Binding of Mercury(II) to Aquatic Humic Substances: Influence of pH and Source of Humic Substances

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Conditional distribution coefficients (K_{DOM}') for Hg(II) binding to seven dissolved organic matter (DOM) isolates were measured at environmentally relevant ratios of Hg(II) to DOM. The results show that K_{DOM}' values for different types of samples (humic acids, fulvic acids, hydrophobic acids) isolated from diverse aquatic environments were all within 1 order of magnitude ($10^{22.5\pm1.0}$–$10^{23.5\pm1.0}$ L kg^{-1}), suggesting similar Hg(II) binding environments, presumably involving thiol groups, for the different isolates. K_{DOM}' values decreased at low pHs (4) compared to values at pH 7, indicating proton competition for the strong Hg(II) binding sites. Chemical modeling of Hg(II)−DOM binding at different pH values was consistent with bidentate binding of Hg(II) by one thiol group (pK_a = 10.3) and one other group (pK_a = 6.3) in the DOM, which is in agreement with recent results on the structure of Hg(II)−DOM bonds obtained by extended X-ray absorption fine structure spectroscopy (EXAFS).

Introduction

Mercury strongly accumulates in aquatic food webs so that, even in remote, pristine ecosystems, top predators can reach critical body burdens (*1*, *2*). Mobility and bioavailability of Hg(II) in aquatic ecosystems are affected by dissolved organic matter (DOM) (*3−9*). For example, Hg(II)−DOM associations may decrease the bioaccumulation of mercury in aquatic food webs by lowering the bioavailability of Hg(II) to methylating organisms (*10*, *11*). Despite the known relevance of DOM for Hg(II) cycling in aquatic systems, quantitative aspects of Hg(II) binding to DOM are still poorly understood, and reliable binding constants at environmentally relevant concentrations, indicators of the strength of Hg(II)−DOM binding interactions, are unavailable.

Determination of conditional distribution coefficients (K_{DOM}') that describe the binding of Hg(II) to DOM is hampered by the heterogeneity of the DOM, the lack of stoichiometric information, and the difficulties presented in determining appropriate distribution coefficients at the low Hg(II):DOM conditions that exist in most aquatic systems. We recently showed that the Hg(II):DOM ratio strongly affects K_{DOM}' values (*12*) in experiments designed to measure the strength of Hg(II)−DOM interactions using DOM isolated

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from the Florida Everglades. These results suggest that distribution coefficients determined at high ratios of Hg(II) to DOM (> approximately 1 µg Hg(II) per mg DOM) are not relevant for natural environments, where Hg(II) to DOM ratios are generally very low (*5*). Unfortunately, most of the published Hg(II)−DOM binding data have been determined at high Hg(II):DOM ratios (e.g. refs *13* and *14*).

In this paper we measured K_{DOM}' values for the binding of Hg(II) to a range of DOM samples isolated from diverse environments, and we evaluated the effects of pH on Hg(II) binding. We hypothesized that Hg(II) is mainly bound to DOM sites containing a reactive thiol group (*12*, *15−18*) and assumed that variations in K_{DOM}' for DOM isolates from different sources would be within a relatively narrow range. Our hypothesis for the pH experiments was that a decrease in pH would cause a significant increase in proton competition for the Hg(II) binding sites (thiols generally have pK_a values higher than the pHs used in these experiments). Therefore, we expected a decrease in binding of Hg(II) to DOM with decreasing pH. Including these data into mercury speciation models should give a more realistic picture of mercury cycling in natural environments than presently available.

Experimental Section

DOM Isolation and Characterization. The hydrophobic organic acid fraction (HPOA) of the DOM were isolated from surface waters associated with a range of environments (Table 1) following the procedures given by Aiken et al. (*19*). The HPOA fraction is operationally defined as that fraction of the DOM that sorbs to Amberlite XAD-8 resin at pH 2 and can be eluted with 0.1 N NaOH, and, is generally comprised of 90−95% fulvic acid with the remainder being humic acid. In brief, surface water was filtered through 0.3 µm glass fiber filters or 0.45 µm silver membranes (Suwannee River isolates only), acidified to pH 2 using HCl, and passed through XAD-8 resin. The HPOA fraction was retained on the XAD-8 resin and then back-eluted with 0.1 M NaOH. The eluate was desalted, proton saturated, lyophilized, and stored for later use. Some hydrophobic acids were further separated into humic acids (HA) and fulvic acids (FA) by acidifying the XAD-8 eluate to pH < 1 with HCl. The humic acids precipitated out, were removed by centrifugation, and freeze-dried. The supernatant containing fulvic acids was then desalted, hydrogen saturated, and freeze-dried. (Table 1).

Elemental compositions of the samples were determined as described by Huffman and Stuber (*20*). The relative content of reduced sulfur (%S_{red}) and thiols+sulfides was measured by sulfur K-edge X-ray absorption near edge structure (XANES) spectroscopy (*21*). Functional group content of the isolates was measured by liquid state ^{13}C NMR. The relative content of aromatic carbons (%Ar) was calculated according to ref *22* by relating the peak area in the 110−160 ppm chemical-shift band to the total peak area. Number-average molecular weights (MW) of the isolates were determined by high-pressure size exclusion chromatography using a Protein-Pak 125 modified silica gel column (Waters, Milford, MA) and polystyrene sulfonate standards according to the method of Chin et al. (*23*).

Reagents. Hg(II) stocks were prepared by dissolving HgCl$_2$ (99.9995%, Alfa Aesar, Ward Hill, MA) in 10% (v/v) HNO$_3$. HgCl$_2$ was used instead of Hg(NO$_3$)$_2$ because the chloride salt is available in higher purity and is not as hygroscopic as the nitrate salt. The small amount of chloride introduced into the system did not play a significant role, because virtually all available Hg(II) was complexed by ethylenedi-

TABLE 1. Site Descriptions for Aquatic Organic Matter Isolates

sample	site description
Suwannee River Humic (SRHA) and Fulvic Acid (SRFA)	Black water river draining the Okeefenokee Swamp; Sampled at Fargo, GA; Vegetation types: Southern Flood plain Forest (*Quercus, Nyassa, Taxodium*); International Humic Substances Society Standard Humic and Fulvic Acids.
Ogeechee River Humic (OGHA) and Fulvic Acid (OGFA)	Small river draining the piedmont in Eastern Georgia. Sampled at Grange, GA. Vegetation types: Oak-Hickory-Pine Forest (*Quercus, Carya, Pinus*).
Coal Creek Fulvic Acid (CCFA)	Small mountain stream draining the Flattops Wilderness Area, CO. Vegetation type: Spruce-Fir Forest (*Picea, Abies*).
F1 Hydrophobic Acid (F1 HPOA)	Eutrophied marshland located in Water Conservation Area 2A in the northern Everglades. Vegetation dominated by cattails (typha latifolia).
2BS Hydrophobic Acid (2BS HPOA)	Relatively pristine marshland located in Water Conservation Area 2B in the northern Everglades. Vegetation dominated by saw grass.

TABLE 2. Chemical Characteristics of Isolated Samples

sample	ash-free elemental composition (wt %)					relative %S_{red}	relative % thiol+sulfide	MW (daltons)	% aromatic C
	C	H	O	N	S				
SRHA	53.4	3.9	40.9	1.1	0.7	55	14	1399[a]	35.1
SRFA	54.2	3.9	38.0	0.7	0.4	48	12	1360[b]	22.9
OGHA	54.6	4.9	36.8	1.6	1.8	37	14	1906	40.8
OGFA	54.0	4.0	38.5	0.9	1.3	44	18	1021	24.7
CCFA	52.8	4.5	38.4	1.0	0.7	47	11	1180	28.0
F1 HPOA	52.2	4.6	39.9	1.5	1.7	60	22	103[a]	25.4
2BS HPOA	52.3	4.8	40.2	1.6	1.2	51	23	953[a]	21.3

[a] Ref *6*. [b] Ref *23*.

aminetetraacetic acid (EDTA) or DOM. Stock solutions of NaClO₄ (inert background electrolyte used to adjust ionic strength) and EDTA were filtered through 0.45 µm membrane filters to exclude the presence of any particles.

For the experiments using DOM isolates, 100 mg L⁻¹ stock solutions were freshly prepared by dissolving a weighed amount of freeze-dried material in deionized water. The solutions were filtered through 0.45 µm PVDF membrane filters to exclude any particles. The concentration of dissolved organic carbon (DOC) in the filtered stock solutions was measured. Solutions containing the isolates were buffered with 0.01 M EDTA, and the pH was adjusted to approximately 7 using NaOH. The experiments with varying pHs also used EDTA as a buffer, and pH was adjusted with NaOH to approximately 4, 5, 6, or 7.

Hg Analysis. Hg(II) was analyzed using a Millennium Merlin mercury analyzer (PSA Analytical, Kent, U.K.). Samples were oxidized overnight by a mixture of KBr, KBrO₃, and HCl to break down all organic Hg(II)−complexes. After oxidation, the samples were prereduced with NH₂OH•HCl to destroy excess bromine, and then Hg(II) was reduced to volatile Hg-(0) with SnCl₂. Hg(0) was separated from solution by purging with high purity argon gas, dried in a semipermeable dryer tube, and detected by cold vapor atomic fluorescence spectrometry (CVAFS). Duplicates and standards prepared from a certified reference material (NIST-3133) were analyzed to assess precision and accuracy of the method. Analysis results for a batch of samples were regarded acceptable if results for standards showed 80−120% recovery and duplicates were within 20% relative difference. Generally, standard recovery was 90−110%, and duplicates were within 10% relative difference.

DOM Analysis. Concentrations of dissolved organic carbon (DOC) were measured using an OI 700 (OI Analytical, College Station, TX) total organic carbon analyzer. DOC-based concentrations were converted to DOM-based concentrations using the organic carbon content of the isolates (Table 2).

Measurement of Hg-DOM Binding. An equilibrium dialysis ligand exchange (EDLE) method was used to determine conditional distribution coefficients for Hg−DOM interactions (*12*). The EDLE method, which combines equilibrium dialysis and ligand exchange, was originally developed to measure metal ion−DOM interactions at nanomolar metal concentrations (*24−27*). In this method, we measured the equilibrium distribution of Hg(II) between solutions inside and outside a dialysis bag (Spectra/Por regenerated cellulose "Biotech" membranes, nominal molecular weight cutoff 3500 Da). The solution inside the bag contained DOM and EDTA, the auxiliary ligand. The solution outside the bag contained only EDTA. The dialysis bag prevented the transport of DOM from inside to outside but allowed the transport of EDTA and Hg(II)−EDTA complexes into the dialysis bag. Initially, Hg(II) was added to the outside solution and allowed to diffuse across the dialysis membrane. The auxiliary ligand EDTA, whose Hg(II)-complexes can pass through the membrane and was added to balance strong binding of Hg(II) to DOM and maintain measurable concentrations of Hg(II) in the external solution. Using known stability constants for the interactions of EDTA with Hg²⁺ (*28*), conditional distribution coefficients (K_{DOM}') for the reaction of Hg²⁺ with DOM can be calculated from the distribution of Hg(II) between inside and outside of the dialysis bag. K_{DOM}' is defined by

$$Hg + DOM = HgDOM \tag{1}$$

$$K_{DOM}' = \frac{[HgDOM]}{[Hg] \times (DOM)} \tag{2}$$

where charges are omitted and 1:1 stoichiometry is assumed. Square brackets denote molar concentrations of HgDOM and Hg²⁺ and round brackets denote the mass concentration of DOM (kg L⁻¹). (DOM) is equivalent to the measured concentration of DOM (in kg L⁻¹) under the condition that the concentration of Hg(II) is much smaller than the concentration of DOM. K_{DOM}' is a mass-related conditional distribution coefficient (units of L kg⁻¹) that is free from any

additional assumptions (e.g., about the molar concentration of DOM or the molar concentration of Hg(II) binding sites). K_{DOM}' was calculated from experimental data, taking into account some leakage of DOM from the inside to the outside compartment (26), following the equation

$$K_{DOM}' = \frac{Q \times \alpha_{Hg}}{(DOM)_{in} - (DOM)_{out} \times (Q+1)} \qquad (3)$$

where $(DOM)_{in}$ and $(DOM)_{out}$ are the concentrations (kg L^{-1}) of DOM inside and outside the dialysis bag. Q is a distribution ratio describing the Hg(II) concentration ratio between inside and outside of the dialysis bag ($[Hg]_{in}$ and $[Hg]_{out}$) defined by

$$Q = \frac{[Hg]_{in} - [Hg]_{out}}{[Hg]_{out}} \qquad (4)$$

α_{Hg} is a measure of complexation of Hg(II) by the auxiliary ligand L and is defined, in general, as

$$\alpha_{Hg} = \frac{[Hg] + \sum_{i=1}^{n}[HgL_i]}{[Hg]} \qquad (5)$$

where i represents the stepwise complexation of Hg(II) by L ($i = 1, 2, ..., n$). Taking the auxiliary ligand and OH$^-$ into consideration, α_{Hg} can be written for $[Hg]_{total} \ll [L]_{total}$ as (24)

$$\alpha_{Hg} = 1 + \sum_{i=1}^{n}\beta_i^L \times [L]^i + \sum_{k=1}^{o}\beta_k^{OH} \times [H]^{-k} =$$
$$1 + \sum_{i=1}^{n}\beta_i^L \times \left(\frac{[L]_{total}}{1 + \sum_{j=1}^{m}[H]^j \times \beta_j^H}\right)^i + \sum_{k=1}^{o}\beta_k^{OH} \times [H]^{-k} \qquad (6)$$

where β_i^L is the stability constant for the step i in the stepwise binding of L to Hg(II), and β_j^H is the stability constant for the step j in the stepwise protonation of L (L+jH$\leftrightarrow_j$L; $j = 1, 2, ..., m$). β_k^{OH} is the overall hydrolysis constant referring to the formation of Hg(OH)$_k$ from Hg^{2+} + kH$_2$O. For a detailed description of this method, including the relevant binding constants for EDTA binding to Hg(II) and protonation constants for EDTA as well as a discussion of DOM leakage through the dialysis membranes, see refs 12 and 24.

Experimental Procedures. The experimental system for measuring Hg(II) binding to DOM consisted of triplicate sets of acid cleaned 125 mL borosilicate glass bottles with Teflon-lined caps containing 93 mL of outside solution and 7 mL of inside solution. Dialysis bags were sealed by tying knots on both ends to separate the inside solutions. The pH value in both compartments was set using EDTA as a buffer, and ionic strength was set at 0.1 M with NaClO$_4$, taking into account ionic strength effects of EDTA. Inside solutions contained 1.0 mg L^{-1} of DOM at the start of the experiments.

Due to differences in the leakage of DOM isolates through the membrane, inside DOM concentrations at the end of the experiments varied. Because DOM concentrations in the actual samples were too low to be measured reliably and because EDTA interferes with dissolved organic carbon (DOC) analysis, DOM concentrations for each isolate were extrapolated from parallel partitioning experiments using initial inside concentrations of approximately 100 mg L^{-1} DOM. This approach gave DOM concentrations at the end of the EDLE experiments of approximately 0.2–0.5 mg L^{-1} (inside
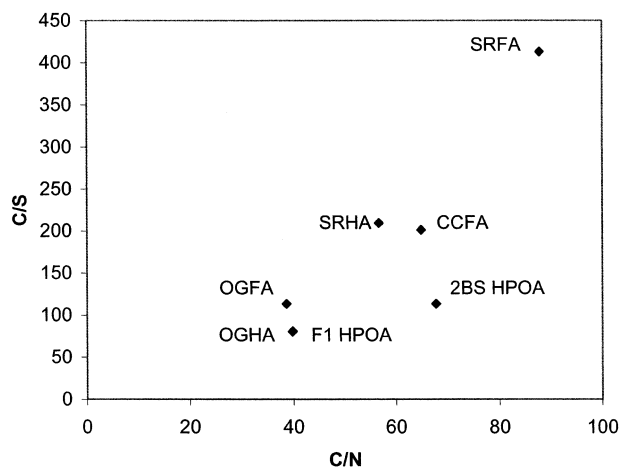
FIGURE 1. Plot showing the atomic C/S ratio versus atomic C/N ratio for the various DOM isolates.

compartment; 7 mL) and 0.02–0.05 mg L^{-1} (outside compartment; 93 mL). Mass balance calculations indicated a 104 ± 4% (mean ± standard deviation) recovery of DOM.

At the start of all experiments, 10 μL of a Hg(II) stock solution (1 mg L^{-1} in 10% HNO$_3$) were added to the outside compartment, and then samples were rotated end-over-end at 22 ± 2 °C. After 18 h, aliquots were taken from the inside and outside compartments and analyzed for Hg(II). Mass balance calculations for mercury indicated a 90 ± 7% (mean ± standard deviation) recovery of mercury. An equilibration time of 18 h was found to be suitable in preliminary experiments measuring Hg(II) and DOM diffusion kinetics across the membranes. Diffusion kinetics in these experiments were slightly faster than measured in previous experiments (cf. ref 12, Figure 1), presumably because the dialysis membranes (same type, but new batch) were thinner than before. The pH value was measured in the outside solution. Parameters that were varied were the type of DOM isolate (seven different isolates, see Table 1) and the pH (from approximately 4–7 for F1 HPOA).

Modeling of Hg(II) Binding to DOM. A discrete site model, which has the advantage of circumventing the use of an electrostatic correction term for modeling stability constants (29), was written using Microsoft Excel. The model simulates a single Hg(II) binding site involving two different types of functional groups. The model started with calculating "simulated" distribution ratios (Q_{sim}) for the distribution of Hg(II) between the inside and the outside of the dialysis membrane at each pH. For this purpose, equation 3 was rearranged to give

$$Q_{sim} = \frac{K_{Hgsite}([site_{free,in}] - [site_{free,out}])}{\alpha_M + K_{Hgsite} \times [site_{free,out}]} \qquad (7)$$

where $[site_{free}]$ denotes the concentration of fully deprotonated binding sites according to

$$[site_{free}] = \frac{[site_{total}]}{1 + 10^{pK_a1} \times [H^+] + 10^{(pK_a1 + pK_a2)} \times [H^+]^2} \qquad (8)$$

The total concentration of strong binding sites ($[site_{total}]$) was calculated from the concentration of DOM and the estimated density of strong sites (5×10^{-9} mol per mg DOM, see ref 12). The deprotonation constant pK_a1 describes the reaction HA$_{(1)} \leftrightarrows$ A$_{(1)}^-$ + H$^+$, where A$_{(1)}^-$ probably represents a thiolate group and pK_a2 describes the reaction HA$_{(2)} \leftrightarrows$ A$_{(2)}^-$ + H$^+$, where A$_{(2)}^-$ probably represents a mixture of functional groups containing O, N, and S.
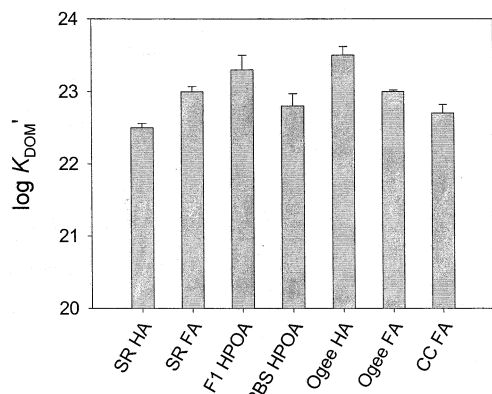
FIGURE 2. Conditional distribution coefficients (log K_{DOM}') measured for the binding of Hg(II) to various DOM isolates. Error bars represent standard deviations calculated from three replicates.

The deviation of Q_{sim} from the measured Q values (eq 4) was calculated for each sample according to

$$\text{fit\_error} = \left(\frac{Q_{sim} - Q_{measured}}{Q_{measured}}\right)^2 \quad (9)$$

The sum of the fit errors for simulations at all pHs was minimized by adjusting K_{Hgsite} and the protonation constants for the thiol group (pK_a1) and the mixed group (pK_a2) within the expected ranges using a Newton–Raphson algorithm.

Results and Discussion

Conditional Distribution Coefficients. The chemical characteristics of the DOM isolates are given in Table 2. The choice of samples was limited by leakage of material through the dialysis membranes used in the EDLE measurements. DOM isolates with lower molecular weights, such as those from Lake Fryxell, Antarctica (MW = 562 daltons), or the Pacific Ocean (MW = 532 daltons), were therefore excluded from this study. Regardless, the isolates used in this study exhibit a number of important differences relevant for understanding Hg(II)–DOM interactions, including heteroatom content and aromaticity. In particular, the samples cover the range of reported values for C/S and C/N atomic ratios for similar materials isolated from surface waters (Figure 1). For each of the samples, S content is low and not all molecules that comprise a given sample contain a S atom. Using elemental, MW, and XANES data presented in Table 2, it can be estimated that thiol functional groups are, at best, present in only one molecule in seven or eight for samples with high S contents (OGHA and F1 HPOA), and only one in 56 for the sample with the lowest S content (SRFA).

The conditional distribution coefficients for strong binding sites determined from the different isolates varied within approximately 1 order of magnitude (Figure 2). There were no clear patterns related to the different types of isolates (FA, HA, HPOA), and there was no significant correlation with either N content or aromaticity of the samples. Overall, the strength of the interactions were comparable to those expected for organic thiols with Hg (*12*). Because there is growing evidence that thiol groups are a major feature of Hg(II) binding sites in DOM (*12, 15–18*), we tried to relate the log K_{DOM}' values to the sulfur content of the isolates (Table 2; Figure 1). With the exception of the SRFA sample, the highest K_{DOM}' values were measured for the samples with the lowest C/S atomic ratios (greater S content). The highest K_{DOM}' values were determined for F1 HPOA and OGHA (C/S about 80), while similar values were measured for both the 2BS HPOA and OGFA sample pair (C/S about 113) and for the SRHA and CCFA sample pair (C/S about 200). These
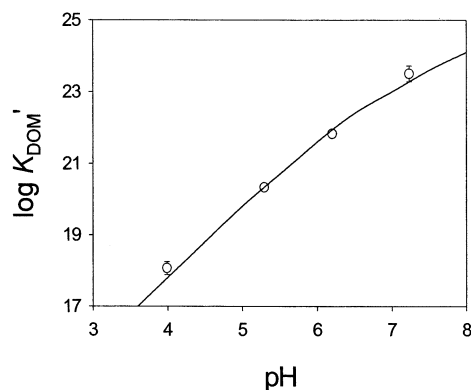

FIGURE 3. Binding of Hg(II) to F1 HPOA as a function of solution pH. Binding is given as conditional distribution coefficient log K_{DOM}' (see eq 2) at $I = 0.1$ M. Circles are the experimental data points ($\pm$ standard deviation). The solid line represents the model fit to the experimental data using parameters in Table 2.

results suggest that samples with greater overall S content have greater probability of having the desired stereochemistry for strong binding of Hg. However, we found no significant correlation between log K_{DOM}' and %S_{red} (squared Pearson correlation coefficient $R^2 = 0.39$, $p = 0.14$) or %thiols+sulfides ($R^2 = 0.36$, $p = 0.16$). This result seems to be contradictory to the hypothesis that thiols are a major feature of Hg(II) strong-binding sites in DOM. However, recent data suggests that only a small fraction (ca. 2%) of reduced sulfur sites actively take part in the binding of mercury (*12, 30*). This means the total percentage of reduced sulfur or thiol+sulfide is probably a crude measure for reactive Hg(II) binding sites on the DOM. Therefore, correlations of log K_{DOM}' with these parameters will likely fail.

It is, perhaps, not surprising that the K_{DOM}' values measured for these samples were clustered within the narrow range reported for thiols. Our previous studies using F1 HPOA indicated the presence of both strong (thiol-like) and weak (carboxyl-like) Hg(II) binding sites (*12*). The strength of the interactions within each type of site was relatively constant over a large range of [Hg(II)]/DOM ratios, and even within a given sample, the range for strong (and weak) interactions was narrow. The results presented here indicate that the strong Hg(II)–DOM binding interactions are similar for different organic matter samples. Our findings and the general concept of relatively little variation in conditional distribution coefficients are in agreement with earlier findings by McKnight et al. (*31*). They found that another relatively soft metal, Cu(II), had conditional binding constants with FA that varied by only a factor of 2 for 18 diverse isolates.

pH Dependency. The binding of Hg(II) clearly decreased with decreasing pH, indicating significant proton competition for the Hg(II) binding sites (Figure 3). Our hypothesis for the pH-dependent binding of Hg(II) to DOM was based upon structural information on Hg(II)–DOM binding provided by extended X-ray absorption fine structure (EXAFS) spectroscopy (*15, 17*) and current knowledge of Hg(II)–DOM binding constants (*18*). We hypothesized that Hg(II) is bound to bidentate DOM sites that contain a thiol group and a second group representing a statistical mixture of functional groups containing oxygen, nitrogen or sulfur. For these binding sites, there should be significant proton competition, because the experimental pH values were clearly lower than the pK_a value of a typical thiol group.

To verify that our hypothesis was consistent with the experimental data, a simple discrete site model was developed to describe the binding of Hg(II) to DOM at different pHs (eqs 6–8). The model was consistent with structural information on Hg(II) binding to DOM provided by extended X-ray

pK_a1	pK_a2	log K_{Hgsite}	site concentration
10.3 ± 0.1	6.3 ± 0.1	28.7 ± 0.1	5×10^{-9} mol mg^{-1}

[a] pK_a1 and pK_a2 are deprotonation constants for the two different functional groups of the bidentate Hg(II) binding site. log K_{Hgsite} is the equilibrium binding constant for the reaction of Hg^{2+} with the fully deprotonated binding site. Errors in binding constants were calculated as a variation in binding constant resulting in a 5% variation of the sum of fit errors. Note that errors reported here only relate to the fitting procedure. Overall errors for absolute binding constants are significantly higher (see ref 12).

absorption fine structure (EXAFS) spectroscopy (15, 17) and with current knowledge of Hg(II)−DOM binding constants (18). Hg(II) generally prefers a two-coordination binding environment (e.g., ref 32). For interactions of Hg(II) with DOM, EXAFS results suggest that one of the electron donors in the first coordination shell is a reduced sulfur group, and the other one may be an oxygen, nitrogen, or sulfur functional group (15, 17). Therefore, our DOM model used a bidentate Hg(II) binding site consisting of one thiol and one "statistically mixed" (O, N, S) group. The pK_a values for the model site were expected to be in the range of approximately 8−12 for the thiol group (33) and less than 10 for the mixed group. The binding constant describing the interaction of Hg^{2+} with the fully deprotonated binding site (Hg^{2+} + site^{2-} = Hgsite) was expected to lie between 10^{25} and 10^{35} (12, 16, 18). Figure 3 shows that this model gave a good fit to the experimental data, despite its simplistic nature. The equilibrium binding constants that result from the best fit of the model to the experimental data (Table 3) were close to binding constants for the interactions of H$^+$ and Hg^{2+} with the corresponding functional groups in simple organic compounds (33). For example, protonation constants for organic thiols are mostly in the range of 10^8−10^{11} (33), and a pK_a value of 6.3 for a mixture of O, N, and S functional groups also seems reasonable. The value of $10^{28.7}$ for the equilibrium binding constant describing the interaction of Hg^{2+} with a fully deprotonated thiol binding site is close to values previously determined with this method (12). It is also interesting that the slope of the line in Figure 3 is ~2. This indicates that two protons are involved in the complexation reaction, giving evidence for the binding model (chelate) used here.

The modeling result is also in good agreement with recent findings by Drexel et al. (18), who estimated Hg^{2+}-thiolate binding constants of 10^{26}−10^{27} from measuring the distribution of Hg(II) in peat/DOM systems. Using soil organic matter (SOM), Skyllberg et al. (16) found Hg^{2+}−thiolate stability constants on the order of 10^{32}. The constants measured in their study may have somewhat overestimated the strength of natural Hg(II)−thiol interactions because high concentrations of bromide were used in the experiments. This can cause the formation of mixed ligand complexes (e.g., SOM−Hg(II)−Br), thus increasing the observed stability constants for Hg(II)−SOM interactions (25). Nevertheless, the magnitude of binding constants reported in their study is in agreement with our data, indicating that Hg(II) binding to organic matter at low ratios of Hg(II) to organic matter is indeed controlled by strong interactions with binding sites containing reactive organic thiol groups.

A recent study by Amirbahman et al. (30) analyzed the binding of methylmercury (MeHg) to DOM. The authors found that the extent of MeHg binding to DOM did not show a strong pH dependence within a pH range of 5−9, which is in contrast to our results with Hg(II). To explain these data by a chemical equilibrium model, they assumed three different types of thiol ligands, having pK_a values of 4, 7, and

10. In their model, the extent of MeHg-binding to each of these sites varied, depending on the pH. Such a model is not suitable for explaining the pH-dependent binding of Hg(II) to DOM, because it lacks the required bidentate binding site, as described above. However, this difference between models for MeHg and Hg(II) binding to DOM is in agreement with their general complexation behavior. Methylmercury complexes almost always possess a coordination number of one (34), and this interaction with DOM is mainly influenced by proton competition for the that site. In contrast, the pH-dependent binding of Hg(II) to DOM has to be governed by the protonation behavior of both functional groups in the bidentate binding site.

Environmental Implications. The speciation of Hg(II) in aquatic systems depends in large part on pH, the strength of DOM interactions, the amount of DOM, the concentrations of inorganic ligands, especially sulfide (S^{2-}), and the distribution of Hg(II) between dissolved and solid phases. The results presented in this paper address the strength of Hg−DOM binding by natural organic matter and the effects of pH on those interactions. The data indicate that the strength of the strong Hg(II)−DOM binding interactions is similar for a suite of DOM isolates. It can be assumed, therefore, that there is consistency in this parameter across ecosystems. Further, our results support the general assumption that the strong binding interactions can be modeled using a bidentate model ligand containing a thiol site. This new information is important for generating chemical speciation models describing interactions of Hg(II) with DOM (e.g., ref 35) and ultimately for taking into account the role of DOM in models describing Hg(II) interactions with biota (e.g., ref 36) and in comprehensive models of mercury cycling in natural waters (e.g., ref 37).

Our data also suggest that the number of strong binding sites present in the DOM in a given sample will be in excess of Hg(II) concentrations, which are often in the 10^{-11}−10^{-12} M range in most aquatic systems (38). Under most environmental conditions, therefore, it can be expected that only the strongest binding sites associated with the DOM will interact with Hg(II). Given the strength of the strongest Hg(II)−DOM interactions ($K_{DOM}' = 10^{23.2 \pm 1.0}$ L kg^{-1}), it follows that, under oxic conditions, Hg(II) binding by DOM should dominate over inorganic speciation.

The speciation of Hg(II) between DOM and S^{2-} in porewaters is of particular interest, especially with regard to the bioavailability of Hg(II). For instance, Benoit et al. (37) have hypothesized that a neutral form of HgS (HgS°) is more likely to partition across cell membranes than charged species. At issue are the number of DOM binding sites available and the relative strength of the interactions between DOM and S^{2-} with Hg(II). Our data indicate that the concentration of strong Hg(II) binding sites on F1 HPOA is 5×10^{-9} mol per mg DOM (12). Given that DOM pore water concentrations can exceed 100 mg L^{-1} (39), the concentration of strong DOM binding sites can reach 5×10^{-7} mol L^{-1}, which approaches the range of sulfide concentrations in anoxic pore waters. The concentration of thiol ligands associated with DOM, therefore, can be comparable to S^{2-} concentrations in many environmental settings. However, S^{2-} has a very strong affinity for Hg(II). We, therefore, determined the speciation of Hg(II) in the presence of DOM and S^{2-} by running speciation calculations in MINEQL+ (40), using a concentration of strong DOM binding sites of 5×10^{-7} mol L^{-1} and the binding constants found in this study (Figure 2). Equilibrium constants for Hg(II)−sulfide interactions were taken from Benoit et al. (41). The calculations indicated that Hg(II)−DOM complexes do not play a role in sulfidic waters. Concentrations of Hg(II)−sulfide species were several orders of magnitude higher than those of Hg(II)−DOM species at any realistic concentration of sulfide in pore

water. These results have to be regarded as tentative, however, because of three reasons. First, the data basis for Hg(II)−sulfide binding constants is relatively weak. For example, published values for the solubility product of HgS(s) vary by 5 orders of magnitude (*41*). Second, the possibility of mixed-complex formation (e.g., DOM−Hg−SH), which may increase the overall binding constants for metal−DOM interactions (*25*), has not been considered in the equilibrium calculations, because no data are available on mixed Hg(II)−sulfide−DOM complexes. Third, it is possible that porewater DOM differs from the surface water isolates used in the present experiments. For example, anoxic conditions might favor higher concentrations of reduced sulfur sites in the DOM. Therefore, a careful experimental study is needed to fully evaluate the significance of Hg(II)−DOM complexes in sulfidic (pore) waters.

Acknowledgments

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