Laboratory simulations of Titan tholins formed by cosmic rays

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Titan is the largest moon of Saturn, which has a dense atmosphere mostly consisted of nitrogen and methane. It has been suggested that Titan's atmosphere is an analogue of that of primitive Earth, which is no more remained. Thus the study of the chemical evolution in Titan's atmosphere could give us many important suggestions, and it draws our special attention from the point of view of astrobiology.
Organic materials were detected in Titan's atmosphere. It indicates that an active organic chemistry occurs due to irradiation by solar UV light, Saturnian magnetospheric electrons and cosmic rays as energy sources. Many simulated experiments have been performed by using these energies. Complex organic materials produced in laboratory simulations have often been called tholins, which contain hydrocarbon, nitrile and heterocyclic aromatic moieties. Tholins could give amino acids after interaction with water.
Most laboratory works have simulated reactions in the higher atmosphere of Titan, where solar UV and Saturnian magnetospheric electrons are considered major energies. In the lower atmosphere, however, cosmic rays could have larger contribution than UV [1]. However, there is not many laboratory simulations using cosmic rays. Taniuchi et al. (2013) studied tholins formed by proton irradiation and the produced tholins were analyzed by SEM, AFM, pyrolysis GC/MS and MALDI-TOF-MS [2]. The tholins yielded a wide variety of amino acid precursors after acid hydrolysis, but the structures of amino acid precursors were little known. In this study, we irradiated gas mixtures simulating Titan atmosphere with high energy protons to investigate possible structures and formation mechanisms of tholins.
We prepared a 700 Torr (93 kPa) of gas mixture of nitrogen (95%) and methane (5%) as a simulated Titan tropospheric atmosphere: The pressure corresponds to that of Titan at an altitude of 10 km. The gas mixture was introduced to a Pyrex tube with a Havar foil window. KBr substrates were also placed in the Pyrex tube to sample the products including insoluble fractions. The gas mixtures were irradiated with protons from a Tandem accelerator at Tokyo Institute of Technology. After proton irradiation, KBr substrates were taken out of the tube and were subjected to FT-IR and XANES analysis. XANES analyses were carried at NewSUBARU synchrotron facility at University of Hyogo. The products on the inside Pyrex tube were collected with several kinds of solvents which has different polar character, and were analyzed by ESI-MS. An Aliquot recovered with each solvent was hydrolyzed and subjected to amino acid analysis by ion exchange HPLC.
ESI-MS indicated that tholins made by proton irradiation contained amino acid precursors such as hexamethylenetetramine. XANES and FT-IR analyses showed that the tholins contained amine groups and aliphatic moieties. No clear evidences of aromatic groups were observed. Characteristics of water soluble fraction of the tholins were difference from those of the whole tholins. Spectroscopic results showed that some O-containing groups in the whole tholins, which suggested that trace amount of water could have contaminated to the gas mixture at room temperature. We are now designing the experiments at low temperature to avoid water vapor contamination.
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Keywords: Astrobiology, Titan, cosmic ray, chemical evolution, ESI-MS, FT-IR spectroscopy

Amino acid formation from simulated midly-reducing primitive atmospheres by spark discharges, UV irradiation and proton irradiation

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Supply of bioorganic compounds such as amino acids were crucial for the generation of life on the Earth. If the primitive Earth atmosphere was strongly reducing, endogenous formation of bioorganic compounds was easy by conventional energies such as thundering [1]. In these days, however, it is commonly thought that the primitive Earth atmosphere was not so strongly reducing but only mildly reducing: Its major constituents could have been carbon dioxide and nitrogen, together with some reducing gases as minor components [2]. In the present study, we examined possible formation of amino acids from mildly reducing gas mixtures by spark discharges (thundering), UV (solar radiation) and proton irradiation (cosmic rays).

A gas mixture of 350 Torr of carbon dioxide + methane with various mixing ratio and 350 Torr of N_2 were introduced to a glass tube with 5 mL of pure water. The gas mixtures were subjected to spark discharge by using a Tesla coil, were irradiated with UV light from a deuterium lamp (Hamamatsu Photonics L1835), or were irradiated with 2.5 MeV protons from a Tandem accelerator (Tokyo Tech, Japan). Estimated total energy deposit to the system was 860 kJ (spark discharges), 140 J (UV irradiation) and 3.2 kJ (proton irradiation), respectively. The resulting products were recovered as aqueous solutions, and were subjected to amino acid analysis by HPLC before and after acid hydrolysis. Hereafter the starting gas mixtures are referred to as their methane molar ratio (r = P_{CH4} / P_{CO2} + P_{CH4} + P_{N2}).

In the unhydrolyzed samples, only traces of amino acids were detected. In the case of spark discharges, hydrolyzed samples gave various amino acids if molar ratio of methane was 15% or more, but we could not detect amino acids in the products when molar ratio of methane was 10% or less. In the case of UV irradiation, small amounts of amino acids were detected in the hydrolyzed sample whose methane ratio was 40 % or more. Thus we can say that there is a threshold of methane molar ratio in the production of amino acid precursors by spark discharges or UV irradiation.

On the other hand, we detected amino acids in the proton irradiation products after hydrolysis, even when methane molar ratio was as low as 0.5 %. Glycine yields by proton irradiation was strongly correlated to the molar ratio of methane, and no threshold of methane ratio for the production of amino acid precursors was observed.

In the case of UV irradiation, none of CH_4, CO_2 or N_2 can be dissociated or ionized efficiently by near UV. Amino acid precursors might be formed by triggered by dissociation of water molecule. Further confirmation of the production of such trace amount of amino acids by UV irradiation is necessary.

On the primitive Earth, the energy flux of thundering is estimated to have been much more than that of cosmic rays, but energy yield (G-value) of amino acids by proton irradiation (cosmic rays) was more than that by spark discharges (thundering). By considering them, it is concluded that thundering was a more important energy source than cosmic rays if the methane molar ratio was quite high. On the other hand, if the methane molar ratio was lower than 10%, we cannot expect the formation of amino acids by thundering, while cosmic rays would have been still effective energy sources in quite mildly reducing atmospheres. Sole solar UV light could not be an effective energy source for the production of amino acids in mildly reducing atmosphere, but its flux was so huge. We should examine possible synergy effect of the solar UV and other energies such as cosmic rays

and thundering.
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Keywords: midly-reducing primitive atmospheres, spark discharge, proton irradiation, UV irradiation, amino acids

Temperature Measurement with the Mechanical Space Thermometer for the Tanpopo Mission

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Temperature in the space is very interesting as astrobiology because it control 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 as shown in Fig.1. Nine times of observation of the thermometer was carried out in 2015. The maximum temperature was 26.4±5 ℃. 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: International Space Station, Tanpopo, Mechanical Space Thermometer

No.	Observation Time GMT (2015/D/T)	Sun Beta Angle (degree)	Max. Temp. (℃)	Min. Temp. (℃)
1	153/01:00 ～ 08:00	73.772 ～ 73.738	−8.8±5	−12.5±5
2	159/09:50 ～ 16:50	53.874 ～ 52.542	−11.1±5	−21.0±5
3	164/13:00 ～ 20:00	29.963 ～ 28.611	16.4±5	−0.6±5
4	170/21:00 ～ 171/06:00	1.747 ～ 0.179	17.5±5	−3.4±5
5	184/00:00 ～ 06:00	−28.325 ～ −28.170	23.9±5	−1.6±5
6	194/17:15 ～ 23:40	0.845 ～ −0.263	20.9±5	3.4±5
7	349/03:30 ～ 11:00	−15.081 ～ −16.486	26.4±5	6.9±5
8	355/11:40 ～ 18:00	−45.075 ～ −46.313	−8.9±5	−20.9±5
9	362/11:40 ～ 18:40	−73.652 ～ −74.172	−27.2±5	−35.3±5

Fig.1 Results of Temperature Measurement

Analysis of mutations of *rpoB* gene in *Deinococcus radiodurans* R1 induced by simulated space conditions

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To investigate the microbial viability and their DNA damage, the radioresistant bacteria *Deinococcus* spp. have been exposed at Exposure Facility of International Space Station (ISS) in Tanpopo mission since May 2015 [1]. The Exposure Panels (EPs) harboring dried-deinococcal cells will return to the ground after about one-, two- and three-year exposure. We are going to analyze the survival rate and DNA damage of dried deinococcal cells using pulsed-field gel electrophoresis, quantitative-PCR and mutation assay. The antibiotics rifampicin binds the RNA polymerase β -subunit, which is encoded by *rpoB* gene, and inhibits the initial step of the transcription activity. Certain mutations in the *rpoB* gene confer rifampicin resistance [2]. Based on the above understanding, we will determine mutant frequency and the mutation spectrum for the *D. radiodurans* *rpoB* gene. From these mutation data, we will estimate major DNA damage induced by space environment. For this purpose, the mutatagenic specificity of the *D. radiodurans rpoB* gene in simulated space conditions was investigated in this study.

The *D. radiodurans* R1 cell-suspension was dropped in the wells of aluminum plates (φ20 mm) and was dried under vacuum (vacuum-dried). The dried cells were exposed to vacuum (< 10^{-5} torr) or UVC_{254nm} under the vacuum conditions. As a control, we analyzed the vacuum-dried cells without additional vacuum incubation. After exposure experiment, the cells were recovered from each well. inoculated to 10 ml of mTGE medium and cultured to show the $OD_{590\ nm}$ to be about 4. The cell suspension was plated on mTGE agar containing 50μg/ml of rifampicin to determine the number of rifampicin resistant colonies (Rif^R), and on mTGE agar without rifampicin to determine the total number of viable colonies.

The rifampicin-resistant mutant frequency of vacuum-dried cells was 1.3 (±0.5) $\times 10^{-8}$. The rifampicin-resistant mutant frequency of the *D. radiodurans* R1 wet cells has been shown to be about 1.5 $\times 10^{-8}$ [3]. The result suggests that the rifampicin-resistant mutant frequencies of vacuum-dried cells and wet cells are comparable for *D. radiodurans* R1. Further, we will report and discuss the rifampicin-resistant mutant frequency and mutation spectra in the *rpoB* gene of rifampicin-resistant cells following exposure to UVC_{254nm} and vacuum (< 10^{-5} torr).

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Keywords: ISS, Tanpopo mission, mutatuion

Attempt to the structure determination of unsaturated archaeol derivatives characteristic for the halophilic archaea lipid-core produced at very high salt concentration

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Archaea has a characteristic lipid-core, archaeol. The structure of archaeol is those in which two C_{20}-saturated isoprenoid are linked to glycerol by ether bond. 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 regiochemisty of the hydrocarbon bonded with glycerol had been determined[1][2]. The C_{25} (long) hydrocarbon is linked with the C-2 of the glycerol.
Existence of archeol derivatives having unsaturated isoprenoid were reported at the lipid-core of psychrophilic[3] and thermophilic[4] archaea. 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[5]. Further, the relation of salinity and the ratio of unsaturated lipid-core was discussed. On the other hands, C_{20} -C_{25} diether and unsaturated derivatives were existed in the lipid-core in this literature. However, the different regiochemical structure 3 were presented (C_{25} hydrocarbon was linked with the C-3 of the glycerol).
During my experiments for the deterimination of the regiochemistry and carbon number of the hydrocarbon of the diether, the general chemical synthetic method for the unsymmetric diether was developed[6]. Therefore, the unsymmetric diether 1 and 2 were prepared in my experiments for the confirmation/determination of the structure of several diether reported at Dawson's literature. Then, 1 and 2 were chemically synthesized according to the reported method of an intermadiate in the synthesis of archael tetraether. The analysis of the mass fragmentation of the TMS derivative, the mass spectrum in Dawson's report was revealed to the isomer 1. The structure of microbiological sample derived from halophilc archaea was confirmed as 1. The determination of precise structure of the varaiety of unsaturated isoprenoid diethers will be presented by the comparision of the mass spectra of 2 with those of Dawson's unsaturated diether.

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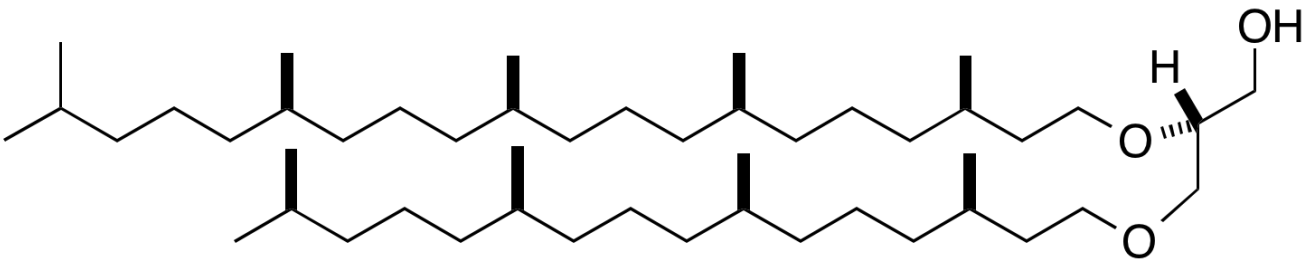
Keywords: halophilic archaea, lipid-core, structure determination



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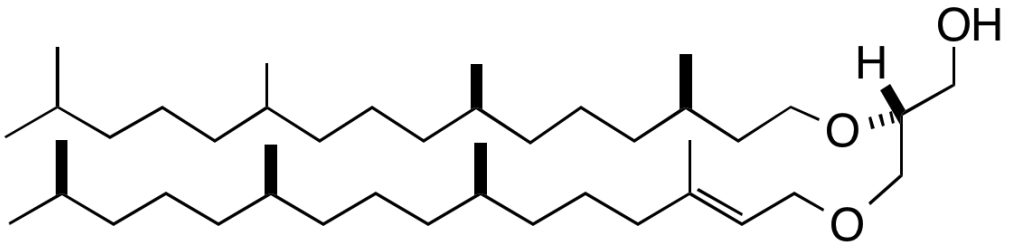

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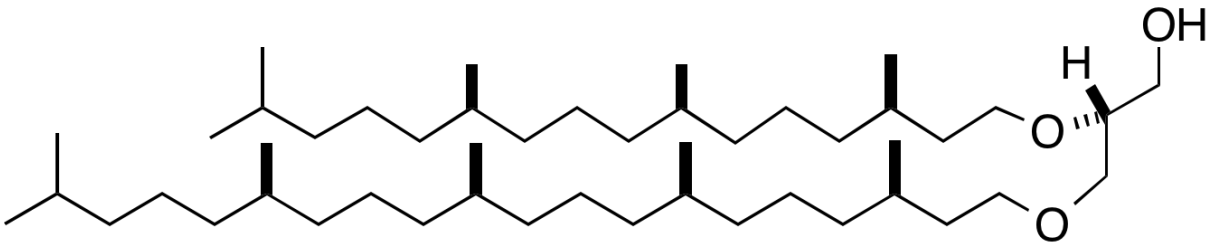

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