渦鞭毛藻Crypthecodinium cohniiにおけるジメチルチオプロピオン酸へのメチオニンの取り込み

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著者	内田, 有恆 大栗, 智昭 石田, 丈博 石田, 祐三郎
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Incorporation of Methionine into Dimethylthiopropanoic Acid in the Dinoflagellate *Crypthecodinium cohnii*

Aritsune Uchida,[*1] Tomoaki Ooguri,[*1,2] Takehiro Ishida,[*1,3] and Yuzaburo Ishida[*1]

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Methionine has been shown to be an efficient precursor for the biosynthesis of dimethylthiopropanoic acid (DMTP) in the dinoflagellate *Crypthecodinium cohnii*. The methyl group, C-3 and C-4 carbons, and sulfur atom of methionine were incorporated into DMTP, whereas C-1 carbon of methionine was not incorporated into DMTP. The results indicate that methionine was converted to DMTP by decarboxylation, deamination, oxidation, and methylation. The order of these conversions has not yet been demonstrated.

The tertiary sulfonium compound dimethylthiopropanoic acid (DMTP) is a precursor of dimethylsulfide (DMS), which is a common volatile sulfur compound excreted by marine planktonic and benthic algae such as rhodophytes,[1] chlorophytes,[1,2] dinophytes,[3,4] and haptophytes.[5,6] Some DMS diffuses as gas from sea water into the atmosphere, where it is photochemically oxidized to sulfate and methane sulfonate acting as nuclei in aerosol formation.[7]

The role of DMTP in osmotic acclimation is well documented for various micro- and macroalgae[5,8–11] and a significant increase in DMTP was observed when cells were up-shocked with external salinity.

Data on the biosynthesis of DMTP are still poor. Although Green[12] first reported that in the green alga *Ulva lactuca* DMTP is synthesized from methionine, its mechanism has not yet been described in detail. It is therefore of interest to determine the biosynthesis pathway of DMTP in microalgae such as dinoflagellates.

Materials and Methods

Organism

The dinoflagellate *Crypthedinium cohnii* (ATCC e32001), which is able to grow heterotrophically, was cultured in an ESW medium (2% glucose and 0.2% yeast extracts in natural sea water) or an A_2E_6 medium[13] at 25°C in dark conditions. In the growth experiments, cells were incubated in 50 m*l* of ESW medium in which sulfate was replaced with 2.5 mM L-cysteine or L-methionine.

Cell growth was determined by measuring optical density at 660 nm.

Chemicals

Sodium[^{35}S]sulfate, L-[^{35}S]cysteine, L-[^{35}S]methionine, L-[methyl-^{14}C]methionine, L-[3,4-^{14}C]methionine, and L-[1-^{14}C]methionine were obtained from Ammersham Life Science, Ltd. DMTP bromide was obtained from Shiono Koryo Kaisha, Ltd. S-Methylmethionine, L-methionine, methylcysteine, L-cysteine, taurine, betaine and sulfate were obtained from Nakarai Chem. Ltd. and Waken Chem. Ltd.

Determination of DMS and DMTP in the Culture Medium

DMS was analyzed using a Shimadzu Model GC-9A gas chromatograph equipped with a specially designed, sulfur-specific, highly sensitive linearized flame photometric detector (FPD) as described previously.[4] DMTP was quantified by alkaline decomposition to yield DMS.[14] Samples of culture media, cell-free supernatants of cell pellets were transferred into 10 m*l* vials. After the vials were sealed with rubber caps, 2 m*l*-aliquots of 1 N NaOH were injected through rub-

*1 Laboratory of Microbiology, Department of Fisheries, Faculty of Agriculture, Kyoto University, Kyoto 606-01, Japan (内田有恆, 大栗智昭, 石田丈博, 石田祐三郎: 京都大学農学部水産微生物学研究室).
*2 Present address: Biochemistry Research Laboratory, Central Research Institute, Taiyo Fishery Co., Ltd., Wadai, Tsukuba, Ibaraki 300-42, Japan.
*3 Present address: Food Research Laboratories, Takara Shuzo Co., Ltd., Otsu, Shiga 520-21, Japan.

ber caps. After 90 min, 50 μl of the gas phase was sampled and analyzed by gas chromatography as described above.

Counting Procedure

Radioactivity measurements on thin layer chromatogram were made with an Alocka Radiochromanyzer.

Isolation and Determination of DMTP

Cells were incubated in 50 m*l* of ESW medium containing 40 μCi of labeled compound at 25°C in dark conditions. Cultured cells collected by centrifugation were suspended in 4% perchloric acid and the suspension was centrifuged at 12,000 × g for 10 min. The supernatant was neutralized with 5 N KOH and centrifuged to remove the potassium salt of perchloric acid. The supernatant fraction was separated by two-dimension cellulose thin layer chromatography (TCL) (Merck) with two different developing solutions (the first developing solution 1, n-butanol : acetic acid : water=4 : 1 : 1 and the second developing solution 2, phenol : water=7 : 3). The air-dried plates were visualized with small amounts of Dragendorff reagent. Only one spot corresponding to the authentic DMTP was observed.

Results and Discussion

Sulfur Sources and Biosynthesis of DMTP

C. cohnii was able to grow in an A_2E_6 medium where sulfate was replaced with L-cysteine. Growth patterns (Fig. 1) suggest that *C. cohnii* cells were

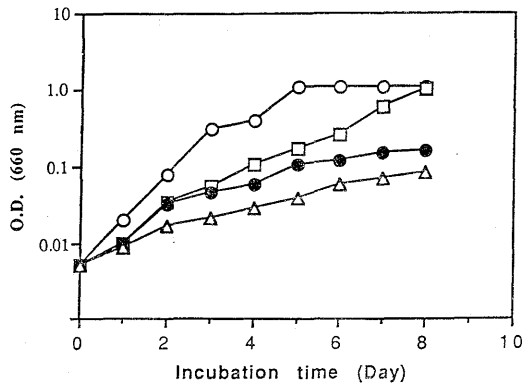


Fig. 1. Growth of *Crypthecodinium cohnii* in an A_2E_6 medium containing different sulfur sources.

Each sulfur compound was added at 5 mM. Symbols: ○, SO_4^{2-}; □, L-cysteine; ●, L-methionine; △, sulfur-free.

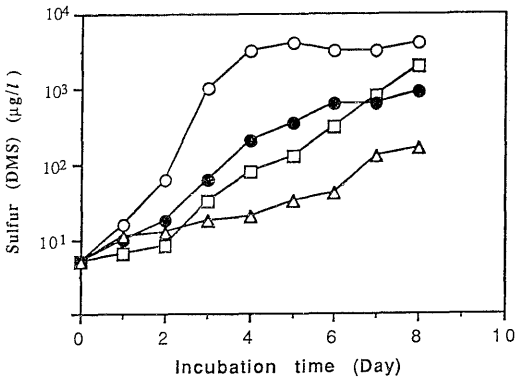


Fig. 2. DMS concentration in the medium during the incubation of *C. cohnii* in an A_2E_6 medium containing different sulfur sources.

Each sulfur compound was added at 5 mM. Symbols: ○, SO_4^{2-}; □, L-cysteine; ●, L-methionine; △, sulfur-free.

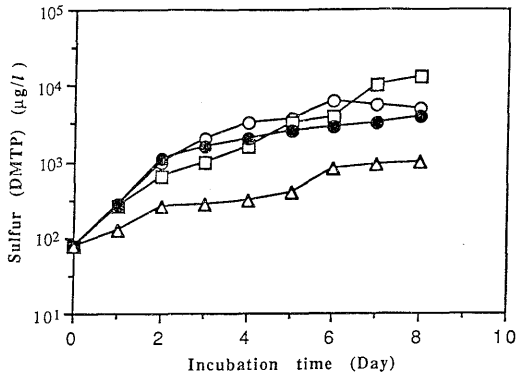


Fig. 3. DMTP concentration in the medium during the incubation of *C. cohnii* in an A_2E_6 medium containing different sulfur sources.

Each sulfur compound was added at 5 mM. Symbols: ○, SO_4^{2-}; □, L-cysteine; ●, L-methionine; △, sulfur-free.

able to utilize L-cysteine as well as sulfate, and that the final cell yields reached almost the same level. However, there was an initial delay period in the medium supplemented with L-cysteine, indicating a possible difference in the uptake mechanism from that of sulfate. In the media supplemented with L-methionine, growth of *C. cohnii* was very poor. This indicated that L-methionine was not able to fill the role of sole source of sulfur. This means that *C. cohnii* may not have a pathway from methionine to cysteine *via* homocysteine as described in higher plants and bacteria, because as shown below *C. cohnii* was able to incorporate L-methionine into the cells. Taurine and methylcysteine were also not able to be come a sulfur

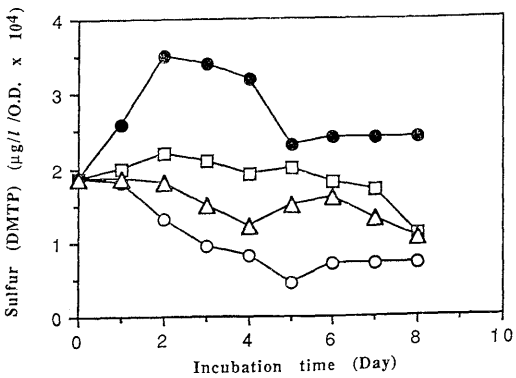


Fig. 4. Ratio of DMTP concentration and O.D. during the incubation of *C. cohnii* in an A_2E_6 medium containing different sulfur sources.
Each sulfur compound was added at 5 mM. Symbols: ○, SO_4^{2-}; □, L-cysteine; ●, L-methionine; △, sulfur-free.

source (data not shown). The DMS and DMTP values in the culture increased in parallel with cell numbers during the logarithmic phase, but the levels stopped increasing in the stationary phase (Figs. 2 and 3). The DMTP levels in cells with incubation time did not vary so much (Fig. 4). The DMTP level was highest in the cells incubated with L-methionine. These results indicated that the L-methionine incorporated was not efficiently utilized as a sulfur source of growth, but was utilized in the biosynthesis of DMTP, and that L-methionine could be a direct or close precursor of DMTP in comparison with L-cysteine.

Incorporation of Labeled Sulfur Containing Compounds into DMTP

The cells were incubated in the ESW medium with sodium-[^{35}S]sulfate, L-[^{35}S]cysteine and L-[^{35}S]methionine, respectively, for 10 days, and the harvested cells were extracted with cold PCA. The

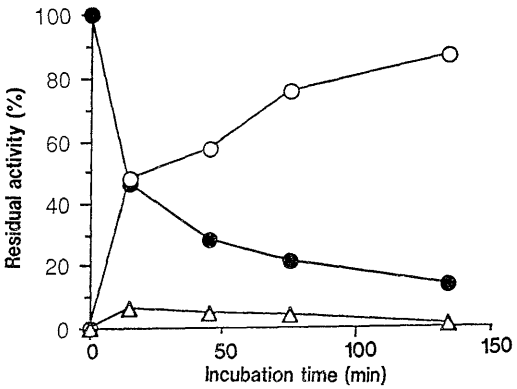


Fig. 5. Distribution of labeled sulfur in the PCA soluble fraction of the intact cells of *C. cohnii*.
The cells were incubated with L-methionine-S^{35}. Symbols: ●, methionine+methionine sulfoxide; ○, DMTP; △, others.

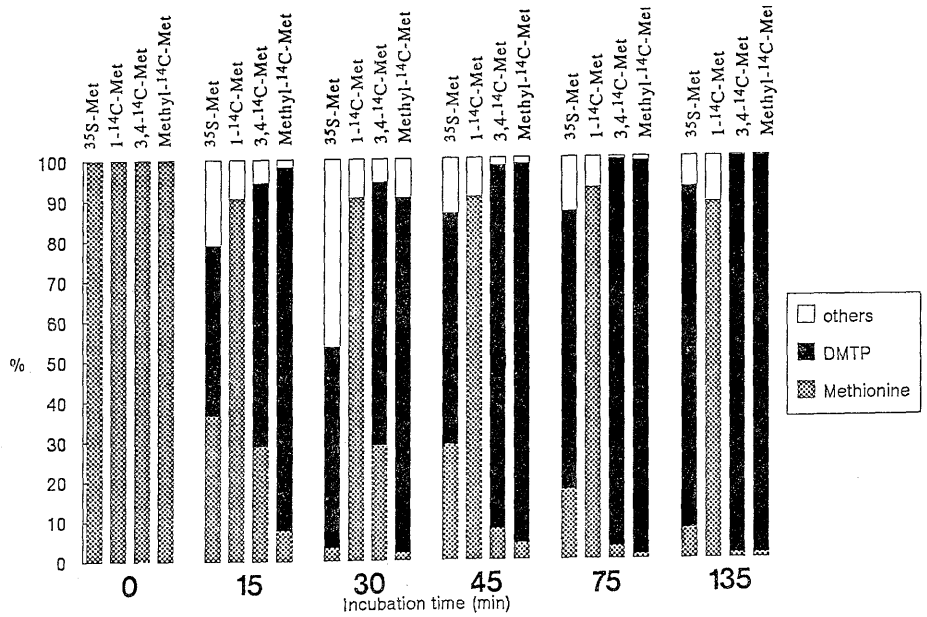
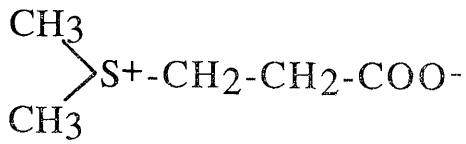
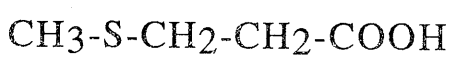


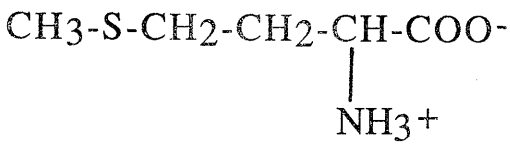
Fig. 6. Incorporation of labeled element of L-methionine into DMTP in the intact cells of *C. cohnii*.



Dimethylthiopropanoic acid(DMTP)



methylthiopropanoic acid(MTP)



Methionine

Fig. 7. Structures of dimethylthiopropanoic acid (DMTP), medthylthiopropanoic acid (MTP), and methionine.

PCA extract was analyzed by TLC. These three labeled sulfur-containing compounds were efficiently incorporated into DMTP, of which methionine was most efficiently incorporated into DMTP. In a short-time incubation (2-hr) experiment, among the low molecular sulfur containing materials DMTP was also the highest labeled with L-[^{35}S]methionine (Fig. 5). In the next experiment various labeled methionine samples whose labeled element was incorporated into DMTP (Fig. 6) were examined. The methyl, C-3 and C-4 carbons, and the sulfur atom of methionine were efficiently incorporated into DMTP. However, C-1 carbon of methionine was not incorporated into DMTP. From the relationship between the structures of methionine and DMTP (Fig. 7), the data presented above shows that the methyl groups and the sulfur of DMTP are derived from the methyl group and the sulfur of methionine, respectively, and that the carboxyl group of DMTP may arise from the C-2 carbon of methionine. These observations are consistent with the hypothesis that methionine is converted to DMTP by decarboxylation, deamination, and methylation. The same conversion pathway has been reported by Green[12] in *Ulva lactuca* thallus.

The incorporation of L-[^{35}S]methionine into DMTP was hardly inhibited by betaine, and a spot corresponding to S-adenosyl-methionine (SAM) was detected after 30 min incubation (data not shown). As shown in Fig. 5, L-[methyl-^{14}C]methionone was the most quickly incorporated into DMTP. These results suggest that SAM may not be an intermediate but may act as a methyl donor like betaine. Methylmethionine sulfonium did not inhibit incorporation of labeled methionine into DMTP either (data not shown). This indicates that methylmethionine is not an intermediate, or in other words that methylation of L-methionine is not the first step of biosynthesis of DMTP in *C. cohnii*. Since these experiments were performed with intact *C. cohnii*, SAM and methylmethionine are not conclusively ruled out as intermediates, because we did not check the permeability of these compounds. In our experiments, very small amounts of methylmethionine and SAM have been detected in *C. cohnii* extracts. Green[12] also reported that incorporation of radioactivity from labeled S-methylmethionine into DMTP was not detected and suggested that this compound was not an intermediate in *U. lactuca*.

There have been some reports concerning decarboxylation of methionine. Mazelis and Ingraham[15,16] have shown that horseradish peroxidase catalyzes oxidative decarboxylation of methionine to yield β-methiopropionamide as a product. Similar enzymes have been purified from *Sterptomyces* sp.[17,18] and from the fern *Dryopteris filix-mas*.[19,20] Although the horseadish enzyme was not specific for methionine, this reaction presented a mechanism for the biosynthesis of methylthiopropanoic acid (MTP). The methyl ester of MTP has been shown to occur in pineapple by Haagen-Smit *et al.*[21]. As for MTP, although the biosynthesis of this closely related compound has not been studied, it is reasonable to assume that it will closely parallel the DMTP pathway. As has been suggested by Cantoni,[22] methyl ester of MTP may arise from DMTP by an intramolecular migration of one of the methyl groups. However, an alternative hypothesis, that MTP arising from the oxidation of methionine is an intermediate in the biosynthesis of both compounds, is equally probable.

The results indicated above show that in the dinoflagellate *C. cohnii* methionine is directly converted to DMTP by decarboxylation, deamination, oxidation, and methylation. Though the

order of these conversions has not yet been clarified, we have found the pyridoxal phosphate-dependent L-methionine decarboxylase in *C. cohnii*.* This enzyme may be a probable candidate for the key enzyme in DMTP synthesis.

Acknowledgments

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