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Influences of Natural Colloids on Metal Bioavailability to Two Marine Bivalves

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We examined the bioavailability of colloid-bound metals [Cd, Cr(III), and Zn] to two marine bivalves (mussel *Perna viridis* and clam *Ruditapes philippinarum*) from subtropical and tropical waters. Natural colloids (between 1 nm and 0.2 μm) were isolated by cross-flow ultrafiltration. Bivalves were then exposed to radiolabeled colloids, and the uptake of colloid-bound metals was compared with the uptake of metals associated with the low molecular weight fraction (LMW, <1 kDa). In general, the bioavailability of colloid-bound Zn to mussels was significantly inhibited as compared to that of the LMW-bound Zn. Its uptake decreased with increasing colloidal organic carbon (COC) concentration. There was no major difference in Cd bioavailability between the LMW and the colloidal treatments, primarily because most of the radiolabeled colloidal Cd remained in the truly dissolved phase during the uptake period. In contrast, the bioavailability of colloid-bound Cr was enhanced in the mussels. In clams, bioavailability of metals was not significantly influenced by colloidal binding, although Zn uptake was slightly enhanced when it was associated with the LMW fraction. The measured dry weight concentration factor (DCF) in bivalve tissue was the highest for Zn, followed by Cd and Cr. Furthermore, DCF was higher in the mussels (20−340) than in the clams (10−35). Colloid-bound metals were mostly accumulated in mussel digestive gland and remaining soft tissue (64−87%), whereas a large fraction (36−73%) of metals was found on the shell of the clams. Coagulation of radioactive tagged colloidal organic matter was insignificant (<9%) for metals in the absence of large suspended particles, indicating that coagulation effects on metal uptake were minimal under our experimental conditions. Thus, our study demonstrated that colloid-bound metals were bioavailable to both the mussels and the clams, but the influences of colloidal binding on metal uptake varied among metals and between the two bivalves.

Introduction

Aquatic colloids are operationally defined as particles in the size fraction between 1 nm and 0.2 μm (*1*). Recent studies have demonstrated that colloidal organic matters are abundant in natural seawater, ranging from 60−70% of dissolved organic matter in estuarine waters to 20−40% in coastal waters (*2*) and can significantly influence the biogeochemical cycling of carbon and metals in marine environments (*2*−*5*). The colloidal-sized macromolecular organic matter, which is polyfunctional and polydispersive (*6*), can strongly bind with certain trace metals and trace organics (*5, 7*−*9*) and thus can influence the bioavailability of trace metals to aquatic organisms (*10*−*12*). Recent studies have shown that a significant but variable fraction of traditionally defined dissolved metals is in the colloidal form (*5, 9, 13*−*15*). The bioavailability of colloid-bound trace metals to aquatic organisms remains largely unstudied but is important for our understanding of the biogeochemical cycling of metals (such as contaminant transport) in aquatic systems. For example, it is still unclear whether the complexation of metals with colloidal organic matter will enhance or reduce the availability of metals to aquatic organisms (*10*). If the colloidally complexed metals can indeed be bioavailable to the organisms, there is a need to examine the transport of metals (e.g., colloidal ingestion, adsorption, pinocytosis), which may be important for the further development of bioaccumulation models (*16*).

Marine bivalves such as mussels and oysters have long been employed as pollution biomonitors in coastal environments due to their exceedingly high pumping activity and their often proportional responses to ambient pollutant concentrations (*17*−*19*). Recently, there have been extensive studies on metal bioavailability and bioaccumulation in bivalves from both particulate and dissolved phases (*20, 21*). It has been shown that the relative importance of metal uptake from the dissolved and particulate phases is greatly dependent on the ambient geochemical conditions as well as the physiological condition of the animals (*22*). For some metals such as Cd, a significant fraction of metals can be obtained from the dissolved phase, whereas for other metals such as Se, almost all the metal is obtained from ingestion of food particles. In these previous studies, the dissolved phase was generally defined as the fraction passing through the 0.2-μm membrane and thus included the colloidal phase (*9*). Given the recent finding that a considerable fraction of metals is associated with the colloids, it is necessary to examine metal uptake from the colloidal phase and from the truly dissolved phase. Furthermore, it is unclear whether bivalves can pump and retain colloidal-sized particles, which may greatly affect metal bioavailability from the traditionally defined dissolved phase.

Past difficulties in studying the bioavailability of colloid-bound metals to aquatic organisms mainly stemmed from the isolation of natural organic colloids and the re-partitioning of colloidal metals while being dispersed into the experimental systems. The use of cross-flow ultrafiltration has made it feasible to isolate large amount of colloids from seawater. Organic colloids can then be labeled with radiotracers and exposed to the organisms (*10*). However, some of the radiolabeled metals may re-partition into the dissolved phase during the exposure period, thus complicating the interpretation of bioaccumulation results. Although Carvalho et al. (*10*) recently concluded that colloidally complexed metals could be bioavailable to brown shrimp, whether metals were re-partitioned between the dissolved and the colloidal phases after the colloids were introduced into the low molecular weight (LMW) seawater remained to be further examined.

The overall objective of this study was to examine the bioavailability of colloidally complexed metals (Cd, Cr, and Zn) to two marine bivalves commonly found in subtropical and tropical waters, including green mussels (*Perna viridis*) and clams (*Ruditapes philippinarum*). We used radiotracer

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and ultrafiltration techniques to measure metal uptake from the colloidal phase. A short-term exposure was employed to measure the kinetics of metal uptake in these bivalves. Coagulation of colloids and partitioning of metals between the colloidal and the 1 kDa ultrafilter passing phases were also monitored during the exposure period.

Materials and Methods

Bivalves. Bivalves used in this study included green mussels (*Perna viridis*) and clams (*Ruditapes philippinarum*). Both species are abundant in the subtropical and tropical waters and have been used as biomonitors of coastal contamination in these regions (*18, 23*). The mussels (3.5 cm shell length) and the clams (3 cm shell length) were collected from intertidal areas in Ma On Shan, Tolo Harbour, Hong Kong (seawater temperature of 27 °C and salinity of 28‰). These bivalves were maintained in the laboratory at 24 °C and 30‰ for about 1 week before the uptake experiments. During this acclimation period, they were fed with the diatom *Thalassiosira pseudonana*. Bivalves were starved overnight to defecate any feces and removed from the aquarium and placed in seawater (<1 kDa ultrafiltered permeate, described below) for about 2 h before the uptake experiments. Gut passage time of food particles in these bivalves was generally <3 h (*24*).

Metals. Three trace metals [Cd(II), Cr(III), and Zn(II)] were considered in our study. Metals added were in the radioactive forms, ^{109}Cd, ^{51}Cr, and ^{65}Zn (from New England Nuclear). These metals are representative of type-B and transitional metals. For example, Cr is considered as a transitional metal that prefers to bind with O-containing ligands while Cd and Zn are transitional or type-B metals that prefer to bind with S-containing ligands rather than with N- or O-containing ligands (*25, 26*). In estuarine environments, considerable fractions of these metals have been found to associate with the colloidal organic matter (*5*) or organic chelated forms (*27*).

Isolation of Colloidal Organic Matter and Radiolabeling. Seawater (with a DOC concentration of 125 μM and a salinity of 30‰) was collected from Tai Chek Mun, Tolo Harbour, Hong Kong, and prefiltered through a 0.2-μm Nuclepore cartridge. Colloids were then isolated from the prefiltered seawater using a 1 kDa spiral-wound cross-flow ultrafiltration cartridge (Amicon S10Y1) (*28, 29*). Briefly, the cartridge was precleaned with 0.05 M NaOH solution, 0.05 N HNO_3, and large volumes of ultrapure water between and after cleaning solutions. The whole ultrafiltration setup was kept in a closed system to minimize the exposure time to the air. A concentration factor of 40 was used to isolate colloidal organic matter (COM), which has been recommended by previous studies (*28, 30*). The isolation of colloidal organic matter was carried out immediately before the radiolabeling. Concentrations of both dissolved (DOC) and colloidal organic carbon (COC) were quantified by a high-temperature combustion method (*31*).

The concentrated COM was spiked with radioisotopes ^{109}Cd, ^{51}Cr(III), and ^{65}Zn in a Teflon jar. The radioactive metals added into the Telfon jar corresponded to 74 nM for ^{109}Cd, 1.5 nM for ^{51}Cr(III), and 74 nM for ^{65}Zn. Because these radioisotopes were carried in acidic 0.1 N HCl solution, microliter amounts of Suprapur NaOH were added to maintain the pH at normal seawater values. After 15 h complexing with COM, the fraction of uncomplexed or the <1 kDa radioactive metals was "squeezed" out by ultrafiltration (1 kDa spiral-wound cross-flow ultrafiltration cartridge, Amicon S10Y1) again to get the radioactive labeled COM for uptake experiments. A concentration factor of 3 was used during the isolation of radiolabeled colloids. The fraction of radioactive metals associated with COM did not increase with time over this equilibrium time. Bivalves were then exposed to spiked colloids at two different COC concentrations (62 and 186 μM, Table 1). In the control or LMW treatment, the unlabeled permeate containing LMW DOC (or truly dissolved DOC with molecular weight <1 kDa) was spiked with the same radioactive metals at similar levels as in the HMW treatments. The metals in the spiked permeate were considered to be in LMW or free ionic form, which was used to compare with the uptake of high molecular weight (HMW) complexed metals. Both the radiolabeled permeate (i.e., LMW fraction) and colloids (or HMW fraction) were immediately used for coagulation and uptake experiments described below.

TABLE 1. Dissolved Organic Carbon (DOC) and Colloidal Organic Carbon (COC) Concentrations Used in Coagulation and Uptake Experiments

group	treatment	background DOC (μM)	COC (μM)
LMW	control	66	
C1	colloidal	64	62
C2	colloidal	61	186

Coagulation of Radiolabeled Colloids. Coagulation of HMW DOM in marine environments is an important physical chemical process (*32*). Indeed, colloidal pumping through coagulation has been considered as an important mechanism in trace metal scavenging in the ocean (*33, 34*). During the uptake experiments, it was possible that colloids might coagulate and form a new particulate phase, thus enhancing metal bioavailability due to the filtering activity of the animals. To examine this possibility, an experiment was first carried out to assess the extent of colloidal coagulation under our experimental conditions. Radiolabeled colloids were dispersed into 200 mL of LMW seawater (with a background DOC concentration of 66 μM) at two different COC concentrations (62 and 186 μM, Table 1). There were two replicates for each treatment. Over a period of 17 h, the fractions of radioisotopes in the >0.2-μm particulate phase due to coagulation were quantified by filtering a 10-mL aliquot through a 0.2-μm polycarbonate filter.

Uptake of Colloid-Bound Metals by Mussels and Clams. There were three different treatments in bivalve uptake experiments: (i) LMW permeate or free ionic treatment (LMW), (ii) HMW treatment with a COC concentration of 62 μM (C1), and (iii) HMW treatment with a COC concentration of 186 μM (C2) (Table 1). Radioisotope additions in both LMW and colloidal treatments corresponded to a concentration of 0.34−0.92 nM for Cd, 11−33 pM for Cr(III), and 0.45−1.50 nM for Zn. No stable metal was added during the uptake experiment. There was a possibility that the amount of stable metals associated with the isolated colloids may have increased the total metal concentration in uptake experiments due to the added COM. However, using a COC concentration of 62 μM as an example, the amounts of stable metals introduced from the added COM were about 2.6 pM for Cd, 5 pM for Cr, and 0.42 nM for Zn based on the average metal concentrations in isolated estuarine COM samples (*35*) and the amounts of COM added. These concentration levels are lower than or similar to those we added as radioactive tracers. Therefore, the stable metal concentrations in the isolated colloids should not significantly change the ambient Cd and Cr concentration after the colloids were re-dispersed into the LMW seawater.

There were five replicate individual mussels or clams in each experimental treatment. Each individual mussel or clam was placed in a 1-L polypropylene beaker containing 400 mL of LMW seawater with different concentrations of radiolabeled colloids (Table 1). At each time interval (2, 4, 6, and 8 h), bivalves were removed and rinsed with unlabeled

permeate seawater, and the radioactivity in each individual bivalve was determined nondestructively. A short-term exposure period was employed to minimize the decline of metal concentration in the exposure medium, the decline of bivalve's filtration activity, and the change of medium chemistry due to excretion of bivalve's metabolites (*21*). Bivalves were then returned to each beaker after measurement of radioactivity. The water was not renewed each time when the radioactivity of bivalves was counted. After the 8-h exposure period, the decline of radioactivity in the water due to bivalve's uptake was minimal, i.e., 2.2−4.9% for Cd, 1.7−3.6% for Cr, and 2.9−8.5% for Zn in the mussel experiment and 1.6−1.9% for Cd, 1.3−2.8% for Cr, and 1.9−3.1% for Zn for clam experiment, in all different treatments. By the end of 8-h exposure, each individual was then dissected into shell, digestive gland, and remaining soft tissue. The radioactivity associated with these body parts was determined. The dry weights of the tissue were quantified after drying at 80 °C for 1 d. The dry weight concentration factor (DCF) of tissue, which represented the *absolute* (or actual) uptake by the bivalve's tissue and can be used to quantify the bioavailability of metals, was calculated at 8 h of exposure, by the following equation:

$$\mathrm{DCF} = C_t/C_w \quad (1)$$

where C_t is the radioactivity of metals in the tissue divided by the dry weight of the tissue (dpm kg^{-1} tissue dry weight) and C_w is the initial radioactivity of metals in the water (dpm L^{-1} water). Previous studies have consistently demonstrated that short-term metal uptake in marine bivalves (including *P. viridis* and *R. philippinarum*) was directly proportional to the metal concentration in the dissolved phase (one rationale underlying the use of bivalves as biomonitors), and thus the calculated concentration factor should be relatively independent of the ambient metal concentration in the environment (*21, 36, 37*).

Because a considerable fraction of metals was associated with the shells during the uptake period, it was not possible to calculate the time-dependent dry weight concentration factor of tissue based on measurements of the radioactivity in whole individual bivalve. However, we calculated the "accumulation index" of whole individual bivalve by the following equation:

$$\text{accumulation index} = C_b/C_w \quad (2)$$

where C_b is the radioactivity of metals in *whole* individual bivalve (dpm), measured by our kinetic study and C_w is the radioactivity of metals in the water (dpm L^{-1} water). It is important to note that the accumulation index calculated from eq 2 also included shell uptake (adsorption and absorption) and cannot be used to indicate the absolute (or true) uptake of metals by the bivalve tissue.

One piece of information necessary for the interpretation of uptake results was the re-partitioning of colloidal metals in the truly dissolved and colloidal phases following resuspension of radiolabeled colloids into the LMW waters. A stirred cell ultrafiltration unit with 1 kDa membranes (Amicon YM1) was used to examine the partitioning of each metal between the colloidal and the 1 kDa ultrafilter passing phases by the end of 8-h exposure period at a pressure of 60 kPa. The concentration factor used was 20. Metals in the >0.2-μm fraction in colloidal treatments were only quantified to evaluate possible coagulation (see previous section) but were not measured in those with animal exposure. However, it has been shown that the presence of bivalves did not contribute to particle formation (>0.2 μm) during a short-term metal uptake period (*21, 37−39*).

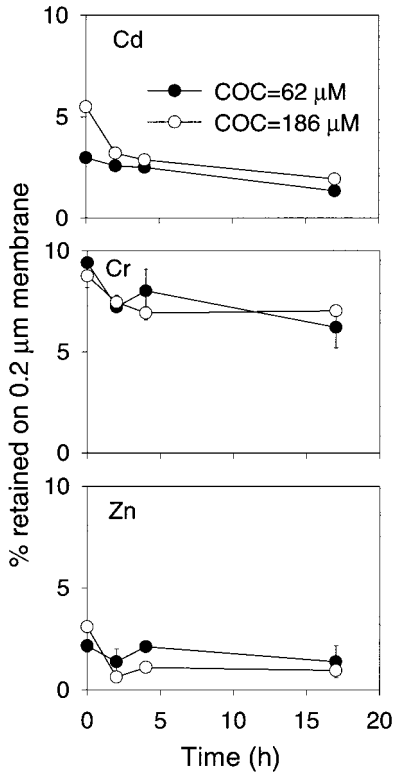

FIGURE 1. Colloidal (1 kDa-0.2 μm) coagulation, quantified by the fraction of metals retained by the 0.2-μm filter, during the 17-h period. COC, colloidal organic carbon. Mean ± SD (n = 2).

Radioactivity Measurements by γ-Counting. Radioactivities of ^{109}Cd, ^{51}Cr(III), and ^{65}Zn were measured by a Wallac 1480 NaI γ-detector or a Canberra NaI well detector. The γ-emission of ^{109}Cd was detected at 88 keV, of ^{51}Cr(III) at 244 keV, and of ^{65}Zn at 1115 keV. Counting times were adjusted to result in a propagated error of <10%.

Results and Discussion

Colloidal Coagulation. Our controlled laboratory experiments showed that colloidal coagulation, quantified by the fraction of radioactive metals retained by the 0.2-μm filter, was minimal or insignificant without the presence of larger particles (Figure 1). There was no detectable increase in the 0.2-μm filter retained fraction during the 17-h exposure period. On average, <5%, 9%, and 3% of Cd, Cr, and Zn was retained by the 0.2-μm filter, respectively (Figure 1). At 17 h, <1.5%, 4%, and 0.5% of Cd, Cr, and Zn was retained by a 1-μm filter, respectively. There was no significant difference in this filter-retained fraction between the two HMW treatments (P > 0.05, t-test). Because the fraction of metals retained by the 0.2-μm filter was relatively constant (or even decreased), it seems likely that this small filter-retained fraction resulted from the coagulation with the filter surfaces during the filtration (*40, 41*).

Possible colloidal coagulation may affect the interpretation of results from metal uptake experiments (*32−34*). Our results however showed that coagulation of radioactive tagged colloids without the presence of larger particles was very slow. Under our experimental conditions, the radiolabeled colloids appeared to be quite stable, similar to the inorganic colloidal system described by Buffle et al. (*6*). The relatively low percentages of Cd, Cr, and Zn retained by the 0.2-μm filter indicated that the effect of colloidal coagulation on metal uptake by bivalves was probably minimal. Consequently, any difference in metal uptake rate determined at different COC concentrations should largely reflect the difference in the metal's distribution between the dissolved

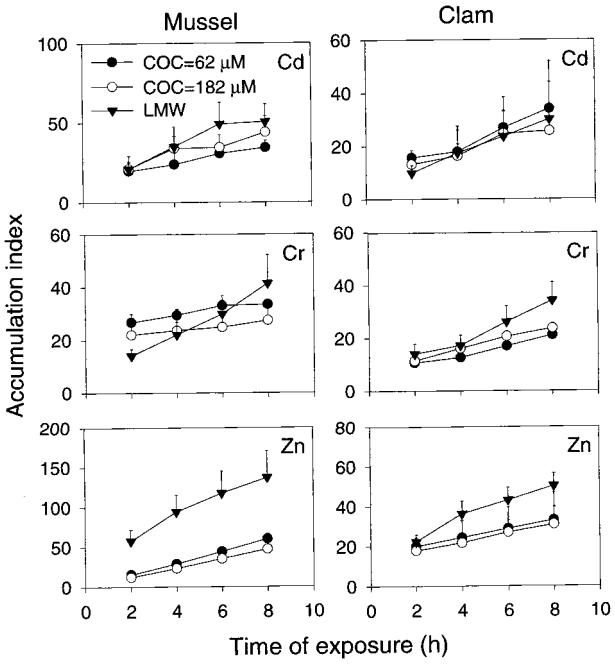

FIGURE 2. Calculated accumulation index of metals in the mussels (*Perna viridis*, left panel) and the clams (*Ruditapes philippinarum*, right panel) during the 8-h uptake period. See text for the calculation of accumulation index, which reflected metal uptake by both soft tissue and shell (adsorption and absorption). LMW, contains no colloids; COC, colloidal organic carbon. Mean + SD ($n = 5$).

TABLE 2. Metal Partitioning between Colloidal (>1 kDa) and 1 kDa Ultrafilter Passing Phases Measured during the Uptake Experiments ($t = 8$ h)[a]

	% in >1 kDa fraction		
metal	LMW	C1	C2
Cd	7	10	6
Cr	9	60	60
Zn	10	64	63

[a] LMW, low molecular weight; C1, COC concentration of 62 μM; C2, COC concentration of 186 μM.

and colloidal phases. In our previous kinetic studies on the uptake of metals from the dissolved phase (<0.2 μm) by marine bivalves, we found that the majority of metals was detected in the dissolved phase during the short-term exposure period, implying that bivalve pumping probably did not contribute to particle formation (*21, 37, 38*). This was however not checked in the present study.

Metal Distribution in Colloids. Apparent partition coefficients of metals were quantified to examine the relationship between colloidal metal uptake in marine bivalves and metal partitioning between colloidal and truly dissolved phases. On average, <10% of Cd, Cr and Zn in the LMW treatments could be retained by the 1 kDa ultrafilter. However, up to 60% of Cr and 64% of Zn in the HMW treatments were found in the >1 kDa colloidal phase in both colloidal treatments (Table 2). The dominant species of Cd in both LMW and HMW treatments was in the LMW form (i.e., the <1 kDa fraction), whereas Cr and Zn were largely complexed by colloids in the HMW treatments. Our results on the partitioning of Cd in the presence of the colloidal phase were similar to previous studies in different marine systems (*14, 15, 42*). The release of metals from the colloidal phase during the exposure period would be important for the interpretation of the uptake results.

The concentrations of the Cd, Cr, and Zn in the natural colloids were not determined in our study. In our radio-

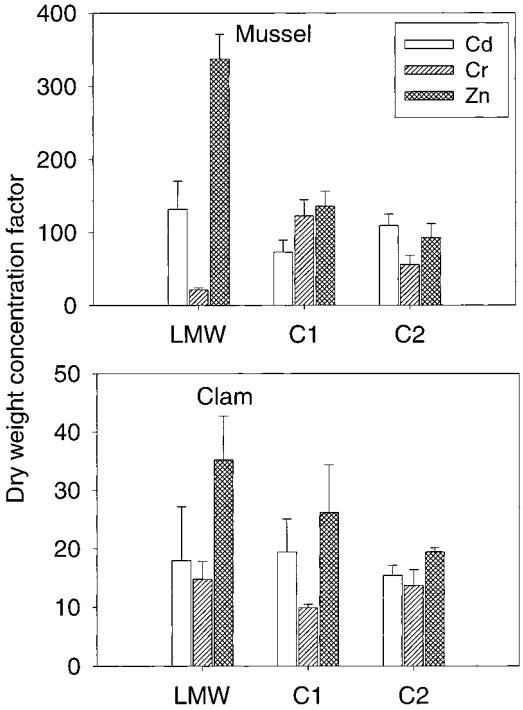

FIGURE 3. Calculated dry weight concentration factors (DCF) of metals in the tissue of the mussels (*Perna viridis*) and the clams (*Ruditapes philippinarum*) after 8-h exposure. LMW, contains no colloids; C1, COC concentration of 62 μM; C2, COC concentration of 186 μM. Mean + SD ($n = 5$).

labeling experiments, the metal concentrations from the added radioisotopes were relatively high. In addition, COM used for the uptake experiments was extracted from the natural seawater, and the final COM concentrations used are within the concentration ranges found in coastal seawater (*2*). Therefore, metals introduced from added COM should not significantly affect the specific activity of added radioisotopes and the overall metal concentrations in the uptake experiments. Furthermore, in our study, we used the bioconcentration factor to quantify the relative uptake of different MW metals, which is relatively independent of the ambient metal concentration or the specific activity of metals in the exposure medium (*21, 37*). The comparable partitioning of metals in the colloidal phase at two colloidal concentrations (Table 2) also indicated that the re-partitioning of isotopes was minimally affected by the specific activity of metals.

Uptake of Colloid-Bound Metals by Mussels and Clams. Metal accumulation in whole individual mussels and clams exposed at different COC concentrations increased approximately linearly between 2 and 8 h of exposure (Figure 2). This was consistent with many previous studies on marine bivalves using a short exposure approach (*39, 43*). In general, the accumulation index, which represented the uptake by both soft tissue and shell, was the highest for Zn in mussels, followed by Cd and Cr. In clams, the accumulation was comparable among the three metals examined. The calculated DCFs in the bivalve tissue, which represented the absolute or actual uptake by bivalve tissue, were generally the highest for Zn after 8-h exposure, followed by Cd and Cr in both mussels and clams in the LMW treatment (Figure 3). DCFs of metals were also much higher in the mussels than in the clams.

Zn accumulation in mussels decreased with increasing COC concentration, whereas Cd and Cr accumulation was more variable between the LMW and HMW treatments. The accumulation of colloid-bound Cr in whole individual mussel (including both tissue and shell) was slower compared with

that of LMW complexed Cr. In general, the bioavailability of HMW metals was lower than that of the LMW metals for Zn in the mussels, whereas the bioavailability of HMW-bound Cr was enhanced, especially at a COC concentration of 62 μM. Thus, an increase in COC concentration significantly reduced the DCFs of Zn in mussels ($P < 0.01$, one-way ANOVA) but significantly increased the DCFs of Cr ($P < 0.01$, one-way ANOVA). In contrast, the DCFs of Cd appeared to comparable at the two high COC concentrations, although they were relatively higher in the LMW treatment than in the HMW treatments.

In clams, the accumulation was similar between the colloid-bound and the LMW-bound Cd and Cr. The accumulation of Zn in the clams was however higher in the LMW treatment than in the HMW treatments, and COC concentration did not greatly affect their accumulation. The colloidal organic carbon concentration did not significantly affect metal uptake in clams ($P > 0.05$, one-way ANOVA), although the DCF of Zn was slightly reduced by colloidal binding.

Our study therefore demonstrated that the presence of colloidal organic carbon and metal phase speciation can influence metal bioavailability to these two marine bivalves. The influence of metal complexation with colloidal organic matter on metal uptake varied among the metals and was highly species specific. For example, Zn bioavailability was considerably inhibited by its complexation with HMW dissolved organic matter. Because Zn speciation appeared to be dominated by HMW complexed species (e.g., Galveson Bay; *5*), its bioavailability to mussels could be controlled by the presence of colloidal organic matter, especially in coastal environments with high COC concentrations. This would be consistent with our recent study on marine plankton in which colloidal complexation also reduced Zn bioavailability to both phytoplankton and zooplankton (*12*).

In contrast to Zn, the bioavailability of colloidal complexed Cd was comparable to or slightly lower than that of LMW Cd. In our stirred cell ultrafiltration experiment, only a small fraction (<10%) of Cd was present in the colloidal phase during the uptake period, even at the highest COC concentration. Consequently, the bioavailability was comparable between the LMW and HMW treatments. Cr however showed an increase in its bioavailability to mussels when associated with the HMW fraction. A further increase in COC concentration reduced the uptake of Cr by the mussels. Because Cr was mostly found in colloidal form, the higher uptake of colloidal Cr may be due to the increased sorption of Cr onto the bivalve's tissue or direct uptake through the pumping of colloidal particles (i.e., ingestion).

The uptake of Cr(III) has been consistently shown to be much lower than the uptake of Cd and Zn (*36, 37, 44*). In our study, the calculated DCF was the highest for Zn, followed by Cd > Cr in both species of bivalves. Such differences may be related to their different binding affinity with SH-containing compounds. Our recent studies have demonstrated that the uptake of Cd and Zn can be considerably influenced by their binding with protein ligands (presumably membrane transport protein) in marine bivalves (*38, 39*). The decrease in the uptake of colloidal Zn compared with their LMW (free ion or inorganic complexes) counterparts by bivalves suggested that the colloidal binding reduced the bioavailability of Zn to bivalves. This was consistent with several previous studies on marine bivalves indicating the significance of free ion in controlling Zn uptake (*39, 45, 46*). It is likely that colloid-bound metals (such as Cd and Zn) may dissociate from colloidal organic matter, followed by complexation with transport ligands before internalization. Because much of the colloidal organic matter is amphiphillic in nature, it cannot be ruled out that some of these colloid-bound metals could have been transported by lipid permeation (*47*).

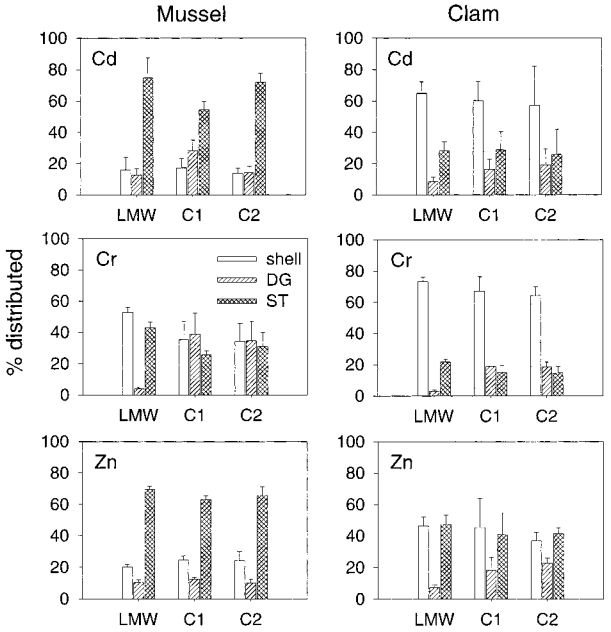

FIGURE 4. Distributions of metals in the digestive gland (DG), remaining soft tissue (ST), and the shell of mussels (*Perna viridis*, left panel) and clams (*Ruditapes philippinarum*, right panel) after 8-h exposure. LMW, contains no colloids; C1, COC concentration of 62 μM; C2, COC concentration of 186 μM. Mean + SD ($n = 5$).

The transport of many class A or transitional metals that prefer to bind with O or N-containing ligands remains less well studied. Whether the transport of Cr was controlled by its free ion concentration is unknown. The degree to which Zn bioavailability was inhibited by colloidally complexation was less pronounced in clams than in mussels. It is unclear whether the difference in metal uptake between these two bivalves was attributable to their difference in pumping activity (siphon pumping in clams vs gill pumping in mussels).

Distribution of Metals in Two Bivalves. Metal distribution in different body parts of the bivalves following 8-h exposure is shown in Figure 4. On average, <20% of Cd in mussels was in the digestive gland or the shell, whereas over 60% was in the remaining soft tissues. For Zn, >60% was in the mussel's remaining soft tissue, and >20% and 10% was in the shell and digestive gland, respectively. The relative distribution of Cd and Zn in mussels was independent of COC concentration or the LMW and HMW treatments. In contrast to Cd and Zn, Cr was almost evenly distributed among the shell, digestive gland, and remaining soft tissue, except for the LMW treatment in which the digestive gland had the lowest percentage of Cr (5%). The fraction of Cr associated with the shells was much higher in the LMW treatment than in the HMW treatments. Comparable distribution pattern between the LMW and HMW treatments indicated that colloidal metals were indeed bioavailable to the mussels.

In contrast to the mussels, a much higher percentage of Cd, Cr, and Zn was associated with the shell of clams (Figure 4). Only about 20% of Cd and Cr were in the remaining soft tissue and the digestive gland of clams. The proportion of metals in the digestive gland was higher in the colloidal treatment than in the LMW treatment, implying that there was a possibility of colloidal ingestion by the clams, probably through the siphon pumping mechanism.

Our study therefore highlights the importance of measuring the partitioning of metals between the colloidal and truly dissolved phases in metal bioavailability studies. In our study (and presumably in any radiotracer study), it was difficult to

maintain all the radiolabeled metals in the colloidal form during the exposure period. Desorption of radiolabeled metals from the colloidal phase to the truly dissolved phase is expected (and cannot be avoided), given the nature of re-partitioning of metals from the colloids to the truly dissolved phase. By the end of 8-h exposure, we found that a large fraction of radiolabeled Cr and Zn was still distributed in the colloidal phase, thus our measurements of the uptake of radiolabeled Cr and Zn may be considered to represent the true uptake of colloidal Cr and Zn. However, the majority of colloid-bound Cd was desorbed and re-partitioned into the truly dissolved phase during the uptake period, thus the uptake of radiolabeled Cd at best reflected the uptake from the truly dissolved phase. To minimize the potential desorption of colloid-bound metals into the truly dissolved phase, one may use inorganic colloids, although it is noted that marine colloids are mostly organic in nature (*32, 48, 49*).

In summary, bioavailability of colloid-bound metals to marine bivalves varied among different metals and bivalve species. Although the bioavailability of Zn to mussels and clams was inhibited by colloidal binding, the bioavailability of colloidal Cr was comparable to or higher than that of Cr bound with the LMW fraction. For Cd, its uptake was not significantly affected by colloidal concentration, largely because most Cd was partitioned into the truly dissolved phase, even at the highest COC concentration. Determination of metal partitioning in colloidal phase during the uptake period was necessary for the interpretation of bioaccumulation data. Colloidal complexation may reduce the bioavailability of certain metals (e.g., Zn) because of the decrease in free or inorganic metal ion concentration. Conversely, colloidal complexation may increase metal bioavailability due to its potential surface sorption or ingestion by aquatic animals. Thus, some colloidal materials may be potentially ingested by aquatic organisms, which may increase metal bioavailability, especially for filter-feeding animals. Our study therefore showed the complexity of colloidal complexation in regulating metal bioavailability to aquatic animals. Given the recent findings of the importance of colloids in metal complexation, it is necessary to develop models which consider the transport of colloidal metals across the biological membranes.

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