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Effects of Sea Salt Anions on the Formation and Stability of Methylmercury

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All forms of mercury are potentially harmful to biota, but monomethyl and dimethyl mercury are particularly neurotoxic. The lipophilic nature of the latter compounds allows them to be concentrated in higher trophic levels and the effects of this biomagnification can be catastrophic (D'ITRI & D'ITRI, 1978). Certain species of microorganisms in contact with inorganic mercury produce methylmercury compounds (JENSEN & JERNELOV, 1969). Environmental factors influence the net amount of methylmercury in an ecosystem by shifting the equilibrium of the opposing methylation and demethylation processes. Methylation is the result of mercuric ion (Hg^{++}) interference with biochemical C-1 transfer reactions (WOOD, 1974). Demethylation is brought about by non-specific hydrolytic and reductive enzyme processes (FURAKAWA & TONOMURA, 1971; 1972a; 1972b). The biotic and abiotic influences that govern the rates at which these processes occur are not completely understood.

Although much of the early work on the cycling of mercury pollutants has been performed in freshwater environments, estuaries are also subject to anthropogenic mercury pollution (BRINCKMAN & IVERSON, 1975). Previous work from this laboratory (BLUM & BARTHA, 1980) has demonstrated a strong negative correlation between the salinity of anaerobic sediments and their ability to form methylmercury from Hg^{++}. As an explanation for this negative correlation the theory was advanced that sulfide, derived by microbial reduction of sea salt sulfate, interferes with Hg^{++} methylation by forming HgS. According to several reports (FAGERSTROM & JERNELOV, 1971; YAMADA & TONOMURA, 1972a; 1972b; 1972c), HgS is not methylated readily. Numerous other explanations, e.g., an instability of methylmercury in the presence of H_2S, an interference of sea salt anions with the methyl transfer from cobalamin to Hg^{++}, and shifts in populations

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or activity of methylating or demethylating sediment bacteria in response to salinity were not excluded. There are several reports in the literature concerning the methylation of Hg^{++} by methycobalamin (WOOD, 1974; BERTILSSON & NEUJAHR, 1971; IMURA, 1971), but none dealing with the methylation of mercuric complexes in seawater or in saline sediments. A thorough analysis of salinity interference with Hg^{++} methylation dictated the performance of initial studies at a reduced level of chemical and biological complexity. This paper describes the abiotic methylation of mercuric ion and mercuric ion-sea salt anion complexes by methylcobalamin under aerobic and anaerobic conditions.

EXPERIMENTAL

Methylcobalamin and aquocobalamin were purchased from Sigma Chemical Co.. Seven Seas Marine Mix (Utility Chemical Co., Paterson, NJ), a chemically defined sea salts mixture, was used in preparing artificial seawater at defined salinity. Other chemicals were of reagent grade. The purity of methylcobalamin and aquocobalamin were judged against the spectral data of DOLPHIN (1971), and by paper chromatography on Whatman No. 1 paper using a solvent system of 1-butanol:2-propanol: acetic acid:water in a ratio of 100:70:1:100 (BERTILSSON & NEUJAHR, 1971). No impurities were found by these methods.

Since all buffers contain anions that could interact with Hg^{++}, the choice of the buffer system required special attention. Many earlier experiments on abiotic methylation of Hg^{++} by methylcobalamin (THAYER, 1981; CHU & GRUENWEDEL, 1977; ROBINSON et al., 1977) were conducted in low-pH acetate buffer. For the purpose of comparison, we used 0.1 M acetate buffer in some of our experiments, but this system is far removed from conditions that prevail in salt marsh sediments. Therefore, in most of our experiments we used a bicarbonate-CO_2 buffer that constitutes the prevalent buffer in marine and estuarine sediments. To assess the effects of the carbonate anion, we also conducted some experiments in unbuffered water, with careful monitoring of the pH both prior to and at the conclusion of the experiments.

The 5% CO_2/4.62 mM sodium bicarbonate buffer was maintained at 25°C in a glove chamber (Labline Instruments) using gas mixtures of 95% air-5% CO_2 for aerobic conditions and 90% nitrogen-5% hydrogen-5% CO_2 (Matheson Gas, East Rutherford, NJ) for anaerobic conditions. A palladium catalyst was used in the chamber under anaerobic conditions to continuously remove traces of oxygen. Using this system a redox potential below -100 mV was routinely achieved. All

solutions for anaerobic work were prepared with water that had been deoxygenated by boiling under oxygen-free nitrogen before being placed in the anaerobic chamber. Sodium bicarbonate was added to the cooled and equilibrated solution to yield a final pH of 6.8. Aerobic or anaerobic conditions , however, were not found to influence the rate or outcome of the reaction studied.

The reaction of 30 µM methylcobalamin and 60 µM mercuric chloride was carried out in two types of reaction vessels and measured by two different methods. A Bausch and Lomb 2000 spectrophotometer was used to measure changes in absorbance at 351 nm and 380 nm, respectively. These are changes characteristic of the transition of methylcobalamin to aquocobalamin (DOLPHIN, 1971). For this study, quartz cuvettes (1 cm path length) tightly stoppered with teflon were used as reaction vessels. This allowed for the maintenance of the gas phase when cuvettes were removed from the glove box for spectrophotometric measurements. The reaction volume was 2.0 mL. Control solutions of methylcobalamin in buffer were included in each experiment to ascertain that changes in absorbance were due only to the specific reaction in question. All incubations were carried out at 25°C in the dark.

Methylmercury formed in the reaction was also measured gas chromatographically. The volatile nature of some reaction products such as dimethylmercury required special enclosures for these experiments. These consisted of 2.0 mL serum vials fitted with teflon lined aluminum crimp caps (Wheaton Glass, Vineland, NJ). These caps were gas tight and allowed for quantitative recovery of volatile as well as soluble forms of methylmercury. Salt solutions were added to the vials and they were closed tightly immediately after the proper concentration of methylcobalamin was added to give a final reaction volume of 1.0 mL. The reaction was quenched at the appropriate time using 200 µL of concentrated HCl injected through the spectrum (IMURA, 1971). The contents were then extracted by 1.0 mL of benzene. This extract was dried using anhydrous sodium sulfate and methylmercuric chloride was quantified by gas chromatography using a Tracor Model-220 instrument with a ^{63}Ni-electron capture detector and on-column injection of 2.0 µL samples. A 183 cm long, 0.73 cm OD glass column packed with 10% silar 10-C on 100/120 mesh Gas Chrom Q (Applied Science, State College, PA) was used under the following operating conditions: 95% argon-5% methane carrier gas at 90 ml/min, inlet at 210°C, oven at 200°C, and detector at 250°C (BLUM & BARTHA, 1980). Peaks were quantified using a Hewlett Packard 2290A Reporting Integrator and appropriate methylmercuric chloride (Matheson, Coleman, and Bell, Norwood, OH) standards. Recovery of monomethylmercury spikes using the same extraction procedure was 90% ± 4%.

RESULTS AND DISCUSSION

An increase in abosrbance at 351 nM and a concommittant decrease in absorbance at 380 nM in the UV-visible spectrum of methylcobalamin during the abiotic transfer of the methyl group to Hg^{++} are characteristic for the loss of the methyl group and formation of aquocobalamin. In experiments monitored by both analytical techniques, gas chromatographic measurements of methylmercury formation were in good agreement with the spectrophotometric measurements of aquocobalamin formation from methylcobalamin at 351 nM. Aerobic versus anaerobic reaction conditions had no measurable effect in any of our experiments on either the methyl transfer rates, the stability of the reactants, or on the reaction products. For this reason, in our data presentation, we have omitted this variable.

In 0.1 M pH 4.5 acetate buffer (Fig. 1) we observed the rapid methyl transfer rates (>90% in 10 min) reported previously by several authors (THAYER, 1981; CHU & GRUENWEDEL, 1977; ROBINSON et al., 1977). It made no measurable difference

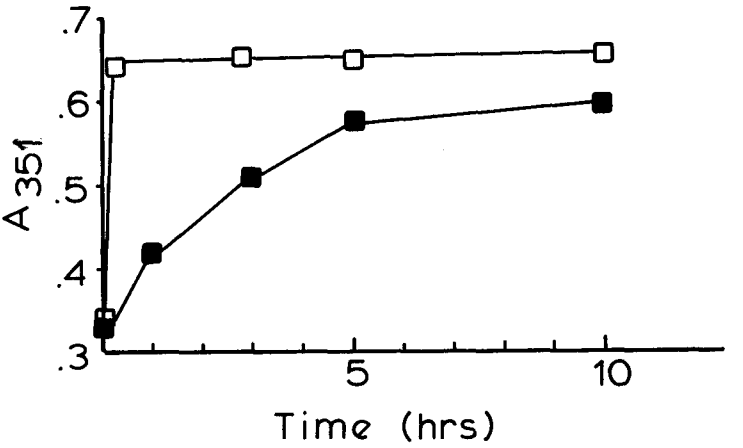

Figure 1. Methylation of Hg^{++} by methylcobalamin in pH 4.5 acetate buffer (□), and in the presence of 0.1 M chloride (■).

whether Hg^{++} was added as $HgCl_2$ or as mercuric acetate. Addition of 0.1 M Cl^- markedly slowed the methyl transfer, a fact also noted in the cited reports. In contrast, transmethylation in unbuffered water at pH 6.8 proceeded much slower (90% complete after 5-10 hrs). At this pH, 0.1 M Cl^- 0.1 M acetate and 4.62 mM HCO_3^- inhibited transmethylation in increasing order (Fig. 2). Nevertheless, since we expected bicarbonate to be present in estuarine sediments, we conducted

all our subsequent transmethylation experiments in bicarbonate buffer.

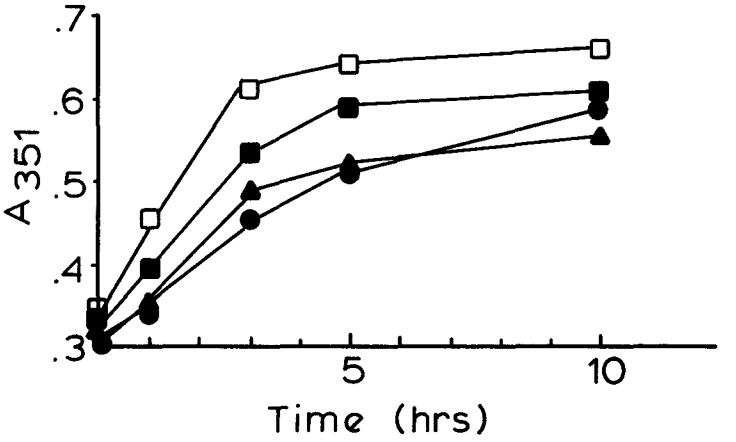

Figure 2. Methylation of Hg^{++} by methylcobalamin in unbuffered pH 6.8 water (□) in the presence of 0.1 M acetate (▲), 0.1 M chloride (■), and 4.62 mM HCO_3^- (●).

Sulfide, added under anaerobic conditions only at 10, 50, and 100 ppm levels as Na_2S, completely prevented the methylation of Hg^{++} by methylcobalamin even at the lowest sulfide concentration tested (Table 1). This result is consistent with the very high affinity of sulfide for Hg^{++} ($K = 10^{-53}$). The same sulfide concentrations had no effect on the stability of CH_3HgCl over a 20 h period, nor had the sulfide any effect on the stability of the methylcobalamin. These experiments clearly established

Table 1. Effects of sulfide on the methylation of Hg^{++} by methylcobalamin and on the stability of reactants and reaction products [1]

Na_2S (ppm)	Formation CH_3HgCl (ppm)	Remaining CH_3HgCl (ppm)	Remaining Me-Co (μM)
0	9.4	9.0	30
10	0	9.3	30
50	0	8.6	30
100	0	8.9	30

[1]Incubated under anaerobic conditions at 25°C in the dark for 20 hours.

that the lack of Hg^{++} methylation in the presence of sulfide was entirely due to the binding of Hg^{++} ions as HgS; methyl-

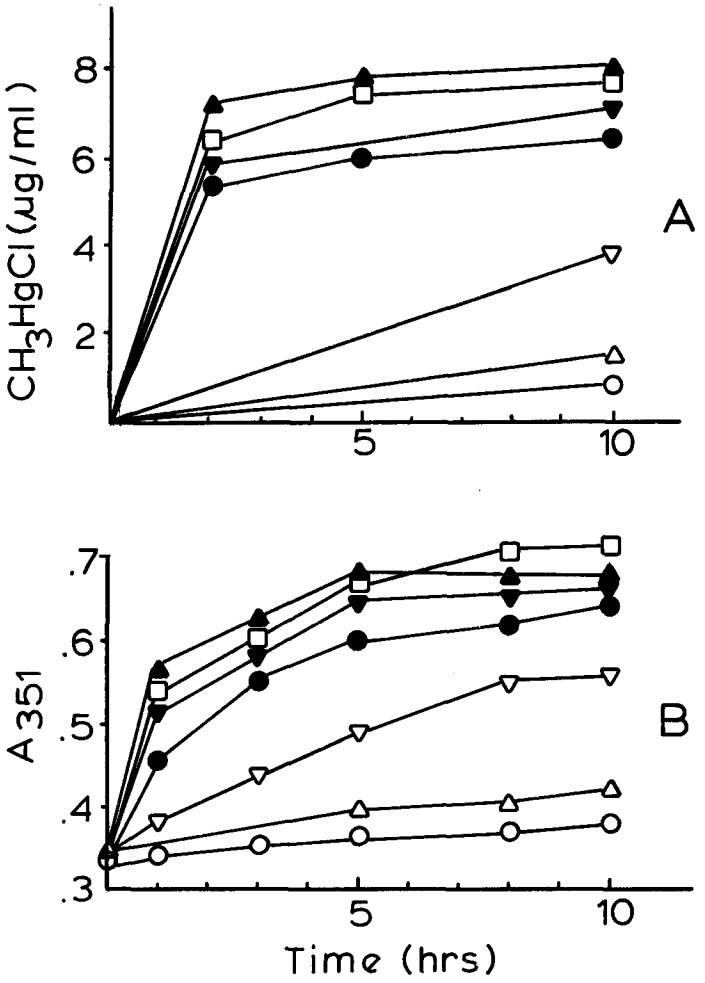

Figure 3. Methylation of Hg^{++} by methylcobalamin in pH 6.8 bicarbonate buffer (□), and in the presence of 0.01 M chloride (▲), 0.1 M chloride (▼), 0.5 M chloride (●), 0.01 M bromide (▽), 0.1 M bromide (△), and 0.5 M bromide (○). Transmethylation was measured gas chromatographically (A) and also spectrophotometrically (B).

cobalamin and preexisting methylmercury remained unaffected by sulfide. The inhibition of methylmercury formation in soils, sediments and bacterial cultures in the presence of sulfide was previously reported by several authors (FAGERSTROM & JERNELOV, 1971; YAMADA & TONOMURA, 1972c; TALMI & MESMER, 1975).

The effect of halogen anions on the methyl transfer process is shown in Fig. 3. In bicarbonate buffer, chloride concentrations ranging from 0.01 to 0.5 M failed to inhibit the methylation of mercury significantly. This was in apparent contradiction to previous reports (THAYER, 1981; CHU & GRUENWEDEL, 1977; ROBINSON et al., 1977) as well as to our own results shown in Fig. 1. However, the excellent agreement of the results obtained by two different measurement techniques and several repeats of the experiment left no doubt about the validity of the finding. The reasons one would expect chloride to interfere with the methyl transfer process are as follows: in the presence of increasing Cl^- concentrations Hg^{++} forms $HgCl^+$, $HgCl_2$, $HgCl_3^-$, and $HgCl_4^{--}$ complexes. Each of these complexes reaches maximal proportions at specific Cl^- concentrations (HAHNE & KROONTJE, 1973). Full strength seawater (3.5% salinity) contains an average concentration of 0.56 M Cl^-. In this environment, soluble mercury exists primarily as $HgCl_3^-$ and $HgCl_4^{--}$ (STOTZKY & BABICH, 1980). Methyl transfer is accomplished by electrophilic attack of the cobalamin by mercury, causing transfer of a carbanion (CH_3^-) (RIDLEY et al., 1977). Thus, the methylation of negatively charged complexes is surprising.

The key to the resolution of the apparent contradiction between the results shown in Fig. 1 and Fig. 3 lies in Fig. 2. Bicarbonate, apparently due to the formation of $HgCO_3$, is more inhibitory to the methyl transfer process than Cl^-. In concentrated stock solutions (but not under our experimental conditions), formation of a precipitate was observed. Addition of either acetate of chloride solubilized the precipitate. Correspondingly, excess acetate or chloride partially reversed the inhibition by bicarbonate buffer. Bicarbonate is a major component of sea salts (TAIT & DE SANTO, 1972) and of estuarine sediments (DADDARIO, 1961). Its negative influence on availability of Hg^{++} for methylation is of importance. We also speculate that other conditions being equal Hg^{++} will be more available for methylation in "soft" than in "hard" (bicarbonate-rich) freshwater systems.

The unexpected effect of chloride prompted us to also investigate the influence of bromide ions (Fig. 3). In bicarbonate buffer, high concentrations of bromide slowed the rate of mercury methylation. The metal-ligand bond between Hg^{++} and Br^- is stronger than the Hg-Cl bond (THAYER, 1981). Hg-Br complexes would be expected to have lower rates of methylation than the corresponding Hg-Cl complexes and unlike Cl^-, they failed to mitigate the effect of bicarbonate. However, the bromide concentration of seawater is rather low (0.83 mM) and, therefore, its effect on methyl transfer is marginal. In bicarbonate buffer, sulfate ions up to their concentration in full strength seawater (0.03 M) had neither positive nor negative effect on methylation. Total sea salts at concentrations approaching full-strength seawater (3.5% salinity) in the presence of bicarbonate did not inhibit Hg^{++} methylation.

CONCLUSIONS

The sulfate component of sea salts is inocuous. However, if reduced to sulfide in anaerobic sediments, it severely reduces the availability of Hg^{++} for methylation by methylcobalamin. The bicarbonate component of sea salts noticably slows the methylation of Hg^{++} under both aerobic and anaerobic conditions. In the presence of bicarbonate, other sea salts anions have no significant influence on the methylation of Hg^{++}. In the dark, monomethylmercuric chloride (CH_3HgCl) is chemically stable in the presence of all tested seawater anions, including sulfide.

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