Micro-spectroscopic Molecular Analyses of Sedimentary Organics and Biofilms in the Deep Subseafloor

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Abstract:

As well as being useful molecular biomarkers that can trace their biological sources, sedimentary organics are sensitive indicators of physical and chemical processes affected by a variety of conditions such as temperature, pressure, redox, pH, and mineralogy. For the second stage of IODP, on the basis of the past achievements, I therefore propose to apply a series of state-of-art micro-spectroscopic techniques, such as synchrotron based x-ray absorption near edge spectroscopy (XANES) combined with scanning transmission x-ray microscopy (STXM), to the advanced organic geochemical survey of deep subseafloor, for further understanding of vast microbial activities within a broad range of extreme conditions. The proposal reseaches below include the application example of STXM-XANES for analyses of the extraterrestrial organics.

Proposed Research Projects:

1. Spectroscopic and isotopic characterization of diagenetic macromolecule (kerogen) in the deep subseafloor sediments.

As a part of Archean Park Project, abundances and distributions of amino acids (Takano et al. 2003, 2004) and fatty acids (Yamanaka and Sakata, 2004) in hydrothermal vents were revealed, providing valuable information on biological activities in the extreme environment. In addition to these biomarkers, sedimentary kerogen is also a significant diagenetic organic product and generally accounts for a major part of total organic carbon. Moreover, the pyrolytic gases from kerogen could be one of the sources of gas hydrates (Pohlman et al. 2005). The diagenetic macromolecular matter in the deep subseafloor sediments including hydrothermal sites, however, has yet to be studied, probably because of low total organic carbon content (< 0.01%) in the environment (Yamanaka and Sakata, 2004) and a difficulty to apply the conventional molecular analytical techniques (e.g., NMR) to such small amounts of organic solids.

In order to understand the peculiar chemical and biological processes in the deep subseafloor systems, one must elucidate the complex structure and isotopic composition of kerogen. This aim will become possible by applying a series of organic microanalytical methods such as synchrotron-based x-ray absorption near edge spectroscopy (XANES) combined with scanning transmission x-ray microscopy (STXM).

The fundamental phenomenon of XANES is that electrons in the inner shells are photo-excited to unfilled molecular orbitals, or quasi-continuum states right above the ionization threshold when soft X-ray is absorbed by light element atoms such as carbon, nitrogen, and oxygen. The energy of these transitions is determined by the type of neighboring atoms and the bonds (functional groups). The STXMs, which provide a focused monochromatic X-ray beam with a spot size on the order of 30 to 40 nm, yields Carbon-XANES spectra of submicron domains and quantifies the electronic structure of carbon molecules. This STXM-XANES technique with high spatial and energy resolution has been shown to be very useful for revealing complex carbon chemistry in various research areas including NASA Stardust mission of comet dust particles sample return (Figs. 1 and 2) (Sandford et al. 2006; Cody et al. 2008).

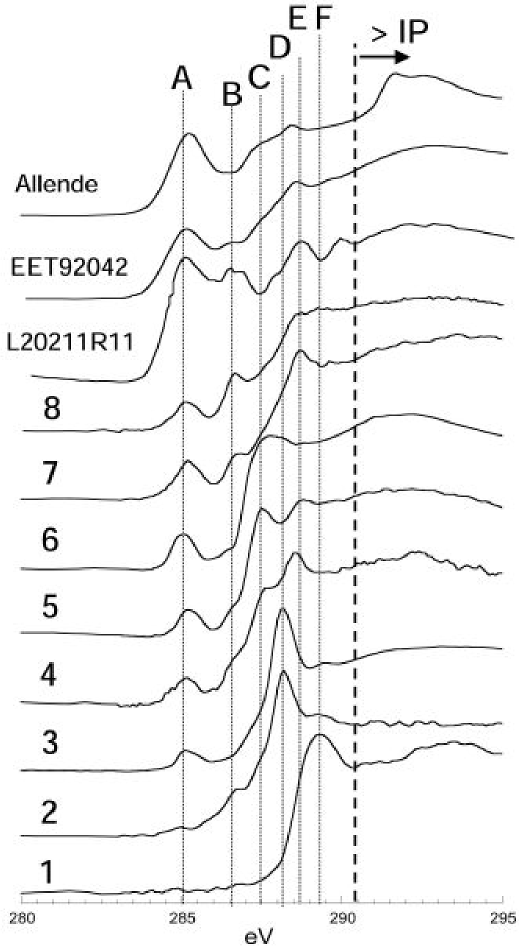

Fig. 1. Carbon-XANES spectra of organics associated with comet dust particles (sample nos 1–8), interplanetary dust particle (L2011R11) and insoluble organic matter isolated from meteorites (EET 92042 and Allende). (Cody et al. 2008). Peak A a 1s-σ* transition of aromatic or olefinic carbon; B a 1s-σ* transition of oxygen substituted double-bonded carbon, C a 1s- ſ*/3p transition of methyl groups; D: a 1s-σ* transition of carbonyl carbon in amide moieties; E: a 1s-σ* transition of carboxyl carbons; and F: a 1s- ſ*/3p transition of alcohol/ether moieties. The carbon 1s ionization threshold is designated with a dashed line (IP) The figure caption is modified.

The submicron-sized spot analysis via the STXM-XANES will enable elucidation of microheterogeneity of the deep subseafloor sedimentary organics which may reflect the diverse activities of the microbes. It is also able to detect structural anomalies which may be novel, potential biomarkers. Moreover, the technique does not require extraction or isolation of organic molecules from samples. Thus, it enables investigation of organic-mineral association which is related to origin, diagenesis, and contribution to biogeological cycle of organic matter in the deep subseafloor.

In addition to STXM-XANES, other complementary techniques, such as micro-Fourier transmission infra red spectroscopy (FTIR), micro-confocal laser Raman spectroscopy, and secondary ion mass spectroscopy (SIMS), would be powerful tools to probe molecular and isotopic characteristics the deep subseafloor sedimentary organics to determine the biological and chemical history under the extreme environment.

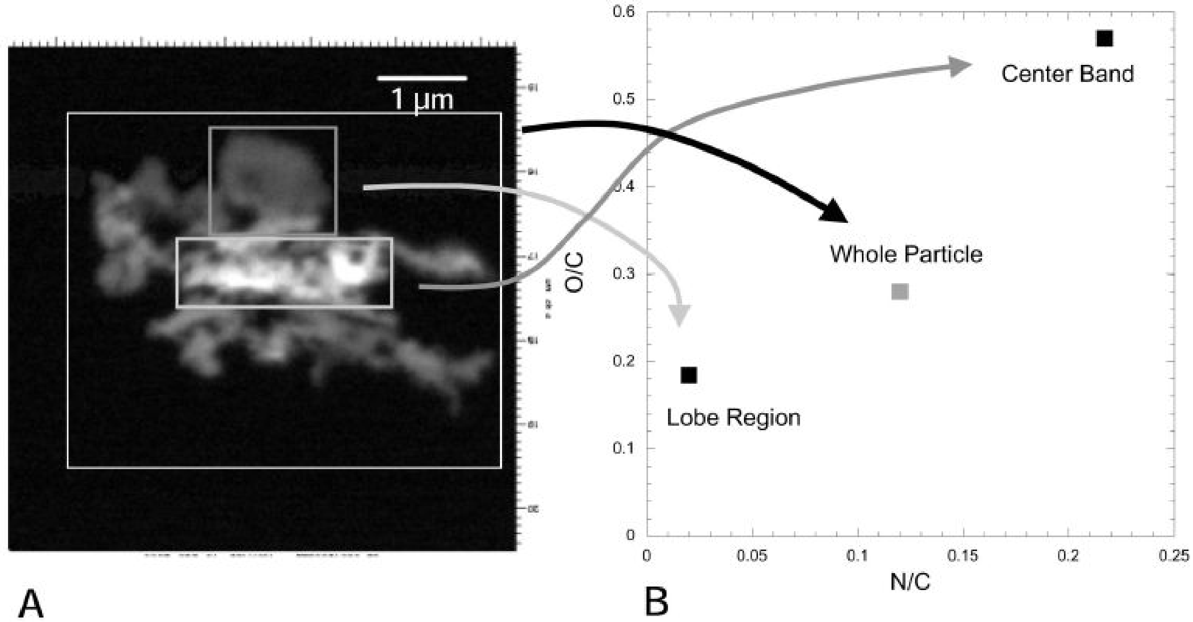

Fig. 2. a) An optical density image on the carbon (1s) absorption edge of the comet dust particle (sample 5). Note that the significant variations in optical density could be due to variations in particle density or chemical heterogeneity. b) Elemental data (N/C and O/C) from the "lobe region" and the "center band" reveal that the variation in optical density observed (left) reflects significant chemical heterogeneity within the sample.(Cody et al. 2008)

2. Molecular and isotopic study of biofilms in the deep subseafloor biosphere.

Biofilms are "a new figure of microbes" in regard to their unique characteristics that multiple microbial species live as communities associated with rock surfaces in structures. The presence of biofilms in hydrothermal environments has been reported through DNA extraction and microscopic cell enumeration (e.g., Cady et al. 1997; Schrenk et al. 2004). Schrenk et al (2004) discusses that the stable isotopic signatures of lipids, fluids and rock samples will be a key to identify important metabolic processes within this novel ecosystem. They also discuss that the abundance of organisms linked to the volatile chemistry at the hydrothermal field hints that similar metabolic processes may operate in the subseafloor. However, the formation mechanisms of biofilm have been poorly understood.

For uncovering the deep sea ecosystem, therefore, it would be important to investigate the biofilm formation from the organic geochemical perspective. Determination of the multiple metabolic systems in biofilms would be possible through the SIMS or nanoSIMS based isotope imaging. Moreover, taking advantage of that the micro-molecular analyses listed above (XANES-STXM, FTIR, and Raman) enables the direct mapping of organic and inorganic components, the interaction between biofilm and minerals would be identified. Such approach will provide new insights into extensive microbial activities and a biogeochemical cycle in the deep subseafloor.

Implication for Planetary Science and Astrobiology:

Deep subseafloor environment including hydrothermal vents may resemble those present on the early Earth. In this regard, the proposed research projects would elucidate what factors may have been favorable for the emergence of life and how deep life can live, as well as the present connection between biology and geochemistry resulting in diversity of the deep biosphere. Furthermore, understanding the deep subseafloor carbon cycle on the Earth may lead to understanding that in the early Mars where tectonics might have existed at that time, which could be a clue to evaluate whether the Martian carbon cycle had provided a habitable environment or not.

References:

Cady, S. L., Blank, C. E., DesMarais, D. J. Walter, M. R. and Farmer, J. D. 1997. Microbial diversity diversity in hyperthermophilic biofilms: Implications for recognizing biogeneity in hydrothermal mineral deposits. *Workshop on Early Mars*, Abstract #3062.

Cody, G. D., Ade, H., Araki, T., Alexander, C. M. O'D., Butterworth, A., Fleckenstein, H., Flynn, G. J., Gilles, M. K., Jacobsen, C., Kilcoyne, A. L. D., Messenger, K., Sanford, S., Tyliszczak, T., Westphal, A. J., Wirick, S. and Yabuta, H. Quantitative organic and light element analyses of Comet Wild 2 particles using C-, N-, and O- micro-XANES. 2008. *Meteoritics and Planetary Science,* 43, 353-366.

Pohlman, J. W., Canuel, E. A., Chapman, N. R., Spence, G. D., Whiticar, M. J. and Coffin, R. B. 2005. The origin of thermogenic gas hydrates on the northern Cascadia Margin as inferred from isotopic ($^{13}C/^{12}C$ and D/H) and molecular composition of hydrate and vent gas. *Organic Geochemistry*, 36, 703-716.

Sandford, S. A. and Stardust organics preliminary examination team. 2006. Organics Captured from Comet Wild 2 by the Stardust Spacecraft, *Science* 314, 1720-1724.

Schrenk, M. O., Kelley, D. S., Bolton, S. A., Baross, J. A. 2004. Low archaeal diversity linked to subseafloor geochemical processes at the Lost City Hydrothermal Field, Mid-Atlantic Ridge. *Environmental Microbiology* 6, 1086-1095.

Takano, Y., Sato, R., Kaneko, T., Kobayashi, K. and Marumo, K. 2003. Biological origin for amino acids in a deep subterranean hydrothermal vent, Toyoha mine, Hokkaido, Japan. *Organic Geochemistry,* 34, 1491–1496.

Takano, Y., Kobayashi, K., Yamanaka, T., Marumo, K. and Urabe, T. 2004. Amino acids in the 308°C deep-sea hydrothermal system of the Suiyo Seamount, Izu-Bonin Arc, Pacic Ocean. *Earth and Planetary Science Letters*, 35, 1105–1120.

Yamanaka, T. and Sakata, S. 2004. Abundance and distribution of fatty acids in hydrothermal vent sediments of the western Pacific Ocean. *Organic Geochemistry*, 35, 573–582.