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Formation of Hg(II) Tetrathiolate Complexes with Cysteine at Neutral pH

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Abstract

Mercury(II) ions precipitate from aqueous cysteine (H_2Cys) solutions containing $\text{H}_2\text{Cys}/\text{Hg(II)}$ mole ratio 2.0 as $\text{Hg}(\text{S-HCys})_2$. In absence of additional cysteine, the precipitate dissolves at pH ~ 12 with the $[\text{Hg}(\text{S},\text{N-Cys})_2]^{2-}$ complex dominating. With excess cysteine ($\text{H}_2\text{Cys}/\text{Hg(II)}$ mole ratio 4.0), higher complexes form and the precipitate dissolves at lower pH values. Previously, we found that tetrathiolate $[\text{Hg}(\text{S-Cys})_4]^{6-}$ complexes form at pH = 11.0; in this work we extend the investigation to pH values of physiological interest. We examined two series of Hg(II)-cysteine solutions in which $C_{\text{Hg(II)}}$ varied between 8 – 9 mM and 80 – 100 mM, respectively, with $\text{H}_2\text{Cys}/\text{Hg(II)}$ mole ratios from 4 to ~ 20 . The solutions were prepared in the pH range 7.1 – 8.8, at the pH at which the initial $\text{Hg}(\text{S-HCys})_2$ precipitate dissolved. The variations in the Hg(II) speciation were followed by ^{199}Hg NMR, X-ray absorption and Raman spectroscopic techniques. Our results show that in the dilute solutions ($C_{\text{Hg(II)}} = 8 - 9 \text{ mM}$), mixtures of di-, tri- (major) and tetrathiolate complexes exist at moderate cysteine excess ($C_{\text{H}_2\text{Cys}} \sim 0.16 \text{ M}$) at pH 7.1. In the more concentrated solutions ($C_{\text{Hg(II)}} = 80 - 100 \text{ mM}$) with high cysteine excess ($C_{\text{H}_2\text{Cys}} > 0.9 \text{ M}$), tetrathiolate $[\text{Hg}(\text{S-cysteinate})_4]^{m-6}$ ($m = 0 - 4$) complexes dominate in the pH range 7.3 – 7.8, with lower charge than for the $[\text{Hg}(\text{S-Cys})_4]^{6-}$ complex due to protonation of some (m) of the amino groups of the coordinated cysteine ligands. The results of this investigation could provide a key to the mechanism of biosorption and accumulation of Hg(II) ions in biological / environmental systems.

Keywords

Mercury(II); Cysteine; EXAFS; ^{199}Hg NMR; Raman

Introduction

The soft thiolate group (R-S^-) has high affinity to mercury(II) ions.¹ The chemistry of Hg(II) bound only to thiolate ligands is dominated by complexes with low coordination

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Supporting Information

Fraction diagram of cysteine species vs. pH; details for least-squares curve-fitting of EXAFS spectra of solutions A – F and G – L; PCA analysis of the EXAFS spectra for solutions G – L; results from fitting linear combinations of simulated EXAFS oscillations for $\text{Hg}(\text{cysteinate})_n$ ($n = 2, 3, 4$) species to the experimental EXAFS spectra for solutions F and G – L; comparison between the EXAFS spectra of solutions F and G; Raman spectra of solutions G – L compared to that of an aqueous cysteine solution; supporting calculations of ^{199}Hg NMR chemical shifts for HgS_2 species in solution F and for HgS_3 species in solution G.

numbers, mainly with linear, trigonal planar and tetrahedral coordination geometries.² While the Hg-S bonds in Hg(II) dithiolate complexes are closely symmetrical ($R_{\text{Hg-S}} \pm 0.02 \text{ \AA}$), the Hg-S bond distances in Hg(SR)₃ and Hg(SR)₄ complexes show larger distributions ($R_{\text{Hg-S}} \pm 0.12 \text{ \AA}$ and $\pm 0.5\text{-}0.6 \text{ \AA}$, respectively).³ According to the Cambridge crystal structure database (CSD), the average Hg-S distance (R_{ave}) increases stepwise by $\sim 0.1 \text{ \AA}$ with increasing number of thiolate groups in the first coordination sphere of the Hg(II) ion: $R_{\text{ave}} = 2.345 \text{ \AA} \pm 0.025 \text{ \AA}$ for Hg(SR)₂, $2.446 \text{ \AA} \pm 0.018 \text{ \AA}$ for Hg(SR)₃, and $2.566 \text{ \AA} \pm 0.047 \text{ \AA}$ for Hg(SR)₄ species.³

Cysteine is the only proteinogenic thiol-containing amino acid and its side chain is the principal coordination site for Hg(II) in proteins and enzymes.² Mercury ions can even become inserted into the disulfide bridges connecting adjoining polypeptide chains.⁴ Such coordination can inhibit the function of enzymes⁵ and folding of proteins,⁶ and is a reason for the high toxicity of mercury. Conversely, the affinity of mercury toward the thiol group is important in developing chelating agents and for mercury detoxification.⁷

Cysteine (H₂Cys) has three functional groups: thiol, amino and carboxylate (Scheme 1). The -SH and -NH₃⁺ groups of cysteine start to deprotonate almost simultaneously around pH 8.5, with the Cys²⁻ ion dominating above pH ~ 10.5 (Figure S-1).⁸ Complexes with Hg(II)/cysteine in mole ratios 1:2 and 1:3 in alkaline aqueous solution had been established by ¹³C NMR measurements.⁹ Our group explored the possibility of formation of complexes with higher coordination number in excess of free cysteinate ions at alkaline pH (11.0), and discovered that the four-coordinated [Hg(*S*-Cys)₄]⁶⁻ complex with the mean Hg-S distance $2.52 \pm 0.02 \text{ \AA}$ can dominate in solutions with the concentration of free cysteinate [Cys²⁻] > 0.1 M.¹⁰ Results from ¹⁹⁹Hg NMR, extended X-ray absorption fine structure (EXAFS) and Raman spectroscopic techniques were combined to estimate the amount of [Hg(*S,N*-Cys)₂]²⁻, [Hg(*S*-Cys)₃]⁴⁻ and [Hg(*S*-Cys)₄]⁶⁻ species present in alkaline solution and to propose the conditional stability constants $\log \beta_n = 40.0, 41.3$ and 42.4 , respectively, for the equilibria: $\text{Hg}^{2+} + n \text{ Cys}^{2-} \leftrightarrow \text{Hg}(\text{Cys})_n$ where $n = 2, 3, 4$.¹⁰

Tri- and tetrathiolate Hg(II) complexes have been also reported to form in cysteine rich *de novo* designed triple-stranded coiled coil TRI peptides,¹¹ and in the human copper chaperone HAH1 bound to Hg(II) ions in solution (pH 9.4).¹² A relevant question is then whether four-coordinated Hg(*S*-cysteinate)₄ species could form in solution at physiological pH (~ 7.4). A recent report on Hg(II) biosorption by *E. Coli* shows that Hg(II) is bound to 2 – 4 thiol groups at the membrane of non-metabolizing cells and also within metabolizing cells in the absence of externally added organic ligands. However, in the presence of cysteine added to the media, Hg(II) ions bound to four thiol groups accumulate within the cytoplasm of metabolizing cells.¹³ Mercury(II)-thiolate clusters have been identified in peat and soil natural organic matter;¹⁴ however, it is not clear how such metallothionein-like clusters are formed. Therefore, exploring the Hg(II) cysteine-thiolate coordination and speciation at physiological pH is of both biochemical and environmental interest.

In the previous study in our group the pH of the solutions was fixed at 11.0, close to the pH at which the Hg(HCys)₂ precipitate completely dissolved at a mole ratio H₂Cys/Hg(II) = 2.2. In the present study, we prepared two series of solutions containing $C_{\text{Hg(II)}} = 8 - 9 \text{ mM}$

and 80 – 100 mM, varying the H₂Cys/Hg(II) mole ratio from 4 to ~20 in both. Each solution was kept at the lowest pH value at which the initial milky Hg(Cys)₂ precipitate dissolved. The pH of the solutions varied from 8.5 to 7.1 in the first series, and from 9.1 to 7.3 in the second (Table 1); the higher the amount of cysteine, the lower the pH for which a clear solution was obtained. Increasing solubility of Hg(HCys)₂ at increasing ionic strength is expected from variations of activity coefficients (γ) in solution, resulting in an increase in the solubility product (K_{sp}),¹⁵ where

Hg L_{III}-edge EXAFS, ¹⁹⁹Hg NMR and Raman spectroscopic techniques were used to evaluate the chemical speciation of the Hg(II)-cysteine complexes in these solutions.

Experimental Section

Sample Preparation

Mercury(II) perchlorate hydrate, Hg(ClO₄)₂•xH₂O ($x = 0.87$), and cysteine obtained from Aldrich were used to prepare two series of Hg(II)-cysteine aqueous solutions containing C_{Hg(II)} = 8 – 9 mM (A – F) or 80 – 100 mM (G – L), with increasing cysteine/Hg(II) mole ratios (Table 1). The solutions were generally prepared by adding an appropriate amount of cysteine dissolved in 3 - 4 mL deoxygenated, boiled water to a solution of (0.05 or 0.5 mmol) mercury(II) perchlorate hydrate in 0.6 mL D₂O under a stream of argon gas. NaOH solutions (6 M, 2 M and 0.2 M) were added dropwise until the initially formed milky precipitate dissolved to a clear solution. The measured final volume varied between 5.0 – 6.2 mL.

¹⁹⁹Hg NMR and Raman Spectroscopy

The spectra were obtained using a Bruker AMX-300 instrument equipped with a 10 mm broadband probe (BB10). The resonance frequency of the acquisition was 53.72 MHz. A saturated solution of HgCl₂ in D₂O was used as an external reference, setting its peak position at –1550 ppm relative to Hg(CH₃)₂ ($\delta = 0$ ppm).^{2, 16} For solutions A – F and G – L, 4000 and 50,000 scans were collected, respectively, using a 90° pulse at 300 K with sweep width 1100 ppm and 1s delay between the scans. All spectra were processed using 10% exponential line broadening.

Raman spectra of solutions G – L placed in glass vials were measured with a 500 mW Bruker RAM II FT-Raman spectrometer equipped with a liquid N₂ cooled Ge detector and YAG laser (1064 nm), co-adding 3286 scans for each solution. Baseline correction was carried out using the OPUS program by subtracting the spectrum of a glass vial containing water.

X-ray absorption spectroscopy

Hg L_{III}-edge X-ray absorption spectra (XAS) were measured at ambient temperature at beam line 7-3 of the Stanford Synchrotron Radiation Lightsource (SSRL) (3.0 GeV, 85 – 100 mA). Higher harmonics from a Si [220] double crystal monochromator were rejected by

detuning the incident beam (I_0) to 50% of its maximum intensity at the end of the scan. Crystalline HgCl_2 powder was used as an external calibration, with the first inflection point of its absorption edge set at 12284 eV. The solutions were placed in a 5 mm Teflon spacer between 5 μm polypropylene windows. For the solutions G – L 3 – 4 scans were collected in transmission mode, and for the dilute solutions A – F 10 – 11 scans were measured in fluorescence mode using a 30-element Ge detector. All scans and channels were compared before averaging, to ensure that radiation damage had not occurred.

Details of the EXAFS data analysis procedures have been explained elsewhere.¹⁰ The crystal structure of $\text{Hg}(\text{NH}_2\text{CH}_2\text{CH}_2\text{S})_2$ was used to create an input file for the FEFF 8.1 program.^{17, 18} Least-squares curve-fitting of the k^3 -weighted EXAFS oscillations was performed in the k -range 2.6 – 13 $\text{\AA}^{-1}$ using the WinXAS 3.1 program,¹⁹ with fixed amplitude reduction factor ($S_0^2 = 0.9$) to allow refinement of the coordination number (N). Principal Component Analysis (PCA), carried out over the k -range 2.6 – 12.5 $\text{\AA}^{-1}$ for the raw EXAFS oscillations of solutions G – L using the EXAFSPAK program package,²⁰ showed contributions from two major and one minor components. Relative amounts of the di-, tri- and tetrathiolate $\text{Hg}(\text{II})$ cysteinate complexes were estimated by fitting the experimental EXAFS spectra of solutions F and G – L to linear combinations of simulated EXAFS oscillations for the $[\text{Hg}(\text{S},\text{N-Cys})_2]^{2-}$, $\text{Hg}(\text{S-cysteinate})_3$ and $\text{Hg}(\text{S-cysteinate})_4$ species over the k -range 4 – 12 $\text{\AA}^{-1}$. The parameters used for simulating EXAFS oscillations were: $\text{Hg}(\text{S-cysteinate})_2$, Hg-S 2.32 – 2.33 $\text{\AA}$, $\sigma^2 = 0.003 \text{ \AA}^2$; $[\text{Hg}(\text{S},\text{N-Cys})_2]^{2-}$, Hg-S 2.34 $\text{\AA}$ ($\sigma^2 = 0.003 \text{ \AA}^2$), Hg-N 2.52 $\text{\AA}$ ($\sigma^2 = 0.010 \text{ \AA}^2$); $\text{Hg}(\text{S-cysteinate})_3$, Hg-S 2.42 – 2.45 $\text{\AA}$, $\sigma^2 = 0.006 \text{ \AA}^2$; $\text{Hg}(\text{S-cysteinate})_4$, Hg-S 2.52 – 2.53 $\text{\AA}$, $\sigma^2 = 0.008 \text{ \AA}^2$.^{10, 21}

Results and Discussions

EXAFS Spectroscopy

The least-squares curve-fitting results for the Hg L_{III}-edge EXAFS spectra of the $\text{Hg}(\text{II})$ cysteine solutions A – F ($C_{\text{Hg}(\text{II})} = 8 - 9 \text{ mM}$) and G – L ($C_{\text{Hg}(\text{II})} = 80 - 100 \text{ mM}$) are shown in Figure 1, with the refined structural parameters in Table 2 (details are provided in Table S-1).

For both series of solutions the average Hg-S distance increases as the $\text{H}_2\text{Cys}/\text{Hg}(\text{II})$ mole ratio and the free cysteine concentration increases. For solutions A – F the average Hg-S distance shows an increase from 2.35 to 2.43 $\text{\AA}$. The corresponding mean Debye-Waller (DW) parameter σ^2 increases from 0.0024 to 0.0093 $\text{\AA}^2$, reflecting a larger variation around the average Hg-S distance in the solutions with higher $\text{H}_2\text{Cys}/\text{Hg}(\text{II})$ mole ratios. The EXAFS spectrum of solution A containing $C_{\text{Hg}(\text{II})} = 9 \text{ mM}$ and $C_{\text{H}_2\text{Cys}} = 37 \text{ mM}$ ($\text{pH} = 8.5$) fitted well to an HgS_2N_2 model with $(\text{Hg-S})_{\text{ave}} = 2.35 \pm 0.02 \text{ \AA}$ and $(\text{Hg-N})_{\text{ave}} = 2.50 \pm 0.02 \text{ \AA}$, and corresponding Debye-Waller parameters $\sigma^2 = 0.0024 \pm 0.001 \text{ \AA}^2$ and $0.029 \pm 0.002 \text{ \AA}^2$ (model II in Table S-1). The high DW parameter for the Hg-N scattering path could be due to partial protonation of the amine group at $\text{pH} = 8.5$ in $[\text{Hg}(\text{S-HCys})(\text{S},\text{N-Cys})]^-$ complexes. Introduction of a Hg-N path in the EXAFS model fitting of the solutions B to F with higher $\text{H}_2\text{Cys}/\text{Hg}(\text{II})$ mole ratios resulted in a very high DW parameter ($\sigma^2 > 0.05 \text{ \AA}^2$) or rejection.

For the more concentrated solutions G – L containing $C_{\text{Hg(II)}} = 80 - 100 \text{ mM}$, the mean Hg-S distance elongates from 2.44 to 2.51 Å, while the DW parameter decreases from $\sigma^2 = 0.011$ to 0.008 Å² as the cysteine concentration increases. The PCA (Principal Component Analysis) of the six spectra showed two major and one minor species (Figure S-2). To estimate the amount of Hg(II)-cysteine complexes with di-, tri- or tetrathiolate coordination, we fitted linear combinations of simulated EXAFS oscillations to the experimental EXAFS spectra for solutions G – L, using the DATFIT program in the EXAFSPAK suite of programs (see *Experimental Section*).²⁰ The simulated EXAFS oscillations for Hg(*S*-cysteinate)₂ (Hg-S 2.32 – 2.33 Å) were consistently rejected in all fittings, and the best fits were obtained using an [Hg(*S,N*-Cys)₂]²⁻ model, and for HgS₃ and HgS₄ coordination the mean distances 2.44 – 2.45 Å and 2.52 – 2.53 Å, respectively. These parameters also produced the best fits in our previous studies.^{10, 21} The average Hg-S distance for the Hg(cysteinate)₃ species (2.44 ± 0.02 Å) is comparable to the average crystallographic distance 2.446 Å ± 0.018 Å for trithiolate Hg(SR)₃ complexes,³ and that of the [Hg(*S*-cysteinate)₄]^{m-6} species (2.52 ± 0.02 Å)¹⁰ is similar to those of the [Hg(*S*-NAC)₄]⁶⁻ and [Hg(*S*-GSH)₄]¹⁰⁻ complexes, all with HgS₄ coordination.^{22, 23}

The fitting results displayed in Tables 3 and S-2, and Figures S-3a,b are consistent with the two major species Hg(cysteinate)₃ and Hg(cysteinate)₄, with the latter dominating in solutions H – L. Solution L contains 70% [Hg(*S*-HCys)₄]²⁻ complexes (assuming all amine groups of the coordinated cysteine ligands are protonated at this pH) at pH = 7.3 and free cysteine concentration ([H₂Cys] + [HCys⁻]) 1.4 M. Excluding the minor dithiolate [Hg(*S,N*-Cys)₂]²⁻ component from the fitting of linear combinations to the EXAFS spectrum resulted in a higher fitting residual for solution G; see Figure S-4. For solution H a minor amount (~ 5%) of Hg(cysteinate)₂ species was included in the fitting, based on the minor band observed at 335 cm⁻¹ in its Raman spectrum (*see below*).

Raman Spectroscopy

The Raman spectra of the Hg(II) cysteine solutions G – L are compared with that of a 0.4 M cysteine solution (pH 7.1) in Figure S-5, with the background subtracted ones shown in Figure 2 (*left*). Two bands can be associated with the Hg(cysteinate)_{*n*} (*n* = 2, 3, 4) complexes. The small band that appears at 335 cm⁻¹ in the spectra of solutions G and H (Figure 2, *right*), is assigned to symmetric S-Hg-S stretching in the [Hg(Cys)₂]²⁻ and [Hg(HCys)(Cys)]⁻ complexes.¹⁰

In the spectrum of solution G a broad feature appears at 278 cm⁻¹, which gradually shifts to 270 cm⁻¹ and gains intensity with increasing amount of cysteine in the solutions. A similar feature previously observed for alkaline Hg(II) cysteine solutions (pH = 11.0) was fitted to two bands at 285 and 265 cm⁻¹, attributed to [Hg(Cys)₃]⁴⁻ and [Hg(Cys)₄]⁶⁻ complexes, respectively.¹⁰ Therefore, higher intensity of the peak shifted towards 270 cm⁻¹ should correspond to higher amount of [Hg(*S*-cysteinate)₄]^{m-6} species in the solutions (*m* is the number of protonated amine group of coordinated cysteine ligands), which is consistent with the EXAFS results (Table 3). The other main peaks in the Figure 2 (*left*) originate from the ClO₄⁻ anion (e.g. 934 cm⁻¹, Cl-O symmetric stretching) and cysteine (685 cm⁻¹, C-S stretching).¹⁰

¹⁹⁹Hg NMR Spectroscopy

All Hg(II)-cysteine solutions (A – L) showed a single resonance in their ¹⁹⁹Hg NMR spectra (Figure 3), as an average for all Hg(II) species that are in fast ligand exchange equilibria (relative to the NMR time scale) in these solutions. The ¹⁹⁹Hg chemical shifts observed for these solutions can be compared with those of Hg(*S*-GSH)₂ (HgS₂; δ_{Hg} = −960 ~ −993 ppm; *R*_{Hg-S} = 2.32 ± 0.02 Å),²³⁻²⁵ [Hg(*S,N*-Pen)₂]²⁻ (HgS₂N₂, δ_{Hg} = −619 ppm) and [Hg(*S,N*-Cys)₂]²⁻ (HgS₂N₂; δ_{Hg} = −609 ~ −656 ppm; *R*_{Hg-S} = 2.34 ± 0.02 Å),^{10, 21} Hg(SCH₂CH₂NH₂)₂ (HgS₂N₂; δ_{Hg} = −608),¹⁷ [Hg(Pen)₃]⁴⁻ (HgS₃N₂; δ_{Hg} = −390 ppm; *R*_{Hg-S} = 2.44 ± 0.02 Å),²¹ the estimated δ(¹⁹⁹Hg) values for Hg(*S*-GSH)₃ and [Hg(*S*-NAC)₃]⁴⁻ (HgS₃; δ_{Hg} ~ −170 ppm; *R*_{Hg-S} = 2.42 ± 0.02 Å),^{22, 23} as well as [Hg(*S*-Cys)₄]⁶⁻ and [Hg(*S*-NAC)₄]⁶⁻ (HgS₄; δ_{Hg} = −340 ~ −350 ppm; *R*_{Hg-S} = 2.52 ± 0.02 Å).^{10, 22} (*abbreviations*: GSH = glutathione; H₂Pen = penicillamine; H₂NAC = *N*-acetylcysteine). For trigonal planar HgS₃ coordination in Hg(*S*-cysteinate)₃ the experimental δ(¹⁹⁹Hg) = −185 ppm was reported.^{12, 26} Also, in combination with PAC (^{199m}Hg perturbed angular correlation spectroscopy), a ¹⁹⁹Hg NMR signal at δ_{Hg} = −348 ppm obtained for Hg(II) bound to a dimeric HAH1 metallochaperone (pH = 8.5 – 9.4) has been assigned to a mixture of distorted (T-shaped) HgS₃ and HgS₄ structures in equilibrium.¹²

The chemical shift for solution A (δ_{Hg} = −565 ppm, H₂Cys/Hg(II) mole ratio 4.1; pH = 8.5) with average *R*_{Hg-S} 2.35 Å ± 0.02 Å from EXAFS, is somewhat shifted relative to that of the [Hg(Cys/Pen)₂]²⁻ species with HgS₂N₂ coordination; see above. The reason could be formation of some amount of [Hg(*S*-HCys)(*S,N*-Cys)]⁻ with HgS₂N coordination, since one amino group could be protonated at this pH, and/ or presence of a minor amount of trithiolate [Hg(HCys)_{*m*}(Cys)_{3-*m*}]^{*m*-4} (*m* = 0 - 3) species with HgS₃N₁₋₂ or HgS₃ coordination, making the average Hg-S distance slightly longer than that of the [Hg(*S,N*-Cys)₂]²⁻ complex.²¹ As the total cysteine concentration increases in solutions B – F (*C*_{Hg(II)} = 8 – 9 mM, H₂Cys/Hg(II) mole ratios 5.2 – 20.0; pH = 8.3 – 7.1) and the average Hg-S distance increases from 2.37 Å to 2.43 Å (Table 2), the ¹⁹⁹Hg NMR resonance shifts to about −386 to −401 ppm, indicating formation of Hg(II) complexes with higher coordination numbers.

In solution F (pH = 7.1; *R*_{Hg-S} = 2.43 ± 0.02 Å) with δ_{Hg} = −401 ppm, it is likely that most of the amino groups in the coordinated cysteinate ligands are protonated, resulting in a mixture of trigonal planar trithiolate [Hg(*S*-HCys)₃]⁻ (HgS₃; δ_{Hg} = −185 ppm; *R*_{Hg-S} = 2.42 ± 0.02 Å as in [Hg(*S*-NAC)₃]⁴⁻ and tetrathiolate [Hg(*S*-HCys)₄]²⁻ complexes (HgS₄; δ_{Hg} ~ −340 ppm; *R*_{Hg-S} = 2.52 ± 0.02 Å as in [Hg(*S*-Cys)₄]⁶⁻), with a minor amount of dithiolate species. Linear combination fitting of models to the EXAFS spectrum of solution F shows that trithiolates (HgS₃) account for more than 50% and tetrathiolates (HgS₄) for about 25% of the Hg(II) ions (Tables 3 and S-2; Figure S-3c). The simulated EXAFS oscillation for digonal HgS₂ coordination with *R*_{HgS} = 2.32 – 2.33 Å was consistently rejected in the fitting model in favor of the EXAFS oscillation for the [Hg(*S,N*-Cys)₂]²⁻ chelate with HgS₂N₂ coordination (Hg-S 2.34 Å; Hg-N 2.52 Å). However, considering the neutral pH of the solution F, it seems more likely that the [Hg(*S,N*Cys)(*S*-HCys)]⁻ or dissolved Hg(*S*-HCys)₂(aq) dithiolate complexes are present. Using the estimated relative amounts of the di-, tri- and tetrathiolates in solution F (Table 3), and the ¹⁹⁹Hg NMR chemical shifts of the

trigonal planar HgS_3 ($\delta_{\text{Hg}} = -185$ ppm),^{12, 26} and tetrahedral HgS_4 ($\delta_{\text{Hg}} = -340$ ppm),¹⁰ a chemical shift between about $\delta_{\text{Hg}} -1140$ and -1200 ppm would be expected for the dithiolate species in solution F (see Appendix I in *Supporting Materials*), which is within the δ_{Hg} range (-800 to -1200 ppm) for mercury(II) dithiolate complexes.²

The similar average Hg-S distances ($2.43 - 2.44$ Å) and chemical shifts, $\delta(^{199}\text{Hg}) = -400$ ppm, of solution G ($C_{\text{Hg(II)}} = 83$ mM, $\text{H}_2\text{Cys}/\text{Hg(II)} = 4.0$, $\text{pH} = 9.1$) and the dilute solution F ($C_{\text{Hg(II)}} = 8$ mM, $\text{H}_2\text{Cys}/\text{Hg(II)} = 20$, $\text{pH} = 7.1$) seem to indicate related Hg(II) speciation (see Table 2 and Figure 3); however, the difference in the pH values should affect the extent of amine protonation of the coordinated cysteine ligands. Figure S-3d shows similar, but not overlapping, EXAFS oscillations of these two solutions. Linear combination fitting of models to the EXAFS spectra for solutions F and G reveals that despite their nearly identical ^{199}Hg NMR chemical shifts, solution G contains higher amount of the tetrathiolate HgS_4 and lower amount of the trithiolate HgS_3 species (Tables 3 and S-2; Figure S-3c). Because of the difference in pH the trithiolate $[\text{Hg}(\text{HCys})_m(\text{Cys})_{3-m}]^{m-4}$ ($m = 0 - 3$) species could have different coordination environment in solution G ($\text{pH} = 9.1$), that is $[\text{Hg}(\text{S},\text{N}-\text{Cys})_2(\text{S}-\text{Cys})]^{4-}$, $[\text{Hg}(\text{S},\text{N}-\text{Cys})_2(\text{S}-\text{HCys})]^{3-}$ (HgS_3N_2) and/ or $[\text{Hg}(\text{S},\text{N}-\text{Cys})(\text{S}-\text{HCys})_2]^{2-}$ (HgS_3N), than the HgS_3 coordination in $[\text{Hg}(\text{S}-\text{HCys})_3]^-$ in solution F at $\text{pH} 7.1$, when all amino groups of the coordinated cysteinate ligands probably are protonated. The presence of long distance, weak Hg... N interactions would influence the shielding of the ^{199}Hg nucleus and its NMR chemical shift. For example, the ^{199}Hg NMR chemical shift of the two-coordinated (HgS_2) dithiolate $\text{Hg}(\text{S}-\text{GSH})_2$ is more shielded than those of the four-coordinated (HgS_2N_2) $[\text{Hg}(\text{S},\text{N}-\text{Pen})_2]^{2-}$ or $[\text{Hg}(\text{S},\text{N}-\text{Cys})_2]^{2-}$ chelate complexes; see above. Also, the estimated ^{199}Hg NMR chemical shifts for the trigonal planar $\text{Hg}(\text{S}-\text{GSH})_3$ and $[\text{Hg}(\text{S}-\text{NAC})_3]^{4-}$ complexes (HgS_3 ; $\delta_{\text{Hg}} \sim -170$ ppm; $R_{\text{Hg-S}} = 2.42 \pm 0.02$ Å) are less shielded than that of the $[\text{Hg}(\text{Pen})_3]^{4-}$ complex ($\delta_{\text{Hg}} \sim -390$ ppm).²¹⁻²³ Based on the ^{199}Hg NMR results, a trigonal bipyramidal HgS_3N_2 structure was proposed for the $[\text{Hg}(\text{Pen})_3]^{4-}$ complex formed in alkaline media ($\text{pH} = 11.0$), in which the three Hg-S bonds occupy the equatorial plane ($R_{\text{Hg-S}} = 2.44 \pm 0.02$ Å) with nitrogen atoms from the amino groups located above and below this plane.²¹ The Hg... N scattering, however, could not be resolved by EXAFS curve-fitting, probably due to the dominating Hg-S scattering and a high σ^2 value for the Hg... N path. It is very likely that the coordination in the $\text{Hg}(\text{cysteinate})_3$ species in the alkaline solution G is similar to that in the $[\text{Hg}(\text{Pen})_3]^{4-}$ complex. Considering the ^{199}Hg NMR chemical shift of solution G (-400 ppm), and those of the chelate $[(\text{S},\text{N}-\text{Cys})_2]^{2-}$ (HgS_2N_2 ; $\delta_{\text{Hg}} = -609$ ppm) and tetrahedral $[\text{Hg}(\text{S}-\text{Cys})_4]^{6-}$ (HgS_4 ; $\delta_{\text{Hg}} = -340$ ppm),¹⁰ and the estimated relative amounts of di-, tri- and tetrathiolates in solution G (Table 3), a chemical shift δ_{Hg} between -376 and -396 ppm can be calculated for the trithiolate species in solution G, comparable with the ^{199}Hg NMR chemical shift of the $[\text{Hg}(\text{Pen})_3]^{4-}$ complex ($\delta_{\text{Hg}} \sim -390$ ppm); see Appendix I in *Supporting Materials*.

Solutions I – L with cysteinate anions in high excess show very similar average Hg-S distances ($2.50 - 2.51$ Å) and also ^{199}Hg NMR chemical shifts ($-347 \sim -357$ ppm) that are close to the estimated values for the $[\text{Hg}(\text{S}-\text{Cys})_4]^{6-}$ (-340 ppm) and $[\text{Hg}(\text{S}-\text{NAC})_4]^{6-}$ species (-350 ppm).^{10, 22} This observation supports our fitting of linear combinations of model oscillations to the experimental EXAFS spectra, which resulted in HgS_4 coordination for at least 70% of the Hg(II) species in these solutions (Tables 3 and S-2).

Conclusions

The combined results from Hg L_{III}-edge EXAFS, ¹⁹⁹Hg NMR and Raman spectroscopic techniques reveal that in dilute mercury(II)-cysteine solutions ($C_{\text{Hg(II)}} = 8 - 9 \text{ mM}$) with moderate excess of cysteine ($C_{\text{H}_2\text{Cys}} \sim 0.16 \text{ M}$), a considerable amount (20 – 30%) of the four-coordinated tetrathiolate $[\text{Hg}(\text{S-cysteinate})_4]^{2-}$ complex is formed at neutral pH 7.1, in equilibrium with a dominating trithiolate $[\text{Hg}(\text{S-HCys})_3]^-$ complex, and minor amount of dithiolate Hg(II) species. Tetrathiolate $[\text{Hg}(\text{S-cysteinate})_4]^{m-6}$ species (m is the number of coordinated cysteine ligands with a protonated amino group) dominate at near physiological pH values (7.3 – 7.8) in more concentrated aqueous solutions ($C_{\text{Hg(II)}} = 80 - 100 \text{ mM}$) with large excess of cysteine ($C_{\text{H}_2\text{Cys}} > 0.9 \text{ M}$). The results are comparable with those from our group's previous study of alkaline mercury(II)-cysteine solutions ($C_{\text{Hg(II)}} \sim 90 \text{ mM}$, pH = 11.0) where lower excess cysteine ($C_{\text{H}_2\text{Cys}} > 0.5 \text{ M}$) was needed for the unprecedented $[\text{Hg}(\text{S-Cys})_4]^{6-}$ complex to dominate,¹⁰ as all cysteine is in its thiolate form at pH = 11.0. Most theoretical models of the logarithmic value of the activity coefficient ($\log \gamma_i$) as a function of ionic strength for an ionic species i (with charge Z) in electrolyte solutions contain a major Debye-Hückel like term with Z^2 dependence:^{15, 27} the higher the charge, the lower the activity coefficient of the ion. The stepwise protonation of the highly charged tetrathiolate $[\text{Hg}(\text{Cys})_4]^{6-}$ complex to $[\text{Hg}(\text{HCys})_m(\text{Cys})_{4-m}]^{m-6}$ ($m = 1 - 4$) species with decreasing pH is expected to substantially increase the activity, and therefore, the stability of the tetrathiolate species and ultimately the $\text{Hg}(\text{HCys})_4^{2-}$ complex that probably dominates near neutral pH values.

The results obtained in the current study clearly show that in presence of excess cysteine, tetrathiolate Hg(II) cysteine species can form even at near physiological pH. Such information may be important in understanding the mechanism of Hg(II) transfer, exchange, absorption and accumulation in biological systems and in the environment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Scheme 1. Structure of cysteine (H₂Cys) in its zwitterionic form

Figure 1.

Least-squares curve-fitting of the k^3 -weighted Hg L_{III}-edge EXAFS spectra (a) of the Hg(II)-cysteine solutions A – F ($C_{\text{Hg(II)}} = 8 - 9 \text{ mM}$) and G – L ($C_{\text{Hg(II)}} = 80 - 100 \text{ mM}$), with their corresponding Fourier-transforms (b); see Table 2.

Figure 2.

Left) Background subtracted Raman spectra of Hg(II) cysteine solutions G – L containing $C_{\text{Hg(II)}} = 80 - 100$ mM with increasing $\text{H}_2\text{Cys}/\text{Hg(II)}$ mole ratios. *Right*) The spectra of solutions G and H prior to background subtraction; for other solutions, see Figure S-5.

Figure 3. ^{199}Hg NMR spectra of Hg(II) cysteine solutions A – F ($C_{\text{Hg(II)}} = 8 - 9$ mM) and G – L ($C_{\text{Hg(II)}} = 80 - 100$ mM) with $\text{H}_2\text{Cys}/\text{Hg(II)}$ mole ratios 4 ~ 20.

Table 1Composition of the Hg^{II}-cysteine solutions^a

Solution	H ₂ Cys/Hg ^{II} mole ratio	[Hg ²⁺] _{tot}	[H ₂ Cys] _{tot}	pH ^b
A	4.1	9	37	8.5
B	5.2	9	47	8.3
C	6.5	8	52	8.1
D	10.7	8	86	7.7
E	15.3	8	122	7.6
F	20.0	8	159	7.1
G	4.0	83	334	9.1
H	5.0	96	482	8.7
I	7.0	91	637	8.0
J	10.0	96	961	7.8
K	15.0	100	1501	7.6 ^c
L	20.8	83	1718	7.3

^aConcentrations in mM^bpH at which the initial Hg(HCys)₂ precipitate dissolved.^cFor solution K, the precipitate dissolved at pH = 7.4

Table 2

Results from least-squares curve fitting of the Hg L_{III}-edge EXAFS spectra obtained for the Hg(II)- cysteine solutions A – F and G – L, and their corresponding ¹⁹⁹Hg NMR chemical shifts (see Figures 1 and 3).^a

Solution (H ₂ Cys/Hg(II))	Hg-S			¹⁹⁹ Hg NMR δ (ppm)	Solution (H ₂ Cys/Hg(II))	Hg-S			¹⁹⁹ Hg NMR δ (ppm)
	<i>N</i>	<i>R</i> (Å)	σ ² (Å ²)			<i>N</i>	<i>R</i> (Å)	σ ² (Å ²)	
<i>C</i> _{Hg(II)} = 8 – 9 mM					<i>C</i> _{Hg(II)} = 80 – 100				
A (4.1) ^{<i>b</i>}	1.7	2.35	0.0024	–565	G (4.0)	3.2	2.44	0.0113	–400
B (5.2)	1.7	2.37	0.0049	–496	H (5.0)	3.5	2.49	0.0090	–366
C (6.5)	1.9	2.37	0.0058	–469	I (7.0)	3.6	2.50	0.0086	–357
D (10.7)	2.6	2.42	0.0093	–413	J (10.0)	3.8	2.50	0.0085	–353
E (15.3)	2.6	2.43	0.0091	–386	K (15.0)	3.9	2.51	0.0082	–347
F (20.0)	2.7	2.43	0.0093	–401	L (20.8)	3.8	2.50	0.0087	–351

^aFitting *k*-range = 2.6 - 13.0 Å⁻¹; *S*₀² = 0.9 fixed; estimated error limits: coordination number *N* ± 20 %, *R* ± 0.02 Å, σ² ± 0.001 Å²

^bthis model also includes a Hg-N path (2.50 Å; σ² = 0.0288 Å²) with its coordination number correlated to that of the Hg-S path (model **II** in Table S-1).

Table 3

Relative amounts of Hg(II)-cysteinate complexes obtained from fitting the experimental EXAFS spectra of solutions F ($C_{\text{Hg(II)}} = 8 \text{ mM}$) and G – L ($C_{\text{Hg(II)}} = 80 - 100 \text{ mM}$) to linear combinations of the simulated EXAFS oscillations for Hg(cysteinate) $_n$ species ($n = 2, 3, 4$); see Figures S-3a-c.^a

Solution (H ₂ Cys/ Hg(II) mole ratio)	pH	Species	% Hg(S,N-Cys) ₂	% Hg(S-Cys) ₃	% Hg(S-Cys) ₄	Average R^b	Mean Hg-S distance (Å) from EXAFS
		Hg-S distance (DW parameter)	2.34 Å ($\sigma^2 = 0.003$ Å ²)	2.44 Å ($\sigma^2 = 0.006$ Å ²)	2.52 Å ($\sigma^2 = 0.008$ Å ²)		
F (20.0)	7.1		17	55	28	2.445	2.43
G (4.0)	9.1		14	40	46	2.463	2.44
H (5.0)	8.7		5 f^c	17	78	2.497	2.49
I (7.0)	8.0			15	85	2.508	2.50
J (10.0)	7.8			8	92	2.513	2.50
K (15.0)	7.6			2	95	2.516	2.51
L (20.8)	7.3			15	85	2.508	2.50
			2.34 Å ($\sigma^2 = 0.003$ Å ²)	2.45 Å ($\sigma^2 = 0.006$ Å ²)	2.53 Å ($\sigma^2 = 0.008$ Å ²)		
F (20.0)	7.1		19	59	22	2.447	2.43
G (4.0)	9.1		16	47	37	2.462	2.44
H (5.0)	8.7		5 f^c	27	68	2.499	2.49
I (7.0)	8.0			28	72	2.508	2.50
J (10.0)	7.8			22	78	2.512	2.50
K (15.0)	7.6			17	83	2.516	2.51
L (20.8)	7.3			28	72	2.508	2.50

^aEstimated error 10 – 15%; f = fixed

^b $R = \sum (\% \text{ of species}) \times (\text{average Hg-S distance in the species})$.

^cEstimated from the Raman spectrum of solution H.