Lead contamination is a serious threat to human health and the environment. Lead poisoning is still one of the most common environmentally caused diseases in the world today. Lead levels are typically measured by using atomic absorption spectroscopy or other related instrumental methods. Probes that can provide rapid, on-site evaluation of the lead content of a sample are very valuable for a variety of applications. A primary challenge is to construct a “turn-on” probe that responds to lead ions with a high selectivity over other heavy metal ions. This has not been achieved with chemically designed small molecules, despite some progress in the last decade. Chemical probes have been developed for the detection of other small molecules, but with limited success for heavy metal ions and paramagnetic metal ions.

An evolution strategy was elegantly applied by Lu and co-workers recently to construct Pb2+ probes based on a DNAzyme. This system is surprisingly selective towards Pb2+ ions. We have adopted a different strategy that takes advantage of the function of metalloregulatory proteins. Nature has evolved numerous such proteins to control the concentrations of beneficial or toxic metal ions with unprecedented sensitivity and selectivity. If the biological sensory events of these proteins were reported with measurable signals, such as fluorescence, practical probes could be obtained for the detection of various metal ions. We have recently invented a method to convert the MerR-family proteins into fluorescent reporters for the detection of Hg2+ and coinage metal ions. Herein we report the successful conversion of a novel Pb2+-regulatory protein in Ralstonia metallidurans CH34 into a fluorescent reporter that exhibits high selectivity and sensitivity for Pb2+ ions. With the aid of this system, the binding of Pb2+ ions to the Pb2+-regulatory protein was also characterized. A surprisingly high binding selectivity of the protein towards Pb2+ is revealed.

**An Exceptionally Selective Lead(II)-Regulatory Protein from Ralstonia metallidurans:**

**Development of a Fluorescent Lead(II) Probe**

Peng Chen, Bill Greenberg, Safiyh Taghavi, Christine Romano, Daniel van der Lelie, and Chuan He*

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**Figure 1.** PbrR regulates the lead-resistance operon (pbr) in Ralstonia metallidurans strain CH34.

**Communications**

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subsequently purified and used in this study (see the Supporting Information). The concentration of PbrR691 was determined by a bovine serum albumin (BSA) assay (Bio-Rad Laboratories, Inc.) that was calibrated by amino acid analysis (Protein Chemistry Laboratory, Texas A&M University).

The addition of PbrR691 to DNA-1 did not cause noticeable changes in the fluorescence of the probe. However, the addition of one equivalent of Pb⁺⁺ ions per PbrR691 dimer triggered a significant fluorescence enhancement within seconds (Figure 3a). The probe is selective, as the addition of a 50-fold excess of Zn⁺⁺, Co⁺⁺, Hg⁺⁺, Cu⁺⁺, or Cd⁺⁺ caused less than 1/20th of the fluorescence increase at λ ≈ 445 nm compared to that with Pb⁺⁺ ions (Figure 3b). We concluded that the fluorescence response of this PbrR691-based probe is over 1000-fold more selective towards Pb⁺⁺ ions than towards the other metal ions in this assay! Thus, PbrR691 appears to exhibit an unprecedented selectivity towards Pb⁺⁺ ions over other metal ions.

When we tried to perform an accurate analysis of Pb⁺⁺ binding to the protein by using this fluorescent system, we discovered that the fluorescence response from this probe is not optimum to give a quantitative assessment of Pb⁺⁺ binding to PbrR691. Instead, we employed 2-aminopurine (2AP), an analogue of adenine, as the fluorescent base. 2AP emits fluorescence at λ ≈ 370 nm when excited between λ = 310 and 320 nm. The incorporation of 2AP into DNA quenches its fluorescence. This quenching is attributed to stacking interactions with nearest neighbor nucleobases and to electron transfer to nearby bases.[16,17] When the base-pairing environment is perturbed, 2AP shows quantitatively enhanced fluorescence; this property makes 2AP an excellent fluorescent probe for studying the structural properties of the DNA duplexes. We incorporated a 2AP base into the central base pair of the PbrR-promoter DNA (DNA-2), as shown in Figure 4a. The response of this new probe towards Pb⁺⁺ binding was tested.

The fluorescence of the 2AP-containing probe is quenched in double-stranded DNA. The addition of the apo-PbrR691 dimer to DNA-2 did not give any significant fluorescence change. The addition of one equivalent of Pb⁺⁺ ions triggered a greater than threefold fluorescence enhancement. The intensity increase is reproducible and the response occurs at both 4°C and room temperature. At room temperature, the detection limit can reach the nanomolar range (50 nM) for free Pb⁺⁺ ions in solution. The probe also shows higher selectivity towards Pb⁺⁺ ions than towards other metal ions (about 1000-fold, Figure 4b). The binding of Pb⁺⁺ ions to

Figure 2. a) Pyrrolo-C (C#) can form a stable base pair with G. The fluorescence intensity of pyrrolo-C is quenched in the duplex DNA. The promoter sequence that PbrR691 binds is used to construct DNA-1; the dyad symmetrical sequence is marked with arrows. b) Binding of Pb⁺⁺ ions to PbrR691 induces base unpairing of pyrrolo-C, which emits strong fluorescence at λ ≈ 445 nm upon excitation.

Figure 3. Responses of the PbrR691-based fluorescent probe towards different metal ions. a) Fluorescence spectra of the probe in the absence and presence of one equivalent of Pb⁺⁺ ions. b) The fluorescence enhancement integrated over λ = 420–500 nm in the presence of Pb⁺⁺ ions (2 µM) and other metal ions (100 µM). The inset presents the fluorescence spectra of the probe in the presence of Pb⁺⁺ (2 µM) and Hg²⁺ ions (100 µM).
PbrR691 appears to be reversible, as the addition of 10 μM ethylenediaminetetraacetate (EDTA) to the Pb²⁺-bound probe restores the original spectrum of the metal-free probe. This new 2AP-based Pb²⁺ probe has several advantages. It is sensitive and highly selective towards Pb²⁺ ions. It shows a quantitative response to various Pb²⁺ concentrations in solution. The fluorescent-reporter system also offers an opportunity to characterize the Pb²⁺-binding properties of PbrR691. The binding of Pb²⁺ ions to PbrR691 was monitored by recording fluorescence spectra as a function of Pb²⁺ concentrations.
To verify the binding constant, isothermal titration calorimetry was performed on the Pb²⁺/PbrR691 system in a solution of 100 mM NaNO₃, 20 mM tris(hydroxymethyl)aminomethane/HNO₃ (Tris-HNO₃), and 5% glycerol at pH 7.[19] The ITC data were fitted by the one-site binding model and they yielded the same 2:1 ratio between PbrR691 and Pb²⁺ ions (see the Supporting Information). The dissociation constant was calculated to be 2.07 ± 0.14 × 10⁻⁵ M, which is consistent with the fluorescence titration result.

Lastly, the Pb²⁺-bound PbrR691 was prepared and analyzed. Excess amounts of Pb(NO₃)₂ (up to a final concentration of 500 μM) were added to a solution of PbrR691 (50 μM). The free and weakly bound Pb²⁺ ions were removed by consecutive dialyses with washing buffer (2 L). Inductively coupled plasma-mass spectrometry (ICP-MS) analysis of this sample showed 1.06 ± 0.02 equivalents of Pb²⁺ ions per PbrR691 dimer. When the protein solution was washed with EDTA (100 μM), almost no Pb²⁺ ions (0.008 equiv) were observed from the ICP-MS measurement. This observation further indicates the binding of one Pb²⁺ ion per PbrR691 dimer.

We have reported herein the characterization of a unique Pb²⁺-regulatory protein, PbrR691. It shows a selectivity of over 1000-fold towards Pb²⁺ ions over other metal ions. By applying a mechanism-based approach, we have successfully converted this Pb²⁺-sensory protein into a selective fluorescent Pb²⁺ reporter. This novel PbrR691-based Pb²⁺ probe has the potential to be used in practical applications after further improvements. The affinity and stoichiometry of the binding of Pb²⁺ ions to PbrR691 were also revealed with the aid of the fluorescent-reporter system and were further confirmed with other methods.

The development of a selective Pb²⁺ chelator for treating lead poisoning is an unachieved challenge.[11] We believe study of the molecular basis for selective Pb²⁺ recognition by PbrR691 may provide fundamental knowledge for designing such agents in the future.

Keywords: fluorescent probes · lead binding · PbrR · regulatory proteins · sensors