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Near-Infrared Fluorescent Probe for Sensitive Detection of Pb(II) Ions in Living Cells

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Abstract

A new near-infrared fluorescent probe (NIR-PbP) for sensitive detection of Pb(II) ions in solution and living cells has been rationally designed and synthesized. The NIR-PbP is inherently non-fluorescent and gains fluorescence in the presence Pb(II) ions. The ion detection is based on Pb(II)-induced unmasking the fluorophore through the opening of the spirocycle, with more than 500-fold fluorescence for sub-micromolar Pb(II) concentration. The NIR-PbP has high sensitivity, good photo-stability, low detection limit, and reversible response to Pb(II) ions.

Graphical abstract

Keywords

Pb(II) ions; Fluorescence; Fluorescent probe; Near-infrared emission

1. Introduction

Lead is one of the most hazardous heavy metal pollutants of the air, water, and soil that plays a key role in biological, environmental and chemical fields. [1–3] It is estimated that millions of workers in the United States are potentially exposed to lead [4–9], and lead exposure is linked with behavioral abnormalities, learning impairment, decreased hearing, high blood pressure, kidney disorders, and neurological impairment [10–12]. A greater worry is that children are the most at-risk for lead exposure which can cause cognitive and neurological impairment [13–15]. Therefore, it is important to keep lead concentration level in our surrounding environment low and precisely monitored. From the various approaches, detecting Pb(II) with fluorescent probes allows for efficient monitoring of lead ions with high sensitivity and low detection limit [16, 17]. Among different fluorescent probes, the turn-on probes allow to detect low concentrations of lead in aqueous media and in living cells because of the Pb⁺²-induced activation of fluorescence [18–22].

Recently, near-infrared (NIR) fluorescent probes have received much attention due to its advantages in deep tissue penetration, low background interference and minimal damage to cells and tissues [23–26]. While significant advances have been made towards detecting cellular targets or small molecules with near-IR probes [24–28], no such probes for Pb(II) detection in living cells were reported.

In this paper, we report a near-infrared fluorescent probe (NIR-PbP) with the spirocyclic structure which is highly sensitive to the Pb⁺² ions in the solutions and in living cells. The NIR-PbP is colorless and non-fluorescence with the intact spirocyclic amide structure in the

absence of Pb^{+2} ions. The fluorescence of the NIR-PbP is triggered by the presence of Pb(II) ions, inducing significant absorbance enhancement at 718 nm and near-infrared fluorescence at 735 nm. The turn-on fluorescence is based on Pb-catalyzed opening of the spirocycle and unmasking of the fluorophore.

2. Experimental Section

2.1 Instrumentation

^1H NMR and ^{13}C NMR spectra were obtained by using a 400 MHz Varian Unity Inova NMR spectrophotometer. ^1H and ^{13}C NMR spectra were recorded in CDCl_3 , chemical shifts (δ) are given in ppm relative to solvent peaks (^1H : δ 7.26; ^{13}C : δ 77.36) as internal standard. Absorption spectra were obtained on a Perkin Elmer Lambda 35 UV/VIS spectrometer. Fluorescence spectra were obtained on a Jobin Yvon Fluoromax-4 spectrofluorometer. Each of the samples was scanned with increments of 1 nm. Fluorescent images were collected with Olympus IX 81 confocal laser scanning microscope.

2.2 Reagents

All reagents and solvents were purchased from commercial suppliers and used without further purification. Air- and moisture-sensitive reactions were conducted in oven-dried glassware using a standard Schlenk line or dry box techniques under the inert atmosphere of dry nitrogen.

2.3 Synthesis

Synthesis of NIR-PbP—To the compound **1** (0.25 g, 0.44 mmol) in anhydrous dichloromethane (20 mL) were added *N*-hydroxysuccinimide (0.06 g, 0.58 mmol), DCC (0.09 g, 0.44 mmol) were added. The resulting reaction mixture was stirred at room temperature for 30 min under nitrogen atmosphere. To this mixture, compound **2** (0.14 g, 0.58 mmol) and triethylamine (0.1 mL) were added, and the mixture was further stirred overnight at room temperature. After the mixture was washed with water (2×30 mL) and brine (2×30 mL), the organic layer was separated, dried over anhydrous sodium sulfate, filtered, and concentrated under vacuum. The crude compound was purified by column chromatography using DCM/EtOH (40:1 to 20:1, v/v) to yield fluorescent probe **A** as a brown solid (0.14 g, 40%). ^1H NMR (400 MHz, CDCl_3): δ 8.44–8.43 (d, $J = 4.0$ Hz, 2H), 7.82–7.80 (d, $J = 8.0$ Hz, 1H), 7.58–7.54 (m, 2H), 7.45–7.36 (m, 5H), 7.17–7.02 (m, 5H), 6.85–6.81 (t, $J = 8.0$ Hz, 1H), 6.61–6.59 (d, $J = 8.0$ Hz, 1H), 6.24–6.15 (m, 3H), 5.36–5.33 (d, $J = 12.0$ Hz, 1H), 3.82–3.73 (m, 4H), 3.43–3.28 (m, 4H), 3.15 (s, 3H), 2.66–2.31 (m, 4H), 1.75–1.71 (d, $J = 16.0$ Hz, 6H), 1.53–1.24 (m, 6H), 1.17–1.13 (t, $J = 8.0$ Hz, 6H). ^{13}C NMR (100 MHz, CDCl_3): δ 168.84, 157.85, 152.94, 151.82, 148.20, 145.57, 139.02, 136.83, 132.31, 132.03, 128.27, 127.92, 123.61, 123.34, 122.97, 122.20, 121.74, 120.41, 119.71, 119.48, 108.55, 105.15, 103.47, 103.42, 97.96, 92.29, 67.08, 60.09, 52.57, 45.64, 44.56, 38.03, 29.90, 29.36, 28.69, 28.50, 25.46, 23.12, 22.20, 12.79. IR (cm^{-1}): 3051.99, 2970.16, 2929.33, 1687.18, 1623.32, 1593.55, 1517.02, 1468.37, 1376.75, 1318.29, 1265.76, 1215.64, 1194.79, 1126.58, 735.03, 701.52. HRMS (FAB) calcd for $\text{C}_{51}\text{H}_{55}\text{N}_6\text{O}_2$ $[\text{M}+\text{H}]^+$, 783.4386; found, 783.4404.

2.4 Optical measurements

All absorption and emission spectra were recorded using a standard 1 cm path length quartz fluorescence cuvette at room temperature. NIR-PbP solution (10 μM) was used each time in the presence of 5 equivalents of different metal ions to determine the selectivity of the probe at room temperature. $\text{Pb}(\text{ClO}_4)_2$ was used as a source of Pb^{+2} for all solution and cell studies. Standard lead nitrate solution (1 M) was prepared freshly for each measurement. For each measurement, the calculated amount of metal ions solution was added to a solution containing 0.5 μM NIR-PbP in an acetonitrile/water (99/1, v/v, organic medium) or 10 % citric acid/ethanol (80/20, v/v, aqueous medium) co-solvent system. After lead ions were added to the solution and mixed homogeneously, its UV-vis absorption and fluorescence spectra were obtained after 10 min. The slit widths of excitation and emission were both set to 5 nm, and the excitation wavelength was set at 620 nm for fluorescence spectroscopies.

2.5 Fluorescent detection of lead ions in living cells

HeLa cells were grown according to ATCC protocol in DMEM (high glucose) medium supplemented with 10% FBS, and 1% strep/pen as a complete growth medium in a 5% CO_2 humidified incubator at 37 $^\circ\text{C}$. Cells were passaged with sub-cultivation of 1:3 every three days. Cells were seeded into the 35 mm glass-bottom culture dishes (F 20 mm) for 1-2 days to reach 70-90% confluence. The cells were washed with DMEM ($\times 3$) and then incubated with 2 mL DMEM containing the NIR-PbP (10 μM) in 5% CO_2 and 95% air atmosphere for 30 min at 37 $^\circ\text{C}$. Cells were then washed with PBS (2×1 mL) at room temperature, and 1 mL was added for imaging. Images were taken with the Olympus IX 81 confocal laser scanning microscope using HC $\times$ PLAPO 60 $\times$ oil objective, with excitation by 635 nm laser, and 675–775 nm emission. For lead measurements, cells were first treated with $\text{Pb}(\text{II})$ (20 μM) in 5% CO_2 and 95% air atmosphere for 30 min at 37 $^\circ\text{C}$. Cells were washed with PBS (3×1 mL), NIR-PbP (10 μM in DMEM, 1 mL) was added, and cells were incubated for another 30 min. The cells were then washed with PBS (2×1 mL) at room temperature, and after addition of 1 mL PBS were imaged with a confocal microscope (635 nm excitation, 675–775 nm emission).

2.6 Cytotoxicity assay

The cytotoxicity of a probe against HeLa cells was measured by using the standard MTS assay. The cells were seeded into 96-well cell culture plate at 5×10^3 /well in complete growth medium. Cells were incubated for 24 h at 37 $^\circ\text{C}$ under 5% CO_2 atmosphere to attach. After removal of the medium and washing with PBS for three times, cells were incubated with fresh medium containing various concentrations of the NIR-PbP for 48 h, respectively. The probe-containing media was then replaced with fresh growth media and CellTiter 96 $\text{\textcircled{A}}$ Aqueous (2 μL /well) was added. After incubating for 2 h, the cell viability was determined by measuring the light absorbance at 490 nm with a microplate reader. The data was collected in triplicates, and each data point was averaged. Data analysis was carried out with respect to measurements for untreated cells (negative control). Cell viability (%) was calculated by comparing the absorbance of the control cells to that of treated cells.

3. Results and Discussion

3.1 Design, synthesis, and turn-on properties of NIR-PbP

For designing NIR-PbP - a near-infrared emissive probe for Pb(II) - we chose a compound **1** (Scheme 1) as a unique dye combining large extinction coefficient, high quantum yield, near-infrared emissions (720 nm), photostability, and the controlled fluorescence on-off switching mechanism [29, 30]. Compound **1** was synthesized following reported procedures [29, 30], and coupled with dipicolylamine derivative (N1,N1-bis(pyridin-2-ylmethyl)ethane-1,2-diamine) (**2**) [31] as a strong electron-donating group that selectively chelates to Pb⁺² ions via an amidation reaction [24]. This reaction triggers the cyclization that forms the closed spirolactam structure and yields the NIR-PbP (Scheme 1). NIR-PbP is colorless, non-fluorescent, and does not absorb in visible and near-infrared regions.

With designing NIR-PbP, we envisioned that Pb(II) ions could be specifically chelated by the dipicolylamine moiety of the probe. Upon Pb(II) binding, the cooperative binding to the carbonyl of the amide moiety would be expected to promote the opening of the spirolactam, and unmasking the fluorophore through the stabilization of the enhanced π -conjugation. We tested our hypothesis by investigating the absorbance responses of NIR-PbP to different concentration of Pb ions in the organic or aqueous medium. Upon addition of Pb(II) ions in to the probe in the organic medium, strong characteristic absorption bands with peaks at 479 nm, 663 nm (shoulder peak) and 718 nm was observed (Figure 1). Gradual increases of Pb(II) ion concentration from 0.4 μ M to 2.3 μ M resulted in significant increase in absorbance at 718 nm accompanied with a shoulder peak at 663 nm (Figure 1a). As a result, the solution of the fluorescent probe displays distinct color changes from colorless to green. The absorbance at 718 nm of NIR-PbP shows a linear response to Pb ion concentration with up to 500-fold enhancement reaching 2.3 μ M Pb(II) indicating a significant sensitivity of NIR-PbP (Figure 1b). The observed alterations in the UV-spectra support the opening of the spirolactam and formation of extended π -conjugation. NIR-PbP was also able to sense Pb(II) in aqueous medium with low percentage of an organic co-solvent (Figure S1, SI).

We investigated the fluorescence responses of NIR-PbP to Pb. Figure 2a shows the fluorescence spectra of NIR-PbP in the absence and presence of Pb(II) ions. NIR-PbP does not exhibit any emission band in the absence of lead due to its non-fluorescent property with the intact spirocycle. The fluorescence emission appears upon addition of Pb(II) at 735 nm. Gradual increase of Pb(II) ion concentration to 2.3 μ M induces more than 500 folds enhancement of the fluorescence intensity. The fluorescence response is in the linear relationship with concentrations (Figure 2b), indicating that NIR-PbP is potentially applicable for quantitative detection of Pb(II) ions using fluorometric assay. We also investigated pH effect on fluorescent NIR-PbP and demonstrated that the probe displays selective responses to Pb⁺² at neutral and basic pH values (Figure S2, SI).

3.2 NIR-PbP exhibits selectivity towards Pb(II) ions

Considering the possibility for fluorescent probes to interaction with other metal ions, we have investigated whether NIR-PbP can selectively sense Pb(II) ions. We studied the absorption and emission responses of NIR-PbP to other metal ions including Zn²⁺, Fe²⁺,

Hg^{2+} , Cd^{2+} , Ca^{2+} , Co^{2+} , Cu^{2+} , Mg^{2+} , Ni^{2+} , Na^+ , and K^+ (Figure 3a). As before, the addition of 2.5 μM $\text{Pb}(\text{II})$ ions to the solution containing 0.5 μM of NIR-PbP led to the appearance of a strong absorption peak at 718 nm. In contrast, under the same condition, other metal ions did not generate this phenomenon, except for Fe^{2+} . However, the absorbance at 718 nm of the solution containing Pb^{+2} was 9-times higher than that of Fe^{2+} , and more than 30-times higher than for other metal ions (Figure 3a). The fluorescence responses of NIR-PbP to different metal ions showed the similar trend. Only $\text{Pb}(\text{II})$ ions were able to induce the significant enhancement of the fluorescence for NIR-PbP in the near-infrared region. The fluorescent intensity of NIR-PbP–Pb complex is more than 150-fold higher than that of Zn^{2+} , Fe^{2+} and any of all other metal complexes (Figure 3b). These results indicate that NIR-PbP has a high binding affinity to Pb^{+2} and can generate selective responses in absorption and fluorescence spectra toward Pb^{+2} over other metal ions. Pb-selectivity of NIR-PbP was also confirmed through co-treatment of the probe with metal ion mixtures (Figure S3), showing that near-IR and fluorescence signals are turned-on only in the presence of lead.

We also examined the photostability of NIR-PbP–Pb complex using a time-dependent fluorescence measurement. For this part, the solution containing fluorescent NIR-PbP and Pb ions was excited continuously at 680 nm for 70 minutes, and fluorescence intensity was measured every 5 minutes. The resulting time-dependent fluorescence (Figure 4a) showed that there was no significant decrease of the fluorescence intensity under 70 minutes excitation for NIR-PbP. We also evaluated the reproducibility of NIR-PbP for sensing Pb ions by using EDTA as a strong Pb^{+2} chelator capable of dissociating the Pb from the probe. After adding equimolar quantities of EDTA to NIR-PbP–Pb complex (Figure S4a), the fluorescence was immediately quenched, indicating the dissociation of the complex and the cyclization of the spiro lactam. However, further addition of Pb^{+2} to this solution restored the strong fluorescence, clearly indicating the reusability of the probe. Also, after six cycles of alternative addition of the equal EDTA and Pb equivalents, only a slight decrease in the fluorescence intensity of the probe was observed (Figure S4b). This reveals that NIR-PbP exhibits high reproducibility, which is crucial for designing reusable Pb sensor devices.

3.6 NIR-PbP as $\text{Pb}(\text{II})$ sensor in live cells

NIR-PbP was further used to detect lead in living cells. First, cytotoxicity of NIR-PbP was established by the MTS assay with HeLa cell line. The cellular viability was estimated to be greater than 85% after 48 h, indicating the lack of cytotoxicity for NIR-PbP at concentrations below 10 μM (Figure S5).

For Pb^{+2} detection in live cells, HeLa cells were treated with NIR-PbP, and Pb^{+2} and fluorescence of the probe was recorded with the confocal fluorescence microscope (Figure 4). When HeLa cells were incubated with NIR-PbP (10 μM), no fluorescence was observed. After additional incubation with Pb^{+2} , red fluorescence appears. An overlay of bright-field and fluorescence images indicates that the fluorescence signals are localized in the intracellular area. These results show that NIR-PbP can be applied to visualize Pb ions within the living cell.

4. SUMMARY

In summary, a new near-infrared fluorescent probe for Pb^{+2} detection was synthesized, and its chemosensing properties were established by absorption and fluorescence spectroscopies. The probe exhibited high selectivity to Pb(II) ions over a wide variety of other metal. Upon addition of Pb(II) , NIR-PbP probe gains absorption in near-IR region of the spectra and changes from colorless to green. It also shows strong “off-on” fluorescence accompanied by a color change from colorless to fluorescent pink in acetonitrile. The spectral changes were observed due to reversible spirocyclic ring-opening mechanism upon binding of Pb(II) ions. The lead-induced absorbance at 718 nm and the fluorescence intensity at 735 nm are proportional to the concentration of Pb(II) ions, indicating that colorimetric or fluorometric method can be used for quantification of lead ions in the solution or living cells. Thus, NIR-PbP can serve as a promising chemosensor for lead in environmental monitoring and medical research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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- The probe shows not any absorption peaks in visible and near-infrared region in the absence of Pb(II) ions.
- Near-infrared fluorescent probe shows no fluorescence in the absence of Pb(II) ion.
- The probe shows significant absorption peak at 718 nm in the presence of Pb(II) ions.
- The probe shows highly fluorescence at 735 nm in the presence of Pb(II) ions.
- The probe responds to Pb(II) reversibly in absorption and fluorescence changes.
- The probe can detect Pb(II) in living cells.

Figure 1.

Responses of NIR-PbP to varied concentrations of Pb(II) ions. **a)** Absorption (UV) spectra for 0.5 μM NIR-PbP upon titration with 0 – 2.3 μM of Pb(II) in acetonitrile:water (99:1). **b)** Plot of absorbance at 718 nm against Pb(II) concentration.

Figure 2.

Fluorescence responses of fluorescent NIR-PbP to different concentrations of Pb(II) ions. **a)** Increase in fluorescence for $0.5 \mu\text{M}$ NIR-PbP upon titration with Pb(II) ions in acetonitrile:water (99:1); **b)** Plot of fluorescence intensity at 735 nm against Pb(II) ion concentrations.

Figure 3.

Selective responses of NIR-PbP to Pb^{2+} over other metal ions. NIR-PbP (0.5. μM) was treated with $Pb(II)$ (2.5 μM) and other metal ions (10 μM). The absorption (a) and fluorescence (b) spectra of the mixture solution were then measured.

Figure 4. Confocal fluorescence images of HeLa cells with NIR-PbP in the absence and presence of Pb(II). a) Bright field with NIR-PbP; b) fluorescence recording for NIR-PbP without Pb(II) ions; c) a/b overlay; d) Bright field for NIR-PbP and Pb(II) ions; e) fluorescence image of NIR-PbP and Pb(II) ions, f) d/e overlay.

Scheme 1.

Synthesis and Pb-induced fluorescence mechanism for NIR-PbP