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Influence of chloride ions on the reduction of mercury species in the presence of dissolved organic matter

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Abstract Mercuric species, Hg(II), interacts strongly with dissolved organic matter (DOM) through the oxidation, reduction, and complexation that affect the fate, bioavailability, and cycling of mercury, Hg, in aquatic environments. Despite its importance, the reactions between Hg(II) and DOM have rarely been studied in the presence of different concentrations of chloride ions (Cl^-) under anoxic conditions. Here, we report that the extent of Hg(II) reduction in the presence of the reduced DOM decreases with increasing Cl^- concentrations. The rate constants of Hg(II) reduction ranged from 0.14 to 1.73 h^{-1} in the presence of Cl^- and were lower than the rate constant (2.41 h^{-1}) in the absence of Cl^-. Using a thermodynamic model, we showed that stable Hg(II)–chloride complexes were formed in the presence of Cl^-. We further examined that H(0) was oxidized to Hg(II) in the presence of the reduced DOM and Cl^- under anoxic conditions, indicating that Hg(II) reduction is inhibited by the Hg(0) oxidation. Therefore, the Hg(II) reduction by the reduced DOM can be offset due to the Hg(II)–chloride complexation and Hg(0) oxidation in chloride-rich environments. These processes can significantly influence the

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speciation of Hg and have an important implication for the behavior of Hg under environmentally relevant concentrations.

Keywords Mercury · Chloride ion · Dissolved organic matter (DOM) · Oxidation–reduction reaction · Complexes

Introduction

The oxidation–reduction (Redox) reactions of mercury (Hg) play an important role in its speciation and bioavailability in anoxic environments (Benoit et al. 2001; Lalonde et al. 2001; Frohne et al. 2012; Lee et al. 2015). It has been shown that mercuric mercury [Hg(II)] was reduced to elementary mercury [Hg(0)], leading to the removal of Hg(0) in lake waters (Mason et al. 1995; Siciliano et al. 2002). It indicates that methylating bacteria (e.g., sulfate- and iron-reducing bacteria) have difficulty methylating Hg(II) to highly bioaccumulated neurotoxin methylmercury (MeHg) (Compeau and Bartha 1985; Kerin et al. 2006; Beckers and Rinklebe 2017).

It has been demonstrated that Hg(II) reduction occurs simultaneously with Hg(0) reoxidation in the presence of reduced dissolved organic matter (DOM) under anoxic conditions (Gu et al. 2011; Zheng et al. 2012). These studies suggested that Hg(II) is reduced to Hg(0) by reduced quinone (semi- or hydro-quinone) in DOM (Alberts et al. 1974; Scott et al. 1998; Ratasuk and Nanny 2007; Uchimiya and Stone 2009). Other studies have shown that Hg(0) is oxidized to Hg(II) by thiol functional groups in reduced DOM through oxidative complexation (Gu et al. 2011; Zheng et al. 2012; Lee et al. 2018). It was recently shown that the small aliphatic structure of thiols, high thiol/Hg ratios, and the presence of electron acceptors enhance the extent and rate of Hg(0) oxidation through the reaction between Hg(0) and thiol model compounds (Zheng et al. 2013; Lee et al. 2018).

The oxidation and reduction of Hg in the presence of chloride ion (Cl^-) have been studied mainly in oxic environments or via photochemical reactions. Previous studies have shown that Cl^- forming a high affinity to Hg(II) is significantly responsible for Hg(0) oxidation (de Magalhães and Tubino 1995; Amyot et al. 1997), followed by the formation of Hg–chloride complexes (Yamamoto et al. 1995; Yamamoto 1996). The Hg(0) was highly oxidized to Hg(II) in the presence of Cl^- and benzoquinone converting photochemically to the reactive compounds (e.g., semiquinones) by ultraviolet-B (UV-B) radiation (Lalonde et al. 2001, 2004). Under the same experimental condition, small fraction of Hg(0) was oxidized to Hg(II) in the presence of the fulvic acid (Lalonde et al. 2001). The author inferred that Cl^- can stabilize Hg(I) and reduce the standard reduction potential of Hg(I) and Hg(0) (Lalonde et al. 2001). However, it has been reported that dissolved Hg(0) is not oxidized to Hg(II) by Cl^- and oxygen (Amyot et al. 2005). To date, it is controversial whether and at what rate the dissolved Hg(0) is oxidized by Cl^-. In addition, Cl^- has been known as the inhibitor for Hg(II) reduction in the presence of DOM (Allard and Arsenie 1991). The extent of Hg(II) reduction has been shown to decrease in the presence of DOM due to the formation of Hg(II)–chloride complexes (Allard and Arsenie 1991). However, it remains unclear that the inhibition of Hg(II) reduction is due to the formation of Hg(II)–chloride or to the oxidation of Hg(0). The objectives in the current study are to evaluate the extent and rate of Hg(II) reduction by DOM at various Cl^- concentrations under dark, anoxic conditions and to elucidate the mechanism of the oxidation and reduction of Hg in the presence of DOM and Cl^- in aquatic environments.

Materials and methods

Preparation of solutions

Clean techniques were used during the preparation of all experiments. The bottles and Teflon tubing were washed with the detergent (trace metal grade), followed by dipping with hydrochloric acid (20% v/v) for more than 24 h. Before using the bottles and Teflon tubing, we thoroughly removed the hydrochloric acid with ultrapure Milli-Q water (> 18 MΩ cm). For the Hg(II) reduction, dissolved Hg(II) was freshly prepared for each experiment. 0.27 g of $HgCl_2$ purchased from Sigma-Aldrich (99.9%) was dissolved in ultrapure Milli-Q water (> 18 MΩ cm), and the mixture was then diluted to a working solution (10 μM). The concentrations of Hg(II) and Hg(0) in the reactors were analyzed, and the final concentration of Hg(II) was typically ~ 10 nM. The recovery of total Hg was 106 ± 5.7% (mean ± SD, *n* = 7), which contains nonpurgeable Hg(II) (99.9 ± 7%) and purgeable Hg(0) (6.3 ± 6%), respectively.

Dissolved Hg(0) stock solutions were prepared according to previously qualified procedures (Whalin and Mason 2006; Gu et al. 2011; Zheng et al. 2013). Briefly, a droplet (30 μL) of Hg(0) was injected onto a piece of silicon tubing enclosed with a Teflon plug. The other end of the silicon tubing was closed with a Teflon plug and then submerged in 150 mL deoxygenated Milli-Q water (> 18 MΩ cm) in amber glass bottles. The Hg(0) in silicon tubing was diffused and equilibrated with the deoxygenated water to produce ~ 80–100 nM dissolved Hg(0). Then, 2.3 mL of the dissolved Hg(0) stock solution was injected into 32.7 mL deoxygenated water to obtain the final concentrations (~ 8 nM). To determine the final concentration of Hg(0) in the reactions, we measured the percentages of dissolved Hg(0) and oxidized Hg(II), which were 97.0 ± 1.7% and 5.1 ± 1.6% of total Hg, respectively. The range of Hg(II) concentrations for Hg(II) oxidation and reduction n was determined in the literature (Amyot et al. 2005; Gu et al. 2011; Zheng et al. 2012). The Hg concentrations (2–10 nM) of liquid Hg droplets were previously observed in hydrothermal vents, gold mining sites, and industrial areas using Hg (Amyot et al. 2005).

We followed the previous method for the preparation of chemically reduced soil humic acid (RSHA) as the reduced DOM (Kappler et al. 2004). Elliot soil humic acid (SHA) obtained from the International Humic Substances Society (IHSS) was left to reduce with the H_2 and Pd processes. About 30 mg L^{-1} of SHA was dissolved in a deoxygenated phosphate buffer (pH 7) at a concentration of 1000 mg L^{-1}. The neutral pH should be maintained during the reduction of SHA since it does not occur under low pH condition. Palladium (99.99% trace metals basis, Sigma-Aldrich) was added to the solution, followed by purging with pure H_2 gas for 25 min. The solution was shaken for 24 h at ~ 25 °C. To remove the particulate matters and hydrogen gas in the solution, the solution was filtered using 0.2 μm cellulose acetate filters and syringes in a glove bag (98% N_2 and 2% H_2) and purged with ultrapure nitrogen gas. The solution without adding SHA was prepared using the same procedure as that used for the control experiments. The amber serum vials containing the solutions were wrapped in aluminum foil and kept in the dark to avoid photochemical reactions. To prepare the purgeable Hg trapping solution, $KMnO_4$ and H_2SO_4 were dissolved in the Milli-Q water to reach 0.6% (wt/v) and 5% (v/v), respectively (Wiatrowski et al. 2006). During oxidation–reduction reaction (4 h), the purgeable Hg(0) in the serum vials was continuously transferred to the $KMnO_4$ trapping solutions through Teflon tubing and subsequently oxidized to Hg(II), which can be dissolved in trapping solutions.

Reduction and oxidation of Hg

The reaction between Hg(II) and reduced DOM was examined at varying Cl^- concentrations. Deoxygenated water for all reagent solutions was prepared by purging with ultrapure N_2 for 2 h. To prevent penetration of the oxygen and the effect of light during the experiments, the solutions were left to continue purging with ultrapure N_2 in amber serum vials. 14.6 g of NaCl was dissolved in 100 mL deoxygenated phosphate buffer (10 mM, pH 7) to reach the final Cl^- concentration (2.5 M), which was diluted in serum vials for use as working solutions. Deoxygenated phosphate buffers (10 mM, pH 7) were added to 35 mL amber glass vials. Various Cl^- concentrations of 0, 0.2, 5, and 500 mM were prepared to the amber glass vials. The control experiment was conducted without the addition of Cl^-. 27 μL of Hg(II) (10 μM) was added to the solutions, and the vials were then tightly capped with rubber stoppers. The final volume of reaction solutions was 25 mL, and the final concentration of Hg(II) was 10 nM. To collect the volatile Hg(0) produced from the reaction, one Teflon tubing (1/8 inch) was connected to the ultrapure N_2 and reaction solution, and the other tubing was connected to the Hg trapping solution (10 mL). After purging with ultrapure N_2 (flow rate of 28 mL min^{-1}), 0.1 mL of reduced DOM (56.1 mg C L^{-1}) was injected into the reaction solutions with syringes without opening the stoppers. To prepare control experiments with identical conditions, the same volume of solution as that in the absence of reduced DOM was added. The final DOC concentration was 0.22 mg C L^{-1}, corresponding to 0.02 mM C in the 10 mM phosphate buffer at pH 7. The Hg trapping solutions were changed for 4 h. Immediately after completion of the experiments, we added 0.2 M BrCl (dissolved in concentrated HCl) at 2% (v/v) to analyze the nonpurgeable Hg in the reactors adsorbed to the wall or reduced DOM for all experiments. However, it is important to note that some earlier studies suggested that Hg loss by adsorption to the wall of the reactor was less than 1% of the total Hg(II) (Wiatrowski et al. 2006; Zheng and Hintelmann 2009; Zheng et al. 2012). Kinetic experiments between Hg(0) and Cl^- in the absence and presence of the reduced DOM were conducted at varying time intervals (10 mM, pH 7). All conditions and materials were identical to those for the experiments for Hg(II) reduction. Considering the volatilization of Hg(0) and the adsorption of Hg(II) during the experiments, we evaluated the mass balance of Hg in the reaction of Hg(0) oxidation.

Analytical methods

The trapping solutions and the remaining solutions in the reactors were treated with 0.1 M BrCl at 2% (v/v). The strong oxidant can play a key role in the preservation of Hg and the oxidation of DOM. After 24 h, the oxidant in the solutions could be effectively neutralized by adding 4 M NH_2OH/HCl at 0.8% (v/v). The solutions were mixed by vortexing to destroy the halogen gas that negatively affects the gold-coated glass traps and equipment. The aliquots in the trapping and remaining solutions were added to glass bubblers, and 0.5 mL of 2% (w/v) $SnCl_2$ was then added. The solutions in the glass bubblers were purged with ultrapure N_2 to adsorb the vapor-phase Hg(0) to a gold-coated glass trap. The adsorbed Hg(0) on the surface of a gold-coated glass trap was thermally desorbed under a flow of N_2 and detected using cold-vapor atomic fluorescence spectroscopy (CVAFS) (Bloom and Fitzgerald 1988; Lee et al. 2011). Dissolved organic carbon (DOC) concentration was determined using a total organic carbon (TOC) analyzer (Vario TOC cube, Germany).

Results and discussion

Hg(II) reduction in the absence of Cl^-

The mass balance of the Hg fractions was conducted at varying molar ratios of reduced DOM and Hg(II) (Table 1). The recovery of total Hg (sum of purgeable and nonpurgeable Hg) ranged from 95% to 102% at DOC/Hg(II) molar ratios from 519:1 to 51,944:1. At a DOC/Hg(II) molar ratio of less than 1870, the purgeable Hg concentrations in the trapping solutions increased when increasing the amount of the reduced DOM concentration, indicating that Hg(II) was reduced to Hg(0) by the reduced DOM. Approximately 76% of Hg(II) was reduced substantially at the molar ratio of DOC/Hg(II) (1870:1), while further enhancement in Hg(II) reduction did not occur at molar ratios of DOC/Hg(II) higher than 1870. This result was consistent with those obtained in previous studies, suggesting that the reduced DOM can be used as a mediator of the oxidation and reduction of Hg (Gu et al. 2011; Zheng et al. 2012). Hg(II) reduction was controlled by semi-quinone via the electron transfer (Scott et al. 1998; Ratasuk and Nanny 2007), while Hg(0) oxidation was affected by the thiol functional groups through oxidative complexation (Gu et al. 2011; Zheng et al. 2012, 2013). In this study, concentrations of semi-quinones ranging from 43.5 to 4350 nM equivalent (0.1–10 mg L^{-1} of the reduced DOM) are sufficient for donating the electrons and then completely reducing 10 nM Hg(II). Consequently, the extent of Hg(II) reduction can be controlled by the thiol contents in the reduced DOM, particularly by the molar ratios of thiol/Hg(II). When the thiol/Hg molar ratio was higher than ~ 1.1, the extent of Hg(II) reduction was decreased (Table 1). At the thiol/Hg(II) molar ratios ranged from 1.1 to 3.2, the extent of nonpurgeable Hg was ~ 34–46%.

The reaction kinetics between Hg(II) and reduced DOM were mostly dominated during the initial periods (30 min) and completed within 4 h of reaction time (Figs. 1, 2). Since the reduced DOM concentrations were present in great excess compared to the Hg(II) concentrations, we assumed that the reduced DOM remained at a constant concentration, leading to the formation of pseudo-first-order reactions:

$$-\mathrm{d}[\mathrm{Hg(II)}]_t/\mathrm{d}t = k_{\mathrm{obs}}[\mathrm{Hg(II)}]_t \qquad (1)$$

where k_{obs} is the pseudo-first-order rate constant, and $[\mathrm{Hg(II)}]_t$ is the Hg(II) concentration at time t. The rate constant of Hg(II) reduction ranged from 0.14 ± 0.01 to 2.41 ± 0.53 h^{-1} which is a similar range to that in abiotic reactions (approximately 0.36–5.76 h^{-1}) (Wiatrowski et al. 2009; Qureshi et al. 2010; Zheng et al. 2012). The rate constants of Hg(II) reduction were 0.03–0.1 h^{-1} in the presence of less reduced DOM (Rocha et al. 2000; Zheng and Hintelmann 2009).

Table 1 Mass balance for Hg fraction at different molar ratio of DOM to Hg(II) in 10 mM phosphate buffer at pH 7 under dark, anoxic conditions

DOM (mg L^{-1})	0.1	0.2	0.4	1	3	10
DOC (mg C L^{-1})	0.06	0.11	0.22	0.56	1.68	5.61
[DOC]/[Hg(II)]	519	1039	1870	5194	15,583	51,944
Semi-quinone (nM equivalent)	43.5	87.0	174.0	435.0	1305.0	4350.0
thiol (nM)	1.1	2.1	4.2	10.5	31.6	105.2
[thiol]/[Hg(II)]	0.1	0.2	0.4	1.1	3.2	10.5
Purgeable Hg	0.38 ± 0.04	0.53 ± 0.01	0.76 ± 0.02	0.70 ± 0.01	0.49 ± 0.01	0.26 ± 0.03
Nonpurgeable Hg	0.60 ± 0.03	0.46 ± 0.04	0.26 ± 0.06	0.34 ± 0.02	0.46 ± 0.01	0.76 ± 0.04
Recovery (%)	0.98 ± 0.01	0.99 ± 0.04	1.02 ± 0.1	1.03 ± 0.01	0.95 ± 0.01	1.02 ± 0.05

Initial Hg(II) concentration was approximately 10 nM, and thiol concentration (HS^-) was calculated using 6% of total sulfur content (Ravichandran 2004; Gu et al. 2011). Error bars represent the standard deviation of triplicate samples

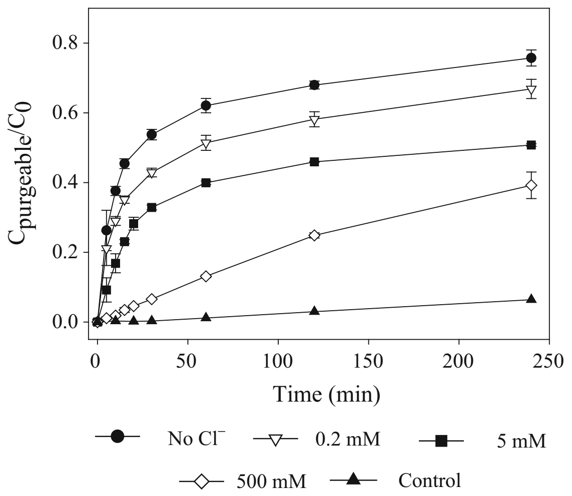

Fig. 1 Reaction between Hg(II) (10 nM) and reduced DOM (0.22 mg C L^{-1}) at varying concentrations of Cl^- in 10 mM phosphate buffer at pH 7 under dark, anoxic conditions. $C_{purgeable}/C_0$ is the ratio of nonpurgeable Hg to the initial Hg(0). Control contains the deoxygenated buffer solution and Hg(II) without Cl^-. Error bars indicate standard deviation of triplicate reactions

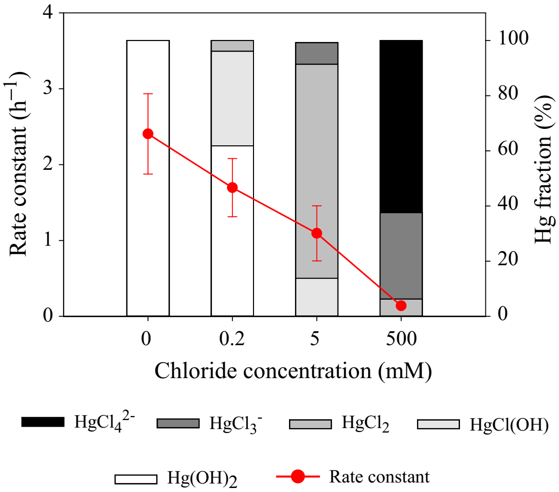

Fig. 2 Hg(II) reduction rate (left axis) and Hg fraction (right axis) at various concentrations of Cl^-. Hg(II) and reduced DOM concentrations were 10 nM and 0.22 mg C L^{-1}, respectively. Reaction time was 240 min. The formation of Hg–chloride complexes was supported by the modeling data, which was estimated using the thermodynamic stability constants (MINEQL +) in the presence of 0–500 mM Cl^- in 10 mM phosphate buffer at pH 7

These rate constants were two orders of magnitude higher than those observed by biotic reactions in natural environments (0.001–0.0001 h^{-1}). Therefore, the rate of Hg(II) reduction was significantly affected by the molar ratio of DOM/Hg(II), the redox potential of DOM, and the abiotic/biotic reactions.

Hg(II) reduction in the presence of Cl^-

The extent of Hg(II) reduction decreased with the increase of Cl^- concentrations from 0 to 500 mM in 10 mM deoxygenated phosphate buffer at pH 7 (Fig. 1). In the absence of Cl^-, 76 ± 2.3% of purgeable Hg was produced in the presence of reduced DOM (0.22 mg C L^{-1}). 67 ± 2.8% of purgeable Hg was recovered in the presence of the reduced DOM and Cl^- (0.2 mM), which is an average concentration of Cl^- in natural freshwater. In the presence of Cl^- (500 mM), 39 ± 3.8% of Hg(II) was formed as the purgeable Hg, whereas Hg(II) mostly remained nonpurgeable Hg (63 ± 10%, data not shown). This result was similar to that observed in previous studies on the decrease in Hg(II) reduction by fulvic acid in the presence of Cl^- (1 and 100 mM) due to the formation of Hg(II)–chloride complexes (Allard and Arsenie 1991; Gabriel and Williamson 2004). In the presence of Cl^-, Hg(II) species is likely to be stabilized as Hg(II)–chloride complexes such as $HgCl_2^0$ ($K = 10^{14}$), $HgCl_3^-$ ($K = 10^{15}$), and $HgCl_4^{2-}$ ($K = 10^{15.6}$) (Morel and Hering 1993; Benoit et al. 2001; Zheng and Hintelmann 2009) which are stable and nonsorbing complexes (Kim et al. 2004). These complexes may inhibit the reaction between Hg(II) and the semiquinones in the reduced DOM. At a low concentration of Cl^- in freshwater, most of the Hg(II) can bind to oxygen functional groups such as carboxyl and phenol functional groups, leading to low stability constants ($K = 10^{7.3}$–10^{10}) (Drexel et al. 2002; Haitzer et al. 2002; Skyllberg et al. 2006), while low amount of Hg(II) might form Hg(II)-reduced sulfur group complexes with high stability constants ($K = 10^{21}$–10^{24}) (Haitzer et al. 2002; Lamborg et al. 2003; Ravichandran 2004; Skyllberg et al. 2006) at the mole ratio of thiol/Hg(II) (~ 0.4). In the absence of sulfide, the dominant Hg(II) species are $Hg(OH)_2$, HgOHCl, and $HgCl_2$ at the neutral pH ranges in freshwater (Ullrich et al. 2010). Hg(II) was mostly bound to Cl^- in marine environments (average concentration of 0.56 M Cl^-) (Allard and Arsenie 1991; Schmerge 2001; Wiatrowski et al. 2009). The formation of Hg(II)–Cl complexes can inhibit the Hg(II) reduction by reduced DOM in chloride-rich environments such as transitional and marine environments.

The formation of Hg–chloride complexes was supported by the modeling data (MINEQL+, Environmental Research Software), which was estimated using the thermodynamic stability constants (Table S1) in the presence of 0–500 mM Cl^- in 10 mM phosphate buffer at pH 7 (Fig. 2). The Hg fraction showed $HgCl_4^{2-}$ (62%), $HgCl_3^-$ (31%), and $HgCl_2$ (6%) in the presence of 500 mM Cl^-, whereas the dominant Hg species was present as $Hg(OH)_2$ (100%) in the absence of Cl^-. This result indicates that the formation of strong Hg–chloride complexes inhibits the reduction of Hg(II) by the reduced DOM. Previous studies showed that the formed Hg(II)–chloride complexes is not readily adsorbed on the surface of electron donors at a range of Hg(II)/Cl^- molar ratio from 1:10^1 to 10^5 (Allard and Arsenie 1991; Wiatrowski et al. 2009). Based on the pseudo-first-order reactions, the rate constants of the Hg(II) reduction ranging from 0.14 ± 0.01 h^{-1} (500 mM Cl^-) to 1.73 ± 0.47 h^{-1} (0.2 mM Cl^-) were lower than those in the absence of Cl^- (2.41 ± 0.53 h^{-1}) (Fig. 2). The decrease in the rate constant of Hg(II) reduction might be attributed to the formation of Hg(II)–chloride complexes, which inhibits the adsorption between Hg(II) and the oxygenated functional groups in the reduced DOM (Kim et al. 2004; Wiatrowski et al. 2009; Pasakarnis et al. 2013). Previous studies suggested that the rate constant of Hg(II) reduction decreased due to the reverse reaction (i.e., Hg(0) oxidation) by the Cl^- (Yamamoto 1996; Lalonde et al. 2001) or thiol functional groups in the reduced DOM (Gu et al. 2011; Zheng et al. 2012, 2013).

Hg(0) oxidation in the presence of Cl^-

Hg(0) reacted with the reduced DOM in the absence and presence of Cl^- to elucidate the Hg(0) oxidation under the same experimental conditions in the Hg(II) reduction (Fig. 3). The extent of Hg(0) oxidation was ~ 15% of the initial Hg(0) in the presence of Cl^- and the reduced DOM. Approximately 9% of the initial Hg(0) was oxidized to nonpurgeable Hg in the presence of the reduced DOM. The rate of Hg(0) oxidation was higher in the presence of the reduced DOM and Cl^- (0.25 ± 0.07 h^{-1}) than in the absence of Cl^- (0.14 ± 0.01 h^{-1}) (Fig. 4). The reaction

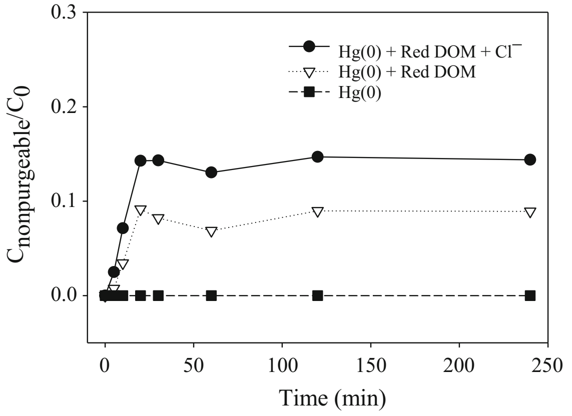

Fig. 3 Reaction kinetic between initial Hg(0) (~ 8 nM) and Cl^- (500 mM), including the reduced DOM (0.22 mg C L^{-1}) in 10 mM phosphate buffer at pH 7 (DOC/Hg = 2344:1). Nonpurgeable Hg concentrations ($C_{nonpurgeable}$) were calculated by the differences between initial Hg(0) (C_0) and purgeable Hg

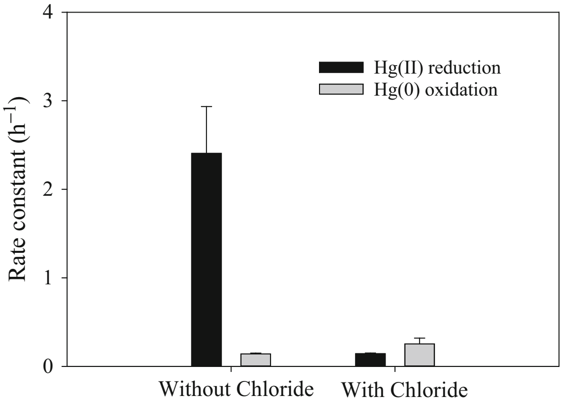

Fig. 4 Comparison of rate constants in the reaction of Hg(II) reduction and Hg(0) oxidation under dark, anoxic conditions. The experiment of Hg(II) reduction was conducted in the presence of 10 nM Hg(II) and reduced DOM (0.22 mg C L^{-1}) in the absence and presence of 500 mM Cl^- in 10 mM phosphate buffer (pH 7). Hg(0) oxidation reacted with 8 nM Hg(0) and reduced DOM (0.22 mg C L^{-1}) in the absence and presence of 500 mM Cl^- in 10 mM phosphate buffer (pH 7). Black and gray bars show the rate constant for Hg(II) reduction and Hg(0) oxidation, respectively. Empirical rate constants for Hg(II) reduction were calculated based on the pseudo-first order. Error bars indicate the standard deviation of triplicate samples

between Hg(0) and Cl^- induced little or no Hg(0) oxidation in the absence of Cl^-. Dissolved Hg(0) was not oxidized at the concentration of 500 mM Cl^- under the oxic condition (20% oxygen) (Amyot et al. 2005). Hg(0) oxidation did not occur in the presence of 500 mM Cl^- and in either the presence or absence of UV-B radiation (Lalonde et al. 2001). On the other hand, this study showed that the extent of Hg(0) oxidation (~ 30%) was observed at the Hg(0)/fulvic acid molar ratio (1:10) in the presence of Cl^- (500 mM) and UV-B radiation, which produces reactive radical compounds such as semi-quinones from fulvic acid. An increase in the semi-quinones allows the electron acceptors formed by the chromophore or thiol groups to receive the electrons released from Hg(0), which facilitates the Hg(0) oxidation, although the standard potentials of semi-quinones (0.7 V) were lower than those of Hg(I) and Hg(0) (1.3 V) (Cartledge 1941; Lalonde et al. 2001; Zheng et al. 2012). Previous studies suggested that Cl^- may play an important role in stabilizing Hg(I) and decreasing the standard potentials of Hg(I) and Hg(0) to readily transfer the electrons released from Hg(0) to the reduced DOM (Rich and Bendall 1980; Lalonde et al. 2001). Given the extent of the inhibited Hg(II) reduction (~ 37%) by the reduced DOM in the presence of Cl^- (500 mM) (Fig. 1), the inhibition of Hg(II) reduction could not be totally explained with the Hg(0) oxidation (~ 15%) (Fig. 3). Therefore, the complexation between Hg(II) and Cl^- could significantly inhibit Hg(II) reduction. The rate of Hg(II) reduction in the presence of Cl^- (0.14 ± 0.01 h^{-1}) was lower than that in the absence of Cl^- (2.41 ± 0.53 h^{-1}) (Fig. 4). The rate of Hg(0) oxidation in the presence of Cl^- (0.25 ± 0.07 h^{-1}) was higher than that in the absence of Cl^- (0.14 ± 0.01 h^{-1}), indicating that Hg(0) oxidation influences the inhibition of Hg(II) reduction by Cl^-. These results suggest that the formation of stable and nonsorbing Hg(II)–chloride complexes and the Hg(0) oxidation may be critical in decreasing the rate of Hg(II) reduction in anoxic environments.

Conclusions

We assessed the extent and rate of Hg(II) reduction in the presence of the reduced DOM at varying concentrations of Cl^- under anoxic conditions. The extents of Hg(II) reduction by the reduced DOM decreased with increasing the concentrations of Cl^-. In addition, the rate of Hg(II) reduction was lower in the presence of Cl^- than in the absence of Cl^-. These results indicate that the interaction between Hg(II) and Cl^- can inhibit Hg(II) reduction in the anoxic and chloride-rich environments. The inhibition of Hg(II) reduction in the presence of the reduced DOM and Cl^- can be due to the Hg(0) oxidation by semi-quinone and Cl^-. Considering the rate of the Hg(II) reduction and Hg(0) oxidation under identical conditions, the decrease in the rate of Hg(II) reduction cannot exclude the inhibition of Hg(II) reduction due to the formation of Hg(II)–chloride complexes. These results suggest that Hg(II) reduction by the reduced DOM can be offset due to the Hg(II)–chloride complexation and Hg(0) oxidation in the anoxic- and chloride-rich environments. Further studies are needed to provide the quantitative comparison of the redox process of Hg and the Hg(II)–chloride complexation. These studies can be expected to elucidate the reason why Hg(II) reduction is predominantly observed in freshwater compared to estuarine and seawater.

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