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Similarities between *N*-acetylcysteine and Glutathione in Binding to Lead(II) Ions

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Abstract

N-acetylcysteine is a natural thiol-containing antioxidant, a precursor for cysteine and glutathione, and a potential detoxifying agent for heavy metal ions. However, previous accounts of the efficiency of *N*-acetylcysteine (H₂NAC) in excretion of lead are few and contradicting. Here we report results on the nature of lead(II) complexes formed with *N*-acetylcysteine in aqueous solution, which were obtained by combining information from several spectroscopic methods, including ²⁰⁷Pb, ¹³C and ¹H NMR, Pb L_{III}-edge X-ray absorption, Ultraviolet-visible (UV-vis.) spectroscopy and electro-spray ionization mass spectrometry (ESI-MS). Two series of solutions were used containing C_{Pb(II)} = 10 and 100 mM, respectively, varying the H₂NAC / Pb(II) mole ratios from 2.1 to 10.0 at pH = 9.1 – 9.4. The coordination environments obtained resemble those previously found for the Pb(II) glutathione system: at a ligand-to-lead mole ratio of 2.1 dimeric or oligomeric Pb(II) *N*-acetylcysteine complexes are formed, while a tri-thiolate [Pb(NAC)₃]⁴⁻ complex dominates in solutions with H₂NAC/Pb(II) mole ratios > 3.0.

Introduction

Lead is an environmental toxin that persists in the environment (air, water, soil) from past and current uses, e.g. in paints, leaded gasoline, batteries, water pipelines, cosmetics, and in many industrial activities such as mining, smelting, soldering, *etc.*¹ Lead exposure can have diverse impacts on human health. It can cause neurotoxicity and brain damage,² affecting cognitive, neurobehavioral and neurophysiological development especially in young children. Blood lead levels (BLL) as low as 10 – 20 µg/dL (0.5 – 1.0 µM) in children aged 4 to 10 years have been associated with reduced scores in developmental tests.³ Lead can induce anemia by inhibiting key enzymes that are involved in the heme biosynthetic pathway, i.e. δ-aminolevulinic acid dehydratase (ALAD), a zinc(II) containing metalloenzyme that facilitates formation of the pyrrole building block porphobilinogen, and

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Supporting Information Available: Crystal structure of complexes for which ²⁰⁷Pb NMR chemical shifts have been reported in Table 2; comparison of the EXAFS and XANES spectra of *c* *N*-acetylcysteine solutions with variable ligand-to-metal ratios; comparing the EXAFS spectra and corresponding Fourier-transforms for solutions A, A* and a Pb(II) glutathione solution with GSH/Pb(II) mole ratio 2 (pH = 8.5); EXAFS curve fitting results for the latter Pb(II) glutathione solution. This material is available free of charge via the Internet at <http://pubs.acs.org>.

ferrochelatase that catalyzes the insertion of an iron(II) ion into the porphyrin ring of protoporphyrin IX.^{4–6} Lead is primarily stored in the human skeleton as $\text{Pb}_3(\text{PO}_4)_2$, and can impact bone cell functions, e.g. inhibiting new bone formation, bone resorption, and skeletal developments.^{6, 7} Succimer (dimercaptosuccinic acid, DMSA), is the first FDA (Food and Drug Administration) approved oral drug for chelating therapy of lead in children with BLL at 45 $\mu\text{g}/\text{dL}$ (2.17 μM) or higher. Chelation treatment, however, is not indicated for children with lower BLL, as it has no neuro-developmental benefits.^{3, 8, 9}

At a molecular level lead toxicity is caused by: 1) its high affinity for cysteine –SH, tyrosine –OH, lysine –NH₂, glutamic / aspartic acid carboxylate (COO[–]), and phosphate groups in proteins, enzymes and cell membranes;^{10–12} 2) its ability to displace essential metal ions such as Ca(II), Fe(II), Zn(II) and Mg(II);^{13, 14} 3) its ability to adopt structurally different coordination environments and binding geometries;¹⁵ and 4) generating oxidative stress.¹⁴ Pb(II) ions have 25 times higher affinity than Zn(II) to bind to ALAD.¹⁶ Lead competes for calcium binding sites, e.g. in bone.⁷ Several mechanisms have been proposed to explain cellular uptake of lead. Pb(II) ions may gain entry to the target cells using existing transport mechanisms, e.g. by substituting Fe(II) and Ca(II) ions.¹⁷ Since Pb(II) has similar ionic radius (1.19 Å) as Ca(II) (1.00 Å),¹⁸ Pb(II) can cross the blood-brain barrier via Ca(II) channels and pumps, such as Ca²⁺-ATPase, and enter neurons and astroglial cells in the brain. Lead can then cause adverse effects on the central nervous system especially during its early development stages, by interrupting intracellular calcium homeostasis and disturbing neurotransmitters like protein kinase C that regulates memory storage, and by apoptosis (programmed cell death) and excitotoxicity.^{14, 17, 19–22}

Lead exposure also creates oxidative stress by altering the antioxidant defense system, not only through inhibition of antioxidative enzymes such as superoxide dismutase and catalase, but also by depletion of antioxidant reserves, e.g. glutathione.¹⁴ Glutathione (GSH) is the main intracellular thiol-containing peptide scavenging reactive oxygen species (ROS) that can damage biomolecules such as proteins, lipids and DNA.²³ In this process, GSH donates an electron from its thiol group to stabilize the ROS, and then converts to glutathione disulfide (GSSG). Under oxidative stress conditions GSH rapidly converts to GSSH and the concentration of GSSH is much higher than free GSH.^{2, 24, 25} Lead(II) can also inactivate GSH by binding to its thiol group.²⁶

N-acetylcysteine is a natural thiol-containing antioxidant that can prevent lead from inhibiting antioxidative enzymes by providing another source of thiol groups.²⁷ Moreover, *N*-acetylcysteine can deacetylate and convert to cysteine, which is a precursor for glutathione.^{28–30} While animal testing shows that *N*-acetylcysteine is capable of coordinating to organic/inorganic mercury and cadmium,³¹ there are contradictory reports about its effect in excretion of Pb(II) ions and its potential use for lead chelation therapy.^{25, 32, 33}

Unlike *L*-cysteine and *D*-penicillamine, *N*-acetyl-*L*-cysteine (H₂NAC; Scheme 1) has only two potential coordination sites: the carboxyl (COOH) and thiol (SH) groups that deprotonate at $\text{p}K_{\text{a}1} = 3.03$ (3.26) and $\text{p}K_{\text{a}2} = 9.51$ (9.64), respectively, at ionic strength $I = 0.2$ M (0.1 M) KNO₃ and 25 °C.^{34, 35}

N-acetylcysteine rarely acts as a bidentate (*S,O*)-donor chelating ligand to form a 6-membered metallocycle with the metal ion.^{36, 37} More often it is monodentate, binding only through its thiolate group to metal ions such as Cr(VI),³⁸ Bi(III),^{39, 40} or bridging between two metal ions like Pt(II) in a dimeric complex,⁴¹ as evidenced by X-ray crystallography or X-ray absorption spectroscopy. Recent reports based on ¹³C-NMR and FT-IR spectroscopic techniques have suggested that Pt(II), Au(I), and Ag(I) ions bind exclusively to the S atom in their *N*-acetylcysteine complexes,^{42–44} while bidentate and even tridentate coordination modes have been proposed for Zn^{II}(*S,O*-NAC),⁴⁵ and R₂Sn^{IV}(NAC) complexes,⁴⁶ respectively.

We recently reported on the nature of Pb(II) complexes formed with the amino acid cysteine,⁴⁷ as well as the chelating agent penicillamine,⁴⁸ both of which have three potential coordination sites: thiol, amine (-NH₂) and carboxyl groups. The current study complements our investigation on Pb(II) complex formation with thiol containing ligands as in *N*-acetylcysteine the amine functionality is blocked as an effective binding site. No formation constants have been reported for binary Pb(II) : *N*-acetylcysteine complexes because of precipitate formation.³⁵ We have combined several spectroscopic techniques, including electro-spray ionization mass spectrometry (ESI-MS), UV-vis., ¹H, ¹³C, ²⁰⁷Pb NMR, and Pb L_{III}-edge extended X-ray absorption fine structure (EXAFS) spectroscopy, to characterize the nature of Pb(II) complexes with *N*-acetylcysteine in aqueous solution.

In this study, we aimed to demonstrate that *N*-acetylcysteine not only can have a therapeutic role as an antioxidant, reversing the oxidative stress caused by lead exposure and normalizing the GSH/GSSG ratio according to earlier studies,⁴⁹ but also can bind to lead(II) ions in a similar way as glutathione. Therefore, further examinations of this natural antioxidant as an alternative treatment for children with BLL < 45 µg/dL, seem motivated, as lead is “unsafe at any level”.⁵⁰

Experimental Section

Sample preparation

N-acetyl-*L*-cysteine, Pb(ClO₄)₂·3H₂O and sodium hydroxide were purchased from Sigma-Aldrich, and enriched lead oxide ²⁰⁷PbO (94.5%) was obtained from Cambridge Isotope Laboratories. All chemicals were used without further purification. Syntheses were carried out under inert argon gas using distilled water deoxygenated by boiling and bubbling argon gas through at cooling. The pH of the aqueous solutions was monitored with a Thermo Scientific Orion Star pH meter, calibrated using standard buffers.

Lead(II) *N*-acetylcysteine solutions

Two series of solutions were prepared with different H₂NAC/Pb^(II) mole ratios for C_{Pb(II)} = 10 and 100 mM, respectively, at the alkaline pH value at which the initial precipitate dissolved (see Table 1). The dilute solutions A – E (C_{Pb(II)} = 10 mM) were freshly prepared for EXAFS measurements by adding Pb(ClO₄)₂·3H₂O (0.05 mmol) to a solution of *N*-acetylcysteine (0.105–0.5 mmol) in deoxygenated water (pH 2 – 2.4). For ²⁰⁷Pb NMR, UV-vis. and ESI-MS measurements of solutions A – E (C_{Pb(II)} = 10 ± 0.2 mM), 50 mM stock solutions of enriched ²⁰⁷PbO (94.5%) and PbO dissolved in 0.15 M HClO₄ were prepared,

respectively. Upon drop-wise addition of sodium hydroxide (1 M) a white precipitate formed, which dissolved at alkaline pH (9.1 – 9.4) giving a clear colorless or pale yellow solution. The final volume for each solution was adjusted to 5.0 mL. These dilute solutions were used for ^1H - and ^{13}C -NMR (prepared in deoxygenated 99.9% D_2O), ESI-MS and UV-vis. measurements. For D_2O solutions, the pH meter reading ($\text{pD} = \text{pH reading} + 0.4$)⁵¹ was 9.4 for solution A and 9.1 for solutions B – E. The more concentrated solutions A* – E* ($C_{\text{Pb(II)}} = 100 \pm 2 \text{ mM}$) were prepared in a similar way using 0.5 mmol of the hygroscopic $\text{Pb}(\text{ClO}_4)_2 \cdot 3\text{H}_2\text{O}$ solid. For solution A* with $\text{H}_2\text{NAC} / \text{Pb(II)}$ mole ratio 2.1, the pH was first raised to ~ 10.4 to dissolve the precipitate completely, and then 0.1 M HClO_4 was added to adjust the pH to 9.4. ^{207}Pb -NMR and Pb L_{III}-edge EXAFS spectra were measured for both sets of solutions (10% v/v D_2O for ^{207}Pb NMR).

Methods

Technical details about instrumentations and related procedures for UV-vis. (Cary 300), ^1H -, ^{13}C - and ^{207}Pb -NMR spectroscopy (Bruker Avance II 400 MHz), as well as EXAFS data collections and data analyses are provided elsewhere.^{47, 48} ESI-MS spectra for solutions A, B and E were measured in both positive-ion and negative-ion on an Agilent 6520 Q-ToF mass spectrometer by direct infusion of the solutions, using water as the mobile phase. The capillary, skimmer and fragmentor voltages were set at 4 kV, 65 V and 120 V, respectively. A continuous injection flow rate of 0.2 mL min^{-1} and a drying gas flow rate of 7 L min^{-1} at 200 C were used. UV-vis. spectra were measured for the dilute solutions A – E, using a 0.5 nm data interval, a quartz cell with 1 mm path length, and a 1.5 absorbance Agilent rear-beam attenuator (RBA) mesh filter in the reference position. ^{207}Pb NMR spectra for solutions A – E enriched in ^{207}Pb were measured at room temperature using a Bruker AMX 300 equipped with a 10 mm broadband probe. ^{207}Pb NMR spectra of solutions A* – E* were collected with a Bruker Avance 400 MHz instrument using a 5 mm broadband probe. For these solutions, the ^{207}Pb NMR chemical shift was externally calibrated relative to 1.0 M $\text{Pb}(\text{NO}_3)_2$ in H_2O , resonating at -2961 ppm relative to $\text{Pb}(\text{CH}_3)_4$ ($\delta = 0 \text{ ppm}$).⁵² The ^1H NMR spectra were internally referenced by means of the $\text{HOD}/\text{H}_2\text{O}$ peak at 4.80 ppm,⁵³ while the ^{13}C NMR spectra were externally calibrated using CH_3OH in D_2O resonating at 49.15 ppm. Approximately 4000 – 71000 scans for the ^{207}Pb NMR, 16 scans for the ^1H NMR and 1500 – 7200 scans for the ^{13}C NMR spectra were co-added.

Pb L_{III}-edge X-ray absorption spectra for the Pb(II) *N*-acetylcysteine solutions were measured under two different operating conditions at BL 7-3 at the Stanford Synchrotron Radiation Lightsource (SSRL). Solutions B* – E* were measured with 100 mA current, using Si(220) ($\varphi = 0^\circ$) monochromator crystals, detuning the incident beam intensity to 50% of the maximum I_0 at the end of the Pb L_{III}-edge scan range, with the beam size adjusted to 2 mm x 2 mm. Solutions A* and A – E were measured with 500 mA current and Si (220) ($\varphi = 90^\circ$) crystals, and the incident beam was detuned by 80% (for solutions A*, A – C) to 90% (for solutions D and E) of maximum I_0 at 13806 eV, and the beam size adjusted to 1 mm x 1mm, to avoid sample decomposition. For solutions containing $C_{\text{Pb(II)}} = 10 \text{ mM}$, 10 scans were measured in both transmission and fluorescence mode, detecting the X-ray fluorescence using a 30-channel Ge detector, while for the more concentrated solutions with $C_{\text{Pb(II)}} = 100 \text{ mM}$, two to three scans were collected in transmission mode, and averaged

after comparing the individual scans. The averaged fluorescence data were noisier than the corresponding transmission data; therefore for all Pb(II) *N*-acetylcysteine solutions, the EXAFS spectra measured in transmission mode were used for curve-fitting. The energy scale was internally calibrated by assigning the first inflection of a Pb foil at 13035.0 eV. The threshold energy E_0 in the X-ray absorption spectra of the Pb(II) *N*-acetylcysteine solutions varied within a narrow range: 13034.1 – 13034.7 eV. Least squares curve-fitting of the EXAFS spectra was performed over the k -range $\sim 3.0 - 12.5 \text{ \AA}^{-1}$ (solutions A* – E) or $\sim 3.0 - 13.7 \text{ \AA}^{-1}$ (solutions B* – E*).

Results

ESI-Mass Spectrometry

The ESI-MS spectra of the dilute lead(II) *N*-acetylcysteine solutions A, B and E ($C_{\text{Pb(II)}} = 10 \text{ mM}$) with ligand-to-metal ratios 2.1, 3.0 and 10.0, respectively, were measured in both positive-ion and negative-ion modes. No mass peak associated with Pb(II) *N*-acetylcysteine complexes could be detected in the negative ion mode. For all the above solutions, positive ion mass peaks were detected at m/z 370.00 and 555.01, corresponding to the Pb(II) *N*-acetylcysteine complex ions $[\text{Pb}(\text{H}_2\text{NAC}) - \text{H}^+]^+$ and $[\text{Na}^+ + \text{Pb}(\text{H}_2\text{NAC})_2 - 2\text{H}^+]^+$ with metal-to-ligand mole ratios 1:1 and 1:2, respectively. A 1:3 complex ion at m/z 740.02 was identified as $[2\text{Na}^+ + \text{Pb}(\text{H}_2\text{NAC})_3 - 3\text{H}^+]^+$ in the ESI-MS spectra of solutions B and E with the $\text{H}_2\text{NAC}/\text{Pb(II)}$ mole ratio 3.0. Only for solution A, a low intensity mass peak for a 2:2 complex ion $[\text{Pb}_2(\text{H}_2\text{NAC})_2 - 3\text{H}^+]^+$ was detected at m/z 738.98. The distinctive isotopic pattern of ^{208}Pb (52.4 %), ^{207}Pb (22.1 %), ^{206}Pb (24.1 %) and ^{204}Pb (1.4 %) facilitated the assignment of lead-containing ions in the ESI-MS spectra. Figure 1 shows a selected region of the ESI-MS spectrum for solutions A and E, with the assignment of significant peaks listed in Table S1.

Electronic Absorption Spectroscopy

UV-vis. spectra for the lead(II) *N*-acetylcysteine solutions A–E ($C_{\text{Pb(II)}} = 10 \text{ mM}$) show an absorption band in the range 324 – 335 nm (Figure 2), assigned to the combination of both $\text{S}^- (3\text{p}) \rightarrow \text{Pb}^{2+} (6\text{p})$ ligand-to-metal charge transfer (LMCT) and intra-atomic $\text{Pb}^{2+} (6\text{s}) \rightarrow \text{Pb}^{2+} (6\text{p})$ transitions.^{54–58} The red shift of the absorption band from $\lambda = 324 \text{ nm}$ (solution A) to 335 nm (solution E) is associated with an increase in the free ligand concentration.

^1H and ^{13}C NMR Spectroscopy

The ^1H and ^{13}C NMR spectra of the Pb(II) *N*-acetylcysteine solutions A – E are compared in Figure 3 with a 0.1 M solution of *N*-acetylcysteine in $\sim 100\%$ D_2O (pH 9.1). Only one set of resonances appears for both Pb(II)-coordinated and unbound *N*-acetylcysteine species, signifying fast ligand exchange on the NMR time scale. Solution A, with the lowest amount of free ligand ($\text{H}_2\text{NAC}/\text{Pb(II)}$ mole ratio = 2.1) in this series, showed the largest change in the ^1H and ^{13}C NMR chemical shifts. The ^{13}C NMR spectrum of this solution shows a deshielding of 3.1 ppm for the C_3 atom bound to the thiolate group (S^-), and only 0.5 ppm shift for the C_1 atom of the carboxylate group (see Table S2). The ^1H NMR signal of the two H_b atoms adjacent to the thiolate group is also clearly deshielded ($\sim 1.0 \text{ ppm}$), compared to the $\sim 0.2 \text{ ppm}$ shift for the H_a atom. The ^{13}C NMR signals for solutions C – E, containing

increasingly higher concentrations of unbound *N*-acetylcysteine, shift toward the corresponding peaks in the ^{13}C NMR spectrum of pure *N*-acetylcysteine.

^{207}Pb NMR Spectroscopy

The ^{207}Pb nucleus has 22.1 % natural abundance, $I = \frac{1}{2}$ spin and a receptivity of 11.7 relative to ^{13}C .⁵² The wide ^{207}Pb NMR chemical shift range (~ 17000 ppm) is sensitive to changes in local structure and electronic environment, providing insight about the nature and local geometry of the donor atoms surrounding the lead ion. Ligands containing S, N, or O donor atoms generally shield the ^{207}Pb nucleus in the order $\text{S} < \text{N} < \text{O}$.^{52, 59–62} Table 2 summarizes reported ^{207}Pb chemical shifts for Pb(II) complexes with sulfur-containing ligands.

Due to the toxicity of tetramethyl lead(IV), ^{207}Pb NMR chemical shifts are usually externally calibrated relative to the signal obtained for a 1.0 M $\text{Pb}(\text{NO}_3)_2$ aqueous solution at $\delta(^{207}\text{Pb}) = -2961$ ppm, relative to that of $\text{Pb}(\text{CH}_3)_4$ ($\delta_{\text{Pb}} = 0$ ppm).⁵² In our earlier reports on Pb(II) complex formation with glutathione, penicillamine or cysteine, we instead used a 1.0 M $\text{Pb}(\text{NO}_3)_2$ solution in D_2O for calibration, and incorrectly set its resonance to -2961 ppm,^{26, 47, 48} a mistake made also in a previous report of the ^{207}Pb NMR chemical shift for a compound with PbSN_2 coordination.⁶⁸ The ^{207}Pb chemical shift for 1.0 M $\text{Pb}(\text{NO}_3)_2$ in D_2O solution appears at -2990 ppm at room temperature, as reported by the Pecoraro group.^{65, 66} In the present work, we have re-calibrated our previously reported ^{207}Pb NMR chemical shifts, which are now referred to the resonance at -2961 ppm of a 1.0 M $\text{Pb}(\text{NO}_3)_2$ aqueous solution.

All lead(II) *N*-acetylcysteine solutions exhibit a single resonance within a narrow range in their ^{207}Pb NMR spectra; see Figure 4. For solutions C – D ($C_{\text{Pb(II)}} = 10$ mM) and B* ($C_{\text{Pb(II)}} = 100$ mM) the peaks were much broader (widths at half-height $\nu_{1/2} \sim 1\text{--}16$ kHz) than for solutions A*, C* – E* ($\nu_{1/2} \sim 10\text{--}300$ Hz; $C_{\text{Pb(II)}} = 100$ mM). The broadening is due to intermediate rate ligand exchange (on the NMR time scale) between different Pb(II) species in solution. For solutions A and B ($C_{\text{Pb(II)}} = 10$ mM, $\text{H}_2\text{NAC}/\text{Pb(II)}$ mole ratios 2.1 and 3.0, respectively) no ^{207}Pb NMR signal could be detected, indicating slower ligand exchange between the Pb(II) *N*-acetylcysteine species,⁷¹ while the sharp peaks obtained for solutions A*, C* – E* and E suggest one dominating complex, or fast exchange between several Pb(II) species in these solutions. The ^{207}Pb NMR signal gradually becomes deshielded from solution C to E ($\delta_{\text{Pb}} = 2735\text{--}2763$ ppm) and from solution B* to E* ($2739\text{--}2762$ ppm), as the free *N*-acetylcysteine concentration increases. Solutions E and E* containing excess ligand show nearly identical ^{207}Pb NMR chemical shifts, which are very close to that of a Pb(II) glutathione complex, $\text{Pb}(\text{S-GSH})_3$ ($\delta_{\text{Pb}} = 2764$ ppm; re-calibrated – see above).²⁶ Surprisingly, solution A* ($C_{\text{Pb(II)}} = 100$ mM, $\text{CH}_2\text{NAC} = 210$ mM, $\text{pH} = 9.4$) with the lowest free ligand concentration in the A* – E* series, shows a sharp signal at 2858 ppm, implying a dominating Pb(II) *N*-acetylcysteine complex with the most deshielded Pb(II) nuclei in these series.

Pb L_{III}-edge X-ray Absorption Spectroscopy

The features in the XANES region (X-ray absorption near-edge structure) are very similar in the two series of Pb(II) *N*-acetylcysteine solutions (A, A*) and (E, E*); see Figure S2. However, the slight divergence between the XANES spectra for solutions A* and E* ($C_{\text{Pb(II)}} = 100 \text{ mM}$; $\text{H}_2\text{NAC}/\text{Pb(II)}$ mole ratio 2.1 and 10.0; pH = 9.4 and 9.1, respectively), indicates some difference in the local structure around the Pb(II) ions (Figure 5, *left*). A similar deviation was previously observed between the XANES spectra of Pb(II) glutathione solutions with a comparable large increase in ligand-to-metal ratios ($C_{\text{Pb(II)}} = 10 \text{ mM}$; GSH/Pb(II) mole ratios 2.0 and 10.0; pH = 8.5).²⁶ The XANES spectrum of a 10 mM lead(II) solution containing 20 mM glutathione (pH = 8.5) is similar to that of solution A with 21 mM *N*-acetylcysteine (pH = 9.4); see Figure 5 (*right*).

A comparison of the Pb L_{III}-edge k^3 -weighted EXAFS spectra for both series of Pb(II) *N*-acetylcysteine solutions ($C_{\text{Pb(II)}} = 10$ and 100 mM , Table 1) shows a gradual increase in the EXAFS amplitude and the corresponding intensities in the Fourier transforms with increasing $\text{H}_2\text{NAC}/\text{Pb(II)}$ mole ratios; Figure 6. Solutions A and A* with the lowest free ligand concentration ($\text{H}_2\text{NAC}/\text{Pb(II)}$ mole ratio = 2.1, pH = 9.4) display the smallest EXAFS amplitude, while solutions E and E* with *N*-acetylcysteine in substantial excess ($\text{H}_2\text{NAC}/\text{Pb(II)}$ mole ratio = 10) show the largest EXAFS amplitude. The amplitude of the EXAFS oscillation previously reported for a 10 mM Pb(II) glutathione solution with GSH/Pb(II) mole ratio = 2.0 (pH = 8.5; measured at room temperature in fluorescence mode) is also comparable to those of solutions A and A* in this study;²⁶ see Figure S3.

All EXAFS spectra fitted well to a model simply consisting of a single scattering Pb-S path, which showed an increase in the refined coordination number from 2.9 – 3.0 for solutions (A, A*) to 3.6 – 3.8 for solutions (E, E*) and a decrease in the corresponding Debye-Waller parameter from 0.01 to $0.006 \pm 0.002 \text{ \AA}^2$; both consistent with the increase in the EXAFS amplitude observed as the ligand-to-metal mole ratio increases for each series of solutions (Figure S4). A mean Pb-S bond distance of $2.65 \pm 0.02 \text{ \AA}$ consistently emerged. Considering the very low relative EXAFS amplitude for solutions A and A* with $\text{H}_2\text{NAC}/\text{Pb(II)}$ mole ratio = 2.1, we assumed a binuclear species with bridging thiolate groups, where the two different Pb-S and Pb-S' scattering paths destructively interfere (*see Discussion section*). The fitting residual of the EXAFS fitting improved for both solutions with two somewhat shorter Pb-S ($2.64 - 2.65 \text{ \AA}$) and one longer Pb-S' ($2.77 - 2.78 \text{ \AA}$) distances (see Table 3).

The results of least squares curve-fitting to the experimental EXAFS oscillations obtained for the Pb(II) *N*-acetylcysteine solutions A – E and A* – E* are shown in Figure 6, with the corresponding structural parameters listed in Table 3. The separate contributions of the scattering paths in the EXAFS curve-fitting for solution A* (Figure 7), show that the two Pb-S and Pb-S' oscillations frequently are out-of-phase, reducing the overall amplitude. The Pb...Pb scattering with an average distance of $3.7 - 3.8 \text{ \AA}$ showed little contribution due to the high disorder parameter ($\sigma^2 = 0.021 - 0.028 \text{ \AA}^2$).

Discussion

When comparing *N*-acetylcysteine to cysteine or penicillamine as ligands to the Pb(II) ion, the blocked amine group greatly influences the coordination mode. This facet of the study is evident from the UV-vis. spectra in Figure 8 of 10 mM Pb(II) solutions containing penicillamine (H₂Pen), cysteine (H₂Cys) or *N*-acetylcysteine with similar ligand to Pb(II) mole ratios. For the Pb(II) cysteine (pH = 9.1 – 10.4) and penicillamine solutions (pH = 9.6), the ligand to metal charge transfer (LMCT) bands are somewhat blue-shifted (298 – 300 nm) relative to those of the Pb(II) *N*-acetylcysteine solutions (324 – 335 nm).^{47, 48} Similar S²⁻ (3p) → Pb²⁺ (6s) LMCT bands (320 – 335 nm) are observed for Pb(II) glutathione solutions with comparable ligand to Pb(II) mole ratios ($C_{\text{Pb(II)}} = 10 \text{ mM}$, pH = 8.5).²⁶

Further experimental evidence is provided by the ²⁰⁷Pb NMR chemical shifts shown in Figure 9 for 100 mM Pb(II) alkaline aqueous solutions, with the $\delta(^{207}\text{Pb})$ recorded for similar ligand to metal ratios of penicillamine (1797 – 1844 ppm) and cysteine (1981 – 2478 ppm) considerably shielded relative to those of *N*-acetylcysteine solutions. The difference is attributed to the ability of cysteine and penicillamine to form chelates as bidentate (*S,N*-Cys) or tridentate (*S,N,O*-Pen/Cys) ligands to the Pb(II) ion,^{47, 48} in which the amine N and the carboxylate O atoms shield the ²⁰⁷Pb nuclei.

Conversely, the lead(II) *N*-acetylcysteine solutions C – E and C* – E* (pH = 9.1) show very similar ²⁰⁷Pb NMR chemical shifts (Figure 4) to those of the corresponding Pb(II) glutathione solutions containing $C_{\text{Pb(II)}} = 10 \text{ mM}$ and GSH/Pb(II) mole ratios 4.0 – 10.0 at pH = 8.5 (2746 – 2764 ppm; re-calibrated – see *Results section*), which have been assigned to a Pb(*S*-GSH)₃ complex with PbS₃ coordination.^{26, 66} The average Pb-S distance for all these Pb(II) *N*-acetylcysteine solutions is $2.65 \pm 0.04 \text{ \AA}$ (Table 3), with a rather consistent Debye-Waller parameter ($\sigma^2 = 0.006 \pm 0.002 \text{ \AA}^2$), like the Pb(II) glutathione solutions.²⁶ The average crystallographic Pb-S distance for mononuclear PbS₄ species is much longer, 2.818 Å.²⁶ A peak at 740.02 amu was observed for a $[2\text{Na}^+ + \text{Pb}(\text{H}_2\text{NAC})_3 - 3\text{H}^+]^+$ ion in the ESI-MS spectrum of solution E. Finally, solutions C – E show similar maximum absorption bands at $\lambda_{\text{max}} = 335 \text{ nm}$ (Figure 2). We conclude that even at free ligand concentrations as low as 10 mM (as in solution C), a mononuclear tri-thiolate Pb(II) *N*-acetylcysteine complex with PbS₃ coordination dominates (Scheme 2, *below*).

For the lead(II) *N*-acetylcysteine solutions A and A* with $C_{\text{Pb(II)}} = 10$ and 100 mM respectively, both with H₂NAC/Pb(II) mole ratio 2.1 and pH = 9.4, the XANES spectra were nearly identical (Figure S2). Their *k*³-weighted EXAFS oscillations had the lowest amplitude within the series (Figure S4), comparable with that of a Pb(II) glutathione solution with rather similar composition ($C_{\text{Pb(II)}} = 10 \text{ mM}$, $C_{\text{GSH}} = 20 \text{ mM}$, pH = 8.5), as shown in Figure S3. In our earlier report,²⁶ we used a PbS₂N₂ model for EXAFS curve-fitting of the glutathione solution to explain the overall amplitude reduction through destructive interference between two out-of-phase Pb-S and Pb-N oscillations. This assumption was partly based on the broad ²⁰⁷Pb NMR signal that we observed at 2687 ppm for a 90 mM Pb(II) glutathione solution with GSH / Pb(II) mole ratio 3.0 (pH = 8.5),²⁶ and the fact that at that time the only reported ²⁰⁷Pb NMR chemical shift for PbS₂N₂ coordination was 2733 ppm for a Pb(II) complex with pyridine as the *N*-donor.⁶⁷ However,

we recently obtained an isotropic ^{207}Pb NMR chemical shift at $\delta_{\text{iso}} = 2105$ ppm for solid $\text{Pb}(\text{S},N\text{-cysteamine})_2$ with the amine group as N -donor ligand.⁴⁷ This chemical shift is considerably more shielded than that of the pyridine adduct with PbS_2N_2 coordination.⁶⁷

In the current study, we were able to observe a sharp ^{207}Pb NMR signal for the $\text{Pb}(\text{II})$ N -acetylcysteine solution A* at 2858 ppm (Figure 4), considerably deshielded (~ 750 ppm) relative to δ_{iso} for the four-coordinated $\text{Pb}(\text{S},N\text{-cysteamine})_2$ compound but close to values reported for three-coordinated PbS_3 and PbS_2N species (see Table 2). Since the amine group in N -acetylcysteine is blocked and not available for coordination, formation of a PbS_2N_2 complex, as previously suggested for $\text{Pb}(\text{GSH})_2$ in a similar $\text{Pb}(\text{II})$ glutathione solution ($C_{\text{Pb}(\text{II})} = 10$ mM, $C_{\text{GSH}} = 20$ mM, $\text{pH} = 8.5$),²⁶ or a PbS_2N complex, is not possible. The ^{207}Pb NMR resonance observed for solution A* at 2858 ppm is comparable to the signal reported earlier by Pecoraro and coworkers at $\delta(^{207}\text{Pb}) = 2806$ ppm for a $\text{Pb}(\text{II})$ bound coiled-coil peptide $\text{Pb}_2(\text{GrandL12AL16L26C})_3^{2-}$.⁶⁵

The observation of a mass peak at m/z 738.98 for $[\text{Pb}_2(\text{H}_2\text{NAC})_2\text{-}3\text{H}^+]^+$ ion in the ESI-MS spectrum of $\text{Pb}(\text{II})$ N -acetylcysteine solution A could be experimental evidence for a dimeric $\text{Pb}(\text{II})$ species in this solution (see Figure 1, Table S1), with two bridging thiolate groups. Even though it is difficult to resolve two similar scattering paths with only 0.12 Å difference with the limited EXAFS k -range available, ~ 10 Å⁻¹, it is the only way we can explain the small EXAFS amplitude for solutions A and A*, and the extra ~ 100 ppm deshielding of the ^{207}Pb NMR signal for solution A* (2858 ppm), relative to those of other $\text{Pb}(\text{II})$ N -acetylcysteine solutions; see Figures 4 and 6.

Therefore, we propose that in the lead(II) N -acetylcysteine solutions A and A* (H_2NAC / $\text{Pb}(\text{II})$ mole ratio = 2.1), binuclear $\text{Pb}_2(\text{S-NAC})_4$ or oligomeric species dominate, in which a S-Pb-S unit shares one of its thiolate ligands with a similar S-Pb-S unit (Scheme 2, *top*), and each $\text{Pb}(\text{II})$ ion has a $\text{PbS}_2\text{S}'$ coordination with two short Pb-S (2.65 ± 0.04 Å) and one slightly longer Pb-S' distance (2.77 ± 0.04 Å); see Table 3. There are only two structures in the Cambridge crystal structure database (CSD code: CAWZIJ and DEWTEE) that contain a $\text{Pb}(\text{II})$ ion with PbS_3 coordination to bulky thiolate ligands, where two of the thiolate groups act as bridges to a neighboring $\text{Pb}(\text{II})$ ion.⁷² In the crystal structure of the trinuclear $\text{Pb}(\text{II})$ complex $[\text{Pb}(\text{SAr}')_2]_3$, where $\text{SAr}' = 2,6$ -diisopropylbenzenethiolate (CSD code: CAWZIJ), the Pb...Pb separation is 4.209 Å.⁷³

Including Pb...Pb scattering in the fitting of the EXAFS spectra for $\text{Pb}(\text{II})$ N -acetylcysteine solutions A and A* showed that this scattering path only gives a minor contribution ($< 5\%$) to those EXAFS oscillations, with the Pb...Pb distance of $3.7 - 3.8$ Å and a high Debye-Waller parameter, $\sigma^2 = 0.021$ Å² (see Figure 7). We previously observed a clear peak for Pb...Pb scattering when analyzing the Fourier transform of the EXAFS spectrum measured at room temperature for the solid lead(II) glutathione compound $[\text{Pb}(\text{GS})](\text{ClO}_4)$, concluding that it has a polymeric structure with PbSO_2 units and the Pb...Pb distance 4.15 ± 0.05 Å ($\sigma^2 = 0.01$ Å²).²⁶ A distinct peak was also observed for Pb...Pb scattering (~ 3.70 Å) in the Fourier transform of the EXAFS spectrum measured at $4-10$ K for crystalline $[\text{Pb}(i\text{-mnt})_2]_2^{4-}$ ($i\text{-mnt} = \text{iso-maleonitriledithiolate}$) with 4 thiolate groups bridging between the two $\text{Pb}(\text{II})$ ions.⁵⁴ However, we were unable to detect any Pb...Pb scattering in the

EXAFS spectrum of the crystalline PbPen (Pb...Pb 4.36 Å), or its corresponding Fourier transform.²⁶ The absence of a detectable contribution of Pb...Pb scattering in the EXAFS oscillations of solutions A and A* (measured at room temperature) is not surprising, as we expect the structural disorder around the Pb(II) ions to be high in solution.

Formation of oligomeric species (Scheme 2, *top left*) in solution seems possible since the initial Pb(II) *N*-acetylcysteine precipitate formed at low pH could only be dissolved at pH > 10.4 when the H₂NAC/ Pb(II) mole ratio was 2.1. A few drops of dilute 0.1 M HClO₄ solution was added to decrease the pH of solution (A*) to 9.4, closer to the pH of the other solutions in the series. At pH < 9.2 the precipitate formed again.

The ¹³C NMR spectrum of solution A (Figure 3) only shows a minor change in its C₁ chemical shift (0.5 ppm; Table S2) relative to that of free *N*-acetylcysteine, indicating that the carboxylate group in the lead(II)-bound *N*-acetylcysteine is not coordinated to the Pb(II) ion. For a Pb(II) penicillamine solution with similar chemical composition (C_{Pb(II)} = 10 mM, C_{H₂Pen} = 20 mM, pH = 9.6), with the carboxylate group of one of the penicillamate ligands in [Pb(*S,N,O*-Pen)(*S-H_n*Pen)]^{2-*n*} (*n* = 0, 1) complexes coordinated to the Pb(II) ion, the ¹³C NMR signal for the carboxylate group shifted ~ 6 ppm relative to the pure ligand.⁴⁸ This suggests that a (*S,O*-NAC) chelate is unlikely in the proposed dimeric Pb₂(NAC)₄ species in solutions A and A*. Coordination of thiolate groups to Pb(II) is proposed to be thermodynamically more favorable than carboxylate coordination.⁶⁶

Considering the similarities between the Pb(II) glutathione solution (C_{Pb(II)} = 10 mM, C_{GSH} = 20 mM, pH = 8.5) and the Pb(II) *N*-acetylcysteine solutions A and A* (H₂NAC/ Pb(II) mole ratio 2.1, pH = 9.4) in the UV-vis. spectra, as well as the EXAFS oscillation and its amplitude, it seems reasonable to assume that oligomeric / dimeric species with similar PbS₂S' coordination also are present in the Pb(II) glutathione solution, rather than the previously proposed PbS₂N₂ coordination.²⁶ EXAFS curve-fitting results for the Pb(II) glutathione solution using different models are presented in Table S3.

The LMCT bands in the UV-vis. spectra of solutions A and B at λ_{max} = 324 and 330 nm, respectively, are blue-shifted relative to those of solutions C – E (335 nm). We made similar observations for the corresponding Pb(II) glutathione solutions at pH = 8.5, which we ascribed based on earlier reports,^{54, 74–76} to be the result of Pb(II) ion coordination to two S-donor atoms (Pb-S₃ → Pb-S₂).²⁶ However, based on the above discussion about the Pb(II) speciation in solutions A and A*, we propose that species with PbS₂S' coordination with two short Pb-S and one slightly longer bridging Pb-S' bonds also show blue-shift (~ 15 nm) in the UV-vis. spectrum, relative to the LMCT band of PbS₃ coordinated complexes.

The ²⁰⁷Pb NMR resonances for the solutions A* – E* (C_{Pb(II)} = 100 mM) in Figure 4 display a shift from 2858 ppm for solution A* to 2762 ppm for solution E*, which has the highest *N*-acetylcysteine concentration (C_{H₂NAC} = 1.0 M). This shift to a more shielded region can be attributed to a conversion from dimeric and/or oligomeric species with PbS₂S' coordination that are in fast ligand exchange in solution A* to the PbS₃ coordinated [Pb(*S*-NAC)₃]⁴⁻ complex in solution E* (Scheme 2). The broad and slightly more shielded ²⁰⁷Pb NMR signal observed for solution B* (H₂NAC/ Pb(II) mole ratio 3.0, pH = 9.1) at 2739

ppm, as well as for solutions C and D ($C_{\text{Pb(II)}} = 10 \text{ mM}$), could be due to a minor amount of a $[\text{Pb}(\text{S-NAC})_2(\text{OH})]^{3-}$ complex with PbS_2O coordination, suppressed by the increasing ligand concentration in solutions C* – E*. While no ^{207}Pb NMR chemical shift has been reported for such a coordination environment, a more shielded chemical shift is expected for PbS_2O than for PbS_3 coordination (2763 ppm), considering the shielding order of the donor atoms $\text{S} < \text{N} < \text{O}$.⁵² The only reported ^{207}Pb NMR chemical shift for PbS_2N coordination (2852 ppm) is that of a pyridine adduct $(2,6\text{-Me}_2\text{C}_6\text{H}_3\text{S})_2\text{Pb}(\text{pyNMe}_2)$ with a highly deshielding *N*-donor ligand ($\text{pyNMe}_2 = 4\text{-dimethylaminorpyridine}$);⁶⁷ see the above discussion on $\delta(^{207}\text{Pb})$ for PbS_2N_2 coordination.

Similarly, for Pb(II) penicillamine solutions ($C_{\text{Pb(II)}} = 100 \text{ mM}$) we explained the shift of the ^{207}Pb NMR resonance from 1797 ppm to 1844 ppm (re-calibrated values; see Figure 9), to be due to an equilibrium between $[\text{Pb}(\text{S},\text{N},\text{O-Pen})(\text{S-H}_n\text{Pen})]^{2-n}$ ($n = 0 - 1$) species with PbS_2NO coordination and a hydroxo complex $[\text{Pb}(\text{S},\text{N},\text{O-Pen})(\text{OH})]^-$ (PbSNO_2).⁴⁸ As the penicillamine concentration increased from 0.2 M to 1.0 M, the amount of the latter hydroxo complex with its more shielding environment for ^{207}Pb nuclei became insignificant.

For the corresponding Pb(II) cysteine solutions ($C_{\text{Pb(II)}} = 100 \text{ mM}$) the situation was more complicated with equilibria between Pb(II) cysteine complexes with PbS_2NO , PbS_2N_2 , PbS_3N and PbS_3 coordination environments. By increasing the cysteine concentration from 0.21 M to 1.0 M, higher amounts of complexes with PbS_3N and PbS_3 coordination formed and shifted the ^{207}Pb NMR signal from 1981 ppm to a more deshielded region at 2478 ppm (re-calibrated values; see Figure 9).⁴⁷

Conclusion

Lead(II) alkaline aqueous solutions containing *N*-acetylcysteine or glutathione show very similar UV-vis. absorption bands, ^{207}Pb NMR chemical shifts, EXAFS oscillations and XANES features. At low $\text{H}_2\text{NAC} / \text{Pb(II)}$ mole ratios (2.1) dimeric / oligomeric Pb(II) *N*-acetylcysteine complexes with $\text{PbS}_2\text{S}'$ coordination form with two short Pb-S ($2.65 \pm 0.04 \text{ \AA}$) and one slightly longer $\text{Pb-S}'$ ($2.77 \pm 0.04 \text{ \AA}$) bonds, and an UV-vis. absorption band at 324 nm. Its ^{207}Pb NMR resonance at 2858 ppm, which is highly deshielded relative to the isotropic chemical shift $\delta_{\text{iso}}(\text{Pb}) = 2105 \text{ ppm}$ for the four-coordinated $\text{Pb}(\text{S},\text{N-cysteamine})_2$ compound,⁴⁷ provided the key to the nature of those Pb(II) *N*-acetylcysteine complexes.

We previously proposed a mononuclear dithiolate $\text{Pb}(\text{GSH})_2$ complex with PbS_2N_2 coordination for a corresponding Pb(II) glutathione solution,²⁶ to explain its low EXAFS amplitude. However, based on the similarity between the UV-vis. and EXAFS spectra obtained in this study for the Pb(II) *N*-acetylcysteine solution ($\text{pH} = 9.4$, $C_{\text{Pb(II)}} = 10 \text{ mM}$, $\text{H}_2\text{NAC}/\text{Pb(II)}$ mole ratio 2.1) and its corresponding Pb(II) glutathione solution ($\text{pH} = 8.5$), we propose that without substantial ligand excess, also glutathione forms dimeric or oligomeric lead(II) species with $\text{PbS}_2\text{S}'$ coordination.

In excess of free *N*-acetylcysteine (as low as 10 mM for mole ratios $\text{H}_2\text{NAC} / \text{Pb(II)} > 3.0$), the tri-thiolate $[\text{Pb}(\text{S-NAC})_3]^{4-}$ complex with PbS_3 coordination dominates, with ^{207}Pb NMR chemical shift 2763 ppm, average Pb-S bond length $2.65 \pm 0.04 \text{ \AA}$, and $\lambda_{\text{max}} = 335$

nm in the UV-vis. region. For corresponding Pb(II) glutathione solutions very similar results showed formation of a Pb(S-GSH)₃ complex.²⁶

The results of the current study strongly support similar coordination behavior of *N*-acetylcysteine and glutathione with respect to Pb(II) binding. Further *in-vivo* tests are needed to establish the usefulness of the natural antioxidant *N*-acetylcysteine as a potential antidote for lead toxicity, in particular for children with BLL < 45 µg/dL for which chelation therapy is not recommended.⁸

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

H₂NAC	<i>N</i> -acetylcysteine
H₂Cys	cysteine
H₂Pen	penicillamine
GSH	glutathione
GSSG	glutathione disulfide
DMSA	dimercaptosuccinic acid
SAr'	2,6-diisopropylbenzenethiolate
<i>i</i>-mnt	<i>iso</i> -maleonitriledithiolate
pyNMe₂	4-dimethylaminopyridine
UV-vis	Ultraviolet-visible
ESI-MS	electro-spray ionization mass spectrometry
EXAFS	extended X-ray absorption fine structure
XANES	X-ray absorption near-edge structure

LMCT	ligand-to-metal charge transfer
RBA	rear-beam attenuator
CSD	crystal structure database
ROS	reactive oxygen species
BLL	blood lead levels
ALAD	δ -aminolevulinic acid dehydratase
SSRL	Stanford Synchrotron Radiation Lightsource

References

1. Tong S, von Schirnding YE, Prapamontol T. Environmental lead exposure: a public health problem of global dimensions. *Bull World Health Organ.* 2000; 78:1068–1077. [PubMed: 11019456]
2. Adonaylo VN, Oteiza PI. Lead intoxication: antioxidant defenses and oxidative damage in rat brain. *Toxicology.* 1999; 135:77–85. [PubMed: 10463764]
3. Rogan WJ, Dietrich KN, Ware JH, Dockery DW, Salganik M, Radcliffe J, Jones RL, Ragan NB, Chisolm JJ, Rhoads GG. The Effect of chelation therapy with succimer on neuropsychological development in children exposed to lead. *N Engl J Med.* 2001; 344:1421–1426. [PubMed: 11346806]
4. Warren M, Cooper J, Wood S, Schooling-Jordan P. Lead poisoning, haem synthesis and 5-aminolaevulinic acid dehydratase. *Trends Biochem Sci.* 1998; 23:217–221. [PubMed: 9644976]
5. Patrick L. Lead toxicity: a review of the literature. Part I: Exposure, evaluation, and treatment. *Altern Med Rev.* 2006; 11:2–22. [PubMed: 16597190]
6. Stefania M, Diana S, Caruso A, Saturnino C. Chemical and biological properties of toxic metals and use of chelating agents for the pharmacological treatment of metal poisoning. *Arch Toxicol.* 2010; 84:501–520. [PubMed: 20386880]
7. Pounds JG, Long GJ, Rosen JF. Cellular and molecular toxicity of lead in bone. *Environ Health Perspect.* 1991; 91:17–32. [PubMed: 2040247]
8. Dietrich KN, Ware JH, Salganik M, Radcliffe J, Rogan WJ, Rhoads GG, Fay ME, Davoli CT, Denckla MB, Bornschein RL, Schwarz D, Dockery DW, Adubato S, Jones RL. Effect of chelation therapy on the neuropsychological and behavioral development of lead-exposed children after school entry. *Pediatrics.* 2004; 114:19–26. [PubMed: 15231903]
9. Gurer H, Ercal N. Can antioxidants be beneficial in the treatment of lead poisoning? *Free Radic Biol Med.* 2000; 29:927–945. [PubMed: 11084283]
10. Abu-Dari K, Hahn FE, Raymond KN. Lead sequestering agents. 1 Synthesis, physical properties, and structures of lead thiohydroxamate complexes. *J Am Chem Soc.* 1990; 112:1519–1524.
11. Srinivas S, Kaul P, Prakash V. Mechanism of interaction of Pb(II) with milk proteins: A case study of α -Casein. *J Agr Food Chem.* 2007; 55:9283–9288. [PubMed: 17924701]
12. Piomelli S. Childhood lead poisoning. *Pediatr Clin North Am.* 2002; 49:1285–1304. [PubMed: 12580366]
13. Godwin HA. The biological chemistry of lead. *Curr Opin Chem Biol.* 2001; 5:223–227. [PubMed: 11282351]
14. Flora G, Gupta D, Tiwari A. Toxicity of lead: A review with recent updates. *Interdiscip Toxicol.* 2012; 5:47–58. [PubMed: 23118587]
15. Kirberger M, Yang JJ. Structural differences between Pb²⁺- and Ca²⁺-binding sites in proteins: Implications with respect to toxicity. *J Inorg Biochem.* 2008; 102:1901–1909. [PubMed: 18684507]
16. Simons TJ. The affinity of human erythrocyte porphobilinogen synthase for Zn²⁺ and Pb²⁺ *Eur J Biochem.* 1995; 234:178–183. [PubMed: 8529638]

17. Bridges CC, Zalups RK. Molecular and ionic mimicry and the transport of toxic metals. *Toxicol Appl Pharmacol*. 2005; 204:274–308. [PubMed: 15845419]
18. Earnshaw, A.; Greenwood, N. *Chemistry of the Elements*. 2. Elsevier Ltd; 1997.
19. Sanders T, Liu Y, Buchner V, Tchounwou PB. Neurotoxic effects and biomarkers of lead exposure: a review. *Rev Environ Health*. 2009; 24:15–45. [PubMed: 19476290]
20. Lidsky TI, Schneider JS. Lead neurotoxicity in children: basic mechanisms and clinical correlates. *Brain*. 2003; 126:5–19. [PubMed: 12477693]
21. Garza A, Vega R, Soto E. Cellular mechanisms of lead neurotoxicity. *Med Sci Monit*. 2006; 12:RA57–65. [PubMed: 16501435]
22. Bradbury MW, Deane R. Permeability of the blood-brain barrier to lead. *Neurotoxicology*. 1993; 14:131–136. [PubMed: 8247388]
23. Halliwell B, Gutteridge JMC. Role of free radicals and catalytic metal ions in human disease: An overview. *Methods Enzymol*. 1990; 186:1–85. [PubMed: 2172697]
24. Ahamed M, Verma S, Kumar A, Siddiqui MKJ. Environmental exposure to lead and its correlation with biochemical indices in children. *Sci Total Environ*. 2005; 346:48–55. [PubMed: 15993681]
25. Ercal N, Treeratphan P, Hammond TC, Matthews RH, Grannemann NH, Spitz DR. In vivo indices of oxidative stress in lead-exposed C57BL/6 mice are reduced by treatment with *meso*-2,3-dimercaptosuccinic acid or *N*-acetylcysteine. *Free Radic Biol Med*. 1996; 21:157–161. [PubMed: 8818630]
26. Mah V, Jalilehvand F. Lead(II) complex formation with glutathione. *Inorg Chem*. 2012; 51:6285–6298. [PubMed: 22594853]
27. Nehru B, Kanwar SS. *N*-acetylcysteine exposure on lead-induced lipid peroxidative damage and oxidative defense system in brain regions of rats. *Biol Trace Elem Res*. 2004; 101:257–264. [PubMed: 15564655]
28. Penugonda S, Ercal N. Comparative evaluation of *N*-acetylcysteine (NAC) and *N*-acetylcysteine amide (NACA) on glutamate and lead-induced toxicity in CD-1 mice. *Toxicol Lett*. 2011; 201:1–7. [PubMed: 21145953]
29. Neal R, Cooper K, Gurer H, Ercal N. Effects of *N*-acetylcysteine and 2,3-dimercaptosuccinic acid on lead induced oxidative stress in rat lenses. *Toxicology*. 1998; 130:167–174. [PubMed: 9865483]
30. Moldéus P, Cotgreave IA. *N*-acetylcysteine. *Methods Enzymol*. 1994; 234:482–492. [PubMed: 7808322]
31. Blanus M, Varnai VM, Piasek M, Kostial K. Chelators as antidotes of metal toxicity: therapeutic and experimental aspects. *Curr Med Chem*. 2005; 12:2771–2794. [PubMed: 16305472]
32. Ottenwälder H, Simon P. Differential effect of *N*-acetylcysteine on excretion of the metals Hg, Cd, Pb and Au. *Arch Toxicol*. 1987; 60:401–402. [PubMed: 3662815]
33. Banner W Jr, Koch M, Capin DM, Hopf SB, Chang S, Tong TG. Experimental chelation therapy in chromium, lead, and boron intoxication with *N*-acetylcysteine and other compounds. *Toxicol Appl Pharmacol*. 1986; 83:142–147. [PubMed: 3952743]
34. Kozłowski H, Urbanska J, Sovago I, Varnagy K, Kiss A, Szychala J, Cherifi K. Cadmium ion interaction with sulfur-containing amino acid and Peptide ligands. *Polyhedron*. 1990; 9:831–837.
35. Quyoom S, Khan B-u-d. Potentiometric and UV spectral studies of binary and ternary complexes of some metal ions with *N*-acetylcysteine and amino acids. *E-Journal of Chemistry*. 2009; 6(S1):S117–S122.
36. Schwab JJ, Wilkinson EC, Wilson SR, Shapley PA. Nitridoosmium(VI) and nitridoruthenium(VI) complexes of cysteine(2-) and related ligands. *J Am Chem Soc*. 1991; 113:6124–6129.
37. Atencio R, Esteruelas MA, Lahoz FJ, Oro LA, Ruiz N. Synthesis and x-ray structure of the unusual cysteine-complex $\text{OsH}_2\{\text{OC}(=\text{O})\text{CH}[\text{NHC}(=\text{O})\text{CH}_3]\text{CH}_2\text{S}\}(\text{P}^i\text{Pr}_3)_2$. *Inorg Chem*. 1995; 34:1004–1006.
38. Brauer SL, Hneihen AS, McBride JS, Wetterhahn KE. Chromium(VI) forms thiolate complexes with γ -glutamylcysteine, *N*-acetylcysteine, cysteine, and the methyl ester of *N*-acetylcysteine. *Inorg Chem*. 1996; 35:373–381. [PubMed: 11666218]

39. Sun H, Li H, Harvey I, Sadler PJ. Interactions of bismuth complexes with metallothionein(II). *J Biol Chem*. 1999; 274:29094–29101. [PubMed: 10506163]
40. Sadler PJ, Li H, Sun H. Coordination chemistry of metals in medicine: target sites for bismuth. *Coord Chem Rev*. 1999; 185–186:689–709.
41. Mitchell KA, Jensen CM. Synthesis, characterization, and reactivity of platinum cysteinato and related thiolato complexes: molecular structure of $\text{Pt}_2(\mu\text{-}N\text{-acetyl-L-cysteine-S})_2(\text{bpy})_2$. *Inorg Chem*. 1995; 34:4441–4446.
42. Corbi PP, Quintão FA, Ferraresi DKD, Lustri WR, Amaral AC, Massabni AC. Chemical, spectroscopic characterization, and in vitro antibacterial studies of a new gold(I) complex with *N*-acetyl-L-cysteine. *J Coord Chem*. 2010; 63:1390–1397.
43. Abbehausen C, Heinrich TA, Abrão EP, Costa-Neto CM, Lustri WR, Formiga ALB, Corbi PP. Chemical, spectroscopic characterization, DFT studies and initial pharmacological assays of a silver(I) complex with *N*-acetyl-L-cysteine. *Polyhedron*. 2011; 30:579–583.
44. Corbi PP, Cagnin F, Massabni AC. Synthesis and characterization of a platinum(II) complex with *N*-acetyl-L-cysteine. *J Coord Chem*. 2009; 62:2764–2771.
45. Adachi Y, Yoshikawa Y, Sakurai H. Antidiabetic zinc(II)-*N*-acetyl-L-cysteine complex: Evaluations of in vitro insulinomimetic and in vivo blood glucose-lowering activities. *BioFactors*. 2007; 29:213–223. [PubMed: 18057552]
46. Pellerito L, Prinziavalli C, Casella G, Fiore T, Pellerito O, Giuliano M, Scopelliti M, Pellerito C. Diorganotin(IV) *N*-acetyl-L-cysteinato complexes: Synthesis, solid state, solution phase, DFT and biological investigations. *J Inorg Biochem*. 2010; 104:750–758. [PubMed: 20421134]
47. Jalilehvand F, Sisombath N, Schell AC, Facey GA. Lead(II) complex formation with *L*-cysteine in aqueous solution. *Inorg Chem*. 2015; 54:2160–2170. [PubMed: 25695880]
48. Sisombath N, Jalilehvand F, Schell AC, Wu Q. Lead(II) binding to the chelating agent *D*-penicillamine in aqueous solution. *Inorg Chem*. 2014; 53:12459–12468. [PubMed: 25385465]
49. Neal R, Yang P, Fiechtl J, Yildiz D, Gurer H, Ercal N. Pro-oxidant effects of δ -aminolevulinic acid (δ -ALA) on Chinese hamster ovary (CHO) cells. *Toxicol Lett*. 1997; 91:169–178. [PubMed: 9217237]
50. Gavaghan H. Lead, unsafe at any level. *Bull World Health Organ*. 2002; 80:82–82. [PubMed: 11884985]
51. Glasoe PK, Long FA. Use of glass electrodes to measure acidities in deuterium oxide. *J Phys Chem*. 1960; 64:188–190.
52. Wrackmeyer, B.; Horchler, K.; Webb, GA. ^{207}Pb -NMR Parameters. In: Webb, GA., editor. *Annual Reports on NMR Spectroscopy*. Academic Press; San Diego, CA, USA: 1990. p. 249–306.
53. Claridge, TDW. Chapter 3 - Practical aspects of high-resolution NMR. In: Claridge, TDW., editor. *Tetrahedron Organic Chemistry Series*. Elsevier; Hungary: 2009. p. 60
54. Magyar J, Weng TC, Stern C, Dye D, Payne J, Bridgewater B, Mijovilovich A, Parkin G, Zaleski J, Penner-Hahn J, Godwin H. Reexamination of lead(II) coordination preferences in sulfur-rich sites: Implications for a critical mechanism of lead poisoning. *J Am Chem Soc*. 2005; 127:9495–9505. [PubMed: 15984876]
55. Wang Y, Hemmingsen L, Giedroc D. Structural and functional characterization of mycobacterium tuberculosis CmtR, a $\text{Pb}^{\text{II}}/\text{Cd}^{\text{II}}$ -sensing SmtB/ArsR metalloregulatory repressor. *Biochemistry*. 2005; 44:8976–8988. [PubMed: 15966722]
56. Ghering A, Miller Jenkins L, Schenck B, Deo S, Mayer A, Pikaart M, Omichinski J, Godwin H. Spectroscopic and functional determination of the interaction of Pb^{2+} with GATA proteins. *J Am Chem Soc*. 2005; 127:3751–3759. [PubMed: 15771509]
57. Vogler A, Nikol H. Photochemistry and photophysics of coordination compounds of the main group metals. *Pure & Appl Chem*. 1992; 64:1311–1317.
58. Claudio, ES.; Godwin, HA.; Magyar, JS. Fundamental coordination chemistry, environmental chemistry and biochemistry of lead(II). In: Karlin, KD., editor. *Prog Inorg Chem*. John Wiley & Sons Inc; Hoboken, New Jersey, USA: 2003. p. 1–144.
59. van Gorkom LCM, Hook JM, Logan MB, Hanna JV, Wasylishen RE. Solid-state lead-207 NMR of lead(II) nitrate: Localized heating effects at high magic angle spinning speeds. *Magn Reson Chem*. 1995; 33:791–795.

60. Harrison PG, Healy MA, Steel AT. Lead-207 chemical shift data for bivalent lead compounds: thermodynamics of the equilibrium $\text{Pb}(\text{O}_2\text{CCH}_3)_2 \rightarrow [\text{Pb}(\text{O}_2\text{CCH}_3)]^+ + \text{O}_2\text{CCH}_3^-$ in aqueous solution in the temperature range 303–323 K. *J Chem Soc Dalton Trans.* 1983;1845–1848.
61. Dmitrenko O, Bai S, Beckmann P, Bramer S, Vega A, Dybowski C. The relationship between ^{207}Pb NMR chemical shift and solid-state structure in Pb(II) compounds. *J Phys Chem A.* 2008; 112:3046–3052. [PubMed: 18351758]
62. Van Bramer SE, Glatfelter A, Bai S, Dybowski C, Neue G, Perry DL. Solid-state ^{207}Pb NMR studies of lead-group 16 and mixed transition-metal/lead-group 16 element-containing materials. *Magn Reson Chem.* 2006; 44:357–365. [PubMed: 16477690]
63. Arsenault JJI, Dean PAW. A preparative and multinuclear nuclear magnetic resonance spectroscopic study of $\text{As}(\text{SPh})_x(\text{SePh})_{3-x}$ ($x=0-3$), $\text{Sb}(\text{SPh})_x(\text{SePh})_{3-x}$ ($x=0-3$), $\text{Bi}(\text{SPh})_3$, $\text{Bi}(\text{SePh})_3$, $[\text{Sn}(\text{SPh})_x(\text{SePh})_{3-x}]^-$ ($x=0-3$), $\text{Pb}(\text{SePh})_3^-$, and $\text{Pb}(\text{SPh})_3^-$ and some related thiolatoplumbates(II). *Can J Chem.* 1983; 61:1516–1523.
64. Dean PAW, Vittal JJ, Payne NC. Discrete trigonal-pyramidal lead(II) complexes: syntheses and x-ray structure analyses of $[(\text{C}_6\text{H}_5)_4\text{As}][\text{Pb}(\text{EC}_6\text{H}_5)_3]$ ($\text{E} = \text{S}, \text{Se}$). *Inorg Chem.* 1984; 23:4232–4236.
65. Neupane KP, Pecoraro VL. Probing a homoleptic PbS_3 coordination environment in a designed peptide using ^{207}Pb NMR spectroscopy: Implications for understanding the molecular basis of lead toxicity. *Angew Chem Int Ed.* 2010; 49:8177–8180.
66. Neupane KP, Pecoraro VL. Pb-207 NMR spectroscopy reveals that Pb(II) coordinates with glutathione (GSH) and tris cysteine zinc finger proteins in a PbS_3 coordination environment. *J Inorg Biochem.* 2011; 105:1030–1034. [PubMed: 21625408]
67. Briand G, Smith A, Schatte G, Rossini A, Schurko R. Probing lead(II) bonding environments in 4-substituted pyridine adducts of $(2,6\text{-Me}_2\text{C}_6\text{H}_3\text{S})_2\text{Pb}$: An x-ray structural and solid-state ^{207}Pb NMR study. *Inorg Chem.* 2007; 46:8625–8637. [PubMed: 17867676]
68. Andersen R, DiTargiani R, Hancock R, Stern C, Goldberg D, Godwin H. Characterization of the first N_2S (alkylthiolate)lead compound: A model for three-coordinate lead in biological systems. *Inorg Chem.* 2006; 45:3–3.
69. Rupprecht S, Franklin SJ, Raymond KN. Synthesis of monothiohydroxamic ligands and their lead complexes. Structures of *N*-methyl-3-pyridothiohydroxamic acid, bis(*N*-methyl-3-pyridothiohydroxamato) lead(II) and bis(*N*-cyclohexyl-phenylacetothiohydroxamato) lead(II). *Inorg Chim Acta.* 1995; 235:185–194.
70. Burnett TR, Dean PAW, Vittal JJ. Thiobenzoates of lead (II) and bismuth (III). The crystal and molecular structures of $(\text{AsPh}_4)[\text{Pb}(\text{SOCPh})_3]$ and $\text{Pb}(\text{SOCPh})_2(\text{S}_2\text{CP}\{c\text{-C}_6\text{H}_{11}\}_3)$. *Can J Chem.* 1994; 72:1127–1136.
71. Aramini JM, Hiraoki T, Yazawa M, Yuan T, Zhang M, Vogel HJ. Lead-207 NMR: a novel probe for the study of calcium-binding proteins. *J Biol Inorg Chem.* 1996; 1:39–48.
72. Allen FH. The Cambridge Structural Database: a quarter of a million crystal structures and rising. *Acta Crystallogr B.* 2002; B58:380–388. [PubMed: 12037359]
73. Hitchcock PB, Lappert MF, Samways BJ, Weinberg EL. Metal (Li , Ge^{II} , Ge^{III} , Sn^{II} , and Pb^{II}) 2,6-dialkylbenzenethiolates; X-ray crystal structures of $\text{Sn}(\text{SAr})_2$ ($\text{Ar} = \text{C}_6\text{H}_2\text{Bu}^{\text{t}}\text{-2,4,6}$) and $[\text{M}(\text{SAr})_2]_3$ ($\text{M} = \text{Sn}$ or Pb , $\text{Ar} = \text{C}_6\text{H}_3\text{Pr}^{\text{t}}\text{-2,6}$). *J Chem Soc Chem Comm.* 1983:1492–1494.
74. Payne J, Horst M, Godwin H. Lead fingers: Pb^{2+} binding to structural zinc-binding domains determined directly by monitoring lead-thiolate charge-transfer bands. *J Am Chem Soc.* 1999; 121:6850–6855.
75. Rousselot-Pailley P, Sénèque O, Lebrun C, Crouzy S, Boturyn D, Dumy P, Ferrand M, Delangle P. Model peptides based on the binding loop of the copper metallochaperone Atx1: selectivity of the consensus sequence MxCxxC for metal ions $\text{Hg}(\text{II})$, $\text{Cu}(\text{I})$, $\text{Cd}(\text{II})$, $\text{Pb}(\text{II})$, and $\text{Zn}(\text{II})$. *Inorg Chem.* 2006; 45:5510–5520. [PubMed: 16813414]
76. Busenlehner L, Weng TC, Penner-Hahn J, Giedroc D. Elucidation of primary ($\alpha_3\text{N}$) and vestigial (α_5) heavy metal-binding sites in staphylococcus aureus pl258 CadC: Evolutionary implications for metal ion selectivity of ArsR/SmtB metal sensor proteins. *J Mol Biol.* 2002; 319:685–701. [PubMed: 12054863]

Figure 1. ESI-MS spectra measured in the positive-ion mode for solutions A (*left*) and E (*right*) with $C_{\text{Pb(II)}} = 10 \text{ mM}$ and $\text{H}_2\text{NAC}/\text{Pb(II)}$ mole ratios 2.1 and 10.0, respectively. The peak at 186.01 amu has 100% intensity. Insets represent selected peaks assigned to lead(II) complexes with distinct isotopic patterns for Pb.

Figure 2.

UV-Vis spectra of alkaline lead(II) *N*-acetylcysteine aqueous solutions containing $C_{\text{Pb(II)}} = 10 \text{ mM}$ and $\text{H}_2\text{NAC/Pb(II)}$ mole ratios 2.1 (A), 3.0 (B), 4.0 (C), 5.0 (D) and 10.0 (E) – see Table 1 – and of a 10 mM *N*-acetylcysteine solution at pH = 9.1.

Figure 3. ^1H and ^{13}C NMR spectra of 0.1 M *N*-acetylcysteine (pH 9.1) and alkaline $\text{Pb}(\text{II})$ *N*-acetylcysteine solutions (99.9% D_2O) containing $C_{\text{Pb}(\text{II})} = 10$ mM and $\text{H}_2\text{NAC}/\text{Pb}(\text{II})$ mole ratios 2.1 (A), 3.0 (B), 4.0 (C), 5.0 (D) and 10.0 (E).

Figure 4. ^{207}Pb NMR spectra of the alkaline aqueous Pb(II) *N*-acetylcysteine solutions C – E enriched in ^{207}Pb ($C_{\text{Pb(II)}} = 10 \text{ mM}$, $\text{H}_2\text{NAC}/\text{Pb(II)}$ mole ratios 4.0 – 10.0) and A* – E* with 10% D_2O ($C_{\text{Pb(II)}} = 100 \text{ mM}$, $\text{H}_2\text{NAC}/\text{Pb(II)}$ mole ratios 2.1 – 10.0). ^{207}Pb NMR chemical shifts were calibrated using a 1.0 M $\text{Pb(NO}_3)_2$ aqueous solution ($\delta_{\text{Pb}} = -2961 \text{ ppm}$; *see text*).

Figure 5.

X-ray absorption near-edge structure (XANES) spectra of Pb(II) *N*-acetylcysteine alkaline aqueous solutions: (*left*) A* ($E_0 = 13034.6$ eV) and E* ($E_0 = 13034.3$ eV) with $C_{\text{Pb(II)}} = 100$ mM, pH = 9.4 and 9.1, H₂NAC/Pb(II) mole ratio 2.1 and 10.0, respectively; E_0 is defined as the first inflection point of the rising edge. (*right*) XANES spectrum of a Pb(II) glutathione solution compared with *N*-acetylcysteine solutions A and E, all containing $C_{\text{Pb(II)}} = 10$ mM.

Figure 6.

Least-squares curve-fitting of k^3 -weighted Pb L_{III}-edge EXAFS spectra and corresponding Fourier transforms for the two series of Pb(II) *N*-acetylcysteine aqueous solutions (pH ~ 9) containing $C_{\text{Pb(II)}} = 10$ mM (A – E), or 100 mM (A* – E*); see Table 3.

Figure 7.

a) Pb L_{III}-edge EXAFS spectrum for solution A* ($C_{\text{Pb(II)}} = 0.1 \text{ M}$, $C_{\text{H}_2\text{NAC}} = 0.21 \text{ M}$, pH = 9.4) measured at room temperature, with separate contributions from 2 Pb-S, 1 Pb-S' and 1 Pb...Pb ($\sigma^2 = 0.021 \text{ \AA}^2$) scattering paths shown below; *b*) corresponding Fourier transforms of the EXAFS oscillations (see Table 3).

Figure 8.

UV-vis. spectra of Pb(II) solutions ($C_{Pb(II)} = 10$ mM) containing *N*-acetylcysteine (solutions A and E), glutathione (GSH), cysteine (H_2Cys) and penicillamine (H_2Pen) with different ligand to metal ratios [Ref. 26, 47, 48]

Figure 9.

Comparison of chemical shifts in the ^{207}Pb NMR spectra of 100 mM Pb(II) solutions containing penicillamine (pH = 9.6 – 11.0), cysteine (pH = 9.1 – 10.4) or *N*-acetylcysteine (pH = 9.1 – 9.4), with different ligand to Pb(II) ratios ($L/\text{Pb} = 2 - 10$) [Ref. 47, 48; ^{207}Pb NMR chemical shifts were re-calibrated using a 1.0 M $\text{Pb(NO}_3)_2$ aqueous solution ($\delta_{\text{Pb}} = -2961$ ppm)].

Scheme 1.
Structure of *N*-acetylcysteine (H₂NAC)

Scheme 2.

Proposed structures for the Pb(II) *N*-acetylcysteine complexes formed in alkaline aqueous solutions with mole ratio $\text{H}_2\text{NAC}/\text{Pb(II)} = 2.1$ (*top*), and in high ligand excess (*below*).

Table 1Composition of Lead(II) *N*-acetylcysteine Solutions

$\text{H}_2\text{NAC/Pb(II)}$ mole ratio	pH	Solution	$C_{\text{Pb(II)}} \text{ (mM)}$ ^a	Solution	$C_{\text{Pb(II)}} \text{ (mM)}$ ^a
2.1	9.4	A	10	A*	100
3.0	9.1	B	10	B*	100
4.0	9.1	C	10	C*	100
5.0	9.1	D	10	D*	100
10.0	9.1	E	10	E*	100

^a Estimated errors are 10 ± 0.2 mM, and 100 ± 2 mM in total lead(II) concentrations $C_{\text{Pb(II)}}$ for solutions prepared from PbO dissolved in 0.15 M HClO₄, and from the hygroscopic Pb(ClO₄)₂·3H₂O compound, respectively.

Table 2²⁰⁷Pb NMR Chemical Shifts Reported for Lead(II) Complexes with *S*-donor Ligands.^a

Coordination environment	Chemical shift ^a (δ, ppm)	Reference
PbS ₃	2763	this work
	2818 – 2868	63, 64
PbS ₃ (peptides)	2548 – 2824	26, 65, 66
PbS ₂ S' ^b	2858	this work
PbS ₂ NS' ^{b,c}	2873	67
PbS ₂ N ^c	2852	67
PbS ₂ N ₂ ^c	2105, 2733	47, 67
PbSN ₂	2328	68
PbS ₂ O ₂	1502 – 1555	69
PbS ₃ O ₃	1422 – 1463	70
PbSNOS' ₂ O' ₂ ^{b,c}	909	48

^a Relative to Pb(CH₃)₄ (δ = 0 ppm); structures of the corresponding Pb(II) complexes are shown in Figure S1.^b S' and O' are bridging donors;^c solid-state isotropic ²⁰⁷Pb NMR chemical shift.

Structural parameters obtained from least-squares curve-fitting of k^2 -weighted Pb L_{III}-edge EXAFS spectra of aqueous Pb(II) *N*-acetylcysteine solutions (see Figure 6).^a

Table 3

Solution	Assumed Coordination	Scattering Path	<i>N</i>	<i>R</i> (Å)	σ^2 (Å ²)
A*	PbS ₃	Pb-S	3.0	2.66	0.0108
		Pb-S	2 <i>f</i>	2.65	0.0075
		Pb-S'	1 <i>f</i>	2.77	0.0086
		Pb ... Pb	1 <i>f</i>	3.76	0.0208
B*	PbS ₃	Pb-S	3.5	2.65	0.0069
C*	PbS ₃	Pb-S	3.8	2.65	0.0068
D*	PbS ₃	Pb-S	3.8	2.65	0.0066
E*	PbS ₃	Pb-S	3.8	2.65	0.0065
A	PbS ₃	Pb-S	2.9	2.65	0.0107
		Pb-S	2 <i>f</i>	2.64	0.0073
		Pb-S'	1 <i>f</i>	2.78	0.0101
		Pb ... Pb	1 <i>f</i>	3.66	0.0284
B	PbS ₃	Pb-S	3.1	2.65	0.0081
C	PbS ₃	Pb-S	3.5	2.65	0.0068
D	PbS ₃	Pb-S	3.7	2.65	0.0068
E	PbS ₃	Pb-S	3.6	2.64	0.0064

^a $S_0^2 = 0.9$ fixed; *f* = fixed; fitting *k*-range ~ 2.8 – 12.5 Å⁻¹ (solutions A*, A – E) and ~ 3.0 – 13.7 Å⁻¹ (solutions B* – E*). Estimated error limits for *R* is ± 0.04 Å and for $\sigma^2 \pm 0.002$ Å²; refined *N* accuracy within ± 20 %.

^b Selected model shown in Figure 6; the fitting residual improved from 27.3 (PbS₃) to 26.9 (PbS₂S' Pb) for solution A, and from 20.7 to 19.0 for solution A*.