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Production and Fate of Methylated Sulfur Compounds from Methionine and Dimethylsulfoniopropionate in Anoxic Salt Marsh Sediments†

RONALD P. KIENE‡* AND PIETER T. VISSCHER§

Marine Sciences Research Center, State University of New York at Stony Brook, Stony Brook, New York 11794-5000

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Anoxic salt marsh sediments were amended with DL-methionine and dimethylsulfoniopropionate (DMSP). Microbial metabolism of methionine yielded methane thiol (MSH) as the major volatile organosulfur product, with the formation of lesser amounts of dimethylsulfide (DMS). Biological transformation of DMSP resulted in the rapid release of DMS and only small amounts of MSH. Experiments with microbial inhibitors indicated that production of MSH from methionine was carried out by procaryotic organisms, probably sulfate-reducing bacteria. Methane-producing bacteria did not metabolize methionine. The involvement of specific groups of organisms in DMSP hydrolysis could not be determined with the inhibitors used, because DMSP was hydrolyzed in all samples except those which were autoclaved. Unamended sediment slurries, prepared from *Spartina alterniflora* sediments, contained significant (1 to 10 μM) concentrations of DMS. Endogenous methylated sulfur compounds and those produced from added methionine and DMSP were consumed by sediment microbes. Both sulfate-reducing and methane-producing bacteria were involved in DMS and MSH consumption. Methanogenesis was stimulated by the volatile organosulfur compounds released from methionine and DMSP. However, apparent competition for these compounds exists between methanogens and sulfate reducers. At low (1 μM) concentrations of methionine, the terminal S-methyl group was metabolized almost exclusively to CO_2 and only small amounts of CH_4. At higher (>100 μM) concentrations of methionine, the proportion of the methyl-sulfur group converted to CH_4 increased. The results of this study demonstrate that methionine and DMSP are potential precursors of methylated sulfur compounds in anoxic sediments and that the microbial community is capable of metabolizing volatile methylated sulfur compounds.

The emissions of volatile methylated sulfur compounds from terrestrial and aquatic environments have been found to be a significant component of the global sulfur cycle (2). Salt marshes in particular are known to have high emission rates of compounds such as dimethylsulfide (DMS), dimethyldisulfide (DMDS), and methane thiol (MSH) (12, 34). The occurrence of MSH, DMS, and DMDS in nature stems from the microbial decomposition of more complex sulfur-containing organic material.

MSH is the predominant methylated sulfur compound released during the decomposition of methionine in bacterial cultures (31), the rumen (30), and anaerobic lake sediments (43). DMS is thought to arise primarily from the enzymatic hydrolysis of the sulfonium compound dimethylsulfoniopropionate (DMSP) (8). DMSP is found in certain species of algae and higher plants, such as *Spartina* spp. (10, 21, 28, 37), and may serve as a compatible solute for osmoregulation. DMSP in algae is thought to be the principle source of DMS in the open ocean (3). DMS is produced during the decay of algal mats (44) and is also a minor product of methionine decomposition (30, 31, 43).

Methylated sulfur compounds have been detected in a wide variety of environments (3, 7, 16, 39). However, relatively little is known about their original sources and biological fates. Furthermore, the mechanisms of methylated sulfur compound metabolism are not well understood. Zinder and Brock (42) observed that the carbon atoms of DMS and MSH could be converted to CH_4 and CO_2 in anaerobic lake sediments and sewage sludge. In a similar study, Zinder and Brock (43) observed that the terminal S-methyl group of methionine was also converted to CH_4, CO_2, and H_2S in lake sediments. Several other studies have found that methanogenesis was stimulated when methionine was added to saline anaerobic sediments (25, 26). Recently, Kiene et al. (18) reported on the methanogenic conversion of DMS, DMDS, and MSH by sediments. These same authors also reported the isolation of a methanogen which was capable of growth on DMS.

We examined the production of methylated sulfur compounds from methionine and DMSP in anoxic sediments from a *Spartina alterniflora* salt marsh. The fate of the methylated sulfur compounds was also studied, and microbial inhibitors were used to assess the involvement of specific groups of sediment microbes in the various transformations. The results of this study indicate that both methionine and DMSP are labile in anoxic sediments and are potential precursors of volatile methylated sulfur compounds. Inhibitor experiments illustrated the involvement of both sulfate-reducing and methanogenic bacteria in the metabolism of reduced methylated sulfur compounds.

MATERIALS AND METHODS

Sediment source and preparation. Sediments were collected from Flax Pond salt marsh, located on the north shore of Long Island, New York. Cores were obtained from peatlike sediments which were composed largely of living

* Corresponding author.

† Contribution number 570 of the Marine Sciences Research Center.

‡ Present address: Rosenstiel School of Marine and Atmospheric Sciences, Division of Marine and Atmospheric Chemistry, University of Miami, Miami, FL 33149-1098.

§ Present address: Department of Microbiology, Ryksuniversiteit, 9751 Haren, The Netherlands.

and dead roots of the cord grass *S. alterniflora*. Sediment slurries were prepared by gently homogenizing the upper 10 cm of cores with an equal volume of filtered (0.45-μm pore size) Flax Pond water (ca. 26‰ salinity; 20 mM sulfate) under a stream of nitrogen. Homogenized slurry was passed through a 2-mm sieve to remove root material and then dispensed to Erlenmeyer flasks or serum bottles, depending on the experiment. The bottles were sealed with black natural rubber (Thomas Scientific Co.) or black butyl rubber (Bellco) stoppers under a stream of N_2 gas. Each bottle was subsequently purged with N_2 for 1 to 2 min to ensure anaerobiosis. Slurry volumes were typically 10 to 25 ml. Additions to the bottles were made and subsamples of the headspace were taken through the rubber stoppers. All incubations were at 23 to 26° C in the dark, and treatments were run in duplicate. Aqueous solutions of DL-methionine, DMSP hydrochloride, and various inhibitors (see below) were added through the rubber stoppers to yield the concentrations indicated. MSH was added as a vapor taken from a bottle containing liquid MSH. The term control, used throughout this paper, refers to sediments dosed with methionine or DMSP but lacking any inhibitor. Sediment samples left unamended are referred to as no-treatment samples.

Determination of gases. Subsamples (100 μl) of bottle headspaces were periodically taken with a Hamilton glass, gas-tight syringe for the analysis of CH_4, MSH, and DMS. Gas samples were injected into a Shimadzu GC-R1A gas chromatograph equipped with a flame ionization detector. The column (2 m by 3 mm) used was stainless steel packed with 80/100-mesh Porapak R. Chromatographic conditions were as follows; oven, injector, and detector, 120°C; N_2 carrier flow, 60 ml/min; H_2, 50 ml/min; air, 400 ml/min. Under these conditions CH_4, MSH, and DMS eluted with retention times of 0.2, 1.0, and 2.5 min, respectively. Peak areas were recorded on a Shimadzu RPR-G1 integrator. Analyses were calibrated with standard curves prepared from authentic materials. Recorder response was linear over at least five orders of magnitude for all the gases studied. The soluble fractions of DMS and MSH were calculated from empirically derived distribution coefficients (concentration in the liquid phase/concentration in the gas phase). The distribution coefficients used for DMS and MSH were 13.77 and 8.82, respectively. These values were similar to those given by Przyjazny et al. (27) and by Dacey et al. (13). Typically, ~25% of the total DMS was in the headspace, while ~35% of the MSH was in the headspace. Unless otherwise indicated, the levels of the compounds reported are expressed as total micromoles per bottle, which includes the gas and liquid phases.

Inhibitor experiments. The role of specific microbial groups in the transformation and consumption of organic sulfur compounds in sediments was studied with various selective inhibitors. Molybdate (sodium salt, 20 mM) was used to inhibit sulfate-reducing bacteria (4, 35), and 2-bromoethanesulfonic acid (BES, 8 mM) was used to inhibit methanogenic bacteria (14). Chloramphenicol (200 μg/ml) was used as a broad-spectrum antibiotic to inhibit procaryotic activity (22). Cycloheximide (200 μg/ml) was used to inhibit eucaryotic organisms (32). Chloroform ($CHCl_3$) was used to inhibit one-carbon metabolism (6). Killed controls were obtained either by addition of 0.5% glutaraldehyde or by autoclaving (28 lb/in^2, 115°C) for 20 min.

Experiments with radiolabeled methionine. Experiments with [*S-methyl*-^{14}C]methionine were used to demonstrate mineralization of the S-methyl group and to determine the relative quantities of $^{14}CO_2$ and $^{14}CH_4$ produced. Sediment slurries (10 ml) were prepared in serum bottles as described above. Samples were preincubated at 25°C for 2 days so that endogenous levels of methylated sulfur compounds would be consumed (R. P. Kiene, submitted for publication). At this time, [*S-methyl*-^{14}C] methionine (0.5 μCi; 55 mCi/mmol; Amersham Inc.) was added, giving a final concentration of ~1 μM added methionine. At selected points over a 3-h time course, 1.0 ml of a 5% glutaraldehyde solution was added (0.5% [vol/vol] final concentration) to terminate biological activity. Samples were then immediately frozen for later analysis, which took place within 2 weeks.

For the analysis of radioactive volatile products of the S-methyl group, samples were acidified with 1 ml of 10 N HCl to liberate CO_2 from the slurry. The headspace of each bottle was then purged with N_2, and the effluent gases were passed through a series of five liquid traps. The first trap contained 5 ml of aqueous 3% $HgCl_2$ (pH <5), which trapped organic sulfide compounds, including DMS and MSH (10), but not CO_2 or CH_4. The next four traps contained 5 ml of a CO_2-absorbing scintillation cocktail (Oxosol; National Diagnostics Corp.). Between traps 3 and 4 the gas sample was passed through an oxidation tube (CuO, >500°C), which oxidized $^{14}CH_4$ to $^{14}CO_2$. Preliminary tests showed that all of the $^{14}CO_2$ was absorbed by the first two Oxosol traps and that no significant amounts of $^{14}CH_4$ were absorbed upstream of the oxidation tube. Therefore, any radioactivity found in traps following the oxidation tube was considered to be $^{14}CH_4$. Trapping efficiency of $^{14}CO_2$ for two traps in series was ~100%. Combustion of $^{14}CH_4$ to $^{14}CO_2$ was found to be >95% efficient. A 10-ml amount of Scintiverse II (Fisher Scientific Co.) was added to $HgCl_2$ traps to form a gel before determining radioactivity by liquid scintillation counting. The Oxosol traps were counted directly.

A separate experiment was designed to test whether the relative distribution of mineralized end products (CO_2 and CH_4) of the S-methyl group depended on the concentration of methionine. Sediment slurries were preincubated for 2 days before additions of [*S-methyl*-^{14}C]methionine (0.5 μCi) were made to each bottle. To these bottles, various levels of unlabeled methionine were added (in duplicate) to give final added concentrations ranging from 1 to 500 μM. The incubation was then allowed to proceed for 2 weeks at 25°C. Samples were then analyzed for mineralization products as described above.

Reagents and chemicals. DMS, DMDS, and DL-methionine (>99% purity) were obtained from Aldrich Chemical Co. Liquid MSH (98% purity) was purchased from Eastman Chemical Co. DMSP hydrochloride was obtained from Custom Chem Laboratory, Livermore, Calif. Analysis of this DMSP by base hydrolysis and quantification of the resulting DMS showed that it was >97% pure. All other chemicals and gases were of reagent quality.

RESULTS

Volatile products from methionine. Methionine additions to sediment slurries resulted in the rapid accumulation of MSH (Fig. 1A). The amount of MSH produced was directly related to the amount of methionine added. However, at the lowest level (3 μM), MSH was not detected, although it was sometimes observed in subsequent experiments (not shown). The accumulation of MSH was transient, and levels declined rapidly after reaching a maximum (Fig. 1A).

DMS was also produced in methionine-treated sediments (Fig. 1B). As with MSH, the amount of DMS formed was related to the amount of methionine added, with higher

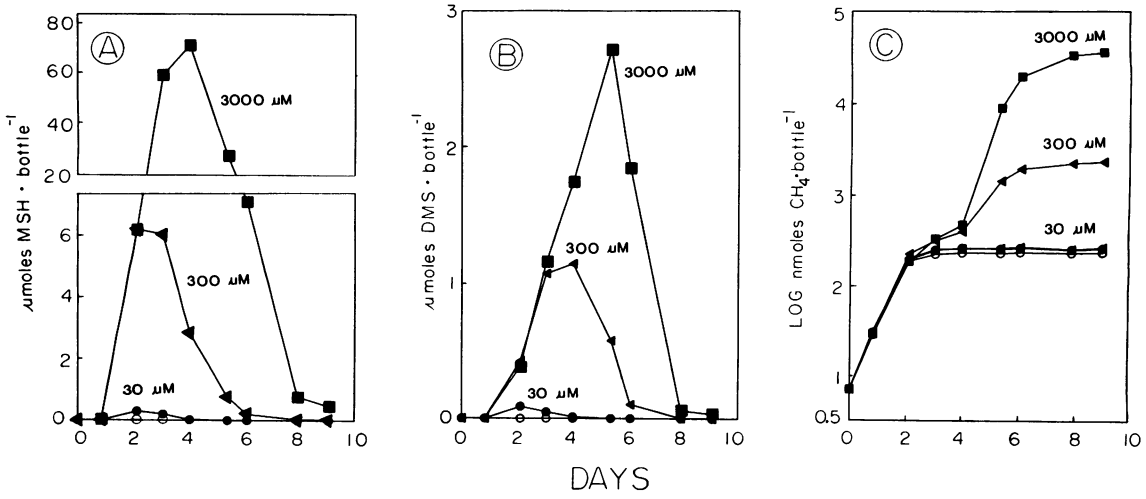

FIG. 1. Time courses of MSH (A), DMS (B), and CH_4 (C) accumulation in anoxic salt marsh sediments (25 ml) which were amended with various concentrations of DL-methionine. Symbols: ○, no treatment; ●, 30 μM; ▲, 300 μM; ■, 3,000 μM. Points represent the mean of two replicates. Standard errors were less than 10% of the means and are not shown.

levels of methionine giving correspondingly higher levels of DMS. The maximum levels of MSH reached during experiments exceeded peaks in DMS by ca. 6-fold in 30 and 300 μM treatments and 30-fold in 3,000 μM treatments.

Methane production was significantly stimulated by 300 and 3,000 μM additions of methionine, but only very slight stimulation was observed at 30 μM (Fig. 1C). No significant stimulation was detected at 3 μM.

Inhibitor experiments. An experiment was carried out to determine which microbial groups were involved in the initial demethiolation of methionine and the subsequent consumption of methylated sulfur compounds (Fig. 2). Glutaraldehyde, autoclaving, and chloramphenicol blocked the production of methylated sulfur compounds from methionine. The inhibitory effect of chloramphenicol was relieved after long incubations (>9 days; data not shown). Cycloheximide had no effect on the time course of MSH production and consumption. BES, an inhibitor of methanogenic bacteria, slightly stimulated the evolution of MSH while the loss of MSH was substantially inhibited (Fig. 2A). MoO_4^{2-} caused inhibition of both MSH production and consumption.

The results for DMS (Fig. 2B) were similar to those for MSH. Cycloheximide had no effect on the DMS time course while chloramphenicol blocked DMS production. A small accumulation of DMS was observed in the chloramphenicol treatment, which may be due to hydrolysis of endogenous sulfur compounds (e.g., DMSP) and the lack of consumptive processes, or possibly by gradual reversal of chloramphenicol inhibition. Inhibition of methanogenesis with BES caused significantly greater accumulation of DMS from methionine than in uninhibited samples, and DMS did not disappear. Molybdate inhibited both DMS production and consumption (Fig. 2B), although DMS eventually disappeared.

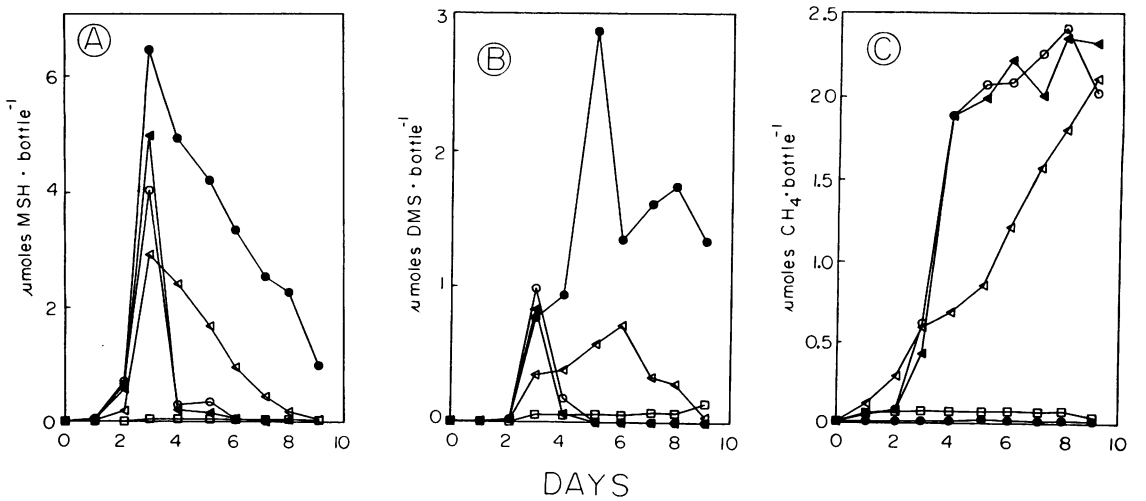

FIG. 2. Effect of various microbial inhibitors on the production and consumption of MSH (A), DMS (B), and CH_4 (C) in anoxic sediments (25 ml) treated with 300 μM methionine. Symbols: ○, methionine alone; □, chloramphenicol; ▲, cycloheximide; △, MoO_4^{2-}; ●, BES. Autoclaved and gluteraldehyde-treated samples (not shown) did not produce MSH, DMS, or CH_4. Points represent the mean of two replicates. Standard errors were less than 15% and are not shown.

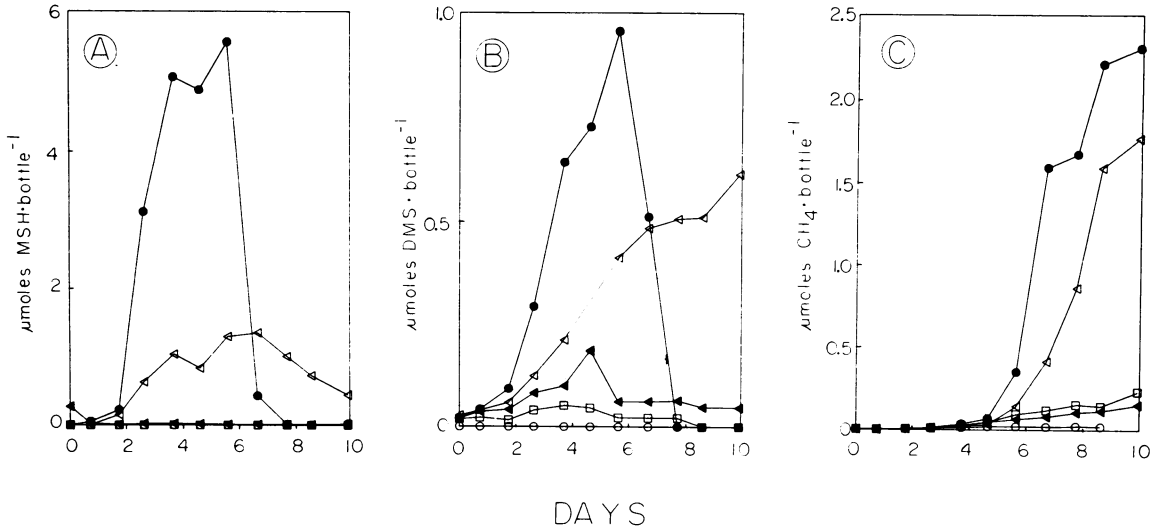

FIG. 3. Effects of molybdate on the production and consumption of MSH (A), DMS (B), and CH_4 (C) in anoxic sediments treated with 300 μM methionine. Symbols: ○, no treatment; ●, methionine alone; □, MoO_4^{2-} alone; △, MoO_4^{2-} plus methionine; ▲, MoO_4^{2-} plus MSH. Points represent the mean of two replicates. Standard errors were less than 15% of the mean and are not shown.

Figure 2C shows the effects of various inhibitors on the production of CH_4 in the presence of 300 μM methionine. Glutaraldehyde, BES, and chloramphenicol completely inhibited methane formation. Cycloheximide had no effect on CH_4 production. In molybdate-methionine samples, methanogenesis was stimulated relative to samples without methionine but inhibited relative to samples with methionine alone (Fig. 2C). CH_4 levels in molybdate-methionine bottles eventually reached those of the uninhibited methionine samples.

The effects of molybdate on methionine decomposition and methylated sulfur compound metabolism were investigated in further detail (Fig. 3). Molybdate significantly inhibited the production and consumption of MSH and DMS (Fig. 3A and B). In this experiment, inhibition by molybdate was more clearly demonstrated than in the previous experiment (Fig. 2A). The addition of molybdate alone (no methionine) caused very slight accumulation of MSH and low but significant levels of DMS (Fig. 3B). To ascertain the origin of DMS during experiments with methionine, a treatment of 0.2 μmol of MSH per bottle (~3 μM) plus MoO_4^{2-} was included (Fig. 3A and B). MSH decreased rapidly to undetectable levels (Fig. 3A), but DMS production was stimulated relative to the molybdate-alone treatment (Fig. 3B).

As previously noted, methionine greatly stimulated methanogenesis (Fig. 3C), but the inclusion of MoO_4^{2-} with methionine caused less CH_4 to be produced. However, both of these treatments produced much more CH_4 than MoO_4^{2-} treatment alone. MSH did not stimulate CH_4 production in this experiment.

Experiments with radiolabeled methionine. Mineralization of the terminal S-methyl group of methionine was verified through the use of [*S-methyl*-^{14}C]methionine. With an added concentration of ~1 μM methionine, the predominant (>99%) product of the S-methyl group was $^{14}CO_2$, and production was nearly linear over a 3-h time course (Fig. 4A). Approximately 30% of the added label was recovered as volatile products during the 3-h incubation. $^{14}CH_4$ was produced, but at levels which were <1% of the $^{14}CO_2$ values (data not shown). The short-term production of labeled organic sulfur compounds from 1 μM [^{14}C]methionine is shown in Fig. 4B. ^{14}C-organic sulfur increased rapidly, reached a maximum at 1.5 h, and subsequently decreased. The amount of radioactivity found in the organic sulfur fraction was only 5% or less of that for $^{14}CO_2$. However, the time course for the production and consumption of labeled organic sulfur was very similar to that observed for unlabeled MSH and DMS produced from higher levels of methionine additions.

An experiment was carried out in which unlabeled methionine was added to sediments over a concentration range of 1 to 500 μM, with radiolabeled methionine added at the same

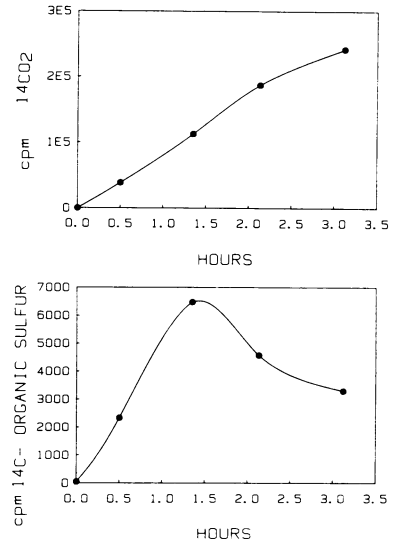

FIG. 4. (Top) Short-term course of $^{14}CO_2$ production from [*S-methyl*-^{14}C]methionine in anoxic salt marsh sediment slurries. Final added concentration of methionine was ~1 μM. Data points represent single bottles in which organisms were killed with glutaraldehyde at the times indicated. (Bottom) Same experiment as above; time course of radiolabeled volatile organic sulfur compounds produced from 1 μM [*S-methyl*-^{14}C]methionine in anoxic salt marsh sediments.

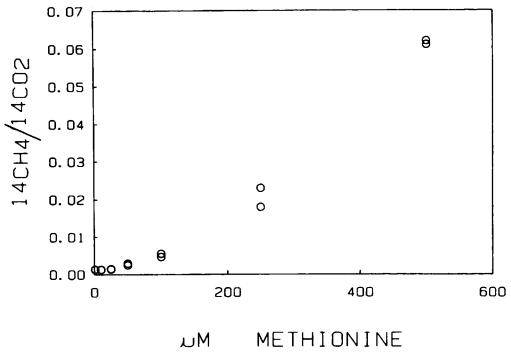

FIG. 5. Effect of different methionine concentrations on the ratio of $^{14}CH_4$ to $^{14}CO_2$ produced from [*S-methyl*-^{14}C]methionine in anoxic salt marsh sediments. Unlabeled methionine was added to give a range of pool sizes from 1 to 500 μM. Results are expressed as the ratio of $^{14}CH_4$ to $^{14}CO_2$. Points represent values for individual bottles at the indicated concentrations.

level (0.5 μCi) to each treatment. The results of this experiment (Fig. 5) revealed that larger pool sizes of methionine gave higher ratios of $^{14}CH_4$ to $^{14}CO_2$, suggesting that as methionine concentrations increase, a greater proportion of the S-methyl group (or free organic sulfur) is utilized by methanogenic bacteria.

DMSP experiments. DMSP was rapidly hydrolyzed to DMS in biologically active samples (Fig. 6). Sediments which were preincubated for 2 days with cycloheximide, chloramphenicol, BES, chloroform, or MoO_4^{2-} showed little or no effects of these inhibitors on the production of DMS from DMSP. DMSP was hydrolyzed at a slow but significant rate in glutaraldehyde-treated samples (Fig. 6). This hydrolysis was faster in samples which had not been preincubated with glutaraldehyde before receiving DMSP (data not shown). Autoclaved samples produced only trace amounts

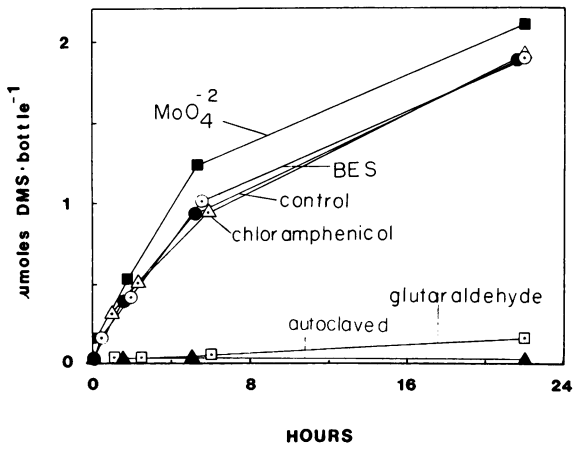

FIG. 6. Effect of various microbial inhibitors on the production of DMS from DMSP in anoxic salt marsh sediments. Sediments were preincubated with the inhibitors for 2 days prior to the addition of DMSP. DMSP was added at 2.7 μmol per bottle (10 ml of sediment slurry). Symbols: ●, DMSP alone; △, chloramphenicol; ■, MoO_4^{2-}; ○, BES; □, glutaraldehyde; ▲, autoclaved. Cycloheximide, chloroform, and MoO_4^{2-}-BES all gave results similar to those for the control. Samples which did not receive DMSP did not evolve significant amounts of DMS relative to the treated samples. Data represent the mean of two replicates. Standard errors were less than 5% and are not shown.

TABLE 1. Effects of various inhibitors on the amounts of DMS, MSH, and CH_4 present after 10 days of incubation in DMSP-treated sediments[a]

Treatment	Amount present at 10 days (μmol/bottle)		
	DMS	MSH	CH_4
No treatment	0	0	0.037
DMSP	0.036	0.044	1.17
Cycloheximide + DMSP	0.007	0.056	1.07
MoO_4^{2-} + DMSP	0	0	3.48
BES + DMSP	0.945	0.366	0.003
MoO_4^{2-} + BES + DMSP	1.59	0.006	0.001
Chloramphenicol + DMSP	1.59	0	0.001
Chloroform + DMSP	1.88	0.023	0.001
Glutaraldehyde + DMSP	1.03	0	0.001
Autoclaved + DMSP	0.033	0.059	0.004

[a] Each 25-ml bottle contained 10 ml of slurry. DMSP was added at 270 μM (2.7 μmol per bottle) 15 min after addition of inhibitors. Added DMSP was hydrolyzed rapidly in all except the autoclaved and glutaraldehyde samples. Autoclaved sediments produced no significant DMS, while glutaraldehyde samples evolved DMS slowly over 10 days. No treatment samples produced insignificant amounts of DMS. All other samples produced a maximum of ~2, μmol of DMS per bottle after 2 days of incubation.

of DMS (DMSP was added after sediments were autoclaved because autoclaving alone results in hydrolysis of DMSP to DMS [R. P. Kiene, unpublished data]).

The amount of DMS, MSH, and CH_4 present after 10 days in sediments which received DMSP and various inhibitors is given in Table 1. In autoclaved samples, the levels of DMS, MSH, and CH_4 did not change during the course of the experiment. Glutaraldehyde-treated sediments slowly accumulated significant quantities of DMS by 10 days, but no metabolism of this DMS was evident (no decrease in DMS and no MSH or CH_4 produced). DMSP was hydrolyzed rapidly in chloroform and chloramphenicol-treated sediments, but no metabolism of the resultant DMS was evident.

DMSP addition stimulated CH_4 production relative to samples which received no sulfur compounds (Table 1), and the DMS was nearly all consumed by 10 days. Results with cycloheximide were similar to those with uninhibited samples. In the case of BES, CH_4 production was inhibited and DMS and MSH levels reamined high after 10 days. With molybdate, DMS was totally consumed and methane production was stimulated relative to DMSP treatment.

DISCUSSION

The decomposition of methionine and DMSP occurred rapidly in anoxic salt marsh sediments, and both of these precursors gave rise to free methylated sulfur compounds. The predominant organic sulfur compound released from DMSP was DMS (Fig. 6), while methionine released predominantly MSH and smaller amounts of DMS (Fig. 1).

Additions of methionine over a broad range of concentrations (3 to 3,000 μM) showed that the patterns of MSH and DMS formation and consumption were independent of the concentrations. At 3 μM methionine, methylated sulfur compounds were not easily detected by headspace gas chromatography. However, experiments with radiolabeled methionine at 1 μM revealed a similar time course for organic sulfur compounds compared with those at the higher levels (Fig. 4B). Inhibitor experiments showed that methionine breakdown was biological and that it involved organisms sensitive to chloramphenicol, probably bacteria. Cycloheximide, a eucaryote inhibitor, had little effect on the

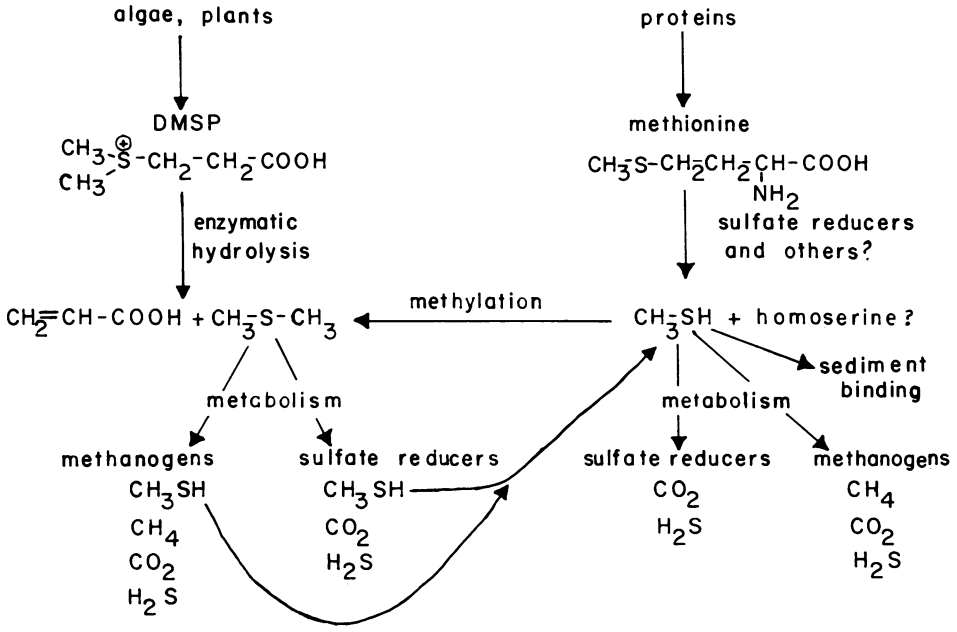

FIG. 7. Working model of the production of DMS and MSH from methionine and DMSP and the fate of these compounds in anoxic salt marsh sediments.

production of methylated sulfur compounds from methionine, suggesting that organisms such as anaerobic ciliates or fungi may not be important in this reaction. Because we have no other data to indicate that cycloheximide was effective against anaerobic eucaryotes or whether the target organisms were present, this conclusion must be considered tentative at this time.

Sulfate reduction and methanogenesis are two important microbial activities in anoxic sediments, and these processes were studied with respect to methionine metabolism. BES did not inhibit MSH formation from methionine, indicating that methanogens are not responsible for demethiolation. Inhibition of sulfate reduction gave a consistently lower production rate and total production of MSH from methionine (Fig. 2A and 3A), suggesting that sulfate-reducing bacteria are carrying out some of the observed demethiolation. Sulfate reduction is the most important anaerobic pathway of organic carbon mineralization in anoxic salt marsh sediments (15, 17). The sulfate-reducing bacteria are known to metabolize a large variety of substrates (F. Widdel, Ph.D. thesis, University of Gottingen, Gottingen, Federal Republic of Germany, 1980), including amino acids (33). The observation that some MSH was produced even with molybdate present at highly inhibitory concentrations (4, 9, 35) indicates that sulfate-reducing bacteria are not the only organisms involved in methionine metabolism. Perhaps anaerobic fermentative organisms also play a role.

The findings of this study are consistent with those of other studies with different systems. Segal and Starkey (31) found that the S-methyl group of methionine was decomposed primarily to MSH by a variety of bacterial cultures. Small amounts of DMS were observed by Segal and Starkey (31). Zinder and Brock (43) also observed that MSH was the predominant sulfur compound released from methionine in anoxic lake sediments, with DMS found only as a minor product. DMDS is sometimes observed during methionine decomposition studies (7, 31). However, DMDS probably forms as a result of MSH oxidation rather than from direct release from methionine. DMDS was not detected in the present study, most likely because DMDS is rapidly reduced to MSH by these anoxic sediments (R. P. Kiene and D. G. Capone, submitted for publication). In relatively oxidized sediments, DMDS may be a more important end product of methionine metabolism.

The appearance of DMS in methionine treatments could be due to the formation of an S-methyl methionine sulfonium compound and subsequent hydrolysis to liberate DMS (10) or to the direct methylation of free MSH or both. Evidence from experiments with MSH additions indicates that the latter explanation is possible, because DMS formed when only MSH was added (Fig. 3). In rumen samples, carbon tetrachloride, an inhibitor of methyl transfer reactions, blocked DMS formation from methionine but did not affect MSH production (30). In the same study, carbon tetrachloride did not affect DMS formation from dimethyl acetothetin (a sulfonium compound). These authors concluded that the DMS in methionine treatments arose from a methyl transfer reaction, while it arose by a different mechanism from dimethyl acetothetin. It is worth mentioning that high levels of ambient methionine, such as those used in our experiments, might favor methylation reactions, due to the potential involvement of *S*-adenosylmethionine in biochemical methylations (10, 23).

DMS formation was observed in both molybdate- and BES-inhibited samples, suggesting that organisms other than sulfate reducers and methanogens can form DMS (20, 31, 41). However, sulfate reducers may play at least a partial role, since DMS production was partially inhibited by MoO_4^{2-}. This, however, must be looked at more closely, because a potential precursor of DMS (MSH) was lower in molybdate-treated bottles (Fig. 2 and 3), and this could have affected the production of DMS. Sulfate-reducing bacteria have been implicated in the methylation of metals such as Hg in sediments (11); therefore, it is possible that they may also be capable of methylating MSH. Methanogens too have been implicated in metal methylation reactions (29). Further work

will be necessary to elucidate the mechanisms of DMS formation from methionine and MSH.

DMSP is found in a variety of algae and higher plants, where it is believed to function as a compatible solute for osmoregulation (28, 37). *Spartina* species have been reported to contain DMSP (21), and *S. alterniflora* leaves release considerable DMS after addition of NaOH (a condition which results in hydrolysis of DMSP [36]). In sediments, DMSP is readily hydrolyzed (Fig. 6), and if DMSP were to be released by plants or detritus in the sediments, it would be rapidly decomposed to DMS. DMSP hydrolysis in biological systems has been found to be enzymatic (8). From the present study it is evident that the formation of DMS from DMSP is biological, since this did not occur in sterile, autoclaved samples. Autoclaving appears to destroy the enzymes responsible for carrying out this reaction in sediments (Fig. 6), but glutaraldehyde only partially inhibits them. The involvement of specific groups of microorganisms in DMSP hydrolysis could not be determined, because DMSP was hydrolyzed in the presence of all the inhibitors tested (Fig. 6). Wagner and Stadtman (38) reported that a strain of *Clostridium propionicum* was capable of hydrolyzing DMSP to give DMS and acrylate. Fermentative organisms such as clostridia may be responsible for breaking down DMSP in sediments and may not be sensitive to the inhibitors used here.

Freshly prepared slurries of *Spartina* sediments contained significant levels of DMS (1 to 10 μM) (R. P. Kiene, submitted), probably arising from the disruption of *Spartina* root tissues and release of DMSP and DMS (16). In situ dissolved concentrations of DMS in Flax Pond sediments are not known but are probably lower than the 1 to 10 μM observed in slurries. Howes et al. (16) found <0.1 μM DMS in undisturbed marsh pore waters from Great Sippewissett marsh. Andreae (1) reported DMS concentrations generally less than 0.1 μM in sediments off the coast of Peru. The low concentrations of DMS in sediment pore waters is probably due to the metabolism of this compound by sediment microbiota (see below).

In addition to DMS, DMSP is known to yield acrylate (CH_2=CH-COO^-) upon hydrolysis (10, 38). Acrylate can be fermented anaerobically to propionate and acetate by species of *Clostridium* (38) and by anaerobic sediments (R. P. Kiene, unpublished data). Thus, DMSP may be a precursor not only of DMS in salt marsh sediments, but also of low-molecular-weight fatty acids. Considering the fact that DMSP is apparently released during handling of salt marsh sediments (16) and is easily hydrolyzed, this release of metabolizable substrates could have significant implications for rate measurements of bacterial activities in marsh sediments. The in situ turnover of DMSP, DMS, and acrylate in undisturbed sediments is a topic for future study.

The accumulation of volatile sulfur compounds after addition of DMSP or methionine was followed by their rapid decrease to undetectable levels in uninhibited samples (Fig. 2A and B, 3A and B, and Table 1). Through the use of selective microbial inhibitors, both sulfate-reducing and methane-producing bacteria have been implicated in the consumption of DMS and MSH. These findings support the earlier conclusions of Kiene et al. (18), who found that sulfate reduction, in addition to methanogenesis, was responsible for metabolizing [^{14}C]DMS in salt marsh sediments. Kiene et al. (18) and Kiene (submitted) have found that sulfate reduction accounts for >80% of DMS metabolism in marsh sediments when DMS is present at concentrations less than 10 μM. At higher DMS concentrations (i.e., ~200 μM), such as those encountered in the experiments with DMSP (Fig. 6, Table 1), sulfate reduction appears to be less important in DMS consumption than methanogenesis. This is illustrated by the results in Table 1 (see also reference 18). When sulfate reduction was inhibited, DMS completely disappeared by 10 days, whereas inhibition of methanogenesis (sulfate reduction active) resulted in the persistence of DMS at 10 days (Table 1).

Methionine greatly stimulated methanogenesis when present at 300 to 3,000 μM (Fig. 1C), but stimulation was less apparent at lower concentrations. Stimulation of methanogenesis in saline sediments has previously been observed by Oremland et al. (26) and Oremland and Polcin (25), who used concentrations of 10 mM methionine.

From isotope experiments with methionine, at a concentration of about 1 μM, >99% of the mineralization products of the S-methyl group was CO_2 (Fig. 4A and 5), indicating an oxidative catabolism, possibly by sulfate respirers or other organisms. Winfrey and Ward (40) found that [*S-methyl*-^{14}C] methionine (<1 μM) was converted entirely to $^{14}CO_2$ and not to $^{14}CH_4$ in marsh sediments from the Brittany coast. It appears that at low (micromolar) concentrations, the S-methyl group of methionine is metabolized to CO_2, while at higher concentrations yields of CH_4 increase (Fig. 5). These findings may explain why MSH at 3 μM did not stimulate methane production in the present study, while stimulation has been observed at higher concentrations in other studies (18).

Methylated sulfur compounds derived from the terminal S-methyl group of methionine may be "competitive" substrates for methanogenesis in sediments (18, 19). The work of Banat et al. (5) supports this conclusion, because they found that sediments inhibited with molybdate produced $^{14}CH_4$ from labeled methionine, while those without molybdate produced only $^{14}CO_2$. Our results are consistent with those of Banat et al. (5) and differ only in that we have implicated the free methylated sulfur compounds as the precursors of CH_4 rather than methionine directly. The concentration effect observed for the conversion of methionine and other methylated sulfur compounds to methane is similar to that described for methanol (19).

Methanogenic bacteria have previously been implicated in the metabolism of MSH and DMS to CH_4 and CO_2 in anaerobic lake sediments and sewage sludge (42). Kiene et al. (18) recently reported on the conversion of DMDS, MSH, and DMS to methane in a variety of anoxic sediments. Additionally, these authors have isolated a pure culture of an estuarine methanogen which grows on DMS. The isolate is incapable of growth on MSH alone, but produces MSH as an intermediate during growth on DMS. The DMS isolate does not demethiolate methionine and cannot grow on this substrate (R. P. Kiene and R. S. Oremland, manuscript in preparation). Thus, it appears that methanogens do not attack methionine directly but are stimulated by the release of methylated sulfur compounds during the metabolism of other organisms. Both MSH and DMS are produced during methionine metabolism in sediments (Fig. 1, 2, and 3), and each of these compounds stimulates methanogenesis when added separately (18). However, both MSH and DMS may be interconverted during sediment metabolism (Kiene and Capone, Microb. Ecol., in press). Thus, it is not clear whether the stimulation of methanogenesis in methionine treatments is from direct conversion of MSH to CH_4 or from metabolism of the DMS formed or both. It is interesting that methanogenesis was inhibited in treatments with methionine plus molybdate (Fig. 2C and 3C). This observation was

unusual in that molybdate generally stimulates methanogenesis (5, 9). Lower methane in MoO_4^{2-} plus methionine-treated samples may be explained by the slower production of the methane precursors MSH and DMS in these samples compared with that after methionine treatment alone.

Figure 7 represents a conceptual model of the production and fate of methylated sulfur compounds from DMSP and methionine in anoxic salt marsh sediments. This model is based on results from the present study as well as related studies (18, 24, 38, 42, 43). Methionine derived from proteins will be metabolized by sulfate-reducing and other sediment bacteria, with the release of MSH. MSH may bind to sediments (24) or be metabolized by sulfate-reducing and methane-producing bacteria to mineralized end products such as CH_4, CO_2, and H_2S. Alternately, MSH may be metabolically transformed to DMS via a methylation reaction. DMS may also arise from the hydrolysis of DMSP, which is ultimately derived from plants and algae such as *Spartina* spp. and *Ulva* spp. DMS is metabolized by both sulfate-reducing and methane-producing bacteria to mineralized products (CH_4, CO_2, and H_2S), but also to MSH, which will be further metabolized as described above. The relative importance of sulfate reduction and methanogenesis in consuming methylated sulfur compounds appears to depend on the concentration of the substrate (higher concentrations favor methanogenesis) and the ambient concentrations of sulfate (low sulfate concentrations, i.e., fresh water, favor methanogenesis). Future studies should be aimed at obtaining quantitative estimates of the rate of turnover of methylated sulfur compounds and their relative contributions as substrates for various microbial activities.

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