APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 1988, p. 2208–2212
0099-2240/88/092208-05$02.00/0

Vol. 54, No. 9

Demethylation of Dimethylsulfoniopropionate and Production of Thiols in Anoxic Marine Sediments†

RONALD P. KIENE[1]* AND BARRIE F. TAYLOR[2]

University of Georgia Marine Institute, Sapelo Island, Georgia 31327,[1] and Division of Marine and Atmospheric Chemistry, Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, Florida 33149-1098[2]

Received 31 March 1988/Accepted 20 June 1988

Dimethylsulfoniopropionate (DMSP) is a natural product of algae and aquatic plants, particularly those from saline environments. We investigated whether DMSP could serve as a precursor of thiols in anoxic coastal marine sediments. The addition of 10 or 60 μM DMSP to anoxic sediment slurries caused the concentrations of 3-mercaptopropionate (3-MPA) and methanethiol (MSH) to increase. Antibiotics prevented the appearance of these thiols, indicating biological formation. Dimethyl sulfide (DMS) and acrylate also accumulated after the addition of DMSP, but these compounds were rapidly metabolized by microbes and did not reach high levels. Acrylate and DMS were probably generated by the enzymatic cleavage of DMSP. MSH arose from the microbial metabolism of DMS, since the direct addition of DMS greatly increased MSH production. Additions of 3-methiolpropionate gave rise to 3-MPA at rates similar to those with DMSP, suggesting that sequential demethylation of DMSP leads to 3-MPA formation. Only small amounts of MSH were liberated from 3-methiolpropionate, indicating that demethiolation was not a major transformation for 3-methiolpropionate. We conclude that DMSP was degraded in anoxic sediments by two different pathways. One involved the well-known enzymatic cleavage to acrylate and DMS, with DMS subsequently serving as a precursor of MSH. In the other pathway, successive demethylations of the sulfur atom proceeded via 3-methiolpropionate to 3-MPA.

Dimethylsulfoniopropionate [$(CH_3)_2S^+$ $CH_2CH_2COO^-$; DMSP] is a widespread organosulfur compound present in many plants and algae, particularly those from marine environments (1, 5, 22, 30). DMSP occurs at very high intracellular concentrations in some plants and probably functions as an osmotic solute (9, 17, 22, 26, 30). DMSP is readily degraded in a variety of biological systems, including bacterial cultures (6, 28), salt marsh sediments (16), and seawater samples containing algae and zooplankton (7). The enzymatic dissimilation of DMSP yields dimethyl sulfide (DMS) and acrylate (4, 28). The production of DMS from DMSP and the biological and geochemical fates of these compounds have been studied intensively over the last few years (3, 7, 14, 16, 29). This interest stems from the fact that DMS emissions from marine-oceanic environments contribute about 60% to the global biogenic-sulfur flux to the atmosphere (2).

In shallow coastal environments, DMSP occurs in the sediments because of the settling of algal detritus, production by benthic algae, and release by rooted plants. Kiene (13) recently measured high levels of DMSP (100 to 200 μmol/liter of sediment) in surface sediments of a salt marsh tidal flat. Some of this DMSP undoubtedly decomposes anaerobically, because coastal sediments are anoxic below the upper few millimeters or centimeters (11). Because DMSP is a potentially important organosulfur compound in coastal marine sediments, we undertook a study of its anaerobic decomposition in sediments from Biscayne Bay, Fla. In particular, our study emphasized the role of DMSP as a source of organic thiols in these sediments. Our findings suggest that DMSP in anoxic sediments is degraded by at least two different pathways. One involves the well-known cleavage of DMSP into DMS and acrylate. The other involves a previously suggested (19) but undocumented demethylation route leading to the formation of 3-mercaptopropionate (3-MPA).

* Corresponding author.

† Contribution no. 609 from the University of Georgia Marine Institute.

MATERIALS AND METHODS

Sediment collection and preparation. Sediment samples were collected from an intertidal mud flat near Bear Cut, Key Biscayne, Fla. The sampling area contained organic-rich sediments (8% weight loss on ignition) and was located near a small mixed bed of *Thalassia testudinum* and *Syringodium* sp. However, no living macrophytes were present where the sediment samples were taken, and decaying root material was only occasionally present. Sediment samples were taken by using aluminum core tubes and were returned to the laboratory for processing within 30 min.

The upper 15 cm of cores was mixed with an equal volume of deoxygenated overlying water. Oxygen-free N_2 was passed over the slurry during preparation to minimize exposure of the slurry to oxygen. The slurry was screened through a 1.5-mm mesh to remove large particles; it was then dispensed into serum bottles in 50-ml portions. The bottles (60 ml) were flushed with N_2 and then sealed with black butyl rubber stoppers and an aluminum crimp. Rubber stoppers were boiled in 0.1 N NaOH before use to minimize the possibility of organosulfur contamination from the stoppers. Tests showed that thiols were not released from the stoppers in measurable amounts.

Physical disturbance of the sediments during preparation of slurries promoted the release of several thiols into the dissolved phase. When thiols were expected as products from experimental additions, slurries were preincubated for 24 to 48 h to allow endogenous removal processes to lower thiols to background levels. Experimental treatments were tested either in duplicate bottles or as single samples. When single samples were used, experiments were repeated at

least twice, and results were reproducible. Samples were maintained in the dark on a rotary shaker (125 rpm; 2.5-cm orbit) at 23 to 26°C.

Experimental additions. DMSP hydrochloride, acrylic acid, DMS, and 3-methiolpropionate were added to the sediment slurries as aqueous solutions. Microbial activity in the sediments was inhibited by adding a mixture of chloramphenicol (CAP) and tetracycline (TET) to final concentrations of 125- and 50-μg/ml slurry, respectively. These broad-spectrum antibiotics, in combination, effectively inhibited biological activity and did not interfere with the analysis of thiols by high-pressure liquid chromatography (HPLC) (see below). CAP-TET was added 2 to 24 h before other compounds. Preincubation with antibiotics was required to achieve maximum inhibitory effects. Sterilization by autoclaving was sometimes used as a control procedure, but this treatment resulted in the release of large amounts of various thiols into the pore water, making it difficult to measure the production of small amounts.

Analytical procedures. Dissolved thiols were determined as fluorescent isoindole derivatives by the HPLC method of Mopper and Delmas (18). By means of an all-polyethylene syringe and a stainless-steel needle, 1 ml of sediment slurry was removed from bottles. The slurry sample was then placed in a polypropylene microcentrifuge tube and was centrifuged at 13,000 × *g* for 1 min. Next, 0.5 ml of the clear supernatant was removed with an autopipette into another microcentrifuge tube and immediately derivatized with 10 μl each of *o*-phthaladehyde (20 mg/ml of methanol) and 2-aminoethanol (20 μl/ml of 0.5 M sodium borate buffer, pH 9.2). Derivatization was allowed to proceed in the centrifuge tube for exactly 1 min before 20 μl of the sample was introduced into the HPLC column via an injection loop.

Fluorescent thiol derivatives were separated on a Waters Radial-Pak reversed-phase column (C-18; 5-μm-diameter particles; inner dimensions, 10 cm by 5 mm;). A binary gradient consisting of 0.05 M sodium acetate buffer at pH 5.7 (solvent A) and 100% methanol (solvent B) was used. The gradient was as follows: isocratic at 10% B for 1 min, 10 to 25% B in 1 min, isocratic at 25% B for 4 min, 25 to 50% B in 7 min, isocratic at 50% B in 6 min, 50 to 60% B in 2 min, 60 to 67.5% B in 4 min, 67.5 to 100% B in 1 min, isocratic at 100% B in 3 min, 100 to 10% B in 2 min, and isocratic at 10% B for 3 min. The flow rate was 1.0 ml/min.

Derivatives were detected by using a Hitachi F1000 fluorometer set at an excitation wavelength of 340 nm and an emission wavelength of 450 nm. Peak areas were recorded by a Shimadzu CR3A integrator. Run times were typically 35 to 40 min. Since nanomolar levels of thiols were extremely sensitive to air oxidation, samples were taken and were run immediately. This procedure greatly limited the number of samples that could be processed, and experiments were planned accordingly. The detection limits for 3-MPA and methanethiol were 0.7 and 1.0 nM, respectively.

Acrylate was measured by ion-exchange chromatography. Centrifuged pore water (20 μl) was injected into a Benson carbohydrate column (inner dimensions, 30 cm by 6 mm; Chromtec, Fort Worth, Fla.). The mobile phase was 0.15 mM H_2SO_4 at a flow rate of 0.5 ml/min, and the detector was a Conductomonitor III (Laboratory Data Control, Riviera Beach, Fla.) operated at 1-μS sensitivity. The detection limit for acrylate was about 5 μM. DMS was determined by gas chromatography as described previously (15).

Reagents and chemicals. DMSP hydrochloride was obtained from Research Plus, Inc., Bayonne, N.J. Purity of the DMSP was >98%, based on ion-exchange chromatography. 3-MPA (>99%) and DMS (>99%) were obtained from Aldrich Chemical Co., Inc., Milwaukee, Wis. Acrylic acid (99%) was from Fluka Chemicals, Hauppauge N.Y. 3-Methiolpropionate was obtained by alkaline hydrolysis of its methyl ester [methyl(3-methylthio)propionate; Aldrich]. Purity of the 3-methiolpropionate was >99% based on ion-exchange chromatography. All other chemicals and solvents were of reagent grade.

RESULTS

The addition of 60 μM DMSP to anoxic sediment slurries resulted in the accumulations of 3-MPA and methanethiol (MSH) as the only detectable thiols (Fig. 1). DMS also accumulated in similar experiments (data not shown), and concentrations reached 2 to 3 μM, compared with 5 and 10 μM for 3-MPA and MSH, respectively (Fig. 1). Acrylate was below our detection limit of 5 μM in experiments in which we added 10 or 60 μM DMSP. When DMSP was added at a relatively high concentration of 700 μM, a small, transient accumulation of 15 μM acrylate was observed (Fig. 2).

MSH was the major thiol product from 60 μM DMSP, with levels reaching about twice those of 3-MPA (Fig. 1). Antibiotics (CAP-TET) prevented the formation of thiols from DMSP and also prevented the disappearance of thiols when thiols were added directly (13). CAP-TET sometimes caused a small accumulation of MSH and 3-MPA in unamended sediments, but the increases were small compared with those in DMSP-treated samples. Some 3-MPA (30 nM) accumulated from acrylate because of the chemical reaction of sulfide with the double bond of acrylate (27). However, this reaction was slow in comparison with the biological conversion of DMSP to 3-MPA (Fig. 1 and 3). When acrylate was added to the CAP-TET-treated slurries, slightly more (70 nM) 3-MPA accumulated than from acrylate alone, but far less than from DMSP. After continued incubation (>48 h), the 3-MPA and MSH which had accumulated after DMSP additions decreased to background levels of <1 nM for 3-MPA and ~10 nM for MSH (data not shown).

Figure 3A shows that at 10 μM levels, 3-methiolpropionate stimulated 3-MPA accumulation at the same rate as DMSP. Acrylate (10 μM) caused only a small, transient accumulations (10 nM) of 3-MPA, which was similar to results found with 60 μM acrylate (Fig. 1A). DMS had no effect on 3-MPA levels in the slurries.

The results for MSH showed that DMSP stimulated MSH production much more than did 3-methiolpropionate (Fig. 3B), but DMS gave the highest MSH accumulation. Although MSH accumulated only slowly from 3-methiolpropionate over the first 15 h of incubation (Fig. 3B), MSH increased significantly after this time (data not shown). This increase may have been due to the selection of microbes capable of demethiolation, or, alternatively, the 3-methiolpropionate could have been transformed to a better precursor of MSH (e.g., DMSP). In all treatments, both 3-MPA and MSH decreased to background levels within 24 h of incubation.

DISCUSSION

Our results indicate that DMSP undergoes degradation by two different pathways in anoxic marine sediments (Fig. 4). One pathway is the established enzymatic cleavage of DMSP to yield DMS and acrylate (4, 28), previously suggested for the decomposition of DMSP in salt marsh sediments (16). DMS and acrylate did not accumulate in large amounts after

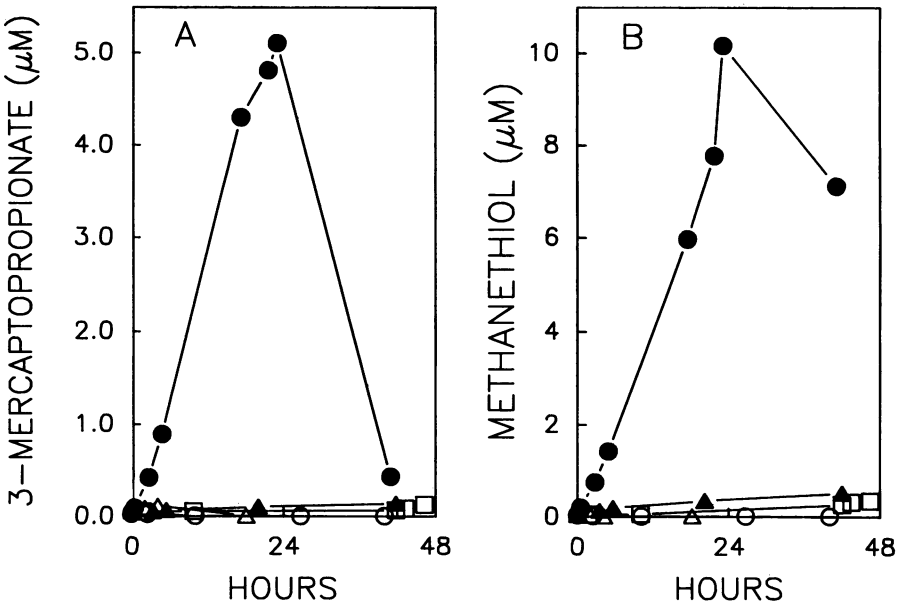

FIG. 1. Accumulations of 3-MPA (A) and MSH (B) in anoxic sediment slurries after the addition of 60 μM DMSP (●), DMSP plus CAP-TET (□), 60 μM acrylate (△), acrylate plus CAP-TET (▲), and CAP-TET alone (○). Untreated samples showed no accumulation of either 3-MPA or MSH. Data are from single bottles of each treatment.

low micromolar additions of DMSP, probably because these products were removed by microbial metabolism. DMS is readily metabolized by sulfate-reducing bacteria in anoxic sediments (13, 14), and acrylate is rapidly metabolized to a mixture of propionate and acetate (R. P. Kiene and B. F. Taylor, *in* E. Saltzman and W. Cooper (ed.), *Biogenic Sulfur in the Environment*, in press), most likely by fermentative organisms similar to the *Clostridium* species isolated by Wagner and Stadtman (28).

A second pathway for DMSP degradation probably involves initial demethylation to give 3-methiolpropionate followed by a second demethylation to give 3-MPA. A demethylation route for the decomposition of DMSP was recently proposed by Mopper and Taylor (19). Our results provide evidence for the existence of this pathway in sediments. Although we did not measure the formation of 3-methiolpropionate during DMSP decomposition, the fact that this compound was converted to 3-MPA at a rate similar to that of DMSP (Fig. 3A) suggests that it is an intermediate in the demethylation pathway. The fate of the methyl group of DMSP in these reactions is unknown. The methyl group could have been metabolized by acetogenic bacteria similar to *Eubacterium limosum*, which demethylates glycine betaine (20), an analog of DMSP. Alternatively, it is possible that DMSP was used as a methyl donor by microorganisms since DMSP has been shown to be a suitable methyl donor

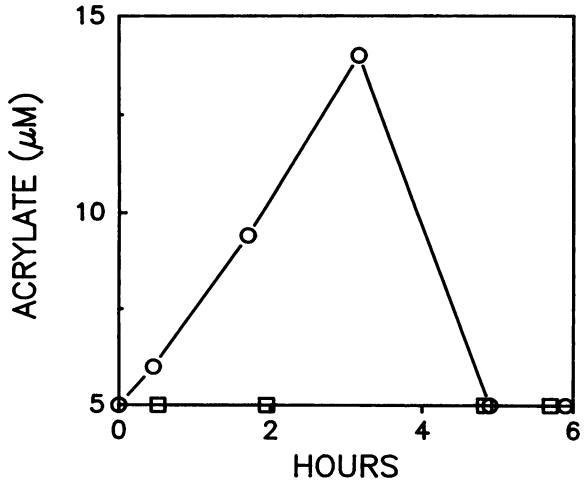

FIG. 2. Accumulation of acrylate in anoxic sediment slurries after the addition of 700 μM DMSP (○) and 700 μM DMSP plus CAP-TET (□). The detection limit for acrylate was 5 μM.

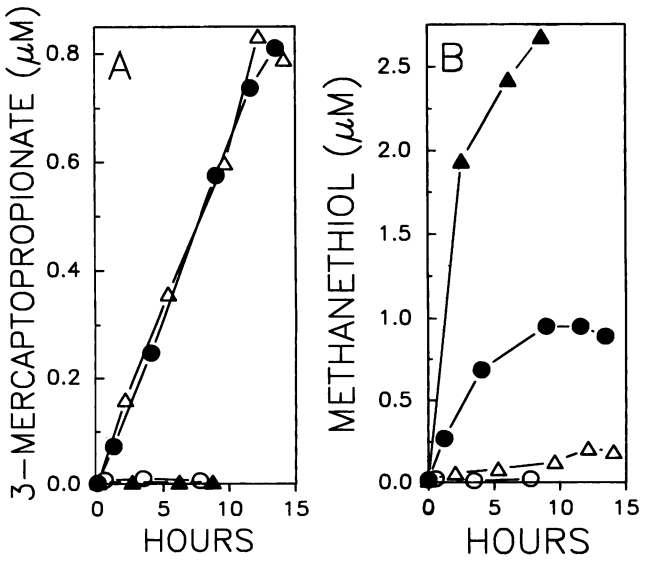

FIG. 3. Accumulations of 3-MPA (A) and MSH (B) in anoxic sediments after the addition of 10 μM DMSP (●), 10 μM 3-methiolpropionate (△), 10 μM acrylate (○), and 10 μM DMS (▲). Untreated samples showed no accumulation of either MSH or 3-MPA. Data are from single sample bottles.

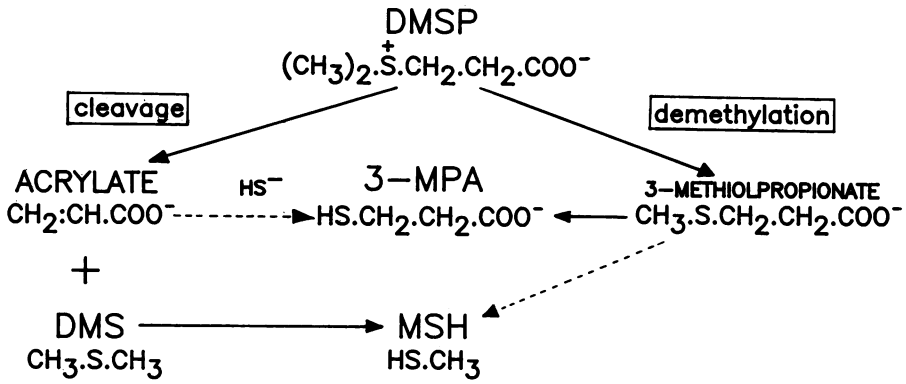

FIG. 4. Predominant (——) and minor (---) pathways by which DMSP may be converted to 3-MPA and MSH in sediments.

for some enzymatic transmethylations (8, 10). The possible role of DMSP in methylation reactions in sediments is worthy of further investigation.

3-Methiolpropionate is an intermediate in the metabolism of methionine in some plants (21) and in rat liver tissues, where it is a precursor of MSH (23, 24). The further demethylation of 3-methiopropionate to give 3-MPA has not been previously reported. Our results indicate that DMSP was a better precursor of MSH than was 3-methiolpropionate (Fig. 3B). Therefore, most of the MSH that arose from DMSP must have come from an immediate precursor other than 3-methiolpropionate. This precursor was probably the DMS formed by enzymatic cleavage since (i) DMS was metabolized rapidly and did not accumulate in large amounts from micromolar levels of DMSP and (ii) DMS was a much better precursor of MSH than was 3-methiolpropionate (Fig. 3B). Several studies have shown that DMS is degraded via MSH in anaerobic sediments (13, 14). Similarly, aerobic bacteria metabolize DMS with MSH as an intermediate (12, 25).

3-MPA and MSH are present in nanomolar to low micromolar concentrations in the pore waters of anoxic sediments (15, 19). Even larger amounts are associated with the sediment particles in an adsorbed or bound form (19; Kiene, unpublished data). DMSP is likely to be a precursor of these thiols in marine sediments, although other precursors such as methionine and homocysteine are also possible (15).

Organic thiols (MSH and 3-MPA) are formed from DMSP by separate pathways (Fig. 4). MSH is formed primarily from the DMS released in the cleavage pathway. 3-MPA on the other hand is formed primarily by a demethylation pathway in which the sulfur remains in the original carbon chain. Whether further metabolism of 3-MPA breaks that C-S bond is unknown. Similar maximal accumulations of 3-MPA and MSH from 10 μM DMSP (Fig. 3) suggest that the two pathways were operating at similar rates. Higher concentrations (60 μM) of DMSP appeared to favor greater MSH production (compare Fig. 1 and 3), suggesting greater importance of the cleavage reaction at high DMSP concentrations. However, these estimates are uncertain because the rate at which 3-MPA or MSH is removed is not known. These matters require further investigation.

Our studies have shown that DMSP is a potential precursor of volatile organosulfur compounds (DMS), thiols (MSH and 3-MPA), and low-molecular-weight fatty acids (acrylate, propionate, and acetate) (Kiene and Taylor, in press; this study). Each of these degradation products is biologically labile in sediments and probably also in other systems. DMSP is widely distributed, and high concentrations are found in sediments and some organisms. Therefore, in addition to being a precursor of DMS and thiols, DMSP should also be considered a potentially significant substrate for microbial metabolism. In addition, it will be interesting to determine whether the demethylation of DMSP occurs in aerobic environments, since this process might lower the potential of DMSP as a source of DMS to the atmosphere.

ACKNOWLEDGMENTS

We thank Kirk Malloy for help with the HPLC assays and Julie McDaniel for reading the manuscript. K. Mopper and A. Vairavamurthy provided helpful discussions on many aspects of this work.

Financial support was obtained from the National Science Foundation (grant OCE-8516020).

LITERATURE CITED

1. **Ackman, R. G., C. S. Tocher, and J. McLachlan.** 1966. Occurrence of dimethyl-β-propiothetin in marine plankton. J. Fish. Res. Board Can. **23**:357–364.
2. **Andreae, M. O.** 1985. The emission of sulfur to the remote atmosphere: background paper, p. 5–25. *In* J. N. Galloway et al. (ed.), The biogeochemical cycling of sulfur and nitrogen in the remote atmosphere. D. Reidel Publishing Co., Boston.
3. **Andreae, M. O., and W. R. Barnard.** 1984. The marine chemistry of dimethyl sulfide. Mar. Chem. **14**:267–279.
4. **Cantoni, G. L., and D. G. Anderson.** 1956. Enzymatic cleavage of dimethylpropiothetin by *Polysiphonia lanosa*. J. Biol. Chem. **222**:171–177.
5. **Challenger, F.** 1959. Aspects of the organic chemistry of sulfur. Butterworths, London.
6. **Dacey, J. W. H., and N. Blough.** 1987. Hydroxide decomposition of DMSP to DMS. J. Geophys. Res. Lett. **14**:1246–1249.
7. **Dacey, J. W. H., and S. G. Wakeham.** 1986. Oceanic dimethylsulfide: production during zooplankton grazing on phytoplankton. Science **233**:1314–1316.
8. **Durell, J., D. G. Anderson, and G. L. Cantoni.** 1957. The synthesis of methionine by enzymatic transmethylation. I. Purification and properties of thetin homocysteine methylpherase. Biochim. Biophys. Acta **26**:270–282.
9. **Edwards, D. M., R. H. Reed, J. A. Chudek, R. Foster, and W. D. P. Stewart.** 1987. Organic solute accumulation in osmotically stressed *Enteromorpha intestinalis*. Mar. Biol. **95**:583–592.
10. **Ishida, Y., and H. Kadota.** 1968. Participation of dimethyl-β-propiothetin in transmethylation reaction in *Gyrodinium cohnii*. Bull. Jpn. Soc. Sci. Fish. **34**:699–705.
11. **Jorgensen, B. B.** 1983. Processes at the sediment water interface, p. 477–509. *In* B. Bolin and R. B. Cook (ed.), The major biogeochemical cycles and their interactions. John Wiley & Sons, Inc., New York.
12. **Kanagawa, T., and D. P. Kelly.** 1986. Breakdown of dimethyl sulphide by mixed cultures and by *Thiobacillus thioparus*.

FEMS Microbiol. Lett. **34**:13–19.
13. **Kiene, R. P.** 1988. Dimethylsulfide metabolism in salt marsh sediments. FEMS Microbiol. Ecol. **53**:71–78.
14. **Kiene, R. P., R. S. Oremland, A. Catena, L. G. Miller, and D. G. Capone.** 1986. Metabolism of reduced methylated sulfur compounds by anaerobic sediments and a pure culture of an estuarine methanogen. Appl. Environ. Microbiol. **52**:1037–1045.
15. **Kiene, R. P., and B. F. Taylor.** 1988. Biotransformations of organosulfur compounds in sediments via 3-mercaptopropionate. Nature (London) **332**:148–150.
16. **Kiene, R. P., and P. T. Visscher.** 1987. Production and fate of methylated sulfur compounds from methionine and dimethylsulfoniopropionate in anoxic salt marsh sediments. Appl. Environ. Microbiol. **53**:2426–2434.
17. **Larher, F., J. Hamelin, and G. R. Stewart.** 1977. L'acide dimethyl sulfonium-3 propanoïque de *Spartina anglica*. Phytochemistry **18**:1396–1397.
18. **Mopper, K., and D. Delmas.** 1984. Trace determination of biological thiols by liquid chromatography and precolumn fluorometric labeling with o-phthalaldehyde. Anal. Chem. **56**:2557–2560.
19. **Mopper, K., and B. F. Taylor.** 1986. Biogeochemical cycling of sulfur: thiols in marine sediments, p. 324–339. *In* M. Sohn (ed.), Organic marine geochemistry. American Chemical Society Symposium Series no. 305. American Chemical Society, Washington, D.C.
20. **Müller, E., K. Fahlbusch, R. Walther, and G. Gottschalk.** 1981. Formation of *N,N*-dimethylglycine, acetic acid, and butyric acid from betaine by *Eubacterium limosum*. Appl. Environ. Microbiol. **42**:439–445.
21. **Pokorny, M., E. Marcenko, and D. Keglevic.** 1970. Comparative studies of L- and D-methionine metabolism in lower and higher plants. Phytochemistry **9**:2175–2188.
22. **Reed, R. H.** 1983. Measurement and osmotic significance of β-dimethylsulfoniopropionate in marine macroalgae. Mar. Biol. Lett. **34**:173–181.
23. **Steele, R. D., and N. J. Benevenga.** 1978. Identification of 3-methylthiopropionic acid as an intermediate in mammalian methionine metabolism *in vitro*. J. Biol. Chem. **253**:7844–7850.
24. **Steele, R. D., and N. J. Benevenga.** 1979. The metabolism of 3-methylthiolpropionate in rat liver homogenates. J. Biol. Chem. **254**:8885–8890.
25. **Suylen, G. M. H., G. C. Stefess, and J. G. Kuenen.** 1986. Chemolithotrophic potential of a *Hyphomicrobium* species, capable of growth of methylated sulfur compounds. Arch. Microbiol. **146**:192–198.
26. **Vairavamurthy, A., M. O. Andreae, and R. L. Iversen.** 1985. Biosynthesis of dimethyl sulfide and dimethyl propiothetin by *Hymenomonas carterae* in relation to sulfur source and salinity variations. Limnol. Oceanogr. **30**:59–70.
27. **Vairavamurthy, A., and K. Mopper.** 1987. Geochemical formation of organosulfur compounds (thiols) by addition of H_2S to sedimentary organic matter. Nature (London) **329**:623–625.
28. **Wagner, C., and E. R. Stadtman.** 1962. Bacterial fermentation of dimethyl-β-propiothetin. Arch. Biochem. Biophys. **98**:331–336.
29. **Wakeham, S. G., B. L. Howes, J. W. H. Dacey, R. P. Schwarzenbach, and J. Zeyer.** 1987. Biogeochemistry of dimethylsulfide in a seasonally stratified coastal salt pond. Geochim. Cosmochim. Acta **51**:1675–1684.
30. **White, R. H.** 1982. Analysis of dimethyl sulfonium compounds in marine algae. J. Mar. Res. **40**:529–536.