

Understanding the generality of terrestrial life

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We only know the life on the Earth. In this presentation I am going to evaluate the system especially genetic system of terrestrial life. Water is needed for life to solubilize the molecules and especially important to maintain the structure of protein. Protein is needed to almost all activity of life, especially catalytic activity that is carried by the protein called enzyme. All terrestrial living organism consists of (a) cell(s), surrounded by membrane. Membrane is maintained by the hydrophobic interaction driven by the hydrogen-bonding network between water molecules. Genetic information is used to form the structure of enzymes to induce catalytic activity, forming the arrangement of amino acid residues. What are the necessities and what are the coincidences? I will present some clues to the questions. These can be useful for searching for life in the Universe.

Keywords: Cell, Organic compounds, Cell membrane, Genetic material

Extremophiles under pressure: Integrating experimental and field studies at deep-sea hydrothermal vent sites

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In the great depths of the Earth's interior, forces capable of moving continents sculpt the seafloor and form volcanoes transferring materials and energy to the ocean. Seawater circulation within these magmatic provinces results in the development of deep-sea hydrothermal vents that are often considered to resemble the conditions on early Earth. It is under these extreme conditions that, by a synergy of chemistry and biology, life thrives in the absence of light and sparse nutrients. Notably, anaerobic chemolithoautotrophic bacteria that inhabit these extreme environments tend to branch deeply in the tree of life, probably retaining relics of early metabolic pathways.

Here, we present data from shipboard continuous-culturing incubations of hydrothermal vent fluids conducted during an R/V Atlantis-ROV Jason/Medea expedition to the deep-sea vents sites (2500 m) at 9°50'N East Pacific Rise (January of 2014). This was accomplished through a multi-disciplinary and multi-institutional collaborative effort to collect; transfer and culture vent fluids from the diffuse flow sites, onboard the vessel under seafloor pressure conditions (250 atm). Experiments were designed to study the cycling of N through the metabolic processes of denitrification and dissimilatory nitrate reduction to ammonia (DNRA) under *in-situ* deep-sea vent temperature and pressure conditions.

In detail, we studied the growth/metabolism of nitrate-reducing microorganisms at mesophilic (30 °C) and thermophilic (60 °C) conditions at pressures ranging from 5 to 250 atm. A high pressure bioreactor was employed onboard the ship to allow direct study of the biological functions of microorganisms collected from diffuse-flow vent fluids (Fig. 1). This experimental approach facilitates continuous culture of microorganisms at temperatures ranging from 25 to 120 °C and pressures up to 680 atm. The system allows incubating microbial communities in medium enriched with dissolved gases, under aerobic or anaerobic conditions, while permitting periodic sampling of the incubated organisms with minimal physical/chemical disturbance inside the reactor.

Experiments conducted simulate the subsurface biosphere environment and the continuous mixing of seawater and hydrothermal fluids in the oceanic crust. Vent fluids were delivered to the bioreactor under high pressure and homogeneously mixed with aqueous media solution enriched in dissolved nitrate, hydrogen, and ¹³C-labeled bicarbonate to facilitate the growth of nitrate-reducing chemolithoautotrophic bacteria. Two distinct sets of experiments were carried out for 356 and 100 hours. During the course of the experiments we monitored the growth of deep-sea microbial communities by measuring cell density and the concentrations of dissolved aqueous species directly involved in nitrate based metabolism, such as NO₃⁻, NH₄⁺, H_{2(aq)} and H_{2S(aq)}. Subsamples were also collected for a number of shore based analyses to determine: i) the ¹⁵N/¹⁴N isotope composition of NO₃⁻/NH₄⁺ and constrain kinetic isotope effects associated with denitrification/DNRA; ii) to study the rates of autotrophic carbon fixation by NanoSIMS; iii) to perform single cell genomics on the microbial populations grown in the bioreactor and (iv) to isolate and characterize novel microorganisms from the communities established in these experiments. In short, experimental results constrain the function and metabolic rates of the native denitrifying microbial communities residing at moderate temperature conditions (30 °C), while DNRA metabolic pathways were identified for the populations residing at higher temperature diffuse flow fluids (60 °C).

Keywords: extremophiles, deep-sea hydrothermal vents, chemolithoautotrophic bacteria, high pressure, continuous culture, nitrate-based metabolism

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Robustness of the thermophilicity of ancient organisms

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All of the modern organisms are thought to have evolved from a single common ancestor named Commonote (1). In order to investigate the environmental temperatures of the ancient organisms, we resurrected amino acid sequences of ancestral nucleoside diphosphate kinases (NDKs) that might be hosted by the last common ancestors of Archaea and of Bacteria. The enzyme catalyzes the transfer of a phosphate from a nucleoside triphosphate to a nucleoside diphosphate. The ancestor of NDK family is thought to have been possessed by the ancient organisms because most extant cells, from bacteria to human, contain the gene(s) that encode a member of this family of proteins. More importantly, the denaturation temperature of a NDK correlates well with the optimal growth temperature of its host. Therefore, we can estimate the environmental temperature of the ancient organisms by reconstruction ancestral NDK's amino acid sequences and characterizing their thermal stabilities. In our previous study (2), the ancestral amino acid sequences of NDK were inferred from two phylogenetic trees with different topologies using a maximum likelihood program. The sequences were then reconstructed and characterized. From thermal denaturation experiments of the reconstructed enzymes, we estimated that the common ancestors of Archaea and of Bacteria lived at 81-97 °C and 80-94 °C, respectively. The Commonote was also likely to be a (hyper)thermophile that lived at a temperature above 75 °C. However, a criticism for our conclusion is that the ancestral sequences have been inferred with an assumption that the amino acid composition has been constant through evolutionary time. Gouy and coworkers (3) predicted the amino acid sequences of ancestral proteins using a Bayesian method that does not assume the constant evolutionary process through time. They estimated the optimal environmental temperature of the ancestral organisms from the amino acid composition of seven amino acid types: leucine, isoleucine, valine, tyrosine, tryptophan, arginine, and glutamate. Based on the analysis, they suggested that the archaeal and bacterial ancestors were thermophilic but the Commonote was not a thermophilic organism. In this study, we re-inferred ancestral NDK sequences using the same Bayesian program. The ancestral NDK sequences were inferred from two phylogenetic trees. One tree was built without constraints and the other with the constraint that Archaea and Bacteria each represent a monophyletic group. The gene encoding the ancestral NDK sequences were synthesized, expressed in *Escherichia coli*, and then the ancestral NDKs were purified. Thermal denaturation measurements showed that the newly inferred ancestral NDK sequences are also extremely thermally stable. Therefore, our conclusion of the (hyper)thermophilic ancestry is robust even if the ancestral amino acid sequences were inferred without the assumption that the amino acid composition has been constant over evolution.

(1) Yamagishi et al. In *Thermophiles: the keys to molecular evolution and the origin of life?* (1998), pp287-295.

(2) Akanuma et al. *Proc. Natl. Acad. Sci. USA* (2013)110, 11067-11072

(3) Boussau et al. *Nature* (2008)456, 942-947

Keywords: resurrection of ancestral proteins, Commonote, nucleoside diphosphate kinase, hyperthermophile

Origin of Eukarya based on phylogenetic trees of aminoacyl-tRNA synthetase

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Woese et al. (1990; PNAS 87:4576-4579) classified all extant organisms into three domains based on the phylogenetic analysis of 16S/18S rRNA. Phylogenetic relationship of these three domains, especially the phylogenetic position of Eukarya has been argued. Besides the three domain hypothesis, the two domain hypothesis has been proposed, where Eukarya are included in Archaea. With recent increase of genome sequences, phylogenetic analyses of all extant organism using concatenate alignment of universal conserved genes supported the two domain hypothesis (Guy & Ettema 2011; Trends Microbiol. 19:580-587, Williams et al. 2012; Proc. Biol. Sci. 279:4870-4879, Williams et al. 2013; Nature 504:231-236, Williams & Martin Embley 2014; Genome Biol. Evol. 6:474-481). However, the closest archaeal species of Eukarya may be more than one in other analyses (Thiergart et al. 2012; Genome Biol. Evol. 4:466-485, Rochette et al. 2014; Mol. Biol. Evol. 31:832-845). Thus, the origin of Eukarya is uncertain. In this study, we performed molecular phylogenetic analyses of 23 aminoacyl-tRNA synthetases (ARSs), and discussed phylogenetic relationship of all extant organisms. Clarifying evolutionary pathways of ARSs may extend our fundamental understanding of early evolution of life. Organellar ARS traced various evolutionary routes different from cytoplasm ARS. Understanding their routes may clarify the origin of organellar and horizontal gene transfer during evolution of Eukarya. Especially, we focused on the phylogenetic position and eukaryal cytoplasmic ARS. Amino acid sequence data of 23 ARSs (117 species: 56 Bacteria: 23 Archaea: 38 Eukarya) were collected. The ARS gene trees were reconstructed by using the maximum likelihood method (RAxML) and the Bayesian method (PhyloBayes). The root of the individual gene tree was inferred from composite tree of several ARSs. Cytoplasm ARSs in 13 trees (LeuRS, GluRS, TrpRS, CysRS, AspRS, GlyRS α_2 type, SerRS, PheRS α subunit, PheRS β subunit, IleRS, ValRS, Class II LysRS, ThrRS) of 23 trees showed monophyly, however, the other 7 trees showed polyphyly (AlaRS, ArgRS, HisRS, MetRS, ProRS, TyrRS, AsnRS). Cytoplasmic ARS is absent in 2 trees of 3 ARSs (Class I LysRS, GlyRS $\alpha_2\beta_2$ type, GlnRS) and is sister group of bacterial group in GlnRS tree. 9 monophyletic cytoplasm ARSs are ingroup of Archaea in 9 trees and 4 monophyletic cytoplasmic ARSs are ingroup of Bacteria in 4 trees. 7 polyphyletic cytoplasmic ARSs are also ingroups of Archaea and Bacteria. These results support the two domain hypothesis. 3 monophyletic cytoplasmic ARSs are closest to TACK superphylum in Archaea and another 3 monophyletic cytoplasmic ARSs is closest to Euryarchaeota in Archaea. The closest species of monophyletic cytoplasmic ARSs in 9 trees showed that Eukarya derived from both TACK superphylum and Euryarchaeota and suggest that the ancestor of Eukarya originated from the fusion between ancestor of TACK superphylum and ancestor of Euryarchaeota. 4 monophyletic cytoplasm ARSs derived from bacteria are closest to different bacterial species, which showed that independent lateral gene transfer occurred from bacterial genome to the genome of Eukaryal ancestor and transferred genes replaced the cytoplasm ARS in the genome of Eukaryal ancestor. Furthermore, 7 polyphyletic cytoplasmic ARSs showed that independent lateral gene transfer from Archaea or Bacteria occurred during evolution of Eukarya and the genes replaced the cytoplasm ARS. Finally, We propose that the ancestor of Eukarya is fused organism between ancestor of TACK superphylum and ancestor of Euryarchaeota and lateral gene transfer of various bacteria from different lineages have been contributed the formation of Eukaryal cells.

Keywords: phylogenetic analysis, aminoacyl tRNA synthetase, early evolution, origin of Eukarya

The First Report of the Tanpopo Mission after Its Arrival to the International Space Station

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To investigate the panspermia hypothesis and chemical evolution, The Tanpopo mission has been developed as Japan's first astrobiology-driven space experiments since 2007 (Yamagishi et al., 2009). This "Tanpopo" mission is launched this spring and it will be likely to start its first-year exposure on the ExHAM pallet onboard the Kibo Exposed Facility of International Space Station (ISS) by the time conference will be held.

The Tanpopo mission is composed of two main experimental apparatus: capture panels and exposure panels. Both will be prepared inside the Kibo module and exposed via airlock with its robot arm up to the maximum of 4 years. The capture panels are to intact capture micrometeoroids, space debris and possible terrestrial aerosols uplifted to the ISS orbit by the world's lowest density silica aerogels exposed to space. If the Tanpopo succeeds to capture terrestrial microbes embedded in the aerosol particles in the aerogel capture panels, it will push the upper limit of existing altitude for terrestrial microbes from the current record of 77 km to 400 km from the ground.

We also test both the survivability of some terrestrial microbes and the chemical alteration of astronomical analog organic compounds in the near Earth space environment. For the former, we emphasize on the importance of cell-aggregates, whose concept is known as "masapanspermia" (Kawaguchi et al., 2013), as the ark for interplanetary transfer of microbes

It is planned that the first samples of both panels will be retrieved back to the Earth in mid-2016, for post-flight analysis initially at ISAS and then at laboratories nationwide by the Tanpopo Team members.

References

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Kawaguchi et al., 2013, Origins of Life and Evolution in Biospheres, 43, 411-428.

Keywords: space experiment, ISS, panspermia hypothesis, chemical evolution

Phosphorus and iron cycles during early diagenesis of sediments under anoxic water mass in a meromictic Lake Kai-ike

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A meromictic lake Kai-ike is located on the northeastern edge of Kami-koshiki island, Japan. Permanent density stratification develops due to seawater infiltration through a gravel bar separating the lake from the ocean. The oxygenated surface water overlies a stagnant, saline, and anoxic deep water containing hydrogen sulfide. Purple sulfur bacteria (*Chromatium sp.*) inhabit the chemocline at 4.5m depth. At the lake bottom, green sulfur bacteria form microbial mat-like structures (Nakajima et al., 2003). Such environment can be regarded as a model for the past anoxic ocean, such as during Oceanic Anoxic Events (Oguri et al., 2003).

We focused on the early diagenesis of phosphorus and iron in the lake. Phosphorus is a bio-essential element and a limiting nutrient for primary production in the ocean on geological time scales. Biogeochemical processes during early diagenesis in sediments play important roles in controlling oceanic P budget, because P fluxes to and from the sediments depend on redox state of the overlying water. Therefore, it is of great importance to understand early diagenetic geochemical behaviors of P in sediments overlain by, for example, oxic, anoxic, and sulfidic (euxinic) water. In this study, we applied sequential extraction procedures to investigate geochemical behaviors of P and Fe in the surface sediments overlain by sulfidic water of the Lake Kai-ike.

A 25 cm-long KAI4 sediment core (Yamaguchi et al., 2010) was used for two sequential extraction methods. SEDEX method (Ruttenberg, 1992) was used for partitioning phosphorus-bearing species into P_{abs} (absorbed), P_{Fe} (iron-bound), P_{auth} (authigenic), P_{det} (detrital), and P_{org} (organic). Iron-bearing species were also divided into Fe_{HCl} (dissolved in HCl), Fe_{carb} (carbonate), Fe_{ox} (oxide), Fe_{mag} (magnetite), Fe_{py} (pyrite), and Fe_{resi} (residue), following Poulton et al. (2005).

Main phosphorus-bearing form was found to be P_{org} . Its content sharply decreases with increasing depth near the sediment surface. Little precipitation of Fe-hydroxides (no significant peak for P_{Fe} or Fe_{ox}) reflects anoxic water condition. Absence of Fe_{py} at the sediment surface implies that syngenetic pyrite did not form. The Fe_{py} content showed downcore increase as the most abundant Fe-bearing phase.

Transformation of P_{org} and P_{Fe} to P_{auth} is called "sink-switching". At sediment surface in an oxygenated ocean, ferric (oxy)hydroxides precipitate to trap phosphate diffusing from deeper-anoxic sediment, and the phosphate concentration in pore water becomes high enough to precipitate authigenic apatite, meaning effective sink-switching (Slomp et al., 1996).

We compared our results with the previous work focusing on early diagenesis under oxic water mass (Ruttenberg and Berner, 1993). Two differences were clearly observed; the abundant P_{org} with sharp downcore decrease just below the sediment surface, and the similar downcore decrease in P_{tot} . We suggest that the amount of ferric (oxy)hydroxides relative to that of P_{org} (P_{Fe}/P_{org}) is a key factor for the retention of phosphorus in the sediments. Further, we quantified the degree of sink-switching, which appears to be related to P_{Fe}/P_{org} ratio. These results suggest the importance of P_{Fe}/P_{org} for sink-switching as an useful proxy to estimate the extent of benthic phosphorus regeneration.

Very low P_{Fe}/P_{org} ratio or absence of P_{Fe} and Fe_{ox} at the sediment surface reflect anoxic water condition, and further, absence of Fe_{py} and very limited abundance of Fe_{carb} indicate ferruginous condition. Anoxic conditions are also indicated by downcore increase in the Fe_{py} content, Fe_{HR}/Fe_{tot} ratios, and DOP (degree of pyritization; $Fe_{py}/(Fe_{py}+Fe_{HCl})$) values.

This study provides useful clues for modeling geochemical cycles of phosphorus and iron in the sediments overlain by photic zone anoxia, and for understanding how Fe- and P-related redox proxies recorded early diagenetic processes of the sediments.

Keywords: phosphorus, iron, early diagenesis, photic zone anoxia, anoxic water mass

Development of Astrobiology with Exoplanet Explorations

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Recent exoplanet researches have advanced significantly. More than 5000 exoplanets including promising candidates have been reported. Large telescopes like the Subaru Telescope have enabled the direct imaging observations of Jovian planets. As a result, it has been shown that various types of stars have various types of planets around them. In particular, the transit observations by the NASA's Kepler mission and the long-term radial-velocity monitoring from the ground have discovered many super-earths and Earth-size planets. However, those planets discovered by the Kepler mission are mostly too far to be followed-up; the low-mass habitable planets around nearby stars have not yet been explored well. In order to study the universality of life under various environments, we study (1) to construct a dedicated facility for long-term planet searches around red dwarfs (M-dwarfs) and (2) to conduct a large-scale survey for Earth-mass habitable planets around red dwarfs using the newly developed spectrometer IRD. Those habitable planets are the best targets for a study of life under non-Earth-like environments (around low-luminosity and with high stellar activities) using the TMT 30 meter telescope (expected FL in 2022).

Keywords: exoplanet, infrared, habitable planet, red dwarf

Search for extrasolar Earth-like planets in the habitable zone using InfraRed Doppler and the Subaru

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Planetary systems around low-mass stars are attractive targets to search for Earth-mass planets in the habitable zone because Doppler signals caused by the planets are relatively large and the habitable zone of the low-mass stars is located at close-in orbits. For the advantages, we have a plan to conduct a planet search program of low-mass stars by the Doppler method using a new InfraRed Doppler (IRD) instrument to search for Earth-like planets. IRD to be mounted on the Subaru telescope in 2015 is composed of a very stable near-infrared high dispersion echelle spectrograph and a laser-frequency comb as a wavelength calibrator of the radial velocity measurements. The main goals of the program are to detect Earth-mass planets in the habitable zone and to understand statistical properties, formation and habitability of extrasolar Earth-mass planets around low-mass stars.

We performed a survey simulation of our planet search program using IRD and the Subaru telescope and estimate expected numbers of detectable planets around low-mass stars based on the results of theoretical population synthesis. In this simulation, we expect more than 50 planets including the more than 30 Earth-mass planets and 10 Earth-mass planets in the habitable zone on a suitable survey plan. And we would detect some transiting planets in the habitable zone, which can characterize planetary atmosphere and may discuss the habitability of the planets by making follow-up observations.

Keywords: Earth-mass planets, low-mass stars, habitable zone

Absorption efficiencies of light-harvesting complexes exposed to the photoenvironment of extrasolar planets

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The detectable size of extrasolar planets, which are planets outside our solar system, is getting smaller. Recently, an Earth-size planet has already been discovered in the habitable zone, the region where a planet can hold liquid water on the surface. By obtaining spectra of exoplanets, a lot of information is derived. For instance, the surface type affects the spectral shape. If we detect the signal of vegetation on exoplanets, it will be a direct evidence of trace of life.

However, trace of vegetation on exoplanets is uncertain when the primal star is different from the Sun. As the first step before detecting trace of vegetation on the planets, it should be examined what kinds of photoenvironments are acceptable for photosynthetic organisms on the earth. Significant processes of photosynthesis, light absorption and excitation energy transfer (EET) processes, occur in light-harvesting complexes (LHCs) that contain photosynthetic pigments. Particularly, we modeled the two processes in the LHC in purple bacteria (LH2), which absorb longer radiation than that in plants, since planets around M dwarfs or M stars (cooler than the Sun) will be the observational targets. We investigated how efficiently the LH2 system absorbs light energies depending on stellar radiation using the quantum chemical calculations.

To begin with photosynthetic pigments, the absorption spectra are calculated to evaluate absorption efficiencies under seven stellar radiation spectra at the top of atmosphere (TOA) of the planets. The pigments and LHC have three main absorption bands: Soret, the Q_x and the Q_y in order of the wavelength. We found that, among the six major pigments, the efficiencies around higher temperature stars, the F, G and K type stars, vary depending on whether Soret bands are placed blueward or redward of 4000 Å break, which is a steep change due to the absorption by some metals in stars below 400 nm. Around the M stars, Soret bands do not contribute the efficiency anymore. Alternatively, Q_y bands affect the efficiencies crucially.

Moreover, the EET process in the aggregation system of LH2 is investigated. The EET velocity becomes double when two pigments in the central antenna are exchanged to the pigments with low excitation energies (from 850 to 890 nm). We also found that the efficiencies using estimated spectrum of the 19 LH2 system are maximized offset from the solar effective temperature (5778 K). The Soret band still has a contribution to the efficiency because the band is just around 4000 Å break.

In order to estimate the efficiencies on planetary surfaces where the organisms inhabit, the planetary atmospheric effects are considered using simple radiation transfer calculations. The contribution of Q_y region is affected due to absorption bands by water vapor. Atmospheric conditions, i.e. oxidizing earth-like or reducing, vary the efficiencies around M stars significantly than those around the Sun. This is particularly because of the spectral overlapping in the Q_y region.

In order to examine conditions which would lead to effective light absorption around M stars, we evaluate the efficiencies with different conformations of the pigments and the LH2 and the solvent. The wavelength of absorption shifts about 120 nm longer in the 19 LH2 system whose central metals of the pigments are exchanged to Pd, compared to that without the metals.

In any conditions as considered the planetary atmospheres, Soret bands contribute the efficiencies due to being enough redward of 4000 Å break. Therefore, in the history of the Earth, there is a possibility for the organisms to have evolved the Soret band to absorb light energies effectively.

Verification of abiotic formation of bioorganic compounds by utilizing Low Earth orbital environment

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In prior to the generation of the first terrestrial life, formation of bioorganic compounds such as amino acids seems to have been essential. There were two possible organic sources for that: Endogenously formed organic compounds and extraterrestrial organic compounds delivered by small bodies. Previously organic compounds could be formed effectively from strongly reducing atmospheres. In these days, however, it was suggested that the endogenous formation was limited since primitive Earth atmosphere would have been only mildly reducing: Conventional energies such as thundering was not effective for the synthesis. On the other hand, laboratory simulations showed that cosmic ray was a possible energy source to form nitrogen-containing organics including amino acids from even only slightly reducing atmospheres. We proposed to expose mildly reducing gas mixture to the environment of low Earth orbit to see chemical evolution toward origins of life could take place without adding artificial energies. Gas mixtures, such as a mixture of methane (5%) and nitrogen (95%) will be sealed in mall gas cells with or without MgF₂ windows, and attached to the Ex-HAM facility equipped on the Exposed Facility of JEM, and exposed to cosmic rays and/or solar UV for more than a year. It is expected the formation of amino acid precursors by the action of cosmic rays and the enhancement of the yield by solar UV. The other possible experiments by using Ex-HAM were also discussed.

Keywords: planetary atmospheres, cosmic rays, Titan, solar ultraviolet light, amino acids, international space station

Formation of nucleobases and amino acids by meteorite impacts on early Earth

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Emergence of biologically important molecules on the prebiotic Earth was a critical step for the origins of life. Miller-Urey type synthesis was once demonstrated the formation of these molecules, such as amino acids and nucleobases. However, reduced atmospheres replicated in those experiments are not realistic considering hot accretion of primitive Earth and hence, this raised questions about how such biologically important molecules were formed. Impact-induced reaction is a potential process that provides these molecules on the early Earth. Formation of a few simple amino acids, amines, and carboxylic acids has been demonstrated in experimental simulation of impacts [1-3]. In this presentation, we report the formation of biologically important organic molecules in experiments simulating reactions induced by extraterrestrial objects impacting on the early oceans. The products contained cytosine and uracil, which are nucleobases found in both DNA and RNA. Proteinogenic amino acids, glycine, alanine, serine, aspartic acid, glutamic acid, valine, leucine, isoleucine, and proline, were also formed as well as non-proteinogenic of β -alanine, sarcosine, α -amino-n-butyric acid, and β -aminoisobutyric acid. Furthermore, methylamine, ethylamine, propylamine, and butylamine were found in products. These organic molecules were formed from impact-induced reactions from iron, nickel, forsterite, water, and ammonium bicarbonate. The yields of produced organics were dependent on the amounts of both metallic iron and ammonium bicarbonate in the starting materials. Estimates based on the experimental results suggest that the formation rate of biologically relevant organics by impacts exceeded the rate supplied by carbonaceous chondrites and was comparable to the flux of interplanetary dust particles (IDPs), although the concentrations of such organics in IDPs remains unclear. These results significantly expand the availability of building blocks of life on the prebiotic Earth.

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Keywords: Origins of life, Astrobiology, Amino acid, Meteorite, Impact, Organic compounds

Collisional growth and the evolution of the internal structure of Archean organic haze particles

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The faint young sun paradox has been discussed for almost four decades. While many theories have been suggested regarding temperatures high enough for primitive life and the existence of liquid water despite the lower luminosity of the Sun during the Archean age, the effect of greenhouse gasses such as CH₄ and NH₃ has especially been paid close attention. In order to ascertain how much contribution the greenhouse gasses made to the warming of the early Earth, a more detailed understanding of the properties of the haze on the Archean Earth, such as its shielding and cooling effects, is essential.

The Archean haze is believed to have been similar to that on Titan. The haze is composed of fractal particles, i.e., fluffy aggregates of monomers. Although monomers collide with each other as they fall and grow as large fractal aggregates, this fractal structure had been ignored and a simplified compact aggregate model has been used in the literature. More recently, Wolf and Toon (2010) conducted simulations considering the fractal structure of the aggregates, but they did not consider compression of the particles accurately. Their assumption of compression is based on the experiment conducted by Onischuk (2003), which is considered to be inappropriate to apply to the haze on the Archean Earth.

Our research is aimed at assessing the properties of organic haze on the Archean Earth based on particle dynamics. We calculate collisional growth and compression of haze particles properly taking into account the physics of collisional and static compression. The result reflects the internal structural change of particles as they fall through the atmosphere after they are formed at higher altitudes.

We find that neither static compression nor collisional compression occur, thus the particles fall with its fractal structure maintained. Simulations based on this assumption show that the haze on the Archean Earth were optically thicker in the UV than the results by Wolf and Toon (2010). Thus, the Archean haze may have had a stronger UV shielding effect than previously expected. Also, the simulations showed that the difference in the internal structure of particles affects the haze mass distribution at each altitude. In the Archean organic haze, the haze is optically thin around the altitude where it is formed, and optically thick haze concentrates below the formation layer.

In conclusion, effective UV shielding by the haze likely protected organics from photolysis and may have helped keep the atmosphere from reducing, which are both favorable for primitive life. The difference in altitude between the haze formation layer and the haze residence layer could be the key to sustainable production of haze on early Earth. Although there remain further questions to affirm that CH₄ or NH₃ provided effective warming effect on the early Earth, our result, combined with the result of Wolf and Toon (2010), supports the argument for greenhouse gasses as the key solution to the faint young sun paradox.

Keywords: the early Earth, haze, fractal particle, the faint young Sun paradox, the internal structure change, the Archean

Polypeptide formation from oligopeptides

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Many scenarios for abiotic polypeptide formation have been proposed for many years, while the research results have shown that both the large peptides and the structure variety of those from amino acids are difficult. Deamidation of asparagine in aqueous solutions and dehydration of aspartic acid in dry conditions give polyaspartic acid upon heating. However, many other amino acids have some difficulties to occupy their residues in backbone structure of the polypeptides. The reason may be considered the diketopiperazine formation, which is carried out from usual linear dipeptides by intramolecular cyclization. This research focuses the polypeptide formation from the oligopeptides containing asparagine, which is an anomalous amino acid. Chemically synthesized dipeptides Gly-Asn, Ala-Asn were heated in the aqueous solution to afford polypeptides with the molecular weight of 3000 to 5000 Da. Asparagine can be produced in the simulative abiotic conditions. If oligopeptides containing asparagine residues had been obtained in the primordial conditions, these oligopeptides would have yielded polypeptides.

Keywords: oligopeptides, polypeptides, asparagine, heating

A reinvestigation of ALH84001 magnetite using SQUID microscopy

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The ~4 Ga Martian meteorite ALH84001 has fine grained magnetite crystals embedded in carbonate blebs along its fracture surfaces (McKay, 1996). Some of these magnetites are strikingly similar (in grain size, morphology, and composition) to those made by magnetotactic bacteria on Earth (Thomas-Keprta et al. 2009). Great debate has raged concerning the origin of the putative biological magnetites. Until recently careful magnetic examination of the magnetite in the carbonate blebs was not possible due to a lack of instrument sensitivity. The ultra-high resolution scanning SQUID microscope (UHRSS) now allows us to study the magnetization of individual carbonate blebs that have been extracted from the meteorite. We are also able to visualize magnetization along fracture surfaces and within the bulk rock by scanning thin slices of the meteorite with the UHRSS.

Two leading hypotheses exist to explain the magnetites found within the carbonate blebs: high-temperature shock deformation leading to the decomposition of iron-bearing carbonate minerals to form magnetite (Treiman and Essene, 2011), and the sedimentary deposition of previously-formed, mature magnetite in an aqueous micro-environment as would be the case for a biogenic origin (Thomas-Keprta, 2009). A well-established paleomagnetic technique which can distinguish between these two hypotheses is the Fuller test of natural remanent magnetization (NRM) (Fuller et al. 1988) which probes the efficiency of the magnetization. If the magnetites grew in a solid state process inside the carbonates (like is suggested by Treiman and Essene (2011) they would be unable to physically rotate as they become stably magnetized and would have a highly efficient magnetization. If the magnetites were detrital (as would be expected from a biological origin scenario) the magnetization would be three orders of magnitude less efficient because the particles would be subject to Brownian motion as they are deposited. Additionally, the susceptibility of Anhyseretic Remanent Magnetization (ARM susceptibility) can be measured for the carbonate blebs. The ARM susceptibility measures the inverse of the effective r.m.s. field strength between magnetic particles (Cisowski 1981). We expect high ARM susceptibility for magnetites formed in situ because they would be evenly spaced as they form and therefore not highly interacting. However, magnetite particles falling through a water column will clump together as they fall (Kobayashi et al. 2006) and will have a low ARM susceptibility.

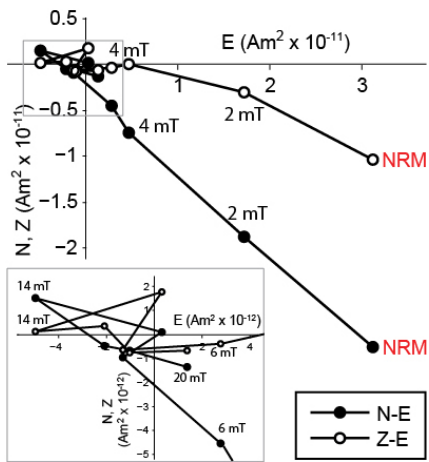
In order to conduct these paleomagnetic tests on the magnetites found within the carbonates and avoid interaction from other magnetic particles in the bulk rock, we extracted the carbonate blebs by carefully flaking them from the fracture surfaces using a non-magnetic needle. We then glued these blebs to magnetically clean microscope slides. We scanned the microscope slides with the UHRSS and were able to observe quantifiable magnetization from the individual blebs. The Fuller test of NRM requires demagnetization of the sample followed by application of an isothermal remanent magnetization (IRM). We have begun demagnetization of the sample. Thus far we observe a clear single magnetic component from NRM to 8 mT followed by chaotic changes in magnetic direction and approximately uniform strength (Figure 1). At this stage it is unclear whether multiple magnetization components will be recovered, our demagnetization is ongoing. Separately, we have measured slices of the ALH84001 bulk rock and observed heterogeneous magnetization, consistent with the work done by Weiss et al. (2000). We have also observed clustering of dipoles within the bulk rock, especially along fracture surfaces. Clustering of dipoles may indicate that multiple deposition or alteration events occurred or that portions of the meteorite have been fractured after emplacement/formation of the carbonates.

Keywords: Martian Life, ALH84001, Rock Magnetism, Paleomagnetism, Biogenic Magnetite, Meteorites

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Were Archean volcanic glasses habitats for microbial organisms?

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Submicron-tube structures have been found in quenched glasses of pillow lavas from Barberton (ca.3.4 Ga) and Abitibi (ca.2.7 Ga) Greenstone belts. These tubes are interpreted as products of bio-alteration (Furnes et al., 2003). However, abiotic processes could form the same structure, and some researchers are questioning biogenic origin of the tube structure (Grosch et al., 2014). Further examination of Archean volcanic glasses is important to constrain habitats of early life.

Here I examined pillow lavas from 2.7 Ga Abitibi Greenstone Belt in Canada. Pillow lavas have clear reaction rims, which were quenched and altered glasses before metamorphism. Typical mineral assemblages in reaction rims are paragonite, chlorite, actinolite, titanite, quartz, calcite, pyrite and epidote. Low metamorphic grade (lower greenschist facies) is suggested by those mineral assemblage. Mineral chemistries suggest that alkaline solutions was responsible for formation of primary minerals, implying alkaline 2.7 Ga ocean.

Titanite occurs in aggregates of fine crystals in chlorite matrix, and often accompanied by unclear tubes. Occurrence of titanite aggregates is identical to bio-alteration features found in other Archean pillow lavas. However, the length and density of tubes are much less compared to others. The examined samples in the present study were less metamorphosed compared to others (upper greenschist facies). Absence of clear tubes in less metamorphosed rocks suggest that tubes in Archean pillow lavas were not products of bio-alteration, but products of metamorphism.

Keywords: Archean, pillow lava, microbes, Abitibi

Mesoarchean pO_2 and pCO_2 based on REE and oxygen isotope geochemistry of BIF from Barberton, South Africa

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A popular mechanism for BIF (Banded Iron Formation) deposition is that Fe-oxides were precipitated in deep-water setting by oxidation of dissolved Fe^{2+} supplied from submarine hydrothermal activity, by dissolved oxygen supplied from oxygenic photosynthesis in the surface ocean. When Fe-oxides precipitated, rare earth elements (REEs) were adsorbed on their surface. REE compositions of seawater have been recognized to reflect redox state of seawater and the extent of input from hydrothermal activity. In this study, we aimed to estimate Mesoarchean seawater chemistry and temperature based on REE signatures of 3.2 Ga old BIFs. These are directly related to pO_2 and pCO_2 in the Mesoarchean atmosphere.

Samples were collected from outcrops of the Mapepe Fm at the bottom of the Fig Tree Group and Msauli Member in the Onverwacht Group, both belonging to the Swaziland Supergroup. Powdered rock samples were analyzed for their major element, REE, and oxygen isotope compositions. Samples with <0.5 wt.% Al_2O_3 are considered to be pure chemical precipitates and thus used for further discussion.

Chondrite-normalized REE patterns of the Mapepe samples show positive Eu anomaly, elevated Y/Ho ratios, and $LREE > HREE$. Furthermore, there exist positive correlations among the extent of positive Eu anomaly, $\sum Fe_2O_3$ contents, and Y/Ho ratios. The maximum Y/Ho ratios are surprisingly comparable to those of the modern ocean. These characteristics suggest a coherent story for BIF deposition; Fe^{2+} emanated from submarine hydrothermal activity was oxidized to Fe^{3+} , which, with enhanced particle reactivity, absorbed dissolved REEs and Y in the 3.2 Ga ocean, producing elevated near-modern Y/Ho ratios. The Msauli samples are mostly enriched in Al_2O_3 and have clastics-dominated REE patterns, suggesting deposition at shallower, more proximal setting.

We also estimate temperature of seawater 3.2 Ga ago from which the BIF precipitated to be around 60-70 °C, based on their oxygen isotope compositions of silicate- and Fe-oxide phases and their binary mixing model. Although crustal heat flux at that time was most likely higher than today, the pCO_2 in the Mesoarchean atmosphere should have been high enough to warm up the seawater under faint young Sun. The pO_2 in the Mesoarchean atmosphere should have been high enough to oxidize dissolved Fe^{2+} supplied from submarine hydrothermal activity.

Keywords: Archean, BIF, REE, Oxygen isotope, South Africa

Birth place of life on Hadean Earth: Geyser model

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Including us human being, life cannot survive with water only. The presence of water itself does not mean the emergence of life automatically. So what is life? Life is composed of three major big molecules, which is (1) carbon (sugar), big monster molecules, (2) metabolism which is the key to get energy. It does not work without P +K which is given from only landmass (provider of nutrients), and (3) self-replication, based on base pair. These 3 molecules are enclosed by membrane. That is life.

So where such molecules for life body are made on Hadean Earth. The most possible place for this process is geyser which locates below the ground. The reason why geyser is thought to be the most suitable site to synthesize building blocks for life body is (1) the ceiling of geyser located below the ground enable gases to be concentrated, such as CO, NH₃, CH₄, HCN, PO₄³⁻ and so on. (2) Amino acid or peptide is possible to be synthesized as necessary material can be provided within cavity of geyser. (3) Periodicity is maintained due to the nature of geyser. (4) Heat source is secured due to the presence of uranium ore deposit which is the alternative heat source instead of solar energy.

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.

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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.

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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

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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.

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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=5dqmFpYQhc&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

