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COMMUNICATIONS

Published on Web 06/29/2006

A Selective Fluorescent Sensor for Detecting Lead in Living Cells

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Received May 1, 2006; E-mail: chrischang@berkeley.edu

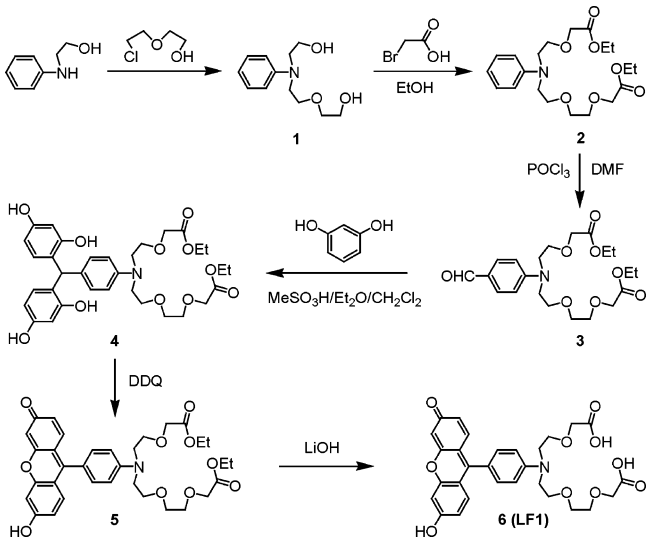
Lead pollution is an ongoing danger to human health and the environment, as most of the 300 million tons of this heavy metal mined to date are still circulating in soil and groundwater.[1] Despite efforts to reduce global emissions, lead poisoning remains the world's most common environmentally caused disease.[2] Once introduced into the body, lead is a potent neurotoxin that can interfere with brain development,[3] slow nerve conduction velocity,[4] and trigger behavioral problems.[5] Plausible molecular targets of lead include calcium- and zinc-binding proteins that control cell signaling and gene expression, respectively,[2,6−10] but mechanisms of lead toxicity remain an open question for study.

Interest in elucidating these pathways, as well as public concerns over toxic lead exposure, provides a need for devising new ways to track Pb^{2+} in natural samples. Whereas standard techniques, such as atomic absorption or anodic stripping voltammetry, can only measure total lead content, fluorescence imaging with Pb^{2+}-sensitive chemosensors can, in principle, provide information on bioavailable lead pools with spatial and temporal resolution. The major challenges to achieving this goal are creating systems that are selective for Pb^{2+} and can function in water. Pb^{2+}-responsive fluorescent probes based on peptide,[11] protein,[12] DNAzyme,[13−16] polymer,[17] and small-molecule[18−27] scaffolds have been reported. However, none of these systems have been utilized successfully for tracking Pb^{2+} in living cells, as current probes can be limited by interfering background fluorescence or nonspecific quenching from competing metal ions, incompatibility with water, and/or ultraviolet excitation that can damage or trigger autofluorescence from biological samples. We now present the synthesis and properties of Leadfluor-1 (LF1, **6**), a new turn-on fluorescent sensor for selective detection of Pb^{2+} in water and in living cells. LF1 features visible wavelength excitation and emission profiles and a ca. 18-fold fluorescence enhancement upon Pb^{2+} binding. Moreover, confocal microscopy experiments establish that LF1 can monitor changes in Pb^{2+} levels within living mammalian cells.

LF1 combines a fluorescein-type scaffold with attractive optical properties and biological compatibility with a dicarboxylate pseudocrown receptor designed to satisfy the size and charge requirements of the Pb^{2+} cation.[28] The synthetic route to LF1 is shown in Scheme 1. Reaction of 2-anilinoethanol with 2-(2-chloroethoxy)ethanol affords diol **1** in 66% yield. Alkylation of **1** with bromoacetic acid followed by acid-catalyzed esterification provides diester **2** in 61% yield. Vilsmeier formylation of **2** using $POCl_3$/DMF followed by basic workup generates aldehyde **3** in 50% yield, which is then coupled with 2 equiv of resorcinol to produce the tetrahydroxy intermediate **4** in 14% yield. DDQ oxidation of **4** furnishes xanthenone **5** in 41% yield. Ester deprotection of **5** under basic conditions proceeds quantitatively to give LF1.

Spectroscopic measurements with LF1 were performed under simulated physiological conditions (20 mM HEPES, buffer pH 7). LF1 displays a characteristic fluorescein-like absorption band in the visible region centered at 490 nm ($\epsilon = 2.5 \times 10^4$ M^{-1} cm^{-1}) and weak fluorescence ($\Phi < 0.001$, $\lambda_{em} = 514$ nm). Upon addition

Scheme 1. Synthesis of Leadfluor-1 (LF1)



of Pb^{2+}, the fluorescence intensity of LF1 increases by ca. 18-fold ($\Phi = 0.013$, Figure 1a) with the same absorption ($\lambda_{abs} = 490$ nm, $\epsilon = 2.8 \times 10^4$ M^{-1} cm^{-1}) and emission maxima ($\lambda_{em} = 514$ nm) as the apo probe. The turn-on response is reversible; treatment with the chelator TPEN restores LF1 fluorescence back to within 5% of baseline levels. Binding assays using the method of continuous variations (Job's plot) are consistent with a 1:1 Pb^{2+}:LF1 complex being responsible for the observed fluorescence enhancement, and the K_d for Pb^{2+} coordination to LF1 is 23 ± 4 μM. LF1 is capable of detecting environmentally relevant concentrations of aqueous Pb^{2+}. The addition of 15 ppb Pb^{2+}, the maximum EPA limit for allowable level of lead in drinking water, to a 5 μM solution of LF1 triggers a 15 ± 2% increase in fluorescence intensity.

LF1 also exhibits a selective turn-on fluorescence response to Pb^{2+} in aqueous solution. Responses of 5 μM LF1 to the presence of various biologically and environmentally relevant metal ions are collected in Figure 1b. The fluorescence profiles of apo or Pb^{2+}-bound LF1 are unchanged in the presence of 2 mM Li^+, Na^+, K^+, Mg^{2+}, and Ca^{2+}, indicating excellent selectivities for Pb^{2+} over these alkali and alkaline earth cations. A series of 3d metal ions, including 75 μM Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, and Zn^{2+}, do not trigger LF1 fluorescence enhancements or interfere with its Pb^{2+} response. Of the first-row transition metal ions, only Cu^{2+} at 75 μM can limit the turn-on Pb^{2+} response of LF1, but lower concentrations of Cu^{2+} (25 μM) do not interfere. LF1 also shows selectivity for Pb^{2+} over the heavy metal ions Hg^{2+} and Cd^{2+}.

Subsequent experiments probed the ability of LF1 to track Pb^{2+} levels in living cells using confocal microscopy. HEK cells incubated with 20 μM of LF1-AM for up to 90 min at 37 °C show negligible intracellular fluorescence (Figure 2a). In contrast, LF1-stained cells exposed to 200 μM Pb^{2+} for 40 min at 37 °C display enhanced cytosolic fluorescence (Figure 2b). Treatment of cells

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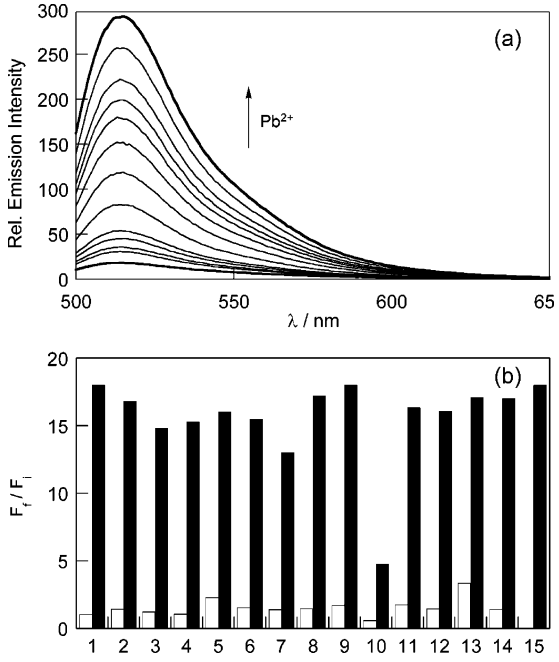

Figure 1. (a) Fluorescence response of 5 μM LF1 to Pb^{2+}. Spectra shown are for Pb^{2+} concentrations of 0, 1, 2, 3, 4, 5, 10, 15, 20, 25, 35, 50, and 75 μM. Spectra were acquired in 20 mM HEPES, pH 7, with excitation at 490 nm. (b) Fluorescence responses of 5 μM LF1 to various metal ions. Bars represent the final integrated fluorescence response (F_f) over the initial integrated emission (F_i). Spectra were acquired in 20 mM HEPES, pH 7. White bars represent the addition of an excess of the appropriate metal ion (2 mM for Li^+, Na^+, K^+, Mg^{2+}, and Ca^{2+}, 75 μM for all other cations except entry 11) to a 5 μM solution of LF1. Black bars represent the addition of 75 μM Pb^{2+} to the solution. Excitation was provided at 490 nm, and the emission was integrated over 500−650 nm. 1, Li^+; 2, Na^+; 3, K^+; 4, Mg^{2+}; 5, Ca^{2+}; 6, Mn^{2+}; 7, Fe^{2+}; 8, Co^{2+}; 9, Ni^{2+}; 10, Cu^{2+}; 11, Cu^{2+}, 25 μM; 12, Zn^{2+}; 13, Cd^{2+}; 14, Hg^{2+}; 15, Pb^{2+}.

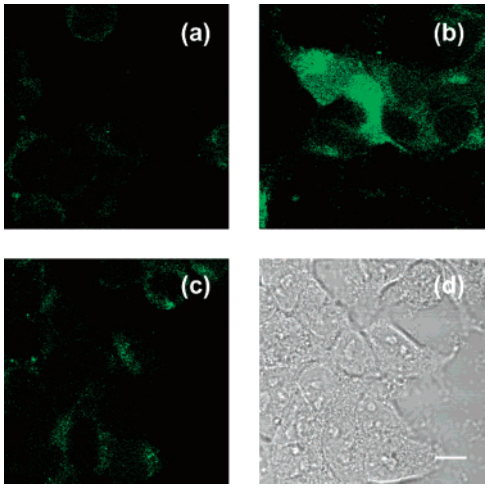

Figure 2. Live-cell imaging of intracellular Pb^{2+} levels by confocal microscopy. (A) Control HEK cells incubated with 20 μM LF1-AM for 90 min at 37 °C. (B) LF1-stained HEK cells exposed to 200 μM $Pb(OAc)_2$ and 1 mM sodium citrate for 40 min at 37 °C. (C) LF1-loaded, lead-supplemented cells treated with 2 mM of the heavy metal chelator TPEN for 5 min at 25 °C. (D) Brightfield image of live HEK cells shown in panel C, confirming their viability. Scale bar = 10 μm.

loaded with LF1 and Pb^{2+} with the heavy metal chelator TPEN (2 mM) for 5 min at 25 °C reverses the observed fluorescence increases (Figure 2c), and further control experiments without dye give no fluorescence over background levels. Finally, brightfield transmission measurements confirm that the cells are viable throughout the imaging studies (Figure 2d). Taken together, these results show that LF1 can respond to changes in intracellular Pb^{2+} levels within living mammalian cells.

In conclusion, LF1 is a new type of synthetic fluorescent sensor for probing Pb^{2+} in living biological samples. Desirable features of this fluorescein-based reagent include a selective turn-on response for Pb^{2+} over competing metal ions in water, sensitivity to EPA limits of lead poisoning, visible excitation and emission profiles to minimize cellular autofluorescence and photodamage, and the ability to track changes in Pb^{2+} levels within living cells. Immediate goals to expand the utility of LF1 and related chemosensor platforms for studies of lead biology include optimization of sensitivities and selectivities to Pb^{2+}, as well as improvement of optical brightness values and dynamic ranges for imaging applications.

Acknowledgment. We thank the University of California, Berkeley, the Camille and Henry Dreyfus Foundation, the Arnold and Mabel Beckman Foundation, the American Federation for Aging Research, and the NSF (CAREER Award CHE-0548245) for funding this work. E.W.M. was supported by a Chemical Biology Interface Training Grant from the NIH (T32 GM066698). We thank Ms. Sarah Bell for preliminary experiments.

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

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JA063029X