Nano-SIMS analysis can highlight the effect of symbiotic zooxanthellae on sulfur utilization of corals

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Despite sulfur is the main component in the ocean as sulfate ions, little is known about utilization in living organisms. Genome analysis has revealed that corals, which are host animals, do not have a system for synthesizing cysteine, essential amino acid and one of the sulfur-containing amino acids (Shinzato et al. 2011, Nature). This suggests that corals may receive cysteine synthesized by zooxanthellae, or may obtain and use it heterotrophically. It has been confirmed that sulfur is concentrated in zooxanthellae by autoradiography using radioactive isotope ^{35}S. In addition, the amount of ^{35}S accumulated in the coral animal tissues, zooxanthellae, and skeletons was examined. As the exposure time to ^{35}S in seawater increased, the amount of radioactive sulfur accumulated in coral tissues and zooxanthellae increased. (Yuyama et al. 2016, Biol Open). In this study, a stable isotope labeling experiment was conducted and analyzed by NanoSIMS (NS 50, Cameca) to clarify how sulfate ions in seawater are taken up by corals and zooxanthellae. In the experiment, a juvenile polyp of genus *Acropora* (approximately 1 month old) was used to create a zooxanthellae symbiotic polyp with infection of *Durusdinium* (Clade D) and an apo-symbiotic polyp without infection of zooxanthellae. Corals were incubated in filtered seawater with $Na_2{}^{34}SO_4$ (sulfur isotope ratio +1000 ‰) for 2 days. Individuals that had been isotopically labeled for two days were returned to normal seawater and incubated for two days. After incubation, juvenile corals were embedded with resin and cutting and polishing were conducted for NanoSIMS analysis. When the sulfur isotopes ^{32}S and ^{34}S were mapped using NanoSIMS, high ^{34}S points were found in the coral soft tissue. This study reconfirmed that coral uptakes sulfur from seawater as sulfate ions. In particular, ^{34}S-labeled sites were found in the presence of zooxanthellae and in coral animals close to seawater. In addition, the aposymbiotic polyps showed a ^{34}S label in the host part, indicating that the host corals assimilate sulfate ions without passing through the symbiotic algae. Furthermore, even after returning to normal seawater for 2 days after the ^{34}S label, the ^{34}S label was clearly seen in both symbiotic and aposymbiotic polyps, indicating that assimilated sulfur was preserved for at least 2 days.

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