

Phosphorus adsorption dynamics and retention capacity in agriculture drainage ditch sediments

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A number of interactions occur between ditch sediment and overlaying water, in which P adsorption is a major process in controlling soluble P concentration in water. Drainage ditches in Kasaoka reclaimed land, Japan is under alkaline conditions (pH >8), because drainage water is diluted with seawater. Our surveys have also demonstrated that ditches were contaminated with high concentration of phosphorus (P). Sediment has a vital role in removing P from discharge water by adsorption. Previous studies have reported sediment P retention capacity under acidic conditions, but little information on phosphorus retention capacity under alkaline conditions was reported. This study aims to evaluate P equilibrium between ditch sediment and overlaying water, and P retention capacity of sediment by determining adsorption parameters under alkaline conditions. Three sub-experiments were carried out in order: (1) adsorption kinetic measurement; (2) adsorption at ambient water P for zero equilibrium P concentration (EPC₀) and P buffering capacity estimation; and (3) sediment P retention capacity determination by using the Langmuir model. Surface sediment (0-10 cm) and overlaying water were collected in three drainage ditches, which receive drainage water from livestock-horticulture area (LHA), livestock area (LA) and grassland area (GLA). In addition, sediment core layers (0-2, 2-4, 4-6, 6-8, 8-10 cm) were taken for evaluation of pore water P concentrations. Results showed that pore-water P of LA and LHA increased with increasing the depth layers, whilst GLA sediment decreased. Sediment particle sizes showed a two-third proportion of silt contents. The kinetic adsorption of sediments consisted of two stages that were quick and slow stages regardless of sites. The quick stage was within the first hour of incubation, and slow stage afterward. The LA drainage ditch, which was more contaminated with P, was the highest the sediment EPC₀, followed by LHA, and GLA ditches. The sediment EPC₀ indicated that three ditch sediments act as a sink for P across sediment surface. The Langmuir models were fitted well with experimental data and adequately describe adsorption isotherms of sediments in this study ($r^2 > 0.95$). Phosphorus retention capacity of sediments by maximum adsorption calculations ranged from 384.2-416.7 mg kg⁻¹, binding energy (K) (0.195-0.263 L mg⁻¹). Our results indicate the importance of ditch sediment in controlling P dynamics discharged from agricultural farms.

Keywords: adsorption, Agricultural drainage ditches, sediment, phosphorus, retention capacity

Survivality of deinococci under space conditions ? Toward the space exposure experiment in "TANPOPO" mission at ISS

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The "panspermia hypothesis" has been proposed as one of the hypotheses on the origin of terrestrial life. In this hypothesis, possible interplanetary migration of microbes has been considered.

To address the question whether interplanetary migration of terrestrial microbes are possible, we have proposed and prepared the space capture and space exposure experiments of terrestrial microbes as two of six sub-themes of "TANPOPO mission" on the Exposure Faculty of Japanese Experiment Module "KIBO" of International Space Station (ISS) (Yamagishi et al., 2008). In this mission, we are going to expose microbes in space for one to three (or more) years. There are harsh environmental factors (vacuum, high UV irradiation, ionization radiation, and so on) in space. We have selected the species that show high tolerance to the space conditions.

We are going to use UV- and radiation-resistant deinococcal species: four stains of *Deinococcus radiodurans* (R1, KH311, rec30, and UVS78), *Deinococcus aereus* TR0125, and *Deinococcus aetherius* ST0316. *D. aereus* and *D. aetherius* were isolated from upper troposphere and lower stratosphere, respectively (Yang et al. 2009, 2010). They showed high tolerance to UV and radiation, similar to or higher than *D. radiodurans* R1. In addition, since the DNA repair systems are known to be the keys of high tolerance to UV and radiation in deinococcal species, space survivability of *D. radiodurans* R1 (wild type strain) will be compared with those of the DNA repair deficient mutant strains of *D. radiodurans*, KH311 (deficient mutant strain of *pprA* gene for non-homologous end-joining (NHEJ) repair), rec30 (deficient mutant strain of *recA* gene for homologous recombination), and UVS78 (deficient mutant strain of *uvrA* and *uvrB* genes for nucleotide excision repair).

In this paper, we summarize survivability of deinococcal species for UV-irradiation, heavy ion-irradiation, high vacuum, and periodical change of temperature. Then, we evaluate survivability of deinococcal species in space after one year.

References

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Keywords: International Space Station, Panspermia, Microbe space exposure experiment, "Tanpopo" mission

Exposure experiments of amino acids and their precursors at the exposure facility on ISS

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Since a diverse suite of amino acids is found in carbonaceous chondrites, exogenous delivery of organic matter could have played an important role for the prebiotic chemical evolution on the early Earth. The interplanetary dust particles (IDPs) are considered to be the major carbon source [1]. However, the organic matter in IDPs is susceptible to the cosmic and solar radiation due to their small nature.

The Tanpopo mission consists of capture experiments and exposure experiments of organic matter and microbes at the Exposure Facility of Japan Experimental Module (JEM) "Kibo" on the International Space Station (ISS), which aims to investigate possible interplanetary migrations of organics and microbes. Here we report the exposure experiments of amino acids and their precursor molecules to the space environment to explore their alteration and survivability.

Selected organic compounds are amino acids (glycine and isovaline), their possible precursors (hydantoin and 5-ethyl-5-methyl hydantoin), and a complex amino acid precursor material synthesized from a mixture of carbon monoxide, ammonia and water by proton irradiation (here after called "CAW"). The amino acid water solutions, hydantoin ethanol solutions, and CAW were put into 3 μ L pits on the exposure panels, and dried under a clean booth. The solid samples were left on the pits ca. 75 nmol each for amino acids and hydantoins, and 15 nmol equivalent to glycine for CAW. Then the samples were covered with hexatriacontane (C₃₆H₇₄) in order to prevent the sample lost during the experiments and transportations. The exposure panels will be launched in this year, and will be recovered after one year, two years and three years of exposure.

[1] Chyba C. and Sagan C. (1992) Nature, 355, 125-132.

Keywords: Tanpopo Mission, origins of life

Tolerance of heavy ions in a terrestrial cyanobacterium, *Nostoc* sp. HK-01

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A terrestrial cyanobacterium, *Nostoc* sp. HK-01, is a candidate species for the Tanpopo Project because of several tolerances to severe Space environments. Heavy ions are especially severe environmental elements and can cause DNA and other serious damages to living organisms. We showed the tolerance to heavy ions in *Nostoc* sp. HK-01.

A dried colony of HK-01 was exposed to heavy ions (He and Ar) in HIMAC. Survival abilities were investigated by FDA cell staining and a growth method. We will present the results on HK-01 as a candidate organism for the Tanpopo Project.

Keywords: Cyanobacteria, Heavy ions tolerance, *Nostoc* sp. HK-01

Biological contamination control for silica aerogels in the Tanpopo mission

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The capture and exposure panels of the Tanpopo mission, in which the panspermia hypothesis and chemical evolution are investigated, are onboard the Japanese Experimental Module (JEM) of International Space Station at about 400 km altitude since this spring. Micrometeoroid space debris and possible terrestrial aerosols are expected to be captured by ultra low-density silica aerogels that would be exposed in space at least one year. After returning back to the earth, the aerogels with "carrot-shaped" tracks formed by hypervelocity impacts of these microparticles should be documented, dissected and delivered to scientists for detailed analysis as soon as possible. During this process, biological contamination as well as chemical one must be avoided (Kiyonaga et al., 2013). Thus, in this study - (1) fabrication of aerogel processing machine called "Yokan Machine", (2) novel evaluation method for biological contamination, and (3) a procedure for pre-flight aerogel surface observation - will be reported from pre-flight simulation in the Tanpopo clean room at ISAS. Time required for the processing of aerogels was estimated from (1). Microbial contamination possibly from human skin during the period was evaluated using several methods including conventional particle counting and chemiluminescence. A novel method using model microbes was also performed. Methods for biological contamination control will be discussed.

Reference

Y. Kiyonaga et al. (2013) Method for Biological Contamination Monitoring During Aerogel Cutting Process in Tanpopo Project Using Bioluminescent Bacteria *Photobacterium kishitani*. In International Astrobiology Workshop 2013, p. 33. LPI Contribution No. 1766, Lunar and Planetary Institute, Houston.

Keywords: Tanpopo mission, ISS, biological contamination

Analysis of Amino Acid Precursors Formed by Particles Irradiation of Possible Interstellar Media

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A wide variety of organic compounds including amino acids have been detected in extraterrestrial bodies such as carbonaceous chondrites and comets. Since it was suggested that complex organics found in the extraterrestrial bodies were formed in extreme cold environments, one of the possible sites for the formation of the organics was in interstellar dusts in molecular clouds. We reported that amino acid precursors could be easily formed from possible interstellar media (H₂O, NH₃, CO, CH₄ and/or CH₃OH) by irradiation with protons or heavy ions. In the present paper, possible interstellar media with various mixing ratios were irradiated with high energy particles, and determined amino acids after acid hydrolysis of the products. We also characterized the products by FT-IR.

Gaseous mixtures of CO, CH₄ and NH₃ with various ratios (total pressure: 700 Torr) with 5 mL of liquid water were introduced in Pyrex tubes with Havar foils. Each Pyrex tube was irradiated with 2.5 MeV protons from a Tandem accelerator (TIT): Total electric quantity was 2 mC each. Liquid mixtures of CH₃OH, NH₃ and H₂O with various ratios were irradiated with 290 MeV/u carbon ions from the HIMAC accelerator (NIRS) for up to 4 hours at the dose rate of 3.75 kGy/h. Amino acids were determined by cation exchange HPLC before and after acid hydrolysis. Products, both before and after acid hydrolysis, were analyzed FT-IR for characterization.

Proton irradiation of the gaseous mixtures yielded only trace of amino acids before hydrolysis, but wide variety of amino acids were detected after hydrolysis. G-value of glycine from 1:1 a mixture of CO and NH₃ with water (CAW) was as high as ca. 0.3. When CH₄ was added to the gas mixture (CMAW), glycine yield was largely decreased, but alanine yield was drastically increased, which was sometimes more than glycine. FT-IR spectra of both CAW and CMAW (before hydrolysis) showed peaks at 1670 cm⁻¹ (amide C=O), and those of CMAW showed peaks at 1750 cm⁻¹ (carboxylic acid or ester C=O). The amide C=O peaks were disappeared after hydrolysis.

Heavy ion irradiation of the liquid mixtures also yielded amino acids after hydrolysis. When ratio of CH₃OH and NH₃ to H₂O were decreased, amino acid yield decreased, but even in the case of CH₃OH: NH₃: H₂O = 10: 1: 37, amino acids were detected. In the HIMAC experiment, the energy of heavy ions were quite high and pass through the target mixtures with only small part of energy was deposited to the target, which is the same situation as what happens in dense clouds. The present results suggested that amino acid precursors can be formed in water-rich ice mantles of interstellar dust particles (ISDs) by the action of cosmic rays.

We are planning to irradiate ice (solid) mixtures simulated interstellar ices with heavy ions, and to compare amino acid yields and structures of the complex organic products with liquid phase irradiation. Characterization of the products will be extended to LC/ESI-MS and XANES.

Effects by pH on the peptide-binding site of two aspartic acid molecules

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Proteins, which have important roles as enzymes in many biological reactions, are consisted of 20 kinds of L- α -amino acids. These amino acids are connected with peptide bonds that combine N in α -amino group to C in α -carboxyl group. There are several proteinogenic amino acids containing two carboxyl groups or two amino groups. Even these amino acids, natural peptide bond found in proteins connects the α -amino group to the α -carboxyl group. The regioselective peptide bounding might have been formed in early stage of chemical evolution because reactive side chain of these amino acids are important for basic functions of proteins. In such case, geological setting or geological events must lead the regioselective peptide bound. In this study, we tried to constrain geological setting for regionselectivity using aspartic acid (Asp) as a model amino acid. Asp has α - and β -carboxyl group that have slightly different pKa. To evaluate favorable geological settings for peptide formation with α -carboxyl carbon, we investigated the effects by different pH at high temperature and high pressure simulating difference in pH of pore water in deep-sea sediments. Asp solutions with pH ranging from 1.5 to 12.1 were heated and compressed for 1 – 8 days at 100 °C and 100 MPa. After incubation, the products were analyzed by liquid chromatography mass spectrometry. We also investigated the effects by pH on decomposition rate of Asp to evaluated suitable pH conditions for α -peptide formation. The decomposition rates of Asp were greater in higher pH and the peptides formed were different with varying pH. These results suggest that environments suitable for α -peptide formation were limited by pH.

Keywords: Aspartic acid, pH, peptides formation

The possibilities for life on Titan - the constraints for methanogenic bacteria with acetylene-based metabolic pathways

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Previous studies have discussed the possibilities for methanogenic bacteria (Methanogens) on Titan. These bacteria were assumed to use chemical reactions which emitted methane to the environment of Titan. We focused on one of these reactions ($C_2H_2 + H_2 \rightarrow 2CH_4$), which hydrogenates acetylene. We calculated the Gibbs free energy which can be obtained from this reaction under the environmental conditions (e.g., temperature and pressure) of Titan. We also discussed the possibilities for these bacteria to survive under the kinetic constraints on Titan applying the method proposed by Seto [2014].

Keywords: Titan, astrobiology, Methanogens, acetylene, bacteria

Studies on analytical methods for amino acids to evaluate biological activities in extreme environments

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Biological activities have been detected in such terrestrial extreme environments as hot springs, submarine hydrothermal systems, deserts and polar environments. Since amino acids are essential constituents for terrestrial organisms, they could be good indicator to evaluate biological activities in extreme environments. In order to analyze amino acids in soil and rock samples, they should be extracted from mineral matrices. Hot water extraction has been widely used to extract amino acids from geological samples, but we previously found that only a small part of amino acids could be released by the method. In the present work, we examined several extraction methods for amino acid analysis, including HF digestion and HCl extraction.

Samples used were (1) soil samples in Yokohama National University (YNU) main campus (ordinary environment), (2) soil samples near the Showa Station, Antarctica, sampled by JARE 49 in 2007-8, and (3) soil samples in Atacama Desert, Chile, sampled in 2002. Procedural blank was prepared by performing the experiment procedure without samples.

Amino acid concentration in Atacama Desert soil and in Antarctica soil (except in Penguin rookeries) was much less than that in YNU campus soils.

In the HF digestion, 0.1 g of sample was heated with 3 mL of 5 M HF-0.1 M HCl in a Teflon vessel at 110 °C for 24 hours, then was acid hydrolyzed in 6 M HCl at 110 °C for 24 hours. In the HCl extraction, 0.1 g of sample was heated with 3 mL of 6 M HCl at 110 °C for 12-48 hours. In the conventional hot water extraction, 0.1 g of sample was first heated in 1.5 mL of water at 110 °C for 24 hours, and then the extract was filtered through a membrane filter: The filtrate was subjected to the HCl hydrolysis for 24 hours. In the modified hot water extraction, 0.1 g of sample was heated with 1.5 mL of water at 110 °C for 24 hours, followed by the acid-hydrolysis without filtration. All the hydrolysates were subjected to amino acid analysis by cation exchange HPLC with post-column derivatization with o-phthalaldehyde and N-acetyl-L-cysteine for fluorometric detection.

The HF digestion, the HCl extraction and the modified hot water extraction gave much higher amount of amino acids than the conventional hot water extraction. The HF digestion, however, showed higher procedural blank than the other methods. In the HCl extractions, heating for longer time yielded less amino acids. Thus the HCl extraction with shorter heating time was recommended for amino acid analysis of geological samples.

Further works, including development of high resolution, high sensitivity method for determination of amino acid enantiomers, will be done.

Keywords: Extreme environment, Amino acid, Biological activity, Extraction method, Antarctica, Atacama Desert

The pH profiles of the catalytic efficiency and thermal stability of resurrected ancestral proteins

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Little is known about the geological setting of the early earth. However, the method to estimate the ancient environment with genetic information has been emerging (Akanuma *et al.*, 2013). The genetic information of ancestral life has been inherited to its descendants. We can therefore infer the amino acid sequence of an ancestral protein by comparing a huge number of extant amino acid sequences that have evolved from a single common ancestor. Because the amino acid sequence of a protein is encoded by DNA sequence, an ancestral protein's amino acid sequence has been also inherited in its descendants, i.e. extant proteins' sequences. We can estimate the ancient environment by reconstructing an ancestral protein and analyzing its physicochemical properties. In addition, physicochemical properties of a protein have often related to the environment of its host.

We have previously estimate the environment of early life by resurrecting ancestral nucleoside diphosphate kinases (NDKs). The amino acid sequences of ancestral NDKs that might be possessed by the last common ancestors of Archaea and of Bacteria were inferred by phylogenetic analyses. The inferred amino acid sequences were reconstructed by using the genetic engineering techniques. Because the ancestral amino acid sequences fold into extremely thermally stable proteins, we concluded that both archaeal and bacterial ancestors were hyperthermophilic. This conclusion is robust because significantly similar characteristics were observed for the ancestral proteins predicted by several different methods. We also estimated that the last universal common ancestor, the Commonote, was a thermophile or a hyperthermophile that thrived at a temperature above 75 °C (Akanuma *et al.*, 2013).

In the current study, we have attempted to estimate the surface pH of early earth. We analyzed the pH profiles for catalytic efficiency and thermal stability of the ancestral NDKs that might exist 3.5-4.0 billion years ago. The specific activities at 70 °C were determined at pHs ranging from 5.5 to 10.0. All of the ancestral NDKs showed the highest activity at pH 9.5 or 10. The same was true for several NDKs of extant microorganisms that grow optimally at an acidic or neutral pH. Therefore, the optimum pH for catalytic efficiency of a NDK does not reflect its host's environment.

We also analyzed the pH dependence of thermal stability of the ancestral and extant NDKs. The extant NDKs from *Sulfolobus tolodaii* and *Thermoplasma acidophilum*, which grow optimally at acidic pHs, are stable at both acid and neutral pHs. In contrast, the NDKs of *Thermus thermophilus* and *Archaeoglobus fulgidus*, which grow optimally at neutral pHs, showed the greatest thermal stability at a neutral pH (pH 6.0 or 7.6) and less stability at an acidic pH (pH 4.5). Because most of the ancestral NDKs also showed the greatest thermal stability at pH 6.0 or 7.6 and were less stable at pH 4.5, we concluded that the ancient organisms such as the archaeal ancestor, bacterial ancestor and the Commonote lived at neutral pHs. However, we cannot rule out the possibility that the ancient organisms lived at an acidic pH because a few ancestral NDKs showed the greatest thermal stability at pH 4.5.

Akanuma *et al.*, *PNAS* 110 11067-11072

Keywords: ancestral protein resurrection, Commonote, nucleoside diphosphate kinase, hyperthermophile

Observations of the cluster of water at vicinity of ice in carbonated water

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We can observe many fine particles in the carbonated water as shown in the photograph (Phot.1). The spiral structure of water is based on planar interconnections with the vertical cohesion. It will form a sphere. Particles made of carbonated water are observed on the ice of the central, and cluster of water with similar size are observed on the surface of the liquid. The cluster of water possessing with spiral structure is able to play an important role for the chemical evolution. The surface of sea water is the candidate of early stage on the chemical evolution.

The carbon dioxide is dissolved in the water at the lower temperature. The carbon dioxide in the early atmosphere was dissolved in the early sea. The bubbles were produced by the carbon dioxide. The floating substances are collided with molecules from the atmosphere. Neighboring atoms can be exchanged by the thermal motion in the substance. Here, there is the adaptability that is caused by the electronic structure. Therefore, complex molecules were synthesized from the floating substance by the energy that comes from outer world such as ultraviolet ray. The compounds of molecules will be evolved through the repetition of production and destruction. The floating substance such as bubbles will accumulate at the surface of water. But, the floating material is small compared with the surface of the sea. There are great amount of sea water. The liquid water is divided to clusters of water molecules. Although life period of the cluster is short, it contributes to the chemical reaction.

The photograph was taken at close distance of 1cm by using a digital camera PENTAX Optio-W90 under the reflected light. Here, the magnification is the maximum. The background was black in order to absorb the light. A shape of fine particle is recognized by a still image. The flicker for human's eye is caused by quick motion of the particle. The movement of particle was observed by frame-by-frame in the pictures of the movie at 30 frames per a second. Please see the movie at website [<https://www.youtube.com/watch?v=5dqmqmFpYQhc&feature=youtu.be>] on the bubble behavior in vicinity of the ice of carbonated water.

[Photograph 1]

Particles made of carbonated water are observed on the ice of the central, and cluster of water with similar size are observed on the surface of the liquid.

Keywords: chemical evolution, cluster of water, spiral structure, carbonated water, bubble, membrane

