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Mechanisms of Cu Solubilization during Deposit Feeding

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Large amounts of Cu in contaminated sediments can be dissolved by digestive fluids of deposit feeders during an in vitro digestion (Mayer et al. *Environ. Sci. Technol.* **1996**, *30*, 2641) and may subsequently become available to those organisms. The mechanisms of this digestive dissolution were investigated by comparing Cu release potentials of modified and unmodified gut fluids. Microwave treatments indicated that complexation rather than enzymatic action likely accounts for enhanced Cu solubilization by gut fluids rich in protein. Solutions of a common protein, bovine serum albumin, with similar AA concentrations mimicked digestive fluids in releasing sedimentary Cu. Chemical modification of histidine residues in gut fluids drastically reduced Cu release, suggesting their important role in Cu bioavailability. Using an ion-selective electrode, we found an abundance of gut ligand sites with conditional stability constants $>10^{10}$, consistent with histidine complexation. Histidine concentrations among various molecular weight fractions of gut fluids can be predictive of the amount of Cu released during in vitro digestion. Complexation-enhanced metal remobilization by gut fluids thus has significant impact on metal availability during deposit feeding.

Introduction

Increasing evidence has shown that ingestion and digestion of food by marine organisms is an important pathway for bioaccumulation of heavy metals from the environment (*2*, *3*). For sedimentary metals to be absorbed, they should be present in soluble form. Measurement of bioavailability of sedimentary metals by harsh chemical attack has given way gradually to the development of more biomimetic or physiology-based methods (*4*, *5*), which are regarded as more relevant to bioaccumulation and risk assessment. In a previous study, we took a more direct approach in which an in vitro digestion was conducted by incubating digestive fluids of marine deposit-feeding organisms with contaminated sediments (*1*). Amounts of metals released during incubation experiments thus could be taken as indicative of their availability in the process of deposit feeding. During those incubation experiments, significant amounts of sedimentary Cu were released from contaminated sediments in comparison to negligible amounts from a clean sediment, while more was extracted by digestive fluids of a polychaete (*Arenicola marina*) than a holothuroid (*Parastichopus californicus*) and least by clean seawater.

We focus here on the agents in gut fluid responsible for the enhanced release of Cu. Neutral gut pH in deposit feeders (*6*) suggests the occurrence of agents other than H^+, while high concentration of dissolved amino acids (*7*, *8*) provides the alternative mechanism of complexation. We have found a striking, positive relationship between concentrations of Cu and dissolved total amino acid (TAA) in gut fluids of eight species of marine invertebrates from diverse uncontaminated habitats over a wide range of gut-fluid amino acid (AA) concentrations (*8*). Although the same study demonstrated the coexistence of Cu and AA over a range of high to low molecular weight (MW) fractions, we did not demonstrate that the coexistence is a result of Cu−AA complexes. The current study thus was designed to test more directly the role of AA in Cu bioavailability during deposit feeding. As high enzymatic activity and high AA concentrations normally appear in the same gut sections (*7*), it is necessary to probe whether Cu release is a result of enzymatic reaction or complexation by AA. We tested this possibility by microwave inactivation of gut enzymes. We then tested whether simple solutions of protein can mimic digestive fluids in the release of sedimentary Cu. Furthermore, by applying a chemical modification technique on gut fluids, we showed that a specific AA (histidine) was primarily responsible for releasing bioavailable Cu. Finally, measurements on Cu-binding constants of gut ligands and histidine concentrations among molecular weight fractions corroborated the role of gut histidine in Cu bioavailability.

Experimental Section

Incubation. Digestive fluids of three species of marine invertebrates, the polychaete *A. marina* and the holothuroids *P. californicus* and *Cucumaria frondosa*, were extracted in the manner described by Mayer et al. (*1*). During dissection, body walls of the animals were cut open with care to avoid rupturing guts. Plastic pipet tips were used to penetrate into guts of *A. marina* and remove luminal fluids. Gut fluids of larger animals, *P. californicus* and *C. frondoa*, were poured directly into a plastic beaker from an opened end of the gut. Sediment particles were removed from gut fluids by centrifugation at 8000*g* and 4 °C for 30 min. Gut fluids from multiple individuals (>10) of each species of organisms were combined and stored in 1.5-mL plastic vials at −80 °C until analysis.

Contaminated sandy, surface (5 mm) sediments were collected in intertidal zones of Boothbay Harbor (BH), ME (total [Cu] ≈ 1110 ppm), and Portsmouth Harbor (PH-1 and PH-2), NH (total [Cu] ≈ 182 and 204 ppm). Excess Cu in sediments presumably originated from nearby shipyard activities.

For in vitro digestion experiments, sediments first were centrifuged to remove most of their original pore water. Wet sediments (about 0.2 g) were then incubated with 500 μL of digestive fluids or seawater (as a control, collected from the Damariscotta Estuary, ME). The mixtures were then held 4 h at room temperature on a shaker. Control experiments also included digestive fluids without sediment. After incubation, fluids were removed from the mixtures by centrifugation at 8000*g* for 30 min at 4 °C. To test the potential role of proteins in Cu bioavailability during deposit feeding, solutions of BSA (Sigma P6529) were incubated with sediments in the same manner as in the in vitro digestion. Concentrated BSA solutions were diluted with 0.85% NaCl to achieve a series of BSA solutions with 0−1667 mM AA, encompassing of the range of TAA concentrations in gut fluids of various invertebrate species found by Mayer et al. (*7*). No contamination of Cu was found in the 0.85% NaCl solution.

Analytical Methods. Concentrations of total dissolved amino acids in gut fluids were analyzed according to Mayer

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et al. (*7*), in which the HCl-hydrolyzed samples were derivatized with orthophthaldialdehyde (OPA) followed by fluorometric detection. To determine histidine concentrations in those HCl-hydrolyzed samples, we adapted a precolumn derivatization method (*9*) in which the OPA-derivatized amino acids were separated by a reversed-phase HPLC column (Alltech Adsorbosphere OPA) and detected fluorometrically (Hitachi L-7480). Copper concentrations in fluids were determined by a graphite furnace atomic absorption spectrophotometer (GFAAS, Perkin-Elmer 5100ZL) with a coefficient of variation of less than 5%.

Ultrafiltration. To assess the relationship between TAA, histidine, and solubilization of Cu by different molecular weight (MW) fractions, a composite gut fluid of *A. marina* ($n = 24$) was separated into four fractions (i.e., >100, 10−100, 2−10, and <2 kDa) by centrifugation sequentially through three sets of ultrafiltration units (SPECTRUM), with MW cutoffs of 100, 10, and 2 kDa, respectively. To avoid clogging the membranes, gut fluids were diluted 10-fold in Nanopure water (Barnstead) before separation. Centrifugation was then conducted at 4 °C, according to the manufacturer's instructions, at 500, 1500, and 2000 g for 100, 10, and 2 kDa membranes, respectively. The separated fractions were then analyzed for AA (including histidine) by HPLC, and Cu contents were analyzed by GFAAS.

Microwave Treatment. To determine the role of enzymatic reactions in Cu release during the in vitro digestion, enzymes in gut fluid of *P. californicus* were heat killed by microwave treatment twice to boiling point. Subsequent enzyme assay (*7*) on the treated gut fluid showed no indication of protease or esterase activities, which are important enzyme species in deposit feeders. Both microwave-treated and untreated gut fluids then were incubated with BH sediments, and Cu concentrations in the digestive fluids were measured following a time course of 0, 2, 60, and 240 min.

Binding Constants and Capacities. Free cupric ion activity and pH during Cu titrations on gut fluids were measured according to ref 10 by a Cu−ISE (Orion 9629) and a pH electrode (Orion 911600) connected to an Orion EA940 ionanalyzer at 22 ±1 °C. Briefly, titration media included pH 7 0.01 M MOPS (4-morpholinepropanesulfonic acid) buffer, 0.01 M $NaNO_3$ (as ionic strength adjuster), and 1% gut fluid, yielding TAA concentrations of about 10^{-3}−10^{-4} M. Controls included Cu titrations into 1% seawater (collected from the Damariscotta Estuary, Maine) and a 1% BSA ([TAA] = 184 mM) sample. The Cu−ISE was calibrated against cupric ion buffers made of various amounts of $Cu(NO_3)_2$ in histidine or glycine (10^{-3} or 10^{-4} M) solutions based on the titration buffer media (*11*). A chemical equilibrium program, MINEQL+, was used to calculate free cupric ion activities in the buffer. No modifications were made to the MINEQL+ thermodynamic database. In no case did the slope of the calibration curve differ significantly from the theoretical 29.3 mV per decade expected at 22 °C.

A Scatchard plot (i.e., $[CuL]/[Cu]_F$ vs $[CuL]$) was generated for each Cu gut-fluid titration experiment (e.g., Figure 1), where [CuL] is the concentration of Cu−ligand complex ($[CuL] = [Cu]_T - [Cu]_F$), $[Cu]_F$ is free cupric ion activity, and $[Cu]_T$ is total Cu concentration. Each linear segment in a Scatchard plot can represent one class of ligand (*12*) according to

$$[CuL^i]/[Cu]_F = K_i' C^i_{Li} - K_i'[CuL^i] \quad (1)$$

where K_i' is the conditional Cu-binding constant of the ith class gut ligand under our experimental conditions (assuming a 1:1 ratio of Cu to L^i), and C^i_{Li} represents the Cu complexation capacity of the ligand. However, this discrete ligand model can be used only to solve unknown parameters for up to three classes of ligands (*12*), which is obviously unrealistic

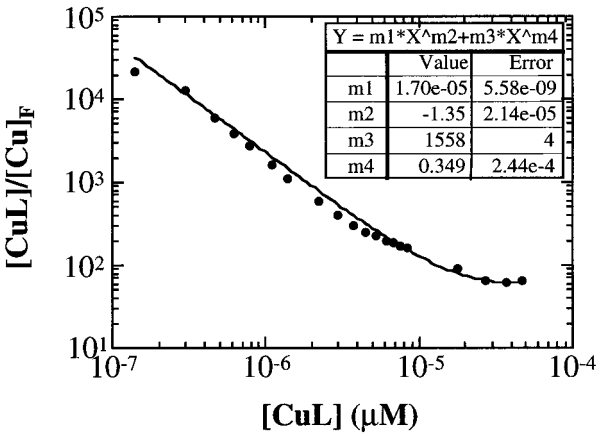

FIGURE 1. Scatchard plot for Cu^{2+} titration on digestive fluid of *P. californicus*. Closed circles = experimental data that were curve-fitted with KaleidaGraph. Curve-fitting parameters are listed in the inset table.

for a complex ligand system such as gut fluids. We modeled the Cu-binding ligands in gut fluids as a suite of binding sites with continuously distributed K values, which is a function of Cu:ligand ratios in titration media. This continuous model has been applied to Cu−humate interactions successfully (*12*). Under such a model, each point of the curve in Scatchard plot could be viewed as one class of ligand described by the tangent at that point, and each ligand can be described by a different equation as in eq 1.

Approximations of the curves in the Scatchard plots were determined by curve-fitting the experimental data with a computer program (KaleidaGraph). We found that among common functions, a double-power function could best fit the curves:

$$Y = aX^b + cX^d \quad (2)$$

where $X = [CuL]$, Y is the fitted value of $[CuL]/[Cu]_F$, and $a-d$ are fitting parameters determined by KaleidaGraph. The tangent of each point on the fitted curve (eq 2) equals the derivative of Y:

$$dY/dX = abX^{b-1} + cdX^{d-1} \quad (3)$$

By considering eq 1 without class i, we arrived at the continuous function $K' = -dY/dX$ and $C_L = ([CuL]/[Cu]_F + K'X)/K'$.

Histidine Blocking. In many metalloenzymes histidine plays an important role in formation of metal-binding and catalytic sites. This role can be identified by selective chemical modification of histidine residues while monitoring enzymatic activity or metal-binding capability (*13*−*16*). One widely used histidine-blocking agent is diethylpyrocarbonate (DEPC), which reacts with histidine residues to form *N*-(carbethoxy)histidine (C-histidine). The modification is relatively specific for histidine residues between pH 5.5 and pH 7.5 (*17*), so we used 0.05 M pH 7.0 phosphate buffer (VWR) as the modification media. A successful modification requires that the molar ratio of DEPC to histidine is >5, and reactions last for ≥15 min (*17*). For the purpose of this study, the molar ratio of DEPC to histidine was set at about 200, and reaction time was 1 h.

Stock DEPC (Aldrich) was freshly prepared every day with dry ethanol, with a concentration of about 300 mM. Digestive fluids of *A. marina* and *P. californicus* were first diluted 500× and 10×, respectively, with pH 7 phosphate buffer, yielding histidine concentrations on the order of 0.01 mM. To start the reaction, 5−10 μL of DEPC stock solution was spiked into 1 mL of the diluted digestive fluids, and the reactants

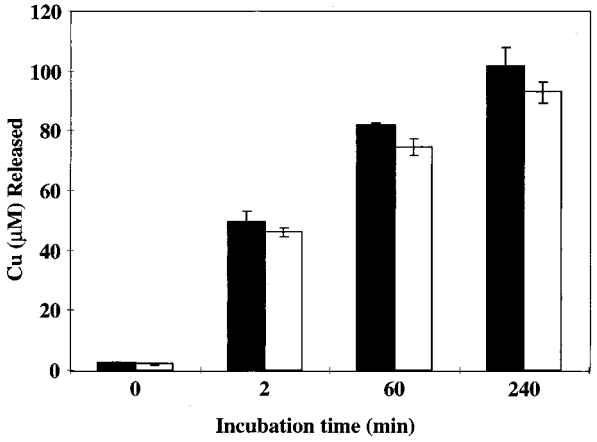

FIGURE 2. Amount of contaminated Cu released by microwave-treated and untreated gut fluids from *P. californicus*. Solid bar = untreated, open bar = treated. Error bars are ±1 SD of the data.

were incubated at room temperature for 1 h. Control treatments included spiking DEPC into 0.01 mM histidine solutions and phosphate buffer. To test the role of histidine in Cu bioavailability during in vitro digestion, 2 mL of either DEPC-treated or untreated fluids were then incubated with 0.08 g of wet BH sediments for 2 h. The high sediment to fluid ratios (>0.4 g wet sediment/mL gut fluids) ensure that the amount of Cu in sediment was sufficient to exhaust the binding sites in incubation fluids within the time frame of incubation (data not shown).

Results and Discussion

The Role of Enzymatic Reactions in Cu Bioavailability. No significant difference (2-way ANOVA, $p = 0.08$) in the amount of Cu extracted was found between microwave-treated (no enzyme activity) and untreated *P. californicus* gut fluids (Figure 2). Therefore, we conclude that the release of bioavailable Cu is likely a simple complexation reaction, which is uncoupled with enzymatic activity per se.

BSA Incubation Experiments. Increase in BSA (i.e., TAA) concentrations led to increased Cu release, with a gradual leveling off at high BSA concentrations to various extents among the three Cu-contaminated sediments (Figure 3). The leveling off of Cu release with increasing TAA concentration, which is most obvious in sediment PH2 (Figure 3c), strongly suggests limitation of one reactant—the sedimentary Cu. Although gut TAA concentrations differed by more than 1 order of magnitude between the polychaete and the holothuroids, the amounts of Cu extracted by gut fluids agree very well with the BSA extractions, although they are usually higher up to a factor of 2 (Figure 3). Because BSA solutions do not contain active digestive agents (i.e., surfactants and enzymes; *7*), this result corroborates our previous interpretation that the close TAA−Cu relationship in gut fluids of invertebrates under field conditions was due to complexation (*8*).

Chemical Modification of Histidine Residues in Gut Fluids. Validity of DEPC in modifying gut histidine residues was tested by incubating 30 μM DEPC-treated and untreated histidine solutions with BH sediment. This experiment showed nearly 100% depression of Cu release (Figure 4) by DEPC treatment, which suggests that DEPC blocked the binding sites of imidazole in histidine and resulted in a C-histidine solution having no more Cu-releasing capability than the pH 7 phosphate buffer. A solution of 30 μM glycine, an amino acid having just a terminal carboxyl and an amino group, dissolved only slightly more Cu than the buffer (data not shown), demonstrating that the most common amino acid functionality is ineffectual in Cu dissolution. DEPC

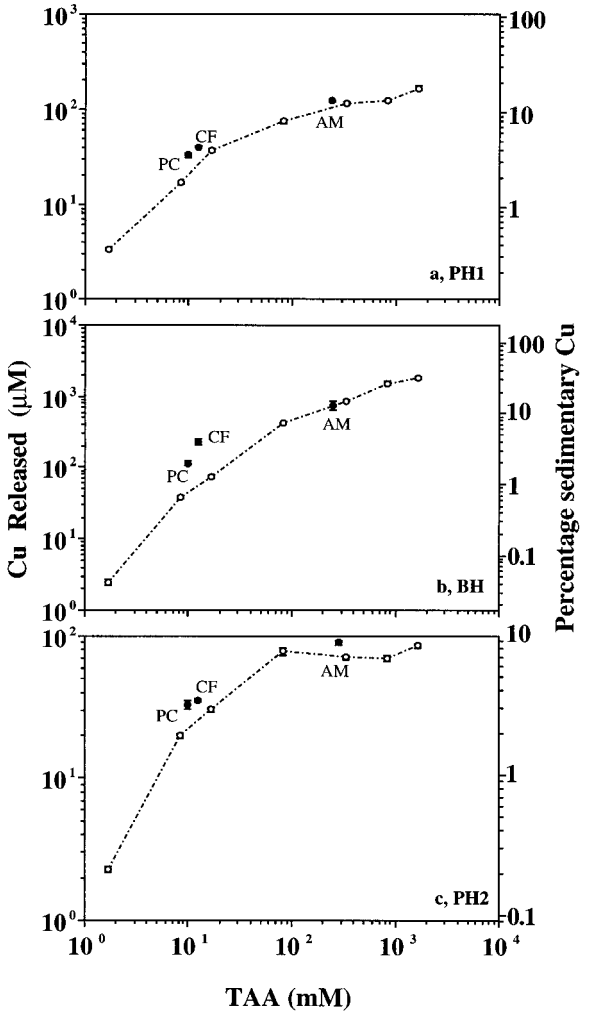

FIGURE 3. Amount of contaminated Cu released after incubation of BSA and gut fluids with Cu-contaminated sediments PH1(a), BH(b), and PH2(c). Open circles = BSA, closed circles = gut fluids. Error bars are ±1 SD of the Cu concentrations. AM, PC, and CF = gut fluids of *A. marina*, *P. californicus*, and *C. frondosa*.

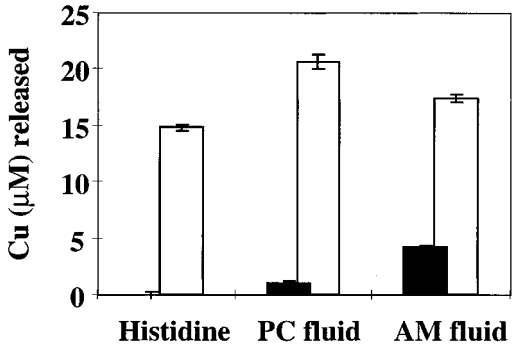

FIGURE 4. Amount of Cu released by DEPC-treated and untreated fluids from contaminated sediment BH. Solid bar = treated, open bar = untreated. Error bars are ±1 SD of the Cu concentrations. Histidine (30 μM) was dissolved with pH 7 phosphate buffer, while digestive fluids of *P. californicus* (PC) and *A. marina* (AM) were diluted to 10× and 500× with the buffer to achieve similar histidine concentrations. Cu release during control experiment of phosphate buffer with sediment was subtracted from each treatment.

treatments of the digestive fluids also led to dramatic declines in the amounts of Cu released (Figure 4), which suggests that histidine residues in gut AA are responsible for 90 and 75% of the released Cu in gut fluids of *P. californicus* and *A. marina*,

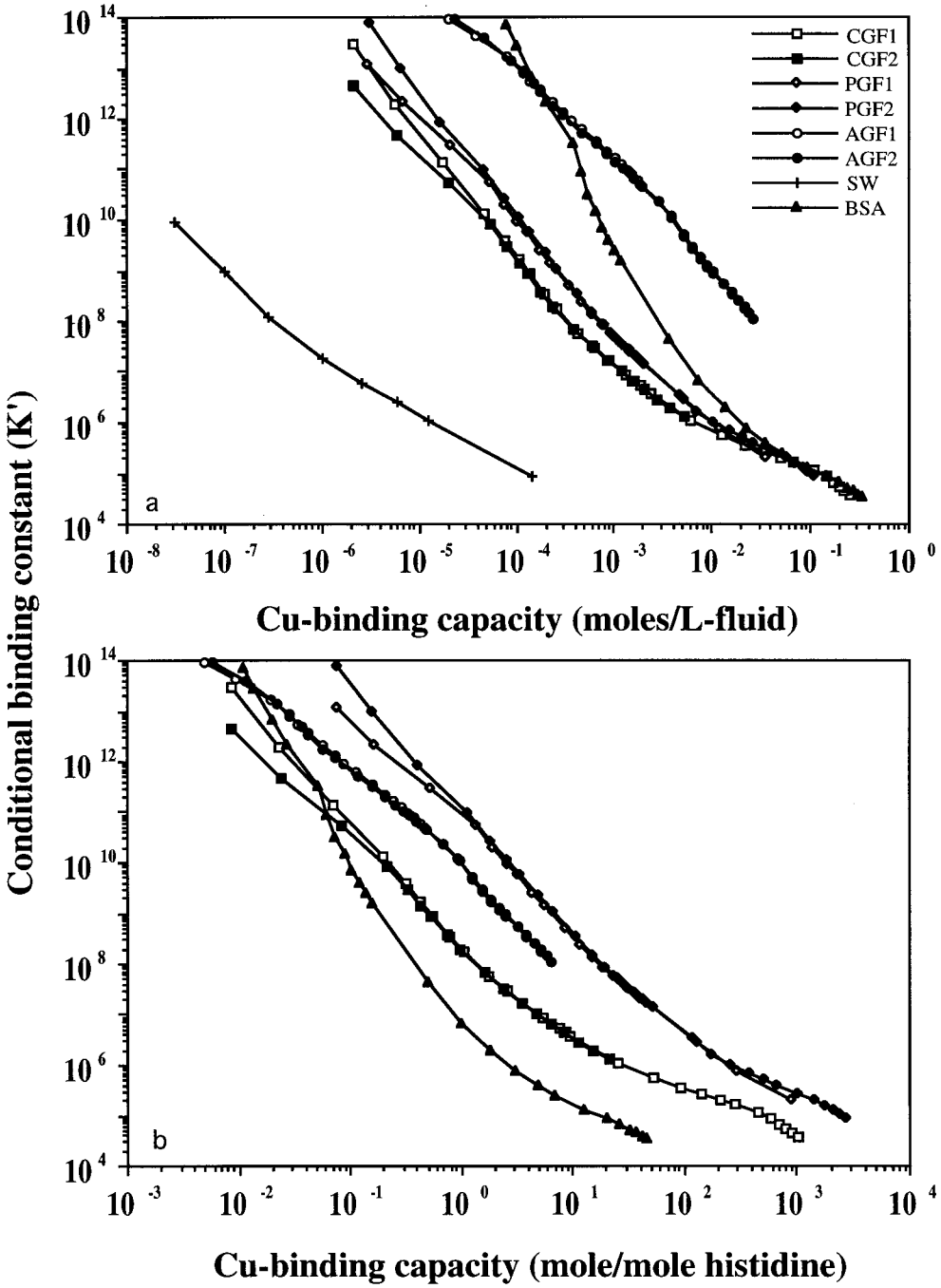

FIGURE 5. Distribution of conditional binding constant and binding capacity in gut fluids determined by Cu titration. The Cu-binding capacity was expressed as moles of Cu per liter of fluids (a) and moles of Cu per mole of histidine (b). Amino acids concentrations in the titration fluids were 12.4 mM for *C. frondosa* (square), 9.5 mM for *P. californicus* (diamond), 242 mM for *A. marina* (circle), and 184 mM for BSA (triangle). Cross = seawater. Open and closed symbols were from replicated experiments.

respectively. Although this experiment indicates the most important AA residue, we cannot exclude the role of other AA residues in Cu solubilization during deposit feeding. The complementary 10 and 25% Cu released by these gut fluids (Figure 4) imply the involvement of either other AA residues or perhaps agents other than AA. For example, chemical modification experiments showed that carboxylate groups present in the cell walls of *Datura innoxia* accounted for significant Cu binding (*18*). Although histidine is likely the most important AA residue for Cu release, other agents or AA residues may be more important in releasing other sedimentary metals such as Pb and Cd.

Cu-Binding Constants in Gut Amino Acids. A plot of Cu-binding capacities (C_L) vs conditional binding constants (K') in fluids shows a continuous inverse trend (Figure 5a,b), indicating the presence of strong binding sites ($K' > 10^{10}$) whose concentrations were lower than those of weak binding sites. Limitation in the sensitivity of our method did not allow detection of low concentrations ($<1.6 \times 10^{-8}$ M) of stronger ligands ($K' > 10^{14}$), while $Cu(OH)_2$ precipitation at high Cu concentrations interfered with the measurement of weaker ligands ($K' < 10^4$). Within our detection window of K' (10^4–10^{14}, Figure 5), Cu-binding capacities (C_L) in the seawater sample were several orders of magnitude lower than in both digestive fluids and BSA for the same K' (Figure 5a). Because digestive fluids have similar salinity (*8*), this result indicates that seawater components contribute a negligible amount of the observed C_L in gut fluids. The slope of the

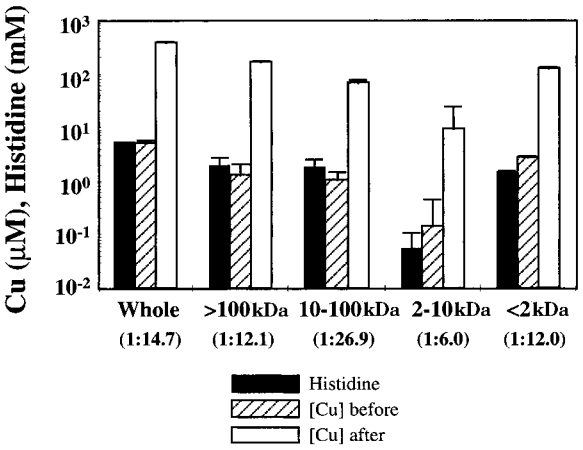

FIGURE 6. Distribution of Cu (μM) and histidine (mM) among various molecular weight fractions in gut fluid of *A. marina* before and after incubation with sediment PH1. Solid bar = histidine, hatched bar = Cu before incubation, open bar = Cu after incubation. Whole = the whole gut fluid. Error bars are ±1 SD of the data. Data in parentheses show the ratios of Cu:histidine in respective molecular weight fractions after incubation.

BSA curve appears much steeper than both gut fluids and seawater samples, suggesting that the ligands in BSA are less diverse than in gut fluids (Figure 5a). C_L values were over 1 order of magnitude higher in polychaete than in holothuroid gut fluids (Figure 5a), which is consistent with the order of TAA concentrations (*8*) and the amounts of Cu released (Figure 3).

Literature results on Cu^{2+}-binding constants of histidine (K') vary mainly according to Cu:histidine ratios, from $10^{10.2}$ (ML) to $10^{18.1}$ (ML_2) (*19*). The detection of strong binding ligands (i.e., $K' \geq 10^{10}$) in gut fluid samples thus corroborates the results of the histidine blocking experiments (Figure 4).

If histidine is the only Cu-binding ligand in gut fluids, the curves in Figure 5a should collapse into a single cluster when K' is plotted against the histidine-normalized C_L (Figure 5b). This normalization does reduce the variation in binding capacities by 1 or 2 orders of magnitude but still leaves a range of as much as 3 orders of magnitude, particularly for strong binding sites. The divergence among $K'-C_L$ curves (Figure 5b) is consistent with the presence of other Cu-binding ligands in gut fluids. In this scenario, there are more non-histidine ligands in gut fluids of *P. californicus* than in *A. marina* or *C. frondosa* (Figure 5b). Alternatively, the results in Figure 5b may imply that histidine is present in different configurations in different gut fluids, with some histidine residues more accessible to Cu ions or more able to join with other, non-histidine ligands than the others. For example, ligands embedded in hydrophobic domains of proteins may not be as effective as those exposed to solution (*20*).

Cu Distribution among MW Fractions. Histidine was found in all of the MW fractions with concentrations of a few millimolar, except in the 2−10 kDa fraction, which was 1 order of magnitude lower (Figure 6a). This pattern is consistent with the distribution of TAA, and histidine accounts for about 2−4% of TAA in each fraction (data not shown). As TAA concentrations in each fraction remained unchanged after incubating with the contaminated sediment PH1 (data not shown), we assume that the histidine distribution in Figure 6a represents its concentration both before and after the incubation experiments.

The distribution of Cu before incubation closely followed that of histidine, with a Cu:histidine molar ratio on the order of $1:10^3$. Assuming that 1 mol of histidine provides 1 mol of Cu-binding site, Cu loading in gut fluids could increase up to $10^3\times$. Incubation of the gut fluid with contaminated sediment resulted in increasing Cu concentrations in all of the MW fractions, but only by about $10^2\times$. This result suggests that more Cu could be solubilized if additional contaminated sediments are added to the system. The amounts of Cu dissolved during an in vitro digestion thus would be determined by both the sedimentary pool sizes and the concentration of gut ligands, probably by the ratios of the two as suggested by BSA incubation experiments (Figure 3). Heterogeneous sediments may also present a wide array of Cu-binding phases (*21*) with perhaps continuously distributed binding constants, as with gut fluids. Redistribution of Cu during an in vitro incubation, in a form of ligand exchange reaction, thus may depend on the concentrations and K' distributions of liquid (gut fluids) and solid (sediment) ligands. Histidine residues represent a family of strong ligands in gut fluids, which may play an important role in outcompeting sedimentary binding sites. Strong Cu-binding phases in sediments may include sulfides (AVS), the concentrations of which are important in the toxicity of Cu, Zn, and Cd (*22*). Thus, Cu bioavailability in sediments may be determined by the concentrations of strong ligands in gut fluids that is a function of the species of organism, the amount of sedimentary Cu partitioned to weakly bound phases, and the kinetics of ligand exchange.

However, an overview of the ISE and MW data suggests that simple complexation of monomeric histidine to Cu cannot fully account for the observed pattern of Cu dissolution. The remnant Cu-binding capacity after DEPC treatment (Figure 4) and the divergence of C_L per histidine among gut fluids (Figure 5b) indicate that the role of nonhistidine residues could be significant.

Implications. Histidine is one of the common 20 AA and makes up about 2% of proteins (*23, 24*, unpublished data). It is well-known that in proteins histidine residues serve as one of the most important ligands for binding borderline metals (*25*). Recent studies on the role of histidine as a nickel carrier in plant systems (*26*) and a Cu-binding site on the surface of bacteria (*27*), in addition to this study, suggest that histidine may play an important role in bioavailability and bioaccumulation in certain biological systems. For example, low MW histidine−metal complexes enhance gut uptake of metal in some organisms (*28*−*30*). In a population of *A. marina*, AA-normalized tissue metal concentrations positively correlated with those in gut fluids (*8*), suggesting the importance of AA in transporting metals between external and internal pools. In addition, we found that gut amino acids appear responsible for detoxification of excess Cu to digestive proteases of deposit feeders (*10*). The DEPC experiments in this study indicate that histidine residues must be primarily responsible for this detoxification.

In summary, solubilization of sedimentary Cu by AA residues in gut fluid of deposit feeders may account for bioavailability and bioaccumulation of Cu and other transition metals via ingestion. Toxic effects derived from this process could further affect the population and biodiversity in this functional group of organisms in situ. Ingestion and digestion of deposit feeders may be an unappreciated link in metal biogeochemical cycling in sediments, given the large magnitude of solubilization in comparison to that found in early diagenesis (*31*).

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Literature Cited

(1)) Mayer, L. M.; Chen, Z.; Findlay, R. H.; Fang, J.; Sampson, S.; Self, R. F. L.; Jumars, P. A.; Quétel, C.; Donard, O. F. X. *Environ. Sci. Techol.* **1996**, *30*, 2641.

(2) Thomann, R. V.; Mahony, J. D.; Mueller, R. *Environ. Toxicol. Chem*. **1995**, *14*, 1989.

(3) Kaag, N. H. B. M.; Foekema, E. M.; Scholten, M. C. T.; van Straalen, N. M. *Environ. Toxicol. Chem*. **1997**, *16*, 837.

(4) Ruby, M. V.; Davis, A.; Kempton, J. H.; Drexler, J. W.; Bergstrom, P. D. *Environ. Sci. Technol.* **1992**, *26*, 1242.

(5) Ruby, M. V.; Davis, A.; Schoof, R.; Eberle, S.; Sellstone, C. M. *Environ. Sci. Technol.* **1996**, *30*, 422.

(6) Plante, C. J.; Jumars, P. A. *Microb. Ecol.* **1992**, *23*, 257.

(7) Mayer L. M.; Schick, L. L.; Self, R. F. L.; Jumars, P. A.; Findlay, R. H.; Chen, Z.; Sampson, S. *J. Mar. Res.* **1997**, *55*, 785.

(8) Chen, Z.; Mayer, L. M.; Sampson, S.; Quétel, C.; Donard, O. F. X.; Self, R. F. L.; Jumars, P. A. Submitted for publication in *Limnol. Oceanogr.*

(9) Jarrett, H. W.; Cooksy, K. D.; Ellis, B.; Anderson, J. M. *Anal. Biochem*. **1986**, *153*, 189.

(10) Chen, Z.; Mayer, L. M. *Environ. Chem. Toxicol.* In press.

(11) Hoyer, B. *Talanta* **1991**, *38*, 115.

(12) Fish, W.; Dzombak, D. A.; Morel, F. M. M. *Environ. Sci. Technol.* **1986**, *20*, 676.

(13) Morgan, W. T. *Biochemistry* **1985**, *24*, 1496.

(14) Drabikowska, A. K.; Wozniak, G. *Biochem. J.* **1990**, *270*, 319.

(15) Blanke, S. R.; Hager, L. P. *J. Biol. Chem*. **1990**, *265*, 12454.

(16) Meyer, S. E.; Cromartie, T. H. *Biochemistry* **1980**, *19*, 1874.

(17) Lundblad, R. L. *Techniques in Protein Modification*; CRC Press: Boca Raton, 1995; Chapter 9.

(18) Drake, L. R.; Lin, S.; Rayson, G. D. *Environ. Sci. Technol.* **1996**, *30*, 110.

(19) Martell, A. E.; Smith, R. M. *Critical Stability Constants, Volume 5: First Supplement*; Plenum Press: New York, 1982; pp 31−32.

(20) Sun, H.; Ma, X.; Wang, T.; Li, R.; Wang, K. *Main Group Metal Chem*. **1994**, *17*, 665.

(21) Luoma, S. N.; Jenne, E. A. In *Biological Implications of Metals in the Environment*; Wildung, R. E., Drucker, H., Eds.; NTIS: Springfield, VA, 1977; pp 213−230.

(22) Ankley, G. T.; Thomas, N. A.; Di Toro, D. M.; Hansen, D. J.; Mahony, J. D.; Berry, W. J.; Swartz, R. C.; Hoke, R. A.; Garrison, A. W.; Allen, H. E.; Zarba, C. S. *Environ. Manage.* **1994**, *18*, 331.

(23) Morgan, W. T. *Biochim. Biophys. Acta* **1978**, *533*, 319.

(24) Thurman, E. M. *Organic geochemistry of natural waters*; Martinus Nijhoff/Dr W. Junk Publishers: Dordrecht; 1985; pp 151−180.

(25) Gregory, D. S.; Martin, A. C. R.; Cheetham, J. C.; Rees, A. R. *Protein Eng.* **1993**, *6*, 29.

(26) Kramer, U.; Cotter-Howells, J. D.; Charnock, J. M.; Baker, A. J. M.; Smith, J. A. C. *Nature* **1996**, *379*, 635.

(27) Mohl, W.; Motschi, H.; Schweiger, A. *Langmuir* **1988**, *4*, 580.

(28) Palida, F.; Waldrop, G.; Ettinger, M. In *Metal Ion Homeostasis: Molecular Biology and Chemistry;* Hamer, D. H., Winge, D. R., Eds.; Alan R. Liss, Inc.: New York, 1989; pp 449−458.

(29) Ashmead, H. D. In *Biological Trace Element Research: Multi-disciplinary Perspectives*; Subramanian, K. S., Iyengar, G. V., Okamoto, K., Eds.; ACS Symposium Series 445; American Chemical Society: Washington, DC, 1991; pp 306−319.

(30) Pecon, J.; Powell, E. N. *Bull. Environ. Contam. Toxicol.* **1981**, *27*, 34.

(31) Aller, R. C. *Am. J. Sci.* **1978**, *278*, 1185.

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