

A Novel Fluorescent Probe for Lead Ions

Ming SUN, Di Hua SHANG GUAN, Hui Min MA*, Li Hua NIE, Xiao Hua LI
Shao Xiang XIONG

Laboratory of Chemical Biology, Center for Molecular Sciences, Institute of Chemistry,
Chinese Academy of Sciences, Beijing 100080

Abstract: A new fluorescent probe for lead ions, *p*-nitrophenyl 3*H*-phenoxazin-3-one-7-yl phosphoric acid (NPPA), has been synthesized by linking resorufin (serving as a fluorophore and electron acceptor) to *p*-nitrophenol (serving as a fluorescence quencher and electron donor) through phosphodiester bonds. When NPPA was irradiated with light, intramolecular fluorescence self-quenching took place due to the PET (photoinduced electron transfer) from the donor to the acceptor. However, upon addition of Pb^{II}, the phosphate ester bonds in the probe were cleaved and the fluorophore was released, accompanying the retrieval of fluorescence.

Key words: Fluorescent probe, lead, phosphodiester, hydrolysis.

Lead is a toxic heavy metallic element that can seriously damage people's health¹, and thus the monitoring of Pb^{II} concentrations *in vivo* is of great significance for further elucidating its toxicology². More recently, the combination of fluorophores with hydrolyzable groups has opened a novel alternative route to the specific determination of analytes with high hydrolytic reactivity³. Based on the fact that lead ions may efficiently hydrolyze some kinds of phosphodiester⁴, in this work an attempt was made on developing a new fluorescent Pb^{II} probe, and our strategies of designing such a probe were also greatly directed by an idea of intramolecular fluorescence self-quenching *via* the process of photoinduced electron transfer (PET)⁵.

Experimental

The preparation of *p*-nitrophenyl 3*H*-phenoxazin-3-one-7-yl phosphoric acid (NPPA, **Figure 1**) started with the reaction of *p*-nitrophenol with phosphorus oxychloride⁶, followed by introducing the resorufin moiety and hydrolyzing the resulting monochloride. Yield 32%; MS (field desorption), *m/z*, 414 [M]⁺ (relative intensity 100%); ¹H NMR (200 MHz, CD₃COCD₃), δ , ppm, 8.17-8.12 (m, 4H), 7.05-6.99 (m, 6H).

Fluorescence measurements were performed on a Hitachi F-2500 spectrofluorimeter. The hydrolytic reaction was performed in a test tube. Typically, to a test solution containing not more than 5.0×10⁻⁷ mol/L of Pb^{II}, 50 μ L of 1.6×10⁻⁵ mol/L acetone solution of the probe and 4.5 mL of 0.05 mol/L KH₂PO₄-NaOH buffer (pH 8.0)

* E-mail: mahm@iccas.ac.cn

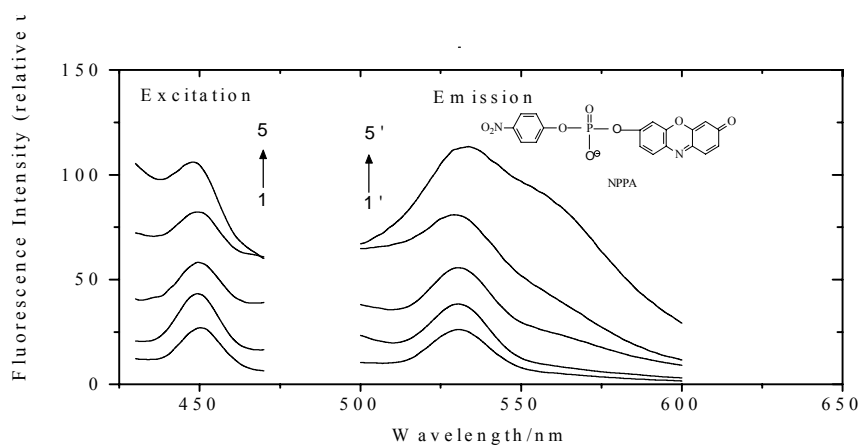
were added and the final volume was adjusted to 5 mL with water. Then, the tube was put into a thermostatic water bath and heated at 60 °C for 4 h with shaking. After cooling to room temperature, a 2 mL portion of the reaction solution was transferred into a quartz cell and the fluorescence was measured at $\lambda_{\text{ex/em}} = 450/531$ nm. In the meantime, a blank solution containing no Pb^{II} was prepared and measured under the same conditions for comparison.

Results and Discussion

Due to the intramolecular fluorescence self-quenching *via* the PET process, NPPA only has a weak fluorescence with a quantum yield of 0.015 (fluorescein as reference, $\Phi=0.95$), whose excitation and emission peaks are located at 450 nm and 531 nm, respectively. However, upon addition of Pb^{II} ions, the fluorescence intensity from NPPA was dramatically enhanced (**Figure 1**), resulting from the cleavage of phosphate ester bonds and thereby causing the fluorophore and the quencher to diffuse away from one another.

Several possible factors affecting the hydrolysis of NPPA by Pb^{II} ions were examined. The obtained optimum conditions are as follows: reacting at 60 °C for 4 h in a pH 8 KH_2PO_4 -NaOH buffer. Under these conditions, the enhancement value (ΔF) of fluorescence intensity in contrast to the blank solution (no Pb^{II} ion) was directly proportional to the Pb^{II} concentration (C) in the range of 50-125 nmol/L with a detection limit of 17 nmol/L ($S/N = 3$), thus showing a sensitive feature. The fluorescence response of NPPA to various ions was also studied to test the selectivity. The results showed that the probe exhibited an excellent selectivity for Pb^{II} ion over a wide range of alkaline earth and other transition metal ions (*e.g.*, Ca^{II} , Cu^{II} , La^{III} , Mg^{II} , Ni^{II} and Sn^{II}), except for Zn^{II} that had a positive but only small interference (+14%). This high selectivity of NPPA is greatly concerned with the Pb^{II} efficient cleavage of phospho-

Figure 1 Fluorescence spectra of NPPA and its cleavage products by Pb^{II} in phosphate buffer (pH 8).



The inset shows the structure of NPPA. Conditions: 160 nmol/L of NPPA was heated at 60 °C for

4 h in the presence of 0 (1, 1'), 50 (2, 2'), 100 (3, 3'), 150 (4, 4'), or 500 nmol/L Pb^{II} (5, 5').

diester bonds, and such a design strategy of combining fluorophores with Pb^{II}-cleavable groups would be beneficial to developing probes for other species. Further studies are being continued to understand the mechanism of the hydrolytic reaction.

Acknowledgment

This work is financially supported by the NNSF of China (No.20175031, No. 20035010).

References

1. M. Y. Liu, H. Y. Lai, B. C. Yang, M. L. Tsai, H. Y. Yang, B. M. Huang, *Life Sci.* **2001**, *68*, 849.
2. S. Deo, H. A. Godwin, *J. Am. Chem. Soc.* **2000**, *122*, 174.
3. A. Berkessel, R. Riedl, *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1481.
4. T. Pan, O. C. Uhlenbeck, *Nature* **1992**, *358*, 560.
5. B. Valeur, I. Leray, *Coord. Chem. Rev.* **2000**, *205*, 3.
6. V. V. Katyshkina, M. Y. Kraft, *Zhur. Obshchei Kim.* **1956**, *26*, 3060. *Chem. Abstract* **1957**, *51*, 8028c.

Received 21 October, 2002