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Microbial Transformations of Methylated Sulfur Compounds in Anoxic Salt Marsh Sediments

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Abstract. Anoxic salt marsh sediments were amended with several methylated sulfur compounds. Sediment microbes transformed the added compounds into other volatile methylated sulfur compounds and eventually mineralized the compounds to CH_4 and presumably to CO_2 and H_2S. The principal methyl-sulfur product of dimethylsulfoniopropionate (DMSP) was found to be dimethylsulfide (DMS), with only small amounts of methane thiol (MSH) produced. By contrast, methionine and S-methyl cysteine were degraded mostly to MSH and to lesser amounts of DMS. Dimethylsulfoxide (DMSO) was biologically converted to DMS. Dimethyldisulfide (DMDS) was rapidly reduced to MSH by the sediment microflora, and some DMS was also produced. DMS, whether added directly or when derived from other precursors, was metabolized with the production of MSH. Methane thiol was also metabolized, and evidence suggests that MSH may be biologically methylated to form DMS. Experiments with selective microbial inhibitors were used to ascertain which microbial groups were responsible for the observed transformations. Based on these experiments, it appears that both sulfate-reducing and methane-producing bacteria may be involved in transforming and mineralizing methylated sulfur compounds. A simple scheme of how methylated sulfur compounds may be transformed in the environment is presented.

Introduction

Volatile methylated reduced sulfur compounds such as methane thiol (MSH), dimethylsulfide (DMS), and dimethyldisulfide (DMDS) are produced by a variety of living organisms [10, 23, 31], and are often liberated during the decay of sulfur-containing organic matter [33, 38]. Organic compounds that have been identified as precursors of volatile methylated sulfur compounds include

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the plant product dimethylsulfoniopropionate (DMSP) [30, 31] and the amino acid methionine [25, 26, 35].

Methylated sulfur compounds have been detected and measured in a variety of terrestrial and aquatic systems [1, 2, 3, 7, 9, 32]. The geochemical distributions of methylated sulfur compounds have received considerable attention recently due to the recognized importance of these compounds in the global sulfur cycle [1, 3, 28]. However, relatively little is known about the biological fate and transformations of these organic sulfur compounds.

The present study considered the metabolism of various methylated sulfur compounds by the microflora of anoxic salt marsh sediments. The purpose of this paper is to describe the various sulfur compound transformations that were observed during sediment incubations with added sulfur compounds. The nature of the transformations and the involvement of specific groups of microbes was studied through the use of selective microbial inhibitors. The results of this investigation showed that methylated sulfur compounds could be readily metabolized by sediment microflora and that methylated sulfur compounds such as DMSP, methionine, DMS, MSH, dimethylsulfoxide (DMSO) and DMDS were transformed to other methylated sulfur compounds by biological activity. In addition, these compounds were consumed and mineralized to CH_4 and presumably to CO_2 and H_2S by the sediment microbiota.

Materials and Methods

Sediment Preparation

Sediments were collected from among stands of *Spartina alterniflora* in the salt marsh at Flax Pond, Old Field, New York. Anoxic sediment slurries were prepared as described elsewhere [18], and 25 ml was dispensed to Erlenmeyer flasks (139 ml total volume) or serum bottles (60 ml total volume). Flasks were sealed with #5 black rubber stoppers (Thomas Scientific, Sweedsboro, NJ), and serum bottles were sealed with black butyl rubber stoppers (Bellco Glass, Vineland, NJ). Use of rubber stoppers in experiments with organic sulfur compounds presents some noteworthy problems due to the solubility of these compounds in the rubber. This aspect will be discussed below. Unless otherwise indicated, experimental treatments were run in duplicate. Incubations were carried out in the dark at 22–26°C with gentle shaking (~100 rpm).

Additions of Methylated Sulfur Compounds

Aqueous solutions of DMSP · HCl, D,L-methionine, S-methyl cysteine, L-cysteine, and dimethylsulfone were added to the sealed anoxic slurries by syringe injection through the rubber stoppers. DMS, DMDS, and DMSO were added as pure liquids with a glass, microliter syringe (Hamilton Inc., Reno, NV). MSH vapor was obtained by placing liquid MSH (0°C) in a serum bottle and removing a portion of the headspace with a 2.5 cc gas-tight syringe (Precision Scientific). The gas was then injected into sample bottles. Sulfur compounds were added at different levels depending on the experiment, and concentrations are indicated in the text and figure legends. In all cases, added concentrations were greater than 100 μM but less than 5 mM.

Analysis of Gaseous Compounds

Methylated sulfur compounds and CH_4 were measured in the headspaces of bottles using a Shimadzu GC R1A gas chromatograph equipped with a flame ionization detector. The column was stainless steel (2 m × 3 mm) packed with Porapak R (80/100 mesh). The oven, injector, and detector temperatures were all maintained at 120°C. Nitrogen (80 ml/min) served as the carrier gas. DMDS did not elute from the Porapak column under the conditions described above. DMDS was measured using a teflon column (0.6 m) packed with Supelpak (a specially treated Porapak QS, Supelco Inc., Bellefonte, PA). The oven temperature was held at 140°C and the carrier flow (N_2) was 70 ml/min.

Flame ionization detection (FID) was chosen rather than sulfur-specific flame photometric detection (FPD) for several reasons. First, FID response to methylated sulfur compounds is linear over at least 5 orders of magnitude whereas the FPD is linear over a much smaller range. Second, the very large quantities (1–2% of gas phase) of H_2S often produced during salt marsh sediment slurry experiments [18] saturate the FPD and make it difficult to quantify peaks that elute after H_2S. Finally, the sensitivity of the FID to methylated sulfur compounds such as DMS and MSH is only slightly less (two-to fourfold) than the FPD. Under optimal conditions, absolute detection limits per sample injection of 1.6 and 3 pmol of DMS and MSH, respectively, have been obtained. Routine detection limits were usually two to three times higher. Typical injection volumes were 100 μl of the sample headspace.

Identification of methylated sulfur compounds was made by retention time analysis and comparison with authentic compounds. Peak identity was supported by using different chromatographic conditions (columns, temperature, flow rate) and retention time analysis. Analyses were calibrated by comparing sample peak areas with standard curves prepared from pure (>99%) compounds. A standard curve could not be prepared using liquid MSH due to difficulties in handling this material. A standard curve for MSH was obtained using MSH (96% purity) from a lecture bottle (Matheson Inc., E. Rutherford, NJ). A DMS standard curve was prepared at the same time and a relative response factor was obtained. This relative response factor between DMS and MSH was routinely used to standardize MSH runs.

Distribution coefficients (concentration in liquid/concentration in vapor) for methylated sulfur compounds were determined empirically by adding known amounts of the compounds to bottles that either contained sediment slurry or did not. The values of the distribution coefficients at 25°C were 11.2, 7.9, and 15.8 for DMS, MSH, and DMDS, respectively. The values obtained were similar to those reported by Przyjazny et al. [22] and Dacey et al. [12]. Typically, 25–35% of the methylated sulfur compounds were in the headspace. All data are reported as μmol/bottle, which includes the totals in the gas and liquid phases.

Inhibitor Experiments

Several microbial inhibitors were used to determine which biological processes were involved in the transformations of methylated sulfur compounds. Chloramphenicol (200 μg/ml) was used to inhibit prokaryotic activity [14]. Sodium molybdate (20 mM) was added to sediments to inhibit sulfate reduction [6, 21, 29]. 2-Bromoethane sulfonic acid (BES) (10 mM) was used as a specific inhibitor of methanogenesis [13]. Killed controls were obtained by adding glutaraldehyde to a final concentration of 0.1%. None of the above-mentioned inhibitors caused transformations or resulted in detectable complexation of free methylated sulfur compounds.

Abiological Absorption of Sulfur Compounds

Because organic molecules, particularly volatile hydrocarbons and methylated sulfur compounds, have significant interactions (absorption and adsorption) with rubber materials, the possibility of

using bottle seals other than the commonly used black or butyl rubber stoppers was investigated. DMS was used to test various other seals. Teflon-faced septa (Supelco) worked well at keeping steady concentrations of DMS in crimp-sealed bottles; however, this held true only when the septa were not pierced by syringe needles. After several samplings, teflon septa gave variable results due to losses of DMS into or through the septa. In addition, teflon enclosures often are unsuitable for anaerobic incubations due to the relatively high permeability of teflon to O_2. Similarly, bottles with Mininert teflon valve caps (Alltech Associates, State College, PA) also lost DMS, but not as rapidly as those with black rubber. Silicone rubber stoppers gave rapid losses of DMS as did gray butyl serum stoppers (data not shown).

Faced with no adequate or convenient means to quantitatively contain methylated sulfur compounds in anaerobic incubations that required repeated sampling, black rubber and butyl rubber stoppers were chosen despite their obvious absorption of methylated sulfur compounds. So that the reader can be aware of the abiological absorption phenomenon, a time course for the disappearance of DMS in a N_2-filled Erlenmeyer flask (~140 ml) sealed with a #5 black rubber stopper is presented in Fig. 1; there was no liquid phase in this bottle. Approximately 54% of the initial DMS was lost from the headspace over a 24 h period. Similar results were observed for MSH and DMDS (data not shown). The loss kinetics of methylated sulfur compounds in sterile, rubber-sealed sample bottles are independent of the initial concentration (data not shown). Stoppers absorb methylated sulfur compounds such as DMS and apparently equilibrate with the ambient levels of the compound. When stoppers that have been exposed to DMS are placed in new bottles containing no DMS, they release DMS (Fig. 1). Similar interactions have been found between CH_4 and rubber stoppers [17]. However, the absorption of CH_4 is much less than for methyl-sulfur compounds. When one considers that the DMS released from the stopper is probably only ~50% of what was absorbed, a significant fraction of the loss of DMS observed in Fig. 1 can be accounted for by absorption into the stopper. This means that in sediment incubations, the methylated sulfur compounds lost by absorption over the initial phase of incubation may be re-released later in the experiment when gas-phase levels of the sulfur compounds are lowered due to metabolism (see results). The DMS that remains unaccounted for could have adsorbed to the glass surface, or could have passed through the stopper. The interactions of methylated sulfur compounds with rubber stoppers make quantitative comparisons difficult. However, useful comparisons may be made despite the abiological losses.

Reagents and Chemicals

DMS, DMDS, dimethylsulfone, L-cysteine, and D,L-methionine were obtained from Aldrich Chemical Co., Milwaukee, WI. MSH was obtained from Eastman Chemical Co., Rochester, NY. DMSP·HCl was obtained from Custom Chem Labs, Livermore, CA. All other reagents and chemicals were of reagent grade.

Results

An experiment in which DMSP, methionine, cysteine, and S-methyl cysteine were added to sediment slurries revealed that MSH was the dominant methyl sulfur product of both methionine and S-methyl cysteine; however, lower levels of DMS were also produced (Fig. 2). By contrast, DMSP yielded mostly DMS and only small amounts of MSH. Cysteine did not yield any significant volatile methyl sulfur compounds. Both MSH and DMS decreased to undetectable levels after their production had ceased. Methane production relative to controls (0.025 μmol/bottle) was stimulated by methionine (0.74 μmol/bottle), S-methyl cysteine (0.62 μmol/bottle), and DMSP (0.61 μmol/bottle), but was unaffected by cysteine (0.022 μmol/bottle).

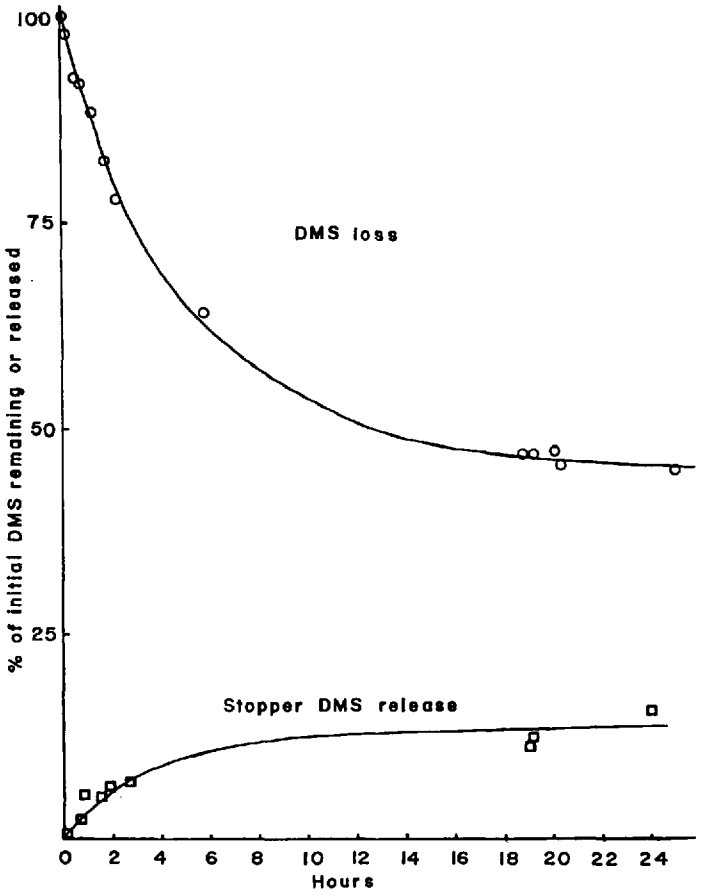

Fig. 1. Time course of the loss of DMS from a sterile, N_2-filled flask sealed with a #5 recessed black rubber stopper. After 26 h the stopper was removed and placed in a fresh flask that contained no initial DMS. The amount of DMS released from this stopper is also shown. Results are expressed as the % of the initial amount present in the first flask. The amount of DMS released from the stopper in the second flask is only a portion of what was absorbed, because some DMS remains in the stopper.

The transformations of individual methylated sulfur compounds (DMSO, DMS, MSH, and DMDS) in uninhibited sediment slurries are illustrated in Fig. 3A, B, C, D. These figures show the time courses of volatile methylated sulfur compounds, as well as methane, during sediment slurry experiments in which the indicated compound was added. In all cases, data values represent excesses above untreated controls. Untreated sediments produced much lower (<0.5 μmol/bottle) quantities of organic sulfur compounds and methane compared with treated flasks. Dimethylsulfone (100 μM) yielded no volatile methylated sulfur compounds and did not have any effect on methane production in marsh sediments (data not shown). Results similar to these have been obtained in many experiments throughout this study.

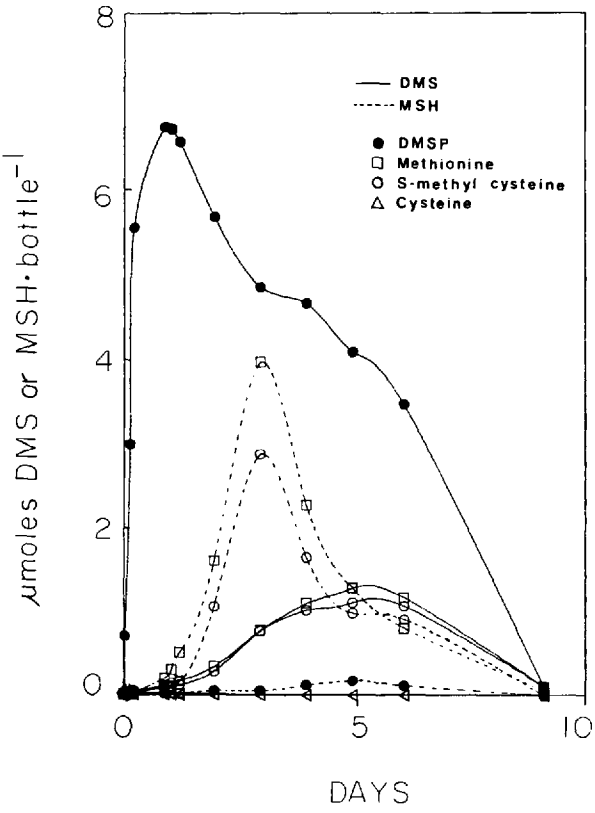

Fig. 2. Production and disappearance of DMS and MSH in anoxic sediments amended with cysteine (△), methionine (□), S-methyl cysteine (○), and DMSP·HCl (●). Solid lines represent DMS and broken lines represent MSH. Compounds were added at 400 μM (10 μmol/25 ml sediment slurry). Values represent single sample flasks. DMS and MSH were not detected in the cysteine treatment.

In order to discern the nature of the observed methyl sulfur transformations (Fig. 3A, B, C, D), experiments were carried out using various microbial inhibitors. The results for each individual sulfur compound are presented below.

DMSO additions gave rise primarily to DMS with the formation of lesser amounts of MSH (Fig. 3A). Methane production was stimulated by DMSO, probably due to the formation of DMS and MSH. The formation of DMS from DMSO did not occur in glutaraldehyde-killed samples (Fig. 4A). Chloramphenicol greatly inhibited DMSO reduction, but a slow steady accumulation of DMS was observed. Addition of 20 mM molybdate to inhibit sulfate reduction resulted in a slightly increased production of DMS, whereas 200 μM NO_3^- (an alternate electron acceptor) caused a delay in DMS production. BES, an inhibitor of methanogenesis, had no effect on the reduction of DMSO to DMS (Fig. 5). Sediments receiving inhibitors but not DMSO did not produce significant levels of DMS compared with those receiving DMSO.

Neither MoO_4^{2-} nor NO_3^- affected the consumption of the DMS evolved in this experiment (Fig. 4A). In chloramphenicol treatments, DMS did not decrease during the 9 day incubation. BES significantly inhibited the loss of DMS formed from DMSO (Fig. 5).

MoO_4^{2-} caused a lower production of MSH compared with uninhibited samples (Fig. 4B), but MSH consumption was not affected. NO_3^- caused a

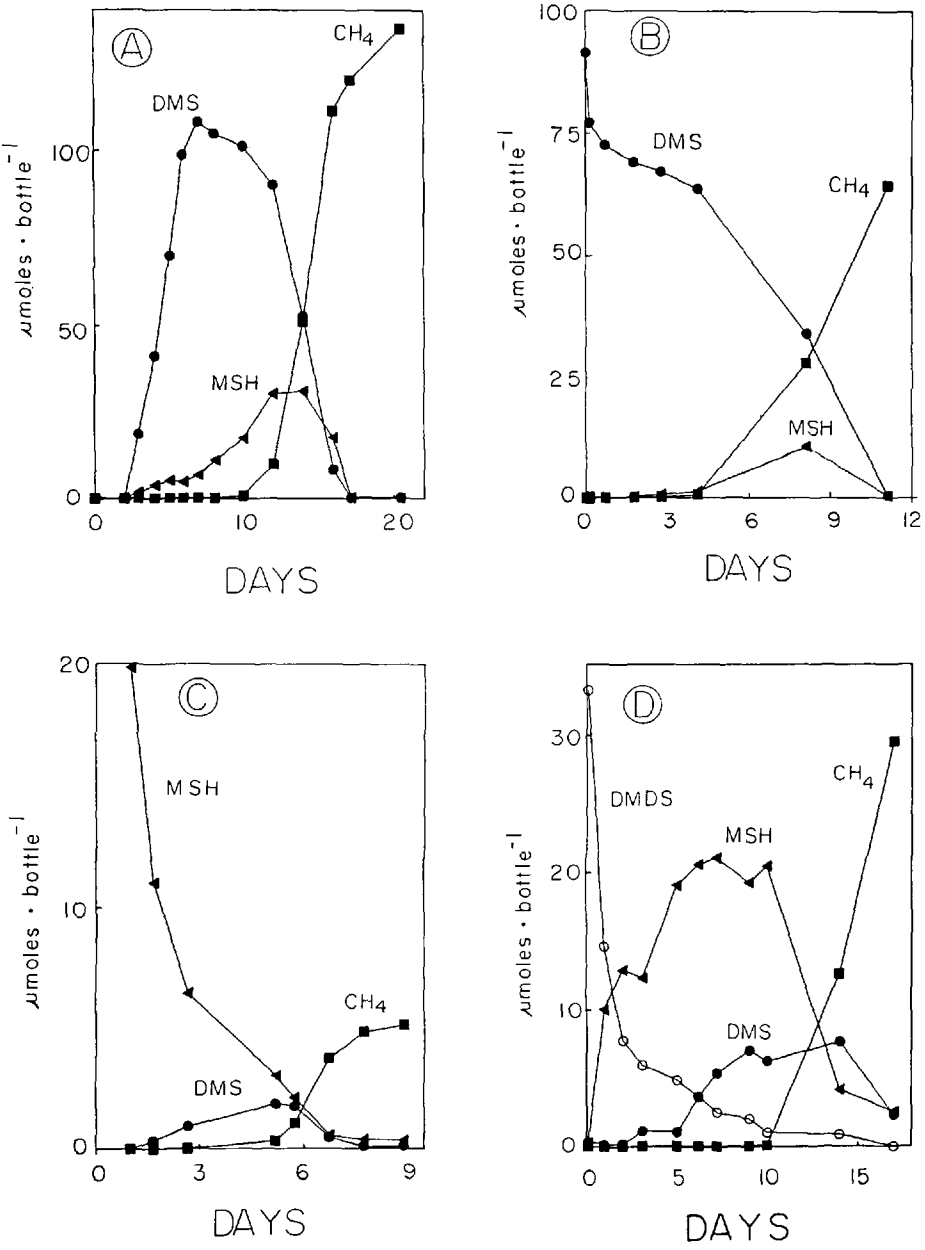

Fig. 3. Time courses of methylated sulfur compounds and CH_4 in anoxic sediments amended with (A) DMSO, (B) DMS, (C) MSH, (D) DMDS. Symbols: (●), DMS; (▲), MSH; (○), DMDS; (■), CH_4. Experiments for each compound were carried out on different dates. Plotted data represent the mean of two replicates. Standard deviations were less than 15% of the means and are not shown.

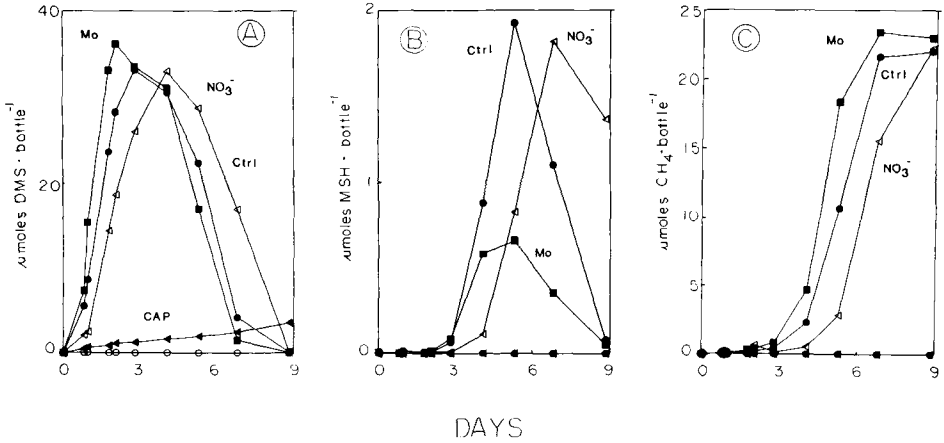

Fig. 4. Effect of various treatments on DMSO transformations in anoxic sediments. Results shown are for DMS (**A**), MSH (**B**), and CH_4 (**C**). Treatments included DMSO alone (Ctrl ●), DMSO + MoO_4 (■), DMSO + NO_3^- (△), DMSO + chloramphenicol (CAP ▲), and DMSO + glutaraldehyde (○). Values represent the mean of 2 replicates (C.V. < 10%). DMSO was added at 70 μmole per flask (25 ml sediment slurry).

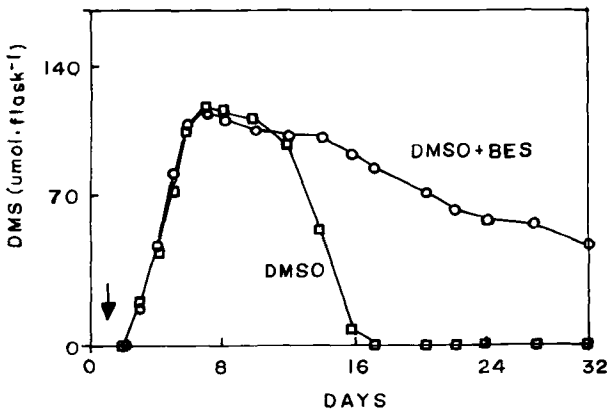

Fig. 5. Effect of BES on the production of DMS in DMSO-treated sediments. Symbols (□) DMSO; (○) DMSO + BES. Variation was less than 10% of the mean and is not shown. The arrow indicates the time of DMSO addition.

delay in MSH production but levels eventually reached those of controls. Nitrate also showed the decrease of MSH. BES significantly inhibited the formation and consumption of MSH (data not shown).

Glutaraldehyde and chloramphenicol (Fig. 4C) and BES (not shown) completely inhibited methane production in the presence of DMSO. Molybdate-DMSO treatments gave slightly higher CH_4 than did DMSO alone and both of these treatments produced much more CH_4 than sediments with molybdate alone. Nitrate-DMSO initially inhibited CH_4 production below endogenous levels, but methanogenesis recovered and eventually reached levels seen in treatments with DMSO alone.

When DMS was added directly, it was consumed by both abiological and biological processes (Figs. 3B, 6). MSH was formed upon DMS addition and

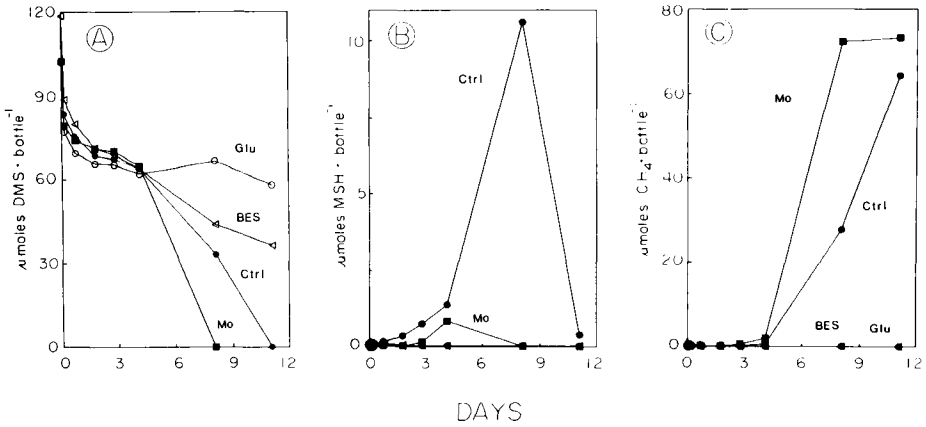

Fig. 6. Effects of several inhibitors on DMS transformations in anoxic sediments. Results shown are for DMS (**A**), MSH (**B**), and CH_4 (**C**). Symbols: (Ctrl ●), DMS; (Mo ■), DMS + MoO_4^{2-}; (△), DMS + BES; (Glu ○), DMS + glutaraldehyde. Values represent the mean of two replicates (C.V. < 15%). Each flask (25 ml sediment slurry) received 80–90 μmol DMS.

CH_4 production was stimulated. The results of an inhibitor experiment with DMS are presented in Figure 6. Glutaraldehyde (Fig. 6A) and chloramphenicol (not shown) prevented the loss of DMS beyond that expected from abiological absorption. BES inhibited the loss of DMS, whereas molybdate accelerated DMS consumption.

The production of MSH in DMS treatments (Fig. 6B) was not observed in the presence of glutaraldehyde or chloramphenicol. Only traces of MSH were observed in BES treatments, and MoO_4^{2-} strongly inhibited MSH formation.

No significant CH_4 was formed in DMS-amended sediments treated with BES, glutaraldehyde, or chloramphenicol (Fig. 6C). Molybdate-DMS treatment resulted in even greater methane production than with DMS alone.

Added MSH disappeared rapidly in sediments (Fig. 3C) due to biological and abiological reactions. DMS was found to be produced in samples treated with MSH, and MSH stimulated methanogenesis. In the inhibitor experiment with MSH (Fig. 7A, B, C) there was a high degree of variability in the amount of MSH added to each treatment due to difficulties in handling MSH. Glutaraldehyde samples received much less MSH than other treatments, and thus it was difficult to distinguish between biological and abiological reactions. It can be seen in Fig. 7A that molybdate resulted in the persistence of MSH for a longer period than in untreated controls or in BES-amended samples. Because the initial amount of MSH was lower in BES treatments, it is difficult to tell from this experiment whether BES significantly affected MSH consumption. However, BES appears to have slightly inhibited the loss of MSH. When molybdate and BES were added in combination, the greatest inhibition of MSH consumption was observed.

DMS production in MSH-treated sediments was slightly inhibited by MoO_4^{2-} or BES (Fig. 7B). When these inhibitors were added in combination, the inhibition was substantial. No significant DMS was formed in glutaraldehyde-

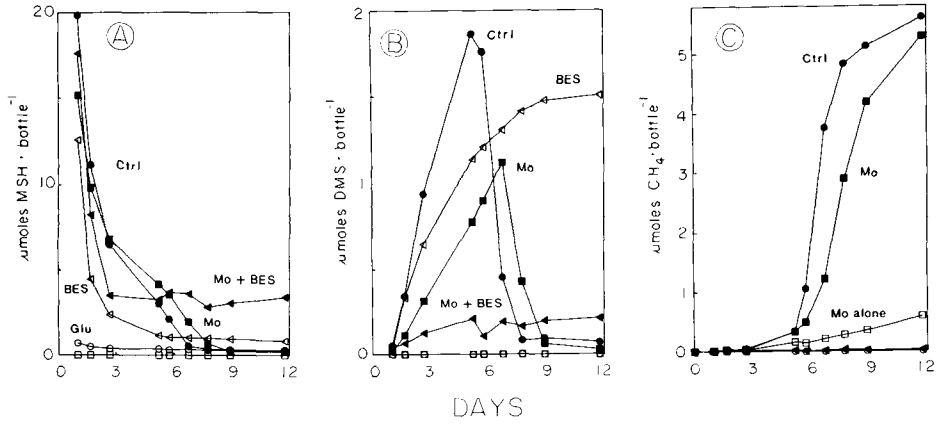

Fig. 7. Effects of several inhibitors on the transformations of MSH in anoxic sediments. Results are for MSH (**A**), DMS (**B**), and CH_4 (**C**). Symbols: (Ctrl ●), MSH; (Mo ■), MSH + MoO_4^{2-}; (△), MSH + BES; (▲), MSH + MoO_4^{2-} + BES; (Glu ○), MSH + glutaraldehyde; (□) MoO_4^{2-}. Values represent the mean of two replicates (C.V. < 15%). Flasks contained 25 ml sediment slurry and received between 12.5 and 20 μmol MSH. Glutaraldehyde flasks received only 1 μmol per flask due to an error in addition.

MSH flasks. Only the addition of BES and molybdate + BES resulted in the persistence of DMS throughout the time course of this experiment.

The stimulation of methane production in MSH-treated sediments was prevented by BES, chloramphenicol, and glutaraldehyde (Fig. 7C). Molybdate + MSH treatments gave slightly less CH_4 than MSH alone, but significantly greater methane production than molybdate alone.

When DMDS was added to sediments there was an abiological and a biological loss of the compound (Figs. 3D, 8A). DMDS was transformed primarily to MSH, but also to DMS. CH_4 production was stimulated by DMDS. Glutaraldehyde and chloramphenicol inhibited consumption of DMDS after abiological losses had occurred (Fig. 8A). The loss of DMDS was initially inhibited by molybdate, but by 9 days, levels had reached those of controls. BES had little effect on DMDS consumption.

No significant MSH was produced from DMDS in the presence of glutaraldehyde, and only small amounts were formed in chloramphenicol treatments (Fig. 8B). BES slightly inhibited MSH formation, whereas MoO_4^{2-} substantially inhibited MSH formation during the first 7 days of incubation, after which time MSH production was stimulated. MSH eventually decreased in control sediments and both BES and molybdate appeared to inhibit the loss of MSH.

DMS was not formed from DMDS in glutaraldehyde or chloramphenicol treatments (Fig. 8C). Molybdate greatly inhibited DMS formation in DMDS-treated sediments, with only a small production occurring after 14 days. BES samples produced DMS at a slower rate than controls but eventually exceeded controls, and levels did not decrease.

Figure 8D shows that after a long delay, methane production in molybdate-DMDS treatments was stimulated above endogenous levels, but to a lesser degree than DMDS alone. Glutaraldehyde, BES, and chloramphenicol prevented CH_4 stimulation by DMDS.

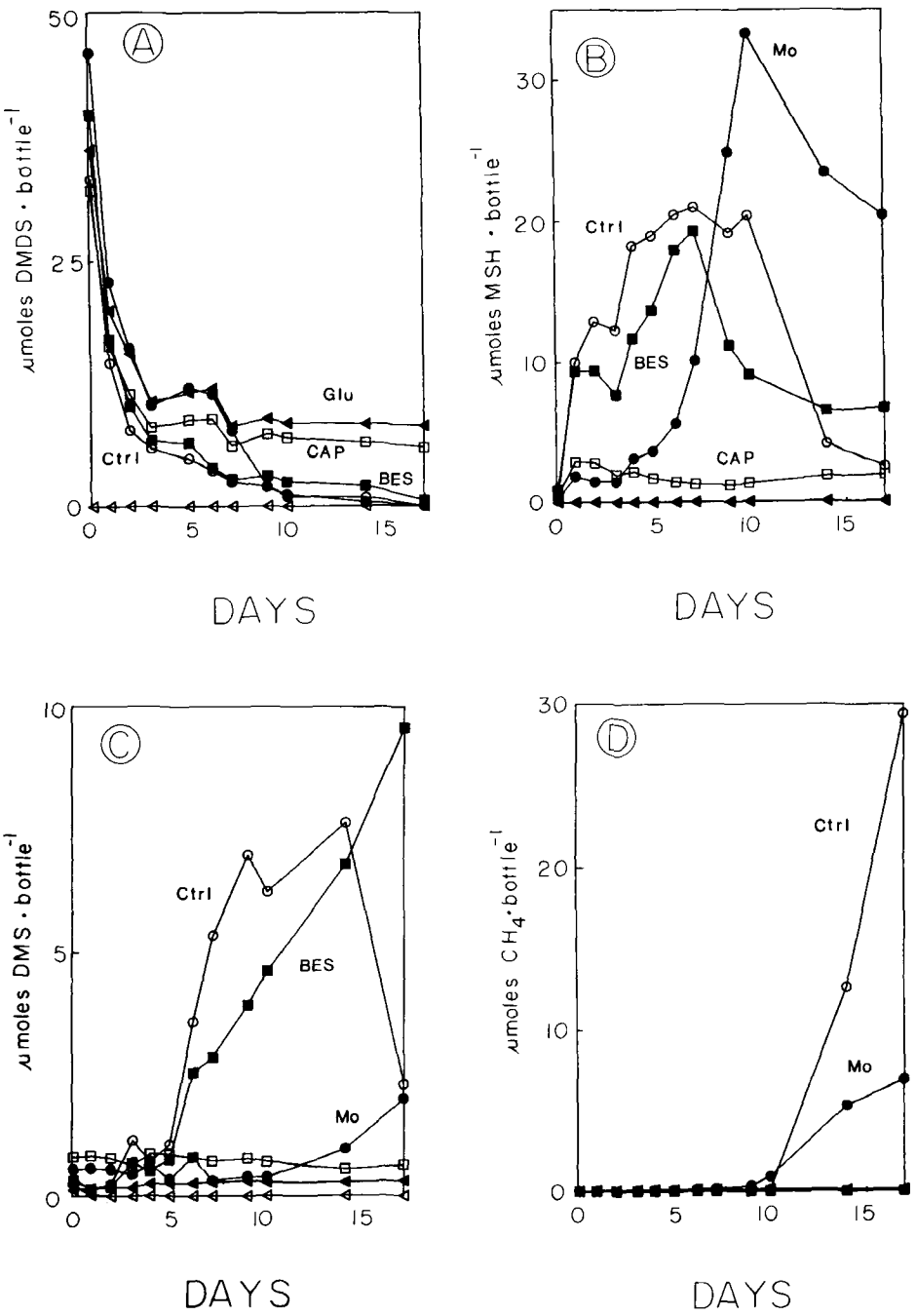

Fig. 8. Effects of several inhibitors on the transformations of DMDS in anoxic sediments. Results are for DMDS (**A**), MSH (**B**), DMS (**C**), and CH_4 (**D**). Symbols: (Ctrl ○), DMDS; (Mo ●), DMDS + MoO_4^{2-}; (BES ■), DMDS + BES; (Glu ▲), DMDS + glutaraldehyde; (CAP □), DMDS + chloramphenicol. Values represent the mean of two replicates (C.V. < 15%). Each flask (25 ml slurry) received between 35 and 45 μmol DMDS.

Discussion

Methylated sulfur compounds such as DMS, MSH, and DMDS have been observed in salt marsh environments as emitted gases [28] and as dissolved constituents in sediment pore waters [15]. The results of the present study show that volatile methylated reduced sulfur compounds may be produced in anoxic salt marsh sediments from precursor molecules such as the plant product dimethylsulfoniopropionate (DMSP), and from methylthio-amino acids such as methionine and S-methyl cysteine (Fig. 2). The resulting volatile methylated sulfur compounds may be consumed by the microbial populations present in anoxic sediments. In contrast to methylated precursors, cysteine did not give rise to volatile methylated sulfur compounds. This finding is consistent with other studies using cysteine [9, 35].

Since it was observed that several methylated sulfur compounds were formed from each precursor, and because very little is known about how compounds like DMS, MSH, DMDS, and DMSO are metabolized in anoxic sediments, a series of experiments was conducted in which each of these compounds was added individually. A detailed study of the production and fate of methylated sulfur compounds from precursor molecules such as methionine and DMSP will be presented elsewhere. The present paper concentrates on the transformations of individual methylated sulfur compounds.

Dimethylsulfoxide (DMSO) was found to be rapidly reduced to DMS in anoxic salt marsh sediments (Fig. 4A). This reduction was biological since it did not occur in the presence of glutaraldehyde, and was greatly inhibited by chloramphenicol. Although no abiological reduction of DMSO was noted in the present study, this phenomenon has been observed in highly sulfidic sediments and in anaerobic growth media containing 2 mM Na_2S and 2 mM cysteine (R. P. Kiene, unpublished results; see also reference 36).

Inhibition of sulfate reduction with 20 mM molybdate did not inhibit DMSO reduction in sediments (Fig. 4A), which implied that sulfate-reducing bacteria did not carry out this reaction. Methanogens did not appear to be involved in DMSO reduction either, since BES did not affect the time course of DMS production (Fig. 5). When 200 μM NO_3^- was included with DMSO in sediments, the production of DMS lagged behind the control for about 24 h, after which time DMS production was similar to the control. DMSO can serve as an electron acceptor for anaerobic respiration in several bacterial isolates [36, 37]. Among the known DMSO-reducing bacteria are *Proteus vulgaris* and *Escherichia coli* strain HB101, both of which are also capable of reducing nitrate [8, 35]. The inhibition of DMSO reduction may be due to the preferential utilization of NO_3^- over DMSO as an electron acceptor. Bilous and Wiener [8] found that DMSO reductase activity in *E. coli* was repressed by the presence of NO_3^-. The resumption of DMSO reduction in nitrate treatments may have been due to the depletion of nitrate which is readily reduced in these sediments [27].

DMSO has been detected in a wide variety of aquatic environments and may arise from the oxidation of DMS or by direct excretion by phytoplankton [2], or possibly from anthropogenic sources. The highest levels detected (0.2 μM) in the water column were found in areas of high primary production [2]. There are presently no data available on DMSO concentrations in sediments; how-

ever, the results of the present study show that anaerobic environments will be sinks for DMSO, since it readily reduced to DMS. Once formed, the DMS from DMSO undergoes metabolism and transformation to MSH and methane in anoxic sediments (see discussion below).

When DMS was added to sediment slurries, it disappeared due to biological consumption and stopper absorption (Fig. 6A). BES partially inhibited the biological loss of DMS which suggested that methanogens consumed DMS. Molybdate caused DMS to disappear more rapidly than in the control, indicating that sulfate-reducing bacteria did not consume significant amounts of DMS at the substrate concentration used in this experiment. Furthermore, it appears that the presence of molybdate allowed DMS-consuming methanogens to proliferate, thereby accelerating DMS consumption (Fig. 6A). These results are very different from those obtained when DMS concentrations are much lower than the 3 mM levels used here. For example, at DMS concentrations less than 10 μM, molybdate greatly inhibited DMS consumption whereas BES was only slightly inhibitory (R. P. Kiene, manuscript in preparation). These findings are consistent with the hypothesis that, in saline sediments, DMS metabolism is dominated by sulfate-reducing bacteria at low (μM) substrate concentrations, and by methanogenic bacteria at high (mM) substrate levels [8]. By contrast, Zinder and Brock [34] found that low (~2.2 μM) concentrations of ^{14}C-DMS were converted mostly to $^{14}CH_4$ and lesser amounts of $^{14}CO_2$ in freshwater lake sediments, even in the presence of 10 mM = added SO_4^{2-}. Since DMS concentrations are generally less than 1 μM in sediments [4, 15], there may be differences in the pathways of DMS degradation between freshwater and saline sediments. CH_4 may be the major mineralized product from DMS in freshwater sediments whereas CO_2 may be the major product in marine systems.

During metabolism of DMS in anoxic salt marsh sediments, MSH was evolved. MSH levels increased initially, then subsequently decreased. This suggests that MSH may be a transient intermediate in the degradation of DMS and that MSH may be metabolized by sediment microflora. MSH production from DMS was inhibited by either MoO_4^{2-} or BES indicating that both methanogens and sulfate reducers produce MSH during DMS metabolism.

Kiene et al. [18] have reported that a pure culture of an estuarine methanogen produces MSH during growth on DMS. Sulfate-reducing bacteria that can metabolize DMS have not yet been identified. Two species of sulfate-reducing bacteria, *Desulfovibrio salixigens* and *D. desulfuricans,* were unable to metabolize DMS [18]. Thus, it is not known whether sulfate reducers can produce MSH from DMS in pure culture. This aspect will await further work on cultures of available sulfate-reducing bacteria and on DMS enrichment cultures. Sulfate reducers such as the methylotrophic (methanol degrading) isolate reported by Nanninga and Gottschal [20] may be likely candidates for the metabolism of methylated sulfur compounds.

When MSH was added directly to sediment slurries, it disappeared rapidly (Fig. 7A). A portion of the observed disappearance was probably due to abiological absorption by the rubber stoppers and perhaps the sediments, but also due to biological consumption. However, due to errors in the addition of MSH, killed controls in this experiment were not adequate to distinguish between biological and abiological reactions. Free thiols may become bound to sediment

particles by forming disulfide associations with sediment-bound sulfhydryl groups or by binding to metals [19]. However, no significant sediment binding of MSH was observed with the relatively high levels of MSH used here (R. P. Kiene, unpublished data). Therefore, stopper absorption was most likely the major abiological loss mechanism for MSH.

The presence of molybdate resulted in the persistence of MSH as compared with control sediments (Fig. 7A), suggesting that sulfate-reducing bacteria are partially responsible for MSH consumption. BES also slightly inhibited the disappearance of MSH, indicating that methanogens might also consume MSH. A combination of both BES and MoO_4^{2-} resulted in the least MSH consumption, thereby substantiating the findings observed for the individual inhibitors. The metabolism of MSH by sulfate-reducing bacteria has not been previously reported. MSH has been observed to stimulate methanogenesis (Fig. 7C; see also reference 18). Furthermore, Zinder and Brock [37] demonstrated that ^{14}C-MSH could be converted to $^{14}CH_4$ and $^{14}CO_2$ in anaerobic lake sediments and sewage sludge. It is not clear at this time whether MSH serves as a direct precursor of CH_4 in sediments. It is possible that the DMS, which is formed from MSH, might be the methane precursor rather than MSH itself. This is suggested by the results of the molybdate-MSH treatment, in which DMS and CH_4 production were lower than in uninhibited sediments. A similar conclusion can be reached from the results using DMDS (Fig. 8; see below). The methanogenic isolate reported by Kiene et al. [18] produced CH_4 and grew on DMS but did not grow or produce CH_4 with MSH as the sole substrate. However, this isolate produced and subsequently consumed MSH during growth on DMS [18]. It is possible that in natural sediments, distinct species or strains of methanogens may be involved in either the production of MSH or the consumption of this compound.

Dimethylsulfide was produced in samples that received MSH (Figs. 3C, 7B). The formation of DMS was biological as it did not occur in killed samples or in those treated with chloramphenicol. DMS may arise from direct methylation of MSH. It is unclear at this time whether methanogens or sulfate-reducing bacteria are involved in the methylation of MSH. Simultaneous inhibition of both these groups substantially decreased production of DMS from MSH (Fig. 7B), whereas inhibition of either sulfate reduction or methanogenesis caused a much smaller inhibition of DMS production; these results suggest that both groups may be involved. Both sulfate reducers and methanogens have been implicated in methylation of metals [11, 24] and it is possible that similar reactions are responsible for MSH methylation.

MSH may also be derived from DMDS (Fig. 3D). After abiological losses of DMDS slowed, the additional decreases could be attributed to biological reduction of DMDS to MSH (Fig. 8A). This is indicated by the fact that no MSH was formed from DMDS in the presence of glutaraldehyde. Sulfate-reducing bacteria may have been involved in the reduction of DMDS since molybdate caused an initial inhibition of DMDS consumption as well as a lag in the production of MSH (Fig. 8A). Based on results with BES, it appeared that methanogens did not contribute significantly to DMDS reduction. Chloramphenicol-treated samples initially produced small amounts of MSH, but

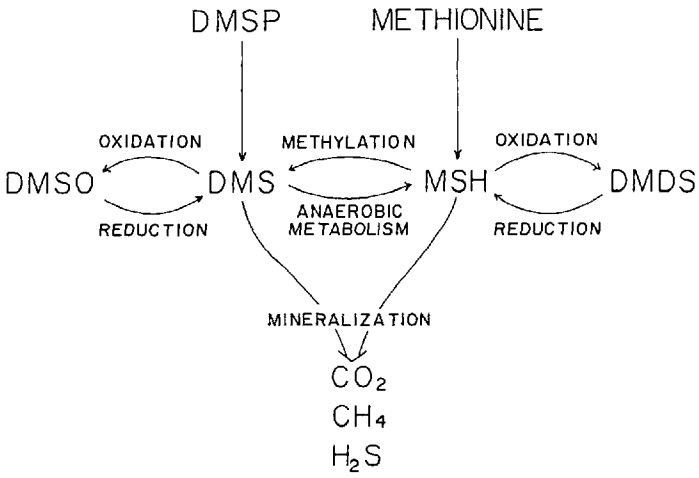

Fig. 9. A simplified scheme of how methylated sulfur compounds may be transformed in the environment.

both DMDS and MSH levels remained relatively constant after the third day of the experiment (Fig. 8A, B). These results suggest that chloramphenicol was not initially effective at blocking DMDS reduction, but that with time, the effectiveness of the inhibitor increased.

DMDS was not detected in anaerobic sediment incubations with any of the other methylated sulfur compounds studied here; this was probably due to the rapid reduction of DMDS in anoxic sediments. Most of the DMDS which is released from salt marshes [5, 28] and from decaying blue-green algal mats [38] probably arises from the oxidation of MSH that is formed under anaerobic conditions.

DMDS additions to sediments stimulated methanogenesis above endogenous levels (Fig. 8D; see also reference 16). However, molybdate appeared to inhibit CH_4 production when compared with sediments containing DMDS alone; this is unusual in that MoO_4^{2-} generally stimulates methane production [21]. Because of the transformations of methylated sulfur compounds which are described above, it is not clear which methylated sulfur compound(s) serve(s) as the substrate(s) for methanogenic bacteria in sediments. The lower amount of methane produced from DMDS in the presence of MoO_4^{2-} may be due to the fact that DMS and MSH were lower in molybdate treatments. The results from methane thiol-molybdate experiments (Fig. 7) suggest that DMS must be formed in order to stimulate methanogenesis.

Figure 9 represents a simple model of how methylated sulfur compounds may be cycled in nature. The methylated sulfur compounds observed in the environment are ultimately derived from relatively complex precursor molecules such as DMSP and methionine, but may also arise from the transformations (both aerobic and anaerobic) of other volatile organic sulfur compounds. The present study illustrates the transformations of several methylated sulfur compounds in anoxic salt marsh sediments. Based on results with selective microbial inhibitors, sulfate-reducing and methane-producing bacteria appear to be involved in the observed transformations, and may be particularly

important in the consumption and mineralization of methylated sulfur compounds. This is contribution number 569 from the Marine Sciences Research Center.

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