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Bioavailability of colloid-bound Cd, Cr, and Zn to marine plankton

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ABSTRACT: The uptake of colloid-bound Cd, Cr, and Zn by marine plankton (including the diatom *Thalassiosira pseudonana*, the dinoflagellate *Prorocentrum minimum*, and the copepod *Paracalanus aculeatus*) was studied using the radiotracer technique. Natural organic colloids (operationally defined as particles between 1 kDa and 0.2 µm) were isolated from Hong Kong coastal seawater using cross-flow ultrafiltration, and radiolabeled with ^{109}Cd, ^{51}Cr, and ^{65}Zn. The uptake of colloid-bound metals by plankton was then compared with the uptake of metals in the low molecular weight fraction (LMW, <1 kDa). Our results indicate that colloids enhanced the initial surface sorption of Cd and Cr onto the algal cells. The rates of subsequent uptake were comparable between the colloidal- and LMW-complexed Cr, whereas the uptake of colloidal Cd and Zn was significantly depressed by colloidal binding. The overall bioavailability of colloidal Cd and Cr to phytoplankton was therefore comparable to or higher than that of LMW metals. In contrast, the bioavailability of colloidal Zn to phytoplankton was significantly lower than that of the LMW Zn. Similar to that for phytoplankton, the bioavailability of colloidal Cr to copepods was significantly enhanced whereas the bioavailability of colloidal Cd to copepods remained relatively unchanged, and the bioavailability of Zn was significantly depressed compared to their respective LMW metals. Direct ingestion of colloidal metals was unlikely because no consistent trend between uptake and colloidal organic carbon concentration was found for Cd, Cr, and Zn. Our study therefore demonstrated that colloidal metals are available to marine plankton and could be actively involved in planktonic food webs. Thus, colloids are important not only in scavenging trace metals, but also in the interactions between metals and the biota in aquatic environments.

KEY WORDS: Colloids · Bioavailability · Cadmium · Chromium · Zinc · Phytoplankton · Copepods

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INTRODUCTION

Aquatic colloids are operationally defined as those sub-micron-sized microparticles and macromolecules with sizes ranging from 1 nm to 0.2 µm (Buffle 1990, Buffle & Leppard 1995, Guo & Santschi 1997). Colloids present in seawater include macromolecular organic matter, viruses, bio-colloids, aggregates of exudates, and clay minerals and oxides of iron, aluminum, and manganese coated with organic matter (Ogura 1977, Tipping 1988, Stumm & Morgan 1996, Guo & Santschi 1997, Buffle et al. 1998). Colloids may play a critical role in regulating the concentration and speciation, and thus the fate and transport of metals in aquatic environments due to their unique physio-chemical properties and large complexing capacity for metals (Honeyman & Santschi 1992, Gustafsson & Gschwend 1997, Santschi et al. 1997). Therefore, there has been an increasing awareness of the importance of colloidal forms and metal-organic complexation in aquatic systems (Wells & Goldberg 1991, Buffle & Leppard 1995, Guo & Santschi 1997, Buffle et al. 1998, Chin et al. 1998, Santschi et al. 1999). Recent studies have demonstrated that a considerable fraction of the traditionally defined 'dissolved' metals (<0.2 or 0.45 µm) in seawater is associated with organic colloids (Martin et al.

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1995, Sanudo-Wilhelmy et al. 1996, Stordal et al. 1996, Wells et al. 1998, Wen et al. 1999). Metals associated with colloids may behave differently in terms of mobility, toxicity, and bioavailability to aquatic organisms compared with those in free ionic forms (Campbell 1995, Carvalho et al. 1999, Doblin et al. 1999). However, little is known about the bioavailability and toxicity of colloidally complexed metals to aquatic organisms (Doblin et al. 1999). Previous studies on the bioavailability (or uptake) of metals to marine organisms mainly focused on the control of metal aqueous chemistry (Campbell 1995). The availability of metals associated with natural colloidal or macromolecular organic matter to marine plankton is a matter of speculation. A few studies have found that pure inorganic colloidal Fe such as Fe_2O_3 and FeOOH can considerably reduce the bioavailability of Fe to marine phytoplankton (Wells et al. 1983, Rich & Morel 1990, Kuma & Matsunaga 1995). The applicability of these studies to natural colloids and other bioactive metals has not yet been tested.

Various biological processes (e.g., excretion, exudation and decomposition of marine plankton) and geochemical processes (e.g., complexation, sorption and coagulation) can contribute to the dynamics of marine colloids (Wells & Goldberg 1991, Aluwihare et al. 1997, Guo & Santschi 1997, Chin et al. 1998). Whether colloids serve as a link to or a sink for bioactive metals in aquatic environments remains largely unknown. Understanding the fate of trace metals and their interactions with plankton is essential to our knowledge of their biogeochemical cycling in the environment. For most trace metals, it is still unknown whether colloidal metals can become bioavailable to aquatic organisms, and if so, whether colloids can enhance or decrease metal bioavailability. Bioavailability can be defined as the potential uptake of metals by aquatic organisms. Such a definition may also include biological surface sorption without transport across the cell membrane (internalization). According to the free ion activity model (Campbell 1995), the free metal ion is the dominant species available for aquatic organisms, and as such, metals bound with macromolecules or colloids will be expected to have a lower bioavailability. Nevertheless, many colloids are both hydrophilic and lipophilic and may be able to diffuse across the biological layer. Lipid permeation has been proposed as an important mechanism for metal uptake, especially for lipophilic metals such as organic mercury (Campbell 1995, Simkiss & Taylor 1995, Mason et al. 1996). Moreover, Phinney & Bruland (1994) have demonstrated that lipophilic metals can indeed be taken up by marine phytoplankton.

The overall objective of this study was to examine the bioavailability of colloidally complexed metals (Cd, Cr and Zn) to marine plankton. Two species of phytoplankton (diatom and dinoflagellate) and 1 copepod were considered in this study. A short-term exposure was employed to measure the kinetics of metal uptake in phytoplankton. Partitioning of metals in the colloidal phase was also monitored during the experiments to determine metal phase speciation. We also quantified the distribution of colloidal metals in the phytoplankton to test whether they can penetrate across the biological membranes.

MATERIALS AND METHODS

Plankton. Axenic cultures of 2 phytoplankton, the diatom *Thalassiosira pseudonana* (clone 3H) and the dinoflagellate *Prorocentrum minimum*, were obtained from Provasoli-Guillard Center for the Culture of Marine Phytoplankton, and were maintained at 18°C and 33‰ seawater collected 10 km off the east coast of Hong Kong. The copepod assemblage (dominated by calanoid *Paracalanus aculeatus*) was collected by net tow (150 µm) from Clear Water Bay, Hong Kong, and was acclimated in the laboratory for 1 d before the uptake experiments. During the acclimation period they were fed with the diatom *T. pseudonana*.

Metals. Cd, Cr, and Zn were chosen in this study because they are representative of toxic and biologically essential metals and because they are available in radioactive forms. Cd and Zn are soft metals that prefer to bind with S-containing ligands while Cr is a transition metal that prefers to bind with O-containing ligands (Nieboer & Richardson 1980). In addition, Cd and Zn have been reported to be mainly associated with colloidal organic carbon (COC) in seawater (e.g., 45% for Cd and 91% for Zn, respectively, in Galveston Bay, Texas, USA; Wen et al. 1999). Radioisotopes, ^{109}Cd, ^{51}Cr(III), and ^{65}Zn, were obtained from New England Nuclear (NEN).

Radiolabeling colloids. Seawater (with a salinity of 30‰) was collected from Tolo Harbor in December 1998, and kept in a cool room before processing. Before ultrafiltration, the seawater was first filtered through a 0.2 µm Nuclepore cartridge. The pre-filtered water was then ultrafiltered to collect colloids using a spiral-wound cross-flow ultrafiltration cartridge (Amicon S10Y1) with 1 kDa cutoff (Guo & Santschi 1996, Guo et al. 2000). The cartridge was cleaned sequentially with 2% Micro detergent, 0.05 M NaOH, and diluted acid solution before use (Guo & Santschi 1996). Colloids were concentrated from about 80 l of seawater to 2 l (with a concentration factor of 40). Concentrations of dissolved organic carbon (DOC) and COC were quantified by a Shimadzu TOC-5000 Total Organic Carbon Analyzer (Guo et al. 1994), and are shown in Table 1.

Table 1. Colloidal organic carbon (COC) concentration and background low molecular weight (LMW, <1 kDa) dissolved organic carbon (DOC) concentration used in this study for coagulation and metal uptake experiments. P: Permeate only; C5: addition of 5 ml radiolabeled colloid; C15: addition of 15 ml radiolabeled colloid; C45: addition of 45 ml radiolabeled colloid

ID	Treatment	Volume (ml)	Background DOC (µM)	COC (µM)
P	LMW	200	65	–
C5	Colloidal	200	64	35
C15	Colloidal	200	61	105
C45	Colloidal	200	53	315

The concentrated colloids were spiked with radioisotopes ^{109}Cd, ^{51}Cr(III), and ^{65}Zn, in a Teflon jar. After 1.5 d of partitioning or equilibrium, the fractions of uncomplexed or the <1 kDa radioactive metals were 'squeezed' out by cross-flow ultrafiltration. Both the radiolabeled permeate (defined as the low molecular weight fraction, LMW) and colloids (defined as the high molecular weight fraction, HMW) were immediately used for uptake and coagulation experiments described below.

Phase speciation and coagulation of radiolabeled colloids. It is important to know if the radiolabeled colloids added to the experimental system remain in the colloidal forms for metal uptake by plankton. Conversely, radiolabeled colloids may form a new particulate phase through coagulation, resulting in possible artifacts in the interpretation of metal uptake results.

To examine colloidal coagulation under our experimental conditions, radiolabeled colloids were dispersed into 200 ml LMW seawater at 3 different COC concentrations (35, 105, and 315 µM). There were 2 replicates for each treatment. Over a period of 47 h, the percentages of radioisotopes retained in the particulate fraction (>0.2 µm) were determined by filtering a 10 ml aliquot through a 0.2 µm polycarbonate membrane. A second 0.2 µm membrane was placed beneath the first membrane to monitor possible radioisotope adsorption onto the polycarbonate membrane during the filtration process.

A stirred cell ultrafiltration unit with 1 kDa membranes was used to monitor the phase speciation of each metal or metal partitioning between the colloidal and the 1 kDa ultrafilter passing phases after 9.5 h of exposure.

Uptake of colloid-bound metals by phytoplankton. The exponentially growing diatom *Thalassiosira pseudonana* and dinoflagellate *Prorocentrum minimum* were collected by filtration onto 3 µm polycarbonate membranes, rinsed and suspended into 30 ml LMW seawater. The cells were added into 200 ml LMW seawater at a cell concentration of 5×10^4 cells ml^{-1} for *T. pseudonana* and 8×10^3 cells ml^{-1} for *P. minimum*. Radiolabeled colloids were added to result in final COC concentrations of 35, 105, and 315 µM (Table 1). For the LMW treatment, only radiolabeled LMW metals were added. There were 2 replicates for each treatment. The 'control' treatment only contained the same concentrations of radiolabeled colloids or LMW metals without the addition of phytoplankton cells. Radioisotope additions corresponded to a metal concentration of 1.6 to 15 nM for Cd, 18 to 160 pM for Cr, and 7 to 66 nM for Zn. At each time interval, the fractions of radioisotopes accumulated in the cells were determined by filtering a 10 ml aliquot onto the 1 µm polycarbonate membranes. The membrane adsorption of the radioisotopes and the possible coagulation were calibrated by filtering 10 ml aliquots from the control treatment onto the 1 µm polycarbonate membranes. However, this fraction was negligible compared to the radioactive uptake by phytoplankton cells. The dry weight based concentration factor (DCF) was then calculated by the following equation:

$$DCF = C/C_w \quad (1)$$

where C is the radioactivity of metals in the cells (dpm kg^{-1} dry wt), and C_w is the radioactivity of metals in the water (dpm l^{-1}).

After 1 d exposure to LMW and HMW metals, the distribution of radioisotopes in the cytoplasm of algal cells was determined as described in Fisher et al. (1983) and Wang & Fisher (1996).

Uptake of colloid-bound metals in zooplankton. Copepods were added to 200 ml LMW seawater at a density of 1 to 2 ind. ml^{-1}. In the LMW treatment, only radiolabeled LMW metals were added. In the HMW treatments, colloids were added at COC concentrations of 35, 105, and 315 µM. There were 3 replicates for each treatment. The zooplankton were placed in the dark for 4 h, after which they were collected by a mesh, rinsed with non-radioactive LMW seawater, and their radioactivity was assayed. We did not measure the kinetics of metal uptake and the distribution of radioisotopes in zooplankton due to the relatively low radioactivity used in the experiments. The DCF of metals was then calculated using Eq. (1). The individual dry weight of copepods was measured by drying the copepods at 80°C for 1 d.

Radioactivity measurements. Radioactivities of ^{109}Cd, ^{51}Cr(III), and ^{65}Zn were measured with a Wallac 1480 NaI gamma detector. The spillover of radioisotopes from high energy window to low energy window was calibrated by the software. Counting times were adjusted to result in a propagated error of <10%. The gamma emission of ^{109}Cd was detected at 22 keV, of ^{51}Cr(III) at 244 keV, and of ^{65}Zn at 1115 keV.

RESULTS AND DISCUSSION

Coagulation of colloidal particles

Colloidal coagulation, quantified by the amount of radioactivity retained by the 0.2 µm polycarbonate membrane, was minimal or insignificant without the presence of larger particles (Fig. 1). Only 2% of the added metals was in the >0.2 µm fraction after 47 h, which was much longer than the exposure time in our uptake experiments (4 to 9.5 h). In general, coagulation, if any, occurred immediately after the addition of colloids to the LMW seawater. The fraction of filter retained metals was almost constant throughout the 47 h exposure period. Among the 3 metals examined, coagulation appeared to be independent of COC concentration for Cr and Zn, but increased slightly with increasing COC concentration for Cd.

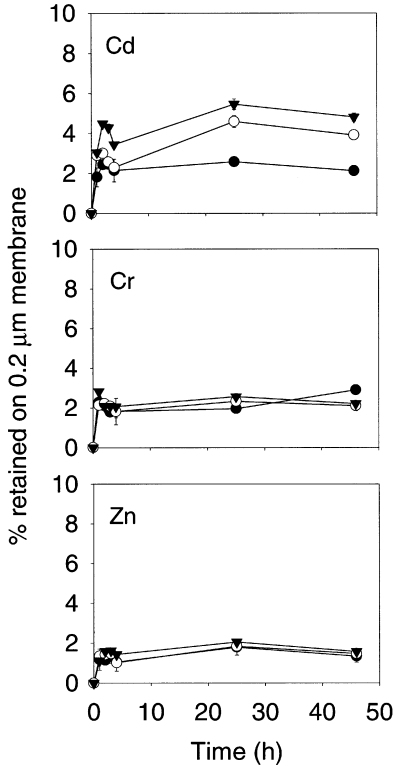

Fig. 1. Percentages of colloidal Cd, Cr, and Zn retained on the >0.2 µm polycarbonate membrane over time at different colloidal organic carbon concentrations (COC). (●) COC concentration of 35 µM; (○) COC concentration of 105 µM; (▼) COC concentration of 315 µM. Values are mean ± SD (n = 2)

It is well-known that large colloids and particles can coagulate following their formation (Jackson 1990, Honeyman & Santschi 1992, Chin et al. 1998). Therefore, possible colloidal coagulation may affect the interpretation of metal uptake experiments. Our results showed that the coagulation of small colloids (in the size range of 1 kDa to 0.2 µm) was extremely slow, in contrast to previous studies which used larger particles (Stordal et al. 1996, Wen et al. 1997, Chin et al. 1998). Colloids (1 kDa to 0.2 µm) appeared to be very stable under our experimental conditions. Similarly, Buffle et al. (1998) suggested that the aggregation of inorganic colloids was generally slow because of the low collision efficiency resulting from the repulsive charge of colloidal particles. Because the fraction of metals detected in the 0.2 µm membrane was maintained relatively constant in our experiments, it may be possible that this filter-retained fraction resulted from the coagulation with the filter surfaces during the filtration (Honeyman & Santschi 1991, Buffle et al. 1992).

Our speciation data indicated that a significant fraction of colloid was re-partitioned following resuspension into the LMW water for 9.5 h (Table 2). For Cd, <14% was associated with the colloids. However, the majority of Cr was associated with the colloidal phase, and 23 to 53% of radiolabeled Zn was partitioned in the colloidal phase following re-equilibrium. The fraction of Zn associated with the colloidal phase appeared to decrease with increasing COC concentration (Table 2).

Bioavailability of colloid-bound metals to phytoplankton

After 1 h of exposure, the diatom and dinoflagellate cells exposed to colloidal metals at different COC concentrations all accumulated more Cd and Cr than the

Table 2. Metal partitioning between colloidal (>1 kDa) and 1 kDa ultrafilter passing phases measured during the uptake experiments. Treatments for different IDs as in Table 1

ID	Cr (%)		Cd (%)		Zn (%)	
	Colloids	<1 kDa	Colloids	<1 kDa	Colloids	<1 kDa
P	9–27	55–82	7–10	84–86	9–15	60–80
C5	54–69	18–34	12–14	77–87	30–53	30–70
C15	50–72	21–36	9–14	76–86	31–42	45–68
C45	38–61	25–53	11–14	77–78	20–23	54–66

cells exposed to LMW metals (Figs. 2 & 3). The uptake patterns were similar between the diatoms and the dinoflagellates. Surface sorption may have occurred within minutes after the addition of the radiolabeled colloids. The initial uptake (within the first 1 h) increased for Cd but decreased for Cr with increasing COC concentration. In contrast, the initial uptake of Zn was lower in the colloidal treatment than in the LMW treatment. By the end of exposure (9.5 h), the DCFs of Zn decreased significantly with increasing COC concentrations ($p < 0.05$, 1-way ANOVA), whereas the DCFs of Cr were comparable among different colloidal treatments ($p > 0.05$). DCFs of Cd in *Prorocentrum minimum* were also significantly higher with increasing COC concentration ($p < 0.05$, 1-way ANOVA).

Between 1 and 9.5 h of exposure, a linear pattern of metal uptake was found for all 3 metals (Figs. 2 & 3). Relative metal uptake in the cells was therefore calculated as the slope of the linear regression between the DCFs of metals in the cells and the duration of exposure (between 1 and 9.5 h, Fig. 4). The relative metal uptake rates between 1 and 9.5 h were comparable for Cr ($p > 0.05$, 1-way ANOVA). For Cd and Zn, their uptake rates decreased significantly with increasing COC concentrations ($p < 0.05$, 1-way ANOVA).

Following the initial surface sorption, metals may have been transported intracellularly (e.g., through internalization). Indeed, colloid-bound metals were found to penetrate into the cytoplasm of diatom and dinoflagellate cells following 1 d exposure (Fig. 5). The amount of metals in the algal cytoplasm was, however, lower in the colloidal treatments than in the LMW treatment, although concentrations of COC did not significantly influence the degree to which a metal penetrated the algal cytoplasm ($p > 0.05$, 1-way ANOVA).

Metal uptake in phytoplankton is generally believed to involve an initial rapid surface sorption (or complexation reaction), followed by a slower step of internalization, e.g., transport across the plasma membrane (Campbell 1995). Our studies clearly showed that metal bioavailability to phytoplankton was either enhanced (Cr) or depressed (for Zn) by the colloidal bind-

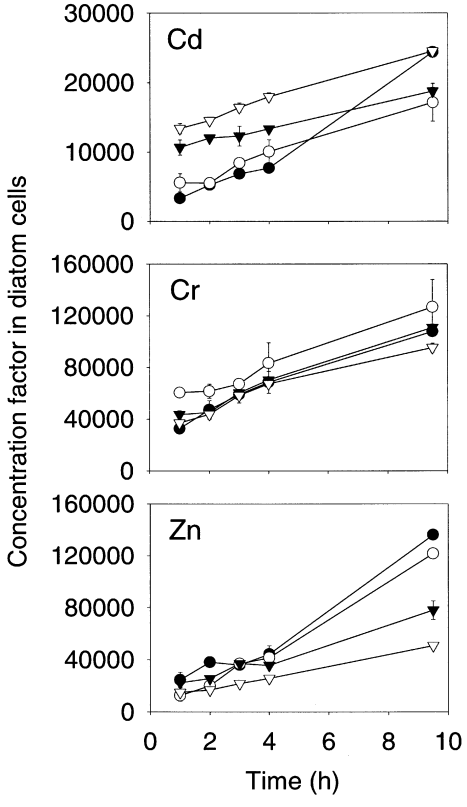

Fig. 2. Dry weight concentration factors (DCFs) of Cd, Cr, and Zn accumulated in the diatom *Thalassiosira pseudonana* over 9.5 h exposure to low molecular weight (LMW) metals or colloid-bound (1 kDa to 0.2 µm) metals at different COC concentrations. (●) LMW metals; (○) COC concentration of 35 µM; (▼) COC concentration of 105 µM; (▽) COC concentration of 315 µM. Values are mean ± SD (n = 2)

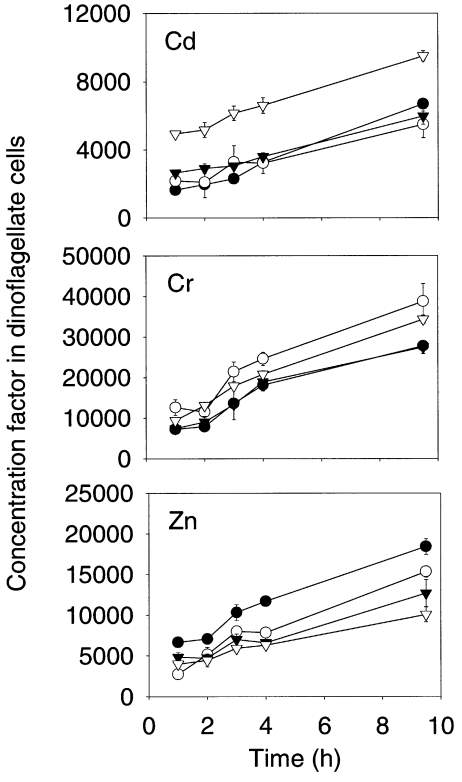

Fig. 3. DCFs of Cd, Cr, and Zn accumulated in the dinoflagellate *Prorocentrum minimum* over 9.5 h exposure to LMW metals or colloid-bound (1 kDa to 0.2 µm) metals at different COC concentrations. (●) LMW metals; (○) COC concentration of 35 µM; (▼) COC concentration of 105 µM; (▽) COC concentration of 315 µM. Values are mean ± SD (n = 2)

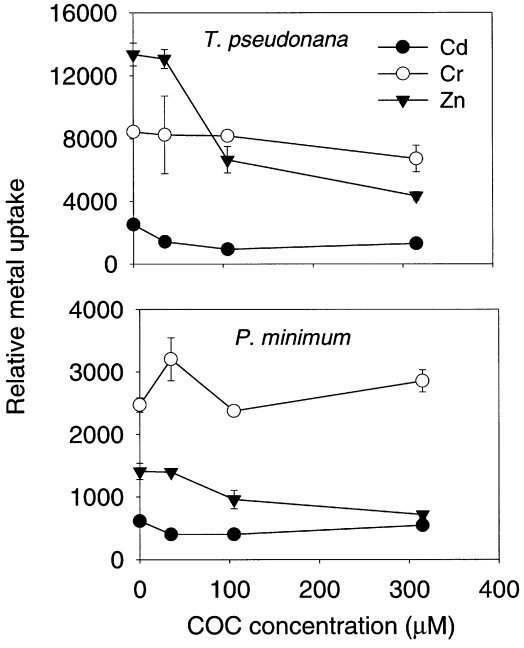

Fig. 4. Relative uptake of Cd, Cr, and Zn by the diatom *Thalassiosira pseudonana* and dinoflagellate *Prorocentrum minimum* as a function of COC concentration. Values are mean ± SD (n = 2). Relative uptake rates were calculated as the slope of the linear relationship between the DCF of metals in the cells and the duration of exposure (between 1 and 9.5 h) (Figs. 2 & 3)

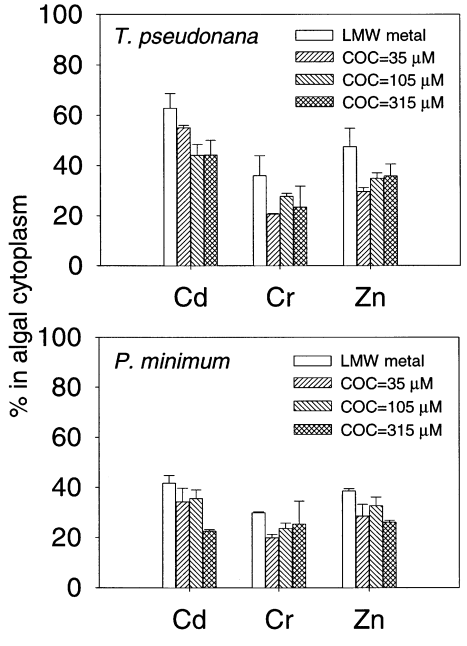

Fig. 5. Percentages of Cd, Cr, and Zn in the cytoplasm of the diatom *Thalassiosira pseudonana*, and the dinoflagellate *Prorocentrum minimum*, following 1 d exposure to LMW metals and colloid-bound metals at different COC concentrations. Values are mean ± SD (n = 2)

ing. The enhancement of the bioavailability of colloidal metals may partly result from the surface sorption when the algae are in contact with the colloids. Given the amphiphilic nature of colloidal particles (both hydrophilic and hydrophobic domains; Buffle 1990), 2 mechanisms may explain the uptake of colloidal metals across the plasma membrane. Some lipophilic colloid-bound metals may have directly crossed the lipid bilayer (Phinney & Bruland 1994). Slightly enhanced or comparable bioavailability of colloidal metals to marine phytoplankton may suggest the direct transport of lipophilic metals. A second mechanism may be involved in the dissociation of metal from the colloids, followed by a complexation on the cell surface with transporters before internalization. This mechanism is also likely because our cell fractionation data indicated that the penetration of colloidal metals into the algal cytoplasm was depressed. Thus, the transport of colloid-bound metals across the plasma membrane may have been slower than the LMW metals. Under such circumstances, the uptake of colloid-bound metals may be kinetically controlled by dissociation and complexation between metals and macromolecules. If this is the case, metals having higher binding efficiency with COC will be depressed in terms of bioavailability to aquatic organisms. Indeed, recent studies have shown that Zn has a higher chance (up to 91%) of binding with colloidal organic matter than Cd (with 45%) in estuarine waters (Wen et al. 1999). Accordingly, less Zn than Cd would be expected to dissociate itself from the colloids and become available to the plankton, which is consistent with our observation in this study. The percentage of Cr associated with marine colloids and their binding efficiency has not been examined. In our experiments, a relatively low percentage of Cd was found in the colloidal phase following resupension of radiolabeled colloids. For Cr and Zn, a much higher percentage of radiolableled metals was associated with the colloids. Consequently, measurements of Cr and Zn uptake by the cells may largely reflect their bioavailability from the colloidal phase.

Campbell et al. (1997) emphasized the significance of direct surface adsorption of dissolved organic matter (DOM) onto live biological surfaces. The molecular weights of the fulvic and humic acids they used as sorbents were >1 kDa, which was above the cut-off size of colloidal particles used in our study. Although our study did not specifically differentiate metal surface adsorption, it appears that surface adsorption is significant for colloid-bound Cd and Cr, which may also lead to the direct uptake of colloidal metals by phytoplankton.

According to the free ion activity model, metal uptake in aquatic organisms is primarily determined by the concentration of metal-cell surface complex, which in turn is dependent on the free ion activity in

the ambient water (Campbell 1995). Our data were in agreement with this model, indicating that colloidal Cd and Zn were accumulated at a slower rate than the accumulation of LMW-bound metals. Such a model requires that the free ion metal is complexed with a surface ligand before internalization. One assumption underlying this model is that metals exist in the exposure solution as hydrophilic species. Lipophilic metal species that may traverse the plasma membrane without first forming a surface complex are not considered in the model (Campbell 1995).

A few studies have examined the bioavailability of colloid-bound Fe to marine phytoplankton (Wells et al. 1983, Rich & Morel 1990, Kuma & Matsunaga 1995). In these studies, a pure phase of well-defined inorganic colloids was synthesized and the growth of diatoms was quantified with the presence of colloidal Fe. Bioavailability of colloidal Fe was either not observed (Rich & Morel 1990) or found to be related to the thermodynamic stability and kinetic lability of the colloidal ferric oxide phase (Kuma & Matsunaga 1995). Wells et al. (1983) observed that the colloidal goethite (α-FeOOH) or hematite (α-Fe_2O_3) did not support the growth of diatoms, whereas the hydrous ferric oxide colloids did. Rich & Morel (1990) proposed that photoreduction or thermal (dark) dissolution is responsible for the availability of various forms of colloidal Fe. Our study on phytoplankton clearly showed that the behavior of Cd, Cr, and Zn associated with natural organic colloids, many of which have biological origins, is different from the pure inorganic colloidal Fe.

Bioavailability of colloidal metals to zooplankton

The calculated DCF in copepods was significantly higher for colloid-bound Cr than for the LMW-bound metals (Fig. 6). In addition, DCF for Cr decreased with increasing COC concentrations, which was consistent with observations in diatoms within the first 1 h of uptake. COC concentration did not significantly affect the DCFs of Cd (1-way ANOVA), whereas the bioavailability of Zn was depressed at the highest COC concentration. The calculated DCF of LMW metals in copepods was highest for Zn, followed by Cd > Cr. The measured DCFs for *Paracalanus aculeatus* were comparable to a previous measurement in another marine copepod *Temora longicornis* (Wang & Fisher 1998). Because the metals exhibited different responses to an increase in COC concentration, it is unlikely that the accumulation of colloidal metals in zooplankton was largely due to the ingestion of colloidal foods. Overall, these results demonstrate that colloid-bound metals were available for the biological uptake by the zooplankton in the marine environments.

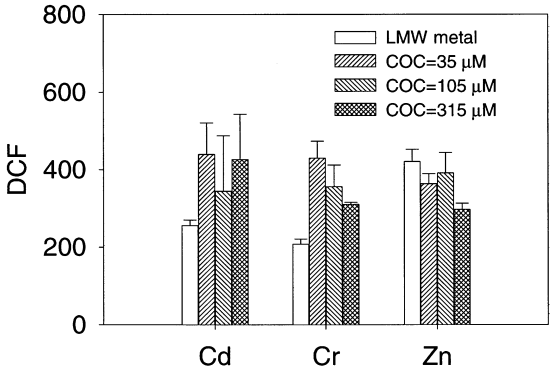

Fig. 6. Measured DCFs of Cd, Cr, and Zn in copepod assemblage dominated by *Paracalanus aculeatus*. Values are mean ± SD (n = 3). The dry weight of copepods was 3.7 μg ind.$^{-1}$

Surface adsorption onto the animal's body surface may account for the higher bioavailability of colloidal metals than the LMW metals, especially for Cr. In aquatic invertebrates, metals can be transported by lipid permeation, passive diffusion, ion channels, active transport, and/or pinocytosis (Simkiss & Taylor 1995). However, the relative importance of each transport pathway for metals remains largely unknown.

In a recent study on the bioavailability of colloid-bound metals to marine shrimps, Carvalho et al. (1999) found that the uptake rates over a 2 wk exposure period were comparable between the LWM and HMW metals for Ag, Cd, Fe, Co, Hg, and Mn, whereas the uptake rates of Ba, Sn and Zn were higher in the LMW fraction. They further suggested that the colloidally complexed metals first accumulated in the shrimps' gills and were subsequently dissociated from the complex before entering the organisms as free ions. Under such circumstances, a kinetic control of metal dissociation may be important in determining metal bioavailability to marine plankton. However, it cannot be excluded that pinocytosis can be a mechanism for metal uptake in aquatic invertebrates.

Our study therefore indicates that colloidal metals are actively involved in biological cycling, in addition to their physico-chemical pathways in aquatic environments. However, the transfer of colloid-bound metals in the marine food chain may be depressed due to the lower rate of metal penetration into the algal cytoplasm, which determines the efficiency of trophic transfer in marine zooplankton (Reinfelder & Fisher 1991). Consequently, the transport of colloid-bound metals can be controlled by both colloidal coagulation (Honeyman & Santschi 1989) and colloidal-biota interaction. Moreover, if metals associated with colloids can indeed be taken up by phytoplankton or zooplankton, the turnover rates of those particle-reactive metals in the ocean may be significantly accelerated, i.e.,

turnover times may become shorter through biological processes. This is consistent with previous studies indicating the short residence time of colloidal particles in the upper ocean (Moran & Buesseler 1992, Santschi et al. 1995). On the other hand, if the bioavailability and toxicity of colloidal metals are significantly different from those of LMW metals, the colloidal phase should be treated as an individual phase in water quality assessment and environmental regulation in coastal environments.

In summary, our study showed the differential bioavailability of colloid-bound metals to marine plankton. Zn exhibited the lowest dissociation kinetics and its bioavailability was significantly inhibited by colloidal binding. Colloidal binding may increase the bioavailability of Cr to marine plankton, presumably due to the surface sorption of colloidal metals. Ligands that bind with metals appear to vary among the 3 metals examined in this study. Our study therefore suggests that colloidal complexation may be important in determining metal bioavailability to aquatic organisms. Further studies are required to examine the influence of colloidal composition and origins on metal bioavailability to aquatic organisms.

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