

RESEARCH ARTICLE

Sulfur utilization of corals is enhanced by endosymbiotic algae

Ikuko Yuyama^{1,2}, Tomihiko Higuchi^{2,3,*} and Yoshio Takei³

ABSTRACT

Sulfur-containing compounds are important components of all organisms, but few studies have explored sulfate utilization in corals. Our previous study found that the expression of a sulfur transporter (*SLC26A11*) was upregulated in the presence of *Symbiodinium* cells in juveniles of the reef-building coral *Acropora tenuis*. In this study, we performed autoradiography using ³⁵S-labeled sulfate ions (³⁵SO₄²⁻) to examine the localization and amount of incorporated radioactive sulfate in the coral tissues and symbiotic algae. Incorporated ³⁵SO₄²⁻ was detected in symbiotic algal cells, nematocysts, ectodermal cells and calicoblast cells. The combined results of ³⁵S autoradiography and Alcian Blue staining showed that incorporated ³⁵S accumulated as sulfated glycosaminoglycans (GAGs) in the ectodermal cell layer. We also compared the relative incorporation of ³⁵SO₄²⁻ into coral tissues and endosymbiotic algae, and their chemical fractions in dark versus light (photosynthetic) conditions. The amount of sulfur compounds, such as GAGs and lipids, generated from ³⁵SO₄²⁻ was higher under photosynthetic conditions. Together with the upregulation of sulfate transporters by symbiosis, our results suggest that photosynthesis of algal endosymbionts contributes to the synthesis and utilization of sulfur compounds in corals.

KEY WORDS: Endosymbiosis, Skeletogenesis, Coral, *Acropora*, *Symbiodinium*, Sulfate ion

INTRODUCTION

Reef-building scleractinian corals harbor endosymbiotic dinoflagellate algae of the genus *Symbiodinium* spp. (referred to as zooxanthellae), which provide coral hosts with their photosynthetic products (Muscatine, 1990). Studies using ¹⁴C-labeled carbon have shown that endosymbiotic algae release photosynthetic products – such as glucose, glycerol and organic acids – that contribute to the coral host carbon requirements (Muscatine and Ceiwichmfu, 1969; Trench, 1971a; Battey and Patton, 1984; Whitehead and Douglas, 2003). Corals also receive various amino acids and fatty acids from symbiotic algae (Trench, 1971b; Wang and Douglas, 1999; Papina et al., 2007). Furthermore, algal photosynthesis results in an increase in the calcification rate in the host skeleton (Al-Horani et al., 2003). Some studies have

demonstrated that the incorporation of Ca²⁺ into coral skeletons is accelerated under light conditions and reduced by a photosynthetic inhibitor (Furla et al., 2000; Al-Horani et al., 2003). Thus, endosymbiotic algae are nutrient sources for corals, and their photosynthetic activities may affect coral growth rates.

Few studies have explored sulfate utilization in corals and their symbionts, although sulfur-containing compounds have been detected in coral soft tissues and skeletons. Atomic force microscopic studies have indicated that early mineralization zones, commonly called ‘centers of calcification,’ contain a high concentration of sulfated polysaccharides, and a layered distribution of sulfate has been observed within the crystal-like fibers (Cuif et al., 2003; Cuif and Dauphin, 2005). Sulfated molecules were also detected in the mucus as sulfated sugars, including oligosaccharide side chains of mucus glycoproteins in *Acropora formosa* (Meikle et al., 1987). Another important group of sulfur-containing compounds are the sulfur-containing amino acids (e.g. cysteine and methionine), which play a key role in the synthesis of essential biomolecules, such as antioxidants, vitamins, and co-factors (Saito, 2004). In symbiotic dinoflagellates, the uptake of sulfate and sulfur-containing amino acids has been observed in *Amphidinium carterae*, *A. klebsii*, and *Symbiodinium microadriaticum* (Deane and O’Brien, 1981), suggestive of a sulfate and sulfur-containing amino acid transport system between corals and symbiotic algae. Investigation of the metabolic processing of sulfates in coral will facilitate elucidation of the relationship between corals and algae.

In a previous study, we identified a gene encoding a sulfate transporter as a symbiotically related gene in *Acropora tenuis* (Yuyama et al., 2011). The expression of this gene was consistently upregulated in symbiotic corals compared with aposymbiotic corals (Yuyama and Watanabe, 2008; Yuyama et al., 2011). Immunoreactive sulfate transporters were identified in mucus cells and in the tissue between the coelenteron and skeleton (Yuyama and Watanabe, 2008). These results suggest that sulfate utilization by the coral was enhanced by its symbiotic associations with algae. Thus, exploring which sulfate is utilized by coral is important to understand the endosymbiotic relationship between corals and algae, as well as coral calcification. The purpose of the present study was to determine how corals and endosymbiotic algae utilize environmental sulfate ions. First, autoradiography was performed using ³⁵S-labeled sulfate ions (³⁵SO₄²⁻) to examine the localization of incorporated sulfate ions. Next, we examined the relative incorporation rate of ³⁵SO₄²⁻ into coral and endosymbiotic algae, and their chemical fractions under dark or light (photosynthetic) conditions.

RESULTS

Autoradiography

After exposure to ³⁵SO₄²⁻, incorporated ³⁵S was detected as brown dots in the coral sections (Fig. 1). Water-soluble compounds were removed from the coral tissues by dehydration. Therefore, ³⁵S-labeled water-insoluble compounds could be detected only in the soft tissues of the coral. Microscopic images of coral sections

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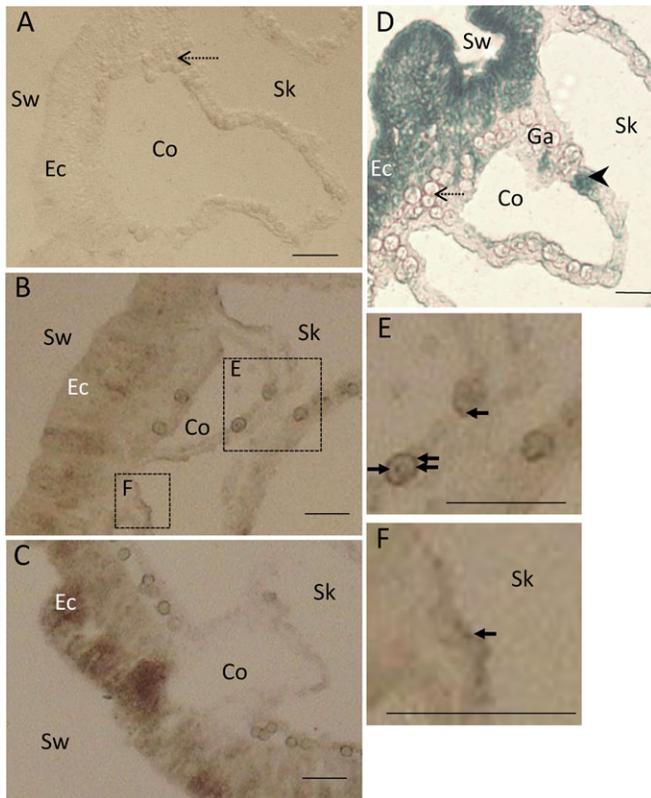


Fig. 1. Light micrographs of decalcified adult coral sections exposed to ^{35}S -labeled sulfate ions ($^{35}\text{SO}_4^{2-}$). Coral sections exposed for 0 (A), 6 (B), and 24 h (C), and non-autoradiographic coral sections stained with Alcian Blue (pH 1.0) (D). Areas surrounded by broken lines in B are enlarged in (E,F). Arrows indicate ^{35}S grains, arrowheads indicate mucocytes, and the dashed-line arrow indicates an endosymbiotic zooxanthellae. Co, coelenteron; Sk, skeleton; Sw, seawater; Ec, ectoderm; Ga, gastrodermis. Scale bars=20 μm .

exposed for 6 and 12 h showed abundant ^{35}S grains in symbiotic algal cells, nematocytes and ectodermal cells (Fig. 1B,C,E). In addition, sulfated GAGs were observed in coral tissues by Alcian Blue staining, which was used to detect the presence of mucocytes (Fig. 1D). Ectoderms and mucus cells in the gastrodermis were strongly stained with Alcian Blue, indicating that these cells were mucocytes, and contained sulfated GAGs.

Measurement of $^{35}\text{SO}_4^{2-}$ incorporation into tissue and chemical fractions

Incorporation of ^{35}S into endosymbiotic algae and coral skeletons tended to increase during the incubation period ($P < 0.05$, ANOVA), but the ^{35}S quantity in coral soft tissue did not change significantly (Fig. 2). Incorporated ^{35}S concentrations in coral soft tissue were generally higher than those in endosymbiotic algae, with the exception of after 2 days of non-exposure (chase). After the 2-day exposure, followed by a 2-day chase period, the ^{35}S concentration in endosymbiotic algae did not change significantly with 2-day pulses, whereas that in the coral soft tissue and skeleton decreased by 89% and 48%, respectively, after the 2-day ^{35}S exposure (pulse).

Incorporation of radioactivity into the principal chemical fractions of the algal cells and animal tissue is shown in Fig. 3. The distribution of ^{35}S radioactivity across the chemical fractions varied significantly between the algae and coral tissue, and showed an increasing trend during incubation experiments. The

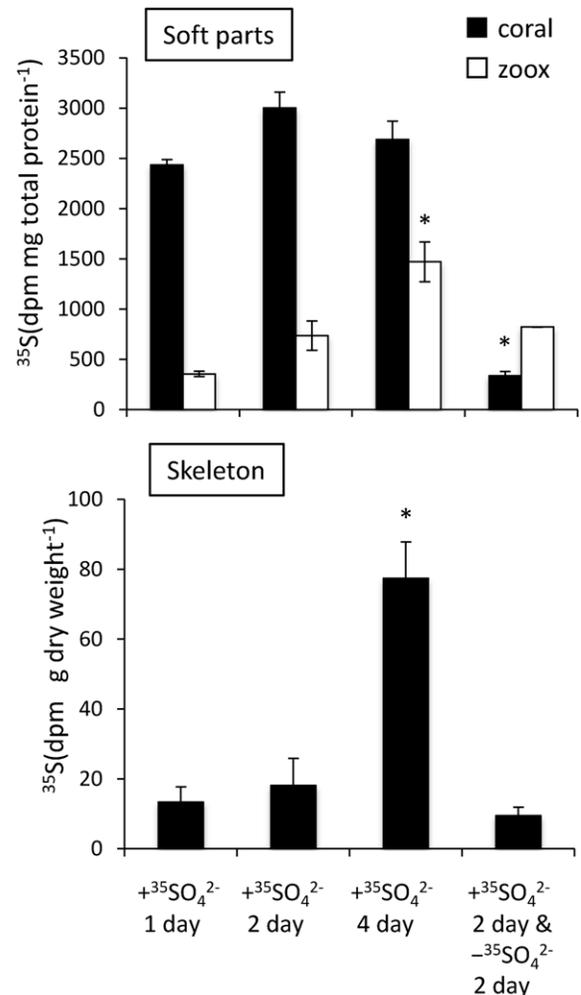


Fig. 2. Incorporation of $^{35}\text{SO}_4^{2-}$ into the soft parts of coral and coral skeleton. Coral tips were incubated for 1, 2, or 4 days in the presence of $^{35}\text{SO}_4^{2-}$, or in the absence of $^{35}\text{SO}_4^{2-}$ for 2 days following a 2-day incubation in the presence of $^{35}\text{SO}_4^{2-}$. Host coral tissues, black bars; zooxanthellae (zoox) cells, white bars. Error bars represent means \pm standard error (s.e.m.) of biological replicates ($n=3$). * $P < 0.05$ compared with other incubation conditions based on a Tukey–Kramer honestly significant difference (HSD) test.

concentration of ^{35}S in GAG fractions was reduced to undetectable levels after the chase. Radioactivity of the lipid fraction after the chase decreased by almost half (62%) that during the pulse period, while the majority remained in algal fractions (101%) after the 2-day pulse. In lipid fractions, the ^{35}S radioactivity in coral was 1.8–3.0-fold higher than in the algae lipid fraction, whereas the radioactivity in protein fractions was higher in algae than in corals.

The effect of light conditions on the incorporation of $^{35}\text{SO}_4^{2-}$

We also compared the incorporation of ^{35}S between light (photosynthesizing) and dark (non-photosynthesizing) conditions (Figs 4, 5). The radioactivity of ^{35}S in soft tissue and algae, and their chemical fractions and skeletons, was greater under photosynthesizing conditions than dark conditions. However, significant differences between dark and light were observed only in the algal total fraction, in which the quantity of incorporated ^{35}S was significantly higher under light conditions (289, 364, 390 dpm mg protein⁻¹) than dark conditions (96, 131, 188 dpm mg protein⁻¹) (Fig. 4). In the coral GAG fractions, ^{35}S radioactivity

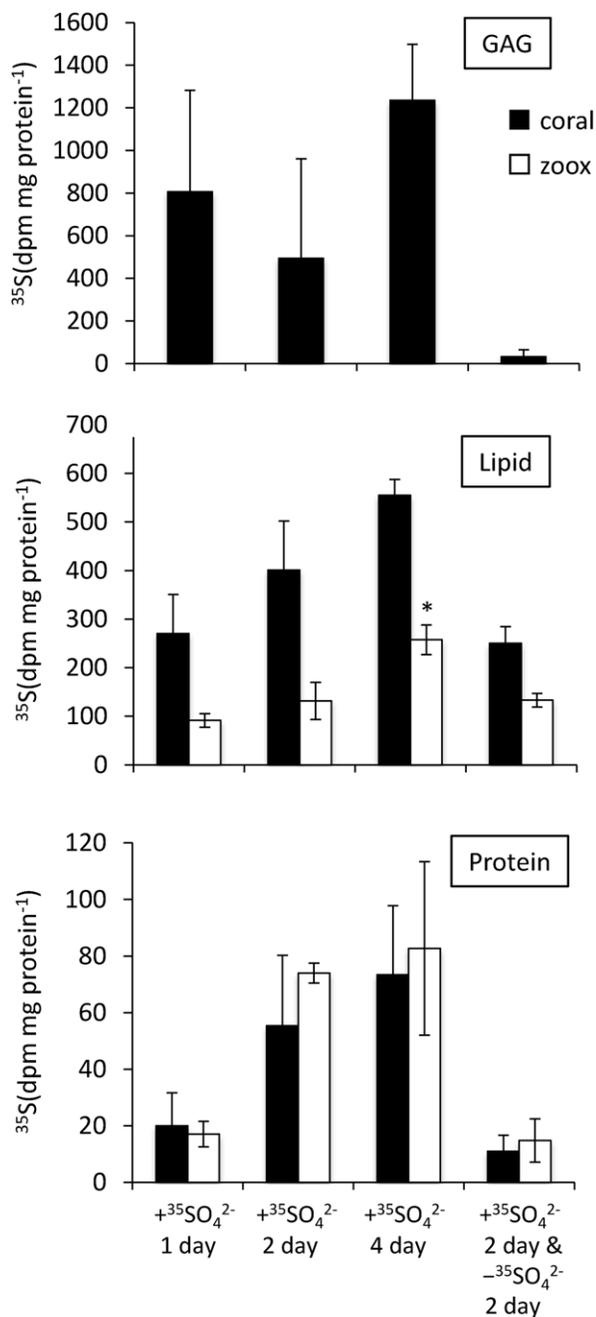


Fig. 3. Incorporation of ³⁵SO₄²⁻ into the glycosaminoglycan (GAG), lipid, and protein fractions of coral tissues and zoox cells. Coral tips were incubated for 1, 2, or 4 days in the presence of ³⁵SO₄²⁻, or incubated in the absence of ³⁵SO₄²⁻ for 2 days following a 2-day incubation in the presence of ³⁵SO₄²⁻. Coral tissues, black bars; zoox cells, white bars. Error bars represent means ± s.e.m. of biological replicates (*n*=3). **P*<0.05 compared with other incubation conditions based on a Tukey–Kramer HSD test.

was detected only in samples incubated under light conditions. The ³⁵S concentrations of the protein fractions from 12-h samples could not be estimated since they showed the same level of radioactivity as the reference samples (³⁵S unexposed corals) (Fig. 5).

DISCUSSION

Based on the results of autoradiography using the labeled sulfate ion, we discuss the possibility that the symbiotic relationship with algae affects the synthesis of sulfur compounds in corals.

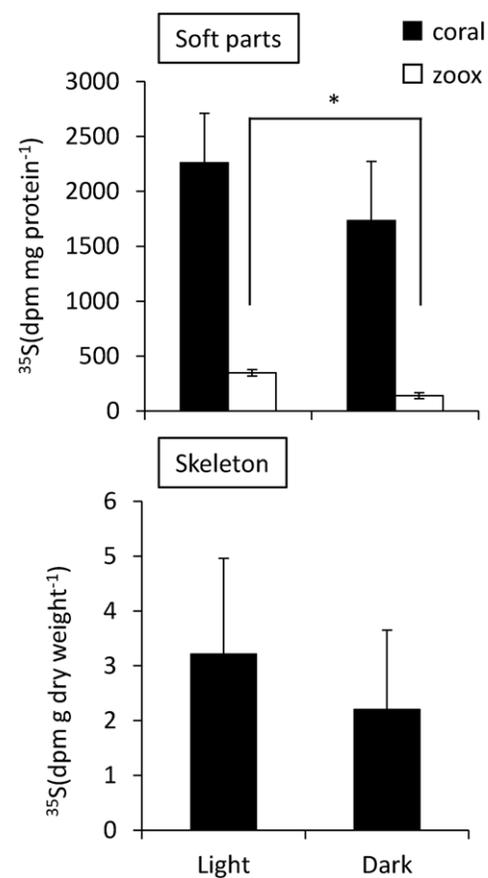


Fig. 4. Effect of light on the incorporation of ³⁵SO₄²⁻ into soft parts of coral and coral skeleton. Coral tips were incubated in the presence of ³⁵SO₄²⁻ for 12 h under light and dark conditions. Host coral tissues, black bars; zoox cells, white bars. Error bars represent means ± s.e.m. of biological replicates (*n*=3). **P*<0.05 between light and dark conditions based on a Student's *t*-test.

The incorporated ³⁵S content of coral skeleton and endosymbiotic algae increased during the experimental period. In contrast, the amount of ³⁵S incorporation into coral soft tissue was not affected by the incubation period. In the light, symbiotic algae incorporated a greater quantity of ³⁵SO₄²⁻ than in the dark (Figs 1, 4). The incorporated ³⁵S was detected around symbiotic algal cells and inside the symbiotic cells (Fig. 1B,E). These results suggest that there is a close relationship between transported sulfate and photosynthesis in symbiotic algae. It was previously hypothesized that endosymbiotic algae promote sulfate utilization of host corals, since higher expression levels of genes coding for a sulfate transporter were found in symbiotic corals compared with aposymbiotic corals (Yuyama and Watanabe, 2008; Yuyama et al., 2011). The results of this study indicate that endosymbiotic algae take up ³⁵SO₄²⁻ from seawater, and the increased overall incorporation rate of coral tissue under photosynthetic conditions supports this hypothesis. Other elements (C, N and Ca) have been investigated in addition to ³⁵S; their rates of incorporation into corals were promoted under light conditions (Muscatine, 1990; Furla et al., 2000; Muscatine and D'Elia, 1978). Thus, endosymbiotic algae provide comprehensive support for the incorporation of various elements by corals.

Autoradiography showed that the transported ³⁵S also accumulated in endodermal cells near the skeleton (Fig. 1F). Moreover, the translocation of ³⁵S into coral skeletons was

sulfolipid, SQDG, is a chloroplast membrane lipid (Garrett et al., 2013; Díaz-Almeyda et al., 2011; Awai et al., 2012). Thus, coral and algae contain their own sulfolipids, but whether these sulfolipids are transported between, and utilized by, coral and algae remains unclear. Synthesis of ^{35}S lipids in coral and algae increased under light conditions (Fig. 5), suggesting that the ^{35}S lipid synthesis pathway is at least in part regulated by algal photosynthesis. Previous studies showed that glucose is one of the lipid precursors transported to the host from algal cells (Whitehead and Douglas, 2003). The photosystem of *Symbiodinium* provides carbon precursors for sulfolipid biosynthesis, which may increase production of ^{35}S lipids under light conditions.

Overall, our results showed that (1) coral and their algal symbionts take up sulfate from seawater for the synthesis of organic sulfur compounds, and (2) the incorporation rate of sulfur compounds is higher under photosynthetic conditions, suggesting that the photosynthesis of algal endosymbionts contributes to the synthesis and utilization of sulfur compounds in corals. Endosymbiotic zooxanthellae are required for coral to efficiently utilize sulfur compounds such as sulfur amino acids, sulfolipids and sulfated GAGs. Sulfur metabolism in coral is an intriguing subject, since many sulfur compounds have redox potential (Mitchell, 2003) and are found in coral skeletons (Cuif et al., 2003; Cuif and Dauphin, 2005; Tanaka and Ohde, 2007). In addition, organic sulfur compounds are potential sources of nutrients for coral-associated bacteria (Raina et al., 2013). To further explore the utilization, transport, and metabolism of sulfur in corals, it is important to understand the mechanisms of the coral defense system and coral growth, as well as the coral–algae endosymbiotic relationship.

MATERIALS AND METHODS

Biological materials

Adult colonies of *Acropora tenuis* were collected around Sesoko Island in Okinawa, Japan, with permission from the Okinawa prefectural government (No. 24–49). The colonies were transferred to the University of Tokyo or Shizuoka University and maintained in circulated seawater at 22°C under a 12 h light:12 h dark cycle at $100\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$. Coral fragments of about 3 cm in length were prepared by cutting branches from the *A. tenuis* colonies; the top of branches (including some polyps) were maintained for 1 week before the start of the experiment. A total of 30 fragments from four adult colonies were randomly selected for the experiment ($n=3$ per treatment).

Autoradiography

Branches of adult *A. tenuis* were put into a glass bottle containing 40 ml of filtered natural seawater with (^{35}S)-sodium sulfate. The (^{35}S)-sulfate ($370\ \text{MBq ml}^{-1}$; GE Healthcare, Little Chalfont, UK) in a total volume of $6\ \mu\text{l}$ was added to filtered natural seawater. The water temperature was set at 22°C; the incubation times were 6 and 24 h under light conditions ($100\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$). After incubation, the coral tip was excised from the plates and washed three times with sterilized seawater. Unexposed coral branches (exposure time to ^{35}S -labeled sulfate, 0 h) were prepared as the experimental control. Branches were transferred to glass bottles containing 40 ml of filtered natural seawater (without addition of ^{35}S) as the experimental treatment. The coral samples were fixed, decalcified and embedded in Paraplast® as described by Yuyama and Watanabe. (2008). De-paraffinized coral tissue sections were then dehydrated through a graded ethanol series (70, 90, and 100%) containing 0.3 M ammonium acetate and air-dried. Thereafter, the sections were dipped in Hypercoat Emulsions (GE Healthcare) and exposed for 3 days in darkness. After development with Kodak D-19 developer (Rochester, NY, USA) and fixation, they were dehydrated. Microscopic observations of the coral sections were performed using a stereomicroscope (SZX-ILLK 100; Olympus, Tokyo, Japan).

Alcian Blue staining

Coral sections not exposed to $^{35}\text{SO}_4^{2-}$ were stained with Alcian Blue to detect sulfated glycosaminoglycans (GAGs). Fixation, decalcification, and embedding were performed as described above. After de-paraffinization, the sections were placed in 0.1 N HCl for 5 min and then stained with 1% Alcian Blue (pH 1.0) for 30 min (Jones and Reid, 1973). Microscopic observations of these sections were made using a stereomicroscope (SZX-ILLK 100; Olympus).

Quantification of incorporation of ^{35}S -labeled sulfate

Branches of adult *A. tenuis* were placed in a glass bottle containing 20 ml of filtered natural seawater with (^{35}S)-sodium sulfate. The (^{35}S)-sulfate ($370\ \text{MBq ml}^{-1}$; American Radiolabeled Chemicals Inc., St. Louis, MO, USA) in a total volume of $3\ \mu\text{l}$ was added to filtered natural seawater. The water temperature was set at 22°C under a 12 h light:12 h dark cycle at $100\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$. In the first experiment, branches were incubated for 1, 2 or 4 days. In the second experiment, branches were incubated in filtered natural seawater for 2 days after a 2-day incubation with (^{35}S)-sodium sulfate. In the third experiment, branches were incubated for 12 h under light ($100\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$) and dark conditions. Replicate groups of three branches were used for each treatment. After incubation, the coral tip was washed three times with fresh seawater. An unexposed coral branch (exposure time to ^{35}S -labeled sulfate, 0 h) was prepared as the experimental control. The counts in the samples incubated without added radioactivity were subtracted from the values of the experimental samples.

Measurement of ^{35}S in endosymbiotic zooxanthellae, coral soft tissue, and skeleton

Soft tissue was removed by using the water-pick method, which stripped the tissue from the coral skeleton into approximately 30 ml of 100 mM phosphate buffer with $10\ \text{g liter}^{-1}$ NaCl (pH=7.0). Removed tissues were homogenized and centrifuged (twice at $600\times g$ for 10 min) to separate the supernatant (coral tissue) and pellets (zooxanthellae). The animal tissue fraction was adjusted to a known volume and the algal pellet was resuspended in 2 ml of 100 mM phosphate buffer with $10\ \text{g liter}^{-1}$ NaCl. The protein content of each homogenate was quantified by Bradford assay (Bradford, 1976). Portions of the algae and coral fractions were re-suspended in hot 80% alcohol and adjusted to a known volume, after which radioactivity was evaluated using a liquid scintillation counter (LSC-5100; Aloka, Tokyo, Japan). The counting efficiency (cpm/dpm ratios) for ^{35}S was calculated to be 0.7. ^{35}S content in seawater was adjusted to $1\times 10^4\ \text{dpm ml}^{-1}$ for all experiments. Skeletons were boiled in 6 N NH_4OH to dissolve and remove residual animal tissue and algae. The tissue-free skeleton was washed in distilled water and the weight of the dried skeleton was measured using an electronic balance. No detection of ^{35}S was confirmed in distilled water for final washing. The clean skeleton was dissolved in cold 6 N HCl, and the HCl fraction containing the ^{35}S was counted after filtration by $0.45\ \mu\text{m}$ PTFE filter.

Fractionation of ^{35}S labeled components of corals and zooxanthellae

Coral and algae fractions were divided for preparation and enumeration into the trichloroacetic acid (TCA) insoluble (protein) fraction (Whitehead and Douglas, 2003), lipid fraction (Bligh and Dyer, 1959), and GAG fraction (Zhang et al., 2001). Samples of the algal and host coral fractions were fixed in 10% TCA for 15 min and then centrifuged at $10,000\times g$ for 15 min. The supernatant was washed with 100% ethanol and then resuspended in $500\ \mu\text{l}$ of 8 M guanidine HCl to detect ^{35}S using a liquid scintillation counter. The lipid fraction was extracted from the coral and algae tissue fractions with 1 ml methanol:chloroform (2:1) for 15 min at room temperature, followed by centrifugation at $1500\times g$ for 15 min. The chloroform layer containing the lipids was separated from the methanol layer by the addition of 0.5 ml of 0.1 M KCl. The chloroform layer was used for counting ^{35}S . To prepare the GAG fraction, an Alcian Blue assay was performed as described by Frazier et al. (2008). The resulting pellet was dissolved in $500\ \mu\text{l}$ of 8 M guanidine HCl and assessed using a liquid scintillation counter. The experiments were performed in triplicate. The GAG fraction was not prepared from the algae

fraction because endosymbiotic *Symbiodinium* in coral tissue sections were not stained by Alcian Blue. Counts in the fractions incubated without added radioactivity were subtracted from the values of the experimental samples.

Statistical analysis

All data were normalized to protein content or skeleton dry weight. Statistical analyses were performed using JMP software (v. 8.0; SAS Institute, Cary, NC, USA); the normalized values were compared with an analysis of variance (ANOVA). *Post hoc* differences were evaluated using the Tukey–Kramer honest significant difference (HSD) test.

Acknowledgements

We are grateful to Dr Akira Iguchi from the University of the Ryukyus for providing *A. tenuis*. We are also grateful to Dr Yasuhisa Ohya and Mr. Toshiyoshi Miyazawa from the Radiochemistry Research Laboratory of Shizuoka University, and Drs Toshiaki Watanabe, Susumu Hyodo, and Taro Watanabe of AORI for their invaluable support.

Competing interests

The authors declare no competing or financial interests.

Author contributions

I.Y. and T.H. designed and conducted the experiments, analyzed data, and wrote the manuscript. Y.T. supported experiments and revised the manuscript. All authors read and approved the final version of the manuscript.

Funding

This work was supported in part by The Japan Science Society Sasagawa Scientific Research Grant [grant number 24-731 to I.Y.], the Fujiwara Natural History Foundation [grant number 15 to I.Y.], a research grant from the Japan Society for the Promotion of Science [grant number 14J40135 to I.Y.] and the Kurita Water and Environment Foundation [grant number 13A055 to T.H.].

References

- Al-Horani, F. A., Al-Moghrabi, S. M. and De Beer, D. (2003). The mechanism of calcification and its relation to photosynthesis and respiration in the scleractinian coral, *Galaxea fascicularis*. *Mar. Biol.* **142**, 419-426.
- Atmaca, G. (2004). Antioxidant effects of sulfur-containing amino acids. *Yonsei Med. J.* **45**, 776-788.
- Awai, K., Matsuoka, R. and Shioi, Y. (2012). Lipid and fatty acid compositions of *Symbiodinium* sp. Proceedings of the 12th inter. coral reef symp., 6A-1, Cairns, Australia (9-13 July 2012).
- Battay, J. F. and Patton, J. S. (1984). A reevaluation of the role of glycerol in carbon translocation in zooxanthellae-coelenterate symbiosis. *Mar. Biol.* **79**, 27-38.
- Battin, E. E. and Brumaghim, J. L. (2009). Antioxidant activity of sulfur and selenium: a review of reactive oxygen species scavenging, glutathione peroxidase, and metal-binding antioxidant mechanisms. *Cell. Biochem. Biophys.* **55**, 1-23.
- Bligh, E. G. and Dyer, W. J. (1959). A rapid method for total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37**, 911-917.
- Bradford, M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248-254.
- Brown, B. and Bythell, J. C. (2005). Perspectives on mucus secretion in reef corals. *Mar. Ecol. Prog. Ser.* **296**, 291-309.
- Cuif, J.-P. and Dauphin, Y. (2005). The two-step mode of growth in the Scleractinian coral skeletons from the micrometre to the overall scale. *J. Struct. Biol.* **150**, 319-331.
- Cuif, J.-P., Dauphin, Y., Doucet, J., Salome, M. and Susini, J. (2003). XANES mapping of organic sulfate in three scleractinian coral skeletons. *Geochim. Cosmochim. Acta.* **67**, 75-83.
- Deane, E. M. and O'Brien, R. W. (1981). Uptake of sulfate, taurine, cysteine and methionine by symbiotic and free-living dinoflagellates. *Arch. Microbiol.* **128**, 331-319.
- Diaz-Almeyda, E., Thomé, P. E., El Hafidi, M., Iglesias-Prieto, R. (2011). Differential stability of photosynthetic membranes and fatty acid composition at elevated temperature in *Symbiodinium*. *Coral Reefs* **30**, 217-225.
- Esko, J. D., Kimata, K. and Lindahl, U. (2009). Proteoglycans and sulfated glycosaminoglycans. In *Essentials of Glycobiology*, Chapter 16, 2nd edn (ed. A. Varki, R.D. Cummings and J.D. Esko). New York: Cold Spring Harbor laboratory Press.
- Frazier, S. B., Roodhouse, K. A., Hourcade, D. E., Zhang, L. (2008). The quantification of Glycosaminoglycans: A comparison of HPLC, carbazole, and alcian blue methods. *Open Glycosci.* **1**, 31-39.
- Fukuda, I., Ooki, S., Fujita, T., Murayama, E., Nagasawa, H., Isa, Y. and Watanabe, T. (2003). Molecular cloning of a cDNA encoding a soluble protein in the coral exoskeleton. *Biochem. Biophys. Res. Comm.* **304**, 11-17.
- Furla, P., Galgani, I., Durand, I. and Allemand, D. (2000). Sources and mechanisms of inorganic carbon transport for coral calcification and photosynthesis. *J. Exp. Biol.* **203**, 3445-3457.
- Garrett, T. A., Schmeitzel, J. L., Klein, J. A., Hwang, J. J. and Schwarz, J. A. (2013). Comparative lipid profiling of the cnidarian *Aiptasia pallida* and its dinoflagellate symbiont. *PLoS ONE* **8**, e57975.
- Jones, R. and Reid, L. (1973). The effect of pH on alcian blue staining of epithelial acid glycoproteins. I. Sialomucins and sulphomucins (singly or in simple combinations). *Histochem. J.* **5**, 9-18.
- Leustek, T. and Saito, K. (1999). Sulfate transport and assimilation in plants. *Plant Physiol.* **120**, 637-644.
- Marshall, A. T., Clode, P. L., Russell, R., Prince, K. and Stern, R. (2007). Electron and ion microprobe analysis of calcium distribution and transport in coral tissue. *J. Exp. Biol.* **210**, 2453-2463.
- Meikle, P., Richards, G. N. and Yellowlees, D. (1988). Structural investigations on the mucus from six species of coral. *Mar. Biol.* **99**, 187-193.
- Mitchell, S. C. (2003). *Biological Interactions of Sulfur Compounds*. Florida, US: CRC Press.
- Muscatine, L. (1990). The role of symbiotic algae in carbon and energy flux in reef corals. *Coral Reefs* **25**, 75-87.
- Muscatine, L. and D'Elia, C. F. (1978). The uptake, retention and release of ammonium by reef corals. *Limnol. Oceanogr.* **23**, 725-734.
- Muscatine, L. and Ceiwichmfu, E. (1969). Assimilation of photosynthetic products of zooxanthellae by a reef coral. *Biol. Bull.* **137**, 506-523.
- Papina, M., Meziane, T. and Van Woessik, R. (2007). Acclimation effect on fatty acids of the coral *Montipora digitata* and its symbiotic algae. *Comp. Biochem. Physiol. Biochem. Mol. Biol.* **147**, 583-589.
- Raina, J.-B., Tapiolas, D. M., Forêt, S., Lutz, A., Abrego, D., Ceh, J., Seneca, F. O., Clode, P. L., Bourne, D. G., Willis, B. L. et al. (2013). DMSP biosynthesis by an animal and its role in coral thermal stress response. *Nature* **502**, 677-680.
- Saito, K. (2004). Sulfur assimilatory metabolism. The long and smelly road. *Plant Physiol.* **136**, 2443-2450.
- Shinzato, C., Shoguchi, E., Kawashima, T., Hamada, M., Hisata, K., Tanaka, M., Fujie, M., Fujiwara, M., Koyanagi, R., Ikuta, T. et al. (2011). Using the *Acropora digitifera* genome to understand coral responses to environmental change. *Nature* **476**, 320-323.
- Shoguchi, E., Shinzato, C., Kawashima, T., Gyoja, F., Mungpakdee, S., Koyanagi, R., Takeuchi, T., Hisata, K., Tanaka, M., Fujiwara, M. et al. (2013). Draft assembly of the *Symbiodinium minutum* nuclear genome reveals dinoflagellate gene structure. *Curr. Biol.* **23**, 1399-1408.
- Tanaka, K. and Ohde, S. (2007). Sulfate content in coral aragonite. *Bull. Soc. Sea Water Sci. Jpn.* **61**, 241-244.
- Trench, R. K. (1971a). The physiology and biochemistry of zooxanthellae symbiotic with marine coelenterates. I. The assimilation of photosynthetic products of zooxanthellae by two marine coelenterates. *Proc. R. Soc. Lond. B Biol. Sci.* **177**, 225-235.
- Trench, R. K. (1971b). The physiology and biochemistry of zooxanthellae symbiotic with marine coelenterates II. Liberation of fixed ¹⁴C by zooxanthellae in vitro. *Proc. R. Soc. Lond. B Biol. Sci.* **177**, 237-250.
- Wang, J. T. and Douglas, A. E. (1999). Essential amino acid synthesis and nitrogen recycling in an alga-invertebrate symbiosis. *Mar. Biol.* **135**, 219-222.
- Whitehead, L. F. and Douglas, A. E. (2003). Metabolite comparisons and the identity of nutrients translocated from symbiotic algae to an animal host. *J. Exp. Biol.* **206**, 3149-3157.
- Yamada, S., Morimoto, H., Fujisawa, T. and Sugahara, K. (2007). Glycosaminoglycans in *Hydra magnipapillata* (Hydrozoa, Cnidaria): demonstration of chondroitin in the developing nematocyst, the sting organelle, and structural characterization of glycosaminoglycans. *Glycobiology* **17**, 886-894.
- Yuyama, I. and Watanabe, T. (2008). Molecular characterization of coral sulfate transporter homolog that is up-regulated by the presence of symbiotic algae. *Fish. Sci.* **74**, 1269-1276.
- Yuyama, I., Watanabe, T. and Takei, I. (2011). Profiling differential gene expression of symbiotic and aposymbiotic coral using a high coverage gene expression profiling (HICEP) analysis. *Mar. Biotech.* **12**, 1436-2236.
- Zhang, L., Lawrence, R., Schwartz, J. J., Bai, X., Wei, G., Esko, J. D. and Rosenberg, R. D. (2001). The effect of precursor structures on the action of Glucosaminyl 3-O-Sulfotransferase -1 and the biosynthesis of anticoagulant heparin sulfate. *J. Biol. Chem.* **276**, 28806-28813.