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Lead(II) Complex Formation with Glutathione

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Abstract

A structural investigation of complexes formed between the Pb^{2+} ion and glutathione (GSH, denoted AH_3 in its triprotonated form) the most abundant nonprotein thiol in biological systems, was carried out for a series of aqueous solutions at pH 8.5 and $C_{\text{Pb}^{2+}} = 10 \text{ mM}$, and in the solid state. The Pb L_{III}-edge EXAFS oscillation for a solid compound with the empirical formula $[\text{Pb}(\text{AH}_2)]\text{ClO}_4$ was modeled with one Pb-S and two short Pb-O bond distances at $2.64 \pm 0.04 \text{ \AA}$ and $2.28 \pm 0.04 \text{ \AA}$, respectively. In addition Pb...Pb interactions at $4.15 \pm 0.05 \text{ \AA}$ indicate dimeric species in a network where the thiolate group forms an asymmetrical bridge between two Pb^{2+} ions. In aqueous solution at the mole ratio GSH / Pb(II) = 2.0 ($C_{\text{Pb}^{2+}} = 10 \text{ mM}$, pH 8.5), lead(II) complexes with two thiolate ligands form, characterized by a ligand-to-metal charge transfer band (LMCT) $\text{S}^- \rightarrow \text{Pb}^{2+}$ at 317 nm in the UV-*vis* spectrum and mean Pb-S and Pb-(N/O) bond distances of $2.65 \pm 0.04 \text{ \AA}$ and $2.51 \pm 0.04 \text{ \AA}$, respectively, from a Pb L_{III}-edge EXAFS spectrum. For solutions with higher mole ratios, GSH / Pb(II) = 3.0, ESI-MS spectra identified a trisglutathionyl lead(II) complex, for which Pb L_{III}-edge EXAFS spectroscopy shows a mean Pb-S distance of $2.65 \pm 0.04 \text{ \AA}$ in PbS_3 coordination, ^{207}Pb NMR spectroscopy displays a chemical shift of 2793 ppm, and in the UV-*vis* spectrum an $\text{S}^- \rightarrow \text{Pb}^{2+}$ LMCT band appears at 335 nm. The complex persists at high excess of glutathione, and also at ~25 K in frozen glycerol (33%) / water glasses for GSH / Pb(II) mole ratios from 4.0 to 10 ($C_{\text{Pb}^{2+}} = 10 \text{ mM}$) measured by Pb L_{III}-edge EXAFS spectroscopy.

Introduction

The toxicology of divalent lead is complex and known to adversely affect the function of organ systems in the human body including the kidneys and developing blood cells, as well as the central nervous and reproductive systems.¹⁻³ The toxic effects are frequently attributed to displacement of essential metals (e.g. Ca^{2+} and Zn^{2+}) by the Pb^{2+} ion, which along with simultaneous binding to structural and catalytic protein sites, disturbs normal biological activity.⁴⁻⁶ One of the major targets identified for the Pb^{2+} ion is δ -aminolaevulinic acid dehydratase (ALAD), a key zinc-containing metalloenzyme in the heme biosynthetic pathway for forming important tetrapyrroles such as heme, corrins, and chlorophyll.⁶ In human erythrocytes, Pb^{2+} ions bind to ALAD with 25-fold higher affinity than Zn^{2+} ions.⁷ Consequently, patients suffering from lead poisoning are often afflicted

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Supporting Information Available. IR and Raman spectra of $[\text{Pb}(\text{AH}_2)]\text{ClO}_4$; crystal structure of D-penicillaminato lead(II), PbPen, used to simulate model EXAFS oscillations; EXAFS fitting of PbPen solid; ESI-MS spectrum of GSH/ $\text{Pb}^{2+} = 10.0$ in the positive ion mode; Table for the assignment of mass ions in positive ion mode; Table for ^1H NMR chemical shift changes for solutions **A – E** relative to free GSH; Table for additional EXAFS fitting results for solutions **A – E** and **A* – E***; structures for Pb^{2+} sulfur-coordinated complexes for which ^{207}Pb NMR chemical shifts are reported; comparison of the EXAFS oscillations of 33% v/v glycerol/water frozen glasses with GSH/ Pb^{2+} mole ratios 2.0, 3.0 and 10.0 ($C_{\text{Pb}^{2+}} = 10 \text{ mM}$, pH 8.5) at LT; CSD survey of crystal structures with PbS_4 , PbS_3 , PbS_3N , PbS_2N_2 , PbS_2O_2 , PbSN_2 , PbS_2N and PbS_2O coordination.

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with anemia.⁸ The native ALAD enzyme has tetrahedral coordination geometry around the Zn^{2+} ion in its active site, while X-ray crystallography of Pb^{2+} -substituted ALAD in yeast reveals that the Pb^{2+} ion coordinates to the thiol groups of three cysteinyl residues in trigonal pyramidal geometry,⁹ which may in part explain the inhibition of ALAD enzymatic activity in the presence of Pb^{2+} ions.¹⁰⁻¹² Spectroscopic studies show that the Pb^{2+} ion also tends to coordinate three thiolate ligands when occupying the domains in sulfur-rich proteins that normally contain Zn^{2+} ions, as well as when bound to the metalloregulatory proteins CadC and AztR found in prokaryotes.¹³⁻¹⁸ In comparison, little information is available on the structure of Pb^{2+} complexes with thiolate ligands in small molecules,¹⁹ which are commonly found within cells or exogenously administered as heavy metal chelating agents.

The Pb^{2+} ion displays a large range of coordination numbers often in an irregular coordination geometry.^{20, 21} At low coordination numbers a void often appears in a distorted (*hemidirected*) structure, a phenomenon that traditionally has been ascribed to an inert, sterically active lone pair of electrons, originating from 6s-6p orbital mixing for the Pb^{2+} ion. However, theoretical DFT calculations have in recent years modified this picture and show that asymmetries in the valence shell electron densities that result from the direct electronic interactions between the cation and its ligands are the cause of the distorted structures in $\text{Pb}(\text{II})$ complexes.²²⁻²⁴ The covalent interaction between filled ligand orbitals (e.g. O 2p) and the Pb^{2+} 6s² and empty 6p orbitals can create bonding and anti-bonding molecular orbitals (MOs).²² A lone pair in such an anti-bonding MO creates a void by repelling the ligands, which increases the ligand-ligand repulsion and counteracts an increase in the coordination number. The combination of those counteracting effects will establish whether a regular (*holodirected*) or a distorted (*hemidirected*) structure correspond to an energy minimum. The regular structure for the solid PbS compound with the Pb^{2+} ion surrounded by six sulfide ions (as in NaCl), in contrast to the distorted structure of PbO with four asymmetrically distributed oxide ions, has been attributed to a weaker electronic interaction due to greater mismatch between the Pb^{2+} 6s and the S 3p atomic orbitals, than with O 2p.²² For high coordination numbers or space-demanding ligands the increase in ligand-ligand repulsion strongly promotes regular (*holodirected*) structures.²¹

Glutathione (γ -L-glutamyl-L-cysteinyl-glycine, GSH, denoted AH_3 in its triprotonated form; Scheme 1) is a ubiquitous tripeptide found in the cells of plants, animals and microorganisms at relatively high concentrations (0.1-10 mM).^{25, 26} Among its many vital roles, GSH protects cellular membranes from the toxic effects of heavy metals (e.g. Pb^{2+} , Hg^{2+} , Cd^{2+}) by complex formation, before they are transferred to higher molecular weight cysteine-rich peptides such as metallothioneins and phytochelatins, which remove the harmful metal ions.²⁷⁻³³ Toxicological studies have shown that patients exposed to lead occupationally obtain decreased levels of GSH in their blood, suggesting that Pb^{2+} -GSH complexes are formed *in vivo*.³⁴⁻³⁶ Gold nanoparticles with glutathione as functional group were recently introduced as a highly selective / sensitive colorimetric probe for detecting Pb^{2+} ions in aqueous environmental / biological samples.³⁷

The microscopic acid dissociation constants for glutathione have been directly determined using ¹H NMR spectroscopy, showing that the two carboxylic acid groups deprotonate simultaneously over the pH range 0.5 – 6 ($\text{pK}_{\text{a}1} = 2.19$ for glutamyl and $\text{pK}_{\text{a}2} = 3.22$ for glycyl), while the cysteinyl thiol group loses its proton concurrently to the ammonium group ($\text{pK}_{\text{a}3} = 8.97$ and $\text{pK}_{\text{a}4} = 9.17$, respectively) over the pH range 7 -12.³⁹

The complex formation between GSH and Pb^{2+} ions in aqueous solution has previously been studied by ¹³C and ¹H NMR methods within the pD range 5.4 – 12.^{40, 41} The ¹H NMR studies suggest that the Pb^{2+} ion binds exclusively to the cysteinyl sulfur donor atom of GSH.⁴¹ However, for a Pb^{2+} -GSH solution with a mole ratio $\text{GSH} / \text{Pb}^{2+} = 2.0$ at pD < 9,

the ^{13}C NMR results indicate minor interactions also between Pb^{2+} and the glycine carboxylate group. Under acidic conditions ($\text{pD} = 2.3 - 5.4$), the appearance of white precipitates restricted solution studies by NMR spectroscopy.⁴⁰ Solid precipitates with the general formula $[\text{Pb}(\text{AH}_2)\text{X}] \cdot \text{H}_2\text{O}$ (where $\text{X} = \text{Cl}, \text{NO}_3^-, \text{CH}_3\text{COO}^-$, or NCS^-) were identified with the cysteinyl thiolate coordinated to the Pb^{2+} ion, as evidenced by the absence of the $\nu(\text{S-H})$ band in the IR spectra.⁴²

An earlier potentiometric study of a dilute Pb^{2+} solution containing $C_{\text{Pb}^{2+}} = 5 \text{ mM}$ and $C_{\text{GSH}} = 10 \text{ mM}$ resulted in a $[\text{Pb}(\text{AH})]$ complex as the major species formed at $\text{pH} \sim 5$,⁴³ while $[\text{Pb}(\text{A})]^-$ and $[\text{Pb}(\text{GSH})_2]^{n-}$ ($n = 2 - 4$) complexes predominate at higher pH values (between 7.0 – 10.0).⁴³ The m/z peaks in ESI-MS (electrospray ionization mass spectroscopy) spectra for $[\text{Pb}(\text{AH}_2)]^+$ and $[\text{Pb}(\text{A})]^-$ species were detected both in positive and negative modes for a solution containing equimolar amounts (0.033 M) of Pb^{2+} and GSH,⁴⁴ while both mononuclear $[\text{Pb}(\text{AH})_2]^{2-}$ and dinuclear $[\text{Pb}_2(\text{AH})_2]$ complexes were proposed to form between pH 5.5 and 7.0 in a polarographic study.⁴⁵

Further insight into the nature and structure of the complexes formed between Pb^{2+} ions and GSH in solution, including the number of donor atoms, their bond distances, and the coordination geometry about the metal center, is clearly needed. In continuation of our systematic structural studies of complexes formed between toxic metal ions and biologically relevant small molecular ligands,⁴⁶⁻⁵¹ the present structural study of Pb^{2+} -GSH complexes was carried out using a combination of spectroscopic techniques, *i.e.* Pb L_{III}-edge XAS (X-ray absorption spectroscopy), ^{207}Pb NMR, ^1H NMR, ESI-MS and UV-*vis*. To promote the formation of higher complexes pH 8.5 was chosen to increase the amount of deprotonated thiolate groups in aqueous solution. The preliminary ^{207}Pb NMR chemical shifts reported in Mah's PhD thesis,⁵² for the Pb^{2+} ion bound to this biologically important thiol-containing ligand showed that ^{207}Pb NMR spectroscopy is a very useful tool for structural characterizations of Pb^{2+} -substituted proteins. This is evidenced in recent reports where ^{207}Pb NMR spectroscopy was used 1) to investigate the Pb(II) environment in thiol-rich proteins,^{53, 54} 2) to propose that a $[\text{Pb}(\text{GS})_3]^-$ complex can form with glutathione in solutions with pH 7.5 to 9.5,⁵⁵ and 3) to reveal that two different trigonal pyramidal PbS_3 environments can be formed in the zinc binding domain in the HIV nucleocapsid protein HIV-CCHC.⁵⁵

Nowadays EXAFS spectra are often measured at low temperature to avoid photo-reduction of the absorbing atom, and to extend the useful k -range of the data by reducing thermal disorder.⁵⁶ Recently we showed for a series of Hg^{2+} -GSH and Cd^{2+} -GSH alkaline solutions that the EXAFS structural parameters obtained from such low temperature (LT) measurements can differ from room temperature (RT) results, corresponding to an increasing stability of higher complexes at LT for strongly exothermic reactions.^{50, 51} In the current study, the effect of temperature on the speciation of Pb^{2+} -GSH complexes formed in solution was evaluated by comparing Pb L_{III}-edge EXAFS spectra obtained at RT with those obtained for frozen glasses at $\sim 25 \text{ K}$ with similar concentration but with 33% v/v glycerol added.

Experimental Section

Sample preparation

GSH, sodium hydroxide and $\text{Pb}(\text{ClO}_4)_2 \cdot 3\text{H}_2\text{O}$ were purchased from Sigma-Aldrich and used without further purification. Syntheses were carried out under inert Ar atmosphere, and the pH of the aqueous solutions was monitored with a Corning Semi-Micro electrode calibrated to standard buffers. All solutions were prepared using deoxygenated water, prepared by boiling distilled water and bubbling Ar through to remove dissolved O_2 .

To a glutathione solution (1 mmol) in O₂-free H₂O (pH 2.7), Pb(ClO₄)₂·3H₂O (0.5 mmol) was added (pH 2.2). Upon dropwise addition of NaOH (2 M) a white precipitate formed, which was collected at pH 2.5. The precipitate was washed with water and ether, and then dried under vacuum. Elemental analysis calculated for [Pb(AH₂)]ClO₄ (PbC₁₀H₁₇N₃O₁₁SCl): C 19.59, H 2.63, N 6.85. Found: C 19.68, H 2.73, N 6.65.

Pb²⁺-glutathione solutions were prepared by dissolving glutathione (0.1 - 0.5 mmol) in deoxygenated water, followed by addition of Pb(ClO₄)₂·3H₂O (0.05 mmol) whereby a white precipitate forming (pH = 2.0 - 2.2). Sodium hydroxide (2 M) was added dropwise to the mixture until the solid dissolved at pH ~5 giving a clear colorless solution. Five solutions with C_{Pb²⁺} = 10 mM and the GSH/Pb²⁺ mole ratios 2.0 (A), 3.0 (B), 4.0 (C), 5.0 (D) and 10.0 (E) were prepared for which the final pH was adjusted to 8.51. Solution I with higher Pb(II) concentration, C_{Pb²⁺} = 90 mM, was prepared to facilitate ²⁰⁷Pb NMR measurements at the mole ratio GSH / Pb²⁺ = 3.0 and pH = 8.5 (Table 1). For the LT (~25 K) Pb L_{III}-edge EXAFS measurements, aqueous GSH solutions containing C_{Pb²⁺} = 15 mM were diluted with 33% v/v glycerol, giving a final C_{Pb²⁺} ~10 mM. Prior to the LT measurement the sample holder containing each diluted solution (A* – E*, Table 1) was immersed in liquid nitrogen, yielding a homogeneous frozen glass, preventing diffraction from crystals during the data collection. The final C_{Pb²⁺} was 10 mM for the samples used for ESI-MS, Pb L_{III}-edge EXAFS at RT and LT (33% v/v glycerol), ²⁰⁷Pb NMR (10 % v/v D₂O), ¹H NMR (100 % D₂O) and UV-*vis* measurements.

Mass spectrometry

Mass spectra were collected in negative and positive ion modes by direct infusion of the Pb²⁺-GSH solutions into the electrospray ionization (ESI) source of a Bruker Esquire 3000 mass spectrometer using a continuous injection flow rate of 0.06 mL/min. The drying gas temperature was 300 °C, at 4 L/min flow rate. The capillary voltage was set at 3.2 kV, for a target mass of 1192 *-m/z*, the skimmer voltage was -47.5 V.

Nuclear Magnetic Resonance Spectroscopy

²⁰⁷Pb and ¹H NMR spectra were collected at 300 K and resonance frequencies of 62.95 MHz and 300.14 MHz, respectively, using a Bruker AMX 300 spectrometer equipped with a 10 mm broadband probe.

The ²⁰⁷Pb chemical shift was externally calibrated relative to 1.0 M Pb(NO₃)₂ in D₂O solution, resonating at -2961.2 ppm relative to Pb(CH₃)₄.⁵⁷ The ²⁰⁷Pb NMR data were acquired using a 90° pulse, a sweep width of 62.8 kHz, and 64 K data points with 1 s recycle delay between scans. Approximately 10000-40000 scans were co-added. Spectra were processed using exponential line broadening between 25-200 Hz (10 % of the line width at half-maximum, Δ*v*_{1/2}).

The ¹H NMR spectra were collected using a 30° pulse, a sweep width of 3.7 kHz, 16 K data points, and a 0.5 s recycle delay between scans. A total of 16 scans were co-added, spectra were referenced internally using the residual HOD/H₂O peak at 4.80 ppm.

Electronic Spectroscopy

UV-*vis* absorption spectra were measured at room temperature using a Cary 300 UV-*vis* double beam spectrophotometer. Samples were loaded into 1 mm quartz cells; distilled water was used as the blank.

Raman and IR Spectroscopy

The Raman spectrum for the [Pb(GS)]ClO₄ solid was measured using a Bruker RAM(II) FT-Raman spectrometer equipped with a liquid N₂ cooled Ge-detector, by means of the 1064 nm line excitation of a YAG laser at 100 mW and 4 cm⁻¹ resolution. The mid-IR spectrum was obtained from a KBr pellet over the range 400 – 4000 cm⁻¹ with 2 cm⁻¹ resolution, using a Nicolet Nexus 470 FT-IR spectrometer.

X-ray Absorption Spectroscopy Data Collection

Pb L_{III}-edge X-ray absorption spectra for the Pb²⁺-GSH solutions **A** – **E** were collected under dedicated conditions at beamline 7-3 at the Stanford Synchrotron Radiation Lightsource (SSRL) (3 GeV, 85-100 mA), and at beamline 12-C at the Photon Factory of High Energy Accelerator Research Organization, Tsukuba, Japan (2.5 GeV, 250-300 mA). The ion chambers I₀ and I₁ (before and after the sample position, respectively) were filled with N₂ gas; ion chamber I₂ (after the calibration foil) with Ar gas. The energy of the X-rays was calibrated by assigning the first inflection point of a Pb foil to 13035 eV. Higher order harmonics were rejected by a Rh coated mirror positioned after the Si(220) double crystal monochromator at SSRL, or by detuning the Si(111) double crystal monochromator to 50% of the maximum I₀ intensity at the end of the Pb L_{III}-edge scan region (13807 eV) at the Photon Factory.

Solutions **A** – **E** were kept in a 5 mm Teflon spacer with 5 μm polypropylene windows for RT EXAFS measurements. The frozen glassy samples (**A*** – **E***) were held in a 2 mm Ti cell with Kapton windows; the sample temperature was maintained at ~25 K by means of a liquid He cryostat. EXAFS spectra were measured by monitoring the Pb L_α fluorescence radiation from the samples using a 19 (Photon Factory) or 30-element Ge detector (SSRL). Between 8 and 14 scans were collected for each sample, and all scans and detector channels were compared to ensure that no radiation damage had occurred during the XAS measurements. EXAFS data for the solid D-penicillaminato lead(II) compound, PbPen, mixed with boron nitride were collected in transmission mode at room temperature at SSRL beamline 9-3, measuring 26 scans to improve the noise level.

X-ray Absorption Spectroscopy Data Analysis

The fluorescence intensity was compared for each channel in all scans to ensure compatibility prior to averaging using the EXAFSPAK suite of programs,⁵⁸ or for the Photon Factory data the RIGAKU REX2000 2.5.6 program.⁵⁹ The EXAFS oscillations were extracted by means of the WinXAS 3.1 program,⁶⁰ using a first order polynomial over the pre-edge region, followed by normalization of the edge step. The energy scale was converted into *k*-space, where $k = [(8\pi^2 m_e / h^2)(E - E_0)]^{1/2}$, using a threshold energy of $E_0 = 13033.7 - 13034.8$ eV; for PbPen solid, $E_0 = 13035.2$ eV.

To extract structural parameters, the EXAFS oscillation, $\chi(k)$, was modeled according to the equation

$$\chi(k) = \sum_i \frac{N_i S_0^2(k)}{k R_i^2} |f_{\text{eff}}(k)|_i \cdot \exp(-2k^2 \sigma_i^2) \cdot \exp[-2R_i / \Lambda(k)] \cdot \sin[2kR_i + \phi_{ij}(k)] \quad (1)$$

where N_i is the number of scatterers at the distance R_i from the absorber in the *i*th shell, the Debye-Waller (or disorder) parameter σ_i^2 is the mean-square variation in R_i , *k* is the scattering variable, $|f_{\text{eff}}(k)|_i$ is the effective amplitude function and $\phi_{ij}(k)$ the total phase-shift of the absorber-scatterer pair; $\Lambda(k)$ is the photoelectron mean free path. The amplitude reduction factor $S_0^2(k)$ is directly correlated to the coordination number N_i .

The effective amplitude function $|f_{\text{eff}}(k)|_i$, the total phase-shift $\phi_{ij}(k)$, and photoelectron mean free path $\lambda(k)$ of the $\chi(k)$ model function were obtained *ab initio* for the relevant atom-pair interactions by means of the FEFF 7.0 program.^{61, 62} The crystal structure of the polymeric D-penicillaminato lead(II) complex PbPen,^{63, 64} which has both short (2.714 Å) and long Pb-S bonds (3.091 Å and 3.464 Å), Pb-(O/N) bonds (2.444 Å, 2.451 Å and 2.720 Å) as well as thiolate bridged Pb...Pb interactions (4.363 and 4.663 Å), was used in the ATOMS program. Least-squares curve-fittings of the $\chi(k)$ model function to the k^3 -weighted experimental EXAFS data for solutions **A** – **E** and samples **A*** – **E*** were performed to refine the structural parameters R , σ^2 , and in some cases also the coordination number N for each type of interaction. The amplitude reduction factor (S_0^2) was fixed to 0.9, as obtained from the EXAFS data analysis of the penicillaminato lead(II) complex PbPen, while ΔE_0 (a common value for all paths in a model) was allowed to float.

Results

Solid [Pb(AH₂)]ClO₄

A white 1:1 Pb²⁺-GSH precipitate formed from the reaction of GSH with Pb(ClO₄)₂·3H₂O in the mole ratio 2:1; an outcome similar to that of the 1:1 D-penicillaminato lead(II) compound that crystallizes as a 1:1 PbPen complex, even in 10-fold excess of the ligand.⁶³ Vibrational spectra (Figure S-1 and Table 2) measured for this precipitate show a $\nu(\text{Cl-O})$ stretch at 930 cm⁻¹ (Raman), as well as $\delta(\text{ClO}_4^-)$ vibrations at 624 cm⁻¹ and 622 cm⁻¹, verifying that ClO₄⁻ was incorporated into the compound with the empirical formula [Pb(AH₂)]ClO₄. The $\nu(\text{S-H})$ stretch of GSH at 2525 cm⁻¹, giving rise to a strong band in the Raman and a weaker band in the IR spectrum, is absent for the solid [Pb(AH₂)]ClO₄ compound, indicating that the cysteinyl thiol group is deprotonated and coordinated to the Pb²⁺ center. The IR spectrum for the solid [Pb(AH₂)]ClO₄ has a broad shoulder at 1708 cm⁻¹ for $\nu(\text{C=O})$ indicating that one of the two GSH carboxyl groups is protonated (-COOH).⁶⁵ Therefore, the coordinated glutathione with only one negative charge (AH₂⁻) in the [Pb(AH₂)]ClO₄ compound that precipitated at pH 2.5 has a Cys thiolate (S⁻), a Glu NH₃⁺, a carboxylic (COOH) and a carboxylate (COO⁻) group at its Gly / Glu ends.

Different structural models were tested in the least-squares curve-fitting procedure for the EXAFS spectrum of the solid [Pb(AH₂)]ClO₄ (Table S-1). The EXAFS oscillation was well modeled with 2 Pb-O, 1 Pb-S and one (or two) Pb...Pb interactions at the mean distances 2.28 ± 0.04 Å, 2.64 ± 0.04 Å and 4.15 ± 0.05 Å, respectively (models 3 and 4 in Table S-1; Figure 1). The relatively large disorder parameter obtained for the Pb-O path, $\sigma^2 = 0.0066 \pm 0.002$ Å², indicates a wide distribution around the mean distance. The short k -range restricts the number of independently refined parameters. When considering two Pb-S paths in the first coordination shell (model 5), or one short Pb-S and one long Pb-S' path (model 6), or two additional long Pb-O' distances as possible bridging ligand atoms, the result was either higher residual, or very high Debye-Waller factors for the longer Pb-(S'/O') paths.

The comparison between different EXAFS fitting models presented in Table S-1 shows that: 1) Including up to three scattering paths in the fitted model is reasonable, but additional paths with longer distance cause strong correlation between the parameters and high σ^2 values for the longer paths; 2) even though the coordination number is difficult to determine (see below) for each type of scattering path (models 2 – 4), there is clearly only one Pb-S path in the first coordination shell of the Pb²⁺ ion, as evidenced by the poor fit for model 5 with the highest residual R ; 3) the refined bond distances are quite consistent for all fitting models (except 5): Pb-O 2.26 – 2.29 Å, Pb-S 2.63 – 2.64 Å and Pb...Pb 4.14 – 4.15 Å.

To test the accuracy of the bond distances obtained for the solid [Pb(AH₂)]ClO₄ compound, the Pb L_{III}-edge EXAFS spectrum of the crystalline penicillaminato lead(II) complex

(PbPen) was measured at room temperature. Its crystal structure (Figure S-2) shows two short Pb-(O/N) bonds at ~ 2.45 Å and one Pb-S thiolate bond at 2.714 Å. There are also longer interactions in the coordination sphere with two additional Pb-S distances to the thiolate sulfur at 3.091 and 3.464 Å giving rise to double bridges in an infinite chain structure with Pb...Pb distances of 4.363 Å, and with one bridging Pb-O distance at 2.720 Å between the chains (with Pb...Pb distances of 4.66 Å).⁶⁴ Least squares curve-fitting of the k^3 -weighted EXAFS oscillation of PbPen over the k -range 2.6 – 9.5 Å⁻¹ was performed with models derived from its crystal structure. A satisfactory fit was obtained with two Pb(N/O) and one Pb-S paths at 2.42 ± 0.04 and 2.68 ± 0.04 Å, respectively, which are slightly (~ 0.03 Å) shorter than the corresponding distances in the crystal structure. Attempts to fit longer paths, *e.g.* Pb-(S'/O'), resulted in very high Debye-Waller factors (~ 0.02 Å²). The amplitude reduction factor (S_0^2) was refined to 0.91, and was later fixed to 0.9 in the EXAFS curve-fitting procedure for [Pb(AH₂)]ClO₄.

Lead(II) Glutathione Solutions

ESI - Mass Spectroscopy

This technique was used for the aqueous solutions **A** – **E** with different GSH/Pb²⁺ mole ratios to study the composition of possible Pb²⁺-GSH complexes. For all five solutions, the ESI-MS spectra showed ions with identical masses, with changes only in the relative intensity of the peaks. The ESI-MS for solution **E** with mole ratio GSH/Pb²⁺ = 10 is shown in Figure 2 with assignments of the prominent peaks for the negatively charged complexes in Table 3.

Strong signals at 305.9 (assigned 100% intensity in Figure 2) and 327.9 $-m/z$ correspond to the monoanions of the free GSH ligand and a Na-GSH complex, respectively. Oxidized glutathione GSSG ($-m/z = 632.9$ and 610.9) and adduct anions of (GSH)_{*n*}, where $n = 2$ ($-m/z = 612.8, 656.9, 678.9$), 3 ($-m/z = 1029.9$) or 4 ($-m/z = 1358.7$ and 1380.7) were observed in the ESI mass spectra. Higher order adducts of (GSH)_{*n*} (where $n = 325$) have also been reported in previous ESI-MS studies of glutathione solutions at acidic pH in the positive ion mode.⁴⁴

Assignments to Pb²⁺-GSH complexes are facilitated by the characteristic Pb isotopic distribution with 52.4% ²⁰⁸Pb, 22.1% ²⁰⁷Pb, 24.1% ²⁰⁶Pb and 1.4% of ²⁰⁴Pb present in natural abundance.⁶⁷ Negatively charged Pb(GSH)₃ complexes were detected for $-m/z$ peaks at 1235.7, 1213.7, and 1191.8 in ion pairs with the expected number of H⁺ or Na⁺ ions paired with the ionizable donor atoms on glutathione (Table 3). In addition, similar negatively charged Pb(GSH) ($-m/z = 511.9$) and Pb(GSH)₂ ($-m/z = 862.8, 840.8$ and 818.8) species were characterized, which may have formed by dissociation of the glutathione ligands in a Pb(GSH)₃ complex.

In the positive ion mode, the ESI-MS spectra were dominated by GSH and (GSH)_{*n*} adducts (Figure S-4, Table S-2). Peaks attributed to Pb(GSH) and Pb(GSH)₂ species had much lower intensity than in the negative ion mode, and no peaks corresponding to Pb(GSH)₃ species could be detected.

¹H NMR Spectroscopy

For Pb²⁺-GSH solutions **A** – **E**, only one set of proton resonances is observed for both coordinated and free GSH molecules, indicative of fast exchange on the NMR timescale (Figure 3). The downfield shift of the Cys β protons for the above solutions, and of the Cys α proton for solutions **A** and **B**, indicates that at pH 8.5 the cysteinyl thiolate group is coordinated to the Pb²⁺ center. The Glu α proton also shows a small downfield shift in particular for solution **A**, which implies possible coordination of Glu COO⁻ or deprotonated

amine groups to the Pb^{2+} ion (Table S-3). The Gly COO^- group of GSH is not coordinated, as the chemical shift of the Gly α protons does not shift downfield relative to the free ligand. Solutions **C** – **E** contain increasingly higher excess of GSH ($C_{\text{GSH}} = 40\text{--}100$ mM), and the ^1H NMR signals begin to resemble that of free GSH.

Electronic Absorption Spectroscopy

In the UV-*vis* spectra for the Pb^{2+} -GSH solutions **A** – **E** (Figure 4), two strong transitions were observed at ~ 280 nm and between 317–335 nm, which have been assigned to combination of both $\text{S}^- 3\text{p} \rightarrow \text{Pb}^{2+} 6\text{p}$ ligand to metal charge transfer (LMCT), and intra-atomic $\text{Pb}^{2+} 6\text{s}^2 \rightarrow \text{Pb}^{2+} 6\text{p}$ transitions.^{1, 17, 18, 69} Similar electronic transitions have been observed for three-coordinate PbS_3 complexes to cysteine-rich metalloregulatory proteins,^{14-16, 18, 70} as well as structural Zn^{2+} binding domains.^{1, 17, 69, 71}

The most blue-shifted absorption spectrum was observed for solution **A** ($\text{GSH}/\text{Pb}^{2+} = 2.0$). The LMCT band had a peak maximum of 317 nm, and a noticeably lower molar absorptivity ($3100 \text{ M}^{-1}\text{cm}^{-1}$) than for solutions **B** – **E** ($3611\text{--}4054 \text{ M}^{-1}\text{cm}^{-1}$, see Figure 4). For solution **B** with the mole ratio $\text{GSH}/\text{Pb}^{2+} = 3.0$, the LMCT transition is slightly blue shifted to 330 nm relative to that of solutions **C** – **E**, with a smaller reduction in the molar absorptivity. Almost identical UV-*vis* spectra were obtained for solutions **C**, **D** and **E** (mole ratios $\text{GSH}/\text{Pb}^{2+} = 4.0, 5.0$ and 10.0 , respectively) with LMCT transition maxima at 335 nm.

^{207}Pb NMR Spectroscopy

The sensitivity of the NMR resonances to changes in the coordination and bonding environment makes it a useful complement to the structural information from EXAFS spectroscopy. To evaluate the coordination environment around the Pb^{2+} ion in solutions **A** – **E**, ^{207}Pb NMR spectroscopy was employed. The ^{207}Pb nucleus has a natural abundance of 22.1 %, $I = 1/2$, receptivity of 11.7 relative to ^{13}C , a large chemical shift range (~ 17000 ppm), giving excellent sensitivity to changes in the metal coordination environment.⁵⁷ Generally, ligands with higher polarizability have less shielding effect on the Pb nucleus. Thus, for biologically relevant donor atoms such as sulfur, oxygen, and nitrogen, the shielding increases in the order, $\text{S} < \text{N} < \text{O}$.⁵⁷ The same trend is observed in the NMR spectra of other heavy metal $I = 1/2$ nuclei such as ^{199}Hg and $^{111/113}\text{Cd}$, with numerous chemical shift values available in the literature for a wide range of different types of $\text{Hg}^{2+}/\text{Cd}^{2+}$ coordination environments.⁷² However, there is still lack of information, in particular for lead (II) thiolate complexes, to make the ^{207}Pb chemical shift scale reliably useful in a similar way, since peptides and proteins containing cysteine are major biological targets for the heavy metal. The ^{207}Pb chemical shifts reported for Pb^{2+} -sulfur coordinated complexes in Figure S-5 are shown in Table 4.

No ^{207}Pb NMR resonance could be detected for solution **A**, not even when a solution with $C_{\text{Pb}^{2+}} = 90$ mM and the same mole ratio $\text{GSH}/\text{Pb}^{2+} = 2.0$ was tested. Also, for solution **B** ($C_{\text{Pb}^{2+}} = 10$ mM and $\text{GSH}/\text{Pb}^{2+} = 3.0$) no ^{207}Pb NMR resonance was detected, but a more concentrated solution **I** ($C_{\text{Pb}^{2+}} = 90$ mM and $\text{GSH}/\text{Pb}^{2+} = 3.0$) produced a very broad ^{207}Pb NMR signal centered at 2716 ppm with a large width at half height, $\Delta \nu_{1/2} \sim 2000$ Hz. The ^{207}Pb NMR spectra for the Pb^{2+} -GSH solutions **C** – **E** ($C_{\text{Pb}^{2+}} = 10$ mM and $C_{\text{GSH}} = 40\text{--}100$ mM) show a single resonance in a relatively narrow range, 2775–2793 ppm (Figure 5).

For lower $\text{GSH}/\text{Pb}^{2+}$ mole ratios the ^{207}Pb NMR chemical shifts became slightly more shielded and increasingly broad. Since lead (II) thiolate bonds are labile, the significant broadening of the ^{207}Pb resonance for concentrated solution, **I** ($C_{\text{Pb}^{2+}} = 90$ mM, $\text{GSH}/\text{Pb}^{2+} = 3.0$), is likely due to intermediate exchange rate between different Pb^{2+} -GSH species at RT. Exchange-broadened line widths have also been previously observed in ^{207}Pb NMR

investigations of the Pb^{2+} -substituted Ca^{2+} -binding proteins parvalbumin and calmodulin.⁷³ A broadening was also observed for the signal at 2777 ppm at pH = 7.5 for a solution containing $C_{\text{Pb}^{2+}} = 5 \text{ mM}$, $\text{GSH}/\text{Pb}^{2+} = 3.0$.⁵⁵

Pb L_{III}-edge XAS at RT

The Pb L_{III}-edge X-ray absorption near edge structure (XANES) region is very similar for solutions **B** – **E** (mole ratios $\text{GSH}/\text{Pb}^{2+} = 3.0$), showing a broad feature at ~13055 eV characteristic of lead (II) thiolate complexes (Figure 6).¹⁷ For solution **A** with lower mole ratio ($\text{GSH}/\text{Pb}^{2+} = 2.0$) the slight shift to 13052 eV indicates a slightly different coordination environment. The k^3 -weighted EXAFS spectra and corresponding Fourier transforms for solutions **A** – **E** are shown in Figure 7. Structural parameters for the Pb^{2+} -GSH complexes formed at RT were obtained by least squares curve fitting of models to the Pb L_{III}-edge EXAFS oscillations, and are presented in Tables 5 and S-4.

The FT magnitude of the EXAFS oscillation for solution **A** ($\text{GSH}/\text{Pb}^{2+} = 2.0$) is noticeably smaller than that of solution **E** ($\text{GSH}/\text{Pb}^{2+} = 10.0$), indicating a lower Pb-S coordination number for **A** (Figure 7). As the $\text{GSH}/\text{Pb}^{2+}$ mole ratio increased from 2.0 (solution **A**) to 3.0 (solution **B**), the EXAFS amplitude gained intensity, and the disorder parameter for the Pb-S path decreased to $\sigma^2 = 0.005 - 0.007 \text{ \AA}^2$. The very similar EXAFS spectra of solutions **B** – **E** were well modeled by three Pb-S bonds at a mean distance of $2.65 \pm 0.04 \text{ \AA}$. The slightly lower Pb-S coordination number for solution **B** is reflected by the reduced EXAFS amplitude compared with that for solutions **C** – **E**.

Pb L_{III}-edge XAS at LT

EXAFS data are commonly collected at reduced temperatures to obtain better signal-to-noise ratios and to avoid sample photo-reduction. Also, earlier observations have shown that for Pb^{2+} ions surrounded by oxygen-donors, the average Pb-O distances from EXAFS spectra measured at RT are usually shorter than those obtained at 10 K.^{80, 81} To study the influence of temperature on the speciation of Pb^{2+} -GSH complexes and the mean Pb-S bond distances, Pb L_{III}-edge X-ray absorption spectra were measured at ~25 K for a series of frozen glycerol/water (33/67 v/v) glasses **A*** – **E*** (Table 1). Least squares curve fitting of the EXAFS spectra for the Pb^{2+} -GSH glassy solutions at LT are shown in Figures 8 (**A***) and 9 (**B*** – **E***), with structural parameters summarized in Table 6, and alternative fitting models in Table S-5.

For the frozen glass **A*** with $\text{GSH}/\text{Pb}^{2+} = 2.0$, the FT magnitude is smaller than that of **E*** with high excess of GSH, indicating a lower Pb-S coordination number for **A***. This is analogous to the smaller FT magnitude for solution **A** compared to **E** at RT. For glass **B*** with mole ratio $\text{GSH}/\text{Pb}^{2+} = 3.0$, the FT magnitude is slightly smaller than for the very similar FTs of **C***, **D*** and **E*** with $\text{GSH}/\text{Pb}^{2+} = 4.0$, 5.0 and 10.0, respectively (Figure S-6).

Discussion

Structure of solid $[\text{Pb}(\text{AH}_2)]\text{ClO}_4$

The vibrational spectra for this solid show that the Pb^{2+} ion is coordinated to GSH via its thiol and one of its carboxyl groups. For GSH, the strong $\nu_{\text{as}}(\text{COO}^-)$ IR band at 1600 cm^{-1} has broadened and shifted to $\sim 1632 \text{ cm}^{-1}$ (IR) for $[\text{Pb}(\text{AH}_2)]\text{ClO}_4$. The $\nu_{\text{s}}(\text{COO}^-)$ band observed at 1397 cm^{-1} in the IR spectrum for solid GSH has shifted to 1387 cm^{-1} (IR) for $[\text{Pb}(\text{AH}_2)]\text{ClO}_4$. These shifts in the COO^- vibrations, with a rather large separation, $\Delta_{\text{IR}} = \nu_{\text{as}}(\text{COO}^-) - \nu_{\text{s}}(\text{COO}^-) = 1632 - 1387 = 245 \text{ cm}^{-1}$, indicate a monodentate complex formation of the coordinated carboxylate group with the Pb^{2+} ion.^{48, 65}

Based on the Pb L_{III}-edge EXAFS data analysis, the first coordination shell in the solid [Pb(AH₂)]ClO₄ compound contains one Pb-S bond at 2.64 ± 0.04 Å and two Pb-O bonds at a mean distance of 2.28 ± 0.04 Å (model 3, Table S-1), forming a distorted trigonal PbSO₂ pyramid with a void over the Pb atom in the apex position created by a stereochemically active electron pair. Recently, it was suggested based on energy minimization calculations that monomeric complexes are formed in [Pb(AH₂)X]·H₂O (X = Cl⁻, NO₃⁻, CH₃COO⁻ and NCS⁻) compounds, where the Pb²⁺ ion coordinates the X ligand together with S and two O atoms from the surrounding Cys thiolate, Gly amide C=O and carboxylic C=O groups, in tetrahedral coordination geometry. Both the Glu and Gly carboxylic groups were assumed to be protonated (-COOH) and the amine group (-NH₂) deprotonated.⁴² Under our experimental conditions (precipitate at pH = 2.5), the amine group is protonated -NH⁺₃, while the partly deprotonated Gly / Glu carboxylate (-COO⁻) group, together with the cysteine thiolate and possibly the weakly coordinating amide C=O groups,⁸² can bind to the Pb²⁺ ion (Scheme 2). Weak coordination of a ClO₄⁻ oxygen atom has been observed in the crystal structure of [PATH-Pb][ClO₄], a biological model for a three-coordinated Pb²⁺ ion with PbSN₂ distorted trigonal pyramidal geometry and a Pb...O-ClO₃⁻ distance of 2.868 Å (PATH = 2-methyl-1-[methyl(2-pyridin-2-ylethyl)amino]propane-2-thiolato).^{77,83} However, the absence of splitting in the IR bands for the ClO₄⁻ ion excludes a short Pb-O-ClO₃ distance in [Pb(AH₂)]ClO₄.

Similar short Pb-S distances as that obtained for solid [Pb(AH₂)]ClO₄, 2.64 ± 0.04 Å, have also been observed in lead (II) complexes with low coordination number. The lead(II) complex with ethanedithiol has two Pb-S bonds at 2.65 – 2.66 Å.⁸⁴ For three-coordinated lead (II) complexes with PbS₃, PbS₂N and PbS₂O geometries, and four-coordinated PbS₂N₂ complexes, the average Pb-S bond distances vary within the range 2.64 - 2.71 Å; in four-coordinated PbS₂O₂ structures the average Pb-S distance is slightly longer: 2.75 Å (see Tables 7 and S-6 for details). The average Pb-(N/O) distances in three-coordinated lead(II) structures are considerably longer (2.50 - 2.51 Å) than the Pb-O distance 2.28 ± 0.04 Å obtained for [Pb(AH₂)]ClO₄. However, similar short Pb-O distances have been obtained in several EXAFS studies for O²⁻, OH⁻ or H₂O ligands; *e.g.* for Pb²⁺ ions adsorbed on Mn(III, IV) (oxyhydr)oxide minerals (2.28 – 2.32 Å),⁸⁵ on biogenic layers of manganese oxide (2.31-2.32 Å),⁸⁶ lead (II) complex formation at a water – Al₂O₃ surface (2.20 – 2.29 Å),⁸¹ and with lignocellulosic biomaterials (2.35 Å).⁸⁷ Also, for crystalline PbO with two short (2.22 Å) and two long Pb-O distances (2.49 Å) in a PbO₄ square pyramidal geometry, the EXAFS spectrum measured at room temperature could only show the closest oxygen atoms at Pb-O_{ave} 2.21 Å.⁸¹

Earlier reports by Manceau et al.⁸⁰ and Bargar et al.⁸¹ on the speciation of Pb²⁺ ions surrounded by ligands with oxygen donor atoms showed that large variations in the Pb-O distances in the first shell, *i.e.* a distorted coordination environment, result in destructive interference between the Pb-O oscillations that reduces the EXAFS amplitude, which is correlated to the Pb-O coordination number. Self-absorption by Pb²⁺ ions was considered to be a less important factor in reducing the EXAFS amplitude (~ 3 - 4%). Modeling the structural disorder with a single scattering path not only increased its σ^2 value, but also made the determination of the correlated coordination number ambiguous. Also, it was not possible to obtain quantitative information about oxygen atoms at longer distances (beyond the first shell) from the Pb L_{III}-edge EXAFS spectra measured at room temperature because of large motion of the atoms. Moreover, both Manceau et al.⁸⁰ and Bargar et al.⁸¹ reported that the Pb-O bond distances obtained from room temperature EXAFS spectra were too short when compared with the data collected at 10 K, again a result of a highly asymmetric distribution of the distances. Manceau et al. had obtained the amplitude functions and phase shifts empirically from model compounds,⁸⁰ while Bargar et al. used the FEFF5 program.⁸¹

The remaining question is whether the $[\text{Pb}(\text{AH}_2)]\text{ClO}_4$ structure is polymeric. PbPen is a polymeric structure with its infinite double chain with $\text{Pb}\cdots\text{Pb}$ distances 4.363 Å,⁶⁴ while the bis(*N*-methylthioacetohydroxamato)lead(II) structure with PbS_2O_2 coordination geometry and a $\text{Pb}\cdots\text{Pb}$ separation of 4.05 Å is regarded as monomeric.⁸⁹ For $[\text{Pb}(\text{AH}_2)]\text{ClO}_4$, an average $\text{Pb}\cdots\text{Pb}$ distance of 4.15 ± 0.05 Å was obtained (Table S-1). Since the coordinated Pb-S and Pb-O bond distances are too short to act as bridging ligand atoms, a chain structure is probably formed, where an AH_2^- ligand connects two Pb^{2+} ions by its carboxylate and thiolate groups. A polymeric structure is also possible, similar to that of the Pb(II) ethanedithiol complex with short Pb-S bonds (2.65 – 2.66 Å) and longer interactions (3.05 – 3.58 Å) in asymmetric bridges to the neighboring Pb^{2+} ions with $\text{Pb}\cdots\text{Pb}$ distances at 4.20 Å.⁸⁴

Structure of the Pb^{2+} -GSH complexes in solution

In the ESI-MS spectra of the lead(II) glutathione solutions **A** - **E**, species with not more than three GSH ligands bound to Pb^{2+} could be detected even with high ligand excess (Figure 2, Table 3). This result differs from that of a recent ESI-MS study in the negative ion mode, where complex formation of the Pb^{2+} ion with three and four GSH ligands were reported for a solution with $C_{\text{Pb}^{2+}} = 10$ mM and mole ratio $\text{GSH}/\text{Pb}^{2+} = 4.0$ at pH 8.⁹⁰

^1H NMR spectra of solutions **A** – **E** showed a significant downfield shift for Cys β_1 and β_2 protons, indicating that the GSH ligands are coordinated to Pb^{2+} ions via their thiol groups (Figure 3, Table S-3). In the UV-*vis.* spectra (Figure 4), the $\text{S}^- \rightarrow \text{Pb}$ LMCT transition appeared as a shoulder at 317 nm for solution **A**, and as a peak with higher molar absorptivity for solutions **B** ($\lambda_{\text{max}} = 330$ nm) and **C-E** ($\lambda_{\text{max}} = 335$ nm). Similar LMCT bands at ~ 330 nm have been reported for Pb(II) complexes of sulfur-rich proteins and peptides containing three or four cysteine residues.^{17, 18, 69, 71, 91} Previous UV-*vis* studies on the coordination of Pb^{2+} ion to metal-binding peptides show a blue shift of the LMCT band from ~330 nm to ~315 nm, when the number of coordinated cysteine residues to the lead(II) center decreases from three to two, respectively.^{71, 91} EXAFS investigations on lead (II) complex formation to the metalloregulatory protein CadC revealed a PbS_3 coordination environment with the mean Pb-S distance of 2.66 Å for the wild type protein, and PbS_2O for the C60G mutant (with similar Pb-S distance of 2.67 Å, and mean Pb-O distance at 2.66 Å). The LMCT bands for these wild type (PbS_3) and mutant (PbS_2O) proteins were found at 350 nm and 325 nm, respectively.¹⁵ Comparisons between the LMCT bands observed for Pb^{2+} -GSH solution and these literature values show that the Pb^{2+} ions are coordinated by two sulfur donors in **A**, two or three in solution **B**, and predominantly three in solutions **C** – **E**.

Considering the large chemical shift range (~17000 ppm) in ^{207}Pb NMR spectroscopy, the chemical shifts observed for solutions **I** (2716 ppm) and **C** – **E** and (2775 – 2793 ppm) are comparable to those reported for lead(II) complexes with PbS_2N_2 , PbS_2N , PbS_3 and $\text{PbS}_2\text{NS}'$ coordination geometries with $\delta(^{207}\text{Pb})$ of 2733, 2852, 2818 - 2868 and 2873 ppm, respectively (Table 4).⁷⁶ A useful complement to the result from ^{207}Pb NMR technique is provided by structural information from X-ray absorption spectroscopy (see below).

GSH / Pb^{2+} mole ratio 2.0—The ^1H NMR spectrum of solution **A** showed the largest change in the chemical shift of the Cys β_1 and β_2 protons, with $\Delta\delta = 0.95$ ppm with respect to free GSH at pH 8.5 (Figure 3 and Table S-3). This is in contrast to a previous ^1H NMR study of a D_2O solution containing $\text{GSH}/\text{Pb}^{2+} = 2.0$ (at pD = 12.9, $C_{\text{Pb}^{2+}} = 5$ mM) where the observed $\Delta\delta$ for the Cys β proton was only 0.23-0.35 ppm.⁴¹ These chemical shift differences may indicate that the speciation of Pb^{2+} -GSH complexes is different in highly alkaline media, where the amino groups are deprotonated, and hydrolysis of the Pb^{2+} ions is also a competing factor.⁹² A closer look at Figure 3 shows that the NMR signal observed for

Glu α proton in free GSH shifts downfield in the spectrum of solution **A**, which can be attributed to the Pb^{2+} coordination to Glu amine or COO^- groups at pH 8.5.

No ^{207}Pb NMR signal could be observed for solution **A**. Line broadening due to chemical exchange is likely the reason for the absence of a resonance, and suggests that mixtures of different Pb^{2+} coordination environments are present for solutions with mole ratios $\text{GSH}/\text{Pb}^{2+} = 2.0$.

The EXAFS spectrum of solution **A** was fitted to different models (Table S-4). When only Pb-S bonds were included in the EXAFS model (I), the coordination number refined to 2.7 at a mean distance of $2.63 \pm 0.04 \text{ \AA}$. As discussed above, the bond distances between Pb^{2+} and its nearest neighbors obtained from EXAFS oscillation Curve-Fitting at RT are slightly shorter than those obtained from crystallography, as previously observed e.g. for the tris(2-mercapto-1-phenylimidazolyl)hydroborato lead complex, with the average Pb-S distance of $2.693 \text{ \AA}$ from the crystal structure,¹⁹ and $2.671 \text{ \AA}$ from EXAFS spectroscopy.¹⁷ Also, the average Pb-(O/N) and Pb-S bond distances obtained from the EXAFS model fitting to the spectrum of solid PbPen in the current study were slightly shorter ($\sim 0.03 \text{ \AA}$) than the corresponding crystallographic distances.

The EXAFS spectrum of solution **A** was also modeled using a combination of Pb-S and Pb-(N/O) scattering pathways, considering the downfield shift of ^1H NMR signal for the Glu α proton in this solution, and the maximum absorption of 315 nm in its UV-*vis*. spectrum. Similar fitting residuals were obtained for $\text{PbS}_2(\text{N/O})$ or $\text{PbS}_2(\text{N/O})_2$ coordination models (II and III in Table S-4), with the mean Pb-S and Pb-(N/O) bond distances at $2.65 - 2.66 \text{ \AA}$ and $2.48 - 2.51 \text{ \AA}$, respectively. The refined Pb-(N/O) distances are considerably longer ($\sim 0.2 \text{ \AA}$) than that of solid $[\text{Pb}(\text{AH}_2)]\text{ClO}_4$ with PbSO_2 coordination geometry, and close to those observed for the three-coordinated bis[2,4,6-tris(trifluoromethyl)thiophenolato] (tetrahydrofuran)lead(II) (PbS_2O ; code: KOYFEJ) and bis(2,6-dimethylphenylthiolato)(4-dimethylaminopyridyl)lead(II) (PbS_2N ; code: XIRCEH) crystal structures, and the four-coordinated PbS_2N_2 structures: benzil bis(thiosemicarbazonato)lead(II) (code: NOGQQQ) and bis(8-mercaptoquinolinolato)lead(II) (code: PBMQNL10); see Table S-6.^{76, 77, 93-95} The large disorder parameter (σ^2) of $0.012 - 0.016 \text{ \AA}^2$ obtained for the Pb-S path represents a wide distribution in the Pb-S bond distances; the formation of mixtures of complexes with $\text{PbS}_2(\text{N/O})$ and $\text{PbS}_2(\text{N/O})_2$ coordination is likely the reason. Fast ligand exchange in such a mixture probably prevented the observation of a ^{207}Pb NMR resonance for solution **A**.

The EXAFS spectrum of the frozen glass **A*** with similar composition ($C_{\text{Pb}^{2+}} = 10 \text{ mM}$, $\text{GSH}/\text{Pb}^{2+} = 2.0$) was fitted with different lead(II) coordination models (Table S-5). For an EXAFS model including only the Pb-S path (model I), the coordination number refined to 2.9 for the mean distance $2.67 \pm 0.04 \text{ \AA}$, which is longer than that obtained for the corresponding solution **A** measured at RT using a similar model ($2.63 \pm 0.04 \text{ \AA}$; Table S-4). The best fit was obtained using a $\text{PbS}_2(\text{N/O})\text{S}'$ coordination model (IV) for which the Pb-S, Pb-(N/O) and the long distance Pb-S' average path lengths refined to $2.67 \pm 0.04 \text{ \AA}$, $2.40 \pm 0.04 \text{ \AA}$ and $3.18 \pm 0.05 \text{ \AA}$, respectively (Figure 8, Table 6). Fixing the Pb-O distance at $2.45 \text{ \AA}$ in the same model fit resulted in very similar refined values and residual, as the contribution of this path is diminished by its high disorder parameter ($\sigma^2 = 0.013 \text{ \AA}^2$). There is no evidence in the literature for Pb(II)-glycerol interactions in such mixtures. However, considering the disordered coordination around Pb^{2+} ions in general, weak long distance interactions that are difficult to detect cannot be ruled out. For the Hg(II)-glutathione system we have shown that the presence of 33% v/v glycerol had no effect on the speciation.⁵⁰

GSH / Pb^{2+} mole ratio 3.0—In the ^1H NMR spectrum of solution **B** ($\text{GSH}/\text{Pb}^{2+} = 3.0$), only the Cys β protons that are close to the GSH thiol group are significantly deshielded

relative to free GSH, with $\Delta\delta = 0.65\text{--}0.69$ ppm (Table S-3). The LMCT band at $\lambda_{\text{max}} = 330$ nm in the UV-vis. spectrum of this solution shows that the Pb^{2+} ions are surrounded by two to three GSH thiol groups (Figure 4).

No ^{207}Pb NMR signal could be observed for this solution, however, by increasing the Pb^{2+} concentration from 10 to 90 mM in solution **I** ($\text{GSH}/\text{Pb}^{2+} = 3.0$), a broad peak with $\delta(^{207}\text{Pb}) = 2716$ ppm appeared that is close to the ^{207}Pb chemical shift of 2733 ppm obtained for the solid $(2,6\text{-Me}_2\text{C}_6\text{H}_3\text{S})_2\text{Pb}(\text{py})_2$ complex with PbS_2N_2 coordination (Table 4). It is also comparable to that of $[\text{Pb}(\text{SPh})_3]^-$ complex with PbS coordination ($\delta(^{207}\text{Pb}) = 2818$ ppm in CD_3OD).⁷⁴ Coordination of O donor atoms to the Pb^{2+} ion are known to cause an upfield shift of the ^{207}Pb NMR resonance (i.e. to lower frequency). For example, in Pb^{2+} complexes to thiohydroxamic ligands with PbS_2O_2 coordination environments, ^{207}Pb NMR resonances have been observed within the range 1493–1555 ppm (in CDCl_3).⁷⁸

A recent ^{207}Pb NMR study on 5 mM enriched $^{207}\text{Pb}(\text{II})$ aqueous solutions with mole ratios $\text{GSH}/\text{Pb}^{2+} = 3.0$ gave a single resonance from 2777 to 2793 to 2797 ppm (converted to the present calibration standard) for pH values from 7.5 to 8.5 to 9.5. This narrow range of chemical shifts was assigned to $\text{Pb}(\text{GSH})_3$ as the major species with trigonal pyramidal PbS_3 coordination geometry.⁵⁵ Increasing the mole ratio to $\text{GSH}/\text{Pb}^{2+} = 6.0$ (pH = 8.0, 25 °C) resulted in a resonance within the same range (2787 ppm).

Least-Squares Curve-Fitting of the experimental EXAFS oscillation obtained for solution **B** using a model with only Pb-S bonds (model I, Table 6) resulted in the mean Pb-S distance 2.65 ± 0.04 Å with a smaller disorder parameter ($\sigma^2 = 0.007$ Å²) than for **A** (0.011 Å²). The refined coordination number of 2.9 for solution **B** is higher than that of solution **A** and is reflected in the larger FT peak (Figure 7). Including a Pb-N path in the models (PbS_2N or PbS_2N_2) resulted in a mean Pb-S distance of 2.65 ± 0.04 Å, while the Pb-N distance varied from 2.45 to 2.49 Å with large disorder parameters ($\sigma^2 = 0.0067$ Å² PbS_2N , 0.0123 Å² PbS_2N_2). Note that oxygen and nitrogen cannot be distinguished by EXAFS spectroscopy; however, due to the considerably deshielded ^{207}Pb NMR chemical shifts obtained for the Pb^{2+} -GSH solutions, Pb^{2+} -O coordination (e.g. OH^- or H_2O) does not seem likely at pH 8.5. The coordinated N-site of glutathione is probably the Glu amine group, as indicated by the small down field shift of the Glu α proton (Figure 3). It is unlikely that the nitrogen atoms of the GSH amide groups coordinate to Pb^{2+} ions: it would require deprotonation and has only been observed for some metal ions at high pH, with crystal field stabilization energy being the driving force;⁸² this is not the case for Pb^{2+} ions.

The combined results from the UV-vis., ^{207}Pb NMR and Pb L_{III}-edge EXAFS spectroscopic studies suggest therefore that solution **B** contains a mixture of Pb^{2+} -GSH complexes with PbS_3 and PbS_2N_2 geometries (Scheme 3), where glutathione coordinates via its $-\text{NH}_2$ and thiol groups to the Pb^{2+} ions, with the mean Pb-S and Pb-N distances 2.66 ± 0.04 Å and 2.49 ± 0.04 Å, respectively.

The EXAFS spectrum at LT for the glassy solution **B*** with similar composition was well modeled by a single shell of Pb-S scatterers with the coordination number 3.4. The mean Pb-S distance 2.65 ± 0.04 Å is the same as that obtained for solution **B** measured at RT, making a PbS_3 coordination environment likely also for the Pb^{2+} -GSH species in **B***. Including the Pb-N scattering path in the fitting model either increased the fitting residual, or resulted in high disorder parameter for this path, which is not reasonable at low temperature (models II and V in Table S-5).

GSH / Pb^{2+} mole ratios ≥ 4.0 —The UV-vis. spectra of solutions **C** – **E** ($\text{GSH} / \text{Pb}^{2+} = 4.0 - 10.0$) display overlapping peaks at 335 nm, showing that three GSH thiol groups are

coordinated to the Pb^{2+} ions.^{17, 71} The ^{207}Pb NMR chemical shifts for solutions **C** – **E** in the range 2775 – 2793 ppm are comparable to the PbS_3 coordinated $[\text{Pb}(\text{SPh})_3]^-$ complex with $\delta(^{207}\text{Pb}) = 2818$ ppm in CD_3OD .⁷⁴

The refined Pb-S distance at 2.65 ± 0.04 Å for solutions **C** – **E** is comparable with average Pb-S bonds in crystal structures of three-coordinated PbS_2O , PbS_2N and PbS_3 , and four-coordinated PbS_2N_2 complexes (range 2.64–2.71 Å; see Tables 7 and S-6) and slightly shorter than that of PbS_2O_2 complexes ($R_{\text{av}} = 2.75$ Å). Fitting the EXAFS spectra of these solutions using a PbS_2N_2 model led to slightly longer Pb-S bond distances and reasonable σ^2 values, although with higher residuals than for the PbS_3 model (Table S-4).

Thus, the combination of UV-*vis.*, ^{207}Pb NMR and EXAFS spectroscopic results confirms that in solutions **C** – **E**, the $\text{Pb}(\text{GSH})_3$ complex is the dominating species with glutathione coordinated exclusively via its thiol group with the mean Pb-S distance 2.65 ± 0.04 Å (Scheme 3). Structural studies of Pb^{2+} complex formation to sulfur-rich sites, including Zn^{2+} in peptides and metalloregulatory proteins, also show preference for a PbS_3 coordination environment, with mean Pb-S distances obtained from EXAFS spectroscopy reported at 2.64 Å and 2.66 Å, respectively.^{15, 17} A PbS_4 coordination geometry is unlikely for the Pb^{2+} -GSH complexes, because in this case the average Pb-S distance should be ~ 2.8 Å (Table S-6), which is more commonly found in polymeric complexes and for Pb^{2+} complexes to dithiocarbamate ligands (not included in Table S-6).

The experimental EXAFS spectra for glasses **C*** – **E*** with similar compositions (mole ratios $\text{GSH} / \text{Pb}^{2+} = 4.0$) were well modeled by three Pb-S bonds at the mean distance of 2.65 ± 0.04 Å, which is the same mean Pb-S distance observed for the Pb^{2+} -GSH solutions **C** – **E** at RT, indicating similar speciation. The smaller Debye-Waller parameter for the Pb-S path, from ~ 0.006 Å² at RT to ~ 0.004 Å² at LT, is expected due to the reduced thermal disorder at low temperature. Addition of a Pb-N path in the fitting model generally did not improve the residual, or resulted in high disorder parameter for this path, which is unexpected at such low temperature (Table S-5).

Conclusion

The vibrational spectra of the white precipitate formed in acidic aqueous solutions of GSH and $\text{Pb}(\text{ClO}_4)_2 \cdot 3\text{H}_2\text{O}$ were consistent with coordination of one GSH ligand to the Pb^{2+} ion through the cysteinyl thiolate and glutamyl / glycyl carboxylate groups in a $[\text{Pb}(\text{AH}_2)]\text{ClO}_4$ compound containing uncoordinated ClO_4^- groups. Its Pb L_{III} -edge EXAFS spectrum was modeled with two Pb-O bonds at 2.28 ± 0.04 Å, one Pb-S bond at 2.64 ± 0.04 Å and with Pb...Pb interactions at 4.15 ± 0.05 Å. Based on these results, a structure with dimeric species with thiolate sulfur atoms forming an asymmetric bridge between the Pb^{2+} centers is proposed.

In dilute solution ($C_{\text{Pb}^{2+}} = 10$ mM) with mole ratio $\text{GSH}/\text{Pb}^{2+} = 2.0$ (pH 8.5), complexes with $\text{PbS}_2(\text{N/O})$ and $\text{PbS}_2(\text{N/O})_2$ coordination predominately form, with the mean Pb-S and Pb-(N/O) distances 2.65 ± 0.04 Å and $2.48 - 2.51$ Å, respectively. The ^1H NMR data support that the Cys thiol and Glu amine or COO^- groups are possible ligands to the Pb^{2+} ion. A ^{207}Pb NMR resonance could not be detected for this solution indicating a mixture of different species, and making it difficult to propose whether Glu amine or the more shielding COO^- groups are coordinated.

At the mole ratio $\text{GSH} / \text{Pb}^{2+} = 3.0$ a mixture of $\text{Pb}(\text{GSH})_2$ and $\text{Pb}(\text{GSH})_3$ complexes with PbS_2N_2 and PbS_3 geometries (Scheme 3) is present, giving rise to a broad ^{207}Pb NMR signal at 2716 ppm ($C_{\text{Pb}^{2+}} = 90$ mM), a $\text{S}^- \rightarrow \text{Pb}^{2+}$ LMCT band at $\lambda_{\text{max}} = 330$ nm, a slight

downfield shift for the Glu α proton, and average Pb-S and Pb-N distances of 2.66 ± 0.04 Å and 2.49 ± 0.04 Å.

For solutions containing mole ratios GSH/Pb²⁺ = 4.0, three-coordinated PbS₃ complexes predominate, with the mean Pb-S distance 2.65 ± 0.04 Å, ²⁰⁷Pb NMR chemical shift 2793 ppm and a LMCT band at 335 nm. The trisglutathionyl lead(II) complex with the general formula [Pb(GSH)₃]²⁻, was also detected by ESI-MS and persists even at high excess of the ligand (mole ratio GSH/Pb²⁺ = 10). This complex probably has trigonal pyramidal PbS₃ geometry (Scheme 3), as the coordination figure mostly is hemi-directed with a stereochemically active inert electron pair for coordination numbers below five. A preference for PbS₃ coordination environments was previously observed for Pb²⁺ bound to large proteins (e.g. cysteine-rich Zn²⁺ structural domains and metalloregulatory proteins).

EXAFS spectra were measured for Pb²⁺-GSH frozen glasses with C_{Pb2+} = 10 mM and mole ratios GSH/Pb²⁺ = 2.0 – 10.0 from solutions with pH 8.5 and with 33% v/v glycerol. The same Pb-S bond distance of 2.65 ± 0.04 Å was obtained at both RT and LT for solutions containing excess GSH where Pb(GSH)₃ species are dominant, indicating that the highest thiol coordinated complex formed at LT (PbS₃) is the same as at RT. This is in contrast to previous results for Hg²⁺-GSH and Cd²⁺-GSH alkaline solutions, for which complexes with higher thiolate coordination numbers were found to form at LT.^{50,51}

These results for the Pb²⁺ complex formation with GSH have implications for the rational design of chelating agents for therapeutic treatment of lead poisoning. Currently the most common compounds used for chelation therapy of Pb²⁺ are meso-2,3-dimercaptosuccinic acid (DMSA), ethylenediaminetetraacetic acid (EDTA), D-penicillamine (DPA), and 2,3-dimercaptopropanol (British Anti-Lewisite, BAL).^{1, 96} One problem associated with these chelating agents is that they are not selective and can also bind essential Fe²⁺, Ca²⁺ and Zn²⁺ metal ions resulting in related toxic effects.⁹⁶ A ¹H NMR study demonstrated that the tridentate [tris(2-mercapto-1-phenylimidazolyl)hydroborate] ligand chelates to the Pb²⁺ ion with 500-fold affinity over Zn²⁺, in the S₃ coordination mode.¹⁹ Given that Pb²⁺ prefers to bind a maximum of three GSH ligands through the Cys thiolate group in aqueous solution, our results suggest that a specially tailored chelating agent with three sulfur donor atoms available for binding could be very efficient in sequestering Pb²⁺ ions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Scheme 1.

The dominant form (AH²⁻) of glutathione at pH ~9.³⁸

Figure 1.

a) Pb L_{III} -edge EXAFS spectrum for the solid $[Pb(AH_2)]ClO_4$ compound with extracted contributions for each scattering path shown below; *b)* corresponding Fourier transforms of EXAFS oscillations (model 3 in Table S-1, including: 2 Pb-O 2.28 ± 0.04 Å, 1 Pb-S 2.64 ± 0.04 Å and 2 Pb...Pb 4.15 ± 0.05 Å).

Figure 2. ESI-MS spectrum measured in the negative ion mode for solution **E**, mole ratio GSH/ Pb^{2+} = 10.0, pH 8.5, $C_{\text{Pb}^{2+}}$ = 10 mM. Peaks assigned to $\text{Pb}(\text{GSH})_3$ species with ^{208}Pb (Table 3) are shown in the inset.

Figure 3. ^1H NMR spectra of solutions A - E with GSH/ Pb^{2+} mole ratios 2.0 (A), 3.0 (B), 4.0 (C), 5.0 (D), 10.0 (E) ($C_{\text{Pb}^{2+}} = 10$ mM) in 100% D_2O , compared with free glutathione³⁹ (as AH $_2^+$ /AH $_2^-$) at $\text{pD} = 8.9$ (measured $\text{pH} = 8.5$).⁶⁸ The chemical shift is referenced relative to the $\text{H}_2\text{O}/\text{HOD}$ peak at 4.80 ppm; changes in the chemical shifts relative to free GSH are given in Table S-3.

Figure 4.

UV-*vis* spectra of Pb^{2+} -GSH solutions A – E with $C_{\text{Pb}^{2+}} = 10 \text{ mM}$ and mole ratios GSH/ $\text{Pb}^{2+} = 2.0$ (A), 3.0 (B), 4.0 (C), 5.0 (D), and 10.0 (E) compared with free glutathione as $\text{AH}_2^-/\text{AH}^{2-}$ at pH 8.5.

Figure 5. ^{207}Pb NMR spectra of aqueous solutions **C**, **D** and **E** (GSH/ Pb^{2+} mole ratios 4.0, 5.0, and 10.0, respectively), containing 10% D_2O , $C_{\text{Pb}^{2+}} = 10$ mM at pH 8.5 and 300 K. A broad signal was obtained for the concentrated solution, **I**, with $C_{\text{Pb}^{2+}} = 90$ mM and GSH/ $\text{Pb}^{2+} = 3.0$ at pH 8.5.

Figure 6. Pb L_{III}-edge XANES spectra at RT for Pb²⁺-GSH solutions **A** (blue; E₀ = 13034.8 eV) and **E** (red; E = 13033.9 eV) with C_{Pb²⁺} = 10 mM, pH 8.5 and GSH/Pb²⁺ mole ratios 2.0 and 10.0, respectively (E₀ is the inflection point of the rising edge).

Figure 7. (Left) Pb L_{III}-edge EXAFS spectra for Pb²⁺-GSH solutions at RT with $C_{\text{Pb}^{2+}} = 10$ mM, pH 8.5 for the mole ratios GSH/Pb²⁺ 2.0 (A), 3.0 (B), 4.0 (C), 5.0 (D) and 10.0 (E). (right) Corresponding Fourier transforms of EXAFS data; see Table 5.

Figure 8.

(a) Pb L_{III}-edge EXAFS spectrum for the frozen glass A* at ~25 K, with separate contributions from the Pb-S, Pb-(N/O) and Pb-S' scattering paths below, $C_{\text{Pb}^{2+}} = 10 \text{ mM}$, $\text{GSH}/\text{Pb}^{2+} = 2.0$, 33 % v/v glycerol, pH 8.5. (b) Corresponding Fourier transforms of the EXAFS oscillations (see Table 6).

Figure 9. (*Left*) Pb L_{III}-edge EXAFS spectra for Pb²⁺-GSH frozen glasses at ~25 K with GSH/Pb²⁺ mole ratios 3.0 (**B***), 4.0 (**C***), 5.0 (**D***) and 10.0 (**E***), $C_{\text{Pb}^{2+}} = 10$ mM, 33 % v/v glycerol, pH 8.5. (*Right*) Corresponding Fourier transforms of the EXAFS (see Table 6 for structural parameters).

Scheme 2.

A possible structure for the $[\text{Pb}(\text{AH}_2)]\text{ClO}_4$ precipitate formed at pH 2.5; the accuracy of bond distances is $\pm 0.04 - 0.05 \text{ \AA}$.

Scheme 3.

Proposed structures for lead(II)-glutathione complexes $\text{Pb}(\text{GSH})_2$ and $\text{Pb}(\text{GSH})_3$ at pH 8.5, with the latter dominating in the solutions containing $C_{\text{Pb}^{2+}} = 10 \text{ mM}$ and $C_{\text{GSH}} > 15 \text{ mM}$ (for the distances, see Table 5).

Table 1Composition of the Pb^{2+} -GSH Solutions Studied at pH 8.5.^a

Solution	GSH/ Pb^{2+}	$C_{\text{Pb}^{2+}}$ (mM)	C_{GSH} (mM)
RT solutions			
A	2.0	10	20
B	3.0	10	30
C	4.0	10	40
D	5.0	10	50
E	10.0	10	100
I	3.0	90	270
LT solutions			
A*	2.0	10	20
B*	3.0	10	30
C*	4.0	10	40
D*	5.0	10	50
E*	10.0	10	100

^aSolutions A* – E* contain 33% v/v glycerol added just before EXAFS measurement; the pH was measured prior to addition of glycerol.

Table 2Selected vibrational bands with assignments for the $[\text{Pb}(\text{AH}_2)]\text{ClO}_4$ precipitate (see Figure S-1)

Raman (cm^{-1})		IR (cm^{-1})		Assignment ⁶⁶
GSH	$[\text{Pb}(\text{AH}_2)]\text{ClO}_4$	GSH	$[\text{Pb}(\text{AH}_2)]\text{ClO}_4$	
2525 s	-	2525 w	-	$\nu(\text{S-H})$
1706 m		1711 s	1708 sh	$\nu(\text{COOH})$
1663 m	1642 m	1661 s		$\nu(\text{C=O})$
1630 m		1627 vs		$\nu(\text{C=O})$
1597 sh	1576 br	1600 vs	1632 br	$\nu_{\text{as}}(\text{COO}^-)$
1537 w		1539 vs	1540 vs	$\nu(\text{C-N}) + \nu(\text{N-H})$
1397 s	1388 m	1397 s	1387 br	$\nu_{\text{s}}(\text{COO}^-)$
	930 s		920 w	$\nu(\text{Cl-O})$
	624 w		622 m	$\delta(\text{Cl-O})$

Table 3

Assignment of Ions Observed in ESI Mass Spectra for the Pb^{2+} -GSH Solutions (pH 8.5, $C_{\text{Pb}^{2+}} = 10 \text{ mM}$) Measured in the Negative Ion Mode, as Shown in Figure 2 for solution **E** ($C_{\text{GSH}} = 100 \text{ mM}$).^a

$-m/z$ (amu)	Assignment	$-m/z$ (amu)	Assignment
1380.7	$[7\text{Na}^+ + (\text{GSH})_4 - 8\text{H}^+]^-$	678.9	$[3\text{Na}^+ + (\text{GSH})_2 - 4\text{H}^+]^-$
1358.7	$[6\text{Na}^+ + (\text{GSH})_4 - 7\text{H}^+]^-$	656.9	$[2\text{Na}^+ + (\text{GSH})_2 - 3\text{H}^+]^-$
1235.7	$[5\text{Na}^+ + \text{Pb}(\text{GSH})_3 - 8\text{H}^+]^-$	632.9	$[\text{Na}^+ + \text{GSSG} - 2\text{H}^+]^-$
1213.7	$[4\text{Na}^+ + \text{Pb}(\text{GSH})_3 - 7\text{H}^+]^-$	612.8	$[(\text{GSH})_2 - \text{H}^+]^-$
1191.8	$[3\text{Na}^+ + \text{Pb}(\text{GSH})_3 - 6\text{H}^+]^-$	610.9	$[\text{GSSG} - \text{H}^+]^-$
1029.9	$[5\text{Na}^+ + (\text{GSH})_3 - 6\text{H}^+]^-$	511.9	$[\text{Pb}(\text{GSH}) - 3\text{H}^+]^-$
862.8	$[2\text{Na}^+ + \text{Pb}(\text{GSH})_2 - 5\text{H}^+]^-$	405.9	$[\text{ClO}_4^- + \text{GSH}]^-$
840.8	$[\text{Na}^+ + \text{Pb}(\text{GSH})_2 - 4\text{H}^+]^-$	327.9	$[\text{Na}^+ + \text{GSH} - 2\text{H}^+]^-$
818.8	$[\text{Pb}(\text{GSH})_2 - 3\text{H}^+]^-$	305.9	$[\text{GSH} - \text{H}^+]^-$
		220.8	$[\text{GSH} - \text{Glu}]^-$

^a Glutathione, GSH ($\text{C}_{10}\text{H}_{17}\text{N}_3\text{O}_6\text{S}$) $m = 307.3$, oxidized glutathione, GSSG ($\text{C}_{20}\text{H}_{32}\text{N}_6\text{O}_{12}\text{S}_2$) $m = 612.6$

Table 4²⁰⁷Pb NMR chemical shifts reported for lead(II) coordination sites containing sulfur ligands.^a

Coordination environment	Chemical shift (δ, ppm)	Ref.
PbS ₃	2818-2868	74, 75
PbS ₃ (peptides)	2577 - 2853	53
PbS ₂ NS' ^{b,c}	2873	76
PbS ₂ N ^c	2852	76
PbS ₂ N ₂ ^c	2733	76
PbSN ₂	2357	77
PbS ₂ O ₂	1506-1555	78
PbS ₃ O ₃	1422-1463	79

^aRelative to Pb(CH₃)₄ (δ = 0), by setting the ²⁰⁷Pb chemical shift for 0.1 M Pb(NO₃)₂ in D₂O at -2961.2 ppm. For information about the types of ligands, see text and Figure S-5

^bS' is a bridging thiolate

^cSolid state ²⁰⁷Pb NMR.

Structural Parameters Derived from EXAFS Least-Squares Curve-Fitting for the Pb^{2+} -GSH Solutions **A** – **E** (pH 8.5, $C_{\text{Pb}^{2+}} = 10 \text{ mM}$) at RT (see Figure 7).^{abc}

Table 5

Solution (GSH/ Pb^{2+} mole ratio)	Pb-S			Pb-(N/O)		
	<i>N</i>	<i>R</i> (Å)	σ^2 (Å ²)	<i>N</i>	<i>R</i> (Å)	σ^2 (Å ²)
A (2.0)	2 <i>f</i>	2.65	0.0156	2 <i>f</i>	2.51	0.0056
B (3.0)	2,9 <i>d</i>	2.65	0.0070			
	2 <i>f</i> ^e	2.66	0.0057	2 <i>f</i>	2.49	0.0123
C (4.0)	3.3	2.65	0.0068			
D (5.0)	3.4	2.65	0.0064			
E (10.0)	3.3	2.65	0.0064			

^aFitting range: 2.9 – 12.4 Å⁻¹; $S_0^2 = 0.9$, *f* = fixed

^bRefined *N* accurate within ± 20 %; estimated error limits: $R \pm 0.04 \text{ Å}$, $\sigma^2 \pm 0.002 \text{ Å}^2$

^calternative EXAFS fitting models are presented in Table S-4

^d PbS_3 model shown in Figure 7

^e PbS_2N_2 model accounting for nitrogen coordination, as shown in Scheme 3.

Table 6

Structural Parameters Derived from EXAFS Least-Squares Curve-Fitting for the Pb^{2+} -GSH Frozen Glasses ($\mathbf{A}^* - \mathbf{E}^*$) at Low Temperature (see Figures 8 and 9).^{abc}

Solution (GSH/ Pb^{2+} mole ratio)	Pb-S			Pb-N(O)		
	N	R (Å)	σ^2 (Å ²)	N	R (Å)	σ^2 (Å ²)
$\mathbf{A}^*$ (2.0)	2	2.67	0.0052	1	2.40	0.0135
	1	3.18	0.0126			
$\mathbf{B}^*$ (3.0)	3.4	2.65	0.0048			
$\mathbf{C}^*$ (4.0)	3.6	2.65	0.0044			
$\mathbf{D}^*$ (5.0)	3.8	2.65	0.0047			
$\mathbf{E}^*$ (10.0)	3.5	2.65	0.0038			

^a Fitting range: $\mathbf{A}^*$ (2.8 – 11.0 Å⁻¹), $\mathbf{B}^* - \mathbf{E}^*$ (3.0 – 13.8 Å⁻¹); f = fixed, $S_0^2 = 0.9f$

^b refined N accurate within $\pm 20\%$; Estimated error limits: $R \pm 0.04$ Å, $\sigma^2 \pm 0.001$ Å²

^c alternative EXAFS fitting models are presented in Table S-5.

Table 7

Survey of three and four coordinated Pb^{2+} complexes containing S donor ligands (from CSD version 5.32 November 2010).⁸⁸ For details, see Table S-6.

Coordination	No. of structures	Pb-S distance range (Å)	Average Pb-S	Pb-(O/N) distance	Average Pb-(O/N)
PbS_4 ^a	3	2.65-3.29	2.82		
PbS_3	6	2.55- 2.86	2.70		
PbS_2N	2	2.61-2.79	2.67	2.43-2.61	2.52
PbS_2O	1	2.64	2.64	2.50	2.50
PbS_2N_2	15	2.60-2.92	2.71	2.40-2.85	2.62
PbS_2O_2	6	2.68-2.86	2.75	2.35-2.50	2.40
PbSN_2	1	2.59-2.60	2.60	2.49- 2.53	2.51

^a Pb(II) complexes to dithiocarbamates, or *S*-donors bound to P or Si were not included