Binding of Mercury(II) to Dissolved Organic Matter: The Role of the Mercury-to-DOM Concentration Ratio

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The binding of Hg(II) to dissolved organic matter (DOM; hydrophobic acids isolated from the Florida Everglades by XAD-8 resin) was measured at a wide range of Hg-to-DOM concentration ratios using an equilibrium dialysis ligand exchange method. Conditional distribution coefficients ($K_{DOM}$) determined by this method were strongly affected by the Hg/DOM concentration ratio. At Hg/DOM ratios below approximately 1 μg of Hg/mg of DOM, we observed very strong interactions ($K_{DOM}' = 10^{32} \pm 1.0$ L kg⁻¹ at pH = 7.0 and $I = 0.1$), indicative of mercury-thiol bonds. Hg/DOM ratios above approximately 10 μg of Hg/mg of DOM, as used in most studies that have determined Hg-DOM binding constants, gave much lower $K_{DOM}'$ values ($10^{10} \pm 1.0$ L kg⁻¹ at pH = 4.9–5.6 and $I = 0.1$), consistent with Hg binding mainly to oxygen functional groups. These results suggest that the binding of Hg to DOM under natural conditions (very low Hg/DOM ratios) is controlled by a small fraction of DOM molecules containing a reactive thiol functional group. Therefore, Hg/DOM distribution coefficients used for modeling the biogeochemical behavior of Hg in natural systems need to be determined at low Hg/DOM ratios.

Introduction

The binding of mercury(II) (denoted as Hg throughout the text) to dissolved organic matter (DOM) strongly affects mobility and bioavailability of Hg in aquatic ecosystems (1–6). While this fact is generally accepted, understanding the effect of DOM on aquatic Hg cycling on a quantitative basis has been limited due to a paucity of reliable Hg/DOM binding constants (7, 8). Literature values span many orders of magnitude and some of the published conditional binding constants lack an exact definition in the form of a chemical reaction equation together with a definition of the concentration basis for DOM used for the calculations (9–14).

Recent spectroscopic studies have found direct evidence of Hg binding to reduced sulfur (Sred) groups in organic matter (15, 16). Because interactions between Hg and compounds containing Sred can be very strong (17), and because the concentration of DOM-bound Sred (DOMSred) in natural systems is generally much higher than the concentra-
tion of Hg (15, 18), strong interactions between Hg and DOMSred are expected under natural conditions. Most laboratory studies determining Hg/DOM binding constants used Hg concentrations in excess of Sred concentrations in DOM (e.g., refs 10 and 14). Under these conditions, it is expected that a small fraction of the total Hg saturates all DOMSred, while the majority of Hg binds to oxygen functional groups that are present in DOM at much higher concentrations than Sred. Because constants for Hg binding to oxygen functional groups are many orders of magnitude lower than constants for Hg–Sred interactions (19), the overall binding constants for Hg/DOM binding at high ratios of Hg to DOMSred are expected to be much smaller than at low ratios. This “metal concentration effect” has been described for the binding of other metals to DOM (20, 21), but according to the hard and soft acids and bases theory (22, 23), the effect is expected to be especially strong for Hg due to its pronounced “soft metal” character and the low concentration of “soft” Sred groups compared to the high concentrations of “hard” oxygen functional groups in DOM.

We therefore hypothesized that the ratio of Hg to DOM strongly affects binding constants and that binding constants at low Hg-to-DOM ratios are in the range of binding constants for Hg–Sred interactions (i.e., much higher than literature values). Similar hypotheses have been discussed by Skyllberg et al. (24) and Hesterberg et al. (16) for the binding of Hg to soil organic matter, but to our knowledge the effect of the Hg-to-organic matter ratio has not been systematically investigated by comparing conditional binding constants measured at different Hg-to-organic matter ratios. To test the hypotheses stated here, we developed a method suitable for measuring conditional Hg–DOM binding constants over a wide range of Hg-to-DOM ratios, including low ratios.

Experimental Section

DOM Isolation and Characterization. This study used the hydrophobic acids fraction (humic and fulvic acids) of DOM isolated from water at the F1 site in the Florida Everglades in July 1997 (sample ID: F1HPoA; for details see ref 3). In brief, surface water was collected, filtered through 0.3 μm fiber filters, acidified to pH 2 using HCl, and passed through XAD-8 resin. The hydrophobic acids fraction was retained on the XAD-8 resin and then back-eluted with 0.1 M NaOH. The eluate was hydrogen saturated, desalted, lyophilized, and stored for later use. For the experiments, 100 mg L−1 stock solutions were freshly prepared by dissolving a weighed amount of freeze-dried material in deionized water. The solution was filtered through a 0.45 μm PVD memory filter (Millipore-HV, Millipore, Bedford, MA) to exclude any particles.

The number-average molecular weight of F1HPoA was 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. (25). Elemental composition of the sample was determined by Huffman Laboratories (Golden, CO) after the method described by Huffman and Stuber (26). The relative content of reduced sulfur was measured by sulfur K-edge X-ray absorption near edge structure (XANES) spectroscopy (27). Carboxyl and phenol groups were quantified by titrating a hydrogen-saturated sample with base. Selected characteristics of F1HPoA are given in Table 1.

Reagents. Hg stocks used for experiments at low Hg concentrations were prepared by dissolving HgCl₂ (99.9995%, Alfa Aesar, Ward Hill, MA) in 10% (v/v) HNO₃. Stocks for
TABLE 1. Selected Characteristics of the F1HPoA Isolate (after 40)

<table>
<thead>
<tr>
<th>molecule-averaged wt (Da)</th>
<th>C (%)</th>
<th>total S (%)</th>
<th>reduced S (%)</th>
<th>carboxyl content (meq/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1030</td>
<td>52.2</td>
<td>1.73</td>
<td>60.0</td>
<td>5.45</td>
</tr>
</tbody>
</table>

* Analyzed on dried samples and reported here on an ash-free basis.

The relative content of reduced sulfur was measured by sulfur X-edge X-ray absorption near edge structure (XANES) spectroscopy (27).

experiments at high Hg concentrations were prepared from Hg(NO₃)₂ (99.99%, Aldrich, Milwaukee, MI) to avoid chloride interference. Stock solutions of buffers, NaClO₃ (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. The pH of EDTA solutions was adjusted to 7.0 using NaOH.

**Hg Analysis.** Hg 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 destroy excess bromine, and then Hg 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, then Hg 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).

**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 F1HPoA (Table 1).

**Measurement of Hg-DOM Binding.** Conditional distribution coefficients for Hg-DOM interactions were measured using an equilibrium dialysis ligand exchange (EDLE) method that was developed to determine distribution coefficients for radionuclide-DOM interactions at nanomolar metal concentrations (28) and later modified to take into account some leakage of DOM across the dialysis membranes (29–31). This technique was adapted to measure the equilibrium distribution of Hg between DOM retained inside a dialysis bag and an outside solution containing no DOM. To avoid Hg depletion in the outside compartment due to strong binding to DOM in the inside compartment and sorption to the container walls, Hg concentrations on both sides of the dialysis membrane were buffered by an auxiliary ligand whose Hg complexes can pass through the membrane. Stability constants for the interactions of the auxiliary ligand with Hg are known, so conditional distribution coefficients (KDOM) for the reaction of Hg with DOM

\[ \text{Hg} + \text{DOM} = \text{HgDOM} \] (1)

(charges omitted, 1:1 stoichiometry assumed) could be calculated from the distribution of Hg between inside and outside of the dialysis bag. KDOM is defined by

\[ K_{\text{DOM}}' = \frac{[\text{HgDOM}]}{[\text{Hg}][\text{DOM}]} \] (2)

where square brackets denote concentrations of HgDOM and Hg in molar (M). The equilibrium concentration of DOM is denoted by round brackets and is equivalent to the measured concentration of DOM (in kg L⁻¹) under the condition that the concentration of Hg is much smaller than the concentration of DOM. KDOM 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 binding sites). KDOM was calculated from experimental data, taking into account some leakage of DOM from the inside to the outside compartment, following the equation (29)

\[ K_{\text{DOM}}' = \frac{Q_{\text{Hg}}}{(\text{DOM})_{\text{in}} - (\text{DOM})_{\text{out}}(Q + 1)} \] (3)

where (DOM)in and (DOM)out are the concentrations (kg L⁻¹) of DOM in the inner and outer analysis compartment (DOM in was always more than 10 times greater than DOM out). Q is a measure for the concentration of Hg in the inner and the outer compartment ([Hg]in and [Hg]out) and is defined by

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

where αHg is a measure of complexation of Hg by the auxiliary ligand L and OH⁻. Under the condition of [Hg]total ≈ [L]total, αHg can be written as

\[ \alpha_{\text{Hg}} = 1 + \sum_{j=1}^{n} \beta_{\text{Hg}}^{-1} [L]^j + \sum_{j=1}^{m} \beta_{\text{OH}}^{-1} [H]^j \]

where \( \beta_{\text{Hg}}^{-1} \) and \( \beta_{\text{OH}}^{-1} \) are the overall stability constants for the stepwise binding of L to Hg and H (protons) to L, respectively. \( \beta_{\text{OH}}^{-1} \) is the overall hydrolysis constant referring to the formation of Hg(OH)₂, from Hg + H₂O. A detailed derivation of the calculation of KDOM is given in Glaus et al. (29).

The EDLE method requires an auxiliary ligand suitable to compete with DOM so that meaningful Hg distribution ratios (Q = [HgDOM]/[HgL]; see eq 4) are reached. In this study, the acceptable range for Q was set to >0.1 (reliably measurable Hg binding to DOM) and <4.0 (approximate diffusion limit of the system after 48 h; see initial slope of curves in Figure 1, parts A and B). Additionally, the precautions must be followed: (1) the ligand should not interfere with Hg analysis; (2) the possibility of mixed-complex formation (DOM—Hg—L) (see Glaus et al. (30)) should be avoided; (3) the ligand should be usable at pH values relevant for most freshwaters (pH 6–8); and (4) reliable binding constants for Hg-L interactions should be available from the literature. Table 2 summarizes the suitability of different ligands for EDLE experiments with Hg and shows that EDTA is the only ligand fulfilling all the conditions for low Hg/DOM ratios. The disadvantage of EDTA is that it is only strong enough to effectively compete with Hg with low concentrations of DOM (about 1 mg L⁻¹ for the F1HPoA isolate used in this study). For EDLE experiments at high Hg/DOM ratios, hydroxide was found to be a suitable ligand. Relevant reactions of these ligands and binding constants for these reactions are summarized in Table 3.
auxiliary ligand of choice (EDTA, molecular weight 292 Da) was much too slow. Experiments checking the membrane diffusion of EDTA and other small organic acids with different amounts of negative charge at pH 7 (acetate, oxalate, citrate) revealed that the diffusion of these small molecules across CE membranes decreased with increasing negative charge, explaining the very slow diffusion of EDTA (mainly EDTAH⁺: at pH 7) and EDTAHg²⁻. Finally, regenerated cellulose “Biotech” membranes (Spectra/Por RCBT, MWCO 3500 Da) were tested. These membranes are free from sulfides and allowed for fast diffusion of Hg–EDTA complexes across the membrane (Figure 1, parts A and B). However, the lowest available molecular weight cutoff is 3500 Da, which raised concern about the ability of these membranes to sufficiently retain DOM. Figure 1 shows that the Hg partitioning equilibrium in an EDLE experiment reached a plateau (Figure 1B) well before the concentration of DOM in the outside compartment reached 10% of the inside concentration (Figure 1C). A comparison of Figure 1, parts A and B, also indicates that the presence of DOM in the inside compartment did not slow the establishment of diffusion equilibrium. This demonstrates that the rate-limiting step in the system was the diffusion across the dialysis membrane and not Hg–DOM complex formation. As shown by Glaus et al. (29), the EDLE method generally allows for reliable measurements of $K_{\text{DOM}}$ under such conditions. Therefore, RCBT 3500 membranes were chosen for the experiments, although these membranes are very fragile and break more easily than RC or CE membranes.

**Experimental Setup.** The experimental system for measuring binding constants consisted of duplicate or 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. To separate the inside solutions, RCBT 3500 dialysis bags (Spectra/Por regenerated cellulose “Biotech” membranes, MWCO 3500 Da), sealed by tying knots on both sides, were used. The pH value in both compartments was buffered using 5 mM phosphate buffer (pH 7), 5 mM acetate buffer (pH 5), or a 10 mM mixture of both buffers (pH 6), and ionic strength was set at 0.1 M with NaClO₄, taking into account ionic strength effects of EDTA and the buffer. Inside solutions additionally contained 1.0 mg L⁻¹ of DOM (F1HPoA isolate) at the start of the experiments. Because of leakage of DOM through the membrane, inside DOM concentrations at the end of the experiments were approximately 0.5 mg L⁻¹. Parameters that were varied (by order of magnitude steps) were total concentration of Hg (0–1000 mg L⁻¹) and concentration of the ligand (EDTA: 10⁻¹⁰–10⁻⁷ M; hydroxide: 10⁻⁹–10⁻⁷ M). At the start of the experiments, 10 or 100 µL of a Hg stock solution (0.01–1000 mg L⁻¹ in 10% HNO₃) was added to the outside compartment, and then samples were rotated end-over-end at 22 ± 2 °C. After 48 h, aliquots were taken from the inside and outside compartments and analyzed for Hg. The pH value was measured in the outside solution. 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 were extrapolated from parallel partitioning experiments using an initial inside concentration of 100 mg L⁻¹ DOM (see Figure 1C). This approach gave final DOM concentrations (after 48 h) for the EDLE experiments of approximately 0.5 mg L⁻¹ (inside compartment; 7 mL) and 0.04 mg L⁻¹ (outside compartment; 93 mL).

**Results and Discussion.** To better understand the partitioning of Hg in the experimental system, we first consider a hypothetical EDLE experiment that uses an “idealized” form of DOM having a single type of Hg-binding site at a concentration in excess of 100 mg L⁻¹. The idealized form of DOM contains a single type of Hg-binding site at a concentration in excess of 100 mg L⁻¹, which is considered to be a high DOM concentration. This approach gives a final DOM concentration of approximately 0.5 mg L⁻¹ (inside compartment; 7 mL) and 0.04 mg L⁻¹ (outside compartment; 93 mL).

**TABLE 2. Suitability of Different Auxiliary Ligands for EDLE Experiments**

<table>
<thead>
<tr>
<th>low Hg: DOM</th>
<th>high Hg/DOM</th>
<th>bronide</th>
<th>iodide</th>
<th>cysteine</th>
<th>EDTA</th>
<th>hydroxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>strong Hg binding</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>0+</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Hg recovery</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>no DOM–Hg = L</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>suitable for pH 6–8</td>
<td>–c</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>reliable log K (Hg–L)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

*a (++) = good, (0) = fair, (? = bad, (? = unknown. *b Chelate formation with DOM is regarded more likely than formation of DOM–Hg–OH. *c HgBr₂ is only competitive with HgDOM at low pH, where H⁺ competition decreases Hg–DOM binding but does not affect Hg–Br binding. *d Based on number of literature values, agreement between data, and IUPAC recommendation.

**TABLE 3. Summary of Binding Constants Used for the Calculation of $K_{\text{DOM}}$**

<table>
<thead>
<tr>
<th>reaction</th>
<th>log $\beta$</th>
<th>ionic strength, temperature</th>
<th>ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hg²⁺ + EDTA⁻ = HgEDTA²⁻</td>
<td>21.8⁰</td>
<td>0.1 M, 20 °C</td>
<td>(41)</td>
</tr>
<tr>
<td>HgEDTA²⁻ + H⁺ = HgEDTAH⁺</td>
<td>3.1⁻⁻⁻</td>
<td>0.1 M, 20 °C</td>
<td>(41)</td>
</tr>
<tr>
<td>EDTA⁻ + H⁺ = EDTAH⁺</td>
<td>10.2²⁻⁻⁻</td>
<td>0.1 M, 20 °C</td>
<td>(41)</td>
</tr>
<tr>
<td>EDTA⁻ + 2H⁺ = EDTAH⁺²⁻⁻⁻</td>
<td>16.4⁻⁻⁻</td>
<td>0.1 M, 20 °C</td>
<td>(41)</td>
</tr>
<tr>
<td>EDTA⁻ + 3H⁺ = EDTAH⁺³⁻⁻⁻</td>
<td>19.1⁻⁻⁻</td>
<td>0.1 M, 20 °C</td>
<td>(41)</td>
</tr>
<tr>
<td>EDTA⁻ + 4H⁺ = EDTAH⁺⁴⁻⁻⁻</td>
<td>21.2⁻⁻⁻</td>
<td>0.1 M, 20 °C</td>
<td>(41)</td>
</tr>
<tr>
<td>Hg²⁺ + OH⁻ = HgOH</td>
<td>10.2⁻⁻⁻</td>
<td>0.1 M, 25 °C</td>
<td>(42)</td>
</tr>
<tr>
<td>Hg²⁺ + 2OH⁻ = Hg(OH)₂</td>
<td>21.2⁻⁻⁻</td>
<td>0.1 M, 25 °C</td>
<td>(42)</td>
</tr>
</tbody>
</table>

*a Overall binding constants. *b Corrections for the experimental temperature of 22 °C using the van’t Hoff equation are not significant. *c IUPAC tentative recommendation (19). *d IUPAC recommendation (19).
of [Hg], and an auxiliary ligand that forms 1:1 complexes with Hg (Figure 2). In such a system, almost all Hg is bound to L at very high concentrations of L so that the distribution ratio Q between Hg bound to DOM and Hg bound to the auxiliary ligand L (Q = [HgDOM]/[HgL]) is indistinguishable from zero (i.e., [HgL] ≫ [HgDOM]). Within a certain range of [L], Q is inversely proportional to the concentration of the ligand (i.e., order of magnitude decreases in [L] cause order of magnitude increases in Q). At low [L], the auxiliary ligand is much less effective in binding Hg than DOM, resulting in distribution ratios limited to a constant value because the amount of Hg diffusing from the outside to the inside compartment during the experimental time is limited. The total amount of Hg added to the system does not affect the distribution ratio Q. This is explained by the fact that, over the entire range of Hg concentrations, Hg is competitively bound to both the auxiliary ligand L and the Hg binding sites on the DOM (assuming that [L] and [sites] are always ≫ [Hg]).

The result of a real EDLE experiment using EDTA as the auxiliary ligand is shown in Figure 3. A nearly “ideal” behavior of the system, as outlined in Figure 2, was only observed for total Hg concentrations ≤ 0.1 μg L⁻¹. At a Hg concentration of 1 μg L⁻¹, order of magnitude decreases in [EDTA] caused only small increases in DOM/ligand distribution ratios. At 10 μg L⁻¹ of Hg, distribution ratios were only slightly above zero. At Hg concentrations ≥ 100 μg L⁻¹, binding of Hg to DOM was no longer detectable by the EDLE method.

To actually quantify the binding of Hg to weak DOM sites, an additional EDLE experiment was carried out. This experiment had to use high concentrations of Hg to make sure that the binding of small amounts of Hg to strong sites did not significantly affect the result. Furthermore, a weak auxiliary ligand suitable to achieve competitive binding of Hg to weak DOM sites had to be used. Figure 5 shows the
binding of 100 μg L⁻¹ of Hg to DOM in the presence of hydroxide as the auxiliary ligand. Competitive binding of Hg to DOM and OH⁻ under these conditions suggests interactions of Hg with weak DOM sites that are present at high concentrations.

To obtain a comprehensive overview of the results, conditional distribution coefficients $K_{DOM}^\prime$ were calculated (eq 3, Table 3) from distribution ratios observed in the EDLE experiments and plotted against the concentration ratio of Hg to DOM (Figure 6). The overall uncertainty of the calculated $K_{DOM}^\prime$ values including statistical errors (i.e., replicate reproducibility) and systematic errors (mainly uncertainty of stability constants for complex equilibria of EDTA with Hg²⁺ and H⁺) was estimated to be 1.0 log units. For an extended discussion of statistical and systematic errors in EDLE experiments, see Glaus et al. (29, 31). Figure 6 shows very strong binding of Hg to DOM at Hg/DOM concentration ratios below approximately 1 μg of Hg/mg of DOM. At higher concentration ratios, we observed a sharp drop in Hg–DOM binding affinity, followed by relatively constant low values for $K_{DOM}^\prime$ at Hg/DOM concentration ratios above approximately 10 μg of Hg/mg of DOM. These data thus confirm our hypothesis of a pronounced effect of the Hg to DOM concentration ratio on the strength of the interactions between Hg and DOM.

Our second hypothesis was that reduced sulfur ($S_{red}$) groups are the dominant Hg binding sites at low Hg/DOM concentration ratios and that Hg is mostly bound to carboxyls at high ratios of Hg to DOM. To verify this hypothesis, we first compared the EDLE-derived concentration of strong Hg binding sites on the DOM to the concentration of $S_{red}$ determined by X-ray absorption near edge structure (XANES) spectroscopy. The concentration of strong DOM ligands was estimated from Figure 6, which shows a constant $K_{DOM}^\prime$ value of approximately $10^{2.32}$ L kg⁻¹ at low Hg/DOM ratios up to a concentration ratio of approximately 1 μg of Hg/mg of DOM. At higher Hg concentrations, a decrease in $K_{DOM}^\prime$ indicates the saturation of strong sites. Therefore estimated the concentration of strong DOM sites to be equal to the molar concentration equivalent of 1 μg of Hg/mg of DOM (i.e., $5 \times 10^{-9}$ mol of Hg/mg of DOM). The concentration of $S_{red}$, calculated from the total sulfur content of 1.73% and the fraction of $S_{red}$ (60.0%) for the DOM isolate (Table 1) at a concentration of 1 mg L⁻¹ was $3.2 \times 10^{-7}$ M. Thus, the molar concentration of $S_{red}$ determined by XANES was 64 times the molar concentration of strong Hg-binding sites derived from EDLE experiments, suggesting that only a small fraction (1.6%) of $S_{red}$ was involved in the strongest interactions between Hg and DOM. This finding agrees well with data reported recently by Amirbahman et al. (32) who, using an equilibrium dialysis method, estimated that only approximately 2% of $S_{red}$ in Suwannee River humic acid took part in strong interactions with methylmercury.

Studies using extended X-ray absorption fine structure (EXAFS) spectroscopy found, however, that interactions between Hg and humic acids included a significant amount of Hg–$S_{red}$ bonds, even at low molar $S_{red}$/Hg ratios of 0.3–4.0 (15, 16). The difference between results from studies measuring strong mercury–DOM interactions (ref 32 and this study) and results from EXAFS spectroscopy may be explained by taking into account that these two methods measure different types of Hg–$S_{red}$ interactions. EXAFS spectroscopy detects sulfur atoms in the vicinity of Hg atoms, without taking into account the binding strength between Hg and $S_{red}$. In contrast, the EDLE method measures Hg–DOM binding in the presence of a competitive ligand and, therefore, only detects Hg interactions with binding sites having the strongest affinity to mercury (probably thiol groups). A comparison of stability constants for Hg binding to thiols (RSH) versus sulfides (RSR) (e.g., cysteine vs methylcysteine or methionine) shows, for example, that constants for mercury–thiol interactions are always many orders of magnitude higher than constants for Hg interactions with similar molecules in which the thiol group is methylated (19). Thus, it is likely that under the competitive conditions of the EDLE experiments, Hg is bound only to a small fraction of the total $S_{red}$ (e.g., thiol groups which are freely accessible), whereas under the conditions of the EXAFS measurements Hg additionally interacted with $S_{red}$ groups that had a much lower affinity for Hg (e.g., sulfide, polysulfide, thiophene).

To further evaluate the hypothesis that thiol groups were the primary strong binding sites for Hg, we compared Hg–DOM binding constants measured at low ratios of Hg/DOM to literature values for binding constants describing Hg binding to simple thiol ligands. Because literature values for binding constants generally refer to the binding of a metal ion to a deprotonated ligand (33), the conditional distribution ratio $K_{DOM}^\prime$ calculated from EDLE experiments had to be converted to binding constants describing Hg²⁺ binding to deprotonated DOM–thiol ligands (Hg²⁺ + RS⁻ = HgRS⁻). This conversion required assumptions about the concentration and protonation constants of DOM–thiol ligands in order to be able to apply the concentration of unprotonated thiol groups instead of the concentration of DOM in eq 3. Using the concentration for strong Hg binding sites found in the
EDLE experiments (5 × 10⁻⁸ mol/mg of DOM; see the previous discussion) and an estimated average value of 10¹⁰ for the protonation constant of an organic thiol group (19), the calculated mean value for conditional binding constants of Hg to deprotonated thiol groups in DOM (K′ = [HgRS⁻]⁻([Hg⁺] × [RS⁻])⁻¹) was 10²⁸.⁵ (at I = 0.1 M). The overall uncertainty for K′ is estimated to be approximately ±2 log units, which is higher than the uncertainty of approximately ±1 log unit for KDOM values, because of additional errors involved in estimating the concentration and an average protonation constant for thiol groups in DOM. The value of 10²⁸.⁵ for K′ is clearly higher than typical stability constants for Hg interactions with oxygen- or nitrogen-containing ligands (19). Binding constants reported in the literature for Hg binding to thiol ligands are sometimes inconsistent, with values describing the interaction of Hg with the same ligand sometimes spanning several orders of magnitude (19). However, most of the values are very high (>10⁹), emphasizing strong interactions between Hg and thiol ligands, as expected for interactions between a soft metal and a soft ligand according to the hard and soft acids and bases theory (22, 23). For example, binding constants of 10⁵⁴, 10⁵⁷, 10⁹⁵, and 10⁹⁷.⁵ were reported for 1:1 binding of Hg to thiosalicylic acid, 2,3-dimercaptopropanol, thioglycolic acid, and cysteine (17, 34, 35). A comparison of these data to the stability constant of 10³⁶, we determined for Hg binding to DOM (assuming that strong DOM ligands were thiolates) indicates that organic thiols in the DOM probably were the primary binding sites at low ratios of Hg to DOM.

To our knowledge, only one study has determined constants for Hg binding to organic matter at low Hg-to-organic matter ratios (24). Using soil organic matter (SOM), the authors found Hg–thiol stability constants on the order of 10³⁵. The constants measured in their study may have somewhat overestimated the strength of natural Hg–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 Br), thus increasing the observed stability constants for Hg–SOM interactions (30). Nevertheless, the magnitude of binding constants reported in this study is in agreement with our data, indicating that Hg binding to organic matter at low ratios of Hg to organic matter is indeed controlled by strong interactions with reactive organic thiol groups.

For high Hg-to-DOM concentration ratios, as used in most studies measuring Hg–DOM binding constants (e.g., refs 10 and 14), we had hypothesized that organic Sred groups are saturated by a small fraction of the total Hg and that carboxyl functional groups are the primary binding sites for Hg. The maximum Hg/DOM concentration ratio of approximately 0.02 to 2 mol of Hg/mg of DOM (12 × 10⁻⁶ mol of Hg/mg of DOM) reached in the EDLE experiment using hydroxide as the auxiliary ligand (Figure 5) shows that, under these conditions, Hg was bound to DOM ligands much more abundant than Sred. This suggests that carboxyls (5.45 × 10⁻⁶ mol of DOM, see Table 1) were the main binding sites for Hg under these conditions. To further evaluate this assumption, binding constants for Hg interactions with weak DOM ligands were compared to literature values for mercury–carboxyl binding constants. For this purpose, KDOM values for interactions between Hg and weak DOM sites were converted to binding constants referring to the binding of Hg⁰ to deprotonated carboxyl groups (see the previous discussion for thiol). Using a carboxyl concentration of 5.45 × 10⁻⁶ mol/mg of DOM (Table 1) and a mean protonation constant of 10⁻¹⁰ for DOM–carboxyls (36) (although this assumption clearly oversimplifies the real protonation behavior of DOM), the mean KDOM value for Hg binding to weak sites (10⁻⁹ L · kg⁻¹) was converted to an average conditional binding constant (K′ = [HgRCOO⁻]⁻([Hg⁺] × [RCOO⁻])) of approximately 10¹⁰. This value is within the range of Hg binding constants for simple organic ligands containing carboxyl groups (e.g., oxalate: 10⁹.⁷, citrate: 10⁹.⁵, salicylate: 10¹⁰.⁷) (19), thus giving further indication that the major Hg–binding ligands at high ratios of Hg to DOM were indeed carboxyl groups.

Thus, our estimations for concentrations and binding strengths of strong and weak Hg–binding DOM ligands were consistent with the hypothesis that organic thiol groups are the primary binding sites for Hg at low ratios of Hg to DOM and that Hg is mainly bound to carboxyls at high Hg/DOM ratios. We also confirmed the hypothesis that Hg–DOM binding constants determined at low ratios of Hg to DOM are much higher than literature values (e.g., refs 10 and 12–14). The concentration ratio of Hg/DOM in most natural systems is even lower than the lowest ratios used in this study. Assuming concentrations of dissolved Hg in natural waters between approximately 1 and 10 ng L⁻¹ (37), a DOM concentration range of 1–100 mg L⁻¹ (38) and 3 × 10⁻⁶ mol of Sred/mg of DOM (see the previous discussion), we can estimate that concentration ratios for Hg to DOM approximately range from 0.01 to 10 ng of Hg/mg of DOM. The corresponding molar Hg/Sred ratios range from 2 × 10⁻⁷ to 2 × 10⁻⁴ mol of Hg/mol of Sred. Therefore, it can be expected that only the strongest DOM binding sites will interact with Hg under natural conditions. Theoretically, it is possible that binding constants for Hg–DOM interactions at very low Hg/DOM ratios are somewhat higher than the values reported in this study, but the fact that KDOM values were constant over a range of Hg/DOM ratios from approximately 0.02 to 1 μg of Hg/mg of DOM (Figure 6) suggests that the upper limit of Hg–DOM binding strength was approached under the experimental conditions of the EDLE experiments.

Finally, it should be noted that Hg–DOM distribution coefficients determined in this study are only valid for a certain fraction of DOM from a certain environment under the given experimental conditions. Further EDLE experiments using different DOM fractions from a wide range of environments at variable pH and ionic strength are currently under way to evaluate the effect of environmental variables on Hg–DOM distribution coefficients. Results from such studies should allow for Hg–DOM interactions to be included in comprehensive metal–DOM binding models (e.g., ref 39), which could eventually be used to estimate Hg speciation in natural waters.

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Supporting Information Available
Exact numbers for the data shown in Figure 6 can be found in the Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

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