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Thiosulfate Enhances Silver Uptake by a Green Alga: Role of Anion Transporters in Metal Uptake

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Short-term (<1 h) silver uptake by the green alga *Chlamydomonas reinhardtii* was measured in the laboratory in defined inorganic media in the presence or absence of ligands (chloride and thiosulfate). In contradiction to the free-ion model of metal uptake, silver accumulation by the alga proved to be sensitive to the choice of ligand used to buffer the free silver concentration. For a low fixed free Ag^+ concentration of 10 nM, silver uptake in the presence of thiosulfate (0.11 μM) was 2× greater than in the presence of chloride (4 mM). When sulfate was removed from the exposure medium (i.e., 81 μM → 0 μM), silver uptake in the presence of thiosulfate was even more markedly enhanced (more than 4× greater than in the presence of chloride). Varying the sulfate concentration in the exposure medium only affected silver uptake if thiosulfate was present. We conclude that silver-thiosulfate complexes are transported across the plasma membrane via sulfate/thiosulfate transport systems and that sulfate acts as a competitive inhibitor of this uptake mechanism.

Introduction

It is generally accepted that the total aqueous concentration of a metal is not a good predictor of its <bioavailability>, i.e., the metal's speciation will affect its availability to aquatic organisms. Qualitatively, complexation of a metal normally leads to a decrease in its bioavailability—in effect, most dissolved ligands that bind metals form *hydrophilic* complexes, $ML_n^{\pm}$, and in such systems metal uptake, nutrition, and toxicity normally vary as a function of the concentration of the free-metal ion in solution (*1*). However, a number of intriguing experiments have been reported in the literature in which the metal's "residual" bioavailability in the presence of hydrophilic $ML_n^{\pm}$ complexes has been found to exceed that which would have been predicted on the basis of the free-metal ion concentration at equilibrium. Most of these apparent exceptions to the free-ion model (FIM) of metal toxicity involve ligands that are assimilable in their own right, and this has led to the suggestion that "accidental" metal transport may occur in their presence (i.e., the ligand is assimilated as a metal−ligand complex and the metal "comes along for the ride") (*2*). In earlier work in this area, we used unicellular algae as our biological model and focused on low molecular weight *organic* ligands such as citrate (*3*, *4*). In the present paper, we extend the concept of "accidental" transport to *inorganic* ligands.

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In principle, the assimilation of intact hydrophilic metal−ligand complexes could occur with inorganic ligands such as phosphate or sulfate. Uptake systems for such essential nutrient anions exist at biological interfaces; if these transport systems could be "fooled" into binding and transporting the intact metal−anion complex, then the metal would find its way into the cell "accidentally". For most metals, the formation of aqueous complexes with phosphate or sulfate anions is thermodynamically unfavorable; in freshwater environments and at environmentally realistic metal and anion concentrations, these complexes exist at levels so low as to rule out a significant role for accidental transport. However, if the metal and the inorganic ligand were to form very stable complexes, this thermodynamic argument would no longer apply.

Silver forms a series of very stable hydrophilic complexes with thiosulfate ($AgS_2O_3^-$, $Ag(S_2O_3)_2^{3-}$: $\log K_1 = 8.82$, $\log \beta_2 = 13.50$) (*5*). Thiosulfate is likely to be of importance in sediment porewaters (*6*), mine tailings ponds (*7*), and photofinishing industry effluents (*8*). The binding of Ag by thiosulfate will reduce the free Ag^+ concentration and, thus, according to the FIM, should reduce silver bioavailability (*9*). However, exactly the opposite results have been reported for Ag accumulation by rainbow trout, *Oncorhynchus mykiss*, in laboratory exposure experiments (*10*, *11*). Neither research group was able to offer a plausible explanation for their results, other than to suggest that the $AgS_2O_3^-$ complex was somehow "bioavailable". We postulated that silver could cross biological membranes as the silver-thiosulfate complex, via an anion transporter, and set out to test this "molecular mimicry" hypothesis (*12*) using a unicellular alga as our biological model. Since algae are known to possess membrane-bound transport systems for the assimilation of sulfate (*13*, *14*), they should be appropriate models for testing the hypothesis that thiosulfate (and silver-thiosulfate complexes) can mimic sulfate and enter the cells via the same pathway (*15*).

Experimental Section

Organism and Culture Conditions. The experiments were carried out with a euryhaline unicellular green alga, *Chlamydomonas reinhardtii* (University of Toronto Culture Collection; UTCC11), in defined inorganic media. Short exposure periods were used to minimize the influence of the algae on their exposure medium. Cells were grown axenically in 100 mL of modified high salt medium [modified from Macfie et al. (*16*); see Table S1, Supporting Information] with an ionic strength of 6 meq L^{-1}. To avoid metal precipitation, culture media were sterilized by autoclaving before the addition of the trace metal mix, which had previously been filter sterilized (0.2 μm polycarbonate membrane; Poretics). Axenic batch cultures were maintained under constant illumination at 100 ± 10 μE m^{-2} s^{-1} (Cool White Fluorescent Tubes), with rotary agitation at 50 rpm and a temperature of 20 °C in 250-mL polycarbonate Erlenmeyer flasks. For regular maintenance, ~2 mL of culture was transferred to a fresh, sterile medium (pH = 7) every week. Cultures were periodically checked for bacterial contamination by plating on nutrient agar (Difco-Bacto agar).

Reagents and Plasticware. All plasticware was soaked for at least 24 h in 10% HNO_3, thoroughly rinsed seven times with ultrapure water (18 MΩcm) and dried under a laminar flow hood prior to use. Teflon containers were used for silver exposure experiments. Salts used for cultures and experiments were of analytical grade or better. Radioactive silver (^{110m}Ag; 136 mCi $mmol^{-1}$) and manganese (^{54}Mn; 1365 mCi

TABLE 1. Exposure Conditions for the Silver Uptake Experiments

medium	$[Ag]_T$ (nM)	$[Ag^+]$ (nM)	$[Cl^-]$ (mM)	$[NO_3^-]$ (mM)	$[SO_4^{2-}]$ (μM)	$[S_2O_3^{2-}]$ (μM)
A	104	10	0.05	5.23	0.0	0.114
B	104	10	0.05	5.07	81.0	0.114
C	104	10	4.0	1.07	81.0	0.0
D[a]	10	10	0.05	5.07	81.0	0.0
E	104	10	0.05	5.23	0−200	0.114
F	104	10	4.0	1.07	0/200	0.0
G	104	10	0.05	5.23	0.0	0.114
H	104	104	0.05	5.23	0.0	0.0
I	104	10	0.05	5.23	120	0.114
J	104	10	4.0	1.23	0.0	0.0

[a] Data from Fortin and Campbell (*17*).

mmol^{-1}) were purchased from Amersham Canada. ^{110m}Ag activity was measured on a Wallac 1414 beta counter using Eco-Lume scintillation cocktail (ICN) whereas the ^{54}Mn activities were measured with a Wallac 1480 gamma counter (Perkin-Elmer Life Sciences, Turku, Finland). A quenching curve was obtained for beta counting by varying the quantity of algae in the presence or absence of filter membranes to reflect the different counting matrixes. Acidic stock solutions of cold and radioactive silver were kept at pH < 2 in the dark at 4 °C.

Experimental Procedures. Silver uptake experiments were performed with radiolabeled ^{110m}Ag, which afforded a low detection limit and allowed us to work at low total silver concentrations and with short algal contact times. For each experiment, cells were initially inoculated at a density of 2500 cells mL^{-1}, allowed to grow for 48 h, and then gently harvested in mid-exponential growth on a 2 μm polycarbonate filter membrane (Poretics) using a vacuum pressure of ≤10 cm Hg. Harvested cells were rinsed five times with 10 mL of sterile simplified culture medium (see Table S1, Supporting Information) containing neither phosphate nor trace metals, and then resuspended in ∼10 mL of the same simplified medium. Sulfate was normally present in this simplified exposure medium (81 μM), except in the sulfate-free experiments. Rapidly, size distribution, average surface area, and density were determined on the cell suspensions using a Coulter Multisizer II particle counter (70 μm orifice tube) and recorded. Cells were then exposed under the conditions outlined for each experiment (Table 1) for a short period of time (15 min, with the exception of one experiment that lasted 60 min). Short exposure times were designed to minimize release by the algal cells of metal-binding peptides that could affect silver speciation in solution and to minimize cell division that would increase cell density during the exposure. Unless otherwise specified, experiments were conducted under ambient laboratory conditions (22 °C and a low light regime of 7 μE m^{-2} s^{-1}; silver uptake was not affected by the decrease from 100 to 7 μE m^{-2} s^{-1} within the time frame of the exposure experiments; data not shown) and with low cell numbers (10 000 cells mL^{-1}), to minimize the effect of the algal cells on the exposure medium (e.g., minimal decrease in dissolved silver concentration, < 5% after 15 min). Finally, cells were recovered on two superimposed polycarbonate filters (2 μm) and rinsed four times with 10 mL of simplified culture medium containing 100 nM nonradioactive Ag. We had previously demonstrated that this rinsing step allowed us to remove the adsorbed silver and thus determine the operationally defined intracellular silver (*17*).

We corrected for passive retention of radioactivity by the polycarbonate filters by counting the lower filter and subtracting its activity from that of the upper filter; uptake values were then normalized for the total surface area of algae. Normalization of uptake in terms of surface area makes comparisons easier between species that have different cell sizes or weights. All uptake experiments were performed at neutral pH (7.0 ± 0.1) without addition of any buffers, to avoid possible effects on silver speciation and on the physiological state of the test alga (*18*). A minimum of three replicates was used unless otherwise indicated.

Silver speciation in the exposure solutions was calculated with the chemical speciation model MINEQL+ (*19*) with an updated thermodynamic database prepared from a reliable source of thermodynamic data (*5*). The database is available at http://www.inrs-eau.uquebec.ca/activites/groupes/biogeo/personal.htm.

Silver Thiosulfate Exposures. Four experiments were carried out. The first experiment involved following silver uptake over time (0, 23, 45, and 60 min) in three exposure media (Table 1, media A, B, and C). Total and free silver nominal concentrations were kept constant at 104 and 10 nM, respectively, for all three media. Thiosulfate (114 nM) was added to media A and B, but medium A differed from B in that it contained no sulfate ($Mg(NO_3)_2$ substituted for $MgSO_4$). In medium C, chloride was used to buffer the free silver concentration (4 mM KCl; the ionic strength was kept constant by reducing the amount of KNO_3; see Table 1).

In the second experiment, short-term silver uptake (15 min) was measured as a function of sulfate concentration in the presence of either chloride or thiosulfate. Again, total and free silver concentrations were kept constant at 104 and 10 nM, respectively, for all media. Six sulfate concentrations were tested using thiosulfate as the silver-binding ligand (medium E: 0, 40, 80, 120, 160, and 200 μM SO_4); only the lowest and highest sulfate concentrations were tested for silver uptake with chloride (4 mM; medium F) as the ligand.

In the third experiment, silver uptake (15 min) was determined in the absence of complexing ligands (medium H) and compared with uptake in three contrasting media: (J) 4 mM Cl, 0 μM SO_4; (G) 114 nM S_2O_3, 0 μM SO_4; (I) 114 nM S_2O_3, 120 μM SO_4. Total silver was held constant at 104 nM in all exposures, and the free silver concentration was also constant 10 nM, except in the noncomplexing medium H that contained neither chloride nor thiosulfate and thus had a free silver concentration virtually equal to the total silver concentration.

The final experiment was designed to confirm that the presence of thiosulfate was not affecting overall membrane transport. To check this point, we measured uptake of Mn^{2+}, a cation with minimal affinity for thiosulfate ($Mn^{2+} + S_2O_3^{2-} \leftrightarrow MnS_2O_3$; $\log K = 1.95$) (*5*), in media G and H. Total Mn concentrations in both media were equal (35 nM) and corresponded to free Mn^{2+}, since Mn speciation is unaffected by the change from 0 to 114 nM thiosulfate at pH 7. Four 50-mL aliquots were retrieved 15 min after inoculation of cells at a density of 40 000 cells mL^{-1}. The cells were collected by gentle filtration onto polycarbonate membranes (2 μm), and adsorbed ^{54}Mn was removed by rinsing with a simplified culture medium solution containing 10^{-4} M EDTA (*20*).

Results

Silver uptake by *C. reinhardtii* was strongly affected by the anions present in the exposure medium (Figure 1), even though the free Ag^+ concentration was kept constant at 10 nM in each medium. Silver accumulation over the interval 0 → 25 min increased in the following order relative to the basic nitrate medium: 1 (NO_3^-; data from Fortin and Campbell (*17*)) < 2.2 (Cl^-) < 5 ($S_2O_3^{2-}$, normal sulfate) < 14 ($S_2O_3^{2-}$, no sulfate).

Increasing the sulfate concentration in the exposure medium decreased silver uptake in the presence of thiosulfate until it reached a concentration of 120 μM SO_4^{2-} (Figure 2),

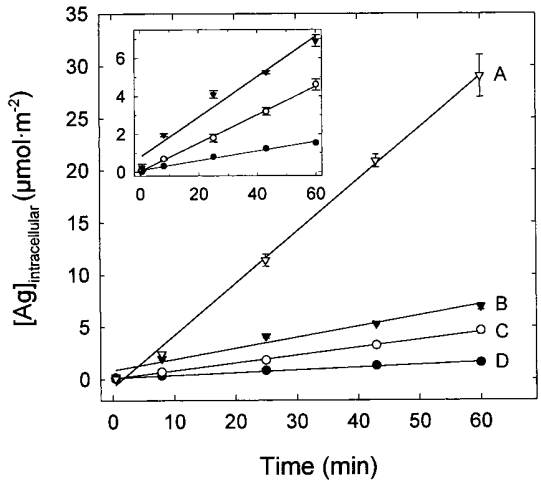

FIGURE 1. Time-course of silver uptake at constant Ag^+ (10 nM). (A) Uptake at low chloride in the presence of thiosulfate but absence of sulfate. (B) Uptake at low chloride in the presence of both thiosulfate and sulfate. (C) Uptake at high chloride in the absence of thiosulfate. (D) Uptake at low chloride in the absence of thiosulfate (data from Fortin and Campbell (*17*)). Error bars represent standard deviations from the average of three measurements. Inset shows uptake curves B, C, and D on a smaller scale.

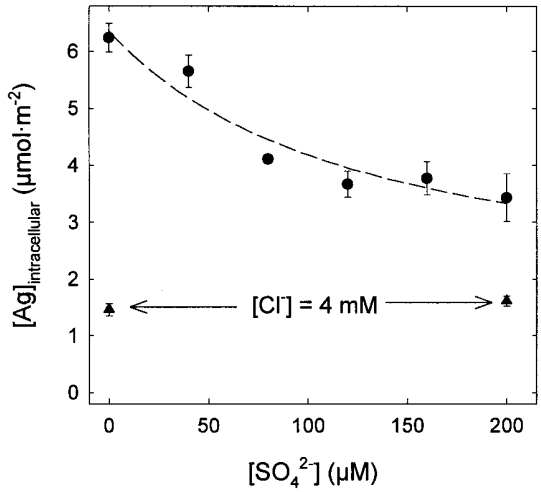

FIGURE 2. Effect of sulfate concentration on silver uptake at constant Ag^+ and Ag_T in the presence of thiosulfate (●) or chloride (▲). Error bars represent standard deviations from the average of three measurements.

after which point there were no further changes in uptake (*t*-test; $P > 0.05$). On the other hand, sulfate concentrations did not affect silver uptake when chloride was used as the complexing ligand (*t*-test; $P > 0.05$). Even at high sulfate concentrations, silver uptake was still higher for thiosulfate than for chloride as a ligand for equal total and free silver concentrations (Figure 2; compare the two right-hand points).

As expected, silver uptake in the presence of chloride (4 mM) was lower than in the noncomplexing medium (0.53; Figure 3, column J ÷ column H), consistent with the anticipated protective effect of chloride complexation. Uptake in thiosulfate/sulfate medium was also less than in the noncomplexing medium, but only slightly so (0.85; Figure 3, column I ÷ column H; *t*-test, $P < 0.05$). Even more remarkably, silver uptake in the sulfate-free thiosulfate medium G was *higher* than in the ligand-free medium H (1.9; Figure 3, column G ÷ column H), revealing an enhancement of silver uptake even though the free Ag^+ concentration for column G was 10× *lower* than for column H.

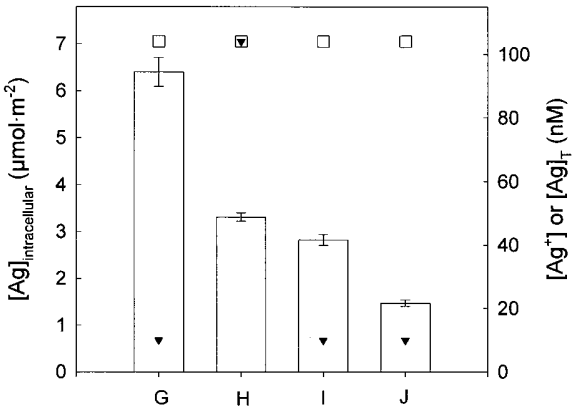

FIGURE 3. Comparison of silver uptake after 15 min of exposure from four exposure media at either 10 or 104 nM Ag^+ (▼) or Ag_T (□). (G) Uptake at low chloride (5 μM) in the presence of thiosulfate (114 nM) but absence of sulfate. (H) Uptake at low chloride (5 μM) in the absence of both sulfate and thiosulfate. (I) Uptake at low chloride (5 μM) in the presence of both sulfate (120 μM) and thiosulfate (114 nM). (J) Uptake at high chloride (4 mM) in the absence of both sulfate and thiosulfate. Error bars represent standard deviations from the average of three measurements.

The presence or absence of thiosulfate had no significant effect on the level of Mn uptake after 15 min of exposure (0.99 ± 0.09 vs 0.95 ± 0.07 nmol Mn m^{-2} with and without thiosulfate, respectively; $N = 4$; *t*-test, $P > 0.05$).

Discussion

Thiosulfate Enhancement of Silver Uptake by *C. reinhardtii*. In previous experiments, we had demonstrated that silver uptake by *C. reinhardtii* was enhanced in the presence of chloride (Figure 1, inset, compare curves C and D). The enhanced uptake observed in the presence of chloride was related to the very high silver uptake rates demonstrated by the test alga, which led to diffusion limitation in the boundary layer surrounding the algal cell (*17*). In such a situation, metal accumulation is proportional to the total metal concentration (i.e., to the concentration gradient between the bulk solution and the algal surface); in the high chloride medium C the total silver concentration was higher than in the low chloride medium D (104 vs 10 nM; see Table 1). This diffusion limitation dissipated at total Ag concentrations greater than 10^{-7} M.

A similar but greater increase in silver uptake was observed in the current experiments in the presence of thiosulfate (Figure 1, compare media A and B with medium C). In this case, however, changes in total silver concentration cannot be invoked to explain the enhanced metal uptake in media A and B, since both total and free silver concentrations were equal in all three media (104 and 10 nM, respectively; see Table 1). Instead, we conclude (i) that the enhanced uptake observed in the presence of thiosulfate is the result of silver-thiosulfate complexes being transported across the plasma membrane via sulfate/thiosulfate transport systems and (ii) that this membrane transport mechanism is affected by the external sulfate concentration.

There are several indications in the literature that sulfate and thiosulfate share a common membrane transport system in bacteria (*21*−*23*) and algae (*15*, *24*, *25*). A competitive effect between sulfate and thiosulfate has been noted in sulfate uptake experiments with unicellular green algae (*24*−*26*), thiosulfate being an efficient inhibitor of sulfate uptake. Several algal species can grow on thiosulfate as a sole sulfur source (*15*, *25*), and thiosulfate reductase activity has been detected in crude extracts of *C. reinhardtii* (*27*, *28*).

Knowing that sulfate and thiosulfate might share a common transport system, and given that exposure medium

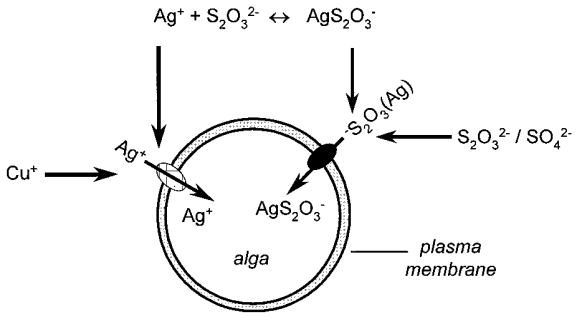

FIGURE 4. Conceptual model of silver interactions with transport systems at the plasma membrane in the presence of sulfate and thiosulfate.

B contained much more sulfate (81 μM) than thiosulfate (0.114 μM), we removed all sulfate from the medium (Figure 1; curve A). Removal of sulfate lead to a greater than 6-fold increase in silver uptake as compared to the chloride exposure medium (curve C). This result clearly supports our contention that a sulfate/thiosulfate transporter is involved in silver uptake in the presence of thiosulfate. Progressive addition of sulfate resulted in a gradual decrease in silver uptake (Figure 2), as would be expected from sulfate/thiosulfate competition for a membrane transport system. These changes in the sulfate concentration in the exposure medium only affected silver uptake if thiosulfate was present; silver uptake in the presence of 4 mM chloride was unaffected ($P > 0.05$; t-test) by the complete removal of sulfate (Figure 2, compare the two filled squares).

As a further demonstration that the effect of thiosulfate on silver uptake was not due to some general physiological effect of the algal cells, we designed a new experiment to demonstrate that this anion has no effect on metal uptake when the metal is not one that forms thiosulfato complexes. We chose Mn^{2+} as a probe to test this prediction because of its low affinity for thiosulfate (<0.001% MnS_2O_3 in the exposure solution). As we would have predicted, the results of this experiment show no significant effect of thiosulfate on Mn uptake (0.99 ± 0.09 vs 0.95 ± 0.07 nmol of Mn m^{-2} with and without thiosulfate, respectively).

We conclude that in the absence of thiosulfate (media C and D) silver is taken up via a cation transporter (probably via a Cu(I) transport system (*17*, *29*, *30*)) and that this transporter is unaffected by changes in ambient sulfate concentrations. In media A and B, however, a second parallel pathway for silver uptake is introduced, involving the accidental transport of silver-thiosulfate complexes via one or more sulfate/thiosulfate transporters. This conceptual model of silver uptake mechanisms is illustrated in Figure 4.

Assuming that Ag^+, $AgS_2O_3^-$, $S_2O_3^{2-}$, and SO_4^{2-} are all at equilibrium with their respective binding sites, and considering that only the sulfate concentration was varied in the upper curve of Figure 2 while all other parameters were constant, we derived a two-binding site model:

$$\varphi_{Ag} = \alpha[Ag^+] + \frac{\beta[AgS_2O_3^-]}{1 + K[SO_4^{2-}]} \quad (1)$$

where the intracellular silver flux rates (φ_{Ag}; μmol m^{-2} min^{-1}) can be expressed by the addition of two terms. The first term is dependent on the free silver concentration while the second term is related to the concentrations (mol L^{-1}) of $AgS_2O_3^-$ and is subject to competition from SO_4^{2-} according to an affinity constant K for the transport site responsible for the uptake of the silver complex. Curve fitting to the experimental data using the least-squares method resulted in the following constants: $\alpha = 10^{6.96}$; $\beta = 10^{6.55}$; $K = 10^{3.89}$ (Figure 2; $r^2 = 0.89$). Extrapolation to infinitely high sulfate concentrations results in uptake levels equal to those observed in the chloride medium. Addition of an excess thiosulfate ($[S_2O_3] \gg [Ag]$) also inhibited silver uptake due to competitive binding between free $S_2O_3^{2-}$ and $AgS_2O_3^-$ complexes for transport sites (see Figure S1 and Table S2, Supporting Information).

The quantitative importance of the silver-thiosulfate uptake pathway can be deduced from Figure 3 (comparison of columns G and H). For equal total silver concentrations (104 nM), silver uptake after 15 min exposure was 2× higher in the presence of thiosulfate (in a sulfate-free medium) than in its absence. Silver uptake is thus not only "greater than would have been expected" on the basis of the free Ag^+ concentration, but is in fact truly enhanced by the presence of thiosulfate. Residual uptake due to the presence of 10 nM Ag^+ in medium G was estimated to be of 0.33 μmol m^{-2} (10% of column H), whereas uptake due to 94 nM $AgS_2O_3^-$ complexes in medium G was 6.1 μmol m^{-2} (column G less 0.33 μmol m^{-2}). Even if the uptake rate for 10 nM Ag^+ is extrapolated linearly to 94 nM (i.e., to a level equivalent to $[AgS_2O_3^-]$), silver uptake after 15 minutes would be only 32 nmol m^{-2} for Ag^+ compared to 65 nmol m^{-2} for $AgS_2O_3^-$. Under these conditions, silver uptake rates via the thiosulfate transport system are thus about twice those through the cation transporter.

The prevailing paradigm for metal uptake by aquatic organisms, i.e., the free ion model or its derivative the biotic ligand model, assumes that metals enter living cells via facilitated cation transport. Most known exceptions to the FIM (*2*) involve ligands that form lipophilic complexes, $M-L_n^o$, which can bypass normal metal transport mechanisms and cross biological membranes by simple diffusion (*31*), assimilable organic ligands (*3*, *4*, *32*), or "chaperone" ligands that are synthesized by living (micro)organisms specifically to complex essential metals and facilitate their eventual uptake (e.g., the role of siderophores in iron nutrition (*33*)). In contrast, evidence of metal uptake through *anion* transport systems is scarce to nonexistent. To our knowledge, the present results represent the first hard evidence for metal transport into cells via an inorganic anion transport system.

Environmental Implications. The environmental implications of our findings will depend on two factors: (i) how likely are metal-thiosulfate complexes to exist in the exposure medium (e.g., in natural waters), and (ii) how widespread are the membrane transport systems involved in the movement of sulfate/thiosulfate across biological membranes.

In synthetic solutions, the speciation of silver is strongly influenced by simple inorganic ligands (chloride, thiosulfate), by polysulfides, and by organic ligands containing reduced sulfur binding groups (thiols); each of these ligand classes can also be found in natural waters. Chloride is one of the major anions in natural waters, and is present in many industrial effluents and in urban runoff. Thiosulfate is less common in natural waters, but is present in effluents from photofinishing plants (*8*) and is also found in the interstitial waters of suboxic sediments (*6*) and in mine tailings ponds (*7*). Fully reduced sulfur(II) species have recently been reported to persist in oxic natural waters, despite their inherent thermodynamic instability in the presence of oxygen (*34*, *35*), and the reported steady-state concentrations exceed normal values for ambient dissolved silver. The nature of these reactive sulfide forms is only beginning to be unravelled, but possible candidate species include dissolved sulfide (stabilized by complexation to Zn or Cu) and polysulfide clusters (*36*). If these reduced sulfur species are accessible, then they would be expected to react with dissolved silver and dominate its speciation in solution. Thiosulfate would not be able to compete effectively with the reduced S(II) species, and silver-thiosulfato complexes would not exist at

appreciable levels in natural waters. If, on the other hand, the reduced sulfur species react only slowly with metals such as silver, then the suggestion that silver-thiosulfate complexes exist in natural waters becomes more plausible.

With respect to the second factor, it is likely that uptake of the anionic Ag-thiosulfato complex could occur in other algal species and in bacteria that have sulfate/thiosulfate transport systems, or even thiosulfate-specific transport systems, but generalization of this uptake mechanism to other species will require further investigation. The case for higher organisms such as fish is less obvious, since little is known about sulfate transport mechanisms in gill cells. However, in laboratory exposure experiments, Ag accumulation by rainbow trout, *Oncorhynchus mykiss*, was inexplicably enhanced in the presence of thiosulfate (*10*, *11*). It is tempting to explain this greater-than-expected silver accumulation by generalizing our conceptual model of silver accumulation, but thiosulfate uptake by fish remains to be demonstrated.

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Supporting Information Available

Composition of the algal culture medium and of the simplified medium used for the exposure experiments (Table S1); thiosulfate concentration dependence of silver uptake (Figure S1); distribution of silver and thiosulfate species in the exposure media (Table S2); and effect of the ratio of free to complexed thiosulfate on silver uptake (Figure S2). This material is available free of charge via the Internet at http://pubs.acs.org.

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