Production of Amino acids by impact reactions using a light-gas gun as simulation experiment of asteroid impacts in space.

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A variety of organic compounds are abiologically produced in space. This is confirmed by the detection of organic compounds from interstellar molecule clouds. This is also confirmed by the identification of organic compounds in carbonaceous meteorites such as the Marchison meteorite. Organic compounds produced by impact reactions can be stored in the surfaces and subsurfaces of planets and scattered into space. Titan, the moon of Saturn, is similar to the primitive Earth and has attracted much attention. In the previous simulation experiments, production of many carbon clusters was confirmed. However, production of amino acids by impacts experiment simulating asteroid impacts has not yet been made clear. Therefore, we carried out the laboratory simulation experiment using a 2-stage light-gas-gun, and tried to find organic compounds such as amino acids from carbon soot under a nitrogen-rich atmosphere [1]. The experiment was carried out at ISAS/JAXA. We used a polycarbonate bullet 7.1 mm in diameter or a stainless steel bullet 3.2 mm in diameter. The bullet was accelerated to about 6.5 km/s and injected into a pressurized target chamber. At the end of the large target chamber, the pressurized target chamber was set. At the end of the pressurized target chamber, an iron target (an ice + iron target or an ice + hexane + iron target or a tholin + iron target) was set. After the impact, the soot was carefully collected. A part of the soot was refluxed in 50 ml of the pure water for 8 hours at 100 ℃. The water was filtered the soot and condensed by heating. A part of the filtered sample was hydrolyzed. A part of the soot was also hydrolyzed, extracted and filtered. Those prepared samples were analyzed by a HPLC. Those HPLC data are compared with those of standard amino acid solution including 17 amino acids and blank. Peaks of glycine and alanine were detected in the sample which was refluxed. Peaks of serine and leucine were detected in the sample which was hydrolyzed. The sample which was extracted from the hydrolyzed soot indicated sharper signals of amino acids. It is estimated that approximately 10^{-6}-10^{-4} g/mg of glycine, alanine, serine and leucine were included in the carbon soot produced by the impact reaction, when the ice + hexane + iron target was used. Because the amount of soot is not uniform, it is better to measure more samples. We think that chains of large organic compounds in the sample were broken down by hydrolysis, and the more peaks were detected. Hydrolysis, in the case of this experiment, may reacts incompletely when the quantity of the soot is too large. The numerical value in quantitative analysis may be loose when the quantity of the soot is too small. A hot plume is produced by the impact under the nitrogen rich atmosphere. In the hot plume, C_2 and N_2 molecules are reacted during the cooling process to produce CN molecules. They could cause reactions to produce amino acids.
Ref.[1] : K. Okochi,T. Mieno et al.: Orig. life. Evol. Biosph (2015) **45**: 195-205.

Keywords: Titan, Impact reaction, Amino acid

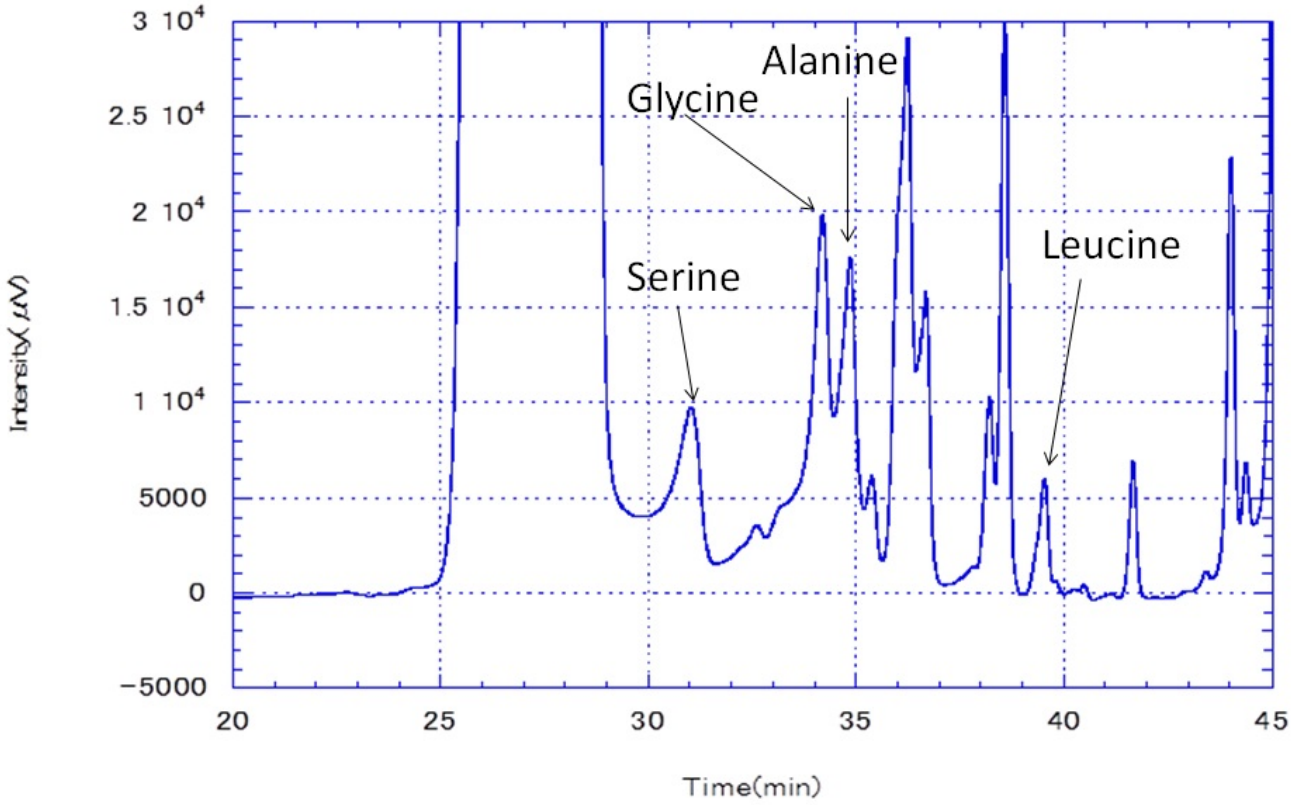

Fig. Example of HPLC data.

Stability of amino acid precursors in various space environments

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Stability of Amino Acid Precursors in Simulated Extraterrestrial Environments

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Amino acids have been detected in such extraterrestrial bodies as carbonaceous chondrites [1]. There have been number of studies to synthesize amino acids in simulated extraterrestrial environments [2]. It seems, however, that most extraterrestrial amino acids are present as precursors or combined forms rather than in free forms, since (i) amino acids in carbonaceous chondrites greatly increased after acid hydrolysis [1], and (ii) laboratory experiments suggested that not free amino acids but amino acid precursors were formed from possible interstellar media by the action of cosmic rays [2]. If these compounds carried to the primitive Earth, we should consider their stability in various extraterrestrial environments including in proto-solar nebula, asteroids, comets and cosmic dusts. For example, organic compounds would have altered in aqueous solution in asteroids by gamma rays from ^{26}Al [3]. High energy particles (cosmic rays) are another possible energy source for alteration of extraterrestrial organics in the solar system. Here we examine the stability of amino acids and amino acid precursors against gamma rays and heavy particles.

Experimental: Target molecules are (i) glycine (Gly, free amino acid), (ii) aminoacetonitrile (AAN; glycine precursor), (iii) hydantoin (Hyd, glycine precursor detected in carbonaceous chondrites [4]), and (iv) complex amino acid precursors “CAW” synthesized from carbon monoxide, ammonia and water by irradiation of 2.5 MeV protons from a Tandem accelerator (Tokyo Tech, Japan). CAW is a model of complex interstellar organics [4].

Aqueous solution of each molecule was sealed in a Pyrex tube, and subjected to 290 MeV/u carbon ions irradiation (HIMAC, NIRS, Japan) or to gamma ray irradiation (^{60}CO source, Tokyo Tech, Japan). Irradiated samples were acid-hydrolyzed (6 M HCl, 110℃, 24 h), and amino acids in the hydrolysates were determined by cation-exchange HPLC (Shimadzu LC-20A).

Results and Discussion: Glycine was determined in both irradiation products from Gly, AAN and Hyd. In the case of CAW, various amino acids were detected in the hydrolysates of the irradiation products, but glycine was predominant. Hereafter we will mainly discuss the relative recovery of glycine in the irradiated samples to reference samples without irradiation.

In the case of carbon ions irradiation, decrease of glycine was limited, but Gly, AAN and hydantoin was largely decomposed after irradiation.

After 5 kGy of gamma irradiation of Gly and Hyd, glycine recoveries from them were 68% and 46%, respectively, but AAN and CAW were hardly decomposed. Hydantoin was less stable than others against gamma irradiation, but still some hydantoin in liquid phase of asteroids could survive in their early stages.

After 15 kGy of carbon ions irradiation, recovery of CAW was highest among all (recovery: 98.5%), followed by glycine (35%) and aminoacetonitrile (18%). Hydantoin was mostly decomposed under the same condition.

It is concluded that complex amino acid precursors (CAW) was more stable than free amino acid (glycine) and small amino acid precursors (hydantoin) against space radiation environments. Aminoacetonitirele

was stable against gamma rays, but not stable against heavy ions.

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Keywords: origins of life, amino acid, stability

Stability of Amino Acid Precursors in Simulated Submarine Hydrothermal Vent Environments

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Submarine hydrothermal vents have been found in various regions of deep oceans since their first discovery in 1977. Submarine hydrothermal systems are regarded as promising sites for prebiotic chemistry toward the generation of life [1]. On the other hand, a wide variety of organic compounds have been detected in extraterrestrial bodies such as meteorites and comets. It seems that such extraterrestrial organic compounds including amino acids were supplied to primordial ocean, and were modified in submarine hydrothermal systems.
Imai et al. [2] showed that peptides were formed from aqueous solution of glycine in a flow reactor simulating submarine hydrothermal systems. Miller and Bada [3] pointed out that amino acids were not stable in hot medium. In their works, free amino acids were used as starting materials. Larger part of the amino acids delivered by extraterrestrial bodies seem to be, however, not free amino acids but amino acid precursors: Laboratory experiments suggested that amino acid precursors were formed from possible interstellar media [4]. Thus it is possible that amino acid precursors in extraterrestrial bodies supplied to primordial ocean.
In the present study, stability of amino acid precursors in simulated submarine hydrothermal system was examined. We selected aminoacetonitrile (AAN) and hydantoin (Hyd) as possible glycine precursors. We also used product obtained by proton irradiation of a gas mixture of carbon monoxide, ammonia and water. This product is hereafter abbreviated as CAW, which is a model of complex amino acid precursors, since glycine and other amino acids were detected after hydrolysis of CAW [4]. In order to simulate reactions in submarine hydrothermal systems, we used the flow reactor (previously introduced as supercritical water flow reactor (SCWFR) [5]).
Experimental: 4 mM each of glycine, aminoacetonitrile and hydantoin aqueous solution was prepared. CAW was prepared by irradiation of a mixture of carbon monoxide (350 Torr) and ammonia (350 Torr) and water water with 2.5 MeV protons from a Tandem accelerator (Tokyo Tech).
Carrier used in the flow reactor was either pure water of 1 mM HCl at the rate of 0.5 mL/min; the latter was used to simulate acid submarine hydrothermal fluid. In the flow reactor, each sample was heated for 2 min and then quenched at 0 ℃. As reference run, each sample was injected to the flow reactor with the heater off. The effluents were collected and subjected to amino acid analysis with Shimadzu LC-20A amino acid analyzer after acid hydrolysis and desalting with Bio-Rad AG-50WX8 cation-exchange resin. Recovery ratio was defined as the ratio of glycine amount in each heated sample to glycine amount in the reference sample with acid-hydrolysis.
Results and Discussion: When Gly, AAN and CAW was heated at 300 ℃, recovery ratios of glycine were less than 1% before hydrolysis, but all the recovery ratio increased after hydrolysis. The runs under the acidic condition gave higher recoveries before and after hydrolysis. This suggested that (i) glycine was decomposed mostly, but some glycine changed to combined species, and (ii) these compounds were more stable under acidic environments rather than in neutral environments. On the other hand, hydantoin’s recovery was about 5% before hydrolysis, and it increased to 20% after hydrolysis. It was shown that ring compounds like hydantoins were more stable than acyclic compounds in submarine hydrothermal systems. Further studies are in progress to examine possible roles of amino acid precursors in prebiotic chemistry in submarine hydrothermal systems.

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Keywords: amino acid precursors, submarine hydrothermal vents, origins of life

Prebiotic Formation of Amino Acid Precursors in Primitive Earth Atmosphere by Cosmic Rays and Solar Energetic Particles

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Since Miller’ s spark discharge experiment in 1953 [1], many experiments have been performed to see how bioorganic compounds such as amino acids were produced in primitive Earth atmosphere. In the earlier experiments, strongly reducing gas mixtures containing methane and ammonia were mainly used, and amino acids were detected after the applying such energies as spark discharges and ultraviolet light. In these days, however, it is estimated that the early Earth atmosphere were less reducing: its major constituents were CO_2 and N_2 , together with small amount of reducing carbon species like CH_4 and/or CO [2]. Simulation experiments suggest, however, that amino acid formation is restricted under these conditions [3]. High-energy charged particles of galactic and solar origins are always penetrating into planetary atmosphere, which could facilitate reactions among atmospheric gases, but they have been ignored as prebiotic energy sources for their lower energy fluxes [4]. We examine possible formation of amino acids from slightly reducing gas mixtures by applying ionizing radiation to simulate the action of galactic and solar cosmic rays.

Gas mixture of N_2, CO_2 and CH_4 of various mixing ratios were introduced to a Pyrex tube together with 5 mL of pure water. The gas mixture was irradiated with 2.5 MeV protons from a Tandem accelerator (Tokyo Tech, Japan). The same composition of gas mixtures were subjected to spark discharges by using a Tesla coil to simulate thudering. Each product was acid-hydrolyzed and was subjected to amino acid analysis by HPLC and GC/MS.

Amino acids were detected in the hydrolyzed products when gas mixtures of N_2, CO_2, CH_4 and H_2O were irradiated with 2.5 MeV protons, even if the molar ratio of methane (r_{CH4}) in the starting gas mixture was as low as 0.5 %. In the case of spark discharges, however, amino acids were not detected when r_{CH4} was lower than 15 %. Considering fluxes of various energies on the primitive Earth [5], galactic cosmic rays appear to be an efficient factor to produce N-containing organics than any other conventional energy sources like thundering or solar UV emission irradiated the early Earth atmosphere.

Besides galactic cosmic rays, frequent solar energetic particles (SEPs) associated with solar explosive events could have served as energy sources for prebiotic chemistry in the atmosphere of early Earth. Frequent superflares have been observed in young sun-like stars [6], which suggests that high energy SEPs produced during solar magnetic storms could have been efficient in supplying energy for efficient production of HCN and N_2O [7]. Solar energetic particle events could have enhanced production of bioorganic compounds in primitive Earth atmosphere. Further experimental studies on such effects are in progress.

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Keywords: Amino acid precursors, Primitive Earth atmosphere, Cosmic rays, Solar energetic particles, Origins of life

Space Exposure of Amino Acids and Their Precursors in the Tanpopo Mission: The First Analysis Report

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Since a wide variety of organic compounds including amino acids have been detected in carbonaceous chondrites [1], it is plausible that organic compounds delivered by extraterrestrial bodies played important roles in the generation of terrestrial life. Cosmic dusts (IDPs) are another candidate of carriers of extraterrestrial organics [2]: Chyba and Sagan [3] suggested that cosmic dusts delivered much more organics to the primitive Earth than meteorites and comets. It is difficult, however, to detect bioorganics in cosmic dusts if they are collected in the terrestrial biosphere.

We initiated the first Japanese astrobiology mission on the International Space Station (ISS) named the Tanpopo Mission in 2015. In the mission, we intended to collect dusts flying in low Earth orbit by using ultra-low density silica gel (aerogel), and to expose organic compounds and microorganisms to space environments [4]. One of the major objectives is to examine possible delivery of organic compounds including amino acids by cosmic dusts. Thus amino acids in captured dusts are analyzed, and stability of selected organic compounds (free amino acids and their precursors) is evaluated in the mission. The first sample returned to the Earth in August 2016 after about 1 year’ s space exposure. Here we report the first analytical results of the organic exposure experiment in the Tanpopo Mission.

Two free amino acids (glycine, and isovaline) and their possible precursors (hydantoin and 5-ethyl-5-methylhydantoin), together with products by proton irradiation of a gas mixture of ^{13}CO, NH_3 and H_2O (hereafter abbreviated as CAW) were selected in the organic exposure experiment: CAW is a mixture of complex organic compounds including amino acid precursors [5]. All the organic materials used were labeled with ^{13}C. Aqueous solution of each of these materials was added to one of dimples on an aluminum plate, and dried. Then the surface of the materials was covered with hexatriacontane to avoid scattering. Each plate for space exposure was covered with a SiO_2 or MgF_2 window. The same kind of plates were prepared for (i) dark controls (exposed in space but no light allowed), (ii) cabin controls (stored in the JEM cabin), and (iii) ground controls.

Alanine thin film was used as a VUV dosimeter based on a dissociation experiment with a 172 nm eximer lamp [6]. Optically stimulated luminescence dosimeter (OSLD) and silver activated phosphate glass dosimeter (RPLD) were used as radiation dosimeters. The dosimeters and the exposure plates were combined together to be an exposure panel, which was attached to an ExHAM module and exposed on the Exposed Facility (EF) of Japanese Experimental Module (JEM) of ISS.

The material in each dimple was collected by using small amount of methanol and water. Amino acids were determined by HPLC (Amino acid precursors and CAW were determined after acid-hydrolysis). The materials were also analyzed by GC/MS and LC/MS.

Preliminary results and discussion will be shown in the poster. We are expecting return of another set of samples in 2017 after 2 years’ exposure.

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Keywords: The Tanpopo Mission, Amino acids, Amino acid precursors, Exposed Facility of the International Space Station, Solar ultraviolet light, Origins of life

Temperature Measurement Results with the Mechanical Space Thermometer for the Tanpopo

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Temperature in the space is very interesting as astrobiology because it controls the life and death of the creature in space environment. The mechanical thermometer using a bimetallic strip coil was developed for the Tanpopo mission. The Tanpopo mission is a multi-year passive exposure experiment for astrobiology exposure and micrometeoroid capture onboard the Exposed Experiment Handrail Attachment Mechanism (ExHAM) at the Japanese Experiment Module ‘Kibo’ (JEM) Exposed Facility (EF) on the International Space Station (ISS). The Tanpopo mission apparatuses were launched by the SpaceX-6 Dragon CRS-6 on April 14 2015, from the Cape Canaveral Air Force Station in the U.S.A. Since its microbial exposure experiment requires recording the maximum temperature that the Tanpopo exposure panel experiences, we have developed a mechanical thermometer with no electric power supplied from the ExHAM. At a given time and orbital position of the ISS, the thermometer indicator was video-imaged by the extravehicular video camera attached to the Kibo-EF and controlled from the ground. With these images analyzed, we were able to derive the temperatures of the Tanpopo exposure panels on the space pointing face of the ExHAM. Temperature measurement results with the mechanical space thermometer are shown in Fig.1. Twelve times of observation of the thermometer was carried out in 2015 and 2016. The maximum and minimum temperature were 26.4±5 ℃ and -41.6±5 ℃, respectively. Now this passive and mechanical thermometer is available to other space missions with no electric supplies required and thus highly expands the possibility of new extravehicular experiments and explorations for both human and robotic missions.

Keywords: Tanpopo, International Space Station, Mechanical Space Thermometer

No.	Year	Date (GMT)	B angle (degree)	Max Temp (℃)	Min Temp (℃)
1	2015	153	74	-8.8	-12.5
2		159	53	-11.1	-21.0
3		164	29	+16.4	-0.6
4		170	1	+17.5	-3.4
5		184	-28	+23.9	-1.6
6		194	1	+20.9	+3.4
7		349	-15	+26.4	+6.9
8		355	-45	-8.9	-20.9
9		362	-75	-27.2	-35.3
10	2016	345	-15	+24.9	+3.5
11		352	-45	-12.6	-27.5
12		360	-75	-32.8	-41.6

Fig.1 Temperature Measurement Results (Error ±5℃)

Discovery of Micrometeoroid Impact Signatures on the Tanpopo Aerogel Panels: Early Report of the Initial Sample Analysis of Its First Year Samples Retrieved back to the Earth

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The “TANPOPO” mission, named after dandelion, has been Japan’s first astrobiology space experiment onboard the International Space Station-Kibo Exposed facility since May 2015, in order to test various aspects of the “quasi-panspermia” hypothesis for exogenesis origin of life precursors and their interplanetary transport. In May and November 2015, the first year samples were installed on a small pallet called “ExHAM” on the handrail of the ISS-Japan Experiment Module (JEM), or Kibo, Exposed Facility (EF) in the duration of 1-3 years. The first year exposed samples were successfully retrieved back to the Earth in August 2016; then the initial sample analysis and curation (ISAC) activity at ISAS had started since late September 2016, by a team of scientists gathered from planetary science to microbiology fields.

By analyzing captured micrometeoroids in the aerogels, one can learn what kinds of extra-terrestrial organic compounds inside micrometeoroids can be transported from parent bodies and how they may be altered in outer space. Also by evaluating retrieved samples of exposed terrestrial microbes and astronomical organic analogs on the exposure panels, one can investigate their survivals and alterations in the duration of interplanetary transport. These samples continue to be returned to ground laboratories after retrieval to the Earth in 2017, 2018 and finally 2019.

The TANPOPO employs blocks of ultra-low dense aerogels on the Capture Panels (CP) that are exposed and retrieved to capture impacting solid microparticles such as organic-bearing micrometeoroids and possible terrestrial particles in the low Earth orbit. In case of microparticles of terrestrial origin impacted into the CPs, one can test if terrestrial microbes (e.g., aerosols embedding microbial colonies) may be present, even temporarily and in “freeze dry” form in the low earth orbit altitudes. Also by evaluating retrieved samples of exposed terrestrial microbes and astronomical organic analogs on the Exposure Panels (EP), one can investigate their survivals and alterations in the duration of interplanetary transport.

The TANPOPO experiment consists of following six sub-themes: 1) capture of microbes in space, 2) exposure of microbes in space, 3) exposure of organic compounds in space, 4) capture of organic compounds in micrometeoroids in space, 5) evaluation of ultra low-density aerogel developed for the Tanpopo mission, and 6) capture of space debris at the ISS orbit. Each will utilize one or more CP and EP samples from various pointing faces onboard the ExHAM as the ISS is a earth gravity gradient three-axis stabilized satellite.

The ISAC procedure has covered from the receipt of retrieved samples, their initial inspection and documentation, processing and distribution of the samples for detailed analyses of each sub-theme,

cataloging for data archiving and to sample storage. For initial inspection and documentation, the Captured Particles Location, Observation and Extraction System (CLOXS) mapped and measured more than 60 hypervelocity penetration tracks and captured particles (e.g., incoming angle, track depth and track volume) on 8 of the first year tanpopo aerogel panels at the ISO-1 level clean environment achieved at the ISAS clean room. Then the CLOXS then processed keystones containing microparticles to be inspected and their penetration tracks for allocation to respective sub-theme researchers, in accordance with their requests for the subsequent detailed analyses within the first 100 days after the Earth sample return, i.e., by January 2017.

Keywords: Microbes, Panspermia, Sample Analysis and Curation

Survivability and DNA damage of *Deinococcus* spp. in cell-aggregates exposed to space in Tanpopo mission

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[Background] The interplanetary transfer of microbes (panspermia hypothesis) is tested in Tanpopo mission on the Exposure Facility of Japanese Experimental Module of ISS [1]. The capture and exposure experiments of terrestrial microbes have started since May 2015. The previous space exposure experiments suggested that microbes inside rocks, which have enougn thickness to shield UV, could survive for a long period in space [2]. On the other hand, we proposed that sub-millimeter cell-aggregate (biofilms) might survive for long time in space (massapanspermia) [3]. We analyzed survival fractions of space-exposed cell-aggregates of *Deinococcus* spp. with various thicknesses. We also investigated DNA damage caused in space environment using DNA repair-deficient mutant strains: *D. radiodurans* UVS78 deficient in the excision repair, rec30 deficient in the homologous recombination repair and KH311 deficient in the non-homologous end-joining.
[Method] Dried deinococcal cell-aggregates in wells of aluminum plates were exposed to space for about one year. The dried cells were resuspended in phosphate buffer and recovered from wells. The cell suspension was inoculated to mTGE agar and incubated at 30ºC before enumerating colonies. The surviving fraction was calculated as the number of viable cells after exposure divided by the number of viable cells without exposure.
[Result and Conclusion] Although the *D. radiodurans* R1 cell-aggregates with less than 100 μm- thickness exhibited a low survival rate, those with more than 500 μm-thickness was well-survived (Fig. 1). It was suggested that DNA damage in the cell-aggregates with more than 500 μm-thickness are readily repaired by homologous recombination and excision repair systems. The surviving fractions of the ground control and the space exposed cell-aggregates with 1000 μm-thickness were comparable. The result might reflect intracellular moisture content that was removed by a long-time space exposure. Low moisture content will help cells to survive in space. From these results, we concluded that the deinococcal cell-aggregate with 500 μm-thickness is sufficient to shield UV, thus surviving for more than one year in space. DNA damage caused in space was mainly base damage such as pyrimidine dimer caused by UV irradiation and double strand breaks.
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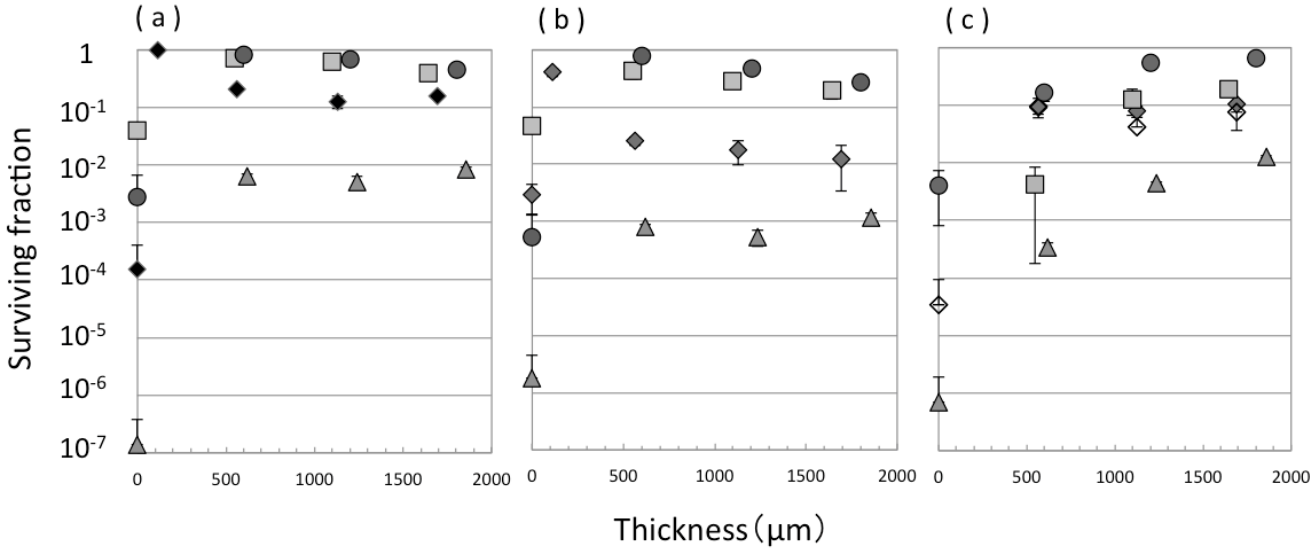
Keywords: Tanpopo mission, Surviving fraction, DNA damage



Analysis of DNA damage in the radiation resistant microbe *Deinococcus radiodurans* R1 exposed to space in Tanpopo mission

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Tanpopo mission is a Japanese astrobiology experiment addressing basic questions on the origin of terrestrial life and panspermia hypothesis (Yamagishi et al., 2009; Kawaguchi et al., 2016). We have started the space experiments at the Exposure Facility of the Japan Experiment Module on the International Space Station (ISS). Capture experiment investigates existence of terrestrial microbes in space. Exposure experiment investigates the microbial survival and DNA damage caused in space. We analyze degree and types of DNA damage in *Deinococcus radiodurans* using following methods: 1) comparison of survival fractions of mutant strains deficient in each of DNA repair systems, 2) analysis of DNA double-strand breaks using pulsed-field gel electrophoresis, 3) estimation of DNA damage using quantitative-PCR (q-PCR), 4) detection of mutation in *rpoB* gene and 5) analysis of DNA base damage using LC-MS/MS. In this work, we quantified DNA damage (double-strand breaks, single-strand breaks, hydrolysis of base, modified base, and so on) in part of the *rpoB* gene using q-PCR.

Methods

Dried deinococcal cell-aggregates with different thickness were exposed to space (space samples) for about one year (space samples). The cells were also stored in the ground laboratory (ground references) and in ISS cabin (ISS references). After exposure or storage, genomic DNA was extracted from each sample and an 887-bp region in the *rpoB* gene was amplified by q-PCR. Intact DNA (%) was determined from the quotient N/N_0, where N = copy number of *rpoB* gene amplified from DNA of exposed or stored cells and N_0 = copy number of *rpoB* gene amplified from freshly prepared DNA.

Results and Discussion

Intact DNA (%) of the cell-aggregates with 100 μm-thickness exposed to space was less than 1% and all cells were dead. Pyrimidine dimer was major DNA damage caused by UV. On the other hand, DNA damage in those with 1000 μm-thickness was similar between the ground references and the space samples (Fig. 1). The result indicates that UV affected only the surface of the cell-aggregates. Intact DNA (%) in the ground references and the space samples (UV> 170 nm) with 500 μm-thickness were about 54%, and that in space samples (UV> 120 nm) with 500 μm-thickness was 46%. Although a significant difference is not recognized between the two samples, UV with shorter wavelength tended to induce more damage in DNA. Intact DNA (%) showed a good correlation with surviving fraction. We will also report the types and degrees of DNA damage using other methods.

Yamagishi, A., et al., (2007) *Biol. Sci. Space* 21: 67–75. , Kawaguchi, Y., et al., (2016) *Astrobiology* 16: 363–376.

Keywords: Panspermia hypothesis, Space exposure experiments, Cell aggregate, DNA damage, Tanpopo mission, Quantitative-PCR

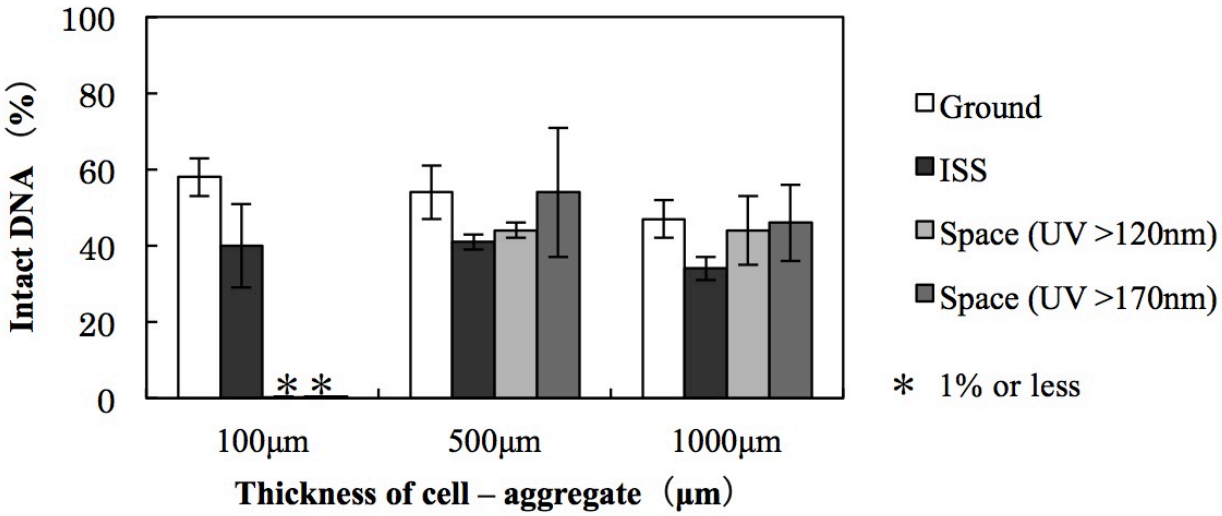

Figure 1 Percentage of Intact DNA

Mutation analysis of the *rpoB* gene in the radiation-resistant bacterium *Deinococcus radiodurans* R1 exposed to space

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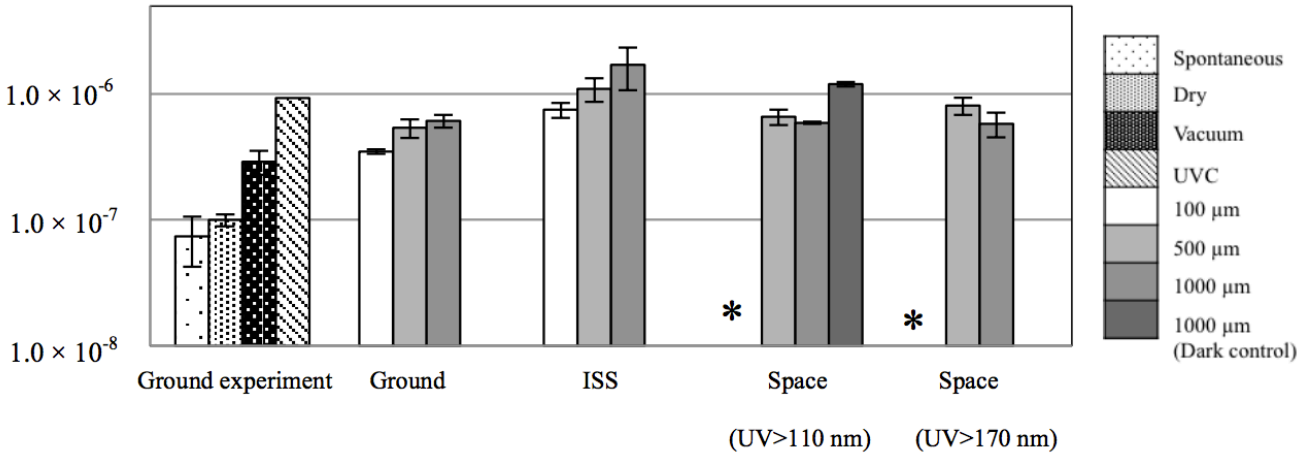
To investigate the microbial viability and their DNA damage, the radiation-resistant bacteria *Deinococcus* spp. have been exposed at the Exposure Facility of the International Space Station (ISS) in Tanpopo mission since May 2015 [1, 2]. The Exposure Panel (EP) harboring dried-deinococcal cells was returned to the ground on August 2016 after about one-year exposure. We analyze the survival rate and DNA damage of dried deinococcal cells using pulsed-field gel electrophoresis, quantitative-PCR and mutation assay. Rifampicin is an antibiotic that binds to RNA polymerase β-subunit encoded by *rpoB* gene, thus inhibiting the initial step of transcription. Certain *rpoB* mutations confer rifampicin resistance to bacteria [3]. On this basis, we determined the mutant frequency and mutation spectrum in the *rpoB* gene of *Deinococcus radiodurans* that was exposed to space. From these data, we estimated major DNA damage induced by the space environment.

D. radiodurans R1 cell suspension was dropped in the wells of aluminum plates and dried under vacuum (vacuum-dried). The dried cells were exposed to space, stored in the ISS cabin or in the ground laboratory. After exposure experiment, the cells recovered from each well were used to mix with 10 ml of mTGE medium and cultured until $OD_{590\ nm}$ reached between 1.1 and 3.0. The culture was plated on mTGE agar supplemented by 50 μg/ml rifampicin to determine the number of rifampicin resistant cells (Rif^R), and on mTGE agar without rifampicin to determine the total number of viable cells. We also determined DNA sequences of the *rpoB* gene extracted from Rif^R.

The mutant frequencies of space exposed cells and ground control were comparable (Fig. 1). The result suggested that the effect of UV on mutation induction was marginal in dried deinococcal cells exposed to space for about one year. Further, we will report and discuss the mutation spectra of the *rpoB* gene in rifampicin-resistant cells obtained from samples exposed to space, stored in the ISS cabin or in the ground laboratory.

[1] Yamagishi, A. et al., (2007) *Bio. Sci. Space* 21: 67–75.
[2] Kawaguchi, Y. et al., (2016) *Astrobiology* 16: 363–376.
[3] Campbell, E. A. et al., (2001) *Cell* 104: 901–912.

Keywords: Deinococcus radiodurans R1, DNA damage, Tanpopo mission, mutation analysis, rpoB gene, rifampicin



*Because samples exposed to the space were dead, mutation couldn't be analyzed.

Fig. 1 Mutant frequency of the *rpoB* gene in *Deinococcus radiodurans* R1

Proto-arc model for ribose and nucleotide genesis: information from Isua Supracrustal Belt

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Proto-arc model has been proposed to explain tectonic evolution of 3.8 to 3.7 Ga Isua Supracrustal Belt, Greenland (ISB; Nutman et al. 2015). Occurrence of tourmaline has been known in ISB (e.g., Appel, 1995, Mishima et al., 2016). Grew et al. (2015) also found tourmaline in various localities in ISB and suggested that the concentration of boron was elevated in a partially isolated basin by hydrothermal processes in proto-arc setting. Initial boron was most likely extracted by deep fluids from TTG and/or accreted sediments. Such deep fluids discharged into oceans as hydrothermal fluids. In addition, Nutman et al. (2016) reported primary evaporite carbonate in ISB. Such carbonate rocks were most likely formed in shallow and partially isolated basin developed in alkaline shallow basin on proto-arc.
I propose that environments created by Hadean proto-arc were ideal not only for TTG genesis but also for prebiotic ribose and nucleotide formations. In isolated and shallow basin on proto-arc, evaporation may have helped to concentrate borate and phosphate, probably precipitating lunebergite. Water in this isolated and shallow basin was alkaline, as indicated by ISB shallow basin. Such alkaline condition is favored to form sugars with the formose reaction. Concentrated borate in such alkaline basin might have helped to form and sequester ribose, selectively. Lunebergite further helps phosphorization of nucleoside (Kim et al., 20016).
Boron-rich (and also phosphate-rich) and alkaline environments also expected locally at around mud volcano on the slop of proto-arc, similar to the model proposed by Holm (2012). Inside of deep marine sediments around the proto-arc would have offered boron and phosphate-rich and alkaline environment (Mishima et al., 2016). Formorse reaction could happen not only at evaporite basin but also in deep marine environments around the Hadean proto-arc. As the result, ribose would have been the major aldopentose in Hadean proto-arc environments.

Keywords: Proto arc, Borate, ribose

The diversity and structure determination of saturated and unsaturated archaeol derivatives characteristic for the halophilic archaea lipid-core

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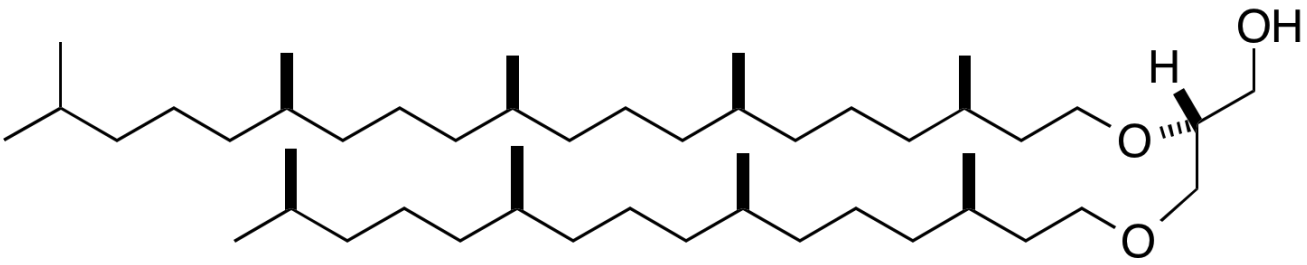
Archaea has a characteristic lipid-core, archaeol. Further, a characteristic diether lipid-core (C_{20}-C_{25} diether (**1**)) which is constructed from one C_{25} and one C_{20} isoprenoid is produced by halophilic archaea. The C_{25} (long) hydrocarbon is linked with the C-2 of the glycerol[1]. Recently, Dawson et al. showed the existence of several unsaturated isoprenoid diethers (such as tentative structure **2**) in the lipid-core of several halophilic archaea which was incubated with very high salt concentration[2].
Then, **1** and **2** were chemically synthesized according to the reported method[3] and the results were presented at the last year's this meeting[4]. The analysis of the mass fragmentation of the TMS derivative, the structure of microbiological sample derived from halophilc archaea was confirmed as **1**. Further, **2** is different from those of Dawson's unsaturated diether.
About the diversities of these unsymmetrical diether, 1) The isomer of the C_{25} (long) hydrocarbon is linked with the C-3 of the glycerol **3** was synthesized and mass fragmentation of the TMS ether of **1** and **3** were observed. Teixidor's report[5] of archaeol derivative from halite were decided to the mixture of **1** and **3** with almost equal amounts. It is suggested that the existence of the unrevealed halophilic archaea which can biosynthesize regioisomeric C_{25}-C_{20} diether in halite and/or the ancient hypersaline environment. 2) The "real" structure of Dawson's unsaturated archaeol derivative were assumed to the structure **4** or **5** from the intermediate of biosynthesis of tetraether lipid in thermophilic archaea[6]. Then, the chemical synthesis and mass fragmentation analysis of **4** and **5** will be presented.

[1] De Rosa *et al., J. Gen. Microbiol.*, **128**, 343 (1982).
[2] Dawson *et al. Org. Geochem.*, **48**, 1 (2012).
[3] Yamauchi *Res. Org. Geochem.*, **29**, 71 (2013).
[4] Yamauchi (2016) *JpGU meeting 2016* BA001-P05.
[5] Texidor *et al.* (1993) *Geochim. Cosmochim. Acta.* **57**, 4479.
[6] Nemoto et al. (2003) *Extremophiles,* **7**, 235.

Keywords: archaea, isoprenoidal dither, structure determination, halophilic, halite



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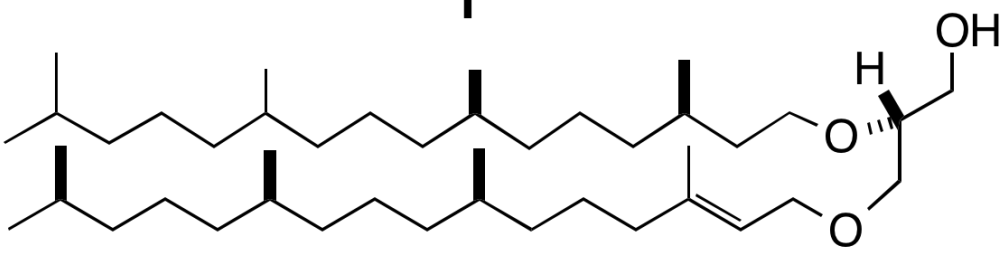

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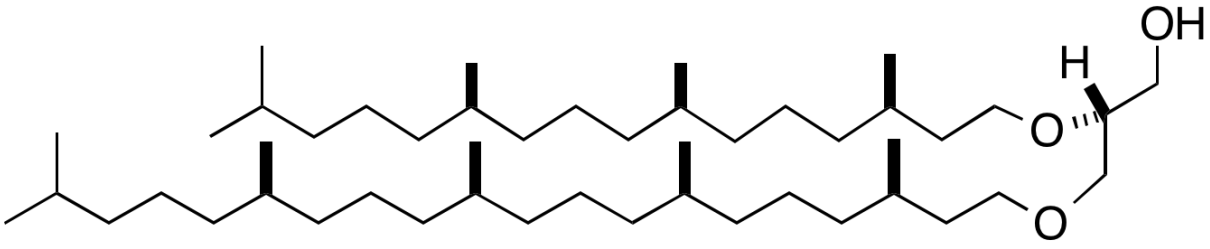

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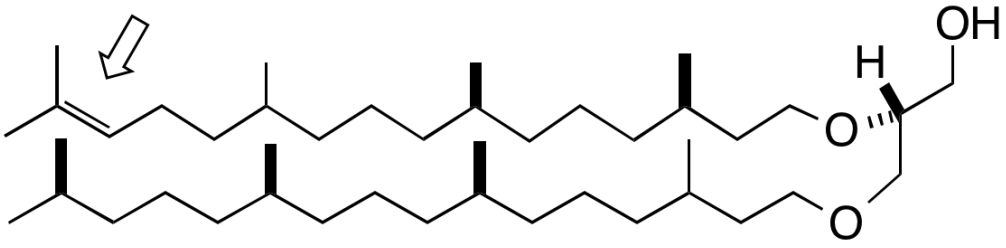

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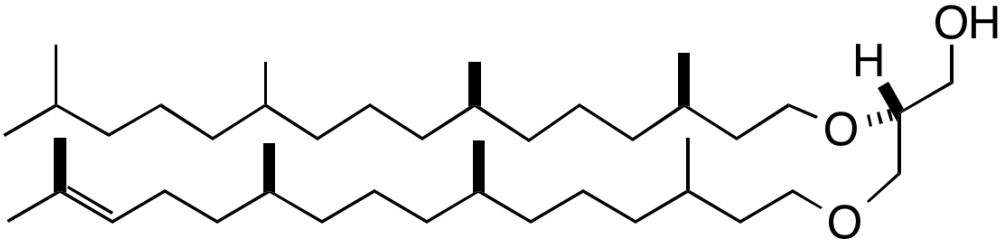

5

Validation on the spiral microstructure formed at interface of the carbonated water in early Earth

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The first stage of molecular bio-history progressed by interactions between the system of intermolecular bonding and the environment. Membrane at interface of water became robust by carbohydrates. Carbohydrates were made from moleules of CO_2 and H_2O. Atmosphere of primitive Earth was comprised of CO_2 and H_2O. Solubility of CO_2 in liquid water under the high pressure is large. From evaluation of Henry's law, 40 bar of CO_2 on initial Earth corresponds with 10^5 times of 0.0004 bar on today's CO_2. However, molecule of CO_2 is soluble in water at only low temperature. 98.3% of the CO_2 in carbonated water is linear molecule. The linear molecule of CO_2 will be incorporated in the through-hole of three-dimensional structure of liquid water as follows.
The molecule of water is described as slightly distorted tetrahedron of sp^3-hybridized four orbitals. Two of short O-H bond lengths are associated with covalent bond. Two of long O--H bond lengths are associated with ionic bond. Crystal of ice is usually hexagonal structure (1h: $P6_3/mmc$ 194). There is spiral alignment, although there exist glide planes alternately in $P6_3/mmc$ structure. The hexagonal symmetry is formed by a spiral alignment. That is, short side of tetrahedron and long side are connected alternately by 3 direction of electric coupling force. So, the growth of ice is much faster at the {100} interface than at the {111} interface [1].
The microstructure of H_2O molecules is formed basing on the plane of interface. There is a space of through-hole in the center of each spiral structure. Linier CO_2 molecule will be inserted to the through-hole, and the spiral structure is rearranged by its flexibility. It is confirmed that lattice structure of α-quartz is an optimum model to represent the microstructure of carbonated water formed at the plane interface [2].
[1] Nada,H., (2009), J. Physical Chemistry B, 113 (2009) 4790-4798.
[2] Karasawa, S., (2016), https://youtu.be/_KRvJ5cClDk

Keywords: Spiral structure, Early Earth, Carbonated water, Crystal growth of ice, α-quartz

