Ⅲ-5

Life, Earth and Planetary Sciences

Highlight

BL4U

A Key Mechanism of Bacterial Pyrite Leaching Identified by Direct STXM Analysis at Cell-Pyrite Interface

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Microbial bioleaching of metal sulfides has been used as a low-cost engineering process for extracting metals from sulfidic ores due to its fast dissolution rate [1]. The microbial bioleaching of metal sulfide also contributes to formation of environmentally detrimental acid mine drainage (AMD), whose acidic nature and heavy-metal constituents cause serious contamination of soil and groundwater in the world. Thus, a better understanding of the mechanisms is of crucial importance for improvement of both industrial bioleaching and AMD formation.

There have been considerable efforts to identify mechanisms of bioleaching. However, little is known on the microbial bioleaching mechanism, because direct chemical speciation of metals and biomolecules at mineral-microbe interface has been difficult due to high spatial resolution in analysis.

Here, we investigated the mechanisms of the bioleaching process in bacterial pyrite leaching by leaching bacteria (*Acidithiobacillus ferrooxidans*) by scanning transmission X-ray microscopy (STXM) based C and Fe near edge X-ray absorption fine structure (NEXAFS) analyses at UVSOR BL4U.

Carbon NEXAFS analysis directly showed that attached *A. ferrooxidans* produces polysaccharide-abundant extracellular polymeric substances (EPS) at the cell-pyrite interface (data not shown, [2-3]). Figure 1 shows the STXM-based merged Fe/C image and Fe 4p NEXAFS of bacteria cells attached to pyrite particles in 2 weeks incubation. The image in Fig. 1a shows that Fe was localized around the surface of the bacteria cells. Considering that C NEXAFS demonstrated the appearance of a polysaccharide-rich EPS layer at the cell-pyrite interface, this suggests that Fe had accumulated in this polysaccharide layer. In the Fe NEXAFS spectra, both the spectra of whole cell and cell-pyrite interface (interface 1 and 2 in Fig. 1b) consist of Fe(II) and Fe(III) peaks. Thus, the Fe species in both cell and cell-pyrite interface were Fe(II) addition to Fe(III) [3].

In previous study, researchers have implied a possible role for the Fe(III) in EPS in whole pyrite bioleaching process, an oxidizing agent for sulfides [1]. That is the Fe(III) in EPS would abiotically oxidize sulfides in pyrite according to following equations.

$FeS_2 + 6Fe^{3+} + 3H_2O \rightarrow S_2O_3^{2-} + 7Fe^{2+} + 6H^+$

$S_2O_3^{2-} + 8Fe^{3+} + 5H_2O \rightarrow 2SO_4^{2-} + 8Fe^{2+} + 10H^+$

However, this interfacial process remained speculative because no Fe(II) in the EPS subsequently formed by this oxidation process had been observed yet. Previous studies determined the Fe species in EPS by indirect methods [1]. The Fe(II) detected by direct STXM based NEXAFS analyses in this study is a first direct evidence supporting the oxidative attack by the Fe(III) in EPS. These findings could be important information for understanding key mechanism of both bacterial mineral leaching and AMD formation.

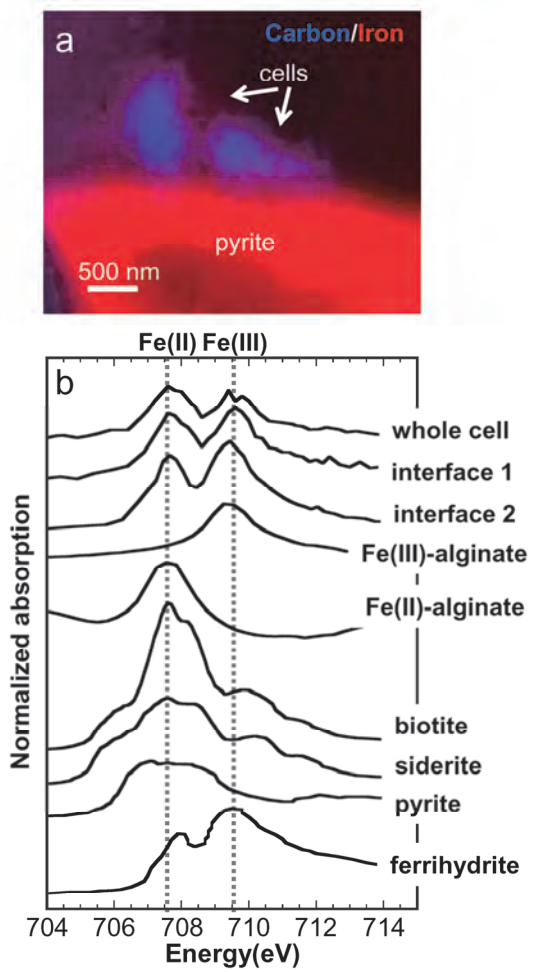

Fig. 1. STXM-based C and Fe images (a) and Fe 4p NEXAFS spectra (b).

[1] W. Sand and T. Gherke, Res. Microbiol. **157** (2006) 49.
[2] S. Mitsunobu *et al.*, Chem. Lett. **44** (2015) 91.
[3] S. Mitsunobu *et al.*, Microbes Environ. (in press).

BL4U

Application of XANES to the Analysis of Carbonaceous Materials in Hayabusa-Returned Samples for Determination of Their Origin

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Carbonaceous particles, those have been found in the sample catcher of Hayabusa spacecraft together with silicate particles, were still under the investigations, including precise determination of their origin. Results of isotopic analysis of H, C and N of the carbonaceous materials by NanoSIMS did not show any signatures of their extraterrestrial origin, e.g. isotopic anomalies against the terrestrial composition [1]. Some of the results of analyses by Transmission Electron Microscopy (TEM, [2]) and X-ray absorption near edge structure (XANES, [3]) indicated relation to the terrestrial material. Thus, those particles might indicate the contamination of terrestrial material into the sample catcher before, during and/or after the operation of Hayabusa spacecraft.

In previous study, a particle from the witness plate exposed to the clean room of Hayabusa2 spacecraft construction, where Hayabusa1 spacecraft was constructed, was investigated by XANES/STXM installed in ALS/LBNL, Berkeley. The shape of XANES spectrum of the particle (green, WP1 in Fig. 2) closely relates to that of a Hayabusa-returned sample, RA-QD02-0180-03 (blue line in Fig. 2), and indicate the relation of them.

In this work, we investigated a particle newly picked up from the same witness plate using XANES/STXM installed in UVSOR, in order to confirm the result of previous study.

Figure 2 shows C-XANES spectra of the new particle (red, WP2) and Hayabusa-returned samples (blue, RA-QD02-0180-03). The shape of the spectra was similar, but position of the peaks of WP2 were different from others.

N-XANES spectrum of the WP2 was also largely different from other two samples. Though particles were picked up from the same witness plate and chemical composition of them was similar, those difference of XANES spectrum indicate the different origin of WP1 and WP2. The fact indicate several paths of the contamination to the sample catcher were possible, and should be considered for the precise determination of the origin of the contaminant.

In future work, we will apply the XANES/STXM analysis to further possible contaminants such as particles of biological materials.

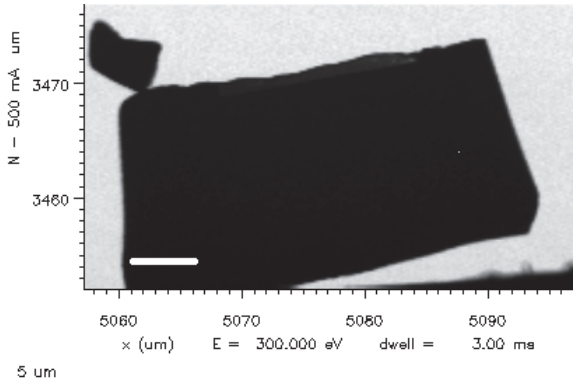

Fig. 1. STXM image of the ultrathin section of WP2 in Fig. 2. The particle was pressed on a gold plate and cut by Focused ion beam to extract ultrathin sections.

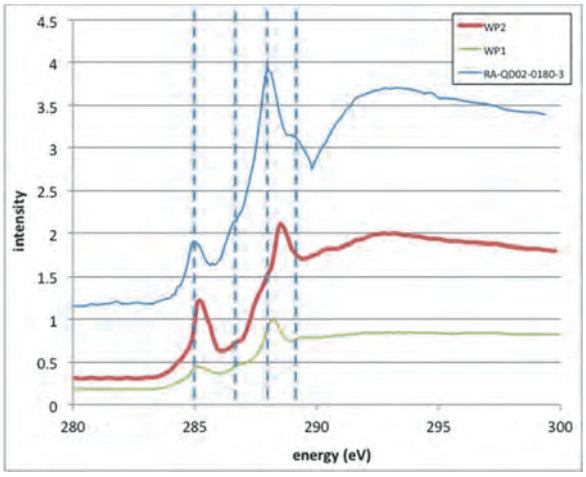

Fig. 2. C-XANES spectra of the particles from the witness plate of clean room of the Hayabusa2 spacecraft construction (green and red), and Hayabusa-returned sample (blue). Peak positions of WP2 were different from those other two samples.

[1] M. Ito *et al*., Earth planet. Space **66** (2014) 91.
[2] M. Uesugi *et al*., Earth planet. Space **66** (2014) 102.
[3] H. Yabuta *et al*., Earth planet. Space **66** (2014) 156.

BL4U

Distribution of DNA and Protein in Mammalian Cell Nuclei Calculated Using NEXAFS Profiles at the N-K and O-K Absorption Edges

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Spectromicroscopy using scanning transmission X-ray microscope (STXM) has been applied to DNA and protein distributions in biological specimens such as chromosome and sperm. Our previous study on the DNA mapping in mammalian cell nuclei was successfully carried out for cultured A549 cells with the aid of different NEXAFS (Near Edge X-ray Absorption Fine Structure) profiles between DNA and protein at the N-K absorption edge as shown in the left panel of Fig. 1 [1]. The results gave rather unexpected, different distributions of DNA and histone in nucleoli. In this study, to confirm these results we extended the analysis to the O-K edge, and also to obtain quantitatively consistent results at different absorption edges, we developed the correction of reference NEXAFS spectra used for the "stack fit" method to obtain molecular distribution.

Human HeLa cell was used because of its large nuclear area. Figure 2 shows transmission and optical density (OD) images of a whole cell taken at 400 eV. Four large nucleoli in the nucleus were identified, and for the enlarged area DNA and histone distributions were calculated using aXis2000 software to fit the specimen spectra to the reference spectra. In the center of nucleoli histone content was rather poor compared with DNA, which was consistent with our previous report [1].

For the extension of the analysis to the O-K edge, reference spectra at the O-K edge (Fig. 1) should be correlated to those at the N-K edge, since the fitting results do not necessarily assure the same quantitative distribution due to the independent measurements at each absorption edge. As a trial of the correlation, first we calculated mass thickness, which is defined as a product of the density and thickness of specimen, from OD by considering mass absorption coefficients at 395 eV. Then the OD of reference spectra at the O-K edge was corrected using this mass thickness at 525 eV. Based on these corrected spectra, DNA and histone distributions were obtained as shown in the lower panels of Fig. 3, indicating similar distributions to those at the N-K edge. Table 1 shows OD range of DNA and histone at the N-K and O-K edges. OD differences and the ratio of DNA and histone were also listed. Similar ratios between the N-K and O-K edges were obtained, although the absolute OD values were not totally the same.

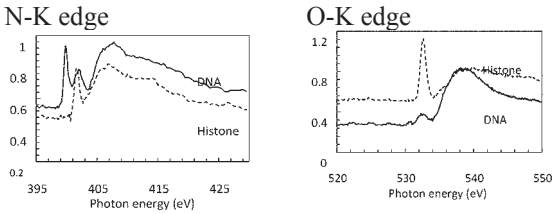

Fig. 1. NEXAFS spectra of DNA and histone at the N-K and O-K edge.

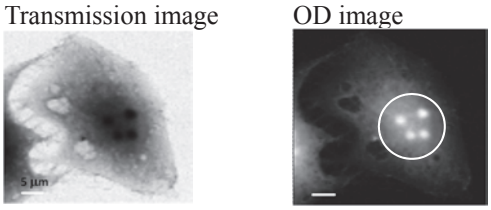

Fig. 2. Transmission and OD images of human HeLa cell at 400 eV. White circle in OD image is enlarged in Fig. 3. Scale bar corresponds to 5 microns.

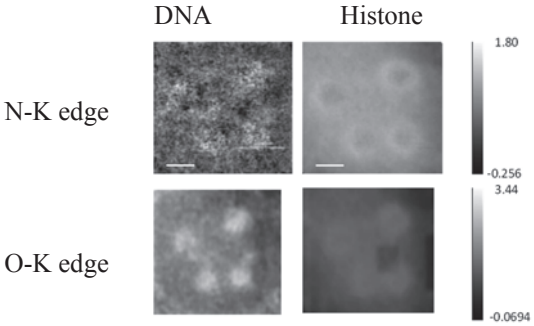

Fig. 3. DNA and histone distributions of nucleus of HeLa cell obtained at the N-K and O-K edges. OD range bar is shown in the right. Scale bar corresponds to 2 microns.

Table 1. Comparison of DNA and histone contents between the N-K and O-K edges.

	OD range	Difference	DNA/Histone
DNA(N-edge)	-0.256~1.80	2.06	2.52
Histone(N-edge)	0.443~1.26	0.817	
DNA(O-edge)	-0.0694~3.44	3.51	2.36
Histone(O-edge)	-0.00413~1.48	1.48	

[1] T. Ohigashi *et al.*, AIP Conference Proceedings **1696** (2016) 020027.

BL4U

Speciation of Sulfur and Calcium in Aerosols by STXM

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Aerosols, defined as small particles or droplets in the atmosphere, have important effects on the environment. Sulfate is the main compound in aerosols (in particular anthropogenic ones) and is emitted primarily by human activities. As suggested by the Intergovernmental Panel on Climate Change, sulfate aerosols can cool the earth in two ways: directly by reflecting sunlight, and indirectly by acting as cloud condensation nuclei. The latter cooling role is made possible by the hygroscopic nature of some sulfates. In the atmosphere, sulfate occurs in several forms including ammonium salts (NH_4HSO_4, $(NH_4)_2SO_4$), calcium salt (gypsum; $CaSO_4 \cdot 2H_2O$), and hydrated species (Takahashi *et al.,* 2006). Among these species, ammonium salts are hygroscopic, while calcium salts are not. Thus, sulfate species should be well identified to precisely evaluate the global cooling effect of sulfate aerosols. Thus, this study conducted speciation of sulfur in aerosols by Scanning Transmission X-ray Microscopy installed at BL4U, UVSOR.

In this study, distributions of sulfur and calcium species within aerosol particles were examined for aerosols collected in Higashi-Hiroshima in May, 2014, which can be a basis for understanding chemical processes to form sulfate aerosols. First, distribution of calcium in a particle with a few μm diameter was examined. Although calcium is distributed in the whole particle, calcium oxalate or calcium sulfate was mainly found at the rim of the particle based on the calcium L_{III}- and L_{II}- edge XANES (Figs. 1 and 2). It is known that concentrations of sulfate and oxalate correlate each other [1]. The present results suggested that both sulfuric acid and oxalic acid react with calcium particle, particularly calcite, in the atmosphere.

Distribution of sulfur was also examined by the sulfur L_{III} edge absorption. It was found that calcium sulfate wad distributed within the rim where calcium oxalate and/or calcium sulfate were found by calcium L-edge absorption (Fig. 1).

All these results support that secondary calcium species produced by the reactions of calcium carbonate with sulfuric or oxalic acids were formed in the atmosphere. In particular, formation of calcium oxalate at the rim of the particle is found for the first time by any microscopic analyses.

Sulfur speciation was also conducted for the particles collected as a fine particle ranging from 0.69 to 1.3 μm. As a result, sulfur species was almost exclusively ammonium salt within the particles based on the XANES analysis, showing that sulfate aerosols smaller than 1.3 μm is not the products of reactions with minerals, but sulfate aerosol possibly produced in droplet mode.

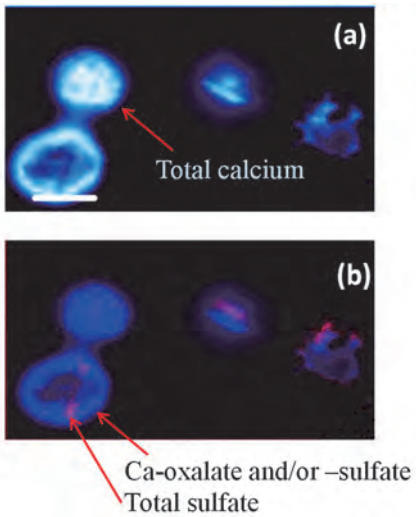

Fig. 1. STXM images of total calcium, Ca-oxalate and/or sulfate, and total sulfate by absorption at Ca and S L-edges.

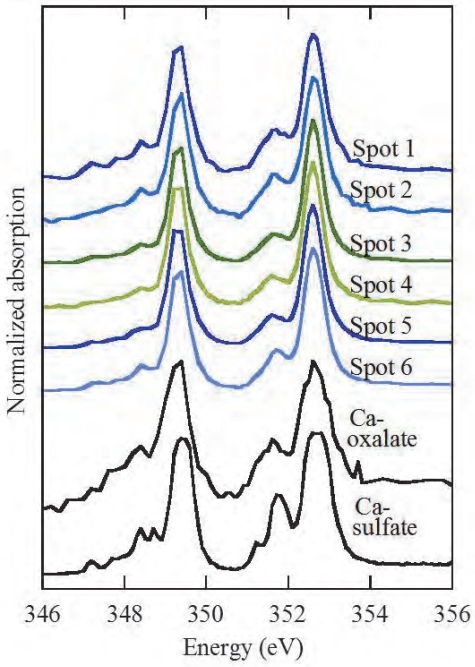

Fig. 2. Calcium L_{III} and L_{II} edges XANES for Ca-oxalate, Ca-sulfate, and Ca in various spots in Fig. 1 (b).

[1] T. Furukawa and Y. Takahashi, Atmos. Chem. Phys. **11** (2011) 4289.

BL4U

Chemical Evolution of Meteoritic Organics during Impact Metamorphism

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The primitive solar system materials such as meteorites, comets, and these-derived cosmic dusts were formed 4.5 billion years ago by accretion of gas and dusts in interstellar cloud. The interstellar dusts are about ten micron-sized solid particles consisting of silicate mineral core, refractory organics, and amorphous ice mantle. Therefore organic molecules in space can be described as one of the major building blocks of our Solar System and Life. To date, molecular and isotopic compositions of organics from the meteorites ranging various groups and petrologic types have been frequently studied in relation to the chemical history in the early Solar System [1, 2].

Shock metamorphism is one of the important processes in the early Solar System, but its relationship with chemical evolution of meteoritic organic compounds has been less frequently studied than aqueous alteration and long-term heating due to radiogenic elements on a meteorite parent body. Yabuta *et al*. (2010) [3] has revealed that molecular and isotopic characteristics of organic macromolecular solids in the shocked meteorites are distinct from those in the long-term heated meteorites. In this study, for understanding chemical behaviors of organic matter in meteorites, molecular analyses of organic matter the experimentally shocked meteorite were conducted.

As a sample, Murchison meteorite powder was used in this study. The shock experiments of a meteorite powder pellet (~180 mg) in a SUS collecting vessel were conducted at 5 and 10 GPa by a single-stage powder gas gun and at 20 and 40 GPa by a double-stage light gas gun at Tokyo Institute of Technology (Fig. 1). After the experiment, the meteorite sample was recovered and treated with CsF/HF-dioxane [4] to purify the insoluble organic macromolecular solid (IOM). The insoluble organic solids from the experimentally shocked meteorites were analyzed by a scanning x-ray transmission microscope (STXM) - x-ray absorption near edge structure (XANES) at beamline 4U, UVSOR.

Carbon-XANES spectrum of organics the experimentally shocked Murchison IOM at 5 GPa (blue spectrum in Fig. 1) showed a peak of aromatic carbon (285 eV) and a peak of carboxyl carbon (288 eV) with a shoulder of aliphatic carbon (297.6 eV), which was very similar to non-shocked Murchison IOM. The C-XANES spectra of IOM at 10 and 20 GPa were also similar to that at 5 GPa. On the other hand, in the C-XANES spectrum of organics at 40 GPa, a peak intensity of carboxyl carbon largely decreased and that of aromatic carbon was slightly developed. This result indicates that modification of IOM by shock heating occurs at 40 GPa and is comparable to their Raman spectra [5].

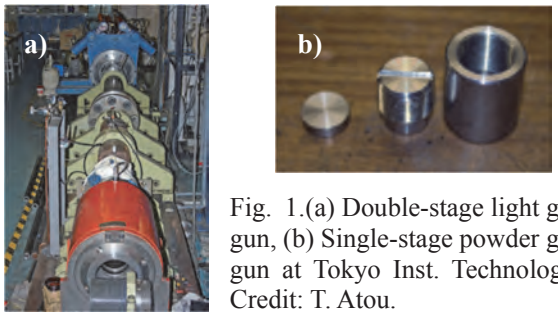

Fig. 1.(a) Double-stage light gas gun, (b) Single-stage powder gas gun at Tokyo Inst. Technology. Credit: T. Atou.

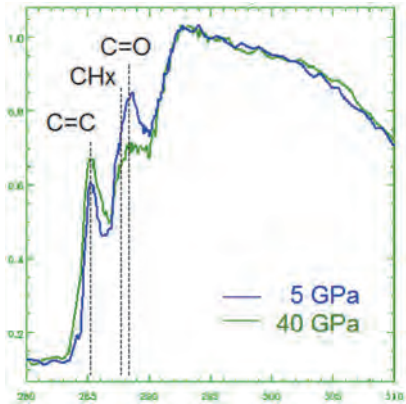

Fig. 2. C-XANES spectra of organic macromolecular solids from the experimentally shocked meteorites. Blue: 5 GPa, Green: 40 GPa.

[1] C. M. O'D. Alexander *et al.*, Geochim. Cosmochim. Acta **71** (2007) 4380.
[2] G. D. Cody *et al*., Earth. Planet. Sci. Lett. **272** (2008) 446.
[3] H. Yabuta *et al.,* Meteor. Planet. Sci. **45** (2010) 1446.
[4] G. D. Cody and C. M. O'D. Alexander, Geochim. Cosmochim. Acta **69** (2005) 1085.
[5] P. Beck *et al.,* Meteor. Planet. Sci. **45** (2012) 1446.

BL4U

Probing Tacrolimus in Human Skin by Soft X-Ray Spectromicroscopy

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Lipophilic drugs, such as dexamethasone and tacrolimus, are commonly used to treat by topical application inflammatory skin diseases. We have investigated recently the penetration of dexamethasone ($C_{22}H_{29}FO_5$), a drug that penetrates readily into human skin, as probed by X-ray microscopy [1]. Adequate penetration is due to its moderate lipophilicity and the relatively low molecular weight of 392 Da, which is well below the 500 Da limit [2]. This limit is known to be of relevance for distinguishing low molecular weight drugs that easily penetrate into skin from heavier ones, such as tacrolimus ($C_{44}H_{69}NO_{12}$, M=804 Da), requiring advanced formulations for sufficient penetration [3]. We have investigated by label-free X-ray spectromicroscopy tacrolimus in the O 1s-regime. The experiments were performed at the BL4U beamline at UVSOR-III using a scanning transmission X-ray microscope (STXM). Intact skin samples from healthy donors as well as tape-stripped skin for thinning the stratum corneum (SC), the top skin penetration barrier, were investigated. For intact skin the drug was dissolved in aqueous ethanol (50%) with a concentration of 10 mg/mL, where 40 µL were applied per cm^2. Tacrolimus was applied to tape-stripped skin by using Protopic ointment 0.1% (Astellas Pharma, Munich, Germany), 30 µg/cm^2. Penetration times ranged between 10 and 1000 min. Chemical selectivity for probing the drug in fixed human skin slices is gained by excitation of the O 1s→π^*-transition at 531.3 eV, which is suitable to suppress the background absorption of fixed human skin (see Fig. 1). This indicates in addition to recent work [1], that X-ray microscopy is a general approach to probe drugs in fixed human skin slices.

The results indicate that in normal skin tacrolimus can only penetrate into the stratum corneum. The drug was not detected in deeper skin layers, such as in the viable epidermis and dermis. However, if the skin is mechanically removed by repeated tape-stripping (50 times) prior to drug exposure, then penetration of tacrolimus is observed, as indicated in Fig. 2. Clearly, the drug is detected in the viable epidermis. It is anticipated that this finding will also apply to drug penetration in barrier deficient inflamed skin, as known for the improvement of atopic dermatitis following the use of tacroliumus ointment [3]. The shape of local tacrolimus distribution is similar to earlier work on dexamethasone [1]. This implies that the transport of both drugs is similar, independent of the molecular weight, once the stratum corneum is passed. We also observe that the drug concentration drops to the detection limit at the basal membrane, implying that in the dermis different transport properties occur. Thus, the basal membrane may form another barrier for transdermal drug penetration, as suggested by present X-ray spectromicroscopy studies.

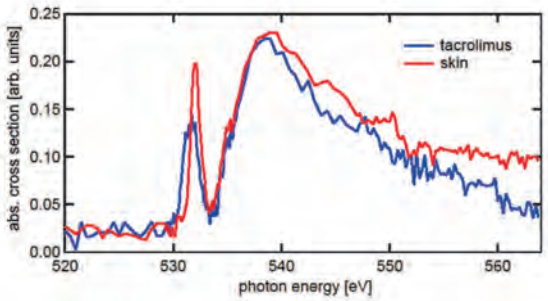

Fig. 1. O 1s-excitation regime of tacrolimus in comparison with fixed human skin. The resonance near 531 eV (O 1s→π^*-transition) is used for selective probing of the drug.

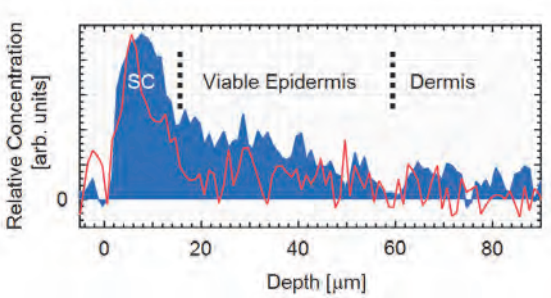

Fig. 2. Penetration of tacrolimus into human skin after tape-stripping. Two different penetration times were studied (10 min: red and 1000 min: blue).

[1] K. Yamamoto *et al.*, Anal. Chem. **61** (2015) 6173.
[2] J.D. Bos *et al.*, Exp. Dermatol. **9** (2000) 165.
[3] M. Lapteva *et al.*, Mol. Pharm. **11** (2014) 2989.

BL4U

Evaluation of FIB Sample Contamination Depends on the Storage Environment Based on XANES Analysis and Application to Organics in Carbonaceous Chondrites

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XANES and high resolution STXM installed in UVSOR BL4U are useful tools for the characterization of molecular structure of the unknown carbonaceous material, and understanding of their micro-distribution. We expected to apply these analyses to the hydrous carbonaceous chondrites, since the extraterrestrial organics have some variety and complex structures those could be evidences of the evolution of parent body of the chondrites. In this aspect, samples preparation must avoid the change of their original microstructure.

It is well known that electron beam of the TEM observation could modify the C-XANES spectrum [1]. In contrast, we reported the sample damage induced by C-XANES in the report of the previous study [2]. TEM observations just before and after XANES analysis showed that NaCl and KCl inclusions in the sample were damaged heavily by the XANES analysis. Before further precise description of the carbonaceous materials in carbonaceous chondrite, damage during XANES analyses and contamination during the sample storage must be evaluated. In this study, we evaluated the contamination during the sample storage by changing the storage environment and the period by XANES/STXM.

We fibbed 8 samples from the matrix of Murchison, one of the hydrous carbonaceous chondrites. The size is about 15x10 um and 100 nm thickness. The samples from #1 to #4 were kept in 4 different conditions for 1 week, and the remaining samples from #5 to #8 were kept for 2 months until the XANES/STXM analysis. #1-4 samples were analyzed again after storage for 3 months. The storage atmospheres were pure Nitrogen and approximately 10^2 Pa vacuumed conditions, and the sample holder made by Si and metal, respectively (Table. 1). C, N-XANES analyses were performed for #1-4 samples after 1 week storage. N, O-XANES spectrum were obtained for #5-8 samples after 2 months storage and for #1-4 samples after 3 months storage.

C-XANES spectrum of #1-4 samples stored for 1 week do not show any changes irrespective of the storage environment. In addition, we can not detect clear N-XANES transition in all samples, even some samples (#1-2, #5-6) were kept in pure N2 atmosphere (Fig. 2). These results suggest that sample storage does not give any contaminations, under the conditions tested in this study.

In future work, we plan to perform C-XANES analysis for these samples to confirm the molecular structures of the carbonaceous materials to be unchanged by a few months storage. Besides, we will apply the XANES/STXM analysis to various hydrous carbonaceous chondrites.

Table 1. Samples and storage conditions.

#	Atmosphere	Holder	Storage period and analyzed spectrum		
			1 week	2 months	3 months
1	N2	Si	C, N		O, N
2		Metal	C, N		O, N
3	Vacuum	Si	C, N		O, N
4		Metal	C, N		LOST
5	N2	Si		O, N	
6		Metal		O, N	
7	Vacuum	Si		O, N	
8		Metal		O, N	

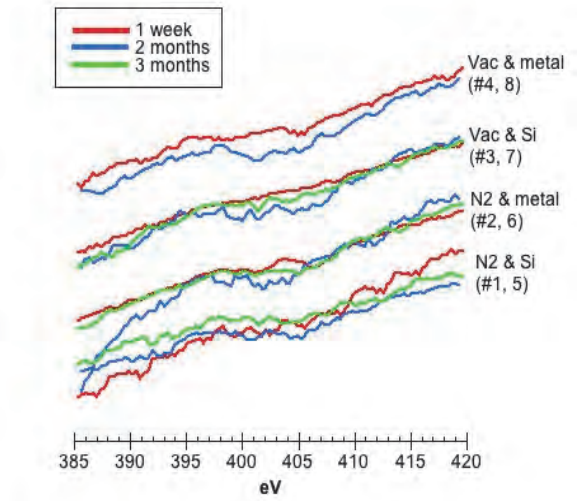

Fig. 1. N-XNAES spectrum of samples #1-8.

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BL6B

Visualization of Spatial Distribution of the Optical Isomers of Amino Acids by Infrared Micro-Spectroscopic Imaging

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Protein, which is one of important components of human bodies, consists of essential amino acids. Although amino acids have optical isomers of L- and D-types except for glycine, living bodies mainly consist of L-amino acids. However, in recent years, it has been revealed that the ratio of D-amino acids increases in living body by racemization reaction with aging. It is considered that an increase of D-amino acids causes the change of the higher-order structure of protein, which is the origin of aging sicknesses such as cataract and Alzheimer's disease [1]. For early prevention of those aging diseases, a simple detection method of D-amino acids is required.

Since physical and chemical properties of D-amino acids are the same as those of L-types, special techniques to separate these isomers, for instance a special chromatography with asymmetric catalysts, are used at present.

In the case of the mixing of L- and D-amino acid molecules, so-called racemates, however, the crystal structure is different from those of homochiral L- and D-molecules. This implies that some infrared (IR) absorption peaks owing to molecular vibrations of racemates are different from those corresponding peaks of the homochiral molecules [2]. Therefore, the IR absorption peak as well as the micro-spectroscopic imaging is a good probe to detect the area and density of racemates, that is of D-amino acids, in the background of L-amino acid molecules.

In this paper, we developed the methodology to detect D-amino acid molecules in L-molecules by using different IR absorption peaks of racemates from those of homochiral molecules as a probe.

Thin film samples of L- and D-alanine, which is one of essential amino acids, on potassium bromide (KBr) substrates were fabricated by a vacuum evaporating method, where L- and D-alanine powders were evaporated separately. A part of evaporation area of these materials was overlapped as shown in Fig. 1(c). In the overlapped area, namely L+D area, the interfacial layer between L- and D-alanine becomes racemates. The IR micro-spectroscopic imaging measurement was performed at the infrared beamline 6B. The imaging measurement was carried out at about 5×5 mm^2 area with 100-μm step (about 3,000 points in total) on the sample.

Figure 1(a) shows an IR absorption peak of L- and D-alanine and L+D overlapping areas. Although the absorption peak shape of pure L and D areas are similar to each other, but the peak of L+D area is different, that looks to have two components, one is the same as pure areas and the other is an extra peak. To clarify the speculation, the peak of the L+D area is well fitted by two Lorentz components as shown in Fig. 1(b). The extra peak originate from the racemate because the same peak has been observed in racemic alanine of a pellet yet. Figure 1(d) shows the spatial distribution of racemic ratio (*R*) evaluated from the ratio of the absorption peak area of the racemic alanine by the fitting at each point performed as the same as that in Fig. 1(b). The image suggests that the *R* values in the L+D area are much different from other homochiral area. This result pronounces that it is possible to visualize the spatial distribution and evaluation of the ratio of D-alanine in L-alanine by the IR imaging. In addition, we could evaluated higher spatial resolution up to 10 μm by using the high-brilliant IR synchrotron radiation (IR-SR). Therefore, the micro-spectroscopic imaging using IR-SR is a suitable tool to detect optical isomers in homochiral amino acids.

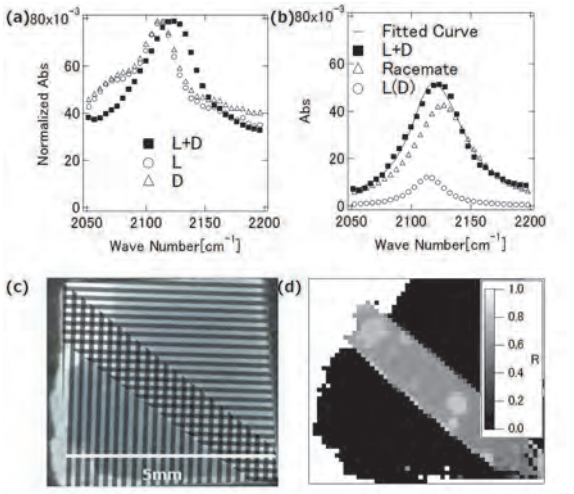

Fig. 1. (a) Infrared absorption spectra of homochiral L-, D-alanine and these overlapping area (L+D). (b) Fitting of the L+D absorption by two Lorentz functions, one is the homochiral spectrum and the other racemic one. (c) Visible image of L-alanine (horizontal line part) and D-alanine (vertical line part) film fabricated on a KBr substrate. (d) Spatial distribution of racemic ratio (R) evaluated by the fitting as shown in (b).

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[2] V. S. Minkov *et al.*, J. Struct. Chem. **51** (2010) 1052.