Pergamon

Geochimica et Cosmochimica Acta, Vol. 65, No. 24, pp. 4445–4451, 2001

0016-7037/01 $20.00 + .00

PII S0016-7037(00)00742-6

Constants for mercury binding by dissolved organic matter isolates from the Florida Everglades

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(*Received August* 22, 2000; *accepted in revised form June* 22, 2001)

Abstract—Dissolved organic matter (DOM) has been implicated as an important complexing agent for Hg that can affect its mobility and bioavailability in aquatic ecosystems. However, binding constants for natural Hg-DOM complexes are not well known. We employed a competitive ligand approach to estimate conditional stability constants for Hg complexes with DOM isolates collected from Florida Everglades surface waters. The isolates examined were the hydrophobic fraction of DOM from a eutrophic, sulfidic site (F1-HPoA) and the hydrophilic fraction from an oligotrophic, low-sulfide site (2BS-HPiA). Our experimental determinations utilized overall octanol–water partitioning coefficients (D_{ow}) for ^{203}Hg at 0.01 M chloride and across pH and DOM concentration gradients. Use of this radioisotope allowed rapid determinations of Hg concentrations in both water and octanol phases without problems of matrix interference.

Conditional stability constants (I = 0.06, 23°C) were log K′ = 11.8 for F1-HPoA and log K′ = 10.6 for 2BS-HPiA. These are similar to previously published stability constants for Hg binding to low-molecular-weight thiols. Further, F1-HPoA showed a pH-dependent decline in D_{ow} that was consistent with models of Hg complexation with thiol groups as the dominant Hg binding sites in DOM. These experiments demonstrate that the DOM isolates are stronger ligands for Hg than chloride ion or ethylenediamine-tetraacetic acid. Speciation calculations indicate that at the DOM concentrations frequently measured in Everglades, 20 to 40 μM, significant complexation of Hg by DOM would be expected in aerobic (sulfide-free) surface waters.

1. INTRODUCTION

Significant binding of Hg by natural dissolved organic matter (DOM) in surface water is suggested by the positive correlation between Hg and DOM that has frequently been observed in lake and river waters (Mierle and Ingram, 1991; Driscoll et al., 1995; Watras et al., 1995, 1998; Babiarz et al., 1998). Although cotransport of Hg and DOM from watersheds can contribute to the Hg load to a water body, Hg-DOM associations may decrease the bioaccumulation of Hg in food webs by lowering the bioavailability of Hg(II) to methylating organisms. For example, Barkay et al. (1997) used a mer-lux bioreporter (a bacterium that contains the mer operon promotor fused to the lux gene so that it produces light proportional to intracellular Hg concentration) to measure the effect of DOM, and they observed that DOM decreased the uptake of Hg(II) by bacteria. Miskimmin et al. (1992) found that methylation in epilimnetic lake water was suppressed by the addition of DOM. Similarly, the uptakes of both inorganic and methylmercury at the base of the food chain are negatively affected by association with DOM, as evidenced by inverse correlations between DOM concentration in water and bioaccumulation factors for seston and zooplankton (Watras et al., 1998). Clearly, reliable stability constants for Hg with natural organic matter are needed to adequately predict and model the transport and fate of Hg in the natural environment.

The few models for Hg speciation that include organic ligands in their formulation indicate that the majority of filterable Hg should be present as dissolved (Dyrssen and Wedborg, 1991; Hudson et al., 1994) or colloidal (Guentzel et al., 1996; Stordal et al., 1996) organic complexes in surface waters except in the low DOM, high chloride environments of the open ocean. Additionally, on the basis of the pore-water Hg speciation model of Benoit et al. (1999a), which uses thioglycolate as a representative organic ligand, complexation of Hg by DOM is predicted to be minimal at detectable sulfide concentration (>0.1 μM). Quantification of Hg binding by natural DOM would greatly improve the predictive power of this and other chemical speciation models for Hg in aquatic ecosystems.

In this work, we determined conditional stability constants for Hg complexes with DOM isolated from surface waters of the Florida Everglades. These isolates differed in their reduced sulfur content and in their reactivity toward $HgS_{(s)}$ (Ravichandran et al., 1998, 1999; Ravichandran, 1999), and we expected to find differences in their Hg stability constants. We investigated changes in the octanol–water partitioning of dissolved Hg across concentration gradients in DOM with chloride present as a competitive ligand. This approach took advantage of the lower octanol–water partitioning coefficients (K_{ow}) for highly charged Hg-DOM complexes compared with $HgCl_2^0$ (0.1 compared with 3.3; this work, Mason et al., 1995). Differences in partitioning allowed determination of the fraction of Hg present as Hg-DOM at any given DOM concentration, which, in turn, allowed calculation of the conditional stability constant for that Hg-DOM complex. This approach represents a new method for the determination of stability constants for Hg complexes that

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Table 1. Characteristics of the DOM isolates used in the experiments.

	Molecular weight (Da)	Carbon content (%)	Sulfur content (%)	S reduced (% total S)	Reduced S mol fraction (mol/mol DOM)	Carboxyl group mol fraction
F1-HPoA	1031	52.2	1.73	22.1	0.12	5.62
2BS-HPiA	862	47.3	1.55	11.3	0.05	2.65

is fast and simple to use. This work is an extension of previous studies into the octanol–water partitioning and bioavailability of Hg in the presence of chloride (Mason et al., 1995, 1996) and sulfide (Benoit et al., 1999b). Although past work used known stability constants to calculate K_{ow} for Hg complexes, the current investigation used the known K_{ow} value for $HgCl_2^0$ to calculate stability constants for Hg binding by DOM.

2. MATERIALS AND METHODS

2.1. DOM Isolation

Details on the isolation and characterization of the Florida Everglades DOM fractions used here have previously been reported (Ravichandran, 1999). In brief, surface water was collected, filtered through 0.3-μm filters, acidified to pH 2, and passed through XAD-8 and XAD-4 resin columns. The columns were back-eluted with 0.1 mol/L NaOH to obtain the hydrophobic acid and the hydrophilic acid fractions, respectively. Eluates were hydrogen saturated, lyophilized, and stored for later use. Selected characteristic of these DOM isolates are given in Table 1 (after Ravichandran, 1999).

2.2. Sampling Sites

Samples were collected at sites F1 and 2BS, two sites in the Florida Everglades that have been extensively sampled during the Aquatic Cycling of Mercury in the Everglades project. Details of the project and the sites are given in Hurley et al. (1998). These sites were studied as part of a nutrient and sulfate gradient that was identified within Water Conservation Area 2B of the northern Everglades. The northerly site, F1, has higher surface water sulfate, pore-water sulfide, sediment sulfate reduction rates, and lower Hg methylation rates than the southerly 2BS (Gilmour et al., 1998). Ravichandran et al. (1998) measured the effect of these isolates on the solubility of metacinnabar in aqueous solutions containing solid-phase mercuric sulfide and DOM at a concentration of 20 mg C L^{-1}. They found ~0.3 μmol dissolved Hg (mg C)$^{-1}$ in solutions containing the hydrophobic fraction from F1 (F1-HPoA) compared with ~0.04 μmol dissolved Hg (mg C)$^{-1}$ in solutions containing the hydrophilic fraction from 2BS (2BS-HPiA). In similar experiments, they investigated the precipitation of metacinnabar in solutions containing 50 μM Hg, 1 mM sulfide, and 10 mg C L^{-1}, and they found that 100% of the Hg remained in solution in the F1-HPoA treatment, whereas only a few percent stayed in solution in the 2BS-HPiA treatment (Ravichandran et al., 1999). We wanted to determine whether the observed differences in reactivity toward cinnabar could be explained by greater complexation of Hg(II), as reflected by different conditional stability constants for Hg-DOM complexes with these two isolates.

2.3. Determinations of Overall Octanol–Water Partitioning Coefficients

Stock DOM solutions were prepared by dissolving known weights of the freeze-dried isolates in distilled, deionized water. After making a small correction for ash and moisture, the molarities of the stocks were calculated by using the average molecular weight of the isolates (Table 1) as determined by high-pressure size exclusion chromatography (Ravichandran et al., 1998). Thus, when referring to the concentration of F1-HPoA or 2BS-HPiA, we are specifying the molarity of total DOM (DOM_T), not the concentration with respect to carbon or any specific functional group. The molar concentration of reduced-S groups was also determined from the average mol fraction S and fraction reduced S for these isolates (Table 1). The F1 isolate had ~2.5-fold higher reduced S content than the 2BS isolate, which is consistent with the environments in which they were produced.

Overall octanol–water partitioning coefficient (D_{ow}) determinations for ^{203}Hg were carried out in 0.04 M phosphate solution, buffered at pH 6.0, with a total Hg concentration of 6 nM (1.2 ng ml^{-1}, ~6000 dpm ml^{-1}) and a chloride concentration that was varied as described below. Aqueous solutions (20-mL volume) in Teflon separatory funnels containing phosphate buffer, chloride, DOM, and ^{203}Hg were shaken for 4 h to equilibrate. An equal volume of octanol was added; then the mixtures were shaken for an additional 1 h. Subsamples of the two phases were transferred to glass vials for counting in a Packard gamma counter. The concentration in each phase was calculated from the dpm per milliliter, and the specific activity of the ^{203}Hg. The octanol–water partitioning coefficient was calculated as D_{ow} = [HgD-octanol]/[HgD-water]. Initial determinations with no DOM indicated that these reaction times were optimal (results not shown). The relationship between D_{ow} and the complex-specific octanol–water partition coefficients (K_{ow}) is given by $D_{ow} = \Sigma\, \alpha_i\, (K_{ow})_i$, where α_i is the fraction of species i and $(K_{ow})_i$ is the octanol–water partition coefficient of species i (Mason et al., 1996).

The K_{ow} value for $HgCl_2^0$ previously determined by Mason et al. (1996) was confirmed by measuring D_{ow} of Hg across a chloride concentration gradient of 10^{-4} to 10^{-1} M. Across this gradient, $HgCl_2$, $HgCl_3^-$, and $HgCl_4^{2-}$ are the dominant chloride complexes. It was assumed that the charged species do not partition into octanol. Although there is a large fraction of Hg present as $Hg(OH)_2$ at 10^{-4} M chloride, K_{ow} for this complex is quite low (0.05, Mason et al., 1995), so it does not contribute significantly to partitioning into octanol.

The precision of the method was verified with ethylenediaminetetraacetic acid (EDTA). The D_{ow} for Hg was determined in 0.04 M phosphate buffer, with 0.01 M chloride, pH 6, and a range in EDTA concentration from 10^{-9} to 10^{-5} M. The fraction of Hg bound to EDTA across this gradient was compared with the predicted fraction on the basis of computer modeling that took into account the degree of protonation of EDTA at this pH. The model included formation of $HgEDTA^{2-}$ and $HgHEDTA^-$, although the former complex dominated under these conditions. All chemical equilibrium modeling was carried out by MINEQL+ (Schecker and McAvoy, 1991) with stability constants from Morel and Hering (1993, and references therein).

Measurement of K_{ow} for the Hg complex formed by each isolate utilized 10^{-5} M DOM in the absence of chloride. Under these conditions, we assumed that all of the Hg was bound by DOM and that the measured D_{ow} equaled K_{ow} for the Hg-DOM complex. At this high DOM concentration it is not likely that inorganic ligands are important, as stability constants for Hg complexation by organic ligands are typically much higher (Morel and Hering, 1993). The K_{ow} for Hg bound to EDTA was determined in the same way.

The first set of DOM binding constant determinations spanned DOM concentration gradients from 10^{-9} to 10^{-5} M for both isolate types. This gradient corresponds to ~0.001 to 10 mg DOM L^{-1}, which spans the environmentally relevant DOM concentration range. Chloride concentration was 0.01 M, so that $HgCl_2^0$ was the dominant complex competing with DOM for Hg binding. A second set of determinations were carried out by F1-HPoA to investigate the effect of pH on Hg binding. Conditions were as given above except that the solutions were buffered at pH 4 and 5 with 0.04 M phosphate buffer.

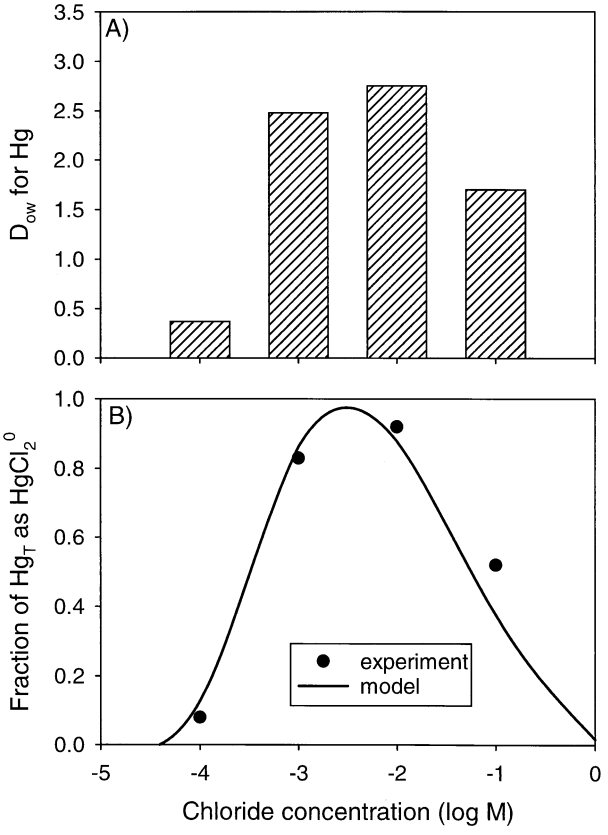

Fig. 1. Experimentally determined D_{ow} for Hg across a chloride gradient (A) and predicted vs. estimated fraction of Hg present as $HgCl_2^0$ (B). The closed circles represent the fraction estimated on the basis of the D_{ow} determinations, and the solid line shows the fraction predicted by thermodynamic equilibrium calculations.

3. RESULTS

Previous determinations that used similar methods showed that the K_{ow} of $HgCl_2^0$ was 3.3 ± 0.3 and that the D_{ow} of Hg changes in a predictable manner with shifts in Hg speciation across a chloride gradient (Mason et al., 1996). Figure 1 shows the predicted fraction of Hg present as $HgCl_2^0$ between 10^{-5} and 1 M chloride at pH 6 and the corresponding measured D_{ow} for Hg. The fraction of Hg present as $HgCl_2^0$ from our D_{ow} data was calculated by use of the Mason et al. (1996) value of K_{ow} for this complex. Close agreement between the experimental and predicted values verify the previously reported K_{ow} for $HgCl_2^0$.

Figure 2 shows the results of EDTA-chloride competition experiments that were performed to provide a measure of the precision of this method. Under the experimental conditions, the dominant chloride complex is $HgCl_2^0$. In the absence of chloride and the presence of 10^{-5} M EDTA, D_{ow} for Hg was 0.1, which we used as the K_{ow} for $HgEDTA^{2-}$. The predicted fraction of Hg present as $HgEDTA^{2-}$ is shown for three potential log K values of the stability constant, all within the range reported for this complex in the literature (21.5 to 23.5; Morel and Hering, 1993; Martell et al., 1998). These lines illustrate how sensitive complex formation predictions are to the value of the stability constant. The close agreement of the

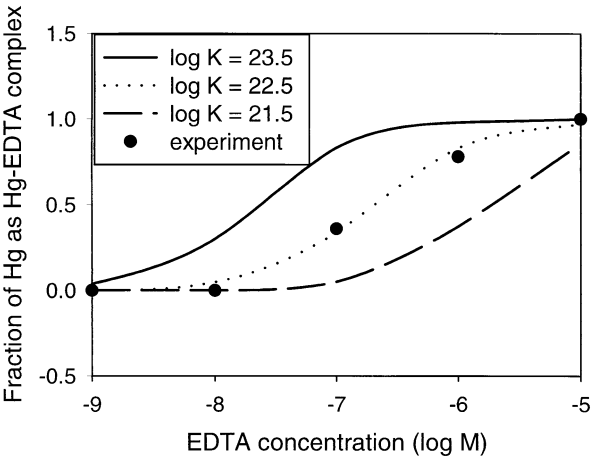

Fig. 2. Validation of the octanol/water distribution method by EDTA. Curves show the fraction of Hg bound by EDTA, as predicted by thermodynamic equilibrium calculations. The solid circles show the fraction estimated on the basis of D_{ow} determinations. Log K for the reaction $Hg^{2+} + EDTA^{4-} = HgEDTA^{2-}$ was varied as indicated.

calculated and predicted speciation for a log K of 22.5 indicates that this method can be used to estimate stability constants to within at least an order of magnitude.

The D_{ow} for Hg in the absence of chloride at 10^{-5} M DOM was 0.12 ± 0.03 (n = 4) for F1-HPoA and 0.10 ± 0.03 (n = 5) for 2BS-HPiA. We used these D_{ow} values as the respective K_{ow} values for the Hg complexes formed with each isolate. Results of D_{ow} determinations for Hg in equilibrium with 0.01 M chloride and varying concentrations of DOM are shown in Figure 3. The observed decline in D_{ow} with increasing DOM concentration is a reflection of an increased fraction of Hg bound to DOM, and the lower K_{ow} of this complex relative to $HgCl_2^0$.

As shown in Figure 4, complete binding of Hg occurred at 1 μM DOM concentration for F1-HPoA and 10 μM for 2BS-HPiA, which indicates that the former is a stronger ligand with

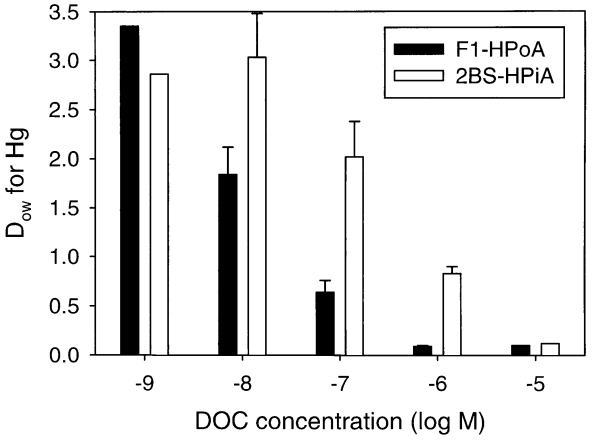

Fig. 3. Experimentally determined D_{ow} for Hg across gradients in two types of DOM from two sites in the Florida Everglades. Determinations were carried out in the presence of 0.01 M chloride and 0.04 M phosphate and buffered at pH 6.

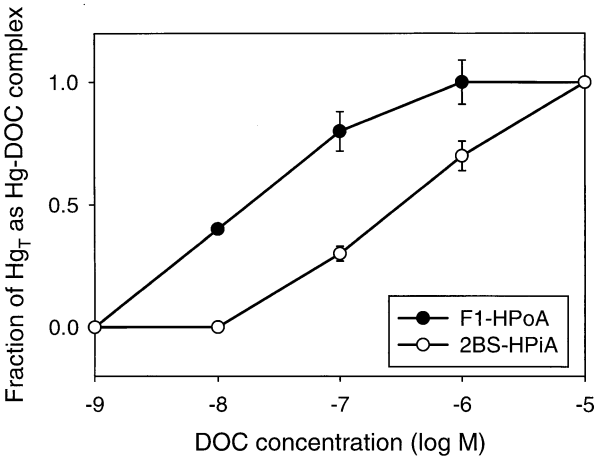

Fig. 4. The fraction of Hg present as Hg-DOM complexes for two types of isolates from the Florida Everglades across DOM concentration gradients in the presence of 0.01 M chloride and 0.04 M phosphate and buffered at pH 6.

respect to Hg. Figure 5 shows the effect of pH on Hg binding by F1-HPoA. Notice that for a given concentration of DOM a smaller fraction of Hg is complexed with DOM as pH decreases. However, the slopes of the lines are similar at all three pH values, as is consistent with a 1:1 complex stoichiometry.

At 10^{-2} M Cl, the concentration of chloride used in the DOM experiments, $HgCl_2^0$ and $HgCl_3^-$, are the dominant chloride complexes. Thus, $[Hg_T] = [HgCl_2^0] + [HgCl_3^-] + [Hg\text{-}DOM]$. At this chloride concentration, $HgCl_3^-$ is always present at 0.128 times the concentration of $HgCl_2^0$, so this equation can be written as

$$1 = 1.128\,x + y, \tag{1}$$

where x is the fraction of Hg_T present as $HgCl_2^0$ and y is the fraction of Hg_T present as Hg-DOM. Overall octanol–water partitioning for Hg_T depends on its complexation, according to

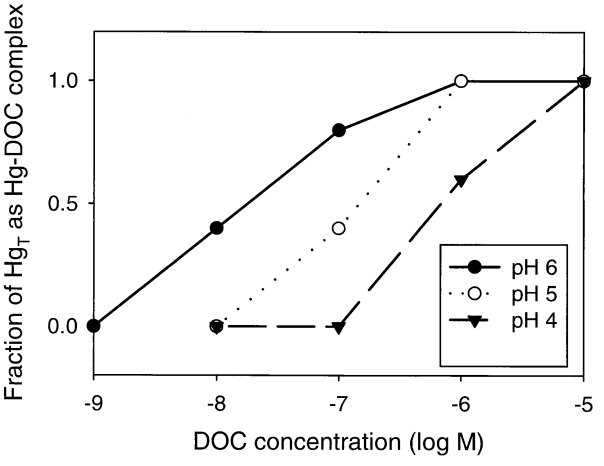

Fig. 5. The effect of pH on Hg binding by DOM isolates. Determinations were carried out in the presence of 0.01 M chloride and 0.04 M phosphate and buffered at pH 4, 5, and 6.

the following equation: $D_{ow} = \Sigma\ \alpha_i\ (K_{ow})_i$, where K_{ow} is the partitioning coefficient of individual chemical species and α is the fraction of Hg present as species i. Because the octanol–water partitioning of $HgCl_3^-$ is negligible, and on the basis of the K_{ow} values for $HgCl_2^0$ and Hg-DOM discussed above, it follows that

$$D_{ow} = 3.3\,x + 0.1\,y, \tag{2}$$

The values of x and y can be determined from each measured D_{ow} by combining Eqn. 1 and Eqn. 2.

We based our stability constant calculations on the following complexation reaction:

$$Hg^{2+} + RXH^{n-} = HgX^{(n-1)-} + H^+, \tag{3}$$

where -XH represents an acidic functional group that is fully protonated at pH 6. The overall charge on the DOM molecule comes from deprotonated carboxyl groups. This proposed reaction indicates a 1:1 DOM:Hg complex and implies a pH dependence of Hg binding. The stoichiometry of the reaction was tested by investigating changes in Hg binding across a several order-of-magnitude gradient in DOM concentration, and the pH dependence was tested at three pH values. However, the existence of 2:1 (DOM:Hg) complexes at higher concentration ratios of DOM to Hg was not tested by this method.

The important competing reaction for Hg binding in these experiments is $2Cl^- + Hg^{2+} = HgCl_2^0$; log K = 14 (Morel and Hering, 1993, and references therein). Ignoring the small amount of Hg bound as $HgCl_3^-$, which introduces a small error into the calculation, the following thermodynamic relationships apply:

$$K' = \frac{[HgRX^{(n-1)-}][H^+]}{[RXH^{n-}][Hg^{2+}]} \tag{4}$$

$$10^{14} = \frac{[HgCl_2^0]}{[Cl^-]^2[Hg^{2+}]} \tag{5}$$

Dividing Eqn. 4 by Eqn. 5

$$K'/10^{14} = \frac{[HgRX^{(n-1)-}]}{[HgCl_2^0]}\frac{[H^+][Cl^-]^2}{[RXH^{n-}]}. \tag{6}$$

Substituting for a chloride concentration of 0.01 M and a pH of 6.0 yields

$$K' = \frac{[HgRX^{(n-1)-}]}{[HgCl_2^0]}\frac{10^4}{[RXH^{n-}]}. \tag{7}$$

The ratio $[HgRX^{(n-1)-}]/[HgCl_2^0]$ is equal to the fraction of Hg as Hg-DOM (y in Eqns. 1 and 2) divided by the fraction as $HgCl_2^0$ (x in Eqns. 1 and 2). The values of x and y can be determined from D_{ow}; therefore, Eqn. 7 allows the calculation of the conditional stability constants (K′) based on the experimental data (Table 2). The conditional stability constants calculated here apply to the molarity of DOM_T as calculated from the average molecular weight of organic compounds present in the isolates. In the experimental solutions, $DOM_T = [RXH^{n-}] + [RX^{(n+1)-}] + [HgRX^{(n-1)-}]$. We ignored the small fraction of total DOM bound to Hg and assumed that pK_a for the thiol was greater than 7, so that the binding group was essentially fully protonated at pH 6. Therefore, $[DOM]_T = [RXH^{n-}]$ in these

Table 2. Calculation of complexation coefficients for DOM isolates F1-HPoA and 2BS-HPiA.[a]

DOM conc. (M)	[Hg-DOM]/[$HgCl_2$]		K′ for Hg-DOM		Log K′ for Hg-DOM	
	F1	2BS	F1	2BS	F1	2BS
10^{-5}						
10^{-6}		3.70		$3.7–10^{10}$		10.6
10^{-6}		2.84		$2.8–10^{10}$		10.5
10^{-7}	6.38	0.37	$6.4–10^{11}$	$3.7–10^{10}$	11.8	10.6
10^{-7}	3.78	0.73	$3.8–10^{11}$	$7.3–10^{10}$	11.6	10.9
10^{-8}	1.05		$1.1–10^{12}$		12.0	
10^{-8}	0.45		$4.5–10^{11}$		11.7	
10^{-9}						
				Average	11.8	10.6
				Standard deviation	0.2	0.2

[a] Determinations were in 40 mM phosphate and 0.01 M chloride at pH 6.0. [Hg-DOM]/[$HgCl_2$] is equal to x/y from Eqns. 1 and 2, calculated as described in the text.

calculations. The validity of this assumption was tested by fitting the data to Eqn. 7.

It was impossible to calculate K′ where the fraction of either Hg-DOM or $HgCl_2^0$ was 0. For both isolates, however, we were able to obtain estimates for K′ at two different DOM concentrations, an order of magnitude apart (Table 2). For both isolates, the calculated values of log K′ were independent of the concentration of DOM. In other words, the ratio [$RX\text{-}Hg^{(n-1)-}$]/[$HgCl_2^0$] declined across the DOM concentration gradient in a manner that was consistent with the stoichiometry of the Hg-binding reaction chosen. If the pK_a of the binding group were 6, then the average log K′ would be increased by 0.3 U, which is within the uncertainty associated with this estimate. The pK_a of the binding group was further investigated by measuring changes in complexation across a range in pH.

The proposed reaction (Eqn. 3) implies a pH dependence on Hg-DOM complex formation—that is, lowered pH should destabilize the complexes due to competition by H^+ for binding sites. Therefore, measurement of D_{ow} across a pH gradient provided another way to test the hypothesized complexation reaction. As predicted, DOM binding was diminished with decreasing pH (Fig. 5). The estimated log K′ values for F1-HPoA binding with Hg were 12.2, 11.9, and 11.8 ± 0.2 at pH 4, 5, and 6, respectively. Again, the observed trends in D_{ow} were consistent with the proposed reaction for Hg complexation because a single value of log K′ (within the error of the estimates) was obtained at three different pH values. Further, the ratio [Hg-DOM]/[$HgCl_2^0$] decreased by a factor of 10 for each pH unit decrease. This result is consistent with the assumption that the pK_a for the reactive group is greater than 7, as is typical for thiols, which have pK_a values between 9 and 11 (Martell et al., 1998). Therefore, within the range of concentrations tested, up to 5 mg/L, the DOM isolates form highly stable 1:1 complexes with Hg at 6 nM, and the binding site is an acidic functional group with a high pK_a. We propose that this functional group is a thiol, although other ligands may also fit the model. The experimental treatments presented here do not specifically identify the functional group or groups responsible for Hg binding. Rather, these K′ values are useful for comparison with other known Hg ligands and for modeling the role of DOM in Hg complexation in aquatic ecosystems when DOM concentration has been measured.

Given the high affinity of organic-matter thiol groups for Hg (Dyrssen and Wedborg, 1986; Schuster, 1991; Xia et al., 1999), we hypothesize that the complexation reaction can be written as $Hg^{2+} + RSH^{n-} = HgRS^{(n-1)-} + H^+$ (after Dyrssen and Wedborg, 1991). To explore the possibility that thiol groups are the important sites of Hg binding on the organic matter in these isolates, specific binding constants were also calculated from [RSH] expressed as the molarity of reduced S. For example, a DOM_T concentration of 10^{-5} M corresponds to a reduced S concentration of 1.2×10^{-6} M for F1-HPoA and 4.7×10^{-7} M for 2BS-HPiA (Table 1). Correspondingly, the average calculated log K′ was 12.4 ± 0.2 for F1-HPoA and 11.6 ± 0.2 for 2BS-HPiA (Table 3). Although this normalization brings the calculated K′ values closer for the two types of DOM, the difference implies that Hg binding cannot be explained in terms of reduced S concentration alone. The reduced S content given in Table 1 includes organic sulfides (R-S-R) and polysulfides that may not be available for Hg binding, and the fraction reduced S present as thiol groups may differ in the two isolates. Additionally, the two isolates were extracted by different resins (Aiken et al., 1992); therefore, they differ in a number of other characteristics, including hydrophobicity, aromaticity, and number of carboxyl groups present, all of which may influence Hg complexation. These extracts are described in more detail in Ravichandran (1999). Dyrssen and Wedborg (1986) investigated Hg binding by natural marine thiols and determined a log stability constant of 41.6 for a 2:1 (thiol:Hg) complex. They further estimated a log stability constant of 12.8 for the 1:1 complex (Dyrssen and Wedborg, 1991), which is very close to the value determined here for the F1 isolate. The sediments at F1 exhibit active sulfate reduction and intense sulfide production (Gilmour et al., 1998), so the dissolved organic S in surface water from this site might contain a higher proportion of thiol groups for Hg complexation. Diagenetic formation of organic thiols by reaction of organic matter with sulfide from sulfate reduction was proposed by Benoit et al. (1999a) to explain observed relationships between dissolved Hg and sulfide in Everglades pore waters. This process has been observed in lacustrine and estuarine sediments (David and Mitchell, 1985; Rudd et al., 1986; Brüchert and Pratt, 1996; Canfield et al., 1998).

The stability constants determined here are conditional in that they apply at the ionic strength of the experimental solu-

Table 3. Stability constants for Hg complexes with DOM isolates from two sites in the Florida Everglades.

Isolate	Reaction	Log K′ (I = 0.06)	Log K (I = 0)
F1-HPoA	$RXH^{5-} + Hg^{2+} = RXHg^{4-} + H^{+}$	11.8	13.2
2BS-HPiA	$RXH^{3-} + Hg^{2+} = RXHg^{2-} + H^{+}$	10.6	11.4
F1-HPoA	$RSH^{5-} + Hg^{2+} = RSHg^{4-} + H^{+}$	12.4	13.8
2BS-HPiA	$RSH^{3-} + Hg^{2+} = RSHg^{2-} + H^{+}$	11.6	12.4

tions (I = 0.06). The correction can be written as follows: log K = log K′ + C, where K is the stability constant at I = 0, K′ is the conditional stability constant, and C is the correction factor. We used the Debeye-Huckel approximation for activity coefficients, $\log \gamma_i = -0.5\ z_i\ I^{0.5}$, where γ_i is the activity coefficient for species i, z_i is the charge on species i, and I is the ionic strength. We based the average charge of the DOM on the number of carboxyl groups per molecule (Table 1). Because pK_a increases with increasing number of carboxyl groups present, we assumed that the pK_a for the sixth carboxyl group on F1-HPoA is much greater than 6, so that this ligand would not be fully deprotonated and would only have a charge of 5+ under the experimental conditions. The reactions considered, log K′, and log K are all given in Table 3. The experiments were performed at 23°C, so the temperature correction to 25°C is not significant.

Stability constants for metal binding by organic ligands are generally reported for the reaction written as: $Hg^{2+} + L^{n-} = HgL^{(n-2)-}$, where L^{n-} represents the fully ionized dissolved organic ligand. To express the constants determined in this study in this way, a pK_a for the Hg binding group is needed. For the sake of comparison, we assumed a pK_a of 10, as is typical for the -SH group of organothiols like cysteine (Morel and Hering, 1993) and thioglycolate (Stricks et al., 1953). Therefore, the constants (log K) for the fully ionized ligands are approximately 23.8 and 22.4 for F1-HPoA and 2BS-HPiA, respectively. These are quite similar to those previously determined for organic matter from lake and river water, where log K ranged from 18.4 to 21.1 (Mantoura and Riley, 1975; Mantoura et al., 1978). They also compare favorably to log stability constant for thioglycolate, 23.3 (Stricks et al., 1953), and for organothiols in seawater, 22.1 (for the reaction $Hg^{2+} + RSH = HgSR^{+} + H^{+}$; Dyrssen and Wedborg, 1991). The procedures used to obtain the DOM isolates may have led to some oxidation of thiol groups; therefore, if thiol groups are the important binding sites for Hg, the constants presented here may underestimate the importance of DOM complexation in natural waters. On the other hand, competition by other divalent metals such as Mn(II) and Zn(II) would lower the apparent stability constant for Hg binding to thiol groups on DOM in natural waters.

The low (<1) K_{ow} value for Hg-DOM complexes formed by these isolates has implications for the bioavailability of Hg bound to organic matter. In general, neutral Hg complexes diffuse more readily across lipid bilayers (Gutknecht, 1981), and octanol–water partitioning has been used as a surrogate for passive uptake of Hg across diatom cell membranes (Mason et al., 1995, 1996). Although other modes of uptake are possible, our studies to date have suggested that passive diffusion is an important uptake mechanism for diatoms in mildly saline waters (Mason et al., 1996) and bacteria in mildly sulfidic environments (Benoit et al., 1999b). In the latter case, Hg speciation shifts from predominantly HgS^0 toward charged disulfide complexes, causes a decrease in D_{ow} of Hg with increasing sulfide concentration (Benoit et al., 1999b). We have proposed that this species shift may lower inorganic Hg uptake by methylating bacteria, thereby explaining the common inverse relationship between sediment methylmercury and pore water sulfide (Benoit et al., 1999a,b). The negative charge and large size of Hg-DOM complexes could similarly limit the passive uptake of Hg, leading to the reduced bioavailability of Hg and MeHg that has been observed in the presence of DOM (Miskimmin et al., 1992; Barkay et al., 1997; Watras et al., 1998). Although significant passive diffusion of large Hg-DOM complexes probably does not occur, there is evidence to suggest that there are other uptake mechanisms for Hg-DOM by diatoms (Mason et al., 1996; Lawson and Mason, 1998). However, the studies done to date suggest that the rate of accumulation is less in the presence of organic matter compared with an equivalent concentration of $HgCl_2^0$.

Given the magnitude of the binding constants presented here (log K = 11.4 to 13.2), chemical speciation calculations indicate that at the DOM concentrations frequently measured in Everglades (20 to 40 μM), significant complexation of Hg by DOM would occur in fully aerobic (sulfide-free) surface waters. However, even nanomolar sulfide concentrations would be expected to out-compete DOM for Hg binding at any realistic ($\leq$1 mM) DOM concentration—for instance, through the reaction $Hg^{2+} + HS^{-} + HgS_{(aq)} + H^{+}$ (log K = 26.5; Benoit et al., 1999a). These results are consistent with the dominance of Hg-sulfide complexes in pore waters in the Everglades, as predicted in the model previously put forward in Benoit et al. (1999a). However, it should be noted that interactions other than simple ligand exchange may influence the reactivity of natural DOM toward Hg in sulfidic environments. For example, Ravichandran et al. (1998, 1999) showed that F1-HPoA enhanced dissolution and inhibited precipitation of $HgS_{(s)}$ to a greater degree (by an order of magnitude) than either 2BS-HPiA or thioglycolate, although our work suggests that the Hg complexes with these ligands have similar stability constants. Therefore, the ligand exchange reactions considered for the aerobic Hg-Cl-DOM chemical system may not adequately reflect the reactivity of DOM in the anaerobic Hg-sulfide-DOM system. Furthermore, a chemical equilibrium approach does not address how the kinetics of Hg-S-DOM interactions may affect the complexation of Hg in sediments with dynamic sulfide and DOM pools.

Acknowledgments—Support was provided by an EPA STAR grant, south Florida Water Management District contract C-7690 and Florida DEP contract SP-434.

Associate editor: D. J. Burdige

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