

Habitable Planet Searches around Red Dwarfs: Ground and Space

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Red dwarfs or M-type stars are the most abundant stars in the Milky Way Galaxy. Therefore, the Earth-like small planet searches around red dwarfs, in particular the habitable Earth-like planet searches, are one of the most important topics in astrobiology. In this talk, I will introduce such searches with ground-based facilities like Subaru/IRD and those with space-based facilities like TESS mission. Then I will outline the future characterization plan of the targets discovered by these projects.

Keywords: exoplanet, IRD, TESS, habitable planet

Exploiting Modern Photoionization Tools to Untangle the Formation of Astrobiologically Relevant Molecules in Extraterrestrial Ices

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Astrobiologically relevant molecules such as the sugar glycolaldehyde are ubiquitous in the interstellar medium, but traditional gas phase astrochemical models cannot explain their formation routes. By systematically exploiting *on line* and *in situ* vacuum ultraviolet photoionization coupled with reflectron time of flight mass spectrometry (PI-ReTOF-MS) and combining these data within infrared spectroscopy (FTIR), we reveal that complex organic molecules - among them astrobiologically relevant species - can be synthesized within interstellar ices that are condensed on interstellar grains via non-equilibrium reactions at temperatures as low as 5K. By probing for the first time specific structural isomers without their degradation (fragment-free), the incorporation of tunable vacuum ultraviolet photoionization allows for a much greater understanding of reaction mechanisms that exist in interstellar ices compared to traditional methods thus eliminating the significant gap between observational and laboratory data that existed for the last decades. With the commissioning of the Atacama Large Millimeter/Submillimeter Array (ALMA), the detection of more complex organic molecules in space will continue to grow - including biorelevant molecules connected to the *Origins of Life* theme - and an understanding of these data will rely on future advances in hard core physical chemistry laboratory experiments.

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Keywords: photoionization, astrobiology, laboratory astrochemistry

The influence of aqueous alteration in carbonaceous meteorites on its soluble organic content

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Carbonaceous meteorites are fragments from the asteroid belt that may be used as time capsules to understand the processes that happened in the early solar system. The analysis of these organic carbon-rich meteorites provide crucial information regarding the chemical reactions that occurred on the meteorite parent bodies, solar nebula or interstellar medium. They contain a rich inventory of extra-terrestrial molecules, present as insoluble organic matter (IOM) [1, 2], and as soluble organic compounds [3-5]. Bulk analysis of the soluble organic fraction of the Murchison meteorite has revealed a high molecular diversity of tens of thousands of different molecular compositions [6]. In addition, different carbonaceous meteorites show different abundances and distributions of their soluble organic content. The reason for this is not fully understood. Aqueous alteration on the meteorite parent body of carbonaceous chondrites may play a role as it is an important alteration process of their mineral, isotopic and volatile content [7-12]. In relation to the soluble organic content, a few studies show that the relative distribution of amino acids in carbonaceous chondrites seems to be influenced by the degree of aqueous alteration on the parent body [13-16]. In this talk I will present the organic inventory of different carbonaceous meteorites, and how the extension of aqueous alteration on the meteorite parent bodies may be related to this. For example, the least aqueously altered CM chondrites have smaller L-enantiomer excess (Lee) values of isovaline [17-19]. The Paris meteorite, one of the most primitive CM chondrites analysed to date has an isovaline Lee close to zero [17]. While aqueous alteration does not create an isovaline asymmetry by itself, it may amplify an L-enantiomeric excess that was originally created by other mechanisms (e.g. ultraviolet circularly polarized light (UV-CPL) photo-processing of interstellar/circumstellar ices [20-25]).

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First year report of the Tanpopo: Capture and Exposure Experiment of Micrometeorite and Microbes on Exposure Facility of International Space Station

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Purpose of Tanpopo mission

Tanpopo, a dandelion in Japanese, is a plant species whose seeds with floss are spread by wind. We proposed this mission to examine possible interplanetary migration of microbes, and organic compounds at the Exposure Facility of Japan Experimental Module (JEM: KIBO) of the International Space Station (ISS).

We are testing the panspermia hypothesis, which proposes the interplanetary transfer of life. We are also testing if the organic compound may be transferred from space before the origin of life on the earth. The Tanpopo mission consists of six subthemes: Capture of microbes in space (Subtheme 1), exposure of microbes in space (Subtheme 2), analysis of organic compounds in interplanetary dust (Subtheme 3), exposure of organic compounds in space (Subtheme 4), measurement of space debris at the ISS orbit (Subtheme 5), and evaluation of ultra low-density aerogel developed for the Tanpopo mission (Subtheme 6).

Apparatus developed for Tanpopo mission

We have developed two types of apparatus used for Tanpopo mission: Capture Panels for aerogel to capture micro-particles and Exposure Panels for exposure of microbes and organic materials. Each Capture Panel contains a silica aerogel block in an aluminum mesh container. Silica aerogel, which is the lowest density solid material, is used to capture micro particles, which may include, micrometeorite, artificial space debris and earth-originated natural particles. We are going to analyze if the particles contain terrestrial microbial cells or not.

Exposure Panels have been developed to expose microbes and organic compounds to the space environment. Several microbial species including, *Deinococcus radiodurans*, *Deinococcus aerius*, *Deinococcus aetherius*, *Nostoc sp.*, *Schizosaccharomyces pombe*, have been exposed to the space environment. These species are expected to be resistant against space environment, vacuum, desiccation, temperature-cycle, UV and ionization radiation. We are testing the survival of these species after one-, two- and three-year exposure in space. Organic compound such as amino acids and the precursors have also been exposed.

Schedule of Tanpopo mission

Tanpopo apparatus was launched on April 2015. The Panels were placed on the Exposed Experiment Handrail Attachment Mechanism (ExHAM) in the ISS. The ExHAM with Panels were placed on the Exposure Facility of KIBO (JEM) with the Japanese robotic arms through the airlock of KIBO on May 2015. The first set of Capture Panels and an Exposure Panel were retrieved on June 2016, contained in plastic bags, and stored in the pressurized area of the International Space Station. They have returned to the

ground in the space capsule, and returned to JAXA, September 2016.

Exposure Panel was separated into each Exposure Unit, each harboring either microbe or organic compound was handed over to the scientist in charge of each microbe or organic compound. Some of the Units are dedicated to the UV or radiation dose measurement.

Each aerogel block of each Capture Panel was examined for the particles captured and the tracks made upon the impact, which were extracted from the aerogel block and handed over to the scientists. The analysis includes, fluorescence microscopic inspection to test if there are microbial cells or not. Particles and tracks will be used for the mineral analysis as well as the analysis of organic compounds.

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Keywords: International Space Station, Exposure Experiment, Microbes, Organic compounds, Aerogel, Micrometeorite

Abiotic syntheses of organic matter and Fe-oxides in submarine hydrothermal plumes in a deep ocean ~3.45 Ga ago

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The evolutions of life and O₂ on the early Earth have long been debated among astrobiologists. Some have suggested that the life did not evolve until ~2.7 Ga, and that the organic matter (OM) in pre-2.7 Ga sedimentary rocks represent OM synthesized abiotically via Fischer-Tropsch-type reactions in submarine hydrothermal environments. The current paradigm for atmospheric evolution is that the atmosphere remained anoxic until ~2.5 Ga because of the presence of MIF-S signatures in pre-2.5 Ga sedimentary rocks. However, some researchers have suggested that the oxygenated atmosphere and the diverse biosphere, including anaerobic and aerobic microbes, have existed since at least ~3.5 Ga. Based largely on nano-scale investigations of the physical and chemical characteristics of OM and Fe-oxides in the Marble Bar Chert/Jasper (MBC) from Western Australia, here we suggest that abiotic hydrothermal synthesis of OM was important and that the diverse biosphere existed in the ~3.5 Ga oceans.

Our investigations of the MBC, utilizing state-of-the-art analytical instruments for nanomaterial sciences (e.g., HRTEM, STEM, EELS, TALOS), have recognized intimate associations of sub-nano- to nano-sized (<0.5 nm – 100 nm) particles of Fe-oxides (FeNP) and organic matter (OM) in the every sample we have examined. Two modes of FeNP-OM associations were recognized. In the first mode, FeNP occurs abundantly both inside and outside of what appear to be “fossils of aerobic Fe-oxidizing microbes”. This mode of FeNP-OM association typically occurs as microbial mats in chert beds formed by low-T hydrothermal fluids (see Watanabe et al., this session).

In the second mode, FeNP and OM occur as a ~30-50 nm-sized aggregate, which is comprised of a spherical- or tear-shaped Fe-rich core (~10-20 nm size) made of FeNP (hematite ± magnetite) with minor OM; the core is surrounded by a ~5 nm-thick ring of OM and then by a ~10-20 nm-thick outer zone comprised of mixtures of sub-nano-sized particles of Fe-oxides and OM. Such aggregates are typically coagulated to form larger clusters of Fe-oxides and OM. Considering the various geochemical data (e.g., Eu anomalies) of the jasper beds that host the FeNP-OM association, we interpret that the Fe-oxides and OM were synthesized abiotically during the mixing in hydrothermal plumes of high-T hydrothermal fluids and ocean bottom water. The abiotic reactions created colloidal Fe²⁺-bearing proteins by utilizing CO₂ from the seawater and Fe²⁺ from the high-T hydrothermal fluids; the colloids were subsequently transformed into mixtures of sub-nano-sized particles of Fe-free OM and hematite (some to magnetite) through further reactions with seawater O₂ and hydrothermal Fe²⁺. These chemical reactions are basically the same as those that produced the OM and Fe-oxides by aerobic Fe-oxidizing bacteria. The main difference is that one is promoted by biochemistry, while the other is promoted by heat.

The abiotic production of OM in the Archean oceans would have been much more important than today because the atmospheric pCO₂ was 100 PAL, the pO₂ was already ~1 PAL, and submarine hydrothermal activity was more extensive than today. The abiotically produced OM would have been more digestible to heterotrophic organisms and more reactive to chemical reactions than the OM produced by autotrophic organisms because of the absence of cell-wall lipids. Therefore, microbial activity would have flourished more during the Archean compared to later times. Thermochemical sulfate reduction by the reactive OM (rich in Fe-bearing proteins) would have generated MIF-S signatures. Decreasing submarine hydrothermal

activity and decreasing atmospheric $p\text{CO}_2$ due to the increasing continental crust size since ~2.5 Ga would have decreased the productions of reactive OM and the MIF-S signatures. The disappearance of MIF-S at ~2.5 Ga does not indicate a change from an anoxic to oxic atmosphere.

Keywords: abiotic organic synthesis, early Earth, MIF-S, O₂ evolution

Syntheses of organic matter and Fe-oxides by aerobic Fe-oxidizing bacteria in a deep ocean ~3.45 Ga ago

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Previous researchers have postulated that anaerobic photoautotrophic Fe-oxidizing bacteria (FeOB) played a major role in the Fe geochemical cycle (especially in the formations of banded iron formations) in Archean. However, no fossil evidence for FeOB has been found in rocks older than ~2.4 Ga. Here we report the morphological, chemical, mineralogical, and isotopic characteristics of the remnants of microbial mats in the Marble Bar Chert/Jasper (MBC) in East Pilbara, Western Australia. We interpret that the mats were developed mostly by aerobic chemolithotrophic FeOB on a 2,000 m-deep ocean-floor during the influence of low-temperature submarine hydrothermal fluids at ~3.45 Ga. We will further discuss implications of our findings on the chemical and biological evolutions of the early Earth.

The major findings and their interpretations from this study include: (a) The intricate nano-scale features of the interface between the OM-rich layers and the underlying minerals suggest that the microbial mats were biochemically bonded to the minerals, rather than simply settling on the minerals; (b) The $\delta^{13}\text{C}$ values (-35 to -21‰) of the kerogens suggest that the kerogens were composed with two populations of primary producers: one that utilized CO_2 via the Calvin-Benson cycle for C-fixation (e.g., cyanobacteria, FeOB, sulfide-OB) and the other involved in the CH_4 related cycle (e.g., methanogens, methanotrophs); (c) Sub-nano- to nano-scale (<0.5 nm – 100 μm) morphologies and chemistries of organic matter (OM) and associated Fe-oxides (mostly hematite) in the MBC closely resemble those of modern aerobic chemolithotrophic FeOB; (d) The close association of nano-crystals of barite with the “microfossils” of FeOB suggests the local production of SO_4 by sulfide-OB; and (e) The $\delta^{34}\text{S}$ values (-4 to +1‰) of pyrite crystals in the benthic mats suggest the activity of sulfate-reducing bacteria (SRB).

Based on the above data we suggest that: (1) Microbial mats in the MBC developed at the interface between CO_2 - and O_2 -rich bottom ocean water and the underlying unconsolidated cherts which were invaded by low-temperature, Fe^{2+} - and H_2S -bearing hydrothermal fluids; (2) Although oxygenic photoautotrophs (cyanobacteria) had evolved by ~3.45 Ga, the involvement of cyanobacteria in the formation of benthic mats in the MBC is unlikely. This is because cyanobacteria could not have been active in the deep (dark) ocean, and the remnants of cyanobacteria in the photic zone could not have accumulated on the deep seafloor (>2,000m) with widely variable thickness in centimeter to meter scales; and (3) The microbial mats were comprised of various autotrophs (primary producers) and heterotrophs. The primary producers were mostly aerobic chemolithotrophic FeOB with minor sulfide-oxidizing bacteria (sulfide-OB) and methanotrophs, and the heterotrophs were mostly Fe-reducing bacteria (FeRB), sulfate-reducing bacteria (SRB), and methanogens. They imply that the global oceans and the atmosphere were already fully oxidized at ~3.45 Ga and the diverse microbial world had evolved by ~3.5 Ga. Our findings of the presence of negative- and positive Ce anomalies and the Y/Ho ratios (up to ~120) of the host cherts also support these implications.

The oldest terrestrial material with life-forming elements

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Besides amino acid detected in meteorites, terrestrial material that preserves information on the Earth's earliest life is extremely poor. The oldest C-isotope record for life has been tracked back to ca. 3.9 Ga (Eoarchean), whereas the oldest solid material (zircon) from the Earth to ca. 4.4 Ga (Hadean). The latter represents a potential target to check evidence for life; nonetheless, total amount of the oldest zircon is highly limited; 3 grains out of 100 thousands dated ones. Despite the recent development in radiometric dating techniques, mineral separation still remains as a major obstacle, particularly in the search for the oldest zircon of the Earth. To improve the efficiency in zircon separation, we newly designed and developed a new machinery, i.e. automatic zircon separator (AZS) that operates in three functions; 1) image processing to choose target individual zircon grains out of all heavy mineral fraction, and 2) automatic capturing of individual zircon grains with micro-tweezers, and 3) placing them one-by-one in a coordinate alignment. A new software for automatic and continuous capturing was also designed/created for continuous mineral picking without human attendance for long hours. We tested the practical efficiency of AZS, by analyzing the Archean Jack Hills conglomerate of the Mt. Narryer complex in Western Australia, i.e. the oldest zircon-bearing rock. Preliminary results are quite positive; we could obtain more than 42 zircons of over 4.0 Ga out of ca. 1,400 checked grains with 4 zircons of over 4,300 Ma with the oldest one of 4,371.1 ± 6.7 Ma. This new AZS system guarantees much higher gain in hunting older zircons. As to the origin of life, we identified tiny mineral inclusions in the oldest zircons, apatite, by Raman spectroscopy. These apatite inclusions naturally contain one of the bioessential element P, halogens (F and Cl), and possibly OH. These indicate that early Earth, at least at 4.37 Ga, has prepared inevitable elements and water potentially for generating the first life in near-surface crust.

Keywords: Hadean, life, zircon, automatic separator, apatite

Microbial nitrogen cycle enhanced by the continental input recorded in the Paleoproterozoic Gunflint Formation

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We report the heterogeneity of nitrogen isotope compositions ($\delta^{15}\text{N}$) observed in the kerogen, to know the complex origins of organic components sealed in a single kerogen of the Gunflint Formation, together with corresponding geochemical data of sedimentary rocks. The Gunflint Formation has been recognized as one of the best geological sections to understand the microbial activity and ocean environments in the Paleoproterozoic era. During the sedimentation of the Gunflint Formation, a significant orogeny event, so-called Penokean orogeny, has occurred which should affect on the change of environment in the sedimentary basin. However, the correlation between the microbial activity and change of the sedimentary environment triggered by the tectonics has not been understood.

The stepwise combustion method was performed on 13 kerogen samples to know the heterogeneities of $\delta^{15}\text{N}$. In this method, components hosted by different carriers that are intimately mixed in a sample and cannot be separated by other physical methods can be resolved based on the combustion temperature. A preliminary study suggested that the temperature dependent $\delta^{15}\text{N}$ heterogeneities were exist in the single kerogen (Ishida *et al.*, 2012, *Geochem. J.*). In the present study, the same isotope heterogeneity was observed among examined kerogen samples. The occurrences of minerals, and major and trace elemental concentrations of bulk rock samples were evaluated to understand the transition of ocean chemistry triggered by the active tectonics in this region.

A positive correlation between $\delta^{15}\text{N}$ values of subset of kerogen, and Pr/Sm ratios of bulk rock was obtained. This relationship indicates that when the terrestrial input increased, the nitrogen isotope composition recorded in the kerogen would become heavy, suggesting the biological nitrogen cycle under the oxic environment was promoted. It is inferred that the increase of terrestrial input promoted the higher productivity of cyanobacteria, making dense-microbial zone in the surface of the ocean. This organic-rich zone secondarily induces the sub-oxic zone beneath it because of consumption of oxygen by decomposing organic matter. As a result, biological nitrogen cycle including nitrification and denitrification was promoted in this zone, resulting to the heavier nitrogen isotope compositions in organic matter.

Our study suggests that the transition of ocean environment can be recorded as unique isotope heterogeneities of nitrogen in kerogen, in the relation to the specific trace elemental concentrations left in the sedimentary rocks. The techniques and evaluation procedures in this study will be largely beneficial to the future research on Precambrian geology.

Keywords: nitrogen, Paleoproterozoic, kerogen

Interaction of Methanogens and Early Earth Environment

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The luminosity of the Sun was 20-25% lower during the Archean (3.8~2.5 Ga) but geological records indicate a generally warmer climate than those of today. The common consensus is that the Archean warm climate was supported by greenhouse effect from CO₂, CH₄, and/or H₂-N₂ collision-induced absorption. It is generally accepted that H₂-using methanogens evolved early. In this work we developed a coupled ecosystem model to study the dynamic relationship between methanogens and their environment on early Earth.

The model shows prior to the development of biological nitrogen fixation, the methanogens biosphere would have little impact on the environment because of limited Net Primary productivity (NPP). After the invention of biological nitrogen fixation, there could be 2 types of interaction patterns. In the case of low hydrogen escape efficiency and high CO₂ weathering rate, both the biomass of methanogens and the environmental variables (temperature, greenhouse gas concentrations, etc.) show cyclic variations around the freezing point. Activities of methanogens are limited by environmental temperature in this case, which is in turn regulated by atmospheric CO₂ and H₂. In the case of high hydrogen escape efficiency and high CO₂ weathering rate, low hydrogen escape efficiency and low CO₂ weathering rate and high H₂ escape efficiency and low CO₂ weathering rate, both the biomass of methanogens and the environmental variables are stable, with the activities of methanogens limited by the availability of H₂, which does not directly influence environmental temperature. We will compare the NPP and atmospheric concentrations of greenhouse gases in the coupled model with results in previous works (Kharecha et al. 2005, Canfield et al. 2006, Wordsworth et al. 2013). We will also discuss the impact of biological nitrogen fixation on the interactions of methanogens and the Archean environment.

Keywords: Early Earth, Methanogens, biological nitrogen fixation

UNKNOWN WIDELY-SPREAD Fe REDOX CYCLING BACTERIA BENEATH THE EAST ANTARCTIC ICE SHEET

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The main objective was to recover bacterial life existing beneath the thick East Antarctic Ice Sheet (EAIS) using the sequencing of bacterial 16S rRNA genes recovered in the ice samples containing bedrock source mineral inclusions. The samples included the accretion (lake water source) ice of the Vostok ice core (the Russian intra-continental station Vostok) containing small numerous mineral inclusions (ice type I). Three ice samples from the same depth horizon (3607-3608 m deep; age about 16 kyr) were obtained from 3 parallel boreholes (5G-1, 5G-2 and 5G-3). Another sample was the glacier ice segment containing numerous big in size reddish rock sediments of moraine source from the D10 ice core (East Antarctic coastal area, nearby the French station Dumont d' Urville). The sample was recovered from 230 m depth and aged by about 20 kyr. The samples were strictly decontaminated and treated under 'clean room' conditions (IGE, CNRS-University Grenoble Alpes).

The comprehensive DNA analyses (constrained by Ancient DNA research criteria) of three Vostok accretion ice samples have confidently revealed three phylotypes of iron-oxidizing beta-proteobacteria belonging to Gallionellaceae. Two related phylotypes from boreholes 5G-2 and 5G-3 samples have had the closest relative at the genus level *Sideroxydans lithotrophicus*, while the remaining phylotype from the borehole 5G-3 sample - *Ferriphaseus amnicola*. The 3rd ice sample originated from the borehole 5G-1 has gave only contaminants.

The similar analysis of the D10 ice core sample has confidently recovered also three phylotypes. The 1st phylotype has proved to be the same bacterium already detected in the Vostok ice core (borehole 5G-3 sample) –the iron-oxidizing bacterium of Gallionellaceae with the closest relative at the genus level *Sideroxydans lithotrophicus*. Two other related phylotypes have showed rather low family level similarity (92%) with the acidophilic thermotolerant facultative anaerobic Fe- and S-oxidizing gamma-proteobacterium *Acidiferrobacter thiooxydans*. However, due to status 'unidentified' they were removed from the further discussion on their possible involvement in the Fe redox cycling.

Thus, three confident phylotypes of iron-oxidizing beta-proteobacteria of Gallionellaceae related to *Sideroxydans lithotrophicus* and *Ferriphaseus amnicola* were revealed in Vostok and D10 ice cores meaning that unknown bacterial Fe redox cycling communities widely exist beneath the EAIS.

Of them, one phylotype (population) related to *Sideroxydans lithotrophicus* was surprisingly found out in both Russian Vostok 5G-3 and French D10 ice cores. The age of both ice sample types is nearly the same while their origin is evidently different - Vostok accretion (lake water source) ice vs. Dumont d' Urville glacier ice. The storage time periods for ice samples (before to be treated in a laboratory) are quite different (0.5 year for Vostok ice samples vs. 40 years for D10 ice sample) as well as the time frame for the ice treatment (in a range of 1-5 years –D10 ice core sample was treated in a year after the last Vostok 5G-3 ice sample) meaning no cross-contamination could happen. The ice coring sites (Vostok and Dumont d' Urville) are far away (more than 1000 km) with no evident hydrological links beneath the EAIS meaning no bacterium 'flow' could occur. How to explain such a coincidence in findings? It seems that the presence of bedrock minerals containing Fe(II) under similar physical-chemical conditions featured by the existence of unfrozen water might provide the plausible scenario.

Keywords: East Antarctic Ice Sheet, Bedrock-originating mineral inclusions, Vostok ice core, D10 ice core, 16S rRNA genes, Iron-oxidizing bacteria

500 μm cell-aggregation of *Deinococcus* spp. was enough thickness to survive after 384 days exposure at ISS orbit in Tanpopo mission

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The concept of panspermia hypnosis is interplanetary transfer of life prospered by solar radio-pressure (Arrhenius, 1903). Previous exposure experiment of microbes in space reveals microbes inside of shielding (e.g. small fragments of rock, mixture of sugar or clay) with efficient thickness to protect from UV irradiation survive in space for a long period (e.g. Onofuri et al., 2012). On the other hand, we proposed interplanetary transfer of cell-aggregation in sub-millimeter to survive at harsh space environment (Kawaguchi et al., 2013). The hypothesis is named massapanspermia. For the investigation of microbial survival and their DNA damage induced in space, dried cells of the radioresistant bacteria *Deinococcus* spp. put in wells of aluminum plates in Exposure Panels (EPs) were exposed in space at the outside of International Space Station (ISS) in Tanpopo mission since May 2015 (Yamagishi et al., 2007; Kawaguchi et al., 2016). EPs are going to be exposed for one, two and three years. The first year's EPs were retrieved into the ISS pressurized room in June 2016 and returned to the ground laboratory in September 2016. Dried-deinococcal cell-aggregations with various thickness from single layer to about 1500 μm were used to expose in space. Dried-deinococcal cells with 100 μm -thickness were dead. However, cell-aggregations with 500 μm -thickness were alive. Intact DNA (%) with 100 μm -thickness was less 1% according to an analysis by quantitative-PCR. The results indicated that a lethal dose of UV reached inside of cell-aggregation in the case of the 100 μm -thickness samples. For 500 μm -thickness samples, UV reached only the surface of cell-aggregation, and the surface of dead cells protected inside of living dried-cells. No remarkable difference was observed in surviving fractions between space exposed samples and laboratory controls in the case of cell-aggregation over 1000 μm -thickness. These results highlight the importance of microbial cell-aggregates as an ark for interplanetary transfer of microbes as we hypothesized in our previous study (Kawaguchi et al., 2013). Global-shaped cell-aggregation of *Deinococcus* spp. with 1 mm-thickness is possible to survive during the interplanetary journey and propagate if water exists in landing planets.

Keywords: panspermia, cell-aggregation, Tanpopo mission

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 °C. 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, C₂ and N₂ 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

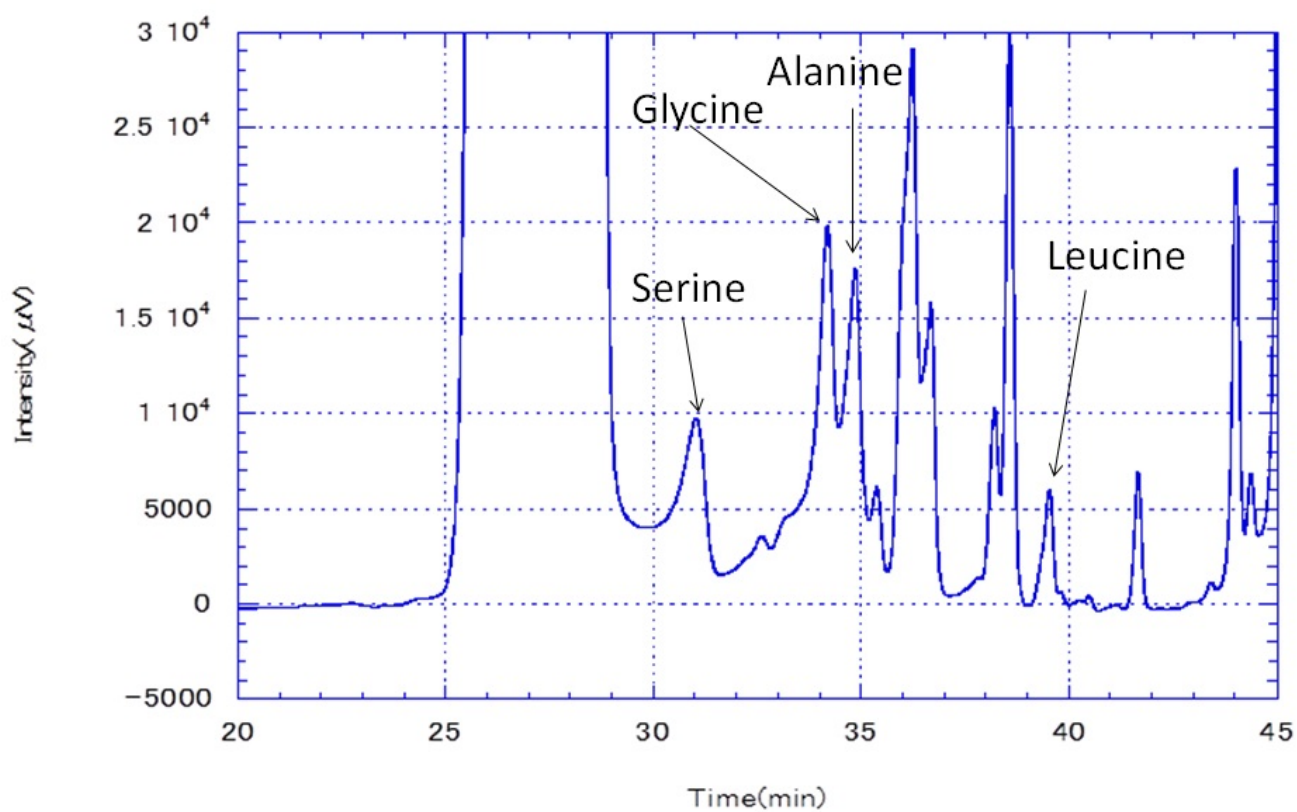


Fig. Example of HPLC data.

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 ²⁶Al [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 (⁶⁰Co source, Tokyo Tech, Japan). Irradiated samples were acid-hydrolyzed (6 M HCl, 110°C, 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. Aminoacetonitrile

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 with 2.5 MeV protons from a Tandem accelerator (Tokyo Tech).

Carrier used in the flow reactor was either pure water or 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 °C. 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 °C, 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 CO₂ and N₂, together with small amount of reducing carbon species like CH₄ 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 N₂, CO₂ and CH₄ 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 thundering. 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 N₂, CO₂, CH₄ and H₂O were irradiated with 2.5 MeV protons, even if the molar ratio of methane (r_{CH_4}) 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_{CH_4} 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 N₂O [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 °C and -41.6 ± 5 °C, 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 (°C)	Min Temp (°C)
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 $\pm 5^{\circ}\text{C}$)

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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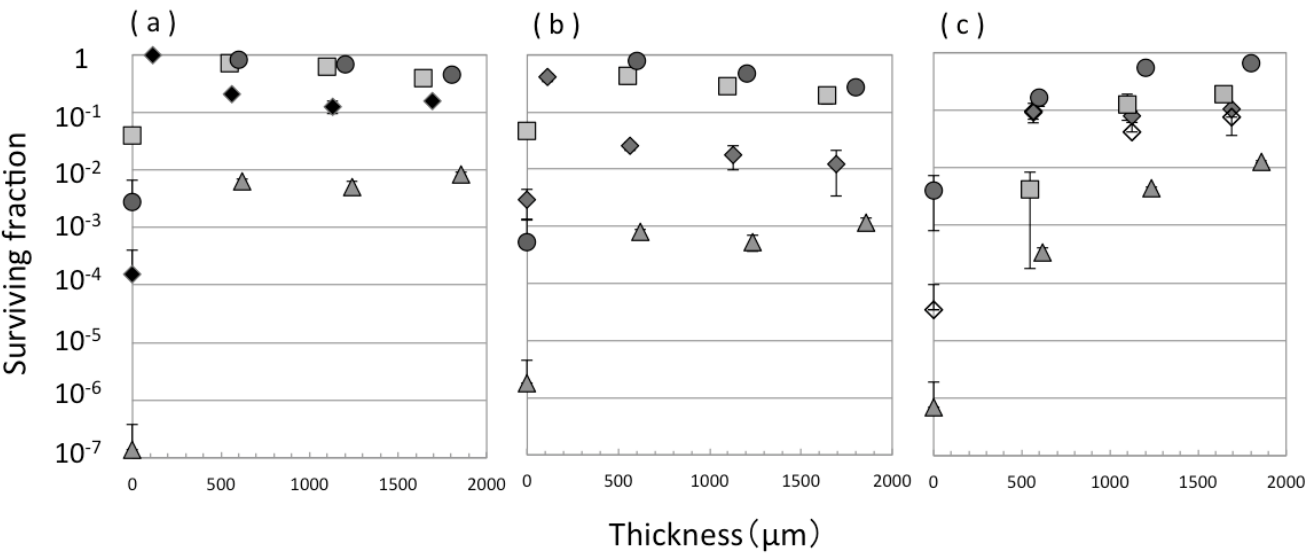
[Background] The interplanetary transfer of microbes (panspermia hypothesis) is tested in Tanpopo mission on the Exposure Facility of Japanese Experimental Module of ISS [1]. The capture and exposure experiments of terrestrial microbes have started since May 2015. The previous space exposure experiments suggested that microbes inside rocks, which have enough 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 30°C 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 were 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.

[References][1] Yamagishi, A. et al., (2007) *Bio. Sci. Space* 21: 67–75; Kawaguchi, Y. et al., (2016) *Astrobiology* 16: 363–367 [2] Onofri, S. et al., (2012) *Astrobiology* 12: 508–518 [3] Kawaguchi, Y. et al., (2013) *Orig. Life Evol. Biosph* 43: 411–428

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 μ m-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 μ m-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 μ m-thickness were about 54%, and that in space samples (UV > 120 nm) with 500 μ m-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

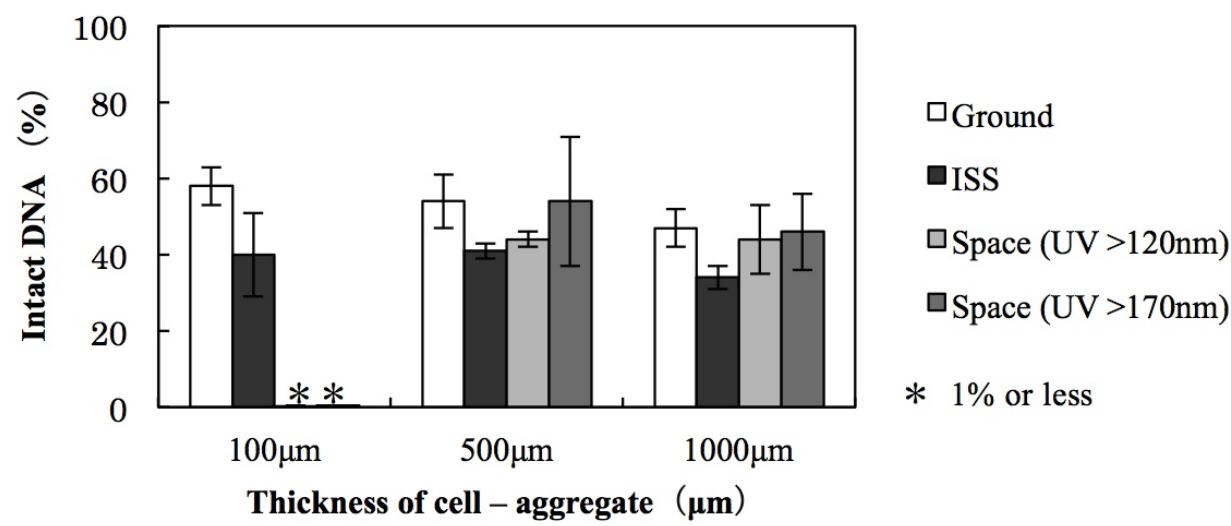


Figure 1 Percentage of Intact DNA

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 μ g/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.

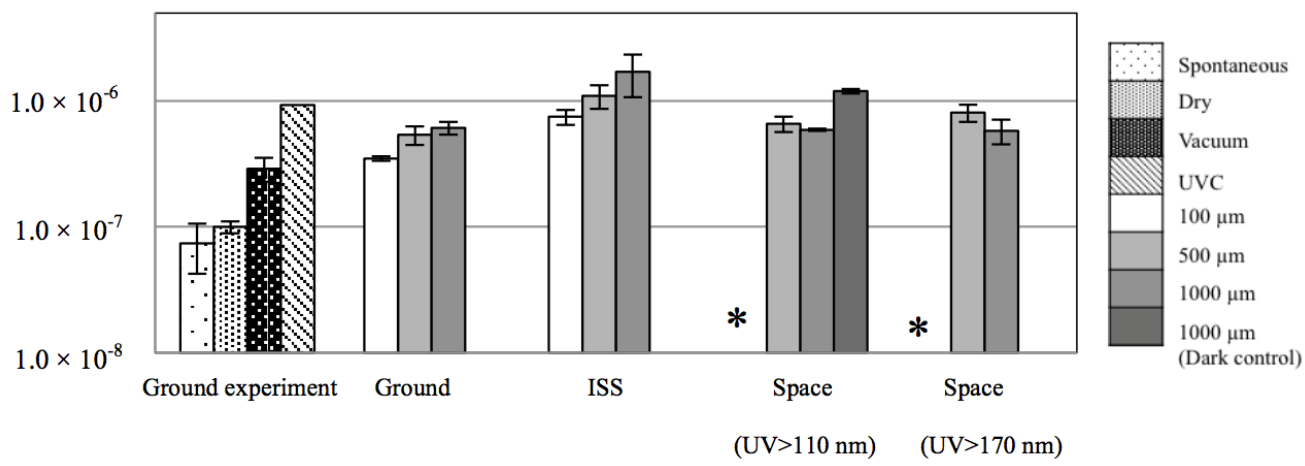
The mutant frequencies of space exposed cells and ground control were comparable (Fig. 1). The result suggested that the effect of UV on mutation induction was marginal in dried deinococcal cells exposed to space for about one year. Further, we will report and discuss the mutation spectra of the *rpoB* 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). Formose 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 halophilic 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).

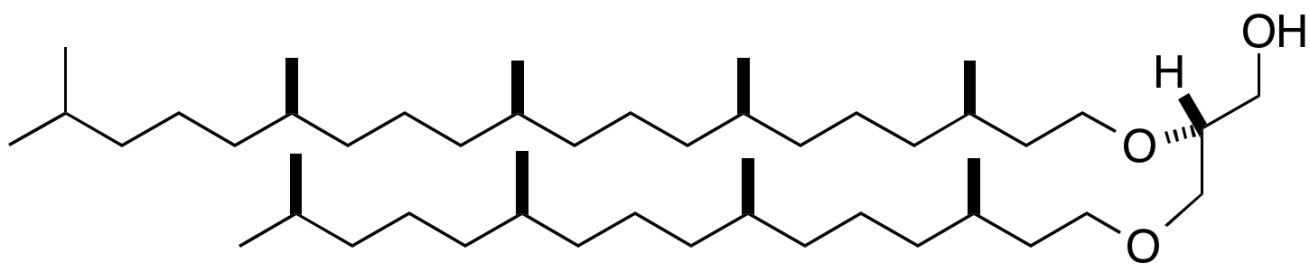
[3] Yamauchi *Res. Org. Geochem.*, **29**, 71 (2013).

[4] Yamauchi (2016) *JpGU meeting 2016* BAO01-P05.

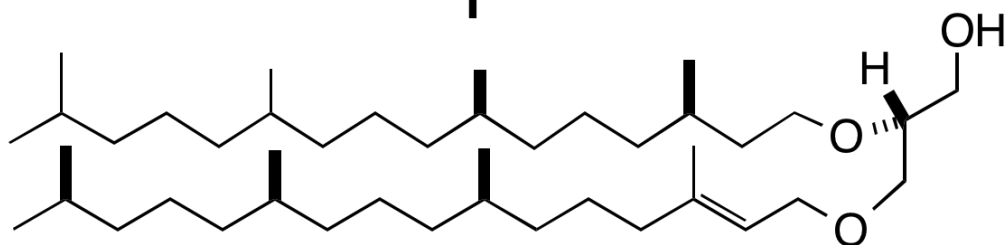
[5] Teixidor *et al.* (1993) *Geochim. Cosmochim. Acta.* **57**, 4479.

[6] Nemoto *et al.* (2003) *Extremophiles*, **7**, 235.

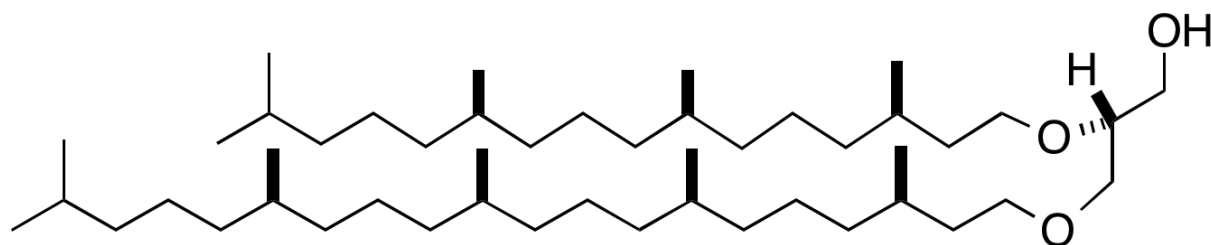
Keywords: archaea, isoprenoidal diether, structure determination, halophilic, halite



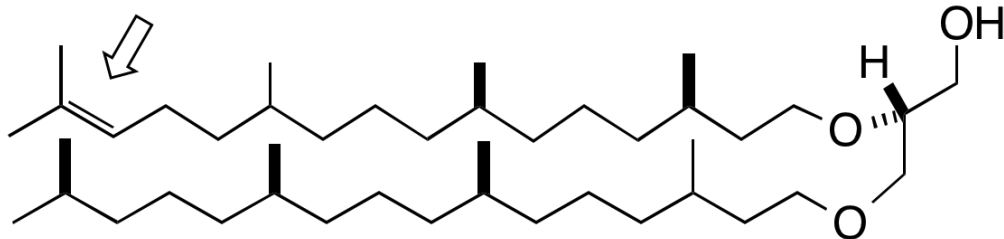
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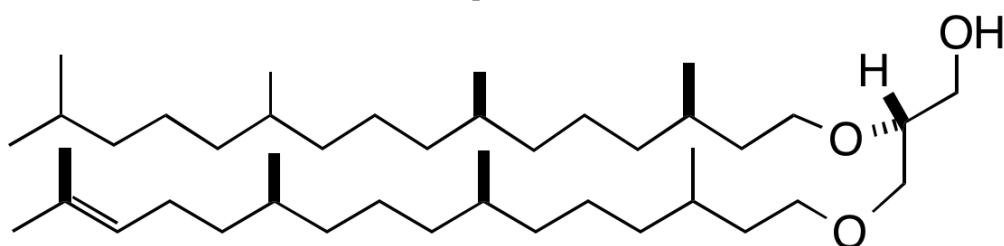
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5

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 molecules of CO₂ and H₂O. Atmosphere of primitive Earth was comprised of CO₂ and H₂O. Solubility of CO₂ in liquid water under the high pressure is large. From evaluation of Henry's law, 40 bar of CO₂ on initial Earth corresponds with 10⁵ times of 0.0004 bar on today's CO₂. However, molecule of CO₂ is soluble in water at only low temperature. 98.3% of the CO₂ in carbonated water is linear molecule. The linear molecule of CO₂ 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 sp³-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: P6₃/mmc 194). There is spiral alignment, although there exist glide planes alternately in P6₃/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 H₂O molecules is formed basing on the plane of interface. There is a space of through-hole in the center of each spiral structure. Linier CO₂ 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/_KRvJ5cCIDk

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