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Intestinal Solubilization of Particle-Associated Organic and Inorganic Mercury as a Measure of Bioavailability to Benthic Invertebrates

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The bioavailability of particle-associated inorganic mercury (Hg_I) and monomethylmercury (MMHg) was evaluated in vitro using digestive fluid of the deposit feeding lugworm, *Arenicola marina*. Digestive fluid, removed from the midgut of the polychaete, was incubated with contaminated sediment, and the proportion of Hg_I or MMHg solubilized by the digestive fluid was determined. Digestive fluid was found to be a more effective solvent than seawater in solubilizing particle-associated Hg_I and MMHg. A greater percentage of MMHg than Hg_I was solubilized from most sediments, suggesting that sediment-associated MMHg is generally more readily available from sediment for biological uptake. The proportion of MMHg released from the sediment was inversely correlated with sediment organic matter content, decreasing exponentially with increasing organic matter content of the sediment. The results for Hg_I were equivocal. MMHg bioaccumulation factors (BAFs) from previous studies showed a similar trend with organic content of sediment, suggesting that solubilization may be the process limiting the bioaccumulation of particle-bound MMHg. It is concluded that in vitro extraction with a deposit feeder's digestive fluid provides a potential tool to study the process of Hg bioaccumulation via ingestion routes, although its application to various sediments and organisms needs further investigation.

Introduction

There is little information concerning inorganic mercury (Hg_I) and methylmercury (MMHg) accumulation by benthic organisms (*1*) even though these contaminants are efficiently bioaccumulated (*2*−*5*) in aquatic food webs, especially in shallow estuarine systems. Although sediments are the dominant site for Hg_I methylation (*6*), MMHg comprises a relatively small fraction of the total Hg in most estuarine sediments (<1%) and in the overlying water (*6*−*8*). However, MMHg is the dominant form of mercury in higher predators in the aquatic food chain (*9*) because of its preferential trophic transfer over Hg_I (*5, 10*).

Recent studies have demonstrated that the bioavailability of sediment-associated metals to benthic organisms is affected not only by chemical factors, such as sediment geochemistry, but also by the physiology of the organism (e.g., the assimilation efficiency for a particular contaminant) (*1, 11, 12*) and biological factors, such as food quality and feeding behavior, which determine the possible and dominant exposure routes (*12*−*14*). Furthermore, it has been suggested that for organisms obtaining most of their contaminants from food, bioavailability is dependent upon the extent of solubilization of the constituent of interest under digestive conditions (*15*). The investigation detailed below aimed to assess whether this was true for inorganic mercury (Hg_I) and monomethylmercury (MMHg). Sediment ingestion appears to be the major route of Hg_I and MMHg exposure to deposit feeders (*12, 16*−*18*) under most environmental conditions, especially in coastal environments where sediments often contain elevated levels of Hg and MMHg (*8, 17, 19*).

The role of digestive processes in controlling bioavailability has only recently received attention (*15, 20*). To complement our previous studies of Hg bioavailability to benthic invertebrates (*11, 21*), we examined the hypothesis that intestinal solubilization is the rate-limiting step in Hg_I and MMHg bioaccumulation. To test this premise, we incubated natural and spiked sediment with the digestive fluid of the lugworm, *Arenicola marina*, as a means of assessing the bioavailability of sediment-associated Hg. This physiology-based method (*22*) contrasts with standard strong acid digestion and other sequential extraction methods, which typically overestimate the fraction of the contaminant that is bioavailable (*15*). The intestinal incubation approach is more relevant to bioavailability and subsequent bioaccumulation and is, in essence, a chemical extraction with a biologically relevant extractant. It should therefore provide a more accurate measure of bioavailability. To examine further the mechanism of solubilization, we compared the efficiency of intestinal fluid to a surrogate, bovine serum albumin (BSA; used by Chen and Mayer (*22*)), in extracting Hg_I and MMHg from sediment.

By mimicking the digestive chemistry of the lugworm, we determine the actual percent of contaminant solubilized by the organism, which is indicative of Hg and MMHg availability during the process of deposit feeding. However, while digestive fluid solubilization is of great value for studying contaminants such as Hg that are strongly associated with particles (*17*) and for which ingestion is a major route of uptake (*11, 16, 21*), the method does not address the subsequent absorption of the solubilized Hg across the gut wall (*15*), and thus places only an upper limit on the proportion of Hg that is likely to be bioaccumulated. Weston and Mayer (*15*) suggest, however, that solubilization is the limiting factor in determining the bioavailability of strongly sediment-associated contaminants.

This experimental approach mimics only digestion and excludes the exposure of live animals to sediment. Therefore, it is not influenced by behavioral and physiological factors that may affect bioaccumulation but are not necessarily inherent components of bioavailability (*15*), and this approach assesses only uptake from particle ingestion. While previous studies show that this is the dominant exposure route for Hg (*11, 16, 21*), water can also be an important uptake route. Our previous laboratory studies included

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TABLE 1. Characteristics of Sediments Used in the Intestinal Solubilization Experiments

sediment	MMHg[a] (pmol g^{-1})	Hg_I (pmol g^{-1})	dry/wet	organic matter (%)	AVS[b] (μmol g^{-1})
Inner Harbor	5.5 (0.55%)	1000	0.54	13	34
Bear Creek	3.0 (10.5%)	28.5	0.26	16	57
Back River	0.4 (0.87%)	45.5	0.72	1.1	1.0
amp exp 4	13.5 (3.2%)	410	0.58	2.3	0.02

[a] Values in parenthese are the percentage of the total mercury as methylmercury. [b] AVS is the acid volatile sulfide concentration; a measure of the amount of reactive sulfide in the sediment.

exposure of deposit feeders to contaminated sediment and subsequent determination of Hg bioaccumulation by calculation of bioaccumulation factors (BAFs) (*11*, *21*). We therefore had the opportunity to compare and contrast the results of the solubilization experiments with those of the bioaccumulation studies.

Materials and Methods

Digestive fluid from the midgut of the polychaete, *Arenicola marina*, was obtained in Maine according to methods outlined in Mayer et al. (*23*). The fluid was frozen at −80 °C, shipped frozen to Maryland, and remained frozen until use. Although freezing may affect the enzymes of the digestive fluid, Chen and Mayer (*22*) assert that Cu solubilization is a result of competitive complexation, rather than enzymatic processes, and that the effectiveness of the intestinal fluid is not compromised by freezing. We hypothesized that the effectiveness of the digestive fluid solubilization for Hg_I and MMHg would also depend on complexation and thus be unaffected by freezing. In addition, experiments using similar methods comparing in vivo and in vitro solubilization of PAHs by *A. marina* showed no significant differences (*24*), again indicating that the methods used here provide a good assessment of bioavailability.

Naturally contaminated sediment from three sites around Baltimore Harbor, MD, and sediment spiked with Hg and MMHg from a previous bioaccumulation experiment (*11*) were used in this study (Table 1). The top few centimeters of naturally contaminated sediments were collected from intertidal areas using a ponar grab. Samples were stored in acid-cleaned plastic storage containers and refrigerated until use.

Incubations. Sediment was incubated with digestive fluid or seawater in acid-cleaned Teflon centrifuge tubes. Each digestive fluid incubation was performed in triplicate; 1.0 (± 0.1) mL of digestive fluid or seawater was added to 1.0 (± 0.2) g of sediment (wet weight), which approximates the solid: solution ratio in deposit feeders (*15*). The slurries were vigorously shaken and held at room temperature for varying amounts of time (0.5, 2, or 4 h) on an orbital shaker. Control incubations included seawater and digestive fluids without sediment. After incubation, fluids were removed from slurries by centrifugation at 2700*g* for 0.5 h at room temperature. The fluids were then transferred to acid cleaned Teflon vials and frozen until subsequent analysis.

For the comparison of digestive fluid and BSA, similar procedures were used. A range of BSA concentrations was obtained by diluting the pH 7.4 phosphate-buffered stock BSA solution in 0.14 M saline solution (Sigma), giving a range of amino acid concentrations that straddled the concentration of amino acids in *A. marina* digestive juice (242 mM; *25*). The BSA experiment was performed using a laboratory spiked sediment of relatively low organic matter content (OM; 1.97%; 207 pmol g^{-1} MMHg).

Sample Analysis. Samples for total Hg were digested in a 7:3 sulfuric acid/nitric acid solution overnight at 60 °C in sealed Teflon vials to ensure complete digestion of organic matter (*26*). These conditions, less stringent than those typically used to digest samples for trace metal analysis, are required to ensure that Hg is not lost by volatilization during digestion. Under cleanroom conditions, the samples were further oxidized with bromine monochloride, and the excess oxidant was neutralized with 10% hydroxylamine hydrochloride. Samples were then reduced with tin chloride and bubbled with argon, and the volatile Hg trapped on a gold column. Mercury was quantified via cold vapor atomic fluorescence detection (CVAFS) (*27*) in accordance with protocols outlined in EPA Method 1631 (*28*). A calibration curve with an r^2 of at least 0.999 was achieved daily. Reported values are averages of duplicate analyses.

Samples for MMHg were distilled after adding a 50% sulfuric acid solution and a 20% potassium chloride solution (*29*). The distillate was reacted with a sodium tetraethylborate solution to convert the nonvolatile MMHg to gaseous methylethylmercury; the volatile adduct was purged from solution and recollected on a graphitic carbon column at room temperature. The methylethylmercury was then thermally desorbed from the column and analyzed by cryogenic gas chromatography with CVAFS (*30*).

Reported values are the averages of triplicate incubations, except for incubations with seawater. The BSA incubations were performed in duplicate. Hg standards, MMHg standards, laboratory duplicates, and standard reference materials of known Hg and MMHg concentrations were analyzed to ensure the accuracy of our results. Both liquid and gas standards were used during the total Hg analysis, and comparison of these provided a measure of the recovery and precision of the total Hg analysis (*9*). Matrix spikes were included in each sample batch when determining MMHg to ensure that suitable recovery was obtained from the distillation process. Duplicate analysis of 10% of the MMHg samples yielded no significant difference, and >90% of all SRM replicates analyzed for Hg and MMHg fell within the certified ranges. Spike recoveries for water and sediment measurements were in the range of 75−120% for Hg and 80−110% for MMHg. Detection limits for Hg and MMHg were based on three standard deviations of the blank measurement (0.3 pmol g^{-1} Hg and 0.07 pmol g^{-1} MMHg, wet weight). Inorganic Hg (Hg_I) was determined by difference (total Hg − MMHg).

Ancillary Analysis. Water content and the organic matter (OM) content in the sediment was determined as in previous experiments (*11*). Water content and percent organic matter determinations were generally analyzed in duplicate, and the error between these duplicates was <10% on average. Acid-volatile sulfur (AVS) analysis was performed using a modified version of the EPA method (*31*). Wet sediments (2.0 g ± 0.2) were digested in a system flushed with nitrogen using degassed cold 6 N HCl. The evolved H_2S was collected in a deaerated solution of zinc acetate and sodium acetate buffer. The precipitated sulfide was then measured using a sulfide probe with a Pb titration. Detection limits for AVS were 0.01 μmol g^{-1} (dry weight).

Results and Discussion

The characteristics of the sediments used in this study are shown in Table 1. The concentration of dissolved Hg in digestive fluid after incubation with sediment was normalized

TABLE 2. Concentrations of Hg_I and MMHg Solubilized during in Vitro Digestion Experiments (0.5 h Incubation)

site	seawater	dig. fluid	dig. fluid/seawater
	Inorganic Mercury[a]		
Inner Harbor	0.3	1.6	5.2
Bear Creek	0.3	2.5	8.3
Back River	0.8	1.1	1.4
exp 4 sed	2.7	24.0	9.1
	Methylmercury[a]		
Inner Harbor	0.10	0.15	1.5
Bear Creek	0.05	0.15	3.0
Back River	0.10	0.20	2.0
exp 4 sed	0.05	1.95	39

[a] Concentration in supernatant (nM).

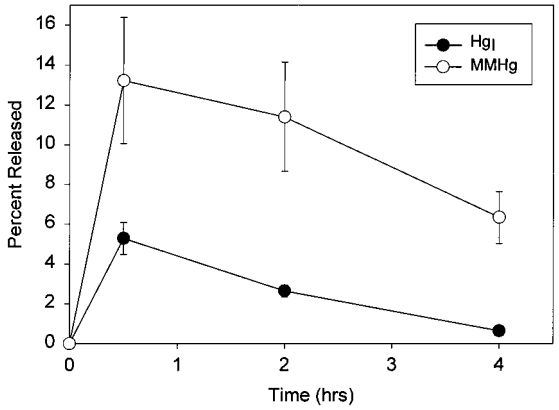

FIGURE 1. Percent release of sediment-associated Hg_I and MMHg by *Arenicola* digestive fluid over time. Each time point represents replicate extractions for the period of time indicated. Values are the mean ± SD ($n = 3$) of replicates.

to the volume of digestive fluid added during the incubation. The data are presented in Table 2 as the concentration of Hg_I or MMHg in the digestive fluid after extraction. The figures show the amount of Hg_I and MMHg released to solution after incubation relative to that of the initial sediment prior to incubation (i.e., as percent released). As can be seen by comparison of the data in Table 2 and in the figures, the percent of MMHg solubilized is higher than that of Hg_I even though the concentration of MMHg in the fluid after extraction is lower. This results from the typically low fraction of the total Hg as MMHg in the sediments (Table 1). The relative MMHg concentration in the Bear Creek sample was atypical. Results from naturally contaminated and spiked sediments are presented simultaneously because, although spiked contaminants have previously been shown to be more bioavailable (*15*), the percent of Hg released from spiked sediment was not substantially higher than from field-contaminated sediment of similar OM in our experiments.

The 0.5-h incubation period was chosen because prior experiments showed that the proportion of PAHs extracted with *Arenicola* digestive fluid was constant from 20 min to 4 h (*15*), and our kinetic experiments showed that the highest proportion of Hg was released from spiked sediment after 0.5 h (Figure 1). This exposure time is similar to the gut retention time of *A. marina* (1−2 h (*32*)) as well as the mean gut passage time of *Leptocheirus plumulosus* (measured to be 95 min by C. Schlekat (unpublished data) using 10-μm plastic beads impregnated with ^{57}Co). Although these incubations were somewhat shorter than the mean gut passage time of the amphipod and lugworm, these values represent the maximum amount of sediment-associated Hg solubilized and available for subsequent bioaccumulation by these organisms. The kinetic data show that the decrease in

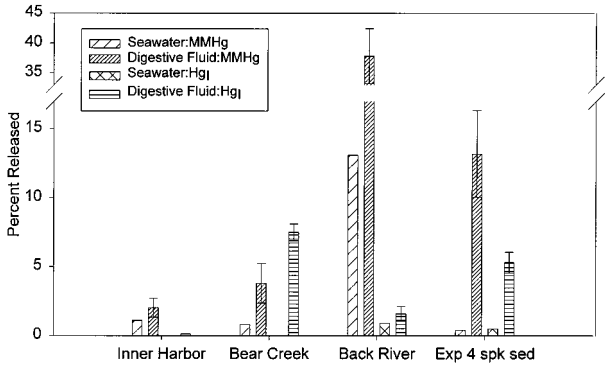

FIGURE 2. Percent release of sediment-associated Hg_I and MMHg after incubation with seawater or digestive fluid. For digestive fluid incubations, mean ± SD ($n = 3$) is shown.

concentration between 0.5 h of incubation and that after 1.5 h is 10−30%, so the error involved in estimating in situ bioavailability, based on a 0.5-h incubation, is comparable to errors associated with the measurements (Figure 1).

Kinetics of Solubilization. There was an initial increase in concentration of both Hg_I and MMHg in the digestive juice, but with continuing incubation the concentration decreased (Figure 1). The decrease in the amount of Hg solubilized over time may be due to partitioning and reabsorption of Hg to particles, coagulation of colloidal material, or adsorption/precipitation of protein onto particulate matter rather than decreased solubilizing power of the digestive fluid. Chen and Mayer (*22*) suggest that digestive dissolution of sediment-associated Cu results from complexation rather than enzymatic action at the neutral pH of digestive fluid. We hypothesize a similar mechanism for Hg_I and MMHg. Therefore, changes in the distribution of complexing agents between dissolved and particulate phases could account for the observed differences in the Hg experiments. Other kinetic experiments (*23*) have indicated that the extent of solubilization for metals did not reach equilibrium in 4 h, and dependent on sediment and/or intestinal fluid, a decrease was seen for both Cu and Cd (*33*). Clearly, the extent of bioavailability to a benthic organism will be influenced by the solubilization kinetics relative to the gut passage time.

Digestive Fluid Solubilization. Digestive fluid was far more effective at solubilizing sediment-associated Hg_I and MMHg than seawater (Figure 2). Seawater extractions, performed in a manner identical to digestive fluid extractions, solubilized 1−13% of the MMHg and only 0−1% of the particle-associated Hg_I. For the field-collected sediment, there was a decrease in the proportion of MMHg extracted by seawater with increasing OM and AVS; however, this trend was not evident for the spiked sediment where seawater extracted little MMHg. Less than 2% of the MMHg from Inner Harbor and Bear Creek sediment, both containing high OM and AVS, was extracted by seawater, whereas 20% of the MMHg from Back River (1.1% OM, 0.02 μmol g^{-1} AVS) sediment was extracted by seawater (Figure 2).

The greater solubilization potential of digestive fluid is apparent from the ratio between the concentrations in the two media (Table 2). The proportion of MMHg and Hg_I released from sediment after incubation with digestive fluid was 2.0−38% and 0.1−7.5%, respectively (Figure 2). The values for MMHg are higher than and those for Hg_I are similar to the proportion of other contaminants released from naturally contaminated sediments after incubation with *Arenicola* digestive fluid (0−14% for Cu, 0−<10% for PAHs, and only 0.3% for Pb) (*22*).

There were dramatic differences among sediments in the proportions of Hg_I and MMHg solubilized by digestive fluid

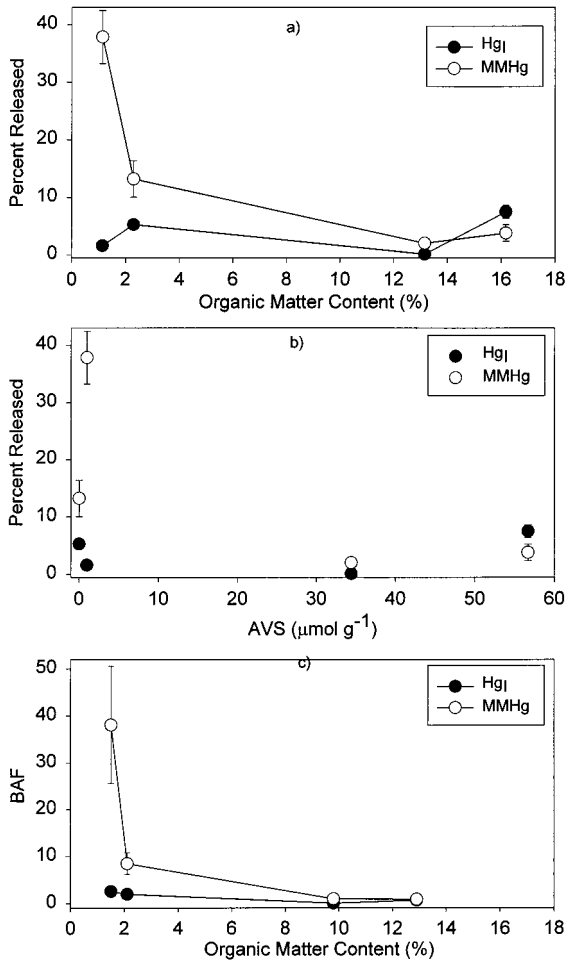

FIGURE 3. (a) Percent of sediment-associated Hg_I and MMHg solubilized by *Arenicola* digestive fluid in relation to (a) sediment organic matter content and (b) acid volatile sulfide. Values are given as the mean ± SD (n = 3) of replicates. The relationship between the bioaccumulation factor for the amphipod *Leptocheirus plumulosus* and sediment organic matter content is shown in panel c. Modified from ref *11*.

(Figure 2). Of the field-contaminated sediments, the highest percent of MMHg released was from Back River sediment (38%), but the highest percent of Hg_I released was from Bear Creek sediment (7.5%). A greater percentage of MMHg as compared to Hg_I was released from all sediments (Figure 2), excluding Bear Creek sediment where the percentages released were similar. This sediment had the highest concentration of AVS (57 μmol g^{-1}) and organic matter content (OM) (16%) (Table 1). The results indicate that sedimentary MMHg is more available for bioaccumulation than Hg_I at intermediate levels of OM in sediments (1.5−7%, i.e., Back River and experimental sediments), but that availability of MMHg and Hg_I is similar but low for high OM sediment (e.g., Bear Creek). Comparison of the effective equilibrium constants of Hg_I and MMHg binding to organic matter (respectively, 10^{19} and $10^{14.6}$ L (kg of C)$^{-1}$ for Aldrich humic acid (*10*, *34*)) support the contention that Hg_I will be more strongly bound to organic matter than MMHg and subsequently less available for extraction by intestinal fluid, except at high OM when all Hg_I and MMHg will be effectively bound to OM.

In corroboration of this, the proportion of MMHg released from the sediments was inversely correlated with OM of the sediments (exponential decay, $y = 2.9 + 116 \exp(-1.1x)$; $r^2 = 0.998$, $p = 0.99$, Figure 3a). A decreasing trend in the proportion of MMHg released with increasing AVS was also indicated; however, the correlation was not significant (Figure 3b). Previous studies have shown that AVS and sediment organic matter content are correlated for Baltimore Harbor sediments (*17*); therefore, it is not surprising that the proportion of MMHg released shows a similar trend with both of these components. A relationship with OM was also seen when investigating assimilation efficiencies of MMHg by a marine deposit-feeding polychaete, *Nereis succinea* (*16*). Furthermore, experimental sequential extraction studies using techniques designed to assess the amounts of Hg bound to organic matter (*35*) have indicated that, in organic-rich sediments, OM is the dominant fraction containing Hg rather than AVS (*35*, Benoit, unpublished data). These studies also suggest that alternative methods of measuring AVS-associated Hg in organic-rich sediments, which do not remove organic matter prior to the HCl extraction step, can lead to an overestimation of the Hg associated with inorganic S phases as the HCl step partially extracts Hg-bound organic matter. The same is likely true for MMHg. Thus, we suggest that our results support the conclusion that OM rather than AVS is the most important factor controlling MMHg bioaccumulation from these sediments (*11*, *17*, *21*).

Over the OM range of these experiments, no trend was seen between proportion of Hg_I solubilized and OM or AVS (Figure 3a,b). The expected, comparable decreasing trend for Hg_I with increasing OM is perhaps not obvious in the current data set. Our complementary laboratory bioaccumulation experiments (*11*) showed marked differences in Hg_I bioaccumulation only at very low OM (<1% OM; levels below those encountered in this experiment) (Figure 3c). Equilibrium modeling suggests that, even at the lowest OM content of the solubilization experiments, most of the Hg_I is bound to OM while this is not true for MMHg. The complexation ability of digestive fluid for Hg_I is apparently insufficient to out-compete the strong Hg_I binding ligands present in the sediment.

To further investigate the mechanisms of solubilization, we incubated laboratory-spiked sediment (1.97% OM) with a protein solution (BSA) at varying concentrations, i.e., comparable experiments to those performed by Chen and Mayer (*22*). These authors concluded that solubilization of Cu by *A. marina* intestinal fluid was the result of complexation of Cu to amino acid ligands, notably histidine, and that intestinal fluid extraction could be mimicked by using a BSA solution of equivalent amino acid content or by direct extraction with histidine (*22*). For Hg_I and MMHg, thiol groups are the most likely complexing ligands, and BSA has a relatively high proportion of thiol-containing residues (6% cysteine, 0.7% methionine; *36*). The amino acid content of *A. marina* intestinal fluid is variable, averaging 242 mM (*25*), and the amino acid concentration of BSA was estimated from the molecular weight (66 400) and total number of residues (583; *36*).

We found an increase in extraction of MMHg from sediment with increasing concentration of BSA (Figure 4). The solubilization of MMHg by BSA was much greater than that of the intestinal fluid. Given the likely variability in amino acid content of intestinal fluid among individuals (up to an order of magnitude; *24*, *25*), the higher solubilization by BSA may be due to very low amino acid concentrations in this batch of gut fluid, which was not measured directly. If solubilization is due to complexation, the differences in solubilization could be partly due to the relatively high concentration of thiols (around 7%; *36*) in BSA as compared to that of intestinal fluid and protein in general (<4%; e.g., refs 25 and 37). However, it is also possible that solubilization of MMHg by BSA is due to hydrophobic solubilization processes within the interior of the globular proteins. In this case, higher solubilization ability of BSA could result from the globular nature of BSA as compared with lower molecular

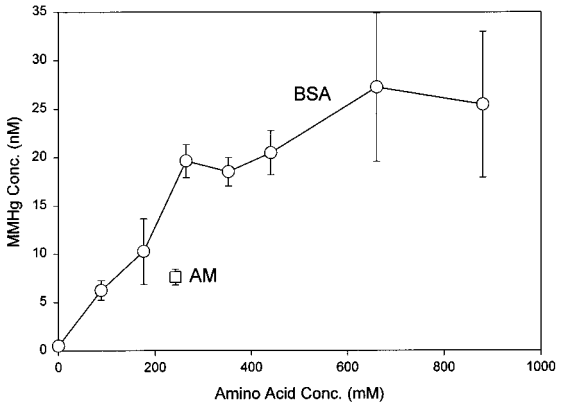

FIGURE 4. Concentration of methylmercury in solution after sediment extraction using various dilutions of bovine serum albumin solution (BSA). A concurrent extraction with the intestinal fluid of *Arenicola marina* (AM) is also shown.

weight peptide material in gut fluids (*25*). Overall, these results indicate the potential importance of proteins in solubilizing MMHg. Further studies are clearly required to separate the importance of complexation as compared to hydrophobic interactions in controlling solubilization.

Comparison of Digestive Solubilization and Bioaccumulation. Our previous laboratory experiments investigated the bioaccumulation of Hg from sediments to the deposit feeding amphipod, *L. plumulosus,* using bioaccumulation factors (BAFs) (the concentration of Hg in the amphipod relative to the concentration of Hg in the sediment on a wet weight basis) as a measure of bioaccumulation (*11*, *21*). Both BAF_{HgI} and BAF_{MMHg} decreased as OM in the sediments increased. At intermediate OM levels (similar to those encountered in these bioavailability experiments), BAF_{MMHg} was greater than BAF_{HgI} by approximately a factor of 10 (*11*, *21*).

To compare the results of the solubilization experiments with our previous bioaccumulation studies using deposit feeding amphipods, BAFs were compared to the proportion of Hg released from sediment incubated with digestive fluid (Figure 3c). At similar OM, there was a consistent trend with OM for BAF_{MMHg} and for the proportion of MMHg released, implying that solubilization experiments are a reasonable surrogate measure for overall bioaccumulation of sediment-associated MMHg by deposit feeders. This has been shown to be true for PAHs as well (*15*). These authors also showed that there was a reasonable relationship between the extent of PAH solubilization and the BAF derived from whole animal exposures to the sediment.

For Hg_I, OM does not correlate well with either the BAF or the proportion of Hg released from sediment. The high percent of Hg_I solubilized at the highest OM content (Bear Creek sediment) appears anomalous. The studies of Wang et al. (*16*), using sediment containing 8.5 and 17% OM, also showed that Hg_I assimilation efficiencies appeared to be unaffected by sediment composition. In agreement with this, the percent of Hg_I released to gut fluid and the BAF from sediment are both relatively constant over this organic matter range (Figure 3c). While other factors in addition to OM and AVS in sediments may be controlling Hg_I solubilization by *A. marina* digestive fluid, we suggest that sediment chemistry (OM and AVS) is controlling solubilization in these experiments.

In summary, *A. marina* digestive fluid is a far more effective solvent than seawater in solubilizing particle-associated Hg_I and MMHg and may provide a measure of potential bioavailability. The greater solubilization of MMHg than Hg_I from sediment by digestive fluid confirms previous observations that MMHg is generally more bioavailable from sediments than Hg_I (*16*). The strong dependence of MMHg desorption from particles on sediment OM is in agreement with our bioaccumulation studies (*11*, *21*) and illustrates the importance of OM in controlling MMHg solubility and hence bioavailability from sediments. Correlation between digestive fluid solubilization and BAFs for MMHg suggests that solubilization is the limiting factor in determining the bioavailability of particle-associated MMHg, and solubilization experiments may potentially be used as a surrogate measure for bioaccumulation of sediment-associated MMHg by deposit feeders. For Hg_I, results are equivocal, but a similar role of OM in governing solubilization appears reasonable based on both the solubilization and the bioaccumulation experiments.

Although amphipod BAFs and a proportion of MMHg solubilized by lugworm digestive fluid were similar, previous studies (*23*) have shown that digestive fluids from different benthic species with similar feeding habits solubilized different amount of metals, indicating that bioavailability varies among species even given a constant mode of uptake. In vitro extraction with a deposit feeder's digestive fluid does however provide a unique tool to study the process of bioaccumulation via ingestion routes, although its broad application needs further scrutiny.

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