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Turn-on ratiometric fluorescent sensor for Pb^{2+} detection

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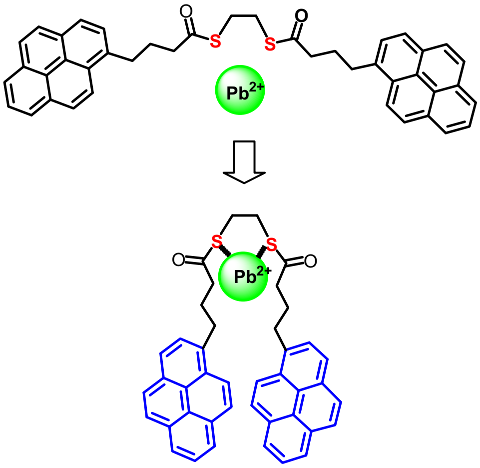
ABSTRACT

We report a ratiometric lead fluorescent sensor (LFS-1) with high affinity to Pb^{2+} that shows considerable selectivity over 12 other physiological related metal cations in aqueous media. Binding induces excimer formation, providing a highly sensitive ratiometric measure of lead concentrations.

As one of the major industrial pollutants, Pb^{2+} causes adverse environmental impacts and human health related problems, particularly for children (where lead poisoning is defined as blood levels above 5 μM).[1] Therefore, developing low-complexity sensing approaches with high affinity for detecting Pb^{2+} remains an active research area.[2] In the past decade, considerable efforts have been devoted to designing and synthesizing fluorescent chemosensors for Pb^{2+} analysis due to their high sensitivity, simplicity, and adaptability to different platforms that facilitate routine screening.[3] Many Pb^{2+}-responsive fluorescent sensors, in which biological molecules, polymers, and small organic scaffolds are utilized as recognition units, have been reported.[4–6] However, compared to other heavy metal ions sensors, the ion specificity and sensitivity of current generation fluorescent sensors for Pb^{2+} limit their utility.[7] Currently, major challenges for Pb^{2+}-responsive fluorescent sensors require increases in both (i) the recognition affinity to Pb^{2+} without interference from other competing metal ions and (ii) the solubility in aqueous media to allow detection of Pb^{2+} in biological samples.[8] Herein, we report the synthesis and properties of a highly soluble turn-on lead fluorescent sensor (LFS-1) that binds Pb^{2+} with high affinity and displays selectivity against other physiological metals in aqueous media. Upon binding Pb^{2+} LFS-1 exhibits a strong excimer peak at 469 nm resulting from contact interactions between pyrene monomers within LFS-1 that are brought together upon metal chelation, which is evident both in model systems and in human blood plasma, indicating the feasibility of using LFS-1 to rapidly screen for Pb^{2+}.

LFS-1 was prepared by using pyrene as the fluorophore, which has been studied extensively because of its strong fluorescence intensity and distinctive excimer emission that results in a large spectral red-shift that facilitates measurements in complex environments.[9] Sensors were designed using the sulfur of a thio-ester moiety as a receptor to recognize Pb^{2+}, thereby facilitating formation of a dimeric interaction between pyrene moieties through a coordinating interaction that is required for excimer formation (Scheme 1). LFS-1 was synthesized by a two-step reaction. 1-Pyrenebutyric acid was refluxed with thionyl chloride for 3 h to give 1-pyrenebutyric chloride. Esterification between 1 equiv 1, 2-ethanedithiol and 2 equiv of 1-pyrenebutyric chloride afforded LFS-1 with 88% yield (Scheme 2). An alternative sensor (i.e., LFS-2) with a shorted linker connecting the thio-ester and pyrene functionalities based on 1-pyrenecarboxylic acid also was prepared



Scheme 1. Fluorescent sensor for Pb^{2+}.

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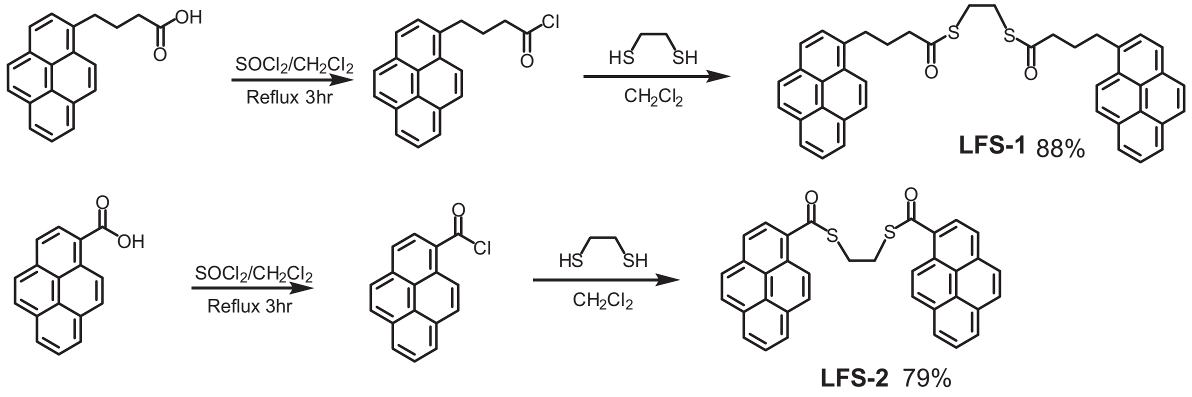

Scheme 2. The synthetic route of LFS-1 and LFS-2.

using the same synthetic route. The structures of LFS-1 and LFS-2 were confirmed by ^{1}H NMR, ^{13}C NMR, and elemental analysis.[10,11]

Spectroscopic measurements were carried out in aqueous buffer (i.e., 10 mM HEPES containing 10% DMSO at pH 7.4). LFS-1 displays characteristic spectral features of pyrene, with absorption bands at 326 and 342 nm and fluorescence emission bands at 375 and 395 nm (λ_{ex} = 355 nm). Upon addition of 10 M equiv of Pb^{2+}, LFS-1(1.0 μM) displays large changes in the absorption spectrum, resulting in decreases in absorption bands at 328 and 342 nm and the appearance of a new peak at 357 nm (Fig. 1A). In the presence of Pb^{2+}, the fluorescence emission spectrum of LFS-1 also exhibits a new band at 469 nm together with a substantial quenching in the 365–425 nm spectral region (Fig. 1B). Observed spectral changes are indicative of the formation of an excimer complex resulting from contact interactions between pyrene monomers in the presence of Pb^{2+} that arise due to π–π stacking interactions. Coupled with the large depletion of fluorescence at 365–425 nm, these results indicate that LFS-1 has the necessary spectral properties to serve as a sensitive ratiometric probe in response to Pb^{2+}.[12] Specifically, as the excimer fluorescence apparent at 469 nm is absent prior to Pb^{2+} binding, it is apparent that the intensity ratio at 469 nm normalized by that at 395 nm (I_{469}/I_{395}) represents a sensitive means to measure Pb^{2+} levels in complex environments using the LFS-1 sensor. While, additional sensitivity is possible by selecting excitation wavelengths that limit excitation of the LFS-1 sensor prior to Pb^{2+} binding, which shifts the absorption spectra toward the red (Fig. 1A), we emphasize the use of LFS-1 under conditions that excite both the free (unbound) and Pb^{2+} chelated species that facilitate ratiometric measurements of the apparent binding affinity.

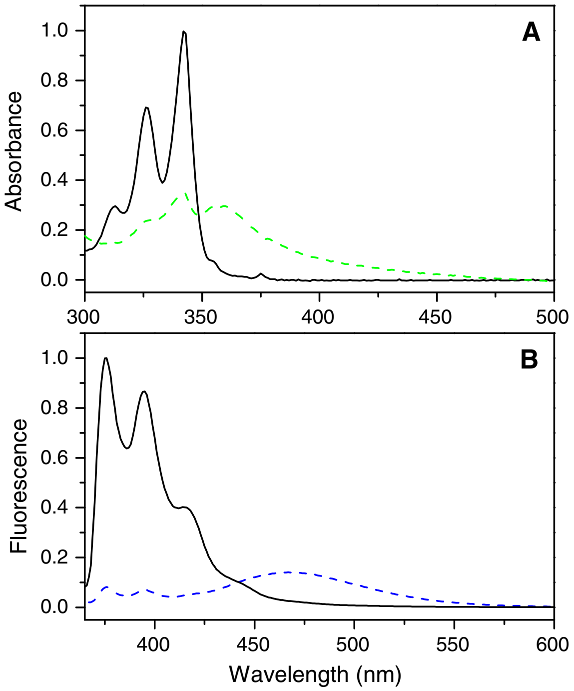

Figure 1. Absorption (A) and fluorescence emission (λ_{ex} = 355 nm) (B) spectra of LFS-1 (1.0 μM) in aqueous buffer (i.e., 10 mM HEPES containing 10% DMSO at pH 7.4), prior to (—) and following addition of 10 μM Pb^{2+} (- - -).

To explore further the binding properties of LFS-1, titration experiments were conducted by using Pb^{2+} with concentrations ranging from 0 to 10 μM (Fig. 2). Upon addition of Pb^{2+} to LFS-1, there is a 92% decrease in the fluorescence of monomer coincident with detection of an excimer fluorescence at 469 nm that provides a high signal-to-noise measurement using 1 μM LFS-1. Based on the titration data, maximal spectral changes are observed upon addition of a near equimolar stoichiometry of Pb^{2+} relative to the LFS-1 (i.e., 1 μM), indicating that the binding affinity of LFS-I for Pb^{2+} is in the submicromolar range needed for practical measurements of Pb^{2+} in drinking water.[13]

The selectivity of LFS-1 to bind Pb^{2+} relative to other common metals was investigated by ratiometric measurements (i.e., I_{469}/I_{395}) in the presence of various biologically and environmentally relevant metal ions. All of measurements were conducted by using the perchlorate of different metal ions (100 μM) and LFS-1(1.0 μM) in aqueous buffer (i.e., 10 mM HEPES containing 10% DMSO at pH 7.4). LFS-1 displayed an extremely selective turn-on response to Pb^{2+} with a maximum I_{469}/I_{395} ratio of 2.1 (no corrections for contributions of scattered light at 469 nm were made). In contrast, upon addition of K^+, Na^+, Ag^+, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Hg^{2+}, and Mg^{2+}, LFS-1 exhibited no significant excimer emission at 469 nm; albeit fluorescence quenching at 375 nm leads to small increases in the calculated I_{469}/I_{395} ratio (<0.5) that can be reduced by correcting for scattered light by using suitable blank subtraction or modifying the detection wavelength used to monitor excimer formation toward the red (Fig. 3A). Especially for Ag^+, Cu^{2+} and Hg^{2+}, the fluorescence at 395 nm was quenched more than 96%. Fluorescence quenching is possible by two mechanisms involving either excimer formation with concomitant decreases in the fluorescence of the pyrene monomer or through spin-orbital coupling that arises from a coupling between metal ions and pyrene.[14,15] Due to the high I_{469}/I_{395} ratio, the fluorescence quenching trigged by Pb^{2+} binding was attributed mainly to the excimer formation rather than in intrinsic quenching. In comparison, some other metals (e.g., Hg^{2+}) exhibit no excimer fluorescence upon binding LFS-1