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Identification of Mercury and Dissolved Organic Matter Complexes Using Ultrahigh Resolution Mass Spectrometry

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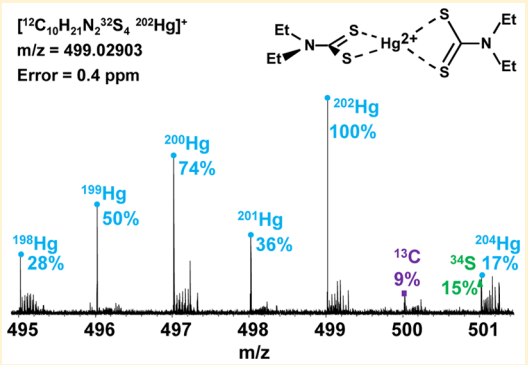
Supporting Information

ABSTRACT: The chemical speciation and bioavailability of mercury (Hg) is markedly influenced by its complexation with naturally dissolved organic matter (DOM) in aquatic environments. To date, however, analytical methodologies capable of identifying such complexes are scarce. Here, we utilize ultrahigh resolution Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) coupled with electrospray ionization to identify individual Hg−DOM complexes. The measurements were performed by direct infusion of DOM in a 1:1 methanol:water solution at a Hg to dissolved organic carbon (DOC) molar ratio of 3×10^{-4}. Heteroatomic molecules, especially those containing multiple S and N atoms, were found to be among the most important in forming strong complexes with Hg. Major Hg−DOM complexes of $C_{10}H_{21}N_2S_4Hg^+$ and $C_8H_{17}N_2S_4Hg^+$ were identified based on both the exact molecular mass and patterns of Hg stable isotope distributions detected by FTICR-MS. Density functional theory was used to predict the solution-phase structures of candidate molecules. These findings represent the first step to unambiguously identify specific DOM molecules in Hg binding, although future studies are warranted to further optimize and validate the methodology so as to explore detailed molecular compositions and structures of Hg−DOM complexes that affect biological uptake and transformation of Hg in the environment.



INTRODUCTION

Dissolved organic matter (DOM) is ubiquitous and represents one of the dominant biochemical compounds affecting mercury (Hg) reduction, oxidation, and species transformation in natural aquatic environments.[1−7] DOM plays key roles in Hg complexation due to its exceptionally strong binding affinities with Hg via reduced sulfur or thiol functional groups.[2,3,8−12] The formation of Hg−DOM complexes influences Hg bioavailability for uptake and production of neurotoxic methylmercury (MeHg) in the environment.[13−15] Knowing exact molecular structures of DOM that form complexes with Hg is thus critical in elucidating factors that may control microbial Hg uptake and methylation. To date, analytical methods capable of identifying such complexes are scarce because DOM is highly complex and contains tens of thousands of molecules of unknown structure and composition.[16,17] Although techniques such as Fourier transform infrared (FTIR) and X-ray absorption spectroscopy (XAS) have yielded significant advances in understanding DOM and revealing the strong complexation of reduced −S and O/N groups with Hg,[11,18−20] they are fundamentally limited because they only provide information about major functional groups or bulk properties of DOM.[17] Under the current analytical paradigm, electrospray ionization (ESI) Fourier transform ion cyclotron resonance−mass spectrometry (FTICR-MS) is the most promising technique to give exact elemental compositions or chemical formulas for individual DOM molecules based on precise mass measurements.[21−25] ESI employs a low energy for analyte ionization, allowing the observation of m/z values corresponding to molecular ions with negligible fragmentation. This technique has been widely used to characterize DOM in water and soils,[21−25] but no study has been carried out to examine the formation of Hg−DOM complexes.

Previous studies have reported the use of ESI with lower-resolution MS to investigate interactions between Hg and simple peptides such as cysteine and glutathione.[26−31] It was demonstrated that ESI-MS can detect Hg-containing mass signals directly.[29,31] Coupled with distinct Hg isotope distribution patterns, characteristic mass peaks of Hg−amino acid or Hg−peptide complexes were identified, mostly under

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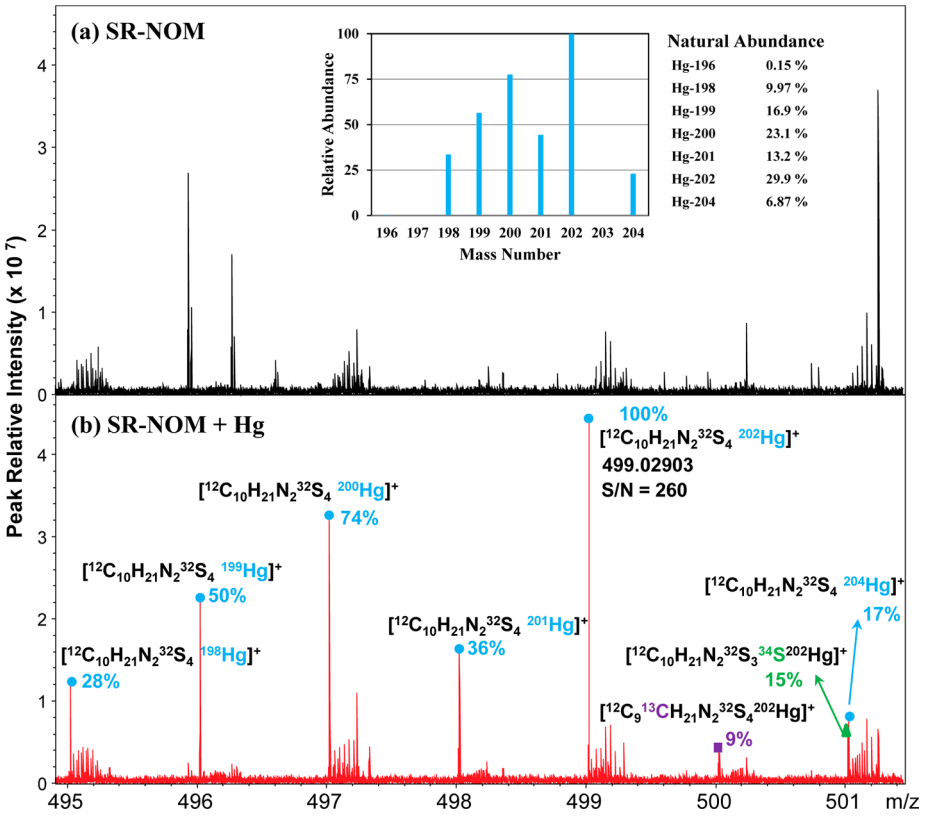

Figure 1. FTICR-MS analyses of SR-NOM by positive mode ESI: (a) before and (b) after addition of Hg (added as $HgCl_2$ at 0.5 μM). The molecular formula $C_{10}H_{20}N_2S_4Hg$ was identified by matching the top six Hg isotopic peaks (^{202}Hg, ^{200}Hg, ^{199}Hg, ^{201}Hg, ^{198}Hg, and ^{204}Hg) and their relative intensities to the natural abundances of Hg in the environment (see inset of panel (a)). The corresponding ^{13}C and ^{34}S isotopic peaks to the dominant $[^{12}C_{10}H_{21}N_2{}^{32}S_4{}^{202}Hg]^+$ peak were also identified and are shown in purple and green, respectively. All labeled peaks in panel (b) are absent in the original SR-NOM spectrum (a).

positive ionization mode.[32,33] The present study was undertaken to explore the feasibility of using FTICR-MS to directly measure and identify specific Hg−DOM complexes in heterogeneous DOM samples. Stable Hg isotope distribution patterns were used to confirm the Hg−DOM mass peaks and their assigned molecular formulas with a resolution of 400,000 (at m/z 400) and an accuracy of <1 ppm.

MATERIALS AND METHODS

Suwanee River natural organic matter (SR-NOM) was obtained from the International Humic Substances Society and stored in a desiccator until use. An additional DOM sample was isolated from East Fork Poplar Creek (EFPC-DOM) in Oak Ridge, Tennessee, using a solid-phase extraction procedure, as described previously.[34] Cysteinylglycine (Cys-Gly) was purchased from Sigma-Aldrich and used as received.

FTICR-MS spectra were collected on a 15T FTICR-MS (Bruker SolariX) outfitted with an ESI interface. Both negative and positive mode data were acquired, in which either negative or positive ions are generated by manipulating the voltage differences during the electrospray process.[35,36] The ESI negative mode favors the detection of molecules with acidic functional groups that deprotonate, whereas positive mode favors basic functional groups such as amines.[36,37] DOM samples were dissolved in high-purity Milli-Q water and filtered through a 0.2-μm filter before use. Hg−DOM complexes were prepared by mixing Hg (0.5 μM, as $HgCl_2$ in water) and DOM at least 24 h prior to MS analysis. The pH of the DOM solution was ~4, similar to the Suwanee River water pH. To avoid introducing additional salts to the sample, no pH adjustments were made. All DOM and Hg−DOM samples were diluted with methanol (1:1 v:v) to a final concentration of 1.67 mM DOC, or a Hg:DOC molar ratio of 3×10^{-4}, so Hg is expected to be mostly complexed with the thiol functional groups in DOM.[2,9,11,12,38] A relatively high Hg:DOC ratio was used here to facilitate the MS detection of Hg−DOM complexes. Additional samples were prepared by adding a model thiol, cysteinylglycine (5 μM) as a competing ligand, to the Hg−DOM solution so that potential formation of Hg-adducts versus true Hg−DOM complexes could be determined. All samples were directly infused using a Hamilton syringe at a flow rate of 2 μL/min. The electrospray voltages were optimized, and the ion current was kept constant for all samples during MS data acquisition.[16,22,25] The ion accumulation time was 0.1 s, with 300 scan averages coadded. The time-of-flight was 0.65 ms, and Q1 was 100 m/z. The instrument was externally calibrated with Agilent ESI-L low concentration tune mix (Agilent Technologies) prior to sample analysis, and the syringes and transfer lines were flushed with 50/50 methanol/water (v/v) between samples.

FTICR-MS spectra were calibrated using a homologous series of organic acids separated by 14 Da CH_2 building blocks in the samples.[24] Molecular formulas were assigned for peaks with a signal-to-noise (S/N) ratio >7 and m/z between 200 and 800, unless otherwise specified, by allowing a mass error of 1.0 ppm between the measured and theoretically calculated mass. Molecular Formula Calculator (v.1.0 ©NHMFL) developed at the National High Magnetic Field Laboratory in Tallahassee,

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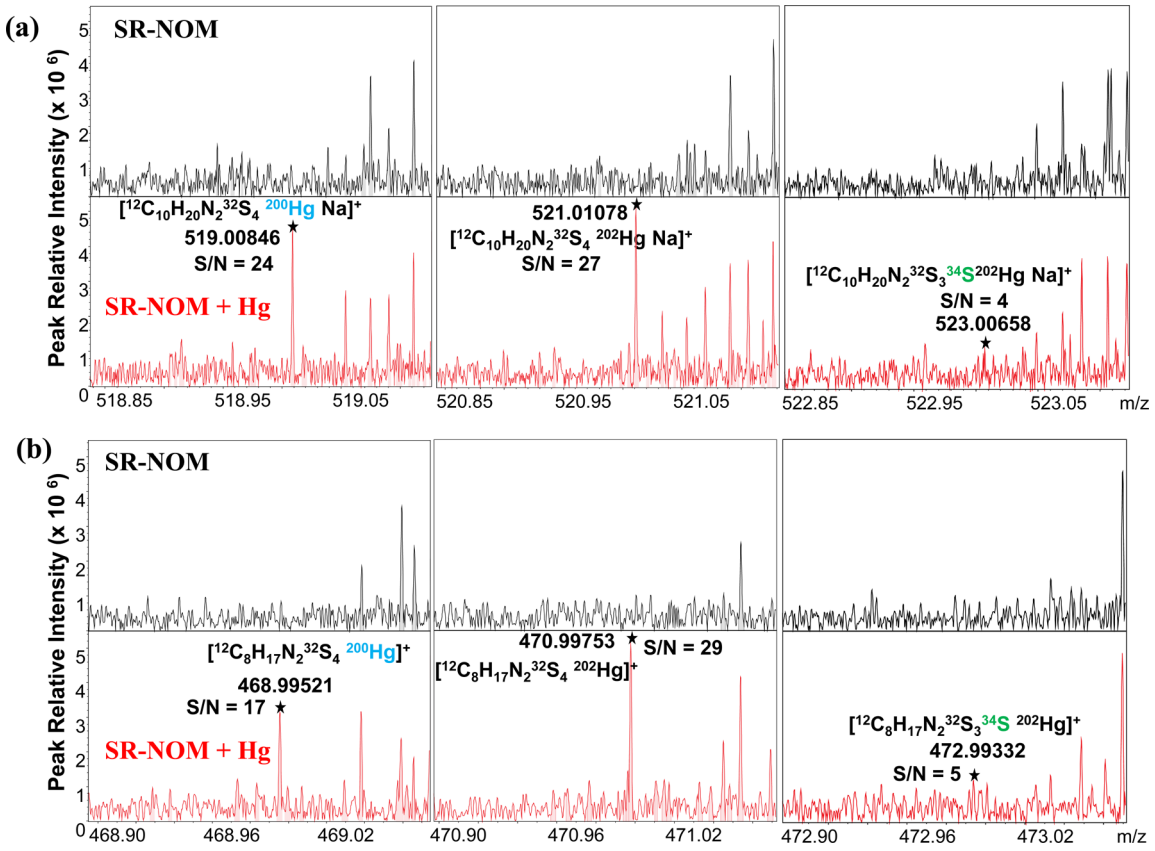

Figure 2. Identification of Hg−DOM formulas by positive mode ESI-FTICR-MS. (a) Na^+ adduct of $C_{10}H_{20}N_2S_4Hg$ and (b) $C_8H_{16}N_2S_4Hg$ in Hg-spiked SR-NOM sample. All the labeled peaks are absent in the original SR-NOM sample [Top panels in (a) and (b)]. See Figure 1 for additional details.

Florida, was used to calculate putative formulas with elemental compositions of C, H, O, N, S, P, Hg, Cl, and Na. MATLAB codes were used to assist with data analysis, as described elsewhere.[39] Isotopic patterns and ratios of the top two most abundant isotopes of ^{12}C vs ^{13}C, ^{32}S vs ^{34}S, ^{202}Hg vs ^{200}Hg, and ^{35}Cl vs ^{37}Cl were used to identify the correct formulas (Supporting Information, Figure S1) when multiple possible molecular formulas were observed for a given mass peak. Because most peaks are singly charged (i.e., $z = 1$), the corresponding isotopically matched peaks can be identified by the mass difference between isotopes of a given element with a detection limit >0.0005 at $m/z \sim 500$.[16,22,25] The ratio of peak intensities between identified isotopes should match the ratio of the natural abundances of the corresponding isotopes of the element and can thus be used to unambiguously identify the correct molecular formulas.[40]

RESULTS AND DISCUSSION

The whole spectra of SR-NOM before and after Hg addition exhibited similar mass profiles (Figure S2), indicating that the presence of low concentrations of Hg (0.5 μM) did not cause significant ion suppression during FTICR-MS analysis. However, the appearance or disappearance of some peaks were noted due to Hg−DOM complexation as well as possible formation of Hg and Cl adducts following $HgCl_2$ addition, as commonly observed in FTICR-MS analysis.[41] Approximately 5500 mass peaks were identified in negative mode and 7000 in positive mode with S/N ratios >7 across a m/z range of 200−800. The spectra are dominated by singly charged peaks, as evidenced by one Thomson difference (1.00335) between the monoisotopic ^{12}C peaks and isotope peaks containing one ^{13}C atom that are typically observed for DOM samples.[21−24] The molecular composition of SR-NOM is also typical of that found in terrestrial and aquatic DOM, with C, H, and O formulas among the most abundant and atomic O:C and H:C ratios between 0 and 1.2 and between 0.3 and 2.5, respectively (Figure S3).[21−24]

We next searched for Hg-containing formulas in SR-NOM with and without added Hg. No Hg-containing formulas were identified either in the initial DOM (without Hg addition) or in the negative-mode spectra (with Hg addition) since DOM could be positively charged when complexed with Hg^{2+}. Three Hg-containing formulas ($C_{10}H_{21}N_2S_4Hg^+$, $C_8H_{17}N_2S_4Hg^+$, and $C_{10}H_{20}N_2S_4HgNa^+$) were identified in the Hg−SR-NOM sample in positive mode (Figures 1 and 2). The formula $C_{10}H_{21}N_2S_4Hg^+$ was the most abundant (S/N = 259), with $C_{10}H_{20}N_2S_4HgNa^+$ as an adduct of $C_{10}H_{21}N_2S_4Hg^+$ that is formed from the same neutral molecule $C_{10}H_{20}N_2S_4Hg$, but with a Na^+ counterion instead of H^+. This phenomenon is common in positive-mode ESI because organic molecules can either be protonated or bind positively charged Na^+ or K^+ ions during ESI.[16,42,43] However, the observed $C_{10}H_{20}N_2S_4Hg$ complex is not an adduct, as confirmed by repeated analyses of Hg−DOM complexes in the presence of a competing thiol ligand, cysteinylglycine, in which both the Hg−cysteinylglycine and $C_{10}H_{20}N_2S_4Hg$ complexes were identified (Figure S4). Additionally, the $C_{10}H_{20}N_2S_4Hg$ complex was identified in multiple MS runs and in replicate SR-NOM samples added with Hg (Figure S5). These results indicate fairly good

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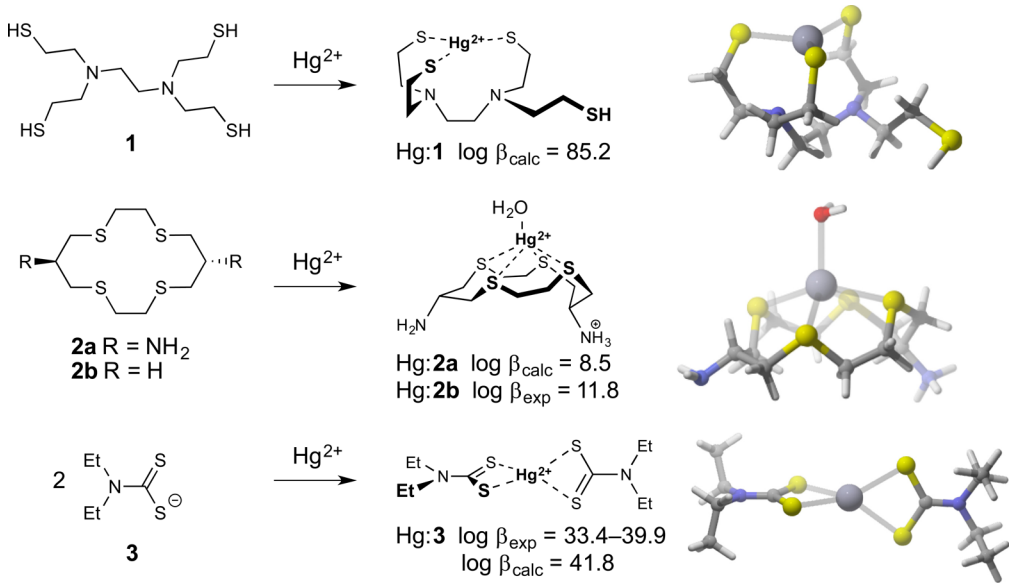

Figure 3. Putative molecular structures for the identified Hg−DOM formula $C_{10}H_{20}N_2S_4Hg$ and corresponding solution-phase geometries and stability constants computed with DFT.

reproducibility, but we emphasize that ESI-FTICR-MS is not a quantitative technique due to variations in ionization efficiency among analytes. Significant changes in peak magnitude or S/N ratios (±30%) are common,[41] especially when samples are prepared or run at different times or on different days.

Unambiguous identification of the three Hg-containing formulas was achieved by strictly matching the exact molecular mass and by comparing the distributions and relative intensities of the ^{200}Hg and ^{202}Hg isotopes with their natural abundances. Here, we assumed that all Hg isotopes react similarly with a given organic molecule such that the resulting Hg−organic complexes differ in molecular formula according to the mass difference between ^{202}Hg and ^{200}Hg. Additionally, the relative peak intensity of the identified formulas should match the natural abundance of the given Hg isotope. For example, the exact mass difference between the isotopes ^{202}Hg and ^{200}Hg is 2.00232, and their natural abundance ratio is 0.77:1 (^{200}Hg:^{202}Hg), which matched well with a peak intensity ratio of 0.74:1 for the identified $C_{10}H_{21}N_2S_4Hg^+$ complex (Figure 1b, Table S1). The deviation between theoretically calculated and experimentally determined ratios was <5% (Table S1). The relatively high peak intensity of $C_{10}H_{21}N_2S_4Hg^+$ also allowed us to identify all of the top six most abundant Hg isotopes (^{202}Hg, ^{200}Hg, ^{199}Hg, ^{201}Hg, ^{198}Hg, and ^{204}Hg), and their relative peak intensities matched well with the natural abundances of these isotopes (Table S1). From all the possible molecular formulas that are consistent with a singly charged ion mass of 499.02903, $^{12}C_{10}H_{21}N_2{}^{32}S_4{}^{202}Hg^+$ is the only formula that matches the pattern to within 1 ppm mass error. The ^{13}C and ^{34}S isotopic peaks corresponding to the dominant $^{12}C_{10}H_{21}N_2{}^{32}S_4{}^{202}Hg^+$ peak were also identified for this compound (Figure 1b), further supporting unambiguous identification of the $C_{10}H_{21}N_2S_4Hg^+$ complex.

Using the same approach, we identified $C_{10}H_{20}N_2S_4HgNa^+$ and $C_8H_{17}N_2S_4Hg^+$ in the SR-NOM sample (Figure 2). $C_8H_{17}N_2S_4Hg^+$ differs from $C_{10}H_{21}N_2S_4Hg^+$ by losing two CH_2 moieties, the most abundant building blocks of DOM.[22,24] However, due to the low peak intensities (S/N = 17−29) observed for these two formulas, only the ^{202}Hg and ^{200}Hg peaks were clearly identifiable. The corresponding ^{34}S isotopic peaks are noted in Figure 2 but cannot be assigned with confidence because of their low S/N ratio and low natural abundance of ^{34}S. Additional peaks were not assigned molecular formulas because they are not associated with Hg. We acknowledge that only a small number of Hg-containing formulas were identified in this work. This observation could be attributed to the following: (1) Hg−DOM complexes were identified strictly based on both the exact mass and isotopic patterns of Hg. (2) Hg likely formed complexes with many DOM molecules with low abundances (due to the low Hg concentration and the strong binding affinities of Hg with the limited number of thiol functional groups in DOM) such that these complexes were not detected by FTICR-MS. (3) Hg−DOM complexes may have dissociated during ESI (e.g., those weakly or singly coordinated with S- or N-containing functional groups).[32,44−46] Therefore, only strong Hg−DOM complexes such as $C_{10}H_{21}N_2S_4Hg^+$ were identified; this formula was identified not only in the Hg−SR-NOM or the mixed SR-NOM and cysteinylglycine samples but also in Hg-spiked EFPC−DOM samples (Figure S6). Alternatively, if the isotope patterns and distributions are not followed, more Hg-containing formulas can be identified from their molecular mass (Table S2). These formulas were identified based on the following criteria: (1) They are new mass peaks after Hg addition. (2) Their corresponding DOM molecules are identifiable in the initial DOM before Hg addition. (3) Their ion mass can be calculated as singly charged formulas. As a result, about a dozen more Hg−DOM formulas were identified, with most of them containing O, S, and N (Table S2).

Importantly, we found that the three identified Hg-containing formulas each contain the subformula $[N_2S_4]$, suggesting that $[N_2S_4]$ may be an important molecular component or motif in DOM for complexing Hg. This observation agrees with previous studies demonstrating strong binding affinities (with conditional stability constant, log K > 22) between Hg and reduced −S functional groups but weak affinities (log K ∼ 10−11) with −O functional groups in DOM.[2,3,10,11] We sought to assign a putative identity to the

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Environ. Sci. Technol. Lett. XXXX, XXX, XXX−XXX

common $[N_2S_4]$ subformula by analogy to Hg^{2+} complexes of known compounds. Although there are numerous possible precursor molecules whose complexes are consistent with the formula $C_{10}H_{20}N_2S_4Hg$, three representative candidates (**1**−**3**, Figure 3) were considered in detail. To qualify as a match, a candidate ligand must satisfy the criterion that its putative HgL complex is singly cationic, either as $[C_{10}H_{20}N_2S_4Hg]^0$ or $[C_{10}H_{21}N_2S_4Hg]^+$ through protonation. Only strongly bound complexes are competitive with other binding ligands (e.g., with added cysteinylglycine) or functional groups in DOM and are therefore likely to be detected, if present. The resulting compounds are symmetric, and each displays a unique binding motif based on its number of titratable protons, which may or may not be displaced by Hg^{2+}. Aqueous phase geometries and cumulative formation constants (log β) for Hg^{2+} complexes of **1**−**3** were predicted with density functional theory (DFT) to assess their binding affinities and competitiveness against a strongly binding ligand, cysteine (log β_{calc} = 35.7).[47] Details of the calculations are provided in the Supporting Information.

Tetrathiol **1**, $C_{10}H_{24}N_2S_4$, is a synthetic chelator of Group 7 cations.[48] DFT calculations predict that the putative dominant nonmetalated $[LH_6]^{2+}$ species displaces five protons upon Hg complexation with both amines and three out of four thiols as $[HgLH]^-$ (log β_{calc} = 85.2, Figure 3). This complex is structurally analogous to Hg−EDTA[49] and is consistent with predictions of tricoordinated soil humates.[50,51] However, it was not identified in either positive or negative mode. Furthermore, neither native nor quasimolecular ligand ions, with or without Hg, were detected in either mode. The lack of MS evidence and failure to meet the protonation criterion disqualify tetrathiol **1** as a potential match.

Thioether **2a**, a soluble derivative of the insoluble, Hg-chelating thiacrown macrocycle **2b**, represents a nonreducible −S candidate ligand. In Hg:**2a**, all four sulfurs suspend the singly hydrated cation, structurally and energetically[52] analogous to Hg:**2b**. Despite the Hg−4S binding motif, the major $[HgLH]^{3+}$ species (log β_{calc} = 8.5) is predicted to be noncompetitive against a strong ligand. Thioethers in general have low Hg^{2+} affinities, e.g., log β = 7.2 for Hg−*S*-methylcysteine.[53] Base peaks matching the expected m/z for the $[LH_2]^{2+}$ and $[LH]^+$ species appear in positive mode before and after Hg addition, although their respective isotopic peaks do not. Furthermore, the only base peak found for the Hg:**2a** complex matches $[HgLH_{-1}]^+$, which has lost an amine proton and is unlikely to occur under positive mode, thus precluding its presence in the Hg−DOM sample.

The putative formulas of Hg:**1**, $[C_{10}H_{21}N_2S_4Hg]^-$, and Hg:**2a**, $[C_{10}H_{23}N_2S_4Hg]^{3+}$, bracket the target formula by one proton, indicating that the ligand may contain only two titratable protons, and that one or both are displaced upon Hg^{2+} binding. We therefore considered whether the overall formula might arise from *bis* ligation of $C_5H_{11}NS_2$, for which dithiocarbamic acid (DTCA, pK_a = 3.4)[53] provides an attractive match. The exceptionally strong $Hg(DTC)_2$ complex has been studied extensively with an array of techniques both in solution[54] and in crystalline forms.[55,56] Encouragingly, large experimental and computed equilibrium constants (33.4−39.9)[57] for $Hg(DTC)_2$ indicate strong Hg binding at magnitudes similar to those found in DOM.[8−12] To test whether the precursor $[C_{10}H_{20}N_2S_4Hg]^0$ could arise from a *bis* complex, we sought evidence for the presence of the monomeric DTCA in solution. We observed the matching formula $C_5H_{12}NS_2^+$ for the DTCA cation in positive mode with and without added Hg (Figure S7), thereby supporting the possible presence of this complex in SR-NOM.

Taken together, we demonstrate the potential for unambiguous identification of Hg−DOM molecular formulas within complex natural DOM samples using ESI-FTICR-MS. The putative structure of the identified formula $C_{10}H_{20}N_2S_4Hg$ was rationalized and proposed to be likely associated with *bis* ligation of DTCA, although experimental validation with techniques such as nuclear magnetic resonance spectroscopy and fragmentation MS/MS spectrometry are warranted. Our finding of the heteroatomic $[N_2S_4]$ subformula in Hg−DOM complexes is consistent with the view that reduced −S molecules in DOM are the most reactive with Hg. Recognizing the limitations of ESI-FTICR-MS (e.g., nonquantitative and potential formation of salt adducts),[41] future studies are suggested to further characterize and validate the presence of various Hg−DOM complexes, so as to fully understand specific roles of DOM in controlling Hg chemical speciation, biological uptake, and methylation in natural aquatic environments.

■ ASSOCIATED CONTENT

Supporting Information

Additional details about materials and methods and supplementary tables and figures mentioned in the text. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.estlett.6b00460.

Materials, methods and supplementary tables and figures mentioned in the text. (PDF)

Calculated molecular geometries and corresponding energies mentioned in the text. (ZIP)

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Notes

The authors declare no competing financial interest.

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Environ. Sci. Technol. Lett. XXXX, XXX, XXX−XXX