Lead Poisoning and the Inactivation of 5-Aminolevulinate Dehydratase as Modeled by the Tris(2-mercapto-1-phenylimidazolyl)hydroborato Lead Complex, {[Tm^{Ph}]Pb}[ClO$_4$]

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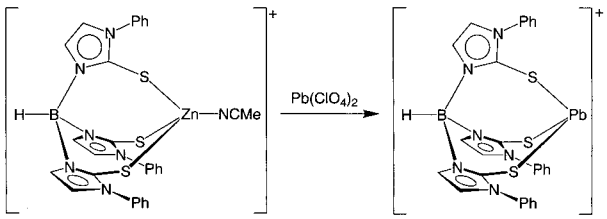
Lead is the most commonly encountered toxic metal pollutant in the environment as a result of its current and previous use in, for example, batteries, gasoline, plumbing, and paints.[1] Considerable effort is, therefore, being directed toward solving this environmental problem.[2] In this regard, the toxicological properties of lead[3] are associated with its interactions with proteins and, in particular, 5-aminolevulinate dehydratase (ALAD).[4−6] The influence of lead on the latter enzyme is particularly harmful because ALAD is responsible for the asymmetric dimerization of 5-aminolevulinic acid (ALA) to porphobilinogen, a monopyrrole which is essential for heme synthesis.[7−9] Thus, not only does inactivation of ALAD result in anemia because it inhibits the formation of heme, and hence hemoglobin, but it also results in a build-up of ALA, a neuropathogenic agent.[7,8] ALAD is a zinc-dependent enzyme,[10] and, in this paper, we address aspects of lead inactivation of ALAD by investigating the reactivity of a synthetic analogue towards Pb^{II}.

Recent crystallographic studies have demonstrated that the catalytic site of yeast ALAD possesses the composition [(Cys)$_3$Zn^{II}(OH$_2$)].[7,11,12] Such a composition must be regarded as truly unusual since the active sites (as opposed to structural sites) of most zinc enzymes include at least one histidine ligand.[13,14] Furthermore, due to the proclivity of sulfur containing ligands to bridge more than one zinc center, mononuclear tetrahedral zinc complexes with sulfur-rich coordination environments that mimic the aforementioned active site of ALAD are not common. Nevertheless, we have recently reported a series of complexes with the requisite motif, which incorporates sterically demanding tris(mercaptoarylimidazolyl)borate ligands, [Tm^{Ar}] (Ar = Ph, Mes), to model the binding of the zinc center to the three cysteine residues, for example, [Tm^{Ph}]ZnX (X = I, NO$_3$) and [Tm^{Mes}]ZnX (X = Cl, I).[15] The availability of such complexes has allowed us to explore the potential for lead to replace zinc in a synthetic system for which the coordination environment mimics that in ALAD. Significantly, we have observed that lead readily displaces the zinc in [Tm^{Ph}]ZnX derivatives. For example, both [Tm^{Ph}]ZnI[15] and {[Tm^{Ph}]Zn(NCMe)}(ClO$_4$)[16] react rapidly with Pb(ClO$_4$)$_2$·*x*H$_2$O to give yellow {[Tm^{Ph}]Pb}(ClO$_4$) (Scheme 1). The latter compound may also be obtained independently by reaction of [Tm^{Ph}]Li with Pb(ClO$_4$)$_2$.

Scheme 1



The molecular structure of {[Tm^{Ph}]Pb}(ClO$_4$) has been determined by X-ray diffraction (Figure 1), illustrating the monomeric trigonal pyramidal nature of the {[Tm^{Ph}]Pb}$^+$ cation with lead at the apex; thus, other than coordination to the three sulfur donors [*d*(Pb−S) = 2.693(2) Å] the next closest interaction is with a disordered perchlorate counteranion at a distance [*d*(Pb···OClO$_3$) ≈ 2.94 Å] which is substantially greater than the sum of the covalent radii of lead and oxygen.[17] Of particular relevance, the structure of {[Tm^{Ph}]Pb}$^+$ is very similar to the active site of Pb^{II}−ALAD.[7] Specifically, neither water nor acetonitrile coordinates to the lead centers in {[Tm^{Ph}]Pb}$^+$ or Pb−ALAD, both of which possess trigonal pyramidal geometry, with very similar average Pb−S bond lengths of 2.7 and 2.8 Å,[7] respectively. The significance of this similarity is underscored by the observation that mononuclear trigonal pyramidal lead complexes with a sulfur coordination sphere are rare.[18,19] Indeed, only one such example, namely [Pb(SPh)$_3$]$^-$,[20,21] is listed in the Cambridge Structural Database (CSD).[22,23]

The trigonal pyramidal structure of the lead center in {[Tm^{Ph}]Pb}$^+$ is in marked contrast to the tetrahedral zinc counterpart {[Tm^{Ph}]Zn(NCMe)}$^+$.[16] Such an observation indicates that, by

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(2) See, for example: Rampley, C. G.; Ogden, K. L. *Environ. Sci. Technol.* **1998**, *32*, 987−993.

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(4) ALAD is also known as porphobilinogen synthase (PBGS).

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(10) A class of magnesium dependent ALAD enzymes is also known. See ref 5 and Frankenberg, N.; Erskine, P. T.; Cooper, J. B.; Shoolingin-Jordan, P. M.; Jahn, D.; Heinz, D. W. *J. Mol. Biol.* **1999**, *289*, 591−602.

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(12) It should be noted that ALAD enzymes contain more than one type of metal binding site. For further discussion of structures of the various sites, see ref 5.

(13) Lipscomb, W. N.; Sträter, N. *Chem. Rev.* **1996**, *96*, 2375−2433.

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(16) {[Tm^{Ph}]Zn(NCMe)}(ClO$_4$) is obtained by reaction of [Tm^{Ph}]Li with Zn(ClO$_4$)$_2$ in MeCN and has been structurally characterized by X-ray diffraction. See Supporting Information.

(17) Furthermore, the Pb···Pb separation of 3.99 Å is significantly greater than twice the covalent radius of lead (3.08 Å).

(18) For a review of the structures of Pb^{II} complexes, see: Shimoni-Livny, L.; Glusker, J. P.; Bock, C. W. *Inorg. Chem.* **1998**, *37*, 1853−1867.

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(21) Simple [Pb(SAr)$_2$] derivatives are polymeric. See, for example (a) Krebs, B.; Brommelhaus, A.; Kersting, B.; Nienhaus, M. *Eur. J. Solid State Inorg. Chem.* **1992**, *29*, 167−180. (b) Rae, A. D.; Craig, D. C.; Dance, I. G.; Scudder, M. L.; Dean, P. A. W.; Kmetic, M. A.; Payne, N. C.; Vittal, J. J. *Acta Crystallogr., Sect. B* **1997**, *53*, 457−465.

(22) Cambridge Structural Database (version 5.18). *3D Search and Research Using the Cambridge Structural Database*; Allen, F. H.; Kennard, O. *Chemical Design Automation News* **1993**, *8* (1), pp 1, 31−37.

(23) The average Pb−S bond length in [Pb(SPh)$_3$]$^-$ (2.65 Å) (ref 20) is comparable to that in {[Tm^{Ph}]Pb} [2.693(2) Å].

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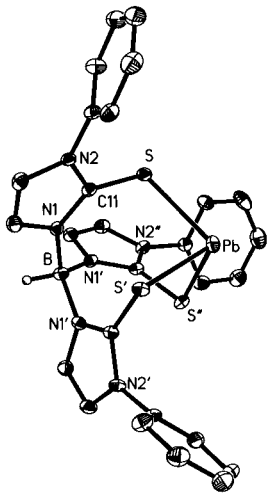

Figure 1. Molecular structure of the cation $\{[Tm^{Ph}]Pb\}^+$.

comparison to zinc, trigonal pyramidal lead centers have a reduced tendency to bind an additional ligand. In this regard, it should be noted that MeCN is present as a solvent of crystallization in $\{[Tm^{Ph}]Pb\}(ClO_4)$, but does *not* bind to the three-coordinate lead center. Consideration of the structures of other zinc and lead complexes reinforces this notion that lead complexes have significantly different structural preferences with respect to tetrahedral and trigonal pyramidal coordination. Thus, whereas tetrahedral coordination is most common for zinc in the solid state,[24] there are no examples of tetrahedral Pb^{II} complexes listed in the CSD; in preference four-coordinate Pb^{II} complexes typically have "hemidirected"[25] geometries, with a "saw-horse" structure.[18] Furthermore, calculations on a variety of four-coordinate Pb^{II} complexes indicates that this geometry is favored over tetrahedral.[18,26]

The reduced Lewis acidity of a trigonal pyramidal Pb^{II} versus Zn^{II} center is presumably associated with the stereochemically active Pb^{II} lone pair which tempers its electrophilicity, and we postulate that this is of significance to the inactivity of Pb^{II}–ALAD. Specifically, of the two mechanisms of action that have been proposed for ALAD, both involve activation of ALA by interaction of the ketone group with the Zn^{II} center.[5,6b] The degree of such activation is clearly a function of the Lewis acidity of the metal center,[27] which is considerably greater for a trigonal pyramidal Zn^{II} center than for a corresponding Pb^{II} center. As such, the formation of a tetrahedral species of the type $[(Cys)_3Pb^{II}$–ALA], a required intermediate in the proposed mechanisms of action of ALAD, would be inhibited.

An understanding of the debilitating effects of lead also requires a comprehension of the factors that distinguish the coordination chemistry of zinc and lead. In particular, it is essential to quantify the ability of lead to replace zinc in an environment which resembles that of ALAD. For this reason, we have determined the preference of $[Tm^{Ph}]$ to bind lead over zinc. Specifically, the reaction between $\{[Tm^{Ph}]Pb\}(ClO_4)$ and $Zn(ClO_4)_2$ in MeCN (equation 1) may be conveniently monitored by ^1H NMR spectroscopy, thereby allowing determination of the equilibrium constant (K).

$$\{[Tm^{Ph}]Pb\}^+ + Zn^{2+} + MeCN \underset{}{\overset{K}{\rightleftharpoons}} \{[Tm^{Ph}]Zn(NCMe)\}^+ + Pb^{2+} \quad (1)$$

The study indicates that the preference of $[Tm^{Ph}]$ to coordinate Pb^{II} over Zn^{II} in this system is ~500:1,[28] a value that is substantially greater than the ~25:1 relative affinity of these metals to reside at the active site of human erythrocyte ALAD.[29] Most interestingly, even though the preference for $[Tm^{Ph}]$ to coordinate Pb^{II} is substantially greater than that for Zn^{II}, zinc may be readily induced to replace lead in $\{[Tm^{Ph}]Pb\}^+$ by addition of NaI. Thus, ^1H NMR spectroscopy demonstrates that addition of NaI to a mixture of $\{[Tm^{Ph}]Pb\}(ClO_4)$ and $Zn(ClO_4)_2$ in acetonitrile results in the formation of $\{[Tm^{Ph}]Zn(NCMe)\}^+$, accompanied by the deposition of yellow PbI_2. In essence, the equilibrium represented by eq 1 is shifted to the right by precipitation of Pb^{II} as PbI_2. The ability to sequester lead by this simple method is of some importance in view of the fact that a completely effective means to ameliorating the toxic effects of lead in the human body is not yet known, despite efforts to develop lead complexing agents.[30]

In summary, the use of the $[Tm^{Ph}]$ ligand has enabled the replacement of zinc by lead to be studied in a synthetic analogue system. Of particular relevance, a quantitative study demonstrates that coordination of $[Tm^{Ph}]$ to Pb^{II} is a factor of ~500 greater than that for Zn^{II}, and that the trigonal pyramidal geometry of the lead center in the cation $\{[Tm^{Ph}]Pb\}^+$ is very similar to the active site of Pb^{II}–ALAD. Finally, despite the fact that Pb^{II} shows a greater preference to bind to $[Tm^{Ph}]$, Zn^{II} may be induced to displace Pb^{II} from $\{[Tm^{Ph}]Pb\}^+$ in the presence of iodide.

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Supporting Information Available: Experimental and crystallographic information (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(24) Bock, C. W.; Katz, A. K.; Glusker, J. P. *J. Am. Chem. Soc.* **1995**, *117*, 3754–3763.

(25) "Hemidirected" geometries are those in which the ligands are distributed over only a portion of the coordination sphere, while "holodirected" geometries are those in which the ligands are distributed more evenly about the whole surface of the coordination sphere. See ref 18.

(26) For example, the "hemidirected" geometry of $[Pb(SH_2)_4]^{2+}$ is 9.5 kcal mol^{-1} higher in energy than the tetrahedral structure. See ref 18.

(27) Vallee, B. L.; Auld, D. S. *Acc. Chem. Res.* **1993**, *26*, 543–551.

(28) The equilibrium constant (K) as defined for eq 1, including MeCN as a reagent, is $1.1(4) \times 10^{-4}$ M^{-1}. If K_{Zn} and K_{Pb} are the respective binding constants for coordination of $[Tm^{Ph}]^-$ to Zn^{II} and Pb^{II} (neglecting MeCN as a reagent), the equilibrium constant for eq 1 may be expressed as $K = K_{Zn}/\{K_{Pb}[MeCN]\}$, thereby allowing the ratio K_{Pb}/K_{Zn} to be determined.

(29) Simons, T. J. B. *Eur. J. Biochem.* **1995**, *234*, 178–183.

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