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Speciation and Microalgal Bioavailability of Inorganic Silver

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Silver accumulation in aquatic organisms is primarily attributed to the bioavailability of the free Ag ion (Ag^+). Some reports suggest that AgCl(aq) is also available for biological uptake, but few studies of Ag bioavailability used the range of chloride concentrations over which $AgCl^0$(aq) is the dominant Ag species. None used environmentally realistic, low Ag concentrations (10−200 pM). To assess the bioavailability of inorganic Ag species and the importance of the low polarity AgCl(aq) complex to biological uptake, we determined the octanol−water partition coefficient of Ag over a range of chloride concentrations (0−50 mM) representative of fresh to brackish waters and measured short-term Ag uptake rates in the euryhaline marine microalga *Thalassiosira weissflogii* exposed to a total silver concentration of 50 pM. Overall octanol−water partition coefficients (D_{ow}) of inorganic silver ranged from 0.02 to 0.06. The K_{ow} of AgCl(aq) calculated using D_{ow} values measured at 0.5, 5, and 50 mM Cl^- and the K_{ow} of Ag^+ (0.03, measured in the absence of Cl^-) was 0.09. Silver D_{ow} and uptake rate constants in phytoplankton were highest at the Cl^- concentration (5 mM) where uncharged AgCl(aq) is the dominant (67%) silver species. Our results demonstrate that AgCl(aq) is the principal bioavailable species of inorganic silver in phytoplankton and suggest that direct uptake of AgCl(aq) is important to the overall accumulation of Ag in aquatic invertebrates.

Introduction

Silver is one of the most toxic trace metals to estuarine phytoplankton (*1*) and invertebrates (*2*) and is a pollutant trace metal of concern in urban/suburban estuaries because it has important anthropogenic sources (*3, 4*). Aquatic organisms can accumulate silver from both dissolved and particulate (by ingestion) phases, but dissolved Ag is the major source of accumulation in marine bivalves (*5, 6*) and copepods (*7*). The speciation and uptake mechanisms of dissolved silver therefore need to be understood in order to predict the fate and effects of Ag in estuarine ecosystems. Compared with other trace metals, however, Ag has not been extensively studied with regard to the relationship between speciation and bioavailability (*8*). While laboratory and field studies have demonstrated high bioaccumulation of Ag in estuarine and marine species (*1, 9*), the mechanism of this accumulation and the chemical species of Ag actually taken up are unknown.

The speciation of dissolved Ag in natural waters, like that of Hg, is dominated by chloride complexation (*10*) and free of strong complexation by dissolved organic ligands (*11, 12*). Although humic acids can bind Ag (*12*), the formation of organic Ag complexes in natural waters will depend on the concentrations of other metals that can react with humic matter, and strong organic Ag complexes have not been observed by electrochemical methods in seawater (*11*). Silver bioaccumulation has been attributed to the accumulation of both Ag^+, which is the dominant species in freshwater, and $AgCl^0$(aq) which is the dominant species in brackish waters with chloride concentrations ranging from 1 to 16 mM (*8, 13, 14*). The relative bioavailabilities of Ag^+ and $AgCl^0$(aq), however, have not been quantified. Furthermore, previous bioavailability studies used silver concentrations that are 2−3 order of magnitude higher than those found in even contaminated natural waters (*3*).

The uncharged chloride complex of inorganic Hg ($HgCl_2$) is somewhat hydrophobic (K_{ow} = 3.3) and as a result was found to accumulate in a marine microalga by passive diffusion (*15*). The results of Mason et al. (*15*) showed that $HgCl_2^0$ was more bioavailable than other inorganic mercury species. Since Ag is similar to Hg in terms of chemical speciation, the low polarity of its Cl^- complexes, and its site of accumulation (membrane) in microalgal cells (*15, 16*), we hypothesized by analogy that Ag bioavailability depends on the formation of $AgCl^0$(aq). Here we examine the speciation and hydrophobicity of inorganic silver and its accumulation in a marine microalga.

Experimental Section

Octanol−Water Partition Coefficients. Overall octanol−water partition coefficients ($D_{ow} = [Ag]_{total-octanol}:[Ag]_{total-water}$) of aqueous inorganic silver were determined over a range of chloride concentrations. The aqueous phase was prepared with ultrapure water and contained 20 nM silver nitrate and various chloride concentrations (NaCl). Background silver concentration in ultrapure water was not detectable by GFAAS (graphite furnace atomic absorption spectrophotometer), using the standard APDC/DDDC extraction method (*17*). The pH of the aqueous phase was adjusted to pH 6 with dilute NaOH. Equal volumes (20 mL) of water-saturated octanol and aqueous phase were mixed in Teflon (PTFE) separatory funnels. Water and octanol phases were allowed to separate for 1 h, after which each phase was removed from the separatory funnel and analyzed for their silver concentrations. Silver in the aqueous phase was measured directly by GFAAS. Silver was extracted from octanol with three volumes (5 mL) of 7 N ultrapure nitric acid. The three nitric acid extracts were combined in a fused quartz crucible and evaporated down to dryness on a hotplate. The residue in the crucible was redissolved in 1 mL of 1% ultrapure nitric acid and analyzed by GFAAS. For these methods, total silver recovery was 94 ± 3.0%, and the detection limit was 0.19 nM (0.02 μg L^{-1}).

Cultures. Experiments utilized the coastal diatom *Thalassiosira weissflogii*, a well-studied euryhaline species. Cultures of *T. weissflogii* were maintained in acid-cleaned polycarbonate bottles containing synthetic ocean water (Aquil; *18*). All cultures were maintained under constant illumination (200 μmol m^{-2} s^{-1}) at 22 °C.

Silver Uptake. Silver accumulation in *T. weissflogii* was measured in short-term uptake experiments (0−4 h) over a range of chloride concentrations (5×10^{-2} to 5×10^{-4} M). Cells were harvested in late exponential growth by filtration on 3-μm polycarbonate membrane filters and resuspended in 900 mL of experimental media containing various chloride concentrations and no added silver to give a final cell density

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of 10^5 cells mL^{-1}. Experimental media consisted of 0.13 M sodium sulfate and 0−0.05 M sodium chloride. Background silver concentration in experimental media was below detection limit (0.74 pM, 80 pg L^{-1}) by GFAAS, using APDC/DDDC extraction method (*17*) with a concentration factor of 250. *T. weissflogii* is a euryhaline species that can grow in low salinity waters. The physiological fitness of *T. weissflogii* in the Na_2SO_4 solution was confirmed by measuring inorganic carbon fixation and microscopic observation of cells that showed photosynthetically active cells and no cell breakage or deformation under these conditions. After a 30-min preincubation period, 100 mL of experimental media containing 500 pM $AgNO_3$ was added to cultures to give a final silver concentration of 50 pM. At each sampling time, 1 mL was decanted for a cell count, and a 200-mL aliquot was filtered through a 3-μm polycarbonate filter and rinsed with experimental medium. Filters were analyzed for particulate silver concentrations by GFAAS after acid digestion. Volume−volume concentration factors (VCF) of Ag in *T. weissflogii* were determined for each chloride concentration according to

$$\text{VCF} = \text{mol of metal } (\mu\text{m of cell})^{-3}/\text{mol of metal } (\mu\text{m of water})^{-3} \quad (1)$$

using a cell volume of *T. weissflogii* of 10^3 μm^3.

Cell Digestion and Analysis. Filters were transferred to 30-mL fused quartz crucibles, and 5 mL of ultrapure concentrated nitric acid (Optima, Fisher) was added. The cells were resuspended in the nitric acid, and the polycarbonate filter was removed. After the nitric acid solution turned clear (20 h at room temperature), it was slowly evaporated to near dryness on a hotplate. After the residue was redissolved in 1 mL of 2% nitric acid, the suspension was centrifuged and the silver concentration of the supernatant was measured by GFAAS. Silver was analyzed with a Perkin-Elmer 4100ZL atomic absorption spectrophotometer fit with a THGA graphite furnace and an AS-71 autosampler, using pyrocoated graphite tubes with L'vov platforms.

Results

Speciation and Hydrophobicity of Inorganic Silver. Inorganic silver speciation is primarily a function of chloride concentration in natural waters and is not sensitive to changes in pH. The effect of chloride concentration over a range of 0.5−50 mM (equivalent to a salinity range of 0.03−3‰) on silver speciation at pH 6 is shown in Figure 1. At low chloride concentrations (<1 mM, pCl > 3.0), the dominant silver species is the free ion, Ag^+. In natural waters where chloride concentrations range from 1 to 16 mM (pCl = 1.8−3.0), typical values of brackish waters, silver speciation is dominated by the uncharged neutral chloride complex, $AgCl^0$(aq). At higher chloride concentrations, higher order chloro complexes of Ag ($AgCl_2^-$, $AgCl_3^{2-}$, and $AgCl_4^{3-}$) become dominant.

Overall octanol−water partition coefficients (D_{ow}) of inorganic silver varied from 0.02 to 0.06 over a range of chloride concentrations (0−5 × 10^{-2} M) (Table 1). The D_{ow} of Ag measured with no chloride in the aqueous phase where all of the silver is present as the free ion was 0.03. The K_{ow} of $AgCl^0$(aq) was estimated using the K_{ow} of Ag^+ (0.03), measured D_{ow} values, and MINEQL speciation calculations (*19*) in

$$D_{ow} = \sum f_i(K_{ow})_i = f_{Ag^+}K_{ow-Ag^+} + f_{AgCl}K_{ow-AgCl} \quad (2)$$

where f_i is the fraction of Ag present as species i. The K_{ow} values of the other inorganic silver species, $AgCl_2^-$ and $AgCl_3^{2-}$, were assumed to be negligible. The D_{ow} measurements made at Cl^- concentrations ranging from 5 × 10^{-4} to 5 × 10^{-2} M yielded estimates of the K_{ow} of AgCl ranging from 0.08 to 0.09 (Table 1).

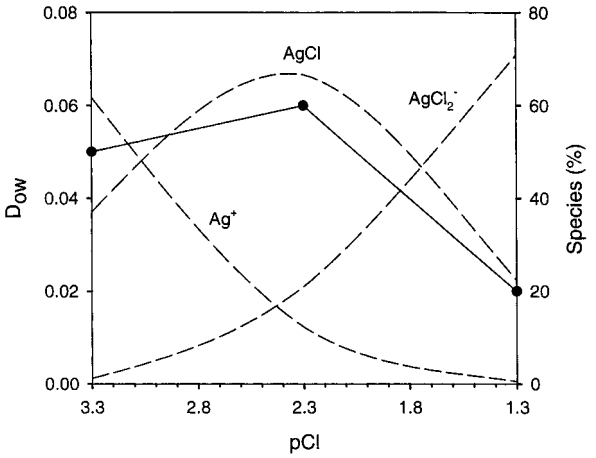

FIGURE 1. Overall octanol−water partition coefficients and speciation (dashed lines) of inorganic silver at pH 6 as a function of chloride concentration (pCl = −log [Cl^-]). Solid line represents the measured D_{ow} where data points (●) are means of triplicates (CV < 6.7%). Silver speciation calculations were made using MINEQL (*19*).

TABLE 1. Inorganic Silver Speciation and Octanol−Water Partition Coefficients at Various Chloride Concentrations (pH = 6 and Ionic Strength = [Cl^-])

	species (%)						
[Cl], M	Ag^+	AgCl	$AgCl_2^-$	$AgCl_3^{2-}$	$AgCl_4^{3-}$	D_{ow}	$K_{ow-AgCl}$
0	100	0	0	0	0	0.03	-
5 × 10^{-4}	61.8	37.0	1.2	0	0	0.05	0.09
5 × 10^{-3}	12.3	66.6	21.0	0.1	0	0.06	0.08
5 × 10^{-2}	0.5	22.4	70.8	5.3	0.9	0.02	0.09

Silver Accumulation. Cultured diatom cells accumulated silver (9.5 ± 1.1 × 10^{-2} amol $cell^{-1}$) from the Aquil medium before the addition of silver. Using the standard APDC/DDDC extraction method (*17*), the total background silver concentration in Aquil medium (post Chelex-100 column) was 15−20 pM. After the 30-min preincubation of diatoms in experimental media, steady-state cellular silver concentrations were inversely related to chloride concentration (Figure 2) showing that more silver was released from the cell surfaces at higher chloride concentration. Short-term uptake of silver in *T. weissflogii* shows rapid accumulation over the first hour with slower accumulation thereafter as steady state is approached. The microalgae cells accumulated Ag most rapidly at a Cl^- concentration of 5 mM (pCl = 2.3), reaching steady state in about 0.5 h (Figure 2).

Volume/volume concentration factors (VCF) calculated after 4-h exposure ranged from $10^{3.4}$ to $10^{3.5}$ for Ag accumulation in *T. weissflogii*, showing that only small differences in cell quotas (0.10−0.12 amol $cell^{-1}$) were observed at all chloride concentrations.

Silver Uptake Kinetics. Silver is a nonessential metal, and its accumulation in phytoplankton does not appear to be regulated (*1*). The uptake of silver by microalgae can therefore be modeled as a reversible, first-order exchange process. Using such a model the rate of change of cellular silver is given by

$$\frac{d[Ag]_{cell}}{dt} = \frac{k_1[Ag]_{water}}{C} - k_2[Ag]_{cell} \quad (3)$$

where $[Ag]_{water}$ is the molar concentration of Ag in the water;

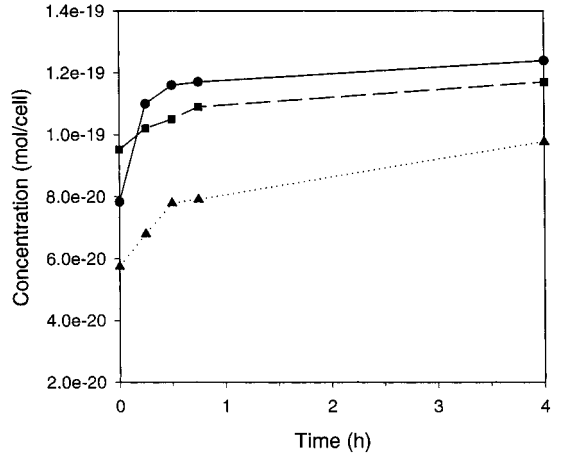

FIGURE 2. Short-term (4 h) silver uptake in the diatom *T. weissflogii* as a function of chloride concentration. Data points are means of triplicates where CV ranged from 0.1 to 25.2% for pCl = 3.3 (■), 2.3 (●), and 1.3 (▲).

TABLE 2. Inorganic Silver Uptake and Loss Rate Constants, Steady-State Influx Rates, and Volume/Volume Concentration Factors in the Marine Diatom *T. weissflogii* Exposed to 50 pM Total Ag

pCl	k_1 (h^{-1})	k_2 (h^{-1})	k_u (mol cell h^{-1})	VCF
3.3	0.304	1.30	1.52×10^{-19}	$10^{3.4}$
2.3	1.16	4.70	5.78×10^{-19}	$10^{3.5}$
1.3	0.222	1.13	1.11×10^{-19}	$10^{3.4}$

$[Ag]_{cell}$ is the cellular concentration of Ag (mol $cell^{-1}$); k_1 and k_2 are the uptake and loss rate constant 5 (h^{-1}), respectively; and C is the phytoplankton cell density (cells L^{-1}). Assuming $[Ag]_{water}$ and C are constant, the analytical solution of eq 3 is

$$[Ag]_{cell(t)} = \frac{k_1}{k_2 C}[Ag]_{water}(1 - \exp^{-k_2 t}) + [Ag]_{cell(0)} \exp^{-k_2 t} \quad (4)$$

where $(k_1/k_2C[Ag]_{water})$ is the steady-state concentration of cellular Ag and $k_1/C[Ag]_{water}$ (k_u, mol $cell^{-1}$ h^{-1}) is the steady-state influx rate. Short-term silver uptake data were fit to the kinetic model in order to determine the uptake and loss rate constants (k_1 and k_2) and the steady-state silver influx rates (k_u) at each Cl^- concentration (Table 2). Silver uptake rate constants ranged from 0.2 to 1.2 h^{-1} and were highest at a pCl = 2.3 (Table 2). Short-term uptake rates of silver in the cells increase with the percent of total silver present as $AgCl^0$(aq) and thus the silver D_{ow} (Figure 3).

Discussion

Speciation and Hydrophobicity of Inorganic Silver. Silver is one of the class B metals that, like Cd and Hg, forms stable complexes with chloride, iodide, and sulfur in aqueous solution (*20, 21*). Silver complexes with organic ligands, which have oxygen and nitrogen donor atoms, are usually unstable in brackish waters due to relatively low stability constants (*11, 12*) and strong binding with chloride. Miller and Bruland showed that silver does not form strong complexes with natural organic ligands in seawater and that organic complexation is relatively unimportant for silver in some estuarine environments (*11*). Silver speciation is therefore strongly affected by chloride concentration, which controls the formation of the strong chloro complexes $AgCl^0$(aq), $AgCl_2^-$, $AgCl_3^{2-}$, and $AgCl_4^{3-}$ in brackish and marine waters (Table 1).

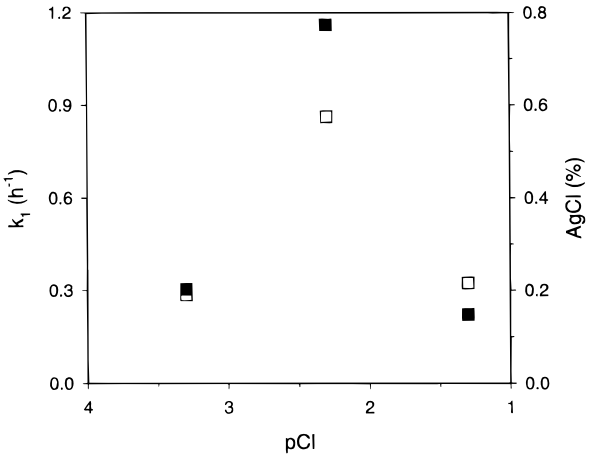

FIGURE 3. Silver uptake rate constants in the diatom *T. weissflogii* (■) and percent Ag present as AgCl(aq) (□) at three chloride concentrations. Percentages of Ag present as AgCl(aq) in the diatom uptake experiments are slightly lower than those in Table 1 because of the formation of $AgSO_4^-$ in the presence of 0.13 M Na_2SO_4.

In brackish waters, uncharged $AgCl^0$ is the dominant Ag species over a Cl^- concentration range of 10^{-3}–$10^{-1.8}$ M (Figure 1). The overall octanol–water partition coefficient (D_{ow}) of inorganic silver is highest in the middle of this range of chloride concentrations (Figure 1), showing that $AgCl^0$(aq) contributes most to the overall hydrophobicity of Ag. Silver D_{ow} values measured with no and $10^{-3.3}$ M Cl^- show some partitioning of Ag^+ into the octanol phase (Table 1). Thus, the free silver ion may contribute to the overall hydrophobicity of silver in freshwaters with low ($<10^{-3}$ M) Cl^- concentrations. However, the contribution of Ag^+ to the overall silver hydrophobicity is less important in brackish waters and saltwaters since its concentration is low and its K_{ow} is about one-third of $AgCl^0$(aq).

The K_{ow} of $AgCl^0$(aq) (0.09) is lower than that of $HgCl_2^0$ (3.3, ref *15*), consistent with the greater covalency (lower polarity) of the $HgCl_2^0$ complex (*22*), but is higher than that of $CdCl_2$ (2×10^{-3}, unpublished). The slight hydrophobicity of $AgCl^0$(aq) may contribute to the tendency of Ag to become associated with hydrophobic phases in estuarine waters such as organic colloidal matter (*23*) and sediment with organic coatings (*24*). Furthermore, the colloidal partitioning of Ag in San Francisco Bay (*25*) and a number of Texas estuaries (*23*) was found to decrease with increasing salinity, consistent with the lower hydrophobicity of higher order chloride complexes of Ag.

Silver Bioaccumulation. The microalgae uptake and octanol–water partitioning results show that the cellular silver accumulation rate is primarily controlled by the hydrophobicity of inorganic silver and is consistent with the passive diffusion of $AgCl^0$(aq) across cell membranes. Uptake rate constants of Ag in *T. weissflogii* were dependent on the overall hydrophobicity of inorganic silver in solution and were highest at a Cl^- concentration of 5 mM (pCl = 2.3) where uncharged AgCl(aq) is the dominant species (Figure 3). Any incidental silver uptake by phytoplankton via essential metal transport sites (e.g., Cd^{2+} uptake via a Mn^{2+} transporter; *26*) is unlikely given silver's low charge density as compared with divalent metal ions and low concentrations in natural waters. Silver uptake in phytoplankton like that of inorganic and methylmercury is a passive process.

Although Ag accumulation rates were dependent on Cl^- concentration, volume/volume concentration factors (VCF) of Ag in *T. weissflogii* were nearly equivalent at all Cl^- concentrations (Table 2). Thus the hydrophobicity of $AgCl^0$(aq) controlled Ag uptake kinetics, but reactivity with cellular components (e.g., protein sulfhydryl groups in the cell

membrane; *16*) controlled steady-state cellular concentrations. The silver VCFs we measured in *T. weissflogii* ($10^{3.4}$–$10^{3.5}$) were lower than those ($10^{4.5}$–$10^{5.8}$) of earlier studies (*1, 27*). Previous studies used relatively high concentrations of total silver (7.4 nM–9.5 μM) as compared to concentrations of total silver found in pristine and contaminated natural waters (10–200 pM) (*3*) and different microalgae species than used in this work. Although there is some variation in bioconcentration factors among different organisms, the significantly lower VCF values from our study indicate that silver accumulation by passive uptake of $AgCl^0$(aq) at environmentally realistic, low silver concentrations (50 pM) results in lower steady-state water–biota concentration factors than occur at higher concentrations.

Dissolved uptake accounts for 50–100% of Ag accumulation in estuarine bivalves (*5, 6*) and crustaceans (*7, 28*). The major accumulation of dissolved Ag in aquatic invertebrates may result from the direct uptake of the neutral AgCl(aq) complex as was suggested by Engel et al. (*14*), who found that Ag accumulation in the grass shrimp, *Palaemonetes pugio*, was best correlated with calculated concentrations of AgCl(aq) rather than those of Ag^+ or $AgCl_2^-$. This mechanism of Ag uptake is consistent with observations of greater accumulation of dissolved Ag by invertebrates at low salinity (10‰, $f_{AgCl} = 6\%$; *29*) than at high salinity (20‰, $f_{AgCl} = 1.7\%$; *24*). The importance of Ag speciation and invertebrate gill and mantle permeabilities of $AgCl^0$(aq) to the kinetics and extents of Ag accumulation in invertebrates is unknown but must be quantified to develop and test models of silver bioaccumulation in aquatic animals.

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