

2段式軽ガス銃を用いた衝突実験におけるアミノ酸分子合成

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

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宇宙空間ではさまざまな有機物が非生物的に合成されている。これは星間分子雲からの有機物の検出によって確認されている。また、マーチソン隕石のような炭素質隕石中に有機物が確認されている。衝突反応によって合成された有機物は、惑星の表面や地下に堆積したり、宇宙へ拡散していく可能性がある。原始地球と類似している、土星の衛星、タイタンが注目されている。タイタンへの小惑星の衝突模擬実験を行った先行研究では、様々な炭素クラスターが合成されていることが確認されている。しかし小惑星衝突を模擬した実験におけるアミノ酸合成はまだ不明確である。そこで本研究では、2段式軽ガス銃による衝突実験を行い、窒素の豊富な大気圏環境下で合成された試料内に、アミノ酸のような有機分子が含まれているか確認することを目的とした。実験はJAXA/ISASが保有する2段式軽ガス銃を使用した。弾は直径7.1 mmのポリカーボネイト弾、または直径3.2 mmのSUS弾を使用し、約6.5 km/sで衝突させた。与圧室がターゲットチャンバー内に設置されており、与圧室の端部にターゲットを固定した。本実験では鉄ターゲット、水+鉄ターゲット、水+ヘキサン+鉄ターゲット、ソーリン+鉄ターゲットが使用された。衝突実験後、与圧室内に堆積した試料を注意深く回収した。回収した試料を超純水50 mlで、約8時間100°Cの還流操作を行った。その後、不純物を取り除くためにろ過を行い、ろ液の熱濃縮を行ったものを分析した。また還流後、加水分解処理した試料の分析も行った。さらに炭素質試料を加水分解し、抽出してからろ過を行う形の分析も行った。試料の分析にはUV/VIS検出器を使った液体高速クロマトグラフィ分析を用いた。還流処理のみを行った試料では、標準アミノ酸の信号と比較することにより、主にグリシンとアラニンの存在を示唆するピークが見られた。加水分解処理を行った試料の分析では、グリシン、アラニンに加えてセリンやロイシンの存在を示唆するピークも見られた。炭素質試料を加水分解した試料は、より多くの鋭いピークが見られた。水とヘキサンと鉄ターゲットを用いた衝突実験により合成された炭素質試料1g中にはグリシンやアラニン、セリン、ロイシンが約 10^{-6} ~ 10^{-4} g含まれていることが見積もられた。炭素質試料の量などに多少のばらつきがあり、それによって数値が変わってしまうため、より多くのデータを使うことが好ましい。また加水分解処理を行うことにより、試料に含まれる大きな有機分子の連鎖が外れ、還流処理試料では検出されなかったピークが検出されるようになったと考えられる。本実験での加水分解は、試料の量が多すぎると完全に反応が行われない可能性があり、少なすぎると定量分析の際に、数値がばらついてしまう可能性があると考えられる。窒素ガスが充填された中で衝突が起きると、高温プルームが形成される。その高温プルーム中で C_2 分子と N_2 分子が反応し、CNが形成され、それがアミノ酸合成を引き起こしていると考えられる。

参照: K. Okochi, T. Mieno et al.: Orig. life. Evol. Biosph (2015) **45**: 195-205

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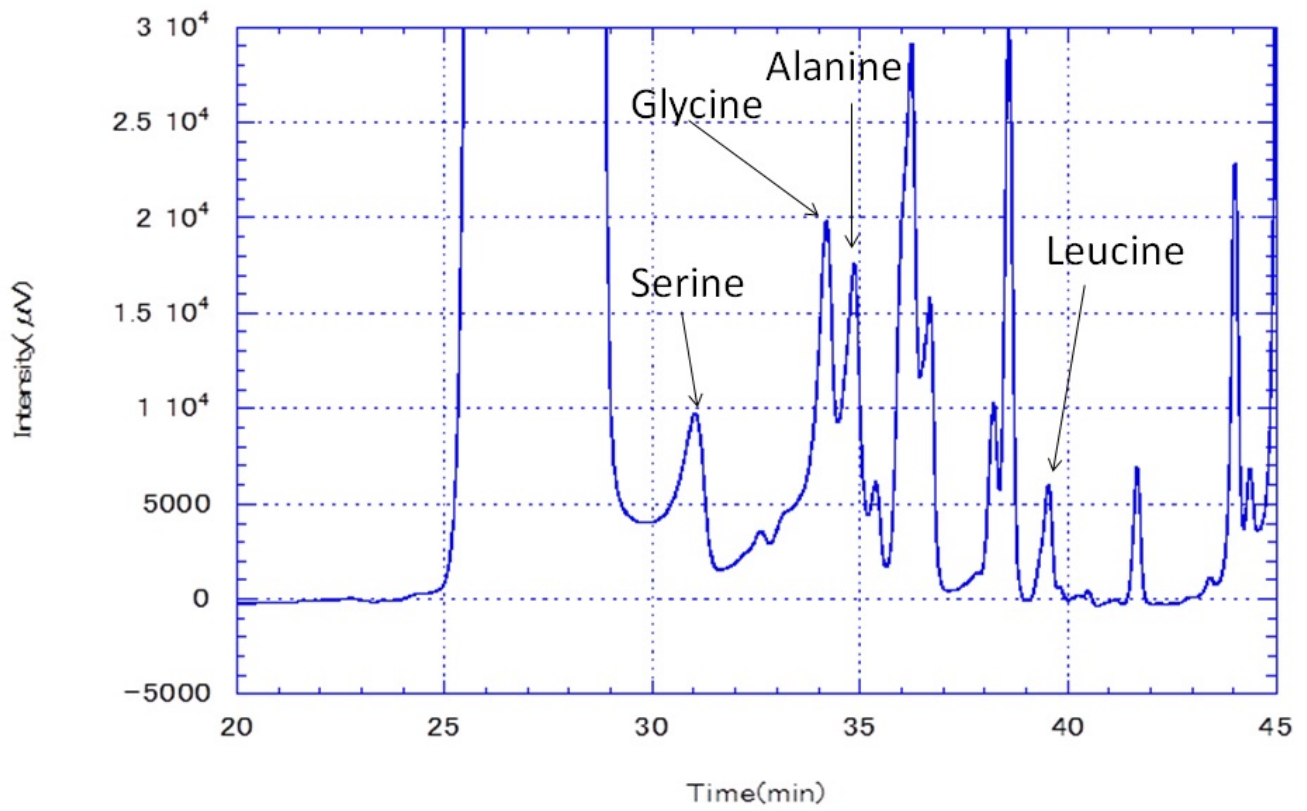


Fig. Example of HPLC data.

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

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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

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

たんぽぽ計画におけるアミノ酸およびその前駆体の宇宙曝露：第1報

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.

キーワード：たんぽぽ、国際宇宙ステーション、機械式宇宙温度計

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 $\pm 5^{\circ}\text{C}$)

たんぽぽエアロゲルパネルに捕集された超高速衝突宇宙塵：初年度地球帰還試料の初期分析結果速報

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.

キーワード : Micrometeoroids、 Space Debris、 Aerogel

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.

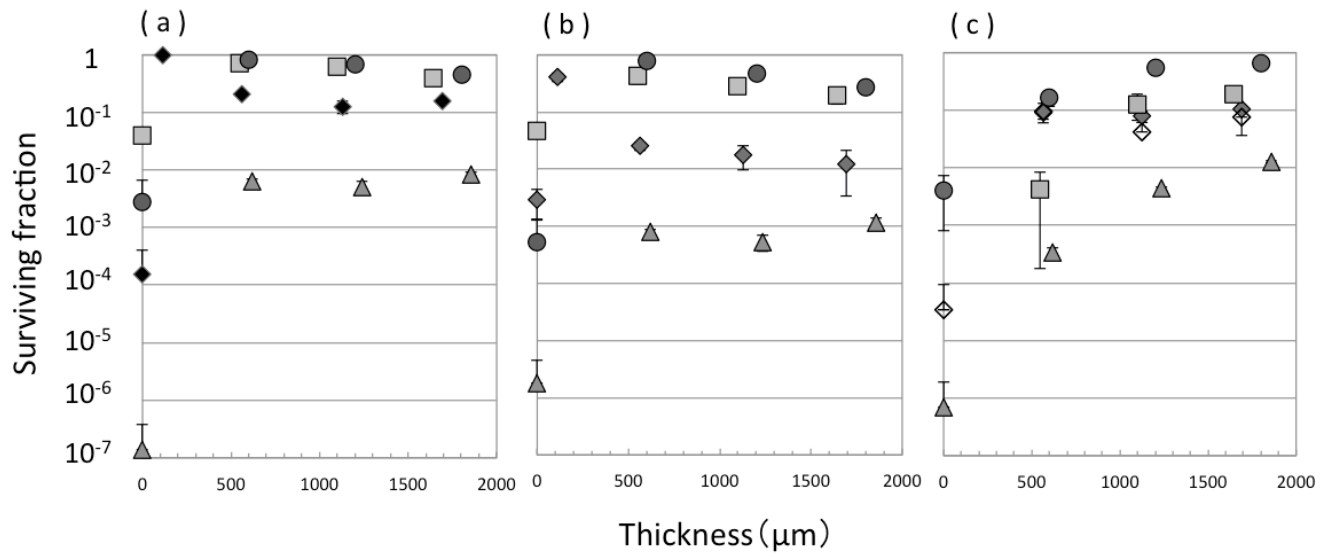
[Method] Dried deinococcal cell-aggregates in wells of aluminum plates were exposed to space for about one year. The dried cells were resuspended in phosphate buffer and recovered from wells. The cell suspension was inoculated to mTGE agar and incubated at 30°C before enumerating colonies. The surviving 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 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.

[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

キーワード：たんぽぽ計画、生存率、DNA損傷

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.

キーワード：パンスペルミア仮説、宇宙曝露実験、凝集体、DNA損傷、たんぽぽ計画、定量PCR

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

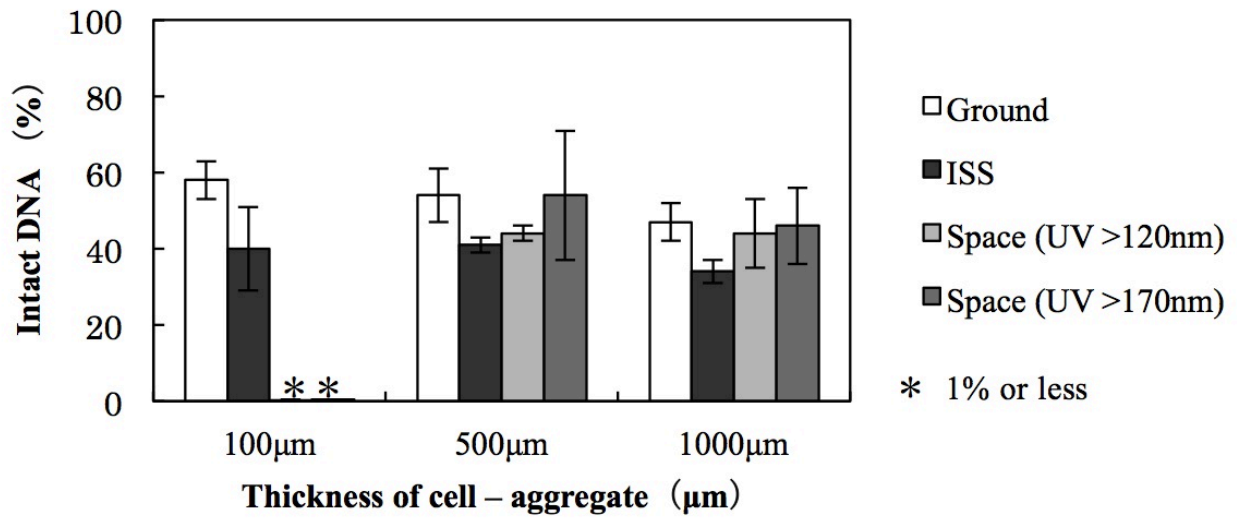


Figure 1 Percentage of Intact DNA

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.

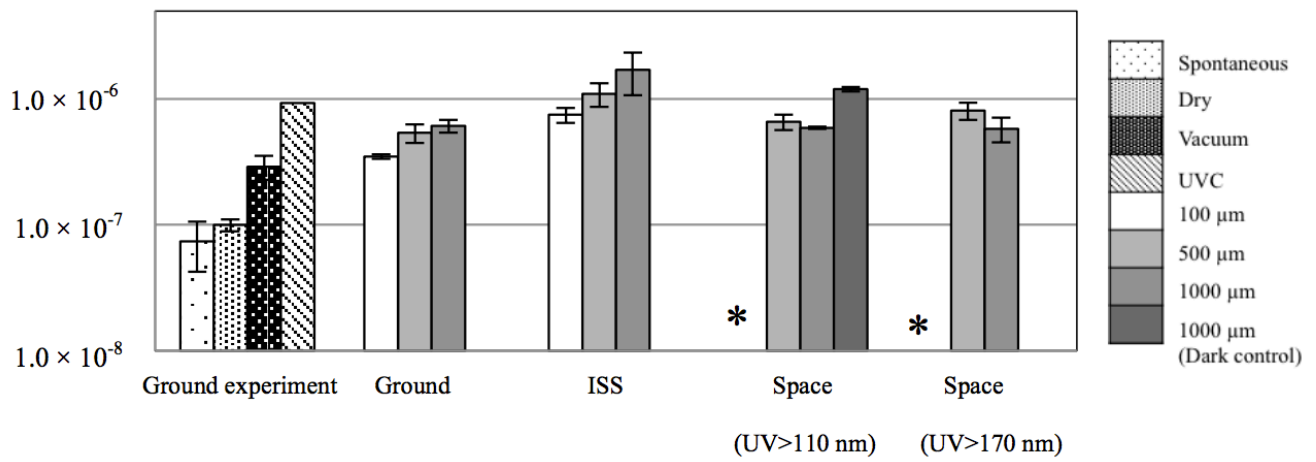
[1] Yamagishi, A. et al., (2007) *Bio. Sci. Space* 21: 67–75.

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[3] Campbell, E. A. et al., (2001) *Cell* 104: 901–912.

キーワード：、、、、

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

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.

キーワード：プロトアーク、ホウ酸、リボース

Keywords: Proto arc, Borate, ribose

好塩性アーキアが生産する脂質コア中の飽和および不飽和アーキオール誘導体の多様性と構造決定の試み

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

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アーキアは全て特徴的な脂質コアであるアーキオール (C_{20} イソプレノイドジエーテル) を持っている。さらに好塩性アーキアは C_{25} イソプレノイドを一つ持つ C_{25} - C_{20} ジエーテル(1)を生産する。これは C_{25} イソプレノイドがグリセロールの二級水酸基側 (C-2) に結合している[1]。また近年Dawsonらは幾つかの超好塩性アーキアでは、アーキオールと C_{25} - C_{20} ジエーテルおよびその不飽和体 (例えば構造 2 が推定されている) が生産され、高塩分培養条件下で不飽和化合物の割合が増加することを報告した[2]。

昨年度本年会にて 1 と 2 を既報[3]に従い合成し、構造解析から 1 の構造は C_{25} イソプレノイドなグリセロールの二級水酸基側 (C-2) に結合していることを確認した。一方構造 2 はそのマスマフラグメントが明らかに異なり、二重結合の位置が異なる異性体が真の不飽和ジエーテルであることが示唆された[4]。

このアーキオール誘導体の多様性について、1) 1 の位置異性体に相当する C_{25} イソプレノイドがグリセロールの一級水酸基側 (C-3) に結合している 3 を調製したところ、Teixidor の報告した岩塩中の C_{25} - C_{20} ジエーテル[5]は 1 と 3 に相当する異性体のほぼ等量混合物であった。これは過去に生育していた、または岩塩中でゆっくりと生育するような、 C_{25} - C_{20} ジエーテルの異性体を膜脂質コアとして利用する、未発見のアーキアが存在する可能性を示唆している。2) Dawsonの不飽和ジエーテルの“真の”構造はテトラエーテル脂質の生合成過程に関する研究結果[6]から、イソプレノイドの末端側に二重結合を持った 4 または 5 であると推定した。化合物 4 および 5 の化学的合成と分析結果の報告を予定している。

[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).

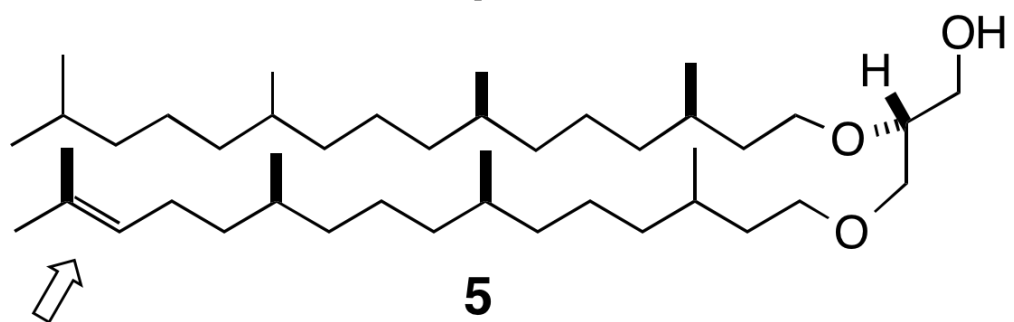
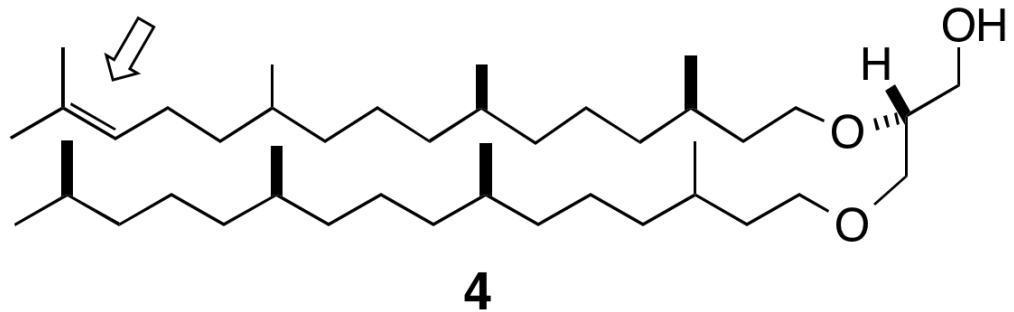
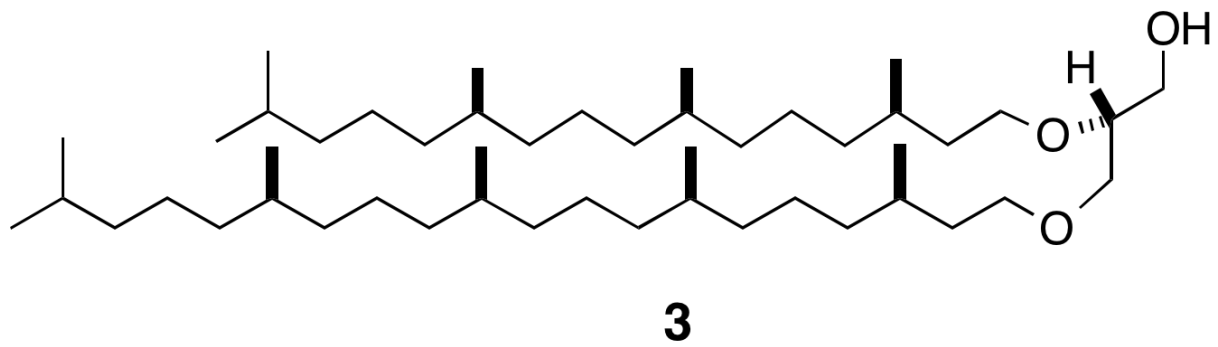
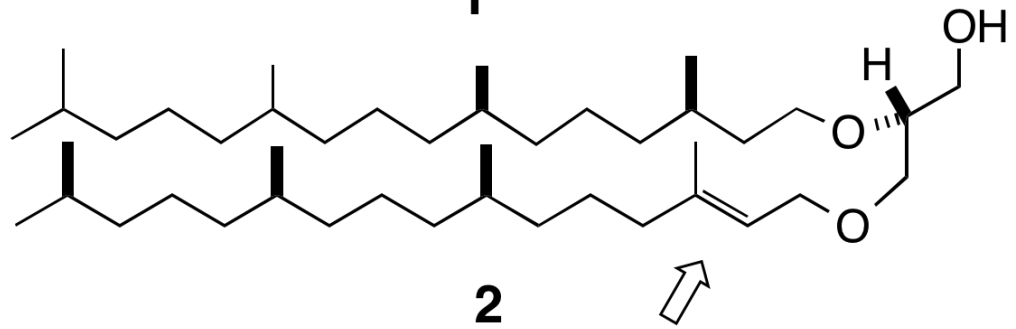
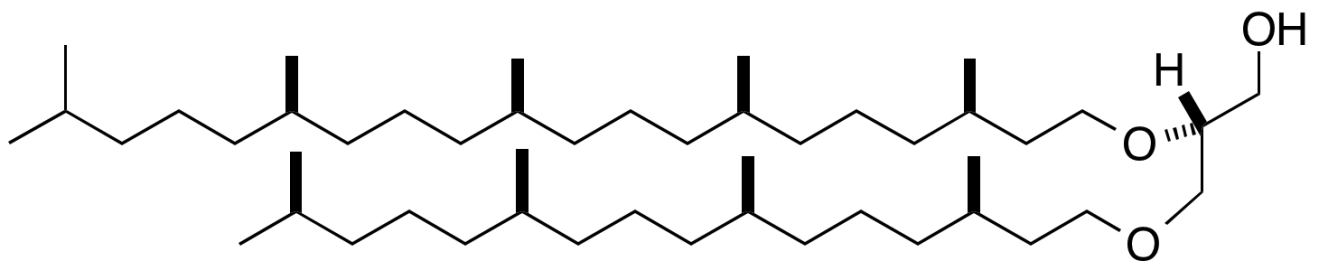
[4] 山内 2016年地球惑星連合大会 BAO01-P05 (2016).

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

キーワード : アーキア、イソプレノイドジエーテル、構造決定、好塩性、岩塩

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



初期の地球で炭酸水の界面に形成されるスパイラル微細構造の検証

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

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分子生物誌の最初の段階は分子間結合の組織と環境間の相互作用により進行しました。水の界面の膜は、炭水化物によって堅牢になる反応性の分子構造です。その炭水化物は、CO₂ と H₂O から作られます。原始地球の大気は、主に CO₂ ガスで構成されていました。高圧下における CO₂ の溶解度が大きく、ヘンリーの法則から、初期の地球の CO₂ の 40気圧が、今日の CO₂ の 0.0004 気圧の 10⁵ 倍に相当します。しかし、CO₂分子は低温でなければ水に溶けません。CO₂ のイオン化の程度は 0.017 です。つまり、炭酸水では CO₂ の 98.3% は線形分子です。線形分子の CO₂ は、次のとおり液体水の三次元構造の貫通孔に組み込まれます。

水の分子は、4 つの sp³ 混成軌道の少し歪んだ四面体として表現されます。短い2 つの O-H 結合部は共有結合性であり、長い2 つの O--H 結合部はイオン結合性です。通常の氷の結晶は六方晶系の (1 h: P6₃/mmc 194) 構造です。P6₃/mmc という構造では交互に滑り平面が有るものの、螺旋状の配置です螺旋状の配置によって、六方晶系の対称性が形成されます。つまり、四面体の短辺と長辺が交互に3 方向の電気的結合力で接続されます。氷結晶の形成では秩序構造の成長が{111} 界面より{100} 界面の方がはるかに速いです[1]。H₂O 分子の微細構造は、界面の面に基づいて形成されます。各スパイラル構造の中心軸に貫通孔の空隙があります。そこに線形のCO₂分子が貫通穴に挿入され、スパイラル構造は炭酸水の格子構造の柔軟性で並び替えられます。こうして、α-水晶の格子構造は平面界面に形成される炭酸水の微細構造を表す最適なモデルであることを検証しました[2]。

[1] Nada, H., J. Physical Chemistry B, 113, (2009), 4790-4798.

[2] Karasawa, S., (2016), <https://youtu.be/azcacA97Qbk>

キーワード：螺旋構造、初期の地球、炭酸水、氷の結晶成長、α水晶

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