The chemical form of sulfur compounds in the Japanese pink coral (*Corallium elatius*) skeleton using μ-XRF/XAS speciation mapping

As established in the pioneering work by Smith *et al*. [1], trace elements in biogenic calcium carbonates are well known as highly valuable records of paleoenvironmental changes since the annual growth band recorded in the hard skeleton is a time stamp of the past and the incorporation of trace elements is closely related with various environmental parameters such as temperature, pH, nutrients, and water salinity. The Mg/Ca and Sr/Ca ratios in biogenic calcium carbonates are good examples of elements with the potential to be used as proxies; these ratios have been successfully used as a seawater thermometer. On the other hand, thus far, the chemical form of trace elements in biogenic carbonates is rarely considered in paleoclimatological investigations, and uncertainties of their chemical form remain. Because an inaccurate understanding of the chemical environment of trace elements may result in an errant interpretation in climate reconstruction, speciation analysis in order to establish the chemical form of trace elements in biogenic carbonates is an active field in paleoclimatology.

X-ray analysis using energy-tunable synchrotron radiation should be an attractive tool for simultaneous elucidation of the chemical form and distribution of trace elements via speciation analysis. X-ray photoabsorption spectroscopy (XAS) is a nondestructive analytical technique to characterize and identify trace elements in compositionally complex natural materials. In particular, XAS analysis of sulfur is an established technique, which is sensitive to the electronic structure, valence state, and local symmetry of the absorbing site and is useful as a fingerprint of the chemical form of elements in materials. Furthermore, the combination of XAS and X-ray fluorescence analysis (XRF) using a micro-focused X-ray beam allows both the chemical state of environmentally important trace elements and their spatial distribution in biogenic carbonates to be determined. In the present study, the μ-XRF/XAS technique was applied to the skeleton of Japanese pink coral (*Corallium elatius*; Fig. 1), to elucidate the chemical form of sulfur and clarify the distribution of chemically distinguishable sulfuric compounds [2].

All experiments were performed at **BL27SU** [2]. Figure 2 shows the XAS spectra of *C. elatius* and reference materials measured at the sulfur *K*-edge. For both coenenchyme and the coral skeleton, the XAS spectra show a major peak at 2481.9 eV. $CaSO_4$ and protamine sulfate are typical standard materials for determining the inorganic and organic sulfate

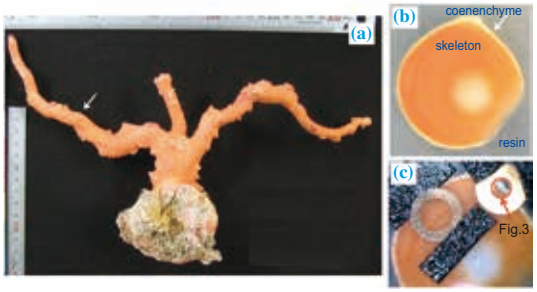

Fig. 1. Morphology of the Japanese pink coral skeleton (*C. elatius*). **(a)** Overview of the coral skeleton. White arrow indicates the analyzed portion. **(b)** Cross section of the slab cut perpendicular to the growth axis. **(c)** Two copper rings (ϕ 1-mm ring for coenenchyme and ϕ 2-mm for the coral skeleton interior) denoting the areas in which the μ-XAS/XRF measurements are performed. Red circle indicates the area in which the speciation mapping analysis is performed (see Fig. 3).

(SO_4^{2-}) content, and the white line at 2481.9 eV is a characteristic fingerprint of sulfate. Therefore, sulfate is the primary species of sulfur in both the coral skeleton and coenenchyme. On the other hand, the XAS spectrum for coenenchyme shows an additional peak at 2472.7 eV (blue curve). Cysteine and methionine are standard organic sulfur materials containing H–S–R and R–S–R bonds, respectively, with their main peaks at 2472.7 eV. These molecules are typical biological molecules containing sulfur. The coenenchyme spectrum suggests the presence of organic sulfur.

Figure 3 presents μ-XRF/XAS speciation mapping of sulfur on the outer edge of the specimen. Figure 3(f) shows a microscope image of the specimen surface for which speciation mapping was performed. The indicated area corresponds to the area labeled with a small Cu ring (ϕ = 1 mm) (Fig. 1(c)). The red area on the right side of the micrograph is the coral skeleton, while the central white area is the coenenchyme. The transparent fragments observed in the coenenchyme are sclerites. The dark area on the left side of the micrograph is the epoxy resin.

The mapping images were obtained by fixing the excitation energy at typical energies for oxidized (2481.9 eV) and reduced sulfur (2472.7 eV). These excitation energies are assigned to the characteristic resonance peaks for sulfate (SO_4^{2-}) and organic sulfur with S-C and/or S-H bonds (Fig. 2). Therefore, these mapping images exhibit the distributions of sulfate and organic sulfur, respectively. The speciation mapping clearly demonstrates that the distribution of sulfur strongly dependents on its chemical form and that the

distributions of sulfate and sulfur-containing organic molecules in the *C. elatius* skeleton differ substantially. Sulfate is identified as the major species in the coral skeleton, whereas organic sulfides are relatively limited (Fig. 3(a)). In contrast, both organic sulfur and sulfate have higher intensities in the coenenchyme (Fig. 3(b)). Furthermore, the distributions of organic sulfur and sulfate in the coenenchyme contrast (Fig. 3(c)), as indicated by the color-coded composite image for the two sulfur compounds. The red and blue areas are attributed to high concentrations of sulfate and organic sulfur, respectively. Figures 3(c) illustrates the sulfate-rich parts of the coral skeleton, embedded with areas of organic sulfur. Figures 3(d) and 3(e) show the elemental mapping images for Mg and P, respectively. The distribution of P is strongly correlated with the distribution of organic sulfur. Thus, organic sulfur is associated with the distribution of biological molecules. Furthermore, in the coenenchyme, the obtained μ-XRF/XAS image indicates that the chemical form of sulfur depends on the biological tissue. Sulfate-rich areas of the coenenchyme are assigned to sclerite, which is composed of a fragmented calcite skeleton distributed in the soft tissues. The distribution of Mg also coincides with that of sulfate in the coenenchyme. Because the major component of sclerite is calcium carbonate, the distribution of Mg in the coenenchyme can be interpreted as substitution for Ca.

A μ-XRF/XAS mapping analyses clarifies that the spatial distributions of sulfur compounds in Japanese pink coral (*C. elatius*) is strongly dependent on the chemical form. Inorganic sulfate species (SO_4^{2-}) are distributed in both the coenenchyme and coral skeleton and the distribution pattern shows dark and bright

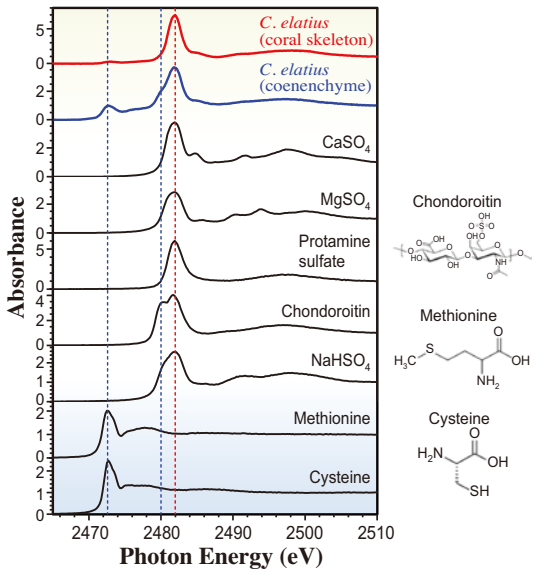

Fig. 2. XAS spectra of Japanese pink coral (*C. elatius*) and sulfur-containing material standards in the sulfur *K*-edge region.

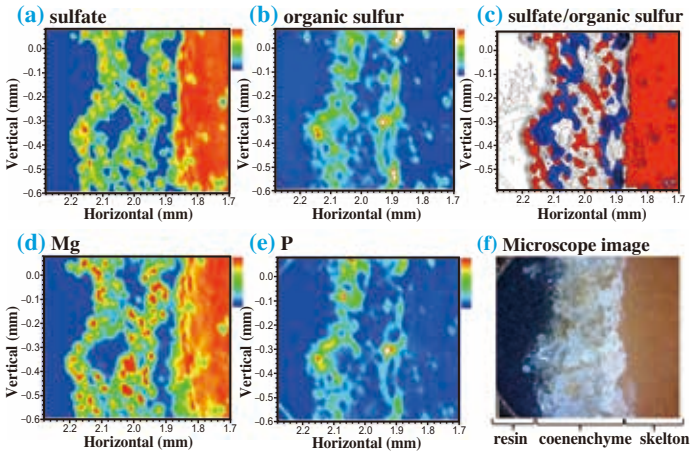

Fig. 3. Sulfur speciation and elemental mapping of Japanese pink coral (*C. elatius*) measured at the coenenchyme. Sulfur-speciation mapping at **(a)** 2481.9 eV (sulfate) and **(b)** 2472.7 eV (organic sulfur). **(c)** Color-coded composite image for two sulfur compounds. Red and blue areas represent areas with high concentrations of sulfate and organic sulfur, respectively. Elemental mapping images of **(d)** Mg and **(e)** P. **(f)** Microscope image of the Japanese pink coral skeleton for which speciation mapping is performed.

bands corresponding to growth bands. In contrast, organic sulfur compounds are mainly concentrated in the coenenchyme. The XAS analysis shows that sulfur can be incorporated into the calcite skeleton as sulfate substituting for carbonate ions, suggesting that sulfur is unsuitable as an indicator of organic matter in *C. elatius* [2]. The sulfate concentration is negatively correlated with the magnesium concentration but positively correlated with that of phosphorus, which is assumed to be mainly present in the organic matrix. The present research demonstrates that speciation analysis will contribute to a more precise understanding of trace elements in biogenic carbonates and will provide significant information on the role of trace elements in biomineralization.

Yusuke Tamenori[a,*], Toshihiro Yoshimura[b] and Nozomu Iwasaki[c]

[a] SPring-8/JASRI
[b] Japan Agency for Marine-Earth Science and Technology (JAMSTEC)
[c] Faculty of Geo-environmental Science, Rissho University

*E-mail: tamenori@spring8.or.jp

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