Environmental and biological influence on seasonal fluctuations of sulfur in a giant clam shell

Bivalves, the shells of which are composed of calcium carbonate ($CaCO_3$), are a geologically important producer of biominerals. The characteristic variations of element profiles in shell $CaCO_3$ are a widely used approach for identifying differences in biological processes and environmental changes associated with element partitioning. A long-lived bivalve, particular giant clams present several advantages for palaeoclimate research, and provide long-term records (up to ~100 years) of the environmental conditions in tropic and sub-tropic oceans [1]. Recently, the dynamics of sulfur incorporation in clam shells are of interest in relation to environmental and physiological parameters.

Sulfate in seawater is the third most abundant ion and sufur concentrations in biogenic $CaCO_3$ range from several hundred to several thousand ppm (e.g., [2]). Sulfur is regarded as an important element in relation to organic compounds, and Cuif *et al.* [3] has proposed a close relationship between skeletal micro-structure and organic matrices. Contrary to the situation with S content in biogenic carbonates which might reflect contamination with organic substances, sulfur in $CaCO_3$ is present as structurally substituted sulfate governed thermodynamic and/or kinetically controlled partitioning [2]. Clarification of how sulfur is hosted in the bivalve shell should elucidate how element incorporation into bivalve shells relates to environmental and biological studies. Furthermore, little attention has been paid to intra-shell seasonal variations of sulfur. Evaluation of the chemical speciation of sulfur is needed to establish reliable paleoenvironmental reconstructions and to better understand details of biomineralization mechanisms.

For this study, we collected a live specimen of the strawberry clam *Hippopus hippopus* (Fig. 1) from Ishigaki Island, Japan (24°33'N, 124°16'E), located in the subtropical northwestern Pacific Ocean. *H. Hippopus* has an aragonitic shell [4]. X-ray absorption near-edge structure (XANES) and μ-X-ray fluorescence analysis (μ-XRF) measurements were carried out at the b-branch of the soft X-ray photochemistry beamline **BL27SU**. μ-XRF mapping data for each element was obtained by using a silicon drift detector. For μ-XRF measurements, the polished slab was fixed on an aluminum sample holder that was then installed in a vacuum chamber and fixed on a motorized XYZ stage. The horizontal and vertical beam size at focus point were 16.3 and 13.7 μm, respectively. The μ-XRF measurements for sulfur and strontium were taken at a photon energy of 2481.3 eV along a transect from the inner shell layer at 8 μm intervals with an acquisition time of 1 s. The spectra of S *K*-edge XANES collected from bivalve shells and S-bearing organic and inorganic reference materials (Fig. 2) indicated that inorganic sulfate was present in marine aragonitic and calcitic bivalve shells (strawberry clam and Japanese scallop *Mizuhopecten yessoensis*). The selected energy range for S XANES measurements was 2460–2510 eV with an energy step of 0.2 eV and an acquisition time of 4 s.

From XRF measurements, we had little direct evidence of the cyclic changes of elements associated with organic components such as phosphorus, with some varying by approximately one order of magnitude (up to >1000 ppm) as observed in sulfur, however. The stacked S *K*-edge XANES spectra collected from reference materials and bivalve shells (Fig. 2) exhibited several features that varied among inorganic and organic reference compounds. The main peaks of the organic reference materials showed large shifts toward higher energies with increasing oxidation state of sulfur. Moreover, the XANES spectra of various inorganic sulfate compounds exhibited a pronounced peak appearing around 2481.5 eV and some small peaks can be confirmed on the higher energy side of main peak. The spectra of the two bivalve shells exhibited similar spectral features to those of sulfate compounds, and the spectrum of the *H. hippopus* and *M. yessoensis* shells showed a significant resonance. A sub-peak at 2484.4 eV that appeared on the high-energy side of the main peak near 2481.5 eV was also apparent in the spectrum of $CaSO_4$ (Fig. 2).

The comparison between the shell structure and the sea surface temperature record shows

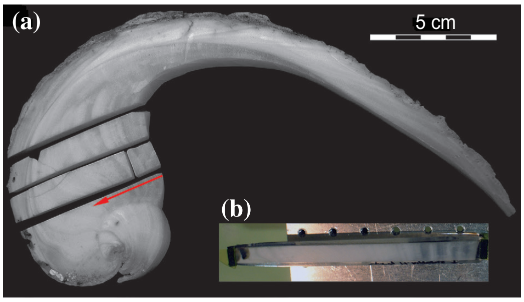

Fig. 1. **(a)** A slab of the *Hippopus hippopus* specimen. A polished slab obtained from the inner shell layer was used for μ-XRF/XAS measurements. The red arrow indicates the measurement transect. **(b)** The polished slab was fixed in an aluminum sample holder with conductive double-sided carbon tape and inserted into the vacuum chamber.

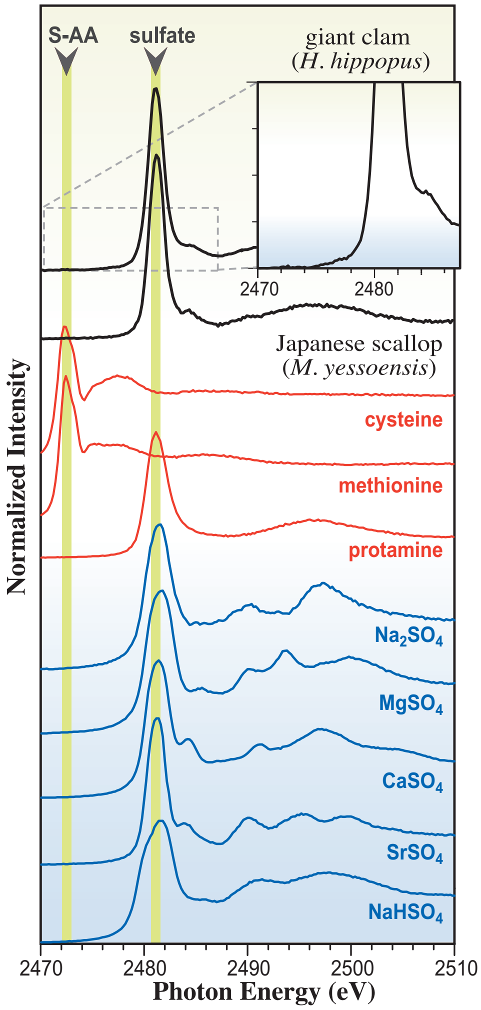

Fig. 2. S *K*-edge XANES spectra of a giant clam shell aragonite (*H. hippopus*), Japanese scallop shell calcite (*M. yessoensis*), and S-bearing reference materials.

that the large increments of light bands correspond to the summers and the thin increments of main dark bands to the winters in *H. hippopus*. Sulfur concentrations in the specimens showed clear annual fluctuations and varied by approximately one order of magnitude, and S maxima and minima of *H. hippopus* shells were associated with the dark and bright bands, respectively, an indication of a negative correlation between sulfate concentrations and water temperature. Supplementary sources of nutrients from symbiotic algae can also maintain the characteristic fast growth of tropical clam shells. The translocation of photosynthates from zooxanthellae, phototrophy is the most significant source of energy to clams, and this source provides sufficient C for growth and metabolic requirements in *H. hippopus* (e.g., [5]). During higher growth periods, carbonate ions (CO_3^{2-}) in calcifying fluids is increased, and at the same time the increased calcium carbonate saturation state induces the higher calcification rates. This process is promoted enzymatically in the calcifying space. The increases in CO_3^{2-} concentrations would negatively influence the sulfate incorporation into $CaCO_3$ by reducing the relative activity of sulfate ions to carbonate ions in the calcifying fluids, with sulfur concentrations being lower during periods of faster growth (Fig. 3). If the seasonal skeletal growth rate and the changes in carbonate ion concentrations in calcifying fluids were positively correlated, it is suggested that environmental factors such as insolation or some influencing factors of shell growth affected sulfate concentrations in their shells.

The large seasonal changes of sulfate concentrations can potentially be an archive of cyclic changes of shell crystallization, which is related to environmental factors (e.g., insolation) and bivalve physiology. In contrast to element partitioning of cations, the mechanisms controlling inorganic anion incorporation are poorly understood. Our analysis and the previous studies suggest that the possible strong controls of crystal structure and aqueous chemistry on sulfate incorporation into $CaCO_3$.

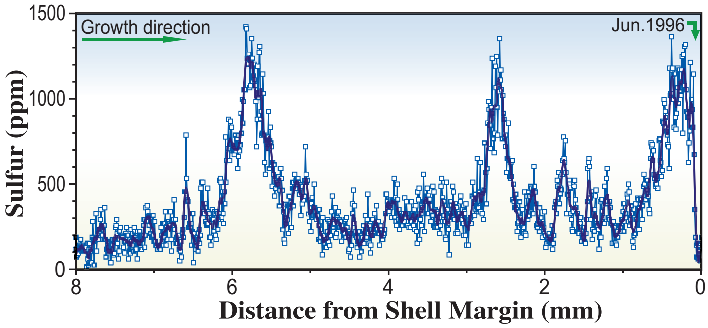

Fig. 3. Sulfur profile from the inner shell layer of *H. hippopus* obtained by the μ-XRF measurement at 8-μm intervals. The spatial resolution of μ-XRF allows the variations of the S content to be examined at a daily timescale (300-850 points per year). The moving average trend (blue line) was calculated by using a 15-point smoothing window.

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References

[1] M. Elliot *et al.*: Palaeogeogr. Palaeoclimatol. Palaeoecol. **280** (2009) 132.
[2] E. Busenberg and L.N. Plummer: Geochim. Cosmochim. Acta **49** (1985) 713.
[3] J.P. Cuif *et al.*: Geochim. Cosmochim. Acta **67** (2003) 75.
[4] *T. Yoshimura, Y. Tamenori, A. Suzuki, R. Nakashima, N. Iwasaki, H. Hasegawa and H. Kawahata: Chem. Geol.* ***352*** *(2013) 170.*
[5] D.D. Klumpp and C.C. Griffiths: Mar. Ecol. Prog. Ser. **115** (1994) 103.