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The AMINO experiment: exposure of amino acids in the EXPOSE-R experiment on the International Space Station and in laboratory

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Abstract: In order to confirm the results of previous experiments concerning the chemical behaviour of organic molecules in the space environment, organic molecules (amino acids and a dipeptide) in pure form and embedded in meteorite powder were exposed in the AMINO experiment in the EXPOSE-R facility onboard the International Space Station. After exposure to space conditions for 24 months (2843 h of irradiation), the samples were returned to the Earth and analysed in the laboratory for reactions caused by solar ultraviolet (UV) and other electromagnetic radiation. Laboratory UV exposure was carried out in parallel in the Cologne DLR Center (Deutsches Zentrum für Luft und Raumfahrt). The molecules were extracted from the sample holder and then (1) derivatized by silylation and analysed by gas chromatography coupled to a mass spectrometer (GC–MS) in order to quantify the rate of degradation of the compounds and (2) analysed by high-resolution mass spectrometry (HRMS) in order to understand the chemical reactions that occurred. The GC–MS results confirm that resistance to irradiation is a function of the chemical nature of the exposed molecules and of the wavelengths of the UV light. They also confirm the protective effect of a coating of meteorite powder. The most altered compounds were the dipeptides and aspartic acid while the most robust were compounds with a hydrocarbon chain. The MS analyses document the products of reactions, such as decarboxylation and decarbonylation of aspartic acid, taking place after UV exposure. Given the universality of chemistry in space, our results have a broader implication for the fate of organic molecules that seeded the planets as soon as they became habitable as well as for the effects of UV radiation on exposed molecules at the surface of Mars, for example.

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Key words: amino acid, GC–MS analysis, HRMS analysis, irradiation, International Space Station, low-Earth orbit, photochemistry, VUV.

Introduction

Organic molecules synthesized in space and delivered to the Earth via carbonaceous meteorites (Cronin & Chang 1993; Cooper *et al.* 2001; Botta & Bada 2002; Sephton 2002; Martins & Sephton 2009; Callahan *et al.* 2011; Martins 2011; Pizzarello *et al.* 2012), micrometeorites (Brinton *et al.* 1998; Clemett *et al.* 1998; Glavin *et al.* 2004; Matrajt *et al.* 2004) and comets (Crovisier & Bockelee-Morvan 1999; Mumma *et al.* 2003; Sandford *et al.* 2006; Elsila *et al.* 2009; Martins *et al.* 2013) were an important contribution to the origin of life on the Earth. It is therefore important to understand the impact of space conditions on the organic molecules during space travel and, in particular, the role of photochemistry on the chemical evolution of organic matter.

Experiment facilities in space provide a unique test bed for astrobiological studies in low-Earth orbit on satellites (Foton capsules or triple cube) or on space stations (Mir or International Space Station (ISS)) and a number of experiments have already been carried out using these platforms.

The experiments Biopan I and Biopan II (Barbier *et al.* 1998, 2001) and UVolution (Guan *et al.* 2010; Stalport *et al.* 2010) were exposed on Foton capsules. The experiment SEVO used a triple-cube satellite (Bramall *et al.* 2012; Mattioda *et al.* 2012), whereas the experiment Perseus took place on the Mir station and the experiments PROCESS, AMINO and ORGANIC on Expose-E or Expose-R onboard the ISS (Boillot *et al.* 2002; Bertrand *et al.* 2012; Cottin *et al.* 2012; Noblet *et al.* 2012). Different organic compounds were thus exposed, including amino acids and their derivatives, peptides, nucleic bases,

polymers (polyoxymethyl, polyacrylonitrile, polycyclic aromatic hydrocarbons PAH, tholins, etc.), acids (phthalic acid, mellitic acid, trimesic acid and the amino acids: glutamic acid, aspartic acid, 2-aminoisobutyric acid and 2-aminobutyric acid), hexamethylenetetramine, aromatic compounds and porphyrins. The compounds were exposed in film or associated with different mineral surfaces such as meteorite powder, clays, martian soil analogue or basalts.

Some of these experiments were carried out in parallel in laboratory facilities (Bertrand *et al.* 2012; Noblet *et al.* 2012). Although it is not possible to accurately reproduce space conditions in the laboratory, ground experiments are an important complementary aid in the interpretation and discussion of the results obtained in space.

In this study, we present the results obtained after a 2-year exposure of organic molecules (amino acids and dipeptide) to space conditions in low-Earth orbit onboard the ISS on the AMINO experiment in the EXPOSE-R facility on the one hand, and to ultraviolet (UV) light in the DLR laboratory on the other hand. All amino acids used in this study have already been detected in Murchison meteorite and in the other carbonaceous meteorites (Cronin & Pizzarello 1983; Pizzarello & Shock 2010), such as in primitive CR meteorites (Martins *et al.* 2007; Pizzarello *et al.* 2012) and have already been exposed in the previous experiment PROCESS in the EXPOSE-E facility (Bertrand *et al.* 2012). The amino acids and dipeptides were chosen for their astrobiological relevance and because of the diversity of their functional groups, an important factor for the formation of macromolecules, such as proteins. The amino acids were chosen on the basis of the alkyl chain length (glycine, alanine and 2-amino butyric acid), the substitution on the α-carbon (alanine and 2-aminoisobutyric acid) or β-carbon (valine), the stability of the functional group (aspartic acid) or the amide bond (for the dipeptide). The objective of this new experiment was to verify previously obtained results and to further our understanding of the chemical reactions involved during exposure in space. Moreover, the aim of comparing reactions to exposure in space with reactions obtained in laboratory conditions was to provide information on the effects of UV radiation and of extraterrestrial electromagnetic radiation.

These results will help understand the effects of the photochemistry on the organic molecules, their preservation in the regolith of Mars and the potential for *in situ* analysis, for instance with Curiosity or the ExoMars 2018 rover. Although the majority of the organic inventory in interplanetary dust particles, meteorites and comets consists of refractory, low-molecular weight aromatic organic molecules, other types of less refractory organic molecules, including amino acids, are also present (Cody *et al.* 2011; Alexander *et al.* 2012; Sephton 2013). Delivered to the surface of Mars, the variety of molecules respond differentially to the physicochemical processes affecting the surface layers: degradation from UV and cosmic radiation, as well as oxidation (Kminek & Bada 2006; Atri & Melott 2014; Hassler *et al.* 2014). The results of our experiments help in understanding the effects of space radiation on a portion of the labile component of meteoritic organic compounds.

Materials and methods

The compounds exposed were the proteic amino acids glycine (Gly), alanine (Ala), valine (Val) and aspartic acid (Asp), the non-proteic amino acids, 2-amino isobutyric acid (AIB) and 2-amino butyric acid (ABA) and the dipeptide dileucine (Leu_2). To exclude any amino acid contamination, the proteic amino acids were used in the D-form (terrestrial proteic amino acids are in the L-form). The amino acids glycine, D-alanine, D-valine, D-aspartic acid, 2-amino isobutyric acid, 2-amino butyric acid and norvaline (used as internal standard) were obtained from Sigma and Aldrich and the dipeptide dileucine from Bachem (purity >98% for all amino acids and the peptide). The methanol used was of 'Plus HPLC' quality purchased from Carlo Erba. The water was of milliQ quality from Millipore. The Allende meteorite obtained from 'meteorite labels', was powdered, washed under stirring with methanol/water (50/50) during 12 h, and then with water during 2 h and finally freeze-dried.

The molecules were exposed both in dry form and mixed with the meteoritic powder to study the effect of mineral surfaces as protection against photolysis in space. The molecules (20 µg of each compound for the molecule mixture samples (a total of 140 µg) and 187.5 µg for the aspartic acid samples), associated with or without meteorite powder (50 mg), were deposited as dry films behind MgF_2 windows, which are transparent to UV and near vacuum ultraviolet (VUV).

Four sets of samples were prepared: (1) a mixture of molecules that were exposed free in triplicate; (2) molecules mixed with meteorite powder and exposed in triplicate; (3) aspartic acid exposed free in duplicate; and (4) aspartic acid mixed with meteorite powder and exposed in duplicate. Some samples were prepared with aspartic acid on its own in order to follow more easily the chemical reactions involved during exposure in the space environment.

A total of five batches of samples were prepared. Two batches were included in the AMINO experiment on EXPOSE-R onboard the ISS, one exposed to solar extraterrestrial UV radiation behind MgF_2 windows, i.e. to photons above 115 nm, one kept in the dark. Three batches were prepared for the DLR laboratory. The first was exposed to a UV lamp emitting photons of 200–450 nm at constant pressure and the other two were kept in the dark with either temperature cycling at constant pressure or with constant temperature and pressure. The temperature cycling in the laboratory reproduces the temperature conditions occurring in space.

AMINO experiment, EXPOSE-R facility onboard the ISS

The Expose-R experiment was transported to the ISS on 26 November 2008 and returned to Earth on 9 March 2011. The samples of the AMINO experiment were installed outside the ISS. One batch of samples was exposed to the solar light for 682 days between 10 March 2009 and 21 January 2011. The second batch stayed in the dark for the duration. The total solar constant hours of exposure were estimated by calculation at about 2843 h. The UV and VUV radiation received by the

Table 1. *Dose (in MJ m^{-2}) recorded in the UV range on the ISS and in the DLR laboratory*

Dose (MJ m^{-2})	UVC (100–280 nm)	UVB (280–315 nm)	UVA (315–400 nm)	UV (100–400 nm)	UV (200–400 nm)
ISS	59.4	159	825	1043.4	
DLR					1128.1

exposed samples were above 115 nm since the MgF_2 windows stop photons below this wavelength value. The fluence was calculated at around 1.043×10^3 MJ m^{-2} from 100 to 400 nm (Table 1). More details about AMINO hardware and the whole experiment configuration can be found in Cottin *et al.* (this issue).

The DLR experimental setup

Three batches of samples were prepared for exposure in the DLR facility. Two batches were exposed to the same temperature cycles according to the flight data received by telemetry from EXPOSE-R during the mission. The experiment in the DLR facility began on 14 December 2009 and ended on 17 October 2011.

One batch was exposed to UV radiation with a UV lamp (range 200–400 nm, with irradiance of 1370.2 W m^{-2}), while the second was kept in the dark. The sample batch exposed to the UV radiation received a dose of 1.128×10^3 MJ m^{-2} corresponding to that received by the samples during the EXPOSE-R mission for the UV range 200–400 nm. The pressure was maintained at 1.7×10^{-3} Pa for both the UV-exposed and the non-exposed batches. A third batch was kept in the dark at a constant 5 °C temperature and 1.7×10^{-3} Pa pressure. The ground exposure to UV radiation in the DLR facility began on 18 July 2011 and ended on 19 August 2011.

Energy received by the samples

The energy received by the samples was similar in the DLR and the ISS experiments (1128.1 and 1043.4 MJ m^{-2}, respectively); the difference between the emitted doses being less than 10%. However, the nature of the emitted radiation was different. In space, UV radiation ranged from 100 to 400 nm, while in the DLR facility, the UV lamp emitted from 200 to 400 nm. In both experiments, the exposure was not continuous. In space, this was due to the variable position of the sun, while at the DLR, the samples were not continuously irradiated in order to avoid temperatures in excess of 40 °C.

Sample preparation

The Allende meteorite powder was carefully rinsed by successive sedimentation steps in distilled water before use. The sedimentation steps were performed to select the finest powder. The meteorite powder was mixed with 400 ml of water in a 500 ml test tube, the magnetic stirring was stopped and after 30 s the supernatant was recovered. This procedure was repeated several times. The supernatants were combined, dried and thus the finest powder was used for the experiments. The absence of amino acids and di-peptides from the meteorite was verified by analysis of several extracts from a single meteorite sample by GC–MS with the same extraction and analysis operating methods and procedures as those used for analysing the exposed samples.

Preparation of the aspartic acid samples

For pure amino acid samples, 75 µl of a 2.5 mg ml^{-1} stock solution of D-aspartic acid in water, corresponding to 187.5 µg, were deposited on the MgF_2 windows with a pipette and then slowly dried at room temperature. For each amino acid/meteorite powder sample, 75 µl of the aspartic acid stock solution were mixed with 50 mg of meteorite powder in a microtube and deposited on the MgF_2 windows and slowly dried at room temperature.

Preparation of all other samples

Stock solutions of glycine, D-alanine, D-valine, D-aspartic acid, 2-amino isobutyric acid and 2-amino butyric acid were prepared in water at a final concentration of 0.267 mg ml^{-1} (2 ml of glycine, of D-alanine, of D-valine, of 2-amino-isobutyric acid and of 2-aminobutyric acid at 2 mg ml^{-1} were mixed with 1.6 ml of D-aspartic acid at 2.5 mg ml^{-1}, 2.67 ml of dileucine at 1.5 mg ml^{-1} and with 0.73 ml of milliQ water). The compound concentration was chosen in function of its water solubility.

For amino acids and dipeptide samples, 75 µl (20 µg) of the stock solution were deposited on the MgF_2 windows and slowly dried at room temperature.

For samples mixed with meteorite powder, 75 µl of the stock solution (20 µg) were mixed with 50 mg of meteorite powder and deposited as described above.

Extraction, sample preparation

After the exposure experiments, the samples, shielded against light and kept in an inert atmosphere (under N_2 or vacuum), were recovered in the laboratory by washing the MgF_2 windows three times with 100 µl of a methanol/water (50/50: v/v) solution. For the samples in pure form, the solvent was evaporated under vacuum and the remaining material solubilized in 500 µl of water. For the samples with meteorite powder, the samples were centrifuged and the supernatant collected. The meteorite powder was washed three times with a methanol/water solution. The supernatants were evaporated under vacuum and the remaining material dissolved in 500 µl of water. For each analysis, 100 µl of sample was collected and functionalized. Two or three derivatizations of each sample were run with a triplicate injection.

Derivatization methods

Silylation was used in order to analyse the amino acids and the dipeptide by GC–MS and increase the sensitivity. The carboxylic and amino groups of the compounds were silylated.

Table 2. *Column 1: t_R, retention time of amino acids; column 2: molecular weight of amino acids; column 3: molecular mass of silylated derivative; column 4: ion fragments used for the quantification*

	t_R (min)	Amino acid (MW)	TBDMS derivatives MW	Ions fragments $[M-159]^+$ and $[M-57]^+$	
Ala	9.6	89.1	317.1	158	260
Gly	10.5	75.1	303.1	147[a]	246
AIB	10.8	103.1	331.1	172	274
ABA	11.2	103.1	331.1	172	274
Val	12.2	117.2	345.2	186	288
Asp	22.8	133.1	475.1[b]	302[a]	418
Leu_2	26.7	244.3	472.3	200[a]	415

[a] Other fragments than $[M-159]^+$.
[b] Tri-silylated compound.

10 µl of an internal standard (I.S., norvaline) at 0.1 g ml^{-1} were added to 100 µl of each sample and the mixture was dried by speed-vacuum. After total solvent evaporation, the compounds were derivatized by silylation with 20 µl of MTBSTFA (*N-tert*-butyldimethylsilyl-*N*-methyltrifluoroacetamide) containing 1% of TBDMSCl (*tert*-butyldimethylchlorosilane) (Fluka) and 60 µl of acetonitrile (Merck, 99.8% purity), shaken by vortex and sonication for 15 min, and then placed at 60 °C for 1 h (see the Table 2 for the molecular weight of compounds and Figure 3 of Bertrand *et al.* 2012 for the derivatization reaction).

GC–MS analysis

After derivatization, 1 µl of each solution was injected into an Agilent 6890 gas chromatograph (GC) equipped with a CP-Sil 19 CB fused-silica capillary column from Varian (length: 30 m, I.D.: 0.25 mm, film thickness: 0.2 µm) coupled to an Agilent 5973 mass spectrometer (MS) as the detector (electronic ionization at 70 eV). GC–MS chromatogram acquisition and data processing were performed with the Agilent MSD ChemStation software. Helium was used as the carrier gas (inlet pressure: 178 kPa) and splitless injection mode was used. A constant flow mode of 1.5 ml min^{-1} was selected. The injector temperature was set to 250 °C, the MS source to 150 °C, and the MS quadrupole to 230 °C. With the CP-Sil 19 CB column, the oven temperature was set to 125 °C for 5 min and then programmed to reach 250 °C at a rate of 5 °C min^{-1}. Identification and quantification of each compound was performed by comparing its chromatographic retention time, integrated chromatogram peak area (Fig. 1) and their mass spectrum in the sample with those of the corresponding standard.

The amino acid tert-butyldimethylsilyl derivatives were characterized by a mass spectrum in which the most intense spectral peak represented the $[M-57]^+$ fragments, corresponding to the molecular ions that have lost a tert-butyl group (57 m/z) (Casal *et al.* 2004; Schummer *et al.* 2009) and the $[M-159]^+$ fragments. Each sample was analysed by MS in both the total ion chromatogram (TIC) and selected ion monitoring

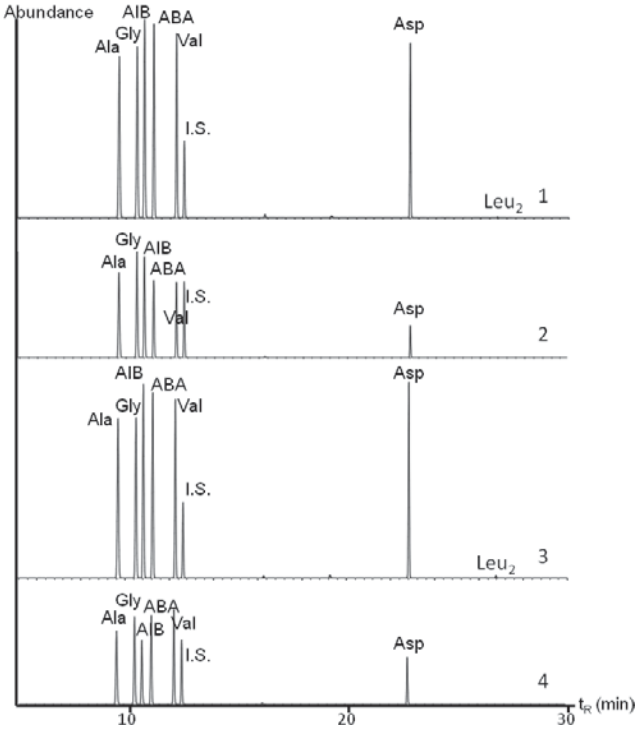

Fig. 1. Chromatograms in SIM mode of silylated extracts analysed on the CP-Sil 19 CB column of (1) a control of the DLR (2) a sample exposed of the DLR (3) a flight dark control of the ISS and (4) a sample exposed on the ISS.

(SIM) acquisition modes. In a TIC, the *Y*-axis corresponds to the sum of all detected ion currents for each scan, while the SIM mode is a data acquisition technique in which only the currents of a small range of selected ion fragments are monitored in order to maximize the sensitivity. The SIM mode with the $[M-57]^+$ and $[M-159]^+$ fragments was chosen to quantify the amino acids (see Table 2).

HRMS analysis

High-resolution mass spectrometry (HRMS) was carried out by injecting 1 µl of each solution (dilution 1/10 in MeOH/H_2O 50/50) in flow injection analysis into a maXis ultra-high-resolution Q-TOF mass spectrometer from Bruker Daltonics equipped with an electrospray ion source. Acquisitions were carried out in positive ion mode over a 50–1400 m/z range. The drying gas flow and temperature were set at 6 l min^{-1} and 200 °C, respectively. External calibration was performed with ESI-L Low Concentration Tuning Mix (Agilent Technologies). ESI–HRMS spectra were processed using DataAnalysis 4.1 software (Bruker Daltonics).

Molecular formulae were generated using the Smart Formula module in DataAnalysis software with a mass tolerance of 2 ppm.

Results

The results obtained in the DLR laboratory and in space on the ISS are shown in Table 3 and Figs. 2 and 3.

Table 3. *Rates of compounds survival measured after exposure in the DLR laboratory and on the ISS for exposed samples in comparison with samples maintained in the dark. The samples were in free form or embedded in meteorite powder*

	DLR		ISS	
	In free form (%)	With meteorite (%)	In free form (%)	With meteorite (%)
Ala	45 ± 4	64 ± 14	58 ± 15	68 ± 5
Gly	51 ± 2	53 ± 7	72 ± 9	74 ± 2
AIB	63 ± 15	93 ± 20	62 ± 13	78 ± 5
ABA	33 ± 2	70 ± 15	59 ± 16	67 ± 7
Val	32 ± 1	76 ± 12	62 ± 19	69 ± 7
Asp	18 ± 12	48 ± 30	24 ± 19	70 ± 12
Leu_2	0	17 ± 37	0	8 ± 22

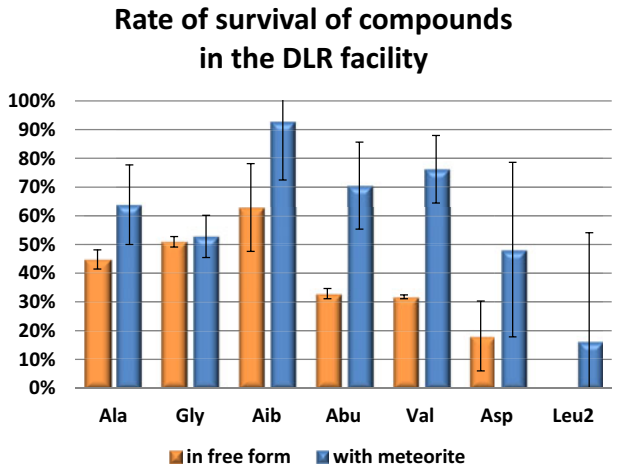

Fig. 2. Rate of remaining compounds measured after exposure in samples exposed to UV in comparison with samples maintained in the dark with or without meteorite powder in DLR laboratory.

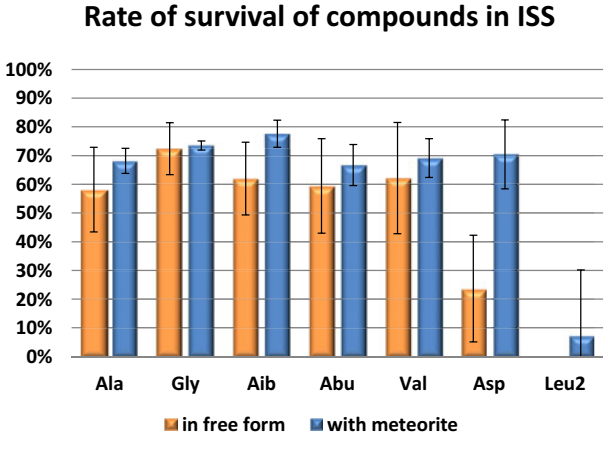

Fig. 3. Rate of survival of compound measured after exposure in samples exposed to UV in comparison with samples maintained in the dark with or without meteorite powder in the AMINO experiment on ISS.

Table 3 shows the results obtained for all samples exposed in free form and with meteorite powder, in the DLR facility and on the ISS. The percentages in the table are the quotient between the mass of remaining compounds in exposed samples and the mass of remaining compounds in control samples (samples maintained in the dark in the same conditions of temperature and pressure as the exposed samples). The same calculation method was used for both experiments (ISS and DLR laboratory).

The data given in Table 3 are the medians of the values obtained in SIM mode for all analyses of three amino acid mixture samples on the ISS and in the DLR laboratory.

GC–MS results

Results of DLR experiment

Figure 2 shows the range of degradation of the amino acids measured in samples irradiated in the free form or embedded in meteorite powder with a UV range of 200–400 nm. Each set of samples was compared to the same batches of controls (samples in the dark with temperature cycling).

Amino acids exposed in the free form were more degraded than those exposed with meteorite powder. The survival rates were from 0 to 63% for those in the free form and from 17 to 93%, depending on the amino acid mixed with meteorite powder. The most stable amino acids were amino isobutyric acid with 93% exposed with meteorite and 63% without, followed by glycine (51 and 53%, respectively) and alanine (45 and 64%, respectively). The least resistant compounds were dileucine and aspartic acid. The survival rates of the molecules exposed with and without meteorite powder differ widely from one molecule to another. The protective effect of the meteorite powder was indeed very significant for amino butyric acid and valine since the survival rate is 30% higher with meteorite powder than without it. The protective effect was more moderate for alanine, the amino isobutyric and aspartic acids, and dileucine but was not demonstrated for glycine.

The variation between the same set of samples was more significant in the sample exposed with meteorite powder than those exposed in free form since the error bars are higher for all amino acids when they were associated with meteorite powder. This difference could be due to difficulties in recovering and extracting the sample when it is mixed with meteorite powder. The variation of the results does not allow a firm conclusion for all the compounds but demonstrates a clear trend.

Results of the Amino experiment onboard ISS

Figure 3 shows the range of degradation of the amino acids measured in samples exposed on the ISS in the free form and with meteoritic powder. Each batch of samples was compared to the same batches of flight dark controls.

The amino acids, alanine, glycine, amino isobutyric acid, amino butyric acid and valine were quite resistant to space conditions and to VUV and UV radiation since more than 59% of the compounds were recovered. The presence of the meteorite powder moderately increases the rate of compound survival for all amino acid and in the case of aspartic acid,

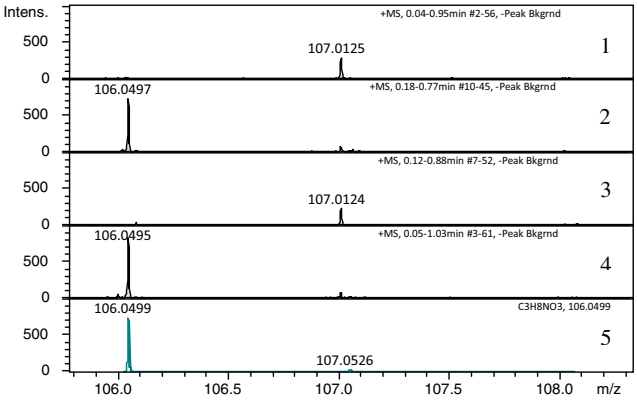

Fig. 4. Spectra of decarbonylation of aspartic acid. (1) Sample maintained in the dark at the DLR; (2) sample exposed to UV light at the DLR; (3) sample maintained in the dark on the ISS; (4) sample exposed to sun on the ISS and (5) theoretical spectrum of $C_3H_8NO_3$.

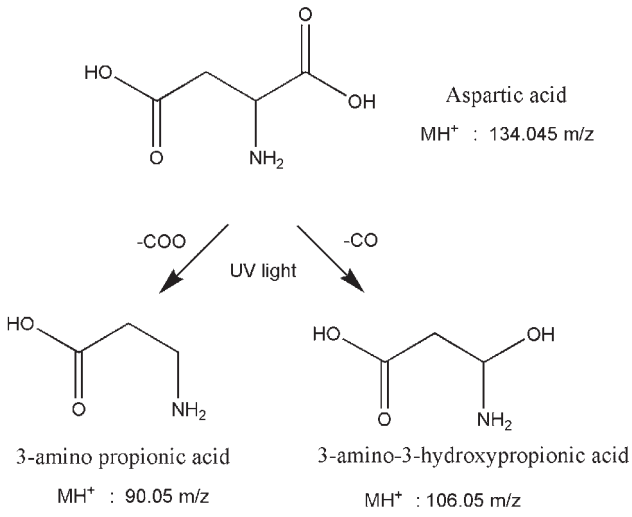

Fig. 5. Proposition of the aspartic acid photoalteration processes: decarboxylation of aspartic acid into 3-aminopropionic acid (β-alanine) and decarbonylation of aspartic acid into 3-amino-3-hydroxypropionic acid.

the increase in survival rate was dramatic being 45% higher with meteorite powder than without, thus bringing its survival rate up to the value of the other amino acids. Dileucine is entirely degraded when it is exposed in free form but is still present when associated with meteorite powder. In this experiment, the variation between the same set of samples is more significant for the samples exposed in the free form.

HRMS results

The samples with aspartic acid in the free form exposed in the DLR laboratory and on the ISS were analysed by HRMS to obtain more information about the photochemical reactions. The blank samples (maintained in the dark) were compared to those exposed to UV.

In the spectra, the peak at 134.0453 m/z corresponds to the exact mass of $C_4H_8NO_4^+$, the MH^+ form of aspartic acid. It is present in all samples, confirming the presence and the resistance to UV of this amino acid. Others peaks were only identified in irradiated samples: a peak at 90.0555 m/z corresponding to the decarboxylation of aspartic acid (loss of CO_2) and a peak at 106.0499 m/z corresponding to aspartic acid decarbonylation (loss of CO). Figure 4 shows the spectra of four samples and a theoretical spectrum: the first is a blank DLR sample, the second an exposed DLR sample, the third a blank ISS sample and the fourth an exposed ISS sample. The fifth is a theoretical spectrum of $C_3H_8NO_3^+$ ion with an isotopic peak at 106.0499 m/z, corresponding to the MH^+ form of aspartic acid with a loss of CO.

Figure 4 shows that the ion at 106.05 m/z is only present in the irradiated samples, demonstrating that the decarbonylation of aspartic acid was produced by UV exposure.

Similar spectra were obtained with the mass of 90.05, corresponding to decarboxylation of the aspartic acid.

Although some chemical reactions took place in samples not exposed to UV light, the molecular signatures of decarbonylation and decarboxylation were only identified in the exposed samples. Thus, the HRMS analyses demonstrate that the decarbonylation and the decarboxylation were chemical reactions caused by the UV exposure.

The VUV radiation absorbed by organic molecules may initiate luminescence, photodestruction and photoionization processes which may lead to damage of molecules. The mean reaction of the UV radiation on amino acids described in the literature is the decarboxylation (Poupko *et al.* 1973; Ehrenfreund *et al.* 2001; Takano *et al.* 2004; Scappini *et al.* 2007). In most of the cases, the UV radiation on molecules leads to radicals even if the mechanism occurred is different in water than on dry films. As demonstrated by Takano (Takano *et al.* 2004), photoalteration processes produce secondary amino acid products via decomposition of the α-carboxylic group. Aspartic acid is thus converted to β-alanine by UV irradiation. In this experiment, α-decarboxylation and α-decarbonylation photolysis of the dicarboxylic amino acid appear to have taken place as suggested in Fig. 5.

The analyses of the samples exposed on the ISS were compared to those of the samples exposed in DLR facility. No notable additional reaction was identified in the analyses, meaning that extraterrestrial radiation caused the same kinds of reactions as UV radiation.

Discussion

The GC–MS results of the ISS and DLR experiments described here show that organic molecules used in this experiment generally resist UV exposure both in space and in simulated space conditions in the laboratory.

Indeed, except for dileucine, all compounds were recovered after UV-exposure. In the DLR experiments, the ratios of remaining compounds in free form (without meteorite powder) were generally lower than in the ISS experiment. This may be explained by the UV-exposure conditions at the DLR facility.

Since the UV lamp used in the DLR exposure does not exactly reproduce the broad UV radiation produced by the sun, exposure in the laboratory was in a narrower UV emission spectrum. Also, samples were UV-exposed for a shorter time period than on the ISS. Without meteorite powder, dileucine and aspartic acid were the most affected compounds as previously described (Bertrand *et al*. 2012).

In the presence of meteorite powder, the behaviour of the products UV exposed in the laboratory or in space was more similar. Except for dileucine, more than 48% of the compounds were recovered. Dileucine somewhat resisted destruction when embedded in meteorite powder. Depending on the samples, between 5 and 54% was recovered, the large variation being explained by the sensitivity of dileucine to irradiation and the degree of destruction depending on the depth of burial by meteorite powder.

The HRMS analyses, by identifying reaction products, allow for the elucidation of the chemical reactions that occur during the photochemical process leading to the amino acid destruction. Two reactions were identified: decarbonylation and decarboxylation. These chemical reactions were only present in the exposed samples (on the ISS and in the DLR facility), demonstrating that they were caused by UV exposure and that solar extraterrestrial UV radiation at wavelengths higher than 110 nm caused the same kinds of reactions as UV at wavelengths higher than 200 nm, as produced by a solar simulator.

No further chemical reaction was identified in the samples maintained in the dark in the ISS experiment compared to the blank samples from DLR. This means that cosmic and other extraterrestrial radiation was too weak to cause apparent modification. The total dose of cosmic radiation during the mission ranged from 225 to 320 mGY, values that were very low (Berger *et al.* this issue) and thus negligible.

In comparison with previous experiments (Boillot *et al.* 2002; Bertrand *et al*. 2012), the GC–MS results of this experiment confirm results previously obtained. In all experiments, in the DLR facility and on the ISS, in the EXPOSE-E and the EXPOSE-R experiments, all compounds were recovered after exposure except for dileucine. The most altered compounds were aspartic acid and dileucine and covering by meteorite powder appeared to have a protective effect for all compounds in all experiments.

Despite a much larger exposure time in the EXPOSE-R experiment than in the EXPOSE-E's (2843 h for AMINO compared to 1958 h for PROCESS), the rate of compound survival was not, on average, significantly lower. However, the large error bars in the rate of compound degradation preclude more precise interpretation, the results depending on each sample. The preparation method using a pipette to deposit samples leads to inhomogeneity of the sample thickness and thus of the effects of UV-exposure between samples. This inter sample variation could be greatly reduced using another method to deposit more homogeneous layers on the MgF_2 Windows. For example, samples prepared by sublimation deposition would to reduce the differences between a same set of samples and improve the interpretation and the understanding of the results. However, the disadvantage with this approach is that it cannot be used to perform compound mixture deposits.

This experiment confirms that dicarboxylic acids, such as aspartic acid, are less resistant to exposure than amino acids with a hydrocarbon chain, as previously demonstrated with aspartic, trimesic, mellitic and phthalic acids (Stalport *et al.* 2010; Bertrand *et al*. 2012; Noblet *et al*. 2012). However, when aspartic acid is associated with meteorite powder, its behaviour is similar to that of other amino acids.

The dileucine dipeptide is the most sensitive compound. It is the least resistant to irradiation since it is totally destroyed when it is not associated with meteorite powder.

Of astrobiological importance, the results show notable differences between blank and exposed samples on both the ISS and in the laboratory exposure. This confirms importance of electromagnetic radiation, such as UV, on the fate of organic molecules and on their subsequent reactivity with the mineral matter, thus influencing the chemical evolution that led to living organisms on Earth and on Mars (Zent *et al.* 1994; Flynn 1996). On Mars, most suggested oxidants, including H_2O_2, are expected to decompose rapidly under martian UV and destroy organic molecules (Quinn & Zent 1999; ten Kate *et al.* 2006; Shkrob *et al.* 2010; Dartnell *et al*. 2012; Quinn *et al*. 2013). The protection of a layer of meteorite powder indicates that organic compounds could occur on Mars, although geological time and depth of protection are important factors to take into account.

Conclusions

The amino acids glycine, D-alanine, D-valine, D-aspartic acid, amino isobutyric acid, 2-amino butyric acid and the dipeptide dileucine were exposed to space conditions on the ISS and in the DLR laboratory, in pure form and embedded in meteorite powder.

The compounds were irradiated and received a total of 1128.1 MJ m^{-2} in the range between 200 and 400 nm in the DLR facility and 1043.4 MJ m^{-2} on the ISS of UV radiation in the range between 100 and 400 nm.

All molecules, with or without meteorite powder, were more or less affected when exposed to UV radiation. The extent of compound degradation was quantified and the chemical reactions of degradation identified. The amino acid with a diacid group (i.e. aspartic acid) was more sensitive to UV radiation than amino acids with hydrocarbon chain and the dipeptide with an amide bond was the most degraded, only surviving when protected by a mineral mixture.

The results confirm that the previously demonstrated resistance to UV radiation depends on the chemical nature of the exposed molecules and the emission spectrum of the UV source, and that a coating of meteorite powder has a significant protective effect on the compounds in all experiments.

The HRMS analyses demonstrate for the first time, that some degradation is due to chemical reactions, such as decarbonylation and decarboxylation caused by exposure to UV radiation. The new compounds formed by losing their carboxylic group were then more resistant to UV radiation

than the initial compound. Comparison of the HRMS analyses of the samples exposed on the ISS and those exposed in DLR facility shows no notable additional reactions.

From the astrobiological viewpoint, our experiments show that some of the UV exposed compounds could be sufficiently stable in space conditions to survive transport in interstellar space, especially if they are embedded in appropriate mineral matter. The photochemistry leading to damage on some molecules, may act as a selective filter to the delivery of extraterrestrial organic molecules to planets. The compounds need energy providing by UV radiation to evolve. This appears thus a necessary ingredient for the origin of life. However, further experiments using other compounds in laboratory and in Earth orbit are necessary to understand the full effects of photochemistry in UV radiation and to demonstrate the link between organic matter synthesized in space and the first living organisms in Earth.

The results provide important information for the fate of organic molecules that have seeded the habitable planets and are interesting from the point of view of searching for organic molecules on Mars. To date, no organic compounds have yet been detected; however, our results show that they can resist the UV radiation and should be present if protected by a mineral matrix, thus supporting the hypothesis that organic matter of recent meteoritic origin could be found in the Mars soil. The present Mars Science Laboratory mission and future ExoMars 2018 mission carry instrumentation capable of detecting organic molecules.

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