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Evidence for the biological turnover of thiols in anoxic marine sediments

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Abstract. The concentrations of methanethiol (MSH) and 3-mercaptopropionate (3-MPA) increased for a period of up to 24 h in fresh slurries of anoxic Biscayne Bay sediments. Other endogenous thiols such as glutathione (GSH) deceased immediately after slurry preparation or were not detectable at all. The maximum concentrations reached by 3-MPA and MSH were sometimes as high as 1 μM, but were usually in the 100 to 300 nM range. After the initial increases, the concentrations of these thiols decreased rapidly to nearly constant levels of ~20 nM for MSH and < 1nM for 3-MPA. In pre-incubated slurries, which had constant levels of thiols, the addition of microbial inhibitors including tungstate, molybdate, chloroform, and a mixture of chloramphenicol plus tetracycline caused MSH and 3-MPA to accumulate steadily. In the presence of inhibitors, accumulation rates of MSH ranged from 18 to 730 $nM \cdot d^{-1}$ and those of 3-MPA ranged from 0 to 185 $nM \cdot d^{-1}$. Tungstate and chloroform generally gave the highest accumulation rates, while molybdate gave the lowest, possibly due to its complexation with sulfhydryl compounds. BES (2-Bromoethanesulfonate) was also tested for its effects, but no 3-MPA and only trace amounts (19 $nM \cdot d^{-1}$) of MSH accumulated with this treatment. However, additions of BES (10 mM) to sulfidic sediments caused significant (~8 $\mu M \cdot d^{-1}$) production of 2-mercaptoethanesulfonate (HS-CoM). Formation of HS-CoM was abiotic and was due to sulfide attack on the bromine atom in BES. The accumulations of 3-MPA and MSH in the presence of several different microbial inhibitors, suggests that these thiols may turn over in anoxic sediments. The relatively low concentrations of thiols observed in pore water profiles may be due to continuous microbial removal of these compounds. Much larger amounts of thiols were associated with sediment particles than present in the pore water. Evidence is presented which suggests that bound thiols may be exchangeable with the porewater, and therefore potentially available for microbial consumption.

Introduction

Thiols have recently been detected in the porewaters of coastal marine sediments (Mopper & Delmas 1984; Mopper & Taylor 1986; Luther et

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al. 1986; Shea & MacCrehan 1988a, b; Sorensen 1988). The principal thiols which have been identified thus far are glutathione (GSH), cysteine, 3-mercaptopropionate (3-MPA) and methanethiol (MSH) (Mopper & Taylor 1986; Shea & MacCrehan 1988; Kiene & Taylor 1988b). These thiols may originate from organic sulfur precursors or from the incorporation of sedimentary sulfide into organic molecules. Kiene & Taylor (1988a, b) and Kiene et al. (1990) demonstrated that 3-MPA and MSH were produced during the anaerobic degradation of dimethylsulfoniopropionate (DMSP), methionine and homocysteine. These authors also reported that cysteine was produced from the degradation of glutathione, and that cysteine was further degraded to mercaptopyruvate, mercaptoethanol and mercaptoacetate (Kiene et al. 1990). Cysteine is also likely to be liberated during protein degradation, but this has not yet been demonstrated in sediments. A recent study by Finster et al. (1990) found that methoxy aromatic compounds may be precursors of MSH through biochemical reactions with sulfide.

The cycling of thiols in natural environments is important because they are biochemically and geochemically reactive (Mopper & Taylor 1986; Luther et al. 1986; Vairavamurthy & Mopper 1987; Kiene & Taylor 1988b). Thiols such as cysteine may be important complexing agents for certain metals in sediments (Shea & MacCrehan 1988b). Furthermore, Vairavamurthy & Mopper (1987; 1989) have recently shown that sedimentary sulfides may react with α, β-unsaturated compounds to form thiols and organic polythionates. These authors concluded that this mechanism may be an important pathway for the incorporation of sulfur into organic matter. Sulfur incorporation into organic matter during early diagenesis is significant (Francois 1988; Kohnen et al. 1987) and is very important with respect to preservation of organic sulfur in fossil fuels (Balzer 1981).

Most studies of thiols in sediments have dealt with their production from organosulfur precursors (Kiene & Taylor 1988a, b; Kiene et al. 1990; Zinder & Brock 1978) or their distributions in sediment pore waters (Sorensen 1988; Shea & MacCrehan 1988; Luther et al. 1986). Few studies exist, which have considered biological cycling of thiols. Here, I present data which suggests that two comon thiols, 3-MPA and MSH, may have significant biological turn over rates in anoxic sediments. Inhibitor experiments suggest that their metabolism is linked to sulfate reduction, but not methanogenesis. Microbial removal of dissolved thiols is undoubtedly responsible for the low (nM to low μM) concentrations of these compounds observed in porewaters. However, reaction with sediment surfaces is also a potentially significant removal mechanism for

thiols. A substantial pool of particle-associated thiols is present in sediments from Biscayne Bay, and this pool may be exchangeable with the porewater and therefore potentially available for diffusion and for metabolism by sediment microbes.

Materials and methods

Sample collection and slurry preparation

Sediment samples were collected from three locations in Biscayne Bay, Florida. The Bear Cut site is an intertidal mud flat located adjacent to a mixed bed of *Syringodium filiforme* and *Thalassia testudinum*. A profile of pore water thiols from this site was presented in Kiene & Taylor (1988b). A second site consisted of more sandy sediments and was located in the center of a shallow *Syringodium* bed. A third site was a muddy sediment located under a canopy of red mangrove (*Rhizophora mangle*).

Sediments were collected in aluminum tubes and returned to the laboratory where they were mixed with an equal volume of deoxygenated overlying water. The resulting slurry was screened through a 1.5 mm mesh and dispensed in 50 ml amounts to serum bottles. The bottles were sealed with butyl rubber stoppers (Bellco, Vineland, N.J.) under an atmosphere of N_2 and were maintained in the dark at 23—25 °C on a shaker table (125 rpm).

A core for profiles of pore water and bound thiols was taken from Bear Cut in a 7 cm (I.D.) polybutyrate core-tube which had pre-drilled holes in the side which allowed discrete depths to be sampled. Pore water was obtained by centrifugation in Centrex filter units (0.45 μm pore size, teflon membrane). All manipulations of the core, including sub-sampling, centrifugation and subsequent derivatization of samples was done in an anoxic chamber (Coy Laboratories).

Analytical determinations

Thiols were measured by the HPLC method of Mopper and Delmas, (1984) as described in Kiene & Taylor (1988a). Briefly, sub-samples (1 ml) of slurry were periodically withdrawn with a syringe and immediately centrifuged for 1 min at 13000 X g in a micro-centrifuge tube. The clear supernatant (0.5 ml) was derivatized with 10 μl each of *o*-phthalaldehyde (20 mg·ml^{-1} methanol) and aminoethanol (20 μl·ml^{-1} sodium borate buffer, pH 9.2). The reagents were mixed with the sample

and the reaction was allowed to proceed for 1 min before injection into the HPLC. Nanomolar levels of thiols are extremely sensitive to air oxidation, therefore samples were processed quickly.

Separation of the fluorescent isoindole derivatives was achieved on a Waters Radial-Pak column (10 cm × 0.5 cm, 5 μm particles C_{18}) using a binary gradient composed of 0.05M sodium acetate (pH 5.7) and 100% methanol. The detector was an Hitachi model F1000 fluorometer set at an excitation wave-length of 340 nm and an emission wavelength of 450 nm. Peak areas were recorded on a Shimadzu CR-3B integrator. The detection limits (20 μl injection) for 3-MPA and MSH were 0.7 nM and 1 nM respectively. Because HPLC runs were ~35 min in length, and because samples could not be preserved, only single sample bottles were used for each treatment (see below).

To measure particle-bound thiols, 0.5 ml of slurry or whole sediment was treated with 0.5 ml of 1% tributylphosphine (TBP) in 2-propanol. TBP, a strong reducing agent, is known to be a specific disulfide cleaving agent (Ruegg & Rudinger 1977) and was previously shown to release sediment-associated thiols into the porewater (Mopper & Taylor 1986). TBP-treated samples were shaken for 1 h, after which they were centrifuged and analyzed for thiols as above.

Experimental

In experiments with microbial inhibitors, slurries were pre-incubated for at least 24 h to allow endogenous thiols to decrease to constant levels (see results). Sodium molybdate (20 mM) and sodium tungstate (20 mM) were used to inhibit sulfate reducing activity in the sediments (Oremland & Taylor 1978; Banat et al. 1984). Sodium 2-bromoethanesulfonate (BES, 10 mM final concentration) was used as a specific inhibitor of methanogenesis (Gunsalus et al. 1972; Oremland & Capone 1988). Chloroform was used as an inhibitor of methyl group metabolism (Bauchop 1967), and was added as a pure liquid to a final concentration of 5 $\mu l \cdot 50\ ml^{-1}$ of slurry. Chloramphenicol and tetracycline (CAP/TET) are broad spectrum antibiotics and were used as general inhibitors of microbial activity. They were added at final concentrations of 125 and 50 $\mu g \cdot ml^{-1}$ of slurry, respectively. Previous studies showed that these concentrations of antibiotics were effective at inhibiting some biotransformations of thiols and their precursors (see Kiene & Taylor, 1988 a, b; and Kiene et al. 1990). However, these same studies showed that some pathways for thiol formation are relatively insensitive to antibiotics. In all experiments, control samples receiving no additions were run. No significant changes in thiol

concentrations were observed in any controls during the experimental incubations.

An experiment was carried out to examine the adsorption of externally-added thiols onto sediment particles. Two identical slurries were preincubated for several days to deplete endogenous thiols. One was then treated with antibiotics (CAP/TET) and the other placed on ice (0 °C) in order to reduce biological activity and minimize biotransformations. Twelve hours after these treatments each of the slurries was amended with a mixture of thiols (1 μM each) including 3-MPA, MSH, ethanethiol (ESH), homocysteine (HCYS), mercaptoethanol (ME) and glutathione (GSH) and the initial concentrations determined. Pore water concentrations of thiols were then measured over a 24 h period after which they approached steady levels. At this time each slurry was diluted 1:1 with a 2mM solution of KHS (pH 7.5) (Morton Thiokol). After dilution, the concentrations of thiols in the pore water were again measured over a period of approximately 6 h. After this time, sub-samples of each slurry were treated with TBP to test whether thiols lost from solution could be recovered from particles.

Results

Almost immediately after the preparation of anoxic sediment slurries from the Bear Cut site, the levels of 3-MPA and MSH began to increase (Fig. 1). Both 3-MPA and MSH reached similar maximal levels of 300 nM, before decreasing to low levels. In prolonged incubations (>24 h) thiol levels always remained constant at 10—20 nM for MSH and $<$ 1nM for 3-MPA. In the experiment presented in Fig. 1, MSH increased faster and disappeared faster than 3-MPA. However, time courses for thiols and the relative amounts of 3-MPA and MSH which accumulated differed in each experiment. In some experiments thiol levels reached as high as 1 μM before declining (data not shown), although 100 to 300 nM levels were more typical. Glutathione was occasionally observed just after slurry preparation, but it quickly decreased to undetectable levels.

3-MPA and MSH accumulated in sediments treated with microbial inhibitors. When tungstate, chloroform or CAP/TET were added to slurries which had reached constant low levels of 3-MPA and MSH, the concentrations of these thiols increased at steady rates (Fig. 2). The accumulation rates of thiols in several experiments and from several sites in Biscayne Bay are summarized in table 1. Molybdate caused thiols to accumulate, but increases were often not linear and were consistently

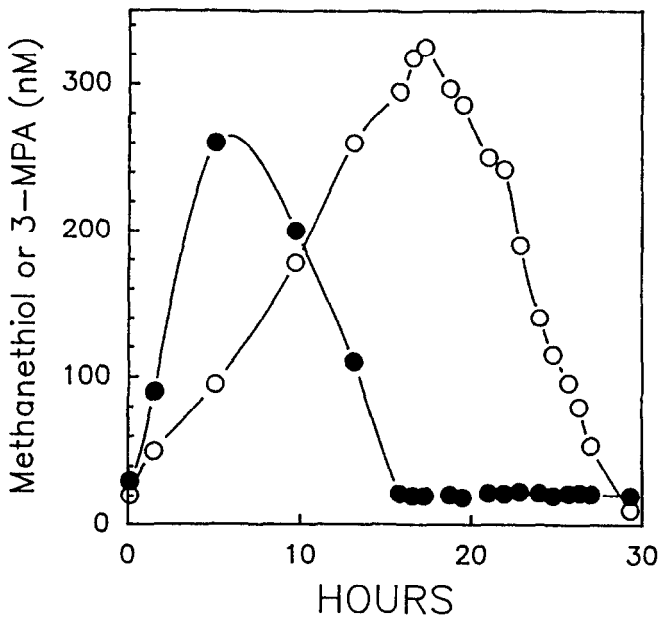

Fig. 1. Time course of endogenous levels of 3-mercaptopropionate (○) and methanethiol (●) in anoxic sediment slurries. Sediments were from Bear Cut in Biscayne Bay, near Miami, Florida. Time zero represents the time that the slurries were prepared. Data are from a single sample bottle, and are representative of many similar observations

lower than with other inhibitors. This may have been due to the complexation of the thiols by molybdate, which forms molybdo-sulfide complexes (Harmner and Sykes, 1980). No clear trends were seen in thiol accumulation rates between the different sediments used in this study (Table 1). For all experiments (Table 1), chloroform gave the highest average accumulations for 3-MPA, followed by tungstate, CAP/TET and molybdate. In contrast, tungstate gave the highest average accumulation rate for MSH followed closely by chloroform and then by CAP/TET and molybdate.

Addition of BES to anoxic Bear Cut sediment slurries resulted in a small accumulation of MSH (19 $nM \cdot d^{-1}$), but 3-MPA was not observed (Table 1). A large unknown thiol peak was observed in BES-treated sediments, and this peak increased steadily during the incubation (data not shown). The unknown thiol coeluted with 2-mercaptoethanesulfonate (HS-CoM). Using the response factor for HS-CoM, the accumulation observed in the sediments was equivalent to 8 $\mu M \cdot d^{-1}$. Subsequent

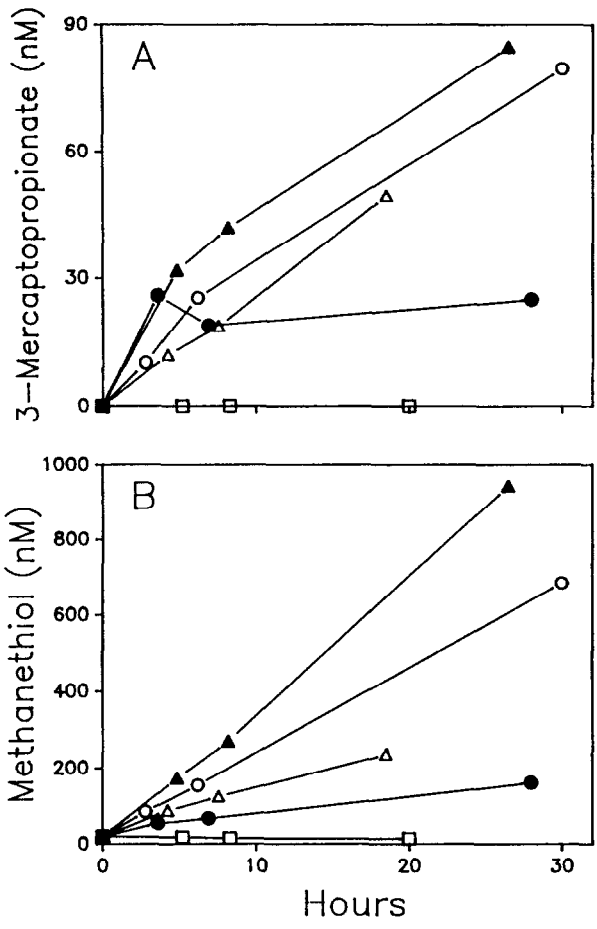

Fig. 2. The accumulation of 3-mercaptopropionate (panel A) and methanethiol (panel B) in anoxic sediments slurries after the addition of several microbial inhibitors. Treatments: 20 mM tungstate (▲); 20 mM molybdate (●); chloroform (○); chloramphenicol plus tetracycline (△); untreated (□). Sediments were from Bear Cut in Biscayne Bay, near Miami, Florida. Slurries were pre-incubated for 24 h prior to the addition of inhibitors (time zero). Data are from individual bottles for each treatment

investigation with pH 7.1-buffered solutions of Na_2S (2 mM) and BES (1 or 10 mM) revealed that HS-CoM was formed at significant rates from these two reactants (Fig. 3). The rate of HS-CoM formation was 9.6 times higher in the 10 mM BES solution than in the 1 mM solution.

Thiols which accumulate in the presence of inhibitors or during slurry preparation could be derived from the degradation of organosulfur

Table 1. Accumulation rates* of 3-mercaptopropionate (3-MPA) and methanethiol in slurries of anoxic Biscayne Bay sediments after the addition of selected microbial inhibitors.

Inhibitor	Sediment Source	Thiol Accumulation Rate ($nM \cdot d^{-1}$)			
		3-MPA	Inhibitor Mean ± Std. Dev.	MSH	Inhibitor Mean ± Std. Dev.
20 mM Tungstate	Bear Cut	41		290	
		120	88 ± 43	730	393 ± 19
	Syringodium Bed	49		330	
	Mangrove	140		220	
20 mM Molybdate	Bear Cut	0		86	
		120		240	
		0	34 ± 47	31	85 ± 80
	Syringodium Bed	2		44	
	Mangrove	50		26	
Chloroform	Bear Cut	190		310	
		98	151 ± 38	600	343 ± 15
	Syringodium Bed	132		250	
	Mangrove	184		210	
CAP/TET	Bear Cut	34		18	
		54		160	
		27		79	
		40	46 ± 16	87	139 ± 11
		60		400	
	Syringodium Bed	33		120	
	Mangrove	73		110	
10 mM BES	Bear Cut	0	—	19	—

* Accumulation rates calculated from linear regression analysis of thiol concentrations vs time.

compounds (e.g. Kiene & Taylor 1988; Kiene et al. 1990), or they could be exchanged into the pore water from particle-bound pools. A profile of dissolved thiols in Bear Cut sediments (Fig. 4A) showed that concentrations of 3-MPA and MSH were low (< 300 nM) throughout the upper 20 cm. GSH on the other hand was present at higher concentrations (up to 600 nM), but was restricted to the upper 5 cm. The pore water concentrations of MSH and 3-MPA increased considerably when sediments were treated with TBP (Fig. 4B), suggesting large particle-associated pools. After TBP treatment, methane thiol concentrations increased 33-206 fold, whereas 3-MPA increased by factors of 2-105 depending on the

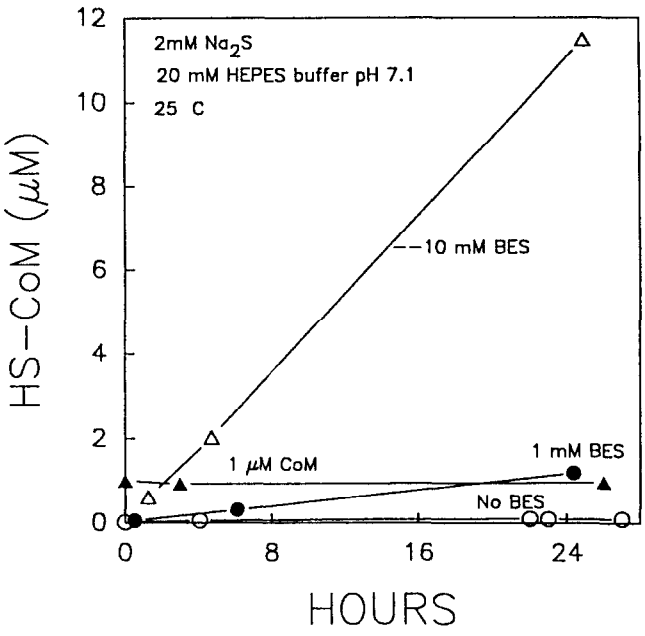

Fig. 3. Production of 2-mercaptoethanesulfonate (HS-CoM) in buffered (pH 7.1) solutions of Na_2S (2 mM) containing 10 mM 2-bromoethanesulfonate (BES) (△), 1 mM BES (●). A control (△) containing 1 μM HS-CoM in Na_2S indicated no abiotic production or loss of HS-CoM. Reaction was carried out at 25 °C in 70 ml serum bottles

depth. Glutathione levels were not substantially increased by TBP in the surface 5 cm, but it increased from undetectable levels to about 500 nM below this depth. The identity of GSH in depths greater than 5 cm should be considered tentative, because interfering peaks near the retention time of GSH were present in these samples.

When a mixture of thiols was added to either chilled or antibiotic-treated sediment slurries, dissolved thiol concentrations decreased rapidly. (Fig. 5). The time courses of thiols were very similar in the two treatments so only the results from the CAP/TET slurry are shown. All thiols decreased over time, but the rate and extent of disappearance differed for each individual thiol (Fig. 5). Glutathione decreased the most rapidly and 3-MPA the least rapidly. After 24 h, these same slurries were diluted 1:1 with 2 mM KHS, which resulted in ~35% lower pore water thiol concentrations (less than 50% because the slurry contained about 30% sediment by volume). After dilution, the concentrations of all thiols, except GSH, increased over a 6 h period. Methane thiol and 3-MPA

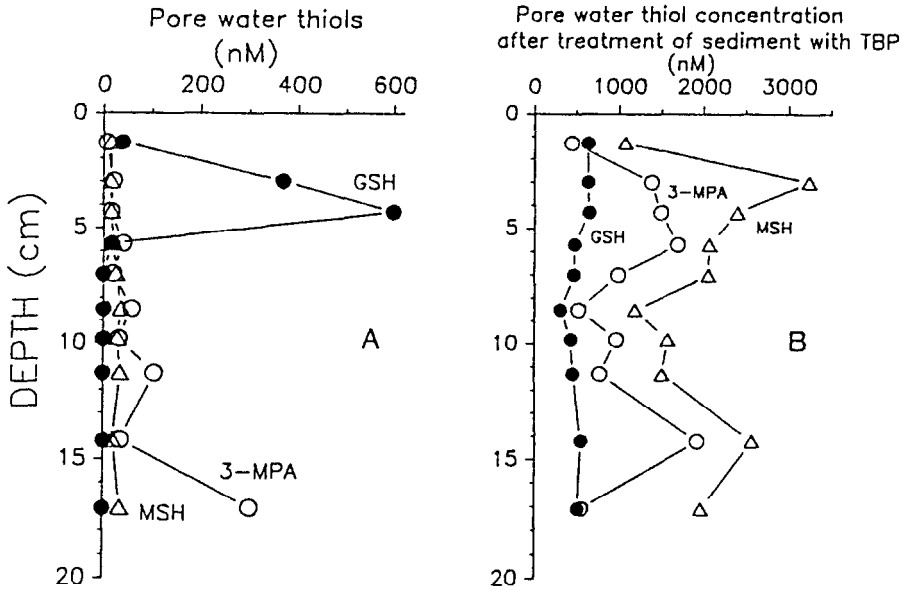

Fig. 4. Concentrations of dissolved pore water thiols (A) and pore water thiols after treatment of sediment with 1% tributylphosphine (B) in a core from Bear Cut, Biscayne Bay, Florida. Symbols: (●), glutathione; (○) 3-mercaptopropionate; (△) methanethiol

increased fastest, whereas mercaptoethanol (ME) increased only slightly. Again, similar results were found with chilled (0°) sediments (not shown). At the end of the experiment, sediments were treated with TBP to test for recovery of the added thiols which had disappeared from solution. For the most part, > 80% of all thiols, except GSH, could be recovered (Table 2). Mercaptoethanol gave only 23% recovery in the CAP/TET treatment, but 137% recovery in the chilled sediment. Recovery values of over 100% indicated that significant endogenous pools of these thiols were present in the sediments.

Discussion

Some thiols, particularly, 3-MPA and MSH are released into the pore water when sediments are slurried (Fig. 1). These findings are similar to those of Kiene (1988) who found that DMS and MSH accumulated in salt marsh sediments after slurry preparation. Initial pulses of sulfur com-

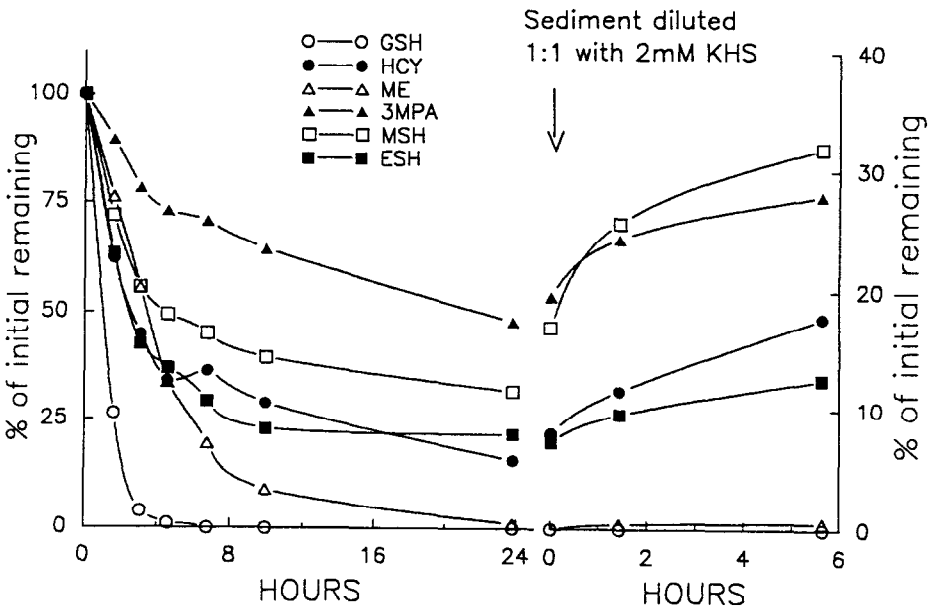

Fig. 5. Time course of dissolved thiol concentrations in a sediment slurry pre-incubated for 12 h with antibiotics (CAP/TET). A mixture of 6 thiols was added at time zero. After 24 h, the slurry was diluted 1:1 with 2 mM KHS, pH 7.5. Similar results were obtained with sediments incubated at 0 °C, but without antibiotics. Abreviations used in the figure: (GLU), glutathione; (HCY), homocysteine; (ME), mercaptoethanol; (3MPA), 3-mercaptopropionate; (MSH), methanethiol; (ESH), ethanethiol

pounds may be due to the release of precursor molecules such as DMSP and amino acids after sediment disturbances (Howes et al. 1985), or possibly due to the release of thiols from particle surfaces (Fig. 5; see also Mopper & Taylor 1986).

Inhibitor experiments and thiol turnover

Several previous studies have shown that the disappearance of 3-MPA and MSH in sediment slurries is due, in part, to microbial activities (Kiene & Taylor 1988a, b; Kiene & Capone 1988). The low steady-state concentrations of MSH (~20 nM) and 3-MPA (<1 nM) observed in sediment slurries after 30 h incubation indicate that microbes are capable of metabolizing MSH and 3-MPA down to very low concentrations. The results presented here suggest that microbial processes are constantly removing thiols (3-MPA and MSH) from the pore water, and that when

Table 2. Percent recovery* of added thiols in sediment sluries after initial adsorption, followed by treatment, with 1% tributylphosphine

Sediment Incubation Condition	1% Recovery of Individual Thiols					
	Glutathione	Homocysteine	2-mercaptoethanol	3-mercaptopropionate	Methanethiol	Ethaneds
0 °C	10	103	137	101	236	166
CAP/TET @ 25 °C	7	85	23	93	360	179

* Calculated as the peak area in simples treated with tributylphine divided by the initial paek area obtained immedistedly after thiol addition (×100). Recovery greated than 100% indicate the presence of endogenous adsorbed thiols. Sediments were preincubated so that dissolved thiols were at before addition of thiol mixture. CAP/TET was added 12 h prior to thiol addition and sediment was chilled 12 h per thiol addition.

microbial processes are inhibited, these thiols accumulate at steady rates (Fig. 2). The accumulation of thiols in the presence of inhibitors may be due to continued production by pathways which are insensitive to the inhibitors, or they may be released from sediment particles. The fact that a variety of different inhibitors, including several with different modes of action, yielded similar results (Table 1), strongly suggests that the accumulations observed were not artifactual i.e. caused by reaction with the inhibitor and the sediment material. Previous studies by others have shown that microbial substrates such as fatty acids (Sorensen et al. 1981; Christensen 1984), amino acids (Parkes et al. 1989) and methylamines (Oremland et al. 1982) accumulate in anoxic sediments when molybdate or BES are added. Inhibitor-addition experiments will give valid estimates of substrate removal rates only if the inhibitor specifically blocks terminal metabolism of the substrate and not its production. While thiol accumulation rates in the presence of inhibitors may provide "order of magnitude" estimates of their turnover, they may not be representative of the true turnover rates. This is because the effects of inhibitors on the formation of thiols from the entire precursor pools are not well understood (see discussion below). Additionally, the fact that thiols may adsorb or bind significantly to sediment particles gives a greater degree of complexity to the determination of *in situ* turnover rates. Further work will be needed to develop techniques which will allow thiol turnover rates to be measured.

The results with molybdate and tungstate suggest that removal of MSH and 3-MPA is linked with sulfate reduction. Molybdate is a potent inhibitor of sulfate reduction (Banat et al. 1984), but it greatly interferes with the thiol analysis, therefore it yielded accumulations which were lower than with tungstate (Table 1). Tungstate (20mM) was probably effective at blocking most of the sulfate reduction (Banat et al. 1984) and it did not substantially interfere with the thiol analysis. Therefore tungstate caused a higher accumulation of both 3-MPA and MSH. A recent study by Heijthuijsen & Hansen (1989) reported that sulfate reducing bacteria from marine sediments could utilize C_1 groups from methylated compounds for growth. Similar organisms could be responsible for metabolizing MSH in sediments.

Chloroform was expected to block the metabolism of MSH because it is a known inhibitor of methyl metabolism (Bauchop 1967). Consistent with this hypothesis, chloroform always gave relatively high accumulations of MSH (Fig. 2; Table 1). Zinder & Brock (1978) also found that MSH accumulated in freshwater lake sediments after the addition of $CHCl_3$. In addition, Zinder and Brock (1978) reported accumulation of n-propane thiol in $CHCl_3$-treated sediment. Propanethiol was not observed in the present study. The accumulation of 3-MPA in the presence of $CHCl_3$ was

not expected but may have been due to interferences in methyl transfer reactions involving methionine, DMSP and homocysteine, which are known precursors of 3-MPA (Kiene & Taylor 1988).

The accumulation rates of thiols in CAP/TET treatments were generally lower than with either tungstate or chloroform (Table 1). Several reasons could be cited to explain this observation, including incomplete inhibition of thiol removal, or partial inhibition of thiol production by the antibiotics. Incomplete inhibition of thiol removal cannot be ruled out because in previous studies, which clearly showed that CAP/TET inhibited metabolism of 3-MPA and MSH (Kiene & Taylor 1988b; Kiene & Capone 1988), it was not possible to determine if the inhibition was partial or complete. Further, the partial inhibition of thiol production by CAP/TET is also likely since these antibiotics blocked the conversion of DMSP to 3-MPA (Kiene & Taylor 1988a), but did not substantially affect conversion of homocysteine and acrylic acid to 3-MPA (Kiene et al. 1990). Similar differential effects of CAP/TET on MSH production from different precursors have been observed (Kiene et al. 1990). Thus, CAP/TET may prevent production of thiols from certain pathways, but not others. Either of these possibilities, or a combination of the two, would result in accumulation rates which are less than the natural turnover rates of these thiols. Compared to CAP/TET, the other inhibitors used (tungstate and chloroform) apparently were better at inhibiting the metabolism of 3-MPA and MSH, or they had less of an effect on the production of these thiols, and therefore yielded greater accumulation rates.

Conversion of BES to HS-CoM

Although BES (2-bromoethanesulfonate) had no effects on the levels of 3-MPA, it caused a slight accumulation of MSH (Table 1), which was much lower than MSH accumulations observed with other inhibitors. This is consistent with the idea that, in SO_4^{2-}-containing sediments, methanogens consume only a small fraction of the total methylated sulfur compounds when these substrates are present at low concentrations (Kiene 1988; Kiene & Visscher 1987).

The observation that 2-mercaptoethanesulfonate (Co-enzyme M) accumulated at significant rates ($\sim 8\ \mu M \cdot d^{-1}$; data note shown) in BES treated sediments is of interest. HS-CoM is unique to methanogenic bacteria and is a critical cofactor involved in the generation of methane (Taylor & Wolf 1974). However, it is not likely that HS-CoM accumulated because its natural turnover was inhibited by BES. More likely, it was formed as a

result of the nucleophilic substitution of the bromine atom of BES by sulfide to form the thiol 2-mercaptoethanesulfonate (HS-CoM).

$$BrCH_2CH_2SO_3^- + HS^- \rightarrow HS - CH_2CH_2SO_3^- + Br^-$$

This reaction was demonstrated in a sediment-free system (Fig. 3). At pH 7.1 and with 2 mM $Na_2S \cdot 9\ H_20$ the rate of reaction was similar to that observed in sediments; 11 $\mu M \cdot d^{-1}$ vs 8 $\mu M \cdot d^{-1}$. Furthermore, the reaction appeared to be first order with respect to BES concentration; the rate at 10 mM BES was 9.6 times the rate at 1 mM BES. BES is widely used as an inhibitor of methanogenesis (see Oremland & Capone 1988). Researchers need to consider that, in the presence of sulfide, BES may actually be converted to the molecule it is designed to mimic. The rate of conversion of BES to HS-CoM in the present study ($0.08\% \cdot d^{-1}$) was relatively slow and the inhibitory effects of BES are not likely to have been compromised. However, nucleophilic substitution reactions are greatly affected by reaction conditions (Vairavamurthy & Mopper 1989; Barbash & Reinhard 1989) and therefore further study of BES conversion to HS-CoM is warranted.

Another important point about the conversion of BES to HS-CoM is that it illustrates that nucleophilic substitution reactions of naturally occurring halogenated compounds may be a mechanism of thiol formation in sulfidic sediments. Similar substitution reactions have been demonstrated for halogenated pollutants in anoxic groundwaters (Schwarzenbach et al. 1985) and substitution reactions involving sulfur nucleophiles have been discussed in detail in Barbash & Reinhard (1989). If the thiols thus formed are more easily degraded than their halogenated precursors, then this type of reaction may facilitate removal of otherwise recalcitrant molecules.

Importance of particle-associated thiols

The profile of pore water thiols presented here (Fig. 4A) is similar to that reported earlier for the same site (Kiene & Taylor 1988). Dissolved thiol concentrations are lower in Biscayne Bay sediments, compared to those reported in Chesapeake Bay sediments (Shea & MacCrehan 1988a) and in a Danish coastal sediment (Sorensen 1988). Sorensen (1988) reported that MSH was present only in the deeper layers of sediments at concentrations up to 1 μM. Comparison among these studies is difficult because of the considerably different environments studied and the different methods used.

The concentrations of thiols dissolved in the pore water may be strongly influenced by the tendency of the sediment to adsorb or bind thiols. It is clear from the present study that, in addition to the dissolved pool of thiols in pore waters, there is also a considerable pool of particle-associated thiols in these carbonate sediments. This has been previously noted by Mopper & Taylor (1986) and also by Guerin & Braman (1985). Several hundred-fold increases in pore water 3-MPA and MSH were observed when whole sediments were treated with TBP (Fig. 4B). No such increases were seen when pore water alone was treated with TBP (RPK unpublished observation; see also Mopper and Taylor, 1986), indicating that these thiols are associated with particles. TBP is known to cleave disulfide bonds (Ruegg & Rudinger 1977), but the exact nature of the thiol adsorption/binding to sediments is not yet known. However, based on the experimental dilution of sediments with 2 mM KHS, it appears that most bound thiols are exchangeable with the pore water or are displaceable by HS^-. In this case HS^- may act as a nucleophile, attacking disulfide bonds of sediment bound thiols and thereby releasing free thiols. This observation, is significant, because it indicates that sediment-associated thiols may be available (under certain conditions) for reaction and metabolism in the pore water. Unfortunately, little is known about the bioavailability of sediment-associated thiols.

Each individual thiol appears to react with the sediment in a different maner (Fig. 5). For example, GSH disappeared very rapidly from solution and it could not be exchanged by addition of KHS, nor could it be significantly recovered by TBP treatment (Fig. 5; Table 2). On the other hand, 3-MPA and MSH disappeared more slowly, and their concentrations increased after dilution of sediment slurry with KHS. These compounds could also be largely recovered by TBP treatment of the sediment. Somewhat lower recovery of several thiols, most notably mercaptoethanol, homocysteine and 3-MPA in the CAP/TET treatment, compared to the chilled samples (Table 2), may be due to some biotransformation of these compounds in the presence of CAP/TET. Kiene et al. (1990), for example, showed that CAP/TET had little effect on the conversion of ME to mercaptoacetate. Nonetheless, the results from these experiments point to significant interactions of thiols with sediment particulate matter. It is important that this interaction be understood, because thiol compounds may be involved in the incorporation and subsequent preservation of sulfur in sedimentary organic matter. Further work will be necessary to understand the nature of the thiol-sediment association, and the potential bioavailability and reactivity of this organic sulfur pool.

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