Thermodynamic stability of mercury(II) complexes with environmentally relevant low molecular mass thiols studied by competing ligand exchange and density functional theory

Van Liem-Nguyen[§], *Ulf Skyllberg*[#], *Kwangho Nam*[§], *Erik Björn*[§*]

[§]Department of Chemistry, Umeå University, SE-901 87 Umeå, Sweden
[#]Department of Forest Ecology and Management, Swedish University of Agricultural Sciences, SE-901 83 Umeå, Sweden
[*]Correspondence to: erik.bjorn@umu.se

Environmental Context (40-70 words)

The chemical speciation of mercury (Hg) largely control its biogeochemical cycling and exposure to biota. Here we investigate the thermodynamic stabilities of complexes formed between inorganic divalent Hg (Hg^{II}) and 15 biogeochemically relevant low molecular mass (LMM) thiol ligands. This information is critical for accurate modeling of the chemical speciation of Hg^{II} which in turn is a prerequisite for clarifying the role of Hg^{II}–LMM thiol complexes in the cycling of Hg in the environment.

Abstract

Inorganic divalent mercury (Hg^{II}) has a very high affinity for reduced sulfur functional groups. Reports from laboratory experiments suggest that Hg^{II} complexes with specific low molecular mass (LMM) thiol (RSH) ligands control rates of Hg^{II} transformation reactions, in particular the formation of neurotoxic methylmercury (MeHg). Because of methodological limitations for precise determination of the very large stability constants of Hg^{II} complexes with LMM thiol ligands, constants reported in the literature remain inconsistent. This impedes accurate modelling of the chemical speciation of Hg^{II} in natural environments, and the possibilities to elucidate the importance of Hg^{II} complexes with LMM thiols for Hg transformation reactions in natural waters, soils and sediment. Here we report values on thermodynamic stability constants for 15 monodentate, two-coordinated Hg^{II} complexes, $Hg(SR)_2$, formed with biogeochemically relevant LMM thiol (RSH) ligands, determined by a 2-step ligand exchange procedure. Specific $Hg(SR)_2$

complexes were quantified by liquid chromatography inductively coupled plasma mass spectrometry (LC-ICPMS) using the iodide ion (step 1) and mercaptoacetic acid or 2-mercaptopropionic acid (step 2) as competing ligands. Determined thermodynamic constants (log β_2) for the investigated $Hg(SR)_2$ complexes ranged from 34.6, N-Cysteinylglycine, to 42.1, 3-mercaptopropionic acid , for the general reaction $Hg^{2+} + 2RS^- = Hg(SR)_2$, where RS^- represents the thiolate group containing compound. Density functional theory (DFT) calculations were carried out to characterize differences in stabilities among the studied Hg^{II}–LMM thiol complexes. The calculations showed that electron-donating carboxyl and carbonyl groups have a stabilizing effect on the Hg^{II}–LMM thiol complexes while electron-withdrawing protonated primary amino groups have a destabilizing effect. Experimental results and DFT calculations demonstrated significant differences in the stability of $Hg(SR)_2$ complexes, depending on the presence of the electron withdrawing or donating functional groups in the vicinity of the RSH group. These differences are expected to be important for the chemical speciation of Hg^{II} and its transformation reactions in environments. where a multitude of Hg^{II}–LMM thiol complexes are present.

Introduction

Mercury (Hg) pollution is of great concern globally, in particular with respect to the formation and bioaccumulation of the neurotoxic methylmercury (MeHg) molecule. Inorganic divalent mercury (Hg^{II}) has a strong affinity for thiolate groups (RS^-), which largely controls its chemical speciation and reactivity in environmental systems.[1] The formation of MeHg is mediated by phylogenetically diverse microorganisms carrying the *hgcA* and *hgcB* gene clusters.[2, 3] Recent laboratory studies have demonstrated that the addition of specific low molecular mass (LMM) thiol (RSH) compounds to bacteria culture systems can greatly enhance cellular uptake and subsequent methylation of Hg^{II}.[4, 5] Current explanation models for these observations are based on the formation of specific $Hg(SR)_2$ complexes with a high bacterial uptake rate, and/or decreased partitioning of Hg^{II} to outer cell membrane functional groups suppressing cellular uptake. It has also been suggested that formation of Hg^{II}–LMM thiol complexes with other coordination than 1:2 Hg:RSH, i.e. $HgSR^+$, $Hg(SR)_3$ and $Hg(SR)_4$, significantly reduces the bacterial uptake rate of Hg^{II}. However, because of uncertainties and variability in thermodynamic constants reported, the

composition of Hg^{II}–LMM thiol complexes in typical Hg^{II} methylation assays remain uncertain. For the same reason, the potential importance of Hg^{II}–LMM thiol complexes for MeHg formation in natural waters, soils and sediment remains elusive. The concentration of such complexes in natural environments are far lower than current detection limits of analytical methods for their direct measurements. The concentrations of Hg^{II}–LMM thiol complexes thus needs to be established by chemical speciation modeling. Such modeling, however, requires accurately determined concentrations of LMM thiol compounds, and accurate thermodynamic stability constants for the corresponding Hg^{II}–LMM thiol complexes. Only quite recently methods capable of detecting different types of LMM thiols in wetlands and marine ecosystems in which MeHg formation is an issue, have been reported. [6-8] Finally, as additional support for constants reported, a theory needs to be established to explain how the thermodynamic stability of Hg^{II}–LMM thiol complexes varies with chemical structure of the thiol ligands. With such a theory the stability of Hg^{II} complexes with today unidentified LMM thiols in natural environments could be predicted to further help explaining the role of LMM thiols for chemical speciation and transformation processes of Hg^{II}.

Recent spectroscopic work, in particular Extended X-ray Absorption Fine Structure (EXAFS) spectroscopy, has established structures for Hg^{II}–LMM thiol complexes. Combined with ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy, EXAFS results show that Hg^{II} forms monodentate bonds with thiol groups belonging to different molecules.[9-12] In a series of works it has been established that $Hg(SR)_2$, $Hg(SR)_3$, and $Hg(SR)_4$ complexes may all form depending on pH and the Hg^{II}/RSH molar ratio. For glutathione (GSH), which is a comparatively large molecule, $Hg(GSH)_2$ is the highly dominant form at acidic and neutral pH values. The proportion of the complexes $Hg(GSH)_2$, $Hg(GSH)_3$ and $Hg(GSH)_4$ is 95:2:3 % at pH 7.4.[10] The ligands N-acetylcysteine (NACCys), penicilamine (Pen) and cysteine (Cys) have a slightly higher tendency to form $Hg(SR)_3$ and $Hg(SR)_4$ complexes, but $Hg(SR)_2$ is still expected to be the highly dominant form in most soils and waters having a pH below 7.[11-13]

Because of the very strong bonding between Hg^{II} and RS^- groups, traditional methods, such as potentiometry, have failed to determine accurate stability constants for Hg^{II}–LMM thiol complexes. The unreasonably wide range (>20 orders of magnitude) of stability constants

reported for Hg^{II}–LMM thiol complexes before 1980 was pointed at by Casas and Jones.[14] They concluded that the log β_2 constant for the formation of $Hg(SR)_2$, the dominant complex in presence of excess of ligand, should be in the range between 40 and 45 for LMM thiols like Cys, Pen and mercaptoacetic acid (MAC).[14, 15] These results were based on experiments with a direct determination of the very low concentration of free Hg^{2+} ions by means of changes in the electrode potential at the surface of a mercury electrode. With a similar approach, Van Der Linden and Beers reported a log β_2 of 39.4 for $Hg(Cys)_2$.[16] A similar magnitude of the stability constant for Hg^{II}–LMM thiol complexes has been reported using a methodology in which the thermodynamics of the Hg^{II} speciation are shifted towards a separable, identifiable, and measurable Hg^{II} complex with the addition of a competing ligand such as iodide (I^-) or bromide (Br^-) ions or the lipophilic thiol dithizone.[9, 17-19] Even with this progress in methodological development it remains a challenge to determine the exceptionally high stability constant for Hg^{II}–LMM thiol complexes, as exemplified by log β_2 constants reported for the $Hg(Cys)_2$ complex in recent literature, ranging between 38.2[9] and 43.5.[18] Thus, there is still a substantial uncertainty remaining before a consensus on Hg^{II}–LMM thiols stability constants can be reached.

In this work, we determine stability constants for Hg^{II} complexes formed with 15 different LMM thiols using a novel methodology based on competing ligand exchange experiments. The investigated LMM thiols have been reported in terrestrial and aquatic ecosystems.[6, 8, 20] The method takes advantage of a selective direct measurement of specific $Hg(SR)_2$ complexes using liquid chromatography inductively coupled plasma mass spectrometry (LC-ICPMS) in competing ligand experiments. Our results were evaluated in light of current knowledge provided by EXAFS spectroscopy measurements of a number of Hg^{II}–LMM thiol complexes.[11-13] Density function theory calculations were used to identify intra-molecular interactions which could explain observed differences in stabilities among Hg^{II}–LMM thiol complexes. The study focuses on the central two-coordinated symmetric type complexes $Hg(SR)_2$, but also considers $HgSR^+$ complexes and hetero complexes R'SHgSR''.

Experimental

Chemicals and reagents

All thiol compounds were purchased from Sigma-Aldrich. Their structures and abbreviations used throughout this paper are given in Figure S1. In addition to a thiolate group, the different LMM thiol ligands contained hydroxyl, carboxyl, carbonyl, primary and/or secondary amino functional groups. Mercury nitrate monohydrate ≥99.99%, ($Hg(NO_3)_2 \times H_2O$), potassium bromide ≥99.5% (KBr), sodium chloride ≥99.5% (NaCl), formic acid (FA), 2-(2-Bis(carboxymethyl)amino ethyl carboxymethyl amino acetic acid (EDTA), sodium perchlorate ≥ 98% ($NaClO_4$) and 1-propanol were purchased from Sigma-Aldrich, analytical grade potassium iodide (KI) from Fisher Scientific and suprapur nitric acid from Merck. Ultrapure water (>18 MΩ×cm) was obtained through a Milli-Q Advantage A10 Ultrapure Water Purification System (Merck Millipore). All stock solutions and reagents were prepared in a glove box with nitrogen (N_2) atmosphere (<100 ppm O_2). Deoxygenated Milli-Q water was prepared by purging with N_2 overnight at a 300 ml min^{-1} flow rate in the glove box. Stock solutions of Hg^{II} (6.5 mM) were prepared from $Hg(NO_3)_2 \times H_2O$ in 0.12 M of HNO_3. The concentration of Hg^{II} stock solution was verified using reverse isotope dilution analysis with ICPMS and using combustion atomic absorption spectrometry (AMA 254 LECO Corporation). Stock solutions of KI (13.5 mM), KBr (100 mM), KCl (100 mM), EDTA (12 mM), $NaClO_4$ (1.0 M) and LMM thiols (5 mM) were prepared in deoxygenated Milli-Q water inside the glove box. Mobile phases for the LC included 1-propanol and Milli-Q water and pH was adjusted by formic acid to the same pH as for the Hg^{II}–LMM thiol sample solutions.

Liquid Chromatography ICPMS

The liquid chromatography (LC) ICPMS instrument consisted of an LC system with two micro pumps (PerkinElmer series 200), a column oven (PerkinElmer series 200), a vacuum degasser (PerkinElmer series 200) and an auto-sampler (PerkinElmer series 200) which were controlled by hard ware units. The temperature of the LC column and sampler tray was thermostated at 25 ^{o}C. The ICPMS (ELAN DRCe, PerkinElmer SCIEX) included a PFA ES-2040-54 nebulizer and a cyclonic spray chamber (thermostated to +4°C) from Elemental Scientific Inc. The nebulizer and auxiliary gas flow rates were set of 0.6 L min^{-1} and 1.2 L min^{-1}, respectively. An ICP RF power of 1350 W

and an ion lens voltage of 10 V were used. The eluting $Hg(SR)_2$ complexes were detected by monitoring the $^{202}Hg^+$ isotope signal intensity.

The LC electrospray ionization mass spectrometry (ESIMS) (Thermo Scientific LCQ Fleet Ion Trap) instrument consisted of quaternary LC pumps, an auto-sampler and a vacuum degasser. The operation parameters of the ESIMS were set 300 ^oC for the capillary temperature, 4.3 kV for the electrospray voltage, 31 V for the capillary voltage, 90 V for the tube lens voltage and 20 and 5 arbitrary units for the sheath and auxiliary gas flow rate, respectively. Both negative and positive ionization modes were used with a mass scan range from 200 to 1000 m/z.

A Phenomenex Kinetic Biphenyl LC column 150×3mm×5µm, with a 4×3.0 mm guard column, was used with mobile phases including Milli-Q water and 1-propanol. 1-propanol was used as organic modifier in the mobile phase due to its low volatility (minimizing excessive solvent loading of the ICP) and enhanced aerosol formation efficiency of the nebulizer, causing increased sensitivity of the ICPMS measurements at a few percentages (2-10%) of 1-propanol in the eluent.[21] The pH of the mobile phase was adjusted by formic acid. A flow rate of 0.4 mL min^{-1} and injection volume of 10 µl were set for the LC. Isocratic elution was used with adjusted mobile phase composition to obtain complete separation of each pair of analyzed $Hg(SR)_2$ complexes. Three different proportions of 1-propanol were used depending on whether MAC (3.5% or 8.5% 1-propanol) or 2-MPA (11% 1-propanol) was used as competing ligand. A post column flow rate of 0.1 mL min^{-1} of an aqueous solution containing 10 ng ml^{-1} Thallium (Tl) was applied to monitor and correct for signal drift of the ICPMS instrument over time. The chromatographic peak areas of the complexes were calculated by OriginPro 9.1.0 from OriginLab Corporation. Stability constants of the complexes were corrected to certain ion strengths by specific interaction theory (SIT) using free software Ionic Strength Corrections for Stability Constants from IUPAC, version 1, 2004.[22] Stability constants were calculated by the software WinSGW from MaJo.[23]

Determination of pKa of the RSH group

The *pKa* values of the RSH group for the 15 investigated LMM thiols were estimated by the Atomic Charges model presented by Ugur et al.[24] The method builds on a linear relationship between computed molecular charge distribution and empirically determined *pKa* values for 25 thiols

compounds with different functional group composition. The data set showed the best linear relationship with Natural Population Analysis (NPA)[25] atomic charges on optimized geometries of the anionic form using the Minnesota functional M06-2X[26, 27] with the 6-311G basis set and the conductor-like polarizable continuum model (CPCM)[28] with default atomic radii.

Step 1: Determination of the stability constant of $Hg(MAC)_2$ and $Hg(2\text{-}MPA)_2$ using I^- as a competing ligand

A series of solutions with I^- concentration of 4, 8, 20, 50, 100, 300, 1000 µM were prepared in solution with pH of 2.9 and 3.6 (adjusted by nitric acid) in 15 ml Falcon tubes, with three ionic conditions. Ionic strength was kept constant at 0.1 M or 1.0 M using $NaClO_4$, or was determined by the I^- and HNO_3 concentrations without addition of extra ionic buffer. The purpose of no addition of extra ionic buffer was to have one set of experiment at low ionic strength condition for the complex formation reactions. For this condition the ionic strength of solutions varied from 0.0003 to maximum 0.003 M, which for the experimental purposes of this study and can be considered close to ion strength of zero (I=0). Samples were prepared at 25°C±1°C with a thermostat in the N_2 filled glove box. To prepare these solutions different volumes of a KI stock solution (13.5 mM) were mixed with 9.6 µl of LMM thiol (5 mM) and shaken for 10 seconds. Then 120 µl of a 100 µM Hg^{II}-nitrate solution, and $NaClO_4$ ionic medium, were added to give final Hg^{II} and LMM thiol concentrations of 4 µM and 16 µM, respectively, in a final sample volume of 3 ml. The samples were rotated (end over end) inside the glove box for two hours prior to measurement to assure equilibrium was achieved. The time required to reach equilibrium for a system with a mixture of two different LMM thiols was shown to be less than 30 min and the formed complexes were shown to be stable up to 5 days or more by replicate injection of the same samples (Figures S2 and S3).

The absolute concentration of $Hg(MAC)_2$ and $Hg(2\text{-}MPA)_2$ complexes were determined by LC-ICPMS using calibration curves and the equilibrium pH of samples was measured after 2 hours. Stability constants for the formation of $Hg(MAC)_2$ and $Hg(2\text{-}MPA)_2$ complexes were calculated for reactions (1a-b) using the WinSGW software, with *Ka* values for the thiol group (reaction 2) of MAC and 2-MPA determined from DFT calculations (Ugur et al., 2014) and well-established

thermodynamic constants for the formation of HgI_n^{2-n} complexes (reactions 3a-d).[24, 29] We also determined the concentration of mixed HgI(MAC, 2-MPA) complexes (reaction 6, supporting information) from the area of those peaks in relation to the peak areas of the $Hg(MAC)_2$ and $Hg(2\text{-}MPA)_2$ complexes. Details on the calculation of the stability constant for the $Hg(MAC)_2$ complex are reported on Page S2 (supporting information).

$Hg^{2+} + 2RSH = Hg(SR)_2 + 2H^+$	$K_{Hg(SR)2}$	(1a)
$Hg^{2+} + 2RS^- = Hg(SR)_2$	β_2	(1b)
$RSH = RS^- + H^+$	K_a	(2)
$Hg^{2+} + I^- = HgI^+$	β_1	(3a)
$Hg^{2+} + 2I^- = HgI_2$	β_2	(3b)
$Hg^{2+} + 3I^- = HgI_3^-$	β_3	(3c)
$Hg^{2+} + 4I^- = HgI_4^{2-}$	β_4	(3d)
$Hg^{2+} + RS^- = HgSR^+$	K_{HgSR+}	(4)
$Hg^{2+} + R'S^- + R''S^- = R'SHgSR''$	$\beta_{R'SHgSR''}$	(5)

Step 2: Determination of the stability constant for $Hg(SR)_2$ complexes using 2-MPA and MAC as competing ligands

The stability constants for $Hg(2\text{-}MPA)_2$ and $Hg(MAC)_2$, as determined by the competition with I^- ions, were first validated against each other. An equilibrated solution of 4 µM $Hg(NO_3)_2$, 8 µM MAC and 8 µM 2-MPA, adjusted to pH 3.0, was examined by LC-ICPMS. First 10 µl of 2.4 mM MAC and 10 µl of 2.4 mM 2-MPA and 2860 µl of pH 3.0 water solution (pH adjusted by HNO_3 acid) were mixed in a 15 ml falcon tube by strong hand shaking for 10 s, and then 120 µl of 100 µM $Hg(NO_3)_2$ was added. The sample was end-end rotated in a glove box filled with N_2 for 2 h. After 2 h of equilibration, concentrations of $Hg(MAC)_2$ and $Hg(2\text{-}MPA)_2$ were determined from LC-ICPMS chromatogram peak areas and stability constants were calculated by use of WinSGW. As shown by Figure S2 the concentrations of $Hg(SR)_2$ complexes did not change in the time window 10 min

to 4 h. Once stability constants for $Hg(MAC)_2$ and $Hg(2\text{-}MPA)_2$ were established, constants for $Hg(SR)_2$ complexes with the other 13 LMM thiols were determined in experiments where MAC or 2-MPA were added as competing ligands at concentrations equal to the studied LMM thiol. By using MAC or 2-MPA as competing ligands, we avoided the interference effects of I^- (suppressing ionization efficiency of Hg in the ICP) and HgI_n^{n-2} complexes (causing enhanced Hg spectral background signals) on the signal of $Hg(SR)_2$ complexes. The signals of I^- and HgI_n^{n-2} were well separated from $Hg(MAC)_2$ and $Hg(2\text{-}MPA)_2$ (as shown in Figure S4c and S4d). Stability constants for $Hg(SR)_2$ complexes were calculated for reactions 1a and 1b. The complete calculation scheme is exemplified in Page S3 (supporting information) using MAC as the competing ligand.

Investigation of the possible formation of one-coordinated HgSR complexes and hetero ligation R'SHgSR'' complexes

The possible formation of one-coordinated Hg^{II} complexes ($Hg^{2+} + RS^- = HgSR^+$, reaction 4) with the LMM thiols Cys, HCys, GSH, MAC, Glyc or NACCys was investigated. Samples were prepared at molar ratios of LMM thiol ligands to Hg^{II} between 1.0 and 10 at pH 3.0 in a constant ionic strength of 0 M (pH 3.0, HNO_3). The samples were rotated inside a N_2 filled glove box for two hours to assure equilibrium was reached. The concentration of $Hg(SR)_2$ complexes were determined by LC-ICPMS. In absence of an apparent peak for the $HgSR^+$ complex (reaction 4), stability constants were calculated from fitting measured (by LC-ICPMS) and modeled (in WinSGW) $Hg(SR)_2$ concentrations at different Hg^{II} to LMM thiol molar ratios using a model including both two- and one-coordinated Hg^{II}–LMM thiol complexes. The existence of possible R'SHgSR'' hetero complexes (reaction 5) was investigated with direct infusion of sample solutions to electrospray ionization mass spectrometry (ESI-MS) and with the use of LC-ESIMS and LC-ICPMS.

Density functional theory modeling of $Hg(SR)_2$ complexes

The initial geometry of each complex was prepared as the structure of $Hg(SR)_2$, in which the two ligands form a linear configuration with protonation state of each functional group at pH 3.0. For each complex, geometry optimizations were performed in gas phase using the B3LYP[30, 31] level of

theory and a mixed basis set comprised of the Def2TZVPP[32] basis set for Hg and 6-31++G(d,p) for all other atoms. The free energy was determined by the frequency calculation at the same level of theory, followed by a single point energy calculation at the B3LYP level of theory with the Grimme's dispersion and Becke-Johnson damping[33] and the basis set consisting of Def2TZVPPD[32] for Hg and 6-311++G(d,p) for all other atoms, respectively. The single point energy at the larger basis set was introduced to correct the energy determined with the smaller basis set used in the geometry optimizations and frequency calculations. The B3LYP functional and the basis sets used in the present work were shown previously to produce results comparable to the CCSD(T) level of theory[34]. The same calculations were performed for Hg^{2+} ion and each ligand, respectively, to determine the free energy of complex formation (in the gas phase). The Gaussian 09[35] program suite was used in all density functional theory (DFT) calculations.

Results and discussion

Spectroscopic methods (i.e. ^{1}H NMR, Hg L_{III}-edge EXAFS) have established that Hg^{II} forms a two-coordinated linear structure with two separate ligand molecules, i.e. $Hg(SR)_2$, in which each thiol group forms a monodentate complex. It has also been shown that three- and four-coordinated Hg^{II}–LMM thiols complexes are possible at neutral to alkaline pH, but not at acidic pH values. Since the primary aim of this study was to characterize two-coordinated $Hg(SR)_2$ complexes, the experimental pH was thus kept at acidic conditions of 2.9 or 3.6 to prevent formation of complexes with higher coordination.[11-13, 36] The two-coordinated structure $Hg(SR)_2$ was verified by LC-ICPMS and ESIMS as the only detectable molecular stoichiometry of Hg^{II}–LMM thiol complexes formed in this study. Electrospray ionization mass spectra showing the molecular mass and isotope pattern of $Hg(SR)_2$ complexes are given in Figure S5.

Calculation of pKa values for LMM thiols

Depending on the pH value, the *pKa* value of the RSH group is of great importance for the stability constant of $Hg(SR)_2$ complexes formed via reaction (1a). The *pKa* value must therefore be known for each thiol compound, and should ideally be determined with a consistent methodology for all compounds to be compared. In the literature, only 10 of the 15 LMM thiols included in this study

have reported *pKa* values for the RSH group. Those values vary due to different ionic strengths and experimental approaches used. Therefore, in order to make our determined stability constants comparable for the different $Hg(SR)_2$ complexes, we determined the *pKa* value for the RSH group of all LMM thiol compounds using the Atomic Charges model developed by Ugur et al.[24] These values, reported in Table 2, are in good agreement with the *pKa* values reported for most of the LMM thiols available in the literature when corrected for ionic strenght.[9, 13, 15, 17-19, 37, 38] The *pKa* value of the RSH group of LMM thiols studied here ranged between 7.3 and 10.8, with CysGly having the lowest and 3-MPA the highest value. Amino groups, particularly the protonated primary amino moiety ($-NH_3^+$), are electron withdrawing and thus decrease the *pKa* value of the corresponding RSH group. Low molecular mass thiols with one primary amino group such as Cys, HCys, Cyst, CysGly, GSH, GluCys and Pen have *pKa* values in the range between 7.3 and 10.3, with an average of 9.1. Low molecular mass thiols without any amino group, such as MAC, 2-MPA, 3-MPA, ETH, Glyc, and SUC, have higher *pKa* values in the range between 9.4 and 10.8, with an average of 10.2. For LMM thiols comprising the same composition of functional groups, compounds with the amino group located closer to the RSH group have smaller *pKa* values: for example, Cys (*pKa* 8.6) versus HCys (*pKa* 9.9).

Determination of stability constants for $Hg(MAC)_2$ and $Hg(2\text{-}MPA)_2$ with I^- as the competing reference ligand

In the first step of our analyses, we determined stability constants of $Hg(MAC)_2$ and $Hg(2\text{-}MPA)_2$ in competition with I^-. In the second step, MAC and 2-MPA were used as competing ligands for the determination of Hg^{II} complexation with the other 13 LMM thiols. The rationale for using I^- as competing ligand in the first step is that, in comparison to $Hg(SR)_2$ complexes, the stability constants for HgI_n^{2-n} complexes are well-established.[29] Other potential competing ligands such as Br^-, Cl^- and EDTA were also investigated. Our results showed that the concentration of $Hg(MAC)_2$ was not significantly lowered even when adding up to 10 mM of those ligands (at $[Hg^{II}]$ = 2 µM and [MAC] = 8 µM) (Figure S6). This demonstrated that these three ligands were all too weak to compete with MAC and 2-MPA to the extent that complexes were detectable by LC-ICPMS. Given the stability constant for $Hg(MAC)_2$ and $Hg(2\text{-}MPA)_2$ are established by competition with I^-, it is

advantageous to determine the stability constants for the remaining 13 $Hg(SR)_2$ complexes in a second step using MAC or 2-MPA as competing ligands. The main reason for selecting MAC and 2-MPA in the first step was that they were well separated in the LC column from both I^- ions (causing signal suppression) and HgI_n^{2-n} complexes (Figure S4). The $Hg(MAC)_2$ and $Hg(2\text{-}MPA)_2$ were also well-separated from all the other $Hg(SR)_2$ complexes. The retention time on the LC column of all investigated $Hg(SR)_2$ complexes are illustrated in Figure S7. Further, the absence of amino groups in MAC and 2-MPA may result in better resolved *pKa* values of the RSH group.

In Figures 1a,b and 2a,b the determined concentrations of $Hg(MAC)_2$ and $Hg(2\text{-}MPA)_2$ are illustrated as a function of I^- concentration. Log $K_{Hg(SR)2}$ and log β_2 (reactions 1a and 1b, respectively) for the formation of $Hg(MAC)_2$ and $Hg(2\text{-}MPA)_2$ were determined according to the calculation scheme on page S2. The constants were calculated for each addition of I^- based on measured concentrations of $Hg(MAC)_2$ and $Hg(2\text{-}MPA)_2$ from LC-ICPMS and established constants for HgI_n^{2-n} complexes (Table S1). Average values for log β_2 for $Hg(MAC)_2$ and $Hg(2\text{-}MPA)_2$ were determined from all experiments and the deviation of data points from the linear equation illustrated in Figures 1c,d and 2c,d were used to calculate uncertainties in this constant, as reported in Table 1. The average log β_2 of $Hg(MAC)_2$ and $Hg(2\text{-}MPA)_2$ were determined to be 40.9±0.2 and 41.5±0.1 (I=0 M), respectively, which are values in fair agreement with previous results by Basinger et al.[15] and Cardiano et al.[19] The formation of a mixed iodide-thiol ligation complex (HgISR) was observed for MAC and 2-MPA when the molar ratio of I^- to Hg^{II} was higher than 25 but not exceeding 250 (the molar ratio of LMM thiol to Hg^{II} was kept constant at 4), as shown in Figure S4. Mixed halide-thiol complexes, in particular HgClSR, has been suggested to form by Hilton et al., as determined by NMR experiments.[39] In this study, HgISR complexes were identified based on observed LC-ICPMS signals of both Hg and iodine, shown in Figure S4. The average log K of HgI(MAC) and HgI(2-MPA) following the reaction $Hg^{2+} + RS^- + I^-$ = HgISR were determined to be 32.2±0.1 and 32.3±0.1, respectively.

Determination of the stability constants for 13 LMM $Hg(SR)_2$ complexes using MAC and 2-MPA as competing reference ligands

346 The stability constants for $Hg(MAC)_2$ and $Hg(2\text{-}MPA)_2$ determined by I^- as competing ligand were
347 cross validated against each other using MAC as competing ligand for $Hg(2\text{-}MPA)_2$. The log β_2
348 (reaction 1b) for $Hg(2\text{-}MPA)_2$ determined with this approach was 41.6, essentially identical to the
349 log β_2 of 41.5 determined by using I^- as the competing ligand. This validation enabled us to use
350 either MAC or 2-MPA as competing ligands for the determination of log β_2 for the remaining 13
351 LMM thiols.
352 In Table 2, stability constants for the formation of $Hg(SR)_2$ complexes between Hg^{II} and all 15 LMM
353 thiols are reported. At low pH, the RSH group is fully protonated and the complex formation with
354 Hg^{2+} is described by reaction 1a: $Hg^{2+} + 2RSH = Hg(SR)_2 + 2H^+$. The log $K_{Hg(SR)2}$ of this reaction varied
355 substantially between 19.6 and 21.0, with $Hg(Cyst)_2$ having the smallest and $Hg(2\text{-}MPA)_2$ the
356 largest value. When the reaction is written with the deprotonated thiolate group (reaction 1b),
357 the log β_2 varied from 34.6 for $Hg(CysGly)_2$ to 42.1 for $Hg(3\text{-}MPA)_2$. The relation between the two
358 constants is log β_2 = log $K_{Hg(SR)2}$ + 2pKa, where pKa relates to reaction (3). The pKa value thus have
359 a strong influence on the value of log β_2. This relatively large variability in thermodynamic
360 constants shows that there are significant differences in the stability of different Hg-LMM thiol
361 complexes depending on the chemical structure of the thiol ligand.
362 Most previous studies reporting stability constants for Hg^{II}–LMM thiol complexes rely on methods
363 which do not directly quantify the $Hg(SR)_2$ complexes, typically electrochemical or radiochemical
364 detection of Hg^{II} in the presence of a competing ligand.[15, 17] Chemical shifts measured by ^1H NMR
365 spectroscopy for LMM thiols with and without addition of Hg^{II} have also been used to calculate
366 stability constants for $Hg(SR)_2$ complexes.[18] Compared to previous studies, reporting log β_2
367 constants on the order of 38 to 44 for $Hg(SR)_2$ complexes, the constants determined in our study
368 are in fair agreement for Hg^{II} complexes with LMM thiols which lack amino groups: $Hg(2\text{-}MPA)_2$,
369 $Hg(SUC)_2$, $Hg(3\text{-}MPA)_2$, $Hg(MAC)_2$, Table 2. For complexes containing amino groups: $Hg(Pen)_2$,
370 $Hg(GSH)_2$, $Hg(Cys)_2$ (Table 2) our determined constants are significantly lower than in these
371 previous studies.[9, 13, 15, 17-19]
372 Our experimental approach, based on a direct quantification of specific $Hg(SR)_2$ complexes, sets
373 a new standard for the determination of thermodynamic constants for Hg^{II}–thiol complexes. The
374 constants determined for the two complexes $Hg(MAC)_2$ and $Hg(2\text{-}MPA)_2$, which are very central

in our methodology since they are used as references for the complexes $Hg(NACPen)_2$, $Hg(NACCys)_2$, $Hg(SUC)_2$, $Hg(3\text{-}MPA)^2$, $Hg(Glyc)_2$, $Hg(GluCys)_2$, $Hg(ETH)_2$, $Hg(Pen)_2$, $Hg(GSH)_2$, $Hg(Cys)_2$, $Hg(CysGly)_2$, $Hg(HCys)_2$ and $Hg(Cyst)_2$. The constants for $Hg(MAC)_2$ and $Hg(2\text{-}MPA)_2$ were determined with comparably large number of data points covering extensive ranges in pH and ionic strength (Figure 1 and 2). The constants of $Hg(SR)_2$ complexes were established with diverse types of LMM thiol containing multitude different functional groups (Figure S1).

Formation of one-coordinated $HgSR^+$ complexes

According to previous studies,[18, 19] model fitting to experimental data suggests the presence of a complex having the 1:1 stoichiometry between LMM thiol ligand and Hg^{2+} ion (reaction 4). Because Hg^{II} prefers two-coordination it is expected that such complexes have a bi-dentate structure where Hg^{2+} is coordinated with one thiol group and with one carboxyl/carbonyl oxygen group, or amino group, from the same LMM thiol molecule.[14, 15, 18, 19] In order to test the existence of such complexes we conducted experiments at RSH to Hg^{II} molar ratios of 1.0. Candidate peaks indicative of $HgSR^+$ complex formation were detected by LC-ICPMS, but with increased broadening as compared to the $Hg(SR)_2$ peak, Figure S8. The reason could be that the relatively weak one-coordinated complexes were partly degraded during the separation on the LC column (possibly by interactions with formic acid in the mobile phase). Because of the relatively large uncertainty in quantifying the area of these small and broad chromatographic peaks, log *K* for $HgSR^+$ complexes were instead determined by fitting the measured concentration of $Hg(SR)_2$ to a model including both $Hg(SR)_2$ and $HgSR^+$ complexes. During the fitting, the log K_{HgSR+} for the reaction $Hg^{2+} + RS^- = HgSR^+$ (reaction 4) was optimized, while keeping log β_2 for $Hg(SR)_2$ complex fixed. Model fitting was done on data for the formation of $Hg(SR)_2$ and $HgSR^+$ in experiments with Cys, HCys, GSH, MAC, Glyc, and NACCys ligands. Figure 3 shows the average $Hg(SR)_2$ concentration for these six $Hg(SR)_2$ complexes measured by LC-ICPMS and calculated by model fits with fixed log β_2 of $Hg(SR)_2$, *pKa* (RSH) (Table 2) and *pKa* (RCOOH). The log *K* for the formation of $HgSR^+$ was successively varied from a value of 29 to 32, with steps of 0.5. The best fit was obtained with a log K_{HgSR+} value in the range between 30.5 and 31. The log K_{HgSR+} for the formation of $HgSR^+$ was

thus approximately 8.5 orders of magnitude lower in size than the average log β_2 of 39.2 for the formation of $Hg(SR)_2$.

This difference in binding strength between Hg^{II} coordinated by two or one RS^- group is in fair agreement with previous results.[14, 15, 18, 19] Due to a lower stability of the $HgSR^+$ complex, as compared to $Hg(SR)_2$, the concentration of $HgSR^+$ is negligible when the molar ratio of RSH to Hg^{II} exceeds 2. This implies that the $Hg(SR)_2$ complex was the by far dominant form of Hg^{II} complexes at the experimental conditions used for the determination of stability constants for reactions 1a and 1b.

Formation of R'SHgSR'' hetero complexes

The thermodynamic stability of R'SHgSR'' hetero complexes (where two different types of LMM RSH groups are involved in the complex formation) relative to $Hg(SR)_2$ complexes is important because the complexity of Hg^{II} speciation models may increase considerably if R'SHgSR'' hetero complexes need to be included in the model. The chromatograms of LC-ICPMS and LC-ESIMS did not include information indicative of any R'SHgSR'' hetero complexes, such as CysHgMAC (Figure S9 and S10). The molar ratio of individual LMM thiols to Hg^{II} was fixed at 2.0 in these experiments (8 µM of Cys and 8 µM of MAC to 4 µM of Hg^{II}). The absence of detectable R'SHgSR''complexes could be due to these complexes being less stable than $Hg(SR)_2$ complexes or that the life-time of hetero complexes being shorter. Pei et al.[40] observed a shift in the retention time of $Hg(GSH)_2$ with increased concentration of Cys in the mobile phase of an LC system and proposed that the CysHgGSH hetero complex was formed. We could observe the signal of R'SHgSR''complexes with direct infusion of sample solutions to ESIMS (without LC column, exemplified in Figure S11). This observation may however be explained by an artificial formation of R'SHgSR'' in the ion-source. In the electrospray ion-source, complex re-formation reactions are common and products with fast kinetics are preferentially formed. The formation of Hg^{II}–LMM thiol complexes have been shown by ^{1}H-NMR to be thermodynamically extremely stable but kinetically very labile.[40-43] In line with the NMR spectroscopy observations, previous studies have shown that Hg^{II} reached equilibrium with LMM thiols within the time frame of 30 s.[40, 42, 43]

Liquid chromatography with ICPMS and ESIMS detection indicated that 100 ± 2% of the Hg^{II} concentration of 4 µM was represented by the $Hg(SR)_2$ complex in our experiments. If it is assumed that maximum 2% of Hg^{II} was in the form of the R'SHgSR'' hetero complex, the stability constant for this complex would be at least 1.5 log units smaller than the average stability constant for the corresponding $Hg(SR)_2$ complexes.

Molecular modeling of $Hg(SR)_2$ complex structure and stability

To gain insights into chemical interactions and structures controlling the differences in the stability constant for Hg^{II}-LMM thiol complexes, molecular modeling based on density functional theory (DFT) was performed for the $Hg(SR)_2$ complexes. There was a good qualitative agreement between the relative stability of Hg^{II}-LMM thiol complexes determined experimentally and predicted by *in silico* modeling of the complexes in the gas phase (except for the $Hg(GSH)_2$ complex). The experimentally determined log $K_{Hg(SR)2}$ values were more or less continuously distributed in the range 19.6 to 21.0 whereas the modeled ΔG values separated the complexes into two groups (Figure 4). The calculated Hg–S bond distances and S–Hg–S bond angles varied between 2.36–2.38 Å and 171–180°, respectively, for all the complexes investigated (Table S2). The Hg–S bond distance for Hg^{II}-thiol complexes has previously been estimated to 2.34 Å[44], which is also a typical bond distance determined by EXAFS spectroscopy for both LMM thiols[10-13] and thiols associated with natural organic matter[45].

Interestingly, the measured and modeled differences in stability of Hg^{II}-LMM thiol complexes were not random but systematically dependent on functional groups neighboring the RSH groups. The DFT calculations indicated two predominant intrinsic intra-complex interactions that can explain these differences. The presence of a primary amino group resulted in weaker Hg^{II}-LMM thiol complexes compared to thiols lacking such functional group (blue versus red colored symbols, respectively in Figure 4). The difference can be understood by the strong electron-withdrawing effect of the $–NH_3^+$ group (protonated at pH < ~10), which lowers the stability of the Hg–S bond by making the $–S^-$ group less negative. By contrast, electron-donating groups, such as the carbonyl and carboxylic groups, can contribute to the stabilization of the Hg^{II}-LMM thiol complexes via additional coordination to Hg^{II} besides the linear S–Hg–S configuration (Figure 5

and Table S2).[46] The opposing effects between the electron-withdrawing primary amino group and electron-donating oxygens on the stability of $Hg(SR)_2$ complexes can be illustrated by comparing the $Hg(Cys)_2$, $Hg(3\text{-}MPA)_2$ and $Hg(2\text{-}MPA)_2$ complexes (Figure 5). The only structural differences between the $Hg(Cys)_2$ and $Hg(3\text{-}MPA)_2$ complexes is the presence of a primary amino group in $Hg(Cys)_2$, contributing to the log $K_{Hg(SR)2}$ for reaction (1a) being half a log unit lower for $Hg(Cys)_2$ than for $Hg(3\text{-}MPA)_2$. The DFT modeling further suggests that an attraction between Hg and carboxylic oxygen in the $Hg(2\text{-}MPA)_2$ complex, but less so in the $Hg(3\text{-}MPA)_2$ complex, results in half a log unit higher log $K_{Hg(SR)2}$ for $Hg(2\text{-}MPA)_2$ compared to $Hg(3\text{-}MPA)_2$. Thus, the degree of destabilization/stabilization differs for the 15 complexes depending on the location of the additional functional groups relative to the –S position in the molecule. The DFT modeling further predicts a lower ΔG value for $Hg(GSH)_2$ compared to the other $Hg(SR)_2$ complexes containing a primary amino group. Since GSH involves many rotatable bonds, free energies estimated based on a single optimized geometry may not be appropriate and multiple geometry sampled by advanced sampling techniques, such as molecular dynamics and Monte Carlo simulations, would enhance accuracy of the determined free energies.

Environmental implications

There are several studies investigating the detailed Hg–S structure in $Hg(SR)_n$ complexes for variable n[11, 44, 47-49], often with the perspective of designing optimized Hg^{II} chelating compounds (at physiological pH). There are few studies investigating the more subtle differences in stability of $Hg(SR)_2$ complexes induced by weaker interactions with O and N functional groups[12, 13] in addition to the S–Hg–S coordination. Our experimental and modeling results show that despite the very strong Hg–S bond there are differences in the stability of $Hg(SR)_2$ complexes ($K_{Hg(SR)2}$ spans 1.5 orders of magnitude, Table 2) that can be explained by the presence of electron withdrawing and electron donating functional groups in the vicinity of the RSH group. These systematic differences may have substantial effects on the chemical speciation of Hg^{II} in environmental and biological systems where several LMM thiol ligands are present in similar concentrations, which in turn affects rates of central Hg transformation reactions, including the formation of the very toxic MeHg molecule. This work thus significantly advances our

fundamental understanding of interactions between Hg^{II} and thiol ligands at the molecular level. The new knowledge and thermodynamic data are critical in order to accurately model the chemical speciation of Hg^{II} in natural environments, and to elucidate the importance of Hg^{II}–LMM thiol complexes for Hg transformation reactions in natural waters, soils and sediment.

Acknowledgements

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Table 1. Thermodynamic constants (i.e. log *β2*, reaction 1b) for the formation of $Hg(MAC)_2$ and $Hg(2\text{-}MPA)_2$ complexes and corresponding constants for mixed complexes including one I^- and one LMM thiol ligand at different ionic strength and pH.

Complexes	Log β_2 (±SD)					
	pH=2.9			pH=3.6		
	I=0 (M)[a]	I=0.1 (M)[b]	I=1.0 (M)[b]	I=0 (M)[a]	I=0.1 (M)[b]	I=1.0 (M)[b]
$Hg(MAC)_2$	40.9 (±0.2)	40.5 (±0.2)	40.1 (±0.1)	40.8 (±0.2)	40.3 (±0.2)	40.2 (±0.2)
$Hg(2\text{-}MPA)_2$	41.5 (±0.1)	40.9 (±0.1)	40.3 (±0.1)	41.5 (±0.2)	40.7 (±0.1)	40.4 (±0.2)
HgIMAC	32.2 (±0.1)	32.0 (±0.1)	29.7 (±0.2)	32.3 (±0.1)	29.8 (±0.2)	29.7 (±0.1)
HgI(2-MPA)	32.3 (±0.1)	32.1 (±0.2)	29.9 (±0.10)	32.3 (±0.1)	29.9 (±0.2)	29.8 (±0.1)

[a]The p*Ka* values of the RSH groups of MAC and 2-MPA at I=0 were determined to 10.2 and 10.3, respectively, based on the model developed by Ugur et al.[24]

[b] The p*Ka* values of the RSH groups were reported by Cardiano et al.[19] to be 10.0 at I=0.1, and 9.8 at I=1.0 M for MAC, and 10.1 at I=0.1 M and 10.1 at I=1.0 M for 2-MPA

Table 2. Thermodynamic constants for the formation of $Hg(RS)_2$ complexes with LMM thiols, as described by reactions 1a and 1b, and *pKa* values for RSH groups by reaction 2. Complexes are sorted according to decreased value on log $K_{Hg(SR)2}$ for reaction (1a) at an ion strength of 0 M and pH=3.0. Literature values are reported with and without correction for ion strength effects and *pKa* values.

Complexes	Thermodynamic constant (± SD)				
	This study, I=0 (M)			Literature[c]	Literature[d]
	log *K* (1a)	log β_2 (1b)	*pKa* (2)		
Hg(2-MPA)$_2$	21.0	41.5 (±0.1)	10.3[b]	42.68[19]	43.2
Hg(NACPen)$_2$	20.9	40.1 (±0.2)	9.6[b]		
Hg(NACCys)$_2$	20.6	40.2 (±0.2)	9.8[a]	41.81[9]	42.0
Hg(SUC)$_2$	20.6	41.7 (±0.2)	10.6[b]	42.92[19]	43.3
Hg(3-MPA)$_2$	20.6	42.1 (±0.2)	10.8[b]	39.54[19]	40.9
Hg(MAC)$_2$	20.6	40.9 (±0.2)	10.2[a]	40.5[15]	40.8
Hg(Glyc)$_2$	20.6	39.4 (±0.2)	9.4[b]		
Hg(GluCys)$_2$	20.5	40.3 (±0.3)	9.9[b]		
Hg(ETH)$_2$	20.5	40.3 (±0.2)	9.9[b]		
Hg(Pen)$_2$	20.3	36.9 (±0.2)	8.3[b]	38.2[9], 43.51[18]	39.0, 44.0
Hg(GSH)$_2$	20.2	38.8 (±0.2)	9.3[a]	40. 36[18]	41.8
Hg(Cys)$_2$	20.1	37.5 (±0.2)	8.6[b]	40.0[17], 43.41[18]	40.3, 44.2
Hg(CysGly)$_2$	19.9	34.6 (±0.3)	7.3[b]		
Hg(HCys)$_2$	19.7	39.4 (±0.2)	9.9[b]		
Hg(Cyst)$_2$	19.6	40.3 (±0.2)	10.3[a]		

[a] Reference from Ugur et al.[24]

[b] Computed from NPA atomic charges on optimized geometrics of the anionic form using M062X/6-311G and CPCM model developed by Ugur et al.[24]

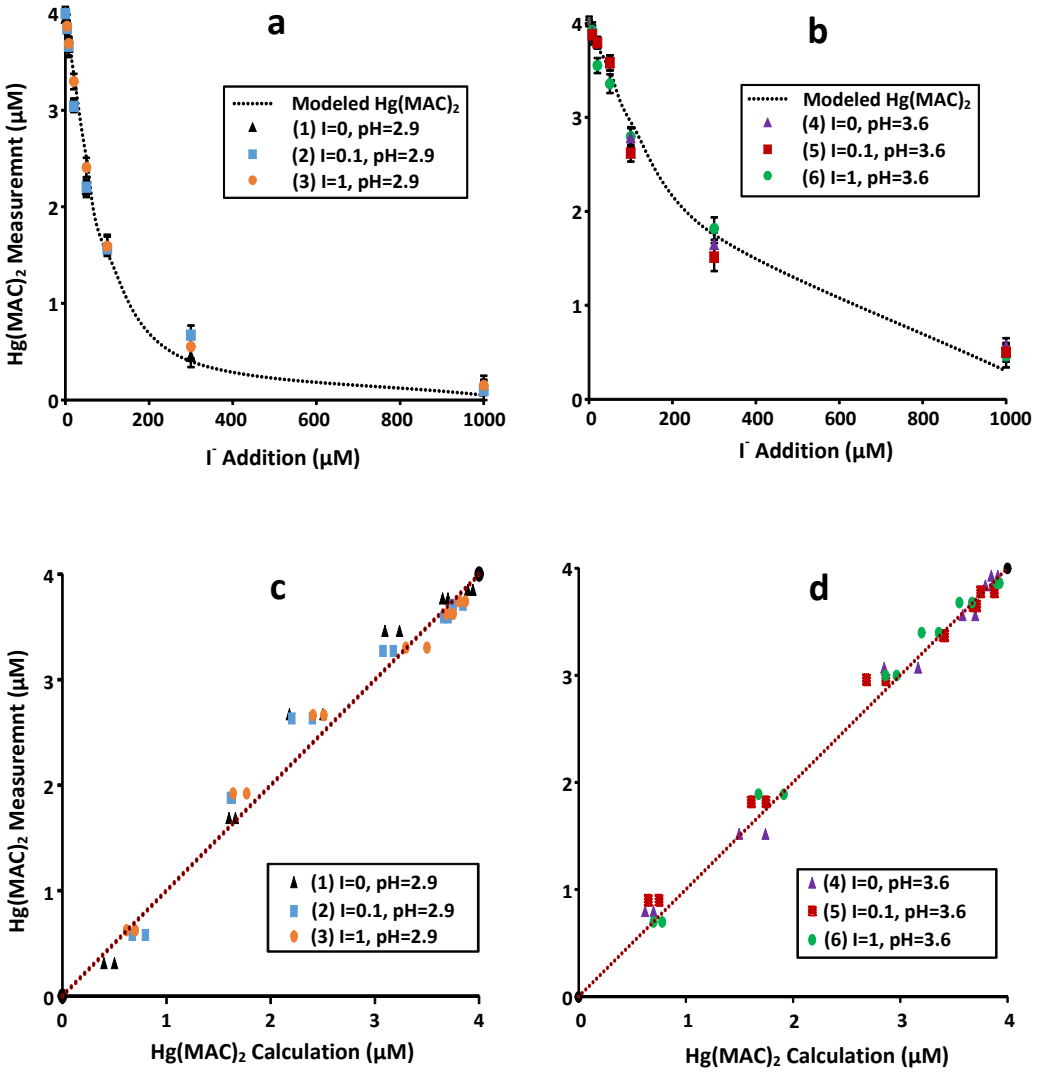
(1a) $Hg^{2+} + 2RSH = Hg(SR)_2 + 2H^+$

(1b) $Hg^{2+} + 2RS^- = Hg(SR)_2$

(2) $RSH = RS^- + H^+$

[c] Literature values without correction for differences in ion strength.

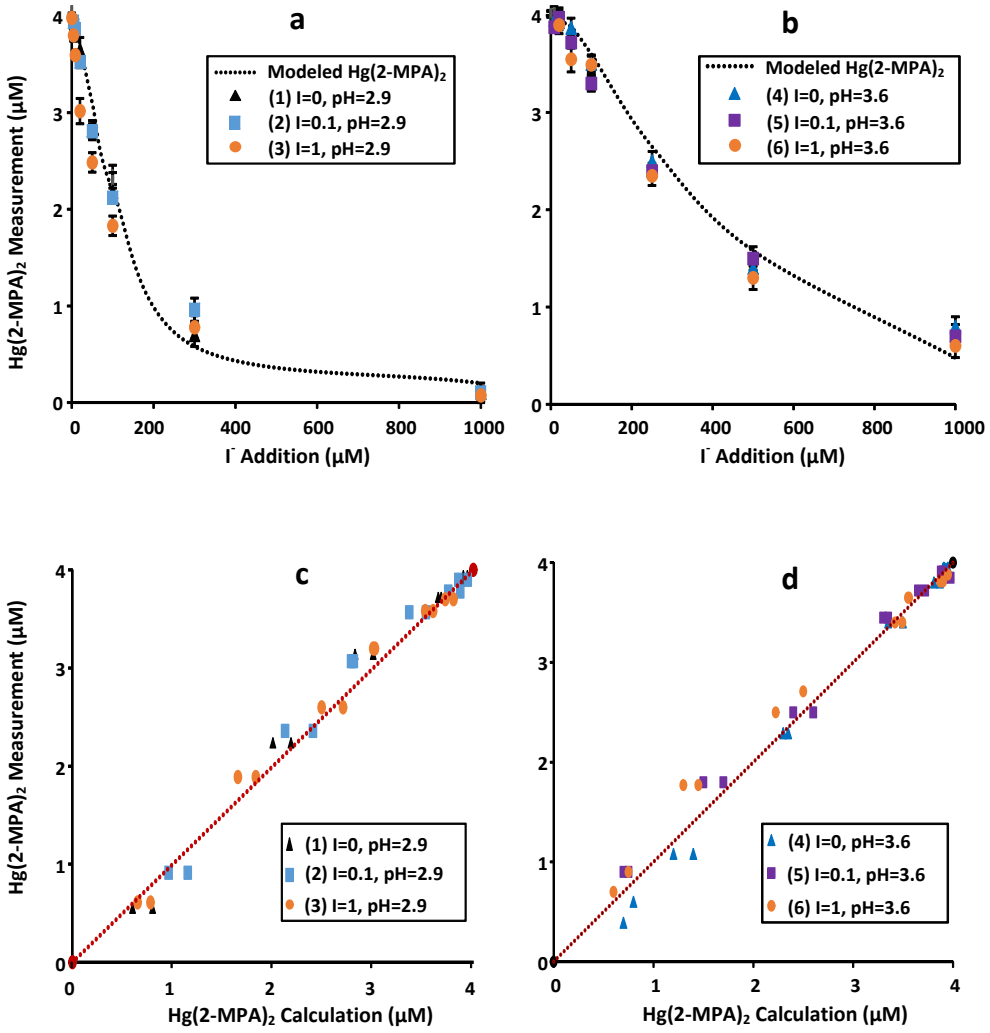
[d] Literature values corrected to I=0 and based on *pKa* values reported by Ugur et al.[24] to be comparable with constants determined in this study.



517

518 **Figure 1.** (a, b) Determined concentration of $Hg(MAC)_2$ as a function of the concentration of competing
519 ligand I^- between 0 µM and 1000 µM. Black dotted lines are the modeled concentration of the $Hg(MAC)_2$
520 complex from WinSGW using the optimized stability constant of log β_2 = 40.9 for $Hg(MAC)_2$. (c, d)
521 Correlation between measured and calculated $Hg(MAC)_2$ concentrations from WinSGW using the
522 optimized stability constant of log β_2 = 40.9 for the formation of $Hg(MAC)_2$ following reaction (1b).
523 Experiments were conducted at T= 25 ^oC with two different pH of 2.9 and 3.6 and three different ionic
524 strengths ($NaClO_4$) of 0, 0.1 and 1 M.

525



526

527 **Figure 2.** (a, b) Determined concentration of $Hg(2\text{-}MPA)_2$ as a function of the concentration of competing
528 ligand I^- between 0 μM and 1000 μM. Black dotted lines are the modeled concentration of $Hg(2\text{-}MPA)_2$
529 complex using the optimized stability constant of log β_2 = 40.9 for $Hg(2\text{-}MPA)_2$. (c, d) Correlation between
530 measured and calculated $Hg(2\text{-}MPA)_2$ concentrations using the optimized stability constant of log β_2 = 40.9
531 for the formation of $Hg(2\text{-}MPA)_2$ following reaction (1b). Experiments were conducted at T= 25 °C with two
532 different pH of 2.9 and 3.6 and three different ionic strengths ($NaClO_4$) of 0, 0.1 and 1 M.

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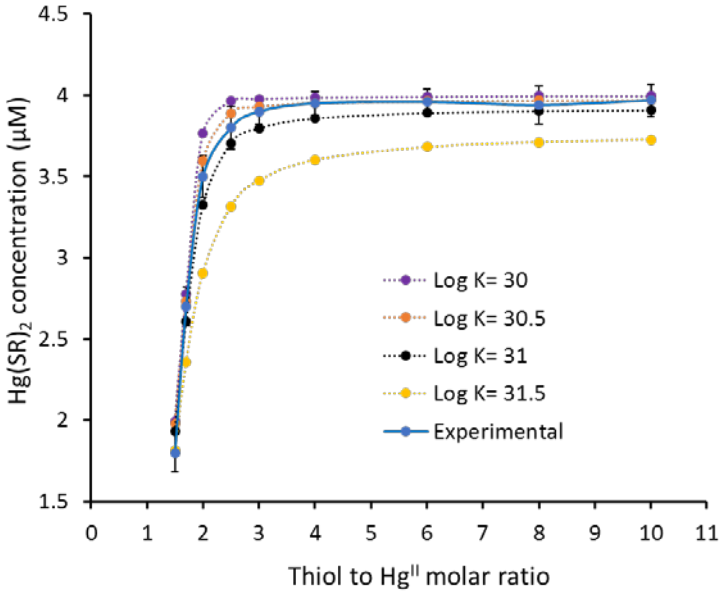


535

536 **Figure 3.** Average concentration of the $Hg(SR)_2$ complex as a function of molar ratio of LMM thiol to Hg^{II}
537 between 1.5 and 10. Experimental data (average ±SD, as represented by the solid blue line) were collected
538 from separate experiments with 4 µM of Hg^{II} and varying concentrations of six different LMM thiols (Cys,
539 HCys, GSH, MAC, Glyc, and NACCys). Dashed lines represent the modeled average concentrations of the
540 $Hg(SR)_2$ complex with log K_{HgSR+} (reaction 4) for the $HgSR^+$ complex varied between 30.0 and 31.5.

541

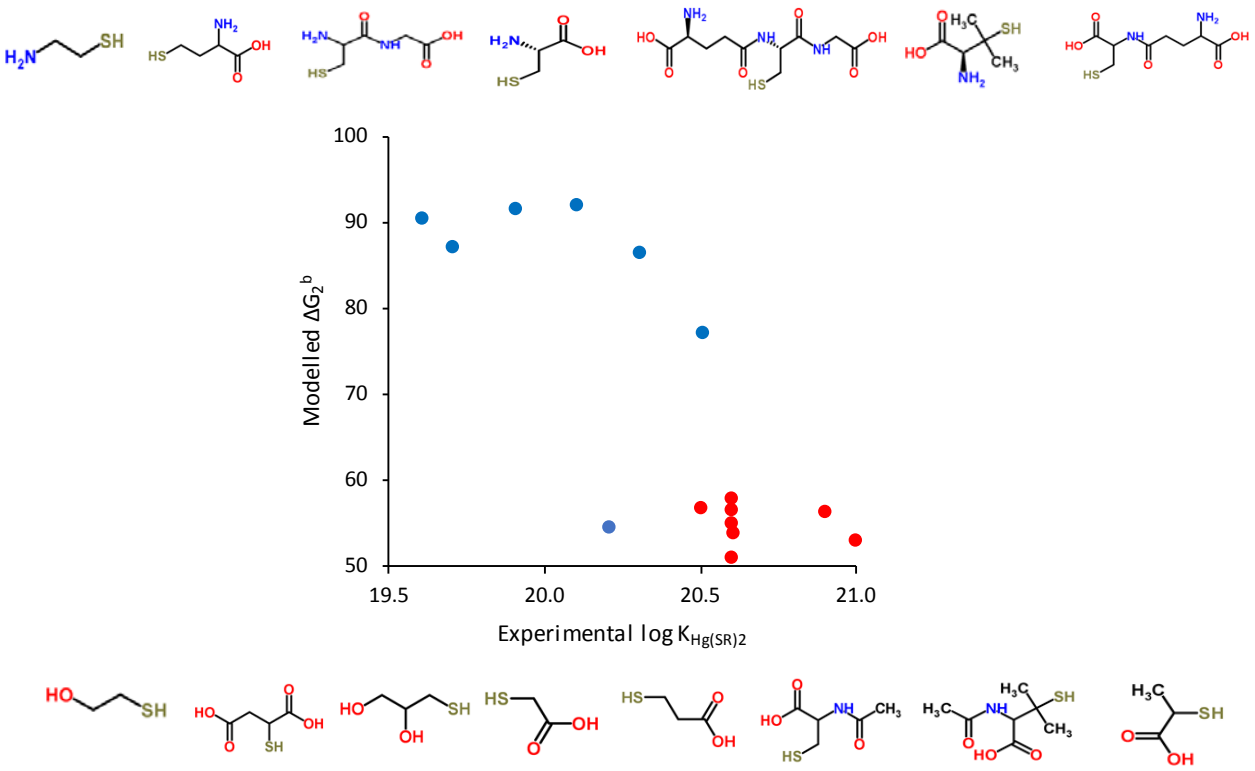
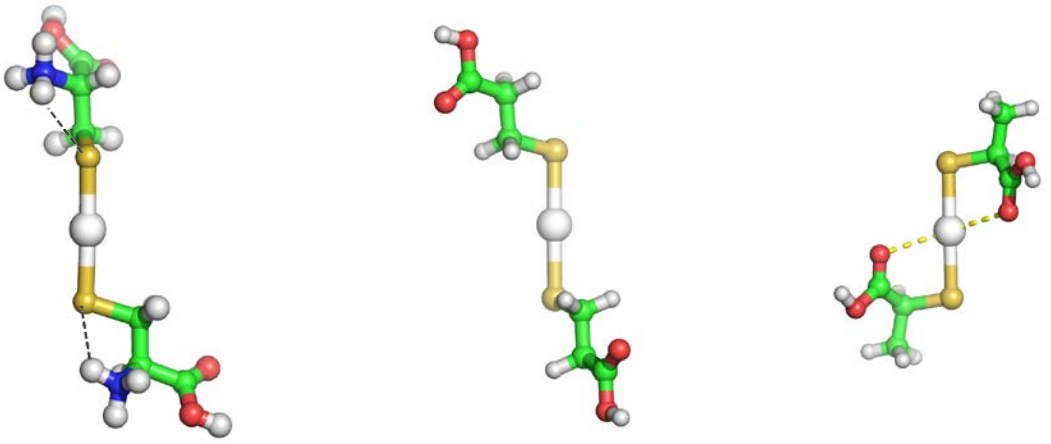


Figure 4. Comparison of the experimentally determined stability constant log $K_{Hg(SR)2}$ and the modeled (gas phase) Gibbs free energy (in kcal/mol) for the formation of the 15 $Hg(SR)_2$ complexes. Thiol ligands with a primary amino group are indicated by blue circles and their chemical structures are displayed at the top of the figure. Thiol ligands lacking a primary amino group are indicated by red circles and their chemical structures are displayed at the bottom of the figure. The thiol structures are arranged from the top left to the bottom right according to increased experimentally determined stability constant for the corresponding $Hg(SR)_2$ complex.



$Hg(Cys)_2$	$Hg(3\text{-}MPA)_2$	$Hg(2\text{-}MPA)_2$
Log *K* = 20.1	Log *K* = 20.6	Log *K* = 21.0

Figure 5. Illustration of intra-complex interactions affecting the stability of Hg^{II}-LMM thiol complexes. The presence of an electron withdrawing primary amino group destabilizes the Hg–S bond as illustrated by comparing the $Hg(Cys)_2$ and $Hg(3\text{-}MPA)_2$ complexes. The only structural differences between the complexes is the presence of a primary amino group in $Hg(Cys)_2$. Coordination to Hg^{2+} of electron donating groups in addition to the linear S-Hg-S configuration enhances the stability of the Hg^{II}-LMM thiol complex as illustrated by comparing the $Hg(3\text{-}MPA)_2$ and $Hg(2\text{-}MPA)_2$ complexes. The DFT modeling suggested that additional coordination to Hg^{II} by carboxylic oxygen is present in the $Hg(2\text{-}MPA)_2$ complex but not in the $Hg(3\text{-}MPA)_2$ complex.

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