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Kinetic Controls on the Complexation between Mercury and Dissolved Organic Matter in a Contaminated Environment

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The interaction of mercury (Hg) with dissolved natural organic matter (NOM) under equilibrium conditions is the focus of many studies but the kinetic controls on Hg−NOM complexation in aquatic systems have often been overlooked. We examined the rates of Hg−NOM complexation both in a contaminated Upper East Fork Poplar Creek (UEFPC) in Oak Ridge, Tennessee, and in controlled laboratory experiments using reducible Hg (Hg_R) measurements and C_{18} solid phase extraction techniques. Of the filterable Hg at the headwaters of UEFPC, >90% was present as Hg_R and this fraction decreased downstream but remained >29% of the filterable Hg at all sites. The presence of higher Hg_R concentrations than would be predicted under equilibrium conditions in UEFPC and in experiments with a NOM isolate suggests that kinetic reactions are controlling the complexation between Hg and NOM. The slow formation of Hg−NOM complexes is attributed to competitive ligand exchange among various moieties and functional groups in NOM with a range of binding strengths and configurations. This study demonstrates the need to consider the effects of Hg−NOM complexation kinetics on processes such as Hg methylation and solid phase partitioning.

Introduction

The fate and transport of mercury (Hg) in aquatic environments is strongly influenced by its interaction with inorganic and organic ligands (*1−3*). Under anaerobic conditions and in the presence of sulfide, inorganic Hg−sulfide dominate the complexation although dissolved natural organic matter (NOM) has also been shown to influence the complexation in these environments (*2, 4−6*). In freshwater systems, when sulfide is not present, complexes between Hg and NOM dominate due to the strong interaction of Hg with reduced sulfur functional groups on the NOM, but this interaction is not completely understood (*2, 7−10*). While the complexation of Hg has been investigated using both NOM isolates (*9, 11−13*) and natural water samples (*7, 8, 14*), these studies assume that equilibrium conditions have established. In many environments, such as systems receiving Hg inputs from mine wastes or industrial discharges, equilibrium conditions may not be established, resulting in misinterpretation of data based on equilibrium thermodynamic models.

In laboratory experiments examining Hg−NOM complexation, solutions are often allowed to equilibrate several hours since it has been realized that the interaction of Hg with NOM is not instantaneous (*5, 8−10, 12*). Wu et al. (*15*) reported that the formation of Hg−NOM complexes in streamwater occurred within 20 s, but these experiments were conducted at high Hg concentrations (30 mg/L) in streamwater containing natural levels of dissolved organic carbon (3−12 mg DOC/L) and likely do not reflect the interaction of Hg with NOM under natural conditions. Two studies measuring the complexation strength of Hg with naturally occurring ligands have shown that the formation of Hg−ligand complexes is not instantaneous and an equilibration time of 9 h is needed for the reaction (*8, 10*). The range of equilibration time used in different studies highlights the need to better understand the rate at which Hg−NOM complexes form since this process will influence the interpretation and determination of Hg−NOM binding mechanisms and stability constants. A simple assumption of equilibrium complexation could result in erroneous predictions regarding the dominant Hg complexes present.

In this study, the formation of Hg−NOM complexes was examined both in a contaminated Upper East Fork Poplar Creek (UEFPC) in Oak Ridge, Tennessee and in laboratory prepared solutions with an NOM isolate and creek water from UEFPC. We test the hypothesis that, in aquatic systems receiving input of inorganic Hg, complexation equilibrium may not be established due to interactions of Hg with different functional groups in NOM. We suggest that the formation rates of Hg−NOM complexes be determined in order to assess the dominant complexes of Hg and its speciation, particularly in contaminated natural aquatic systems.

Experimental Section

Field Sampling. Creek water samples were collected on February 25, 2009 at 7 sites along the upper 2.5 km of UEFPC. Three operationally defined Hg fractions were analyzed, including unfiltered (Hg_T), 0.2 μM filterable (Hg_F), and reducible (Hg_R), defined as Hg(II) reduced to Hg^0 with stannous chloride ($SnCl_2$). The headwaters of this creek consist of water from an industrial outfall (OF200) mixed with water from Melton Hill Lake (MHL), which is used to manage the water flow in this contaminated system. Under base flow conditions in February 2009, the headwaters of UEFPC were comprised of approximately 80% MHL and 20% OF200. Sample locations in the creek were labeled according to the kilometers downstream from the headwaters (e.g., Station 0.6 was 0.6 km from the headwaters). Between October 2008 and March 2009, extensive sampling of the surface water was conducted in the upper 2.5 km of UEFPC which included measurements of pH, oxidation−reduction potential (ORP), and dissolved oxygen (DO). Measured ORP values in the surface waters of this shallow creek ranged from 96 to 226 mV and DO concentrations were always >8 mg/L. Under the high oxidizing conditions dissolved sulfide species are unstable and are unlikely to be present (*16*). For Hg_R measurements, samples were first filtered through 0.2 μm cellulose nitrate disposable filters, and Hg_R was determined within 30 min of collection in the field. To examine the loss of Hg_R in UEFPC water, a water sample from Station 0.9 was also filtered, and Hg_R measured at various time intervals following its collection.

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Laboratory Experiments. *Equilibrium Experiment.* The Hg_R concentration in solution with several inorganic and low molecular weight (LMW) organic ligands was examined under equilibrium conditions. Solid phase extractions (SPE) were also performed on a subset of the solutions. Solutions were prepared in 0.1 M sodium perchlorate, added to adjust the ionic strength, and a phosphate buffer (10 mM total phosphate) so that the pH could be adjusted between 7.5 and 7.8. Each ligand solution contained 20 μM of sodium chloride, sodium acetate, sodium citrate, thioglycolic acid, thiosalicylic acid, or cysteine. Solutions were also prepared with Suwannee River natural organic matter (SR NOM), an unfractionated reference NOM obtained from the International Humic Substances Society (IHSS). SR NOM was isolated using reverse osmosis (*17*), and is well characterized and has been used previously to examine the interaction of Hg with NOM (*5*). Experimental NOM solutions were prepared from a concentrated (800 mg C/L) solution, which was filtered through a 0.2 μm filter before use. Unless otherwise noted, all NOM solutions were held overnight in a refrigerator before the addition of Hg. Mercury was added to all solutions to obtain a final concentration of 100 ng/L using an unacidified inorganic working standard (1 μg/L), and the solutions were equilibrated for a minimum of 24 h before Hg_R analysis. The working standard was prepared from a 1000 mg/L stock $Hg(NO_3)_2$ (Ricca Chemical Company, preserved with 3% nitric acid) and the concentration of the working standard was tested with each experiment. For all experiments, Hg_T in samples were taken throughout the experiment so that corrections could be made for the potential loss of Hg to the container walls. Experiments were conducted in amber glass bottles that were held in the dark for the duration of the experiment. Before use, all bottles were combusted at 500 °C followed by a 24 h soak in 10% HCl to remove organics and Hg, respectively.

Kinetic Experiments. Kinetic experiments were conducted to examine the influence of NOM concentration on the rate of Hg−NOM complexation using SR NOM. Solutions of NOM were prepared as described above using three concentrations of SR NOM (1, 8, and 16 mg C/L). Hg from the working standard was added to the experimental solutions (to a final concentration of 100 ng/L), and Hg_R and Hg_T were measured several times over a 26 h period. Experiments were also conducted to examine the formation of Hg−NOM complexes in water collected from UEFPC. Water was collected at the exit of the pipe transporting the water from MHL to the creek and at Station 0.2 and was filtered in the laboratory using 0.2 μm Supor filters. The water was held overnight to allow the background Hg to reach equilibrium complexation. Using the working standard, Hg was added to the creek water, and Hg_R and Hg_T were measured several times over 26 h. Filtered water from MHL was also used to examine the influence of Hg concentration on the formation of Hg−NOM complexes using three concentrations of Hg (50, 100, and 200 ng/L).

Solid Phase Extractions. Solid phase extractions (SPE), using Supelclean ENVI-18 cartridges (0.5 g C_{18} resin), were used to examine the formation of hydrophobic Hg−NOM complexes in laboratory experiments examining the kinetics of complexation in UEFPC water. To clean the cartridges, 10 mL of 0.1 M HCl in 10% methanol was passed through the cartridge followed by 50 mL of nanopure water. The cartridges were equilibrated with 10 mL of the sample solution followed by the sample extraction, at 2 mL/min, using 25 mL of experimental solution. The Hg_T concentration was measured in the whole sample and in solution after passing through the cartridge during sample extraction. The hydrophobic Hg, operationally defined as the Hg extracted by SPE, was determined by the difference between the Hg in the whole sample and Hg in the cartridge-passing fraction.

Mercury Analysis. Mercury analysis was conducted using cold vapor atomic fluorescence spectroscopy (CVAFS) detection of Hg^0 (*18, 19*). For Hg_R measurements, hydrochloric acid (0.5% HCl) was added to the samples followed by immediate reduction using 500 μL of $SnCl_2$ (20% w/v solution in 10% HCl). The Hg^0 was purged from solution using ultra high purity argon and collected on gold coated sand traps. The mercury was thermally desorbed under a flow of argon and detected using CVAFS. A purge time of 15 min was used for all Hg_R analyses based on preliminary experiments examining the time required to reach a steady state concentration of Hg_R (Supporting Information (SI) Figure S1). Using purge times ranging from 1 to 20 min, Hg present as an inorganic $Hg(OH)_2$ complex was completely reduced and purged from solutions within 2.5 min. When complexed to LMW organic ligands, a reaction/purge time of 10 min was required to completely remove the Hg from solution. A minimum purge time of 10 min was also required to reach a consistent and reproducible concentration of Hg_R in a solution containing NOM or UEFPC water. For total mercury (Hg_T) analysis, bromine monochloride was added to samples for a minimum of 24 h. Hydroxylamine hydrochloride was added to the sample just prior to the analysis by an automated CVAFS system (Tekran 2600). Sample duplicates, spikes and reference materials were routinely analyzed for quality controls. For Hg_T analysis, relative standard deviations of duplicate samples were less than 5%, and average spike recoveries were 100 ± 3%. A digested sediment reference material (NIST 8407) was also routinely measured and values were within 97 ± 3% of the reported value. Relative standard deviations of Hg_R samples were less than 5%.

Ancillary Measurements and Hg Speciation Modeling. The dissolved organic carbon (DOC) concentration in filtered UEFPC water samples (0.2 μm) and laboratory solutions was determined using a Shimazu TOC-5000A total organic carbon analyzer. A Dionex ICS-1500 ion chromatography system was used to measure the concentration of anions (chloride, sulfate, and nitrate). Filtered samples (0.2 μm) were kept in a refrigerator until analysis for anions. For TOC analysis samples were acidified to pH ∼ 2 with hydrochloric acid. Equilibrium complexation calculations were performed using the chemical speciation program MINEQL+ and formation constants in the MINEQL database (*20*) for inorganic Hg complexes. Constants for Hg−NOM and Hg−LMW ligand complexes (SI Table S1) were selected based on a recent review of previously reported data for Hg bound to LMW thiol compounds and NOM (*6*). Reported complexation constants for Hg−NOM at environmentally relevant Hg/NOM ratios vary multiple orders of magnitude ($\log K = 21.6-47.7$). However this variability does not appear to significantly influence the speciation calculations conducted in this study (see discussion below).

Results and Discussion

Reducible and C_{18}-Extractable Hg−NOM Complexes. Ionic Hg(II) complexed to inorganic ligands such as chloride (Cl^-) and hydroxide (OH^-) was completely reducible by $SnCl_2$ under the conditions used in this study (SI Table S2). Hg in solution with SR NOM, however, was only partially reducible, yielding a small fraction (≤11%) of Hg_R. Although small, the presence of Hg_R suggests that some fraction of the Hg is either present as inorganic complexes or complexed to functional groups on the SR NOM that are reducible by $SnCl_2$. Similarly, only 10% of the Hg added to UEFPC water (Station 2.5) was reducible after a 24 h equilibration period. Hg complexed to five LMW model organic ligands was completely reducible. In solutions containing oxalate or citrate, equilibrium calculations predict that Hg−hydroxide complexes should dominate even when these ligands are present at micromolar concentrations because of the weaker binding strength of oxalate and citrate. Interestingly, Hg complexed to thioglycolic acid, cysteine, and thiosalicylic acid ligands are also completely reducible even though these thiol-containing ligands are known to form strong complexes with Hg and exhibit complexation strengths similar to those of NOM (*6*). A similar observation has been reported with EDTA (*8*), a nonthiol containing ligand that forms a bidentate complex with Hg. These observations demonstrate that reducible Hg measurements are not solely a function of complexation strength, and caution needs to be used in the interpretation of Hg_R data. Additionally, in natural waters, it is likely that Hg associated with some functional groups on NOM or with naturally occurring LMW molecules is reducible by $SnCl_2$.

Solid phase extractions using C_{18} provide another means of measuring Hg−NOM complexes in UEFPC. When complexed to inorganic ligands or cysteine, <15% of the Hg was retained by the C_{18} column (i.e., present as hydrophobic fraction) (SI Table S2). When Hg was added to creek water and allowed to equilibrate for 24 h, 94% of the Hg was present as hydrophobic complexes. The differences in the reducibility and C_{18} extractability of equilibrated Hg−NOM complexes and Hg complexed to inorganic or LMW organic ligands enabled these techniques to be used in monitoring changes in Hg−NOM complexation.

Kinetics of Hg−NOM Complexation. For all laboratory experiments, equilibrium speciation calculations predict that all of the Hg(II) should be complexed to thiol functional groups on NOM. The binding sites available for Hg complexation on NOM were estimated using the formula of 0.15% of DOC on a mass basis as reported in Skylberg (*6*). This was derived from spectroscopic analysis of NOM (*21, 22*) and is consistent with other estimates of Hg binding sites on NOM (*13, 14*). Because of the presence of excess amounts of NOM, Hg(II) complexation with thiol functional groups on NOM should dominate at equilibrium, even if a complexation constant on the low end of the reported range of values (log $K = 22.4$) is used in the speciation model. Greater than 85% of the Hg in solution with the NOM is anticipated to be present as a nonreducible Hg−NOM complexes under equilibrium conditions (SI Table S2).

The reaction kinetics between Hg(II) and NOM in MHL and UEFPC Station 0.2 waters were examined by measuring the loss of Hg_R and the formation of hydrophobic Hg−NOM complexes after the addition of inorganic Hg(II). The Hg_R measurements and C_{18} extractions provided two independent analyses demonstrating that the formation of Hg−NOM complexes is kinetically controlled (Figure 1). Within the first hour of the reaction, the Hg_R concentration dropped by 28% and 37% in the MHL and in Station 0.2 water, respectively. This was followed by a gradual decrease in the Hg_R concentration as the hydrophobic Hg concentration increased. The decrease in reducible Hg and the increase in C_{18} extractable Hg throughout the experiment indicate that Hg is being transferred from inorganic complexes or reducible Hg−NOM complexes to stronger nonreducible/hydrophobic Hg−NOM complexes.

The influence of the Hg:NOM ratio on Hg−NOM complexation kinetics was further examined using MHL water and solutions containing SR NOM. In the first, three concentrations of Hg (50, 100, and 200 ng/L) were added to filtered MHL water and the rate of Hg_R loss was monitored over time. In the second experiment, one concentration of Hg (100 ng/L) was used and the NOM concentration was varied using SR NOM. In all experiments, a significant loss of Hg_R (16−65%) occurred within the first hour, as a result of an initial rapid interaction between Hg and the NOM, with the largest losses occurring in the treatments with the highest NOM concentration (Table 1). In a solution containing 8 mg C/L SR NOM spiked with 100 ng/L Hg, the loss of Hg_R was also measured at 5 min intervals for the first hour in an attempt to quantify this initial reaction rate. However, measured Hg_R concentrations within the first hour (47−60%) were comparable to that observed at 1 h, suggesting that the initial interaction of Hg with the NOM is very rapid. We were unable to quantify this initial reaction rate due to limitations of using the $SnCl_2$ reduction and purging technique. This initial rapid drop was followed by a slower decrease in Hg_R as observed in the UEFPC water, demonstrating that the process occurring in natural water can be simulated using a NOM isolate. To ensure that the loss of Hg_R was not a result of the degradation or oxidation of the organic ligands in 0.1 M sodium perchlorate, a separate experiment was conducted to examine the loss of Hg_R in two solutions (containing 8 mg C/L SR NOM), which were equilibrated for different lengths of time (18 and 42 h) before the addition of Hg (120 ng/L). If the organic ligands in the NOM solution were degrading, we would expect differences in the concentration of Hg_R in the two treatments, which was not observed (SI Figure S2). While perchlorate is a strong oxidizer, it is known to be exceedingly stable in water in the environment due to its high kinetic barrier (activation energy at ~120 kJ/mol) (*23, 24*) and as a result unlikely to oxidize the NOM. Therefore, the loss of Hg_R during the experiments could be attributed to

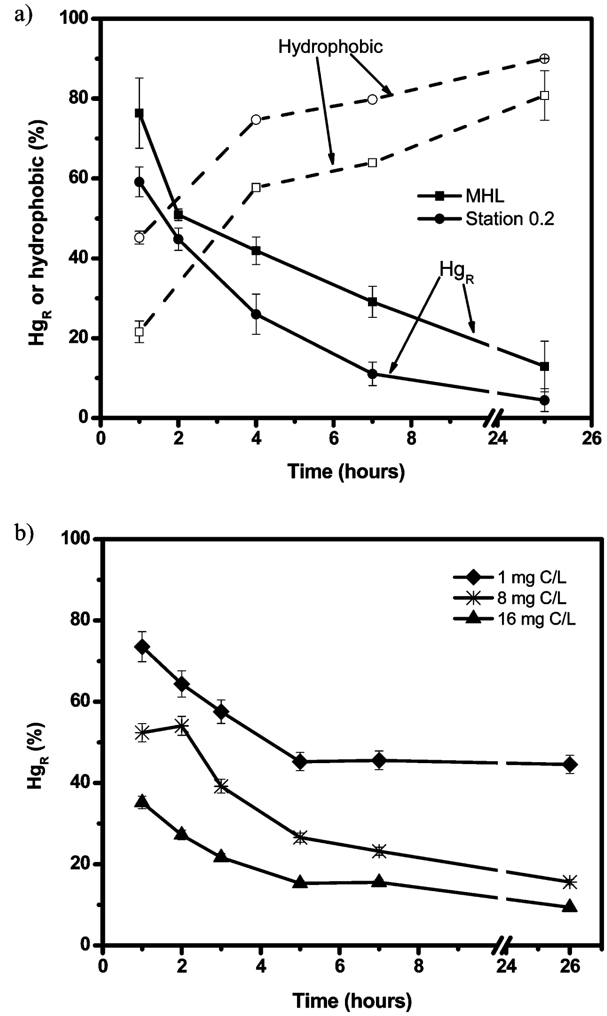

FIGURE 1. (a) Changes in the fraction of mercury present as reducible Hg (Hg_R; solid symbols) or hydrophobic complexes (open symbols) over time in water collected from Station 0.2 in Upper East Fork Poplar Creek (●) and Melton Hill Lake water (■), and (b) Changes of Hg_R in solutions containing 1 (♦), 8 (*), or 16 (▲) mg C/L Suwannee River NOM. All solutions were spiked with 100 ng/L Hg, and error bars represent the range of values in duplicate treatments.

TABLE 1. Pseudo-First Order Rate Constants (k_{NOM}) and Changes of Reducible Hg_R for the Complexation of Hg with Varying Sources and Concentrations of NOM (Expressed as DOC) from Laboratory Experiments

solution	Hg_T (ng/L)	DOC (mg C/L)	Hg:DOC mole ratio ($\times 10^6$) [a]	k_{NOM} (hr^{-1})[b]	% Hg_R at 1 h	% Hg_R after 24 h [c]
Station 0.2	100	1.5	4.0	0.29 ± 0.03	62	4
MHL	50	1.4	2.2	0.20 ± 0.03	56	17
MHL	100	1.4	4.3	0.14 ±0.03	54	16
MHL	200	1.4	8.6	0.05 ± 0.01	84	10
SR NOM	100	1.0	6.0	0.09 ± 0.02	74	45
SR NOM	100	8.0	0.8	0.15 ±0.02	52	16
SR NOM	100	16.0	0.4	0.18 ± 0.05	35	9

[a] Molar ratio based on molar carbon concentration in the DOM. [b] Error values represent one standard deviation in the slope of the line used to calculate the rate constant. [c] Final Hg_R measurements were made 24−26 h after the addition of Hg.

changes in the complexation of Hg with NOM rather than the degradation of organic ligands.

The loss of Hg_R during the course of the experiments demonstrates that the interaction of Hg with NOM is complex and likely involves the competitive interactions and ligand exchanges among different functional groups on the NOM. In a previous study (*8*), first order kinetics was used to describe the loss of Hg_R added to natural water but only one water sample was used and the amount of Hg added to the sample was not reported. Neither first-order nor second-order kinetics provided a good fit for all the data presented here because of the initial rapid reaction followed by a slow decrease of Hg_R. However, if a subset of the data between 1 and 7 h is used, the data appeared to follow the pseudo-first order kinetics, and the rate constant (k_{NOM}) associated with the formation of nonreducible Hg−NOM complexes can be determined using the integrated rate equation (eq 1):

$$\ln[Hg_R/Hg_T]_t = -k_{NOM}t + \ln[Hg_R/Hg_T]_1 \quad (1)$$

The rate constants were subsequently calculated and provided insights into the interactions between Hg and NOM (Figure 1; Table 1). We realize that excluding the reduction of Hg_R in the first hour could underestimate the overall reaction rates of Hg with NOM. Furthermore, the Hg_T concentration, which is equivalent to Hg_F in these experiments since all solutions were filtered before the addition of Hg, decreased during the experiment as a result of the sorption of Hg to the container walls, as observed in previous studies (*8*). The loss of Hg_T to container walls was generally <15% except for experiments with SR NOM where approximately 25% loss occurred during the first 7 h of the experiments. The loss of Hg_R from the solutions is therefore a function of the association of Hg with NOM and the sorption to the container wall. To minimize the influence of Hg loss to container wall in the determination of the rate constant of Hg−NOM formation, the ratio of reducible Hg to total Hg ($[Hg_R/Hg_T]_t$) at each time point (t) starting at one hour ($[Hg_R/Hg_T]_1$) in the experiment was used in the rate calculation (eq 1). Utilizing this ratio in the rate equation assumes that all the Hg lost to the container wall is Hg_R, which is probably not true, leading to a slight underestimation of k_{NOM}.

The calculated rate constants (Table 1) varied depending on the Hg:DOC ratio and the NOM source but in all treatments first order kinetics provided a good fit ($r^2 > 0.85$) for the data and could be used to describe the interaction of Hg with the NOM in the 1−7 h time frame. This suggests that a similar process is occurring in all treatments even though the source and concentration of NOM and the concentration of Hg were varied. The Hg_R concentration measured after 24 h is higher than would be predicted based on first order kinetics and in some treatments the deviation from first order kinetics can be seen as early as 7 h. These observations again indicate the complex and multiple interactions between Hg and NOM, which contains both LMW and macromolecular components, before equilibrium is established. The initial rapid decrease of Hg_R in the first hour is likely a result of the interaction of Hg with all available functional groups including those strongly bound reduced thiols, which are present in low abundance in NOM, and those weakly bound carboxyl and amine functional groups, which are present in high abundance. However, over the course of reactions (e.g., 1−7 h), those Hg−NOM complexes formed initially with functional groups with lower binding energies are slowly replaced by multiple transfers of the Hg to stronger binding sites within the NOM macromolecular structure (*25, 26*).

Such complex ligand exchange and competitive interactions can be expected considering the heterogeneous nature and complex molecular structure and binding environments within NOM which effect the stability and thus reactivity of complexed Hg(II). For example, the hydrophobic fragments of NOM with highly conjugated aromatics may provide a microenvironment that limits the exchange and/or competitive interactions with other ligands in the system. Similarly, previous studies have shown that different moieties or subfractions of NOM competitively interact with metals and/or metal oxides because of their varying binding strengths (*26, 27*). The chemical structure and binding modes of the NOM (e.g., monodentate versus bidentate) also may influence the complexation energy and thus reactivity of the Hg(II). The higher Hg_R concentrations and slower rate of Hg−NOM complexation at a high Hg concentration would support this argument since, as the ratio of Hg(II)/NOM increases, the easily accessible binding sites, including those with low binding energies, are rapidly saturated with Hg(II). The binding of organic or inorganic ions with NOM is also known to cause changes in the molecular configuration such as folding and agglomeration of NOM in the region surrounding the binding site. For example, humic substances have been shown to exhibit micellar behavior in solution and can be precipitated by multivalent ions such as Ca^{2+} and Fe^{3+} (*28, 29*). This in turn might influence subsequent competitive interactions and exchange kinetics as Hg(II) re-equilibrates and binds to sites with higher binding energies over time. The faster rate of exchange of Hg(II) complexed to LMW organic ligands versus natural ligands has been proposed to explain the high reducibility of Hg complexes involving LMW ligands (*8*). A combination of mono and multidentate Hg−NOM complexes with thiols within the macromolecular structure of the large NOM molecules could also results in different reducibility, although the reducibility of bidentate complexes between Hg and macromolecular NOM have not been quantified. Studies on soil humics show that Hg(II) forms bidentate complexes with thiols (*21, 30, 31*) but the dominance of this type of complex has not been confirmed with aquatic NOM because of insufficient detection limits associated with spectroscopic methods.

Reducible Mercury in UEFPC and Environmental Implications. To evaluate the environmental relevance of the kinetic controls on the Hg−NOM complexation and species

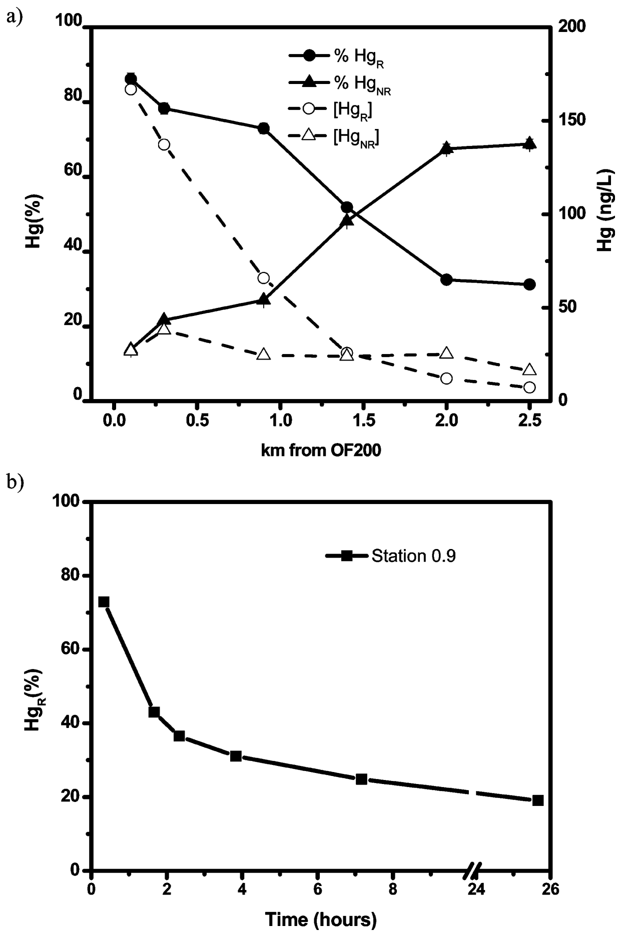

FIGURE 2. (a) Reducible (Hg_R; ●) and nonreducible (Hg_{NR}; ▲) mercury in filtered surface water collected in the upper 2.5 km of Upper East Fork Poplar Creek (UEFPC) starting at the headwaters (OF200) expressed as percentage (solid symbols) and concentration (open symbols) (b) changes of Hg_R over time in filtered creek water from Station 0.9 in EFPC. Filtration and Hg_R measurements were conducted within 30 min of sample collection. Error bars represent the range of values in replicate measurements.

distribution, the Hg_F and Hg_R concentrations were determined in UEFPC (Figure 2a; SI Table S3), where the headwaters receive a constant input of high concentrations of reducible Hg(II) from an industrial outfall (OF200). At the headwaters of UEFPC, Hg_R from Outfall 200 mixes with MHL water containing 1.5 mg /L DOC at a 1:4 ratio resulting in a Hg_R concentration of approximately 200 ng/L immediately after mixing. Under base flow conditions, it takes approximately 1.5 h for water entering UEFPC to reach Station 2.5. The majority of the Hg_F entering UEFPC from OF200 was present as Hg_R (90%) but moving downstream both Hg_F and Hg_R decrease (Figure 2a). Even at the site sampled furthest downstream (Station 2.5), 29% of the filtered Hg was present as a reducible complex. Complexes between Hg and NOM in the filtered phase should dominate at all sites in UEFPC if equilibrium is established on the basis of the complexation calculations using the pH, DOC, and anion concentrations measured within this system (SI Table S4). The pH, DOC, and anion concentrations in UEFPC downstream of the mixing of OF200 and MHL water did not vary substantially resulting in no changes in the predicted equilibrium complexation of Hg throughout the creek. In equilibrium with water from UEFPC, Hg_R should be $\leq$10% (SI Table S2) therefore, the presence of high concentrations of Hg_R in the upper reaches of UEFPC indicates that dissolved phase equilibrium conditions were not established in this contaminated system.

The lack of equilibrium in the filterable fraction of the water from UEFPC is potentially important in overall cycling of Hg in this system. As Hg forms complexes with NOM the concentration of nonreducible Hg (Hg_{NR}) should increase moving downstream in UEFPC. Interestingly, the fraction of the Hg present as a nonreducible complex increases downstream but the actual concentration of the Hg_{NR} did not change (Figure 2a). This is a result of the decrease in the Hg_F concentration as Hg become particle associated. This again highlights that kinetics are important in the complexation of Hg in the filterable fraction of water in this system. The decrease in Hg_R concentration without an increase in the Hg_{NR} concentration potentially indicates that the reducible fraction of Hg is more particle reactive than the nonreducible fraction. An alternate explanation is that the nonreducible Hg−NOM complexes are sorbing onto the particles and that the kinetics controlling the formation of nonreducible Hg−NOM complexes is similar in magnitude to the rate of partitioning of these complexes onto particles. This suggests that the lack of equilibrium, which is responsible for the presence of reducible Hg complexes, is important in controlling the cycling of Hg in this system.

Measuring the Hg_R concentration in a sample collected from Station 0.9, filtered and held in the dark for 26 h, also demonstrated the lack of Hg complexation equilibrium in UEFPC (Figure 2b). At the time of collection, the filtered Hg was 73% Hg_R and decreased to 19% after 26 h. The formation of Hg−NOM complexes, as indicated by the decrease in Hg_R, is consistent with the explanation that the NOM responsible for the formation of nonreducible Hg complexes is present in the creek water, but the formation of nonreducible Hg (presumably those of strongly bound Hg−NOM complexes) is kinetically hindered.

This research demonstrates that in aquatic systems receiving input of inorganic Hg, the kinetics of Hg−NOM complexation needs to be evaluated in order to determine the dominant complexes of Hg in the system. Equilibrium conditions cannot be assumed in such systems due to the heterogeneity of NOM and the competitive interactions among various functional moieties of NOM for binding with Hg. Although the concentration of Hg used in the laboratory and field observations were above the levels observed in most natural systems, there is evidence that similar interactions are occurring in uncontaminated systems. For example, when rainwater containing 15−60% Hg_R was equilibrated with a high DOC river water for 6 h, the reducible Hg was 50% higher than would be expected based on the reducibility of the background Hg in the river water (*32*). This suggests that reaction rates between Hg and NOM are slow and can also influence the complexation of Hg in uncontaminated aquatic systems. Kinetics therefore needs to be taken into account in evaluating processes such as Hg partitioning between particles and solution phases, bacterial uptake and methylation, and Hg oxidation and reduction. Furthermore, it is likely that interactions between Hg, sulfide, and NOM are also kinetically influenced in anaerobic environments. Lastly, $SnCl_2$ reactivity is only useful as a tool for assessing Hg complexation if measurements are done quickly after sample collection, since the reactivity will change if the samples are held for several hours.

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Note Added after ASAP Publication

Reference 6 was modified in the version of this paper published ASAP October 7, 2009; the corrected version published ASAP October 14, 2009.

Supporting Information Available

Additional data on the reactivity of different Hg complexes and mercury (Hg_T, Hg_D, and Hg_R) and ancillary data (anions, DOC and pH) from EFPC.This material is available free of charge via the Internet at http://pubs.acs.org.

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