degrades RNA bases. UV present in the solar spectral irradiations were absorbed by the bases of the RNA and can involve photo-induced processes.

The distribution in size of the various RNA samples was characterized by denaturing polyacrylamide gel

electrophoresis (Fig. 2). The samples number 1, 2 and 3 exposed to the Sun in flight showed no intact RNA bands. A weak trail indicates a large size distribution resulting from very strong damage to the RNA. Samples not exposed to the Sun in flight displayed intact RNA bands followed by well-specified trails due to large distributions of lower-sized products. Thus, the exposition to the solar radiation has a strong degradation effect on the size distribution of RNA.

On ground, the samples maintained at 5 °C or subjected to temperature cycles displayed very weak intact RNA bands, followed by weak trails. Large distributions, bushy in small size, indicate strong damaging.

The sample on ground maintained at −20 °C mainly presented strong intact RNA bands (lanes number 13, 14 and 15).

Staining by ethidium bromide results from interactions of the dye with the bases and no biological compound can interfere with such interaction (Le Pec & Paoletti 1966). Consequently, staining by ethidium bromide of the lower-sized components indicates that the bases were still present. Thus, staining reveals that bases of RNA were conserved even in small-sized products as shown by the conservation of the absorption ratio of the samples. One notices that the prebiotic synthesis of adenine was obtained thanks to UV bombardment (Oro 1961). The overall experiment demonstrated an important effect on the size of the RNA. We should mention that in 10% polyacrylamide gels the marker bromophenol blue comigrates with fragments of double stranded DNA of 20–45 nucleotide pairs (Molecular Cloning – Laboratory manual). Thus, electrophoresis did not reveal smaller nucleic acid fragments.

The presence of the higher content of crystallized NaCl in contact with RNA resulted in a very weak intact RNA band are shown in lanes 9 and 12. NaCl did not change the absorption ratio of the bases but induces a slight protective effect against the degradation of RNA. Thus, NaCl has protective effect on the ribose phosphate backbone.

The RNA molecules not exposed to the Sun mainly conserved the bases but were affected by a reduction of the size by degradation involving the ribose phosphate backbone. On ground the comparison between the sample at various temperatures and the sample conserved at −20 °C shows that the temperature has a strong effect on the size distribution process. This backbone is probably the most sensitive part of the molecule to the thermal agitation and can be cleaved.

The comparison between flight not exposed, and on ground at various temperatures indicates that the sample in flight was better preserved.

The humidity might lead to the degradation of the ground samples. In space, the environment is very dry and the exposed samples were subsequently damaged by UV, whereas non-exposed samples showed less degradation. From ground samples (at variable and constant temperatures), the degradation of RNA could be due to humidity during transportation and/or conditioning preparation as compared to the intact well-preserved ground samples maintained at −20 °C.

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References

Beuselinck, T. & Bavinchove, C.V. (2011). EXPOSE: environmental history by calculation: EXPOSE-R simulation results, REDSHIFT company. Report under ESA contract 4000102520.

Bossa, J.B., Duvernay, F., Theule, P., Borget, F., d'Hendecourt, L. & Chiavassa, T. (2009). Methylammonium methylcarbamate thermal formation in interstellar ice analogs: a glycine salt precursor in protostellar environments. *Astron. Astrophys.* **506**, 601–608.

Callahan, M.P., Smith, K.E., Cleaves, H.J. II, Ruzicka, J., Stern, J.C., Glavin, D.P., House, C.H. & Dworkin, J.P. (2011). Carbonaceous meteorites contain a wide range of extraterrestrial nucleobases. *Proc. Natl. Acad. Sci. USA* **108**(34), 13995–13998.

Cech, T.R. (1987). The chemistry of self-splicing RNA and RNA enzymes. *Science* **236**, 1532–1539.

Cornée, A., Vergne, J., Guyot, F., Goffe, B., Rouchy, J.M. et Maurel, M.C. (2004). Effet protecteur des cristaux de NaCl pour des macromolécules biologiques (ARN). Approche expérimentale. Résumés en français et en anglais, Atelier *Microbialithes et communautés microbiennes dans les systèmes sédimentaires*, SGF, ASF et PNEC, Paris, 6–9 septembre 2004, 31–34.

Cottin, *et al.* (2014). Personal communication.

Delan-Forino, C., Deforges, J., Benard, L., Sargueil, B., Maurel, M-C. & Torchet, C. (2014). Structural analyses of Avocado sunblotch viroid reveal differences in the folding of plus and minus RNA strands. *Viruses* **6**, 489–506.

Delsemme, A.H. (2000). 1999 kuiper prize lecture cometary origin of the biosphere. *Icarus* **146**, 313–325.

Demets, R. *et al.* (2014). Window contamination on EXPOSE-R. *Int. J. Astrobiol*, volume **13**, in press.

Dundas, I. (1998). Was the environment for primordial life hypersaline? *Extremophiles* **2**, 375–377.

El-Murr, N., Maurel, M-C., Rihova, M., Vergne, J., Hervé, G., Kato, M. & Kawamura, K. (2012). Behavior of a hammerhead ribozyme in aqueous solution at medium to high temperatures. *Naturwissenschaften* **9**, 731–738.

Eschenmoser, A. & Loewenthal, E. (1992). Chemistry of potentially prebiological natural products. *Chem. Soc. Rev.* **21**, 1–16.

Gesteland, R.F. & Atkins, J.F. (1993). *The RNA World*. Cold Spring Harbor Laboratory Press, New York.

Gilbert, W. 1986. Origin of life: the RNA world. *Nature* **319**, 618.

Guerrier-Tanaka, C., Gardiner, K., Marsh, T., Pace, N. & Altman, S. (1983). The RNA moiety of ribonuclease P is the catalytic subunit of the enzyme. *Cell* **35**, 849–857.

Hui-Bon-Hoa, G., Kaddour, H., Vergne, J., Kruglik, S.G. & Maurel, M-C. (2014). Raman characterization of Avocado Sunblotch viroid and its response to external perturbations and self-cleavage. *BMC Biophys.* **7**, 2–15.

Kargel, J.S. (1998). The salt of Europa. *Science* **280**, 1211–1212.

Knauth, L.P. (1998). Salinity history of the Earth's early ocean. *Nature* **395**, 554–555.

Kobayashi, K., Kaneko, T., Saito, T. & Oshima, T. (1998). Amino acids formation in gas mixtures by high energy particle irradiation. *Orig. Life Evol. Biosph.* **28**, 155–165.

Le Pec, J.B. & Paoletti, C. (1966). A new fluorometric method for RNA and DNA determination. *Anal. Biochem.* **17**, 100–107.

Maurel, M-C. (1992). RNA in evolution: a review. *J. Evol. Biol* **5**, 173–188.

Maurel, M-C. & Décout, J-L. (1999). Origins of life: molecular foundations and new approaches. *Tetrahedron* **55**, 3141–3182.

Maurel, M-C. & Haenni, A-L. (2005). The RNA world: hypothesis, facts and experimental results. In *Lectures in Astrobiology*. eds. Berlin: Springer-Verlag, pp. 571–594.

Maurel, M-C. & Ninio, J. (1987). Catalysis by a prebiotic nucleotide analog of histidine. *Biochimie* **69**, 551–553.

Maurel, M-C. & Zaccai, G. (2001). Why biologists should support the exploration of Mars. *Bioessays* **23**, 977–978.

Meinert, C., Filippi, J.-J., Marcellus, P.d., d'Hendecourt, L.L.S. & Meierhenrich, U.J. (2012). N-(2-Aminoethyl)glycine and amino acids from interstellar ice analogues. *ChemPlusChem* **77**, 186–191.

Meli, M., Vergne, J., Décout, J-L. & Maurel, M-C. (2002). Adenine-aptamer complexes. A bipartite RNA site which binds the adenine nucleic base. *J. Biol. Chem*. **277(3)**, 2104–2111.

Meli, M., Vergne, J. & Maurel, M-C. (2003). *In vitro* selection of adenine-dependent hairpin ribozymes. *J. Biol. Chem*. **278**, 9835–9842.

Miller, S.L. (1953). Production of amino acids under possible primitive Earth conditions. *Science* **117**(3046), 528–529.

Miyakawa, S., Murasawa, K., Kobayashi, K. & Sawaoka, A.B. (1999). Cytosine and uracil synthesis by quenching with high-temperature plasma. *J. Am. Chem. Soc*. **121**, 8144–8145.

Oro, J. (1961). Mechanism of synthesis of adenine from hydrogen cyanide under possible primitive Earth conditions. *Nature* **191**, 1193–1194.

Oro, J., Mills, T. & Lazcano, A. (1992). Comets and the formation of the biochemical compounds on the primitive Earth. *Orig. Life Evol. Biosph*. **21**, 267–277.

Powner, M.W., Gerland, B. & Sutherland, J.D. (2009). Synthesis of activated pyrimidine ribonucleotides in prebiotically plausible conditions. *Nature* **459**, 239–242.

Rabbow, E. *et al.* (2014). The astrobiological mission EXPOSE-R on board of the International Space Station. *Int. J. Astrobiol*, volume **13**, in press.

Saiagh, K., Cloix, M., Fray, N. & Cottin, H. (2014). VUV and Mid-UV photoabsorption cross sections of thin films of adenine: application on its photochemistry in the Solar System. *Planet. Space Sci*. **90**, 90–99.

Sutherland, J.D. & Whitfield, J.N. (1997). Prebiotic chemistry: a bioorganic perspective. *Tetrahedron* **53**, 11595–11626.

Tehei, M., Franzetti, B., Maurel, M-C., Vergne, J., Hountondji, C. & Zaccai, G. (2002). The search for traces of life: the protective effect of salt on biological macromolecules. *Extremophiles* **6**, 427–430.

Vergne, J., Dumas, L., Décout, J-L. & Maurel, M-C. (2000). Possible prebiotic catalysts formed from adenine and aldehyde. *Planet. Space Sci*. **48**, 1139–1142.

Vergne, J., Cognet, J.A., Szathmáry, E. & Maurel, M-C. (2006). *In vitro* selection of halo-thermophilic RNA reveals two families of resistant RNA. *Gene* **371**, 182–193.