

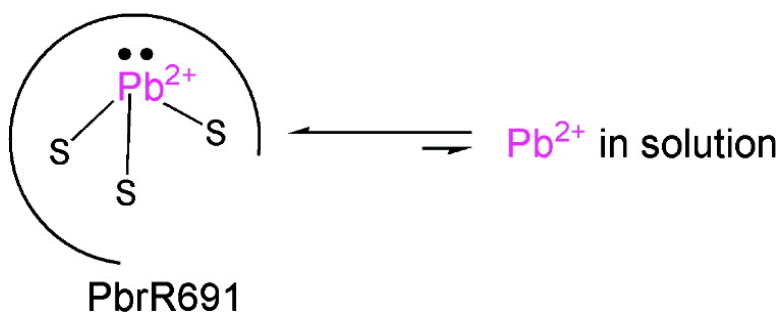
Communication

**Spectroscopic Insights into Lead(II) Coordination
 by the Selective Lead(II)-Binding Protein PbrR691**

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Spectroscopic Insights into Lead(II) Coordination by the Selective Lead(II)-Binding Protein PbrR691

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Developing molecules that can recognize various metal ions with high selectivity is a challenging problem in chemistry. Nature's solution is to evolve metalloproteins that preorganize binding geometries to recognize specific metal ions with high selectivity and sensitivity. We have been interested in elucidating mechanisms used by these proteins. Recently, we have shown that a lead(II)-regulatory protein, PbrR691 from *Ralstonia* (or *Cupriavidus*) *metallidurans* CH34, binds lead(II) almost 1000-fold more selectively over other metal ions such as mercury(II), cadmium(II), zinc(II), cobalt(II), nickel(II), copper(I), and silver(I).¹ This protein, together with its homologues in the same bacterium, are the only known lead(II)-specific binding proteins found in nature.² The unprecedented selectivity exhibited by PbrR691 prompted us to study the underlying molecular mechanism.

PbrR691 belongs to the MerR family transcriptional factors that regulate the concentrations of a range of toxic or essential metal ions in bacteria.^{3,4} The prototype is the Hg²⁺-binding MerR itself that uses three highly conserved Cys residues to selectively bind Hg²⁺ in a proposed trigonal geometry.⁵ A sequence alignment indicates that the three Cys residues are conserved as Cys78, Cys113, and Cys122 in PbrR691 (Figure S1). Structural studies of the copper(I)- and zinc(II)-binding MerR type proteins also suggest that these residues may form the metal binding pocket in PbrR691.^{6,7} It is not a surprise at all that Cys residues are used to recognize the soft lead(II) ion, but how PbrR691 discriminates other soft metal ions from binding is unclear.

The coordination chemistry of lead(II) is quite unique. The hybridization of s and p orbitals gives rise to a stereochemically active lone pair in Pb²⁺ that is resistant to engage in bonding to ligands.^{8,9} Thus, low coordinate (3- or 4-coordinate) lead(II) complexes tend to adopt a hemidirected geometry with all of the ligands clustered on one side of the metal, and the other face is occupied by the stereochemically active lone pair (Figure 1A).^{8–10} We wondered if it is the protein folding that enforces such a unique geometry which enables PbrR691 to selectively bind lead(II) and exclude other soft metal ions. In this scenario (Figure 1B) other metal ions would stay in solution to avoid paying high energetic penalties to enter the metal binding site in PbrR691, whereas lead(II) can adopt both geometries and could be preferentially recognized by PbrR691 owing to the chelate effect. The similar competition (in protein vs in solution) argument helped to explain the preferred binding of zinc(II) by zinc fingers, although mechanistically the two cases are different in that the ligand field stabilization energy (LFSE) effect underlies the selectivity for zinc fingers.^{11,12}

To confirm the hypothesis we performed spectroscopic studies on the lead(II)-loaded PbrR691. We have shown that the dimer of

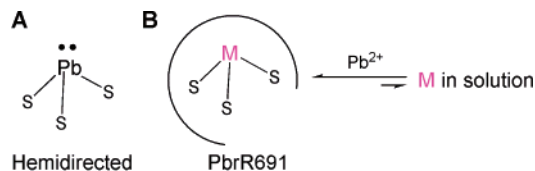


Figure 1. (A) Lead(II) prefers a hemidirected geometry in 3- or 4-coordinate complexes; (B) a proposed mechanism for the selective lead(II) recognition by the PbrR691 protein.

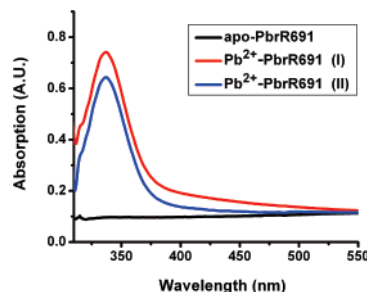


Figure 2. UV-vis spectra of two independently prepared samples of the Pb²⁺-PbrR691 complex (sample I, dimer concentration of 0.18 mM, extinction coefficient = 3589 M⁻¹ cm⁻¹ at 337 nm; sample II, dimer concentration of 0.15 mM; extinction coefficient = 3660 M⁻¹ cm⁻¹).

PbrR691 binds 1 equiv of lead(II).¹ The UV-vis spectra of two independently prepared samples were obtained (Figure 2). The extinction coefficient (3625 M⁻¹ cm⁻¹) observed at 337 nm, a peak representing a thiolate-lead(II) charge-transfer band, not only showed the involvement of Cys residues but also suggested the engagement of three Cys residues as ligands to Pb²⁺ (Table S1).^{13,14} To confirm this observation and to probe the coordination environment of the Pb²⁺ center in PbrR691, we performed X-ray absorption spectroscopic studies of the L_{III} edge of Pb²⁺.^{15,16}

The Pb²⁺-PbrR691 L_{III} edge data are shown in Figure 3A, along with the data from two model complexes, the hemidirected PbS₃,¹⁷ and the holodirected PbS₆.^{15,18} (Figure S2). Comparing the edge data of the two model complexes studied previously,¹⁵ there is a difference in shape and intensity at 13061 eV. Although it is tempting to use this difference as an indication of the local site geometry around the photoabsorber, the PbS₆ data in this region may be affected by multiple scattering and potential Pb-Pb interactions that are greatly reduced or absent in PbS₃. Thus, caution should be exercised in using this region as a marker for ligand directionality. Nonetheless, the Pb²⁺-PbrR691 sample closely resembles edge data of the PbS₃ model and that of a peptide containing a hemidirected Pb²⁺ center,¹⁵ which suggests that Pb²⁺ in PbrR691 is most likely hemidirected.

More evidence comes from the EXAFS data and the Fourier transform, along with the best fit shown in Figure 3B and Table 1. The Fourier transform spectrum of Pb²⁺-PbrR691 has a single

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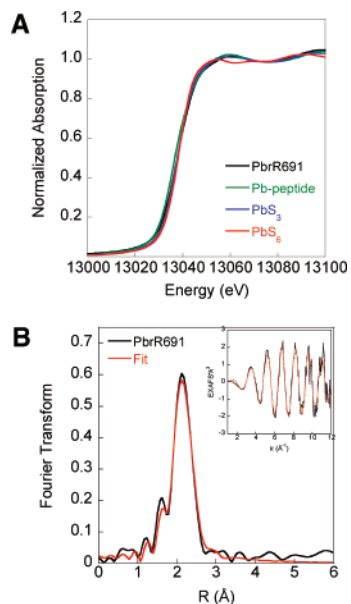


Figure 3. (A) Pb L_{III}-edge data of Pb²⁺–PbrR691 and the model complexes PbS₃, PbS₆, and a Pb²⁺-bound peptide (CP–CCCC¹⁵). (B) Nonphase shift-corrected Fourier transformed EXAFS data from PbrR691 and the three-coordinate fit with sulfur ligation described in the text. The four-coordinate fit with sulfur ligation is almost identical and is given in Figure S3. Note the lack of multiple scattering ($R = 3.0$ – 4.5 Å) indicative of longer range structure as would be present in, for example, histidine ligation. The inset shows the EXAFS data of Pb²⁺–PbrR691 and the three-coordinate fit with sulfur ligation.

Table 1. Selected EXAFS Fits of Pb²⁺–PbrR691

fit no.	ligation	R (Å)	σ^2 (Å ²)	ΔE_0 (eV)	error ^a
1	3 S	2.67	0.00517	–15.4	0.12
2	4 S	2.67	0.00698	–15.0	0.13
3	2 S	2.72	0.00690		
	2 O	2.52	0.00212	–4.7	0.12
4	2 S	2.71	0.00583		
	2 N	2.53	0.00164	–7.5	0.12

^a The error is given by $\sum[(\chi_{\text{obsd}} - \chi_{\text{calcd}})^2 k^6] / \sum[(\chi_{\text{obsd}})^2 k^6]$.

strong feature at around 2.1 Å (non-phase shift corrected) and no distinctive longer distance features. Multiple models of the PbrR691 active site result in similar fits to the data. A single shell of three or four sulfur ligands at 2.67 Å successfully fits the data with similar error values (fits 1 and 2, Table 1). The presence of oxygen- and nitrogen-based ligands were also investigated. A model with two sulfur ligands at 2.72 Å and two oxygen-based ligands at 2.52 Å (fit 3, Table 1) or two nitrogen-based ligands at 2.53 Å also successfully fit the data (fit 4, Table 1). However, the lack of long-range interactions in the Fourier transform spectrum beyond the nearest neighboring shell makes this an unlikely result in a biological system. Ultimately, all fits describing a six-coordinate site failed, having either a significantly larger error or unreasonable Debye–Waller factors (fits 6–10, Table S2). To test the validity of the fits with large $|\Delta E_0|$, the EXAFS data of PbS₃ model complex were fit and compared with the crystal structure. The resulting fit of three sulfur ligands at 2.71 Å compares well with the distance determined from crystallography (2.69 Å) and exhibits a ΔE_0 (–14.9 eV) similar to that of the all-sulfur fits to PbrR691. Moreover, forcing mixed sulfur and oxygen into the fit to the PbS₃ data results in a ΔE_0 value of –7.9 eV. Thus, the PbrR691 EXAFS fits with mixed ligation of sulfur and nitrogen/oxygen that exhibit ΔE_0 of –4 to –10 eV are inconsistent with the PbrR691 data. Therefore, the EXAFS data indicate that three to four ligands are involved in Pb²⁺ binding, and they are most likely Cys residues.

To summarize, the spectroscopic results and the common structural features of the MerR family proteins led us to conclude that three Cys residues are involved in lead(II) binding in PbrR691. A possible fourth ligand could not be excluded based on the current data. With such a low coordination number, Pb²⁺ predominantly prefers hemidirected geometries;^{8,9} it is known that the hemidirected geometry is favored by more than 5 kcal/mol versus tetrahedral in four-coordinate lead(II) complexes from an ab initio analysis.⁹ The edge spectrum of Pb²⁺–PbrR691 also closely resembles those of a PbS₃ model and a peptide with bound Pb²⁺ in a hemidirected geometry (Figures 3A and S4).¹⁵ Thus, all evidence supports a hemidirected geometry used by PbrR691 to recognize lead(II). In other words, the MerR protein may preorganize a trigonal geometry to selectively recognize mercury(II), whereas PbrR691 perhaps preorganizes a hemidirected geometry to selectively bind lead(II) with the same or similar set of ligands.

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Supporting Information Available: Experimental details; Figures S1–S4; Tables S1–S3. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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