A tryptophan-containing fluoroionophore sensor with high sensitivity to and selectivity for lead ion in water†

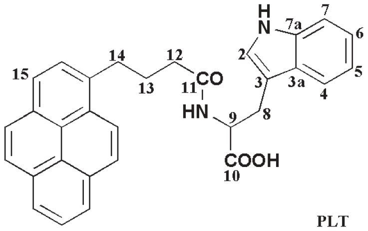
Li-Jun Ma, Yi-Fu Liu and Yuqing Wu*

Received (in Cambridge, UK) 30th March 2006, Accepted 8th May 2006
First published as an Advance Article on the web 23rd May 2006
DOI: 10.1039/b604623a

We report herein a fluoroionophore sensor derivated from tryptophan that shows high sensitivity (detection limit up to 0.15 μM) and specific selectivity for lead ion (Pb^{2+}) over Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, K^+, Mg^{2+}, Na^+, Fe^{2+}, Mn^{2+}, Ni^{2+} and Zn^{2+} in aqueous solution.

Fluoroionophore chemosensors are becoming increasingly popular due to their easy use in solution as well as their high sensitivity to and selectivity for trace analytes.[1] Many efficient fluoroionophores have been developed for the specific recognition of metal ions including alkali metals, alkaline earth metals and zinc ions.[2] However, the development of fluoroionophores for Pb^{2+} detection has been limited due to the high fluorescence quenching abilities of heavy metal ions. The detection of Pb^{2+} in the environment and in biological systems is important because of its toxicity.[3] Hayashita *et al.* reported a podand fluoroionophore[4a] and a PD-18C6/Triton X-100 complex sensor[4b] that both exhibited high selectivities for lead ion in water, but the sensitivities were insufficient for practical Pb^{2+} analyses. Two other fluorescent chemosensors developed by Métivier *et al.*[4c] and Kwon *et al.*[4d] exhibited much higher sensitivities and selectivities for Pb^{2+}, but required a high concentration or a pure organic medium for analysis. Therefore, development of a specific fluoroionophore for the real-time and *in situ* detection of Pb^{2+} in water needs to be explored for practical analytical applications.

Pyrene, with its defined emission spectrum and aggregation capability, is often employed as a fluorescent probe.[5] In addition, noncovalent binding forces within the metal ion-indole ring of tryptophan (Trp, W) have recently been the subject of much investigation due to the functional and structural importance of such interactions in chemistry and biology.[6] Various types of unique metal–indole bonds, dependent upon both the binding-site environment of the indole and the nature of the metal ion, have been identified.[7] Here we report a fluoroionophore sensor for lead ion, *N*-[4(1-pyrene)-butyroyl]-L-tryptophan (**PLT**; Fig. 1), which was synthesized by combining a pyrene-containing fluorophore, 4-(1-pyrenyl)-butyric acid, with a versatile functional tryptophan (see ESI†). Although **PLT** is structurally simple and easily synthesized, it not only displays a strong interaction between the indole moiety and lead ion, but also distinguishes Pb^{2+} from other metal ions and exhibits a very high sensitivity (0.15 μM) in aqueous solution. Another advantage afforded by **PLT** is that its interactions with metal ions closely mimic those of a natural tryptophan-containing peptide or protein. Thus, these studies using **PLT** as a model will provide a foundation for future biophysical examination of recognition mechanisms between proteins and metal ions.



PLT

Fig. 1 The chemical structure and atom numbering of *N*-[4(1-pyrene)-butyroyl]-L-tryptophan (**PLT**).

Fluorescence and NMR spectra were used to assess the cooperative interactions between metal ions and the tryptophan moiety in **PLT**. Fig. 2(a) shows changes in the fluorescence spectrum of **PLT** with the addition of lead ion in 98% water–2% DMSO (v/v; pH 6.4). Obvious differences in fluorescence spectra were observed in the presence of different concentrations of Pb^{2+}. The increase in the broad emission band at 470 nm corresponding to pyrene excimer,[4] and the concomitant decrease in monomer emission in the region of 370–410 nm, are clearly observed. In the presence of Pb^{2+}, the absorption spectrum of **PLT** shows characteristic features (see ESI†) attributed to ground-state interactions between two pyrene rings.[4,8,9] These results strongly demonstrate that a pyrene dimer is formed upon binding of Pb^{2+} to **PLT**.

Fig. 2(b) shows plots of the fluorescence intensity ratio (I_{470}/I_{377}) as a function of metal ion concentration. If it is assumed that the fluorescence change is induced solely by 2 : 1 complex formation between **PLT** and the metal ion (M^{2+}), the fluorescence intensity ratio (I_{470}/I_{377}) can be expressed by eqn (1),[4,9] where $[\mathrm{PLT}]_0$ is the initial concentration of the fluoroionophore, and ϕ_{f1} and ϕ_{f2} are the fluorescence quantum yields for **PLT** at 470 and 377 nm, respectively. Similarly, ϕ_{c1} and ϕ_{c2} are the quantum yields for the 2 : 1 complex at 470 and 377 nm, respectively. The value of ϕ_{f1}/ϕ_{f2} can be evaluated from the intensity ratio (I_{470}/I_{377}) when $[M^{2+}] = 0$ M.[9] K_{21} [eqn (2)] is the apparent 2 : 1 binding constant of **PLT** with M^{2+}. As shown in Fig. 2(b), the observed I_{470}/I_{377} values for Pb^{2+} are well-fitted by eqn (1) (curved line), with a binding constant of $1.09 \times 10^6\ M^{-2}$ ($R^2 = 0.9935$).

Key Laboratory for Supramolecular Structure and Material of Ministry of Education, Jilin University, 2699 Qianjin Street, Changchun, P. R. China. E-mail: yqwu@jlu.edu.cn; Fax: +86-431-5193421; Tel: +86-431-5168730

† Electronic supplementary information (ESI) available: Synthesis and characterization of **PLT** and **PLP**, spectroscopic and NMR data of **PLT** with Pb^{2+}, effect of pH and competing metal ions on the interaction of **PLT** and Pb^{2+}. See DOI: 10.1039/b604623a

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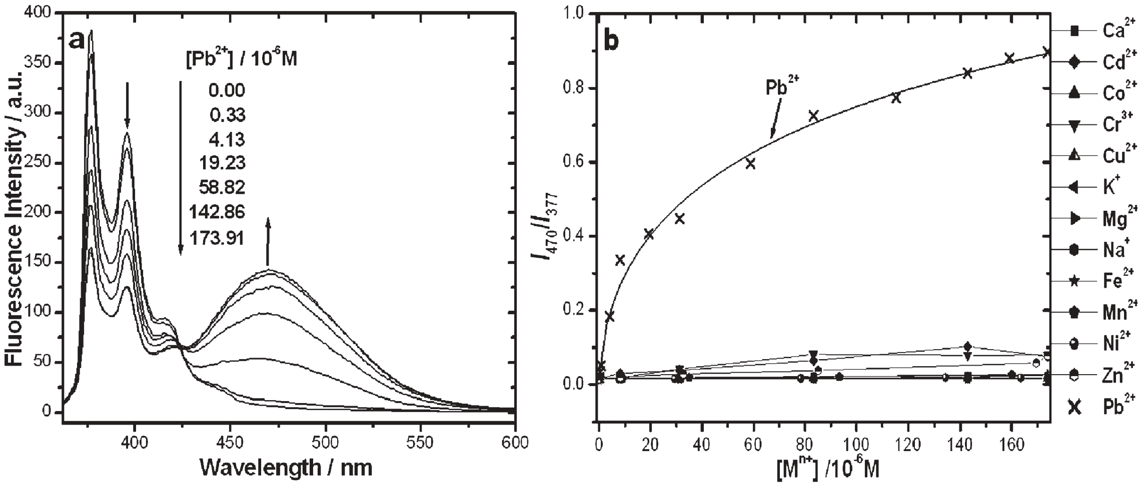

Fig. 2 (a) Fluorescence spectra of **PLT** (5.0×10^{-6} M in 98% water–2% DMSO (v/v)) with concentration changes of Pb^{2+}. Excitation wavelength is 340 nm. (b) Dependence of I_{470}/I_{377} on the concentration of various metal ions.

$$\frac{I_{470}}{I_{377}} = \frac{4\frac{\phi_{f1}}{\phi_{f2}} + \frac{\phi_{c1}}{\phi_{f2}}\left(-1+\sqrt{1+8K_{21}[\mathrm{M}^{2+}][\mathbf{PLT}]_0}\right)}{4+\frac{\phi_{c2}}{\phi_{f2}}\left(-1+\sqrt{1+8K_{21}[\mathrm{M}^{2+}][\mathbf{PLT}]_0}\right)} \quad (1)$$

$$K_{21} = \frac{[\mathrm{M}^{2+}(\mathbf{PLT})_2]}{[\mathrm{M}^{2+}][\mathbf{PLT}]^2} \quad (2)$$

As also shown in Fig. 2(b), the fluorescence spectra of **PLT** upon addition of Ca^{2+}, Co^{2+}, Cu^{2+}, K^+, Mg^{2+}, Na^+, Fe^{2+}, Mn^{2+} and Ni^{2+} were characterized by certain extent quenching and a lack of dimer peak at 470 nm. Only a very weak I_{470}/I_{377} response was observed for **PLT** upon addition of Cd^{2+}, Cr^{3+} and Zn^{2+} (see Fig. 2(b) and ESI†). Therefore, it can be concluded that **PLT** exhibits high selectivity for Pb^{2+} over the other examined metal ions in water. In addition, it should be noted that high sensitivity for Pb^{2+} (detection limit up to 0.15 μM) is achieved by **PLT** in the present study. This represents the lowest reported detection limit for Pb^{2+} by a fluoroionophore in aqueous solution and is well within the practical range (0.02–1.0 μM) for Pb^{2+} analysis.[3] The interference of the potentially competing metal ions on lead ion detection has been also investigated in the presence of other kinds of metal ions and the results demonstrate that the presence of Cr^{3+} or Cu^{2+} reduces its sensitivity, while no obvious interference of other metal ions as Mg^{2+}, Ca^{2+}, Fe^{2+}, Zn^{2+}, Mn^{2+}, Cd^{2+} *etc.* (in a concentration of 10 μM) can be observed (see ESI†). Meanwhile, pH-dependent study on the interaction between PLT and lead ion indicates that pH around 6.4 is the ideal condition because low pH prevent the formation of COO^-, while high pH will cause precipitation of metal ions (see ESI†).

When the indole moiety in **PLT** was changed to benzene, only detectable low-region fluorescence quenching other than the 470 nm emission response was detected (see ESI†). This result demonstrates that indole moiety of **PLT** plays a crucial role in the specific recognition of Pb^{2+}.

NMR experiments were performed to explore the coordination mechanism of **PLT** and Pb^{2+}. Fig. 3 shows ^1H and ^{13}C NMR spectra of indole before and after the addition of Pb^{2+} (10 equivalents). Indole protons shifted downfield upon Pb^{2+} binding, and obvious chemical shifts were also observed for carbons.[10] These findings not only are consistent with a direct coordination interaction between indole and Pb^{2+}, but also

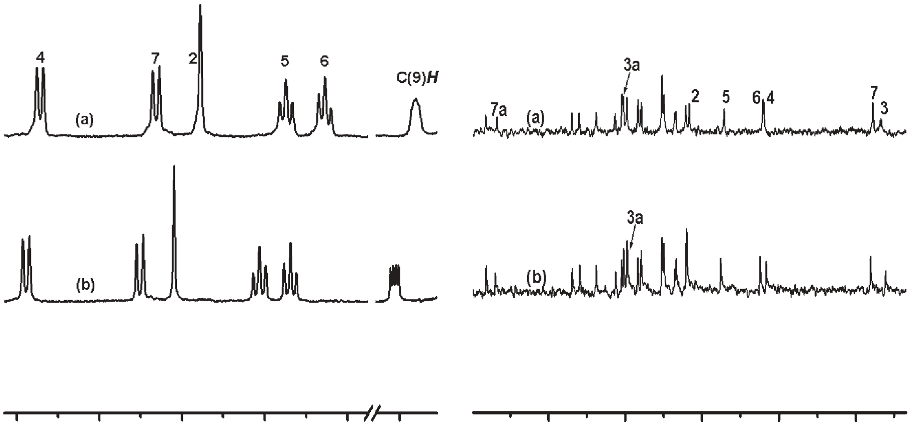

Fig. 3 Region of the ^1H NMR (left) and ^{13}C NMR (right) spectra of (a) **PLT** (5.5 mM), (b) **PLT**/Pb^{2+} in D_2O–DMSO-d_6 (1 : 2, v/v) solution.

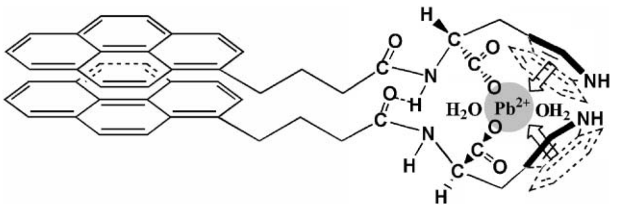

Fig. 4 Proposed Pb^{2+}–indole interaction model for Pb^{2+}–**PLT**.

indicate that the entire indole ring binds to the Pb^{2+} in a noncovalent interaction (possibly due to spatial positioning). Because the chemical shift of Trp indolyl C(3) is exquisitely sensitive to changes in the torsion angles of the side-chain, the observed chemical shift ($\Delta\delta_{C3}$ = 0.318 ppm) reflects substantial reorientation mobility of the indole unit.[11] Chelation of Pb^{2+} with carboxylate leads to reorganization of the indole unit to achieve Pb^{2+} coordination, leading to changes in the torsion angles of the Trp indole group (Fig. 4). This coordination mechanism was further supported by the downfield shift of α-carbon C(9) proton ($\Delta\delta_{H9}$ = 0.066 ppm) and its splitting into four peaks (Fig. 3) due to the influence of nonequivalent hydrogen $C(8)H_2$, with the rotatory movement of the indole ring being limited upon binding of Pb^{2+} with carboxylate. Furthermore, the chemical shifts of C(12) ($\Delta\delta_{C12} = -0.2$ ppm) and C(11) ($\Delta\delta_{C11}$ = 0.416 ppm) are consistent with the formation of a hydrogen bond between the carbonyl oxygen and amide hydrogen (Fig. 4) based on the similar proposed model.[12] Thus, the electrostatic interaction between Pb^{2+} and carboxylate induced coordination of Pb^{2+} within the Trp moiety and subsequent hydrogen bonding between amide groups, thereby generating a pyrene dimer. The formation of dimer induced a C(14) upfield shift of 2.142 ppm due to the high shielding of the pyrene (see ESI†).

In conclusion, we have characterized a fluoroionophore sensor, **PLT**, that shows high selectivity for Pb^{2+} over Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, K^+, Mg^{2+}, Na^+, Fe^{2+}, Mn^{2+}, Ni^{2+} and Zn^{2+} in aqueous solution. The sensor exhibited changes in its dimer/monomer fluorescence emission ratio (I_{470}/I_{377}) in response to Pb^{2+} in water, allowing highly sensitive Pb^{2+} detection (detection limit 0.15 μM). The high sensitivity and selectivity observed for **PLT** are very important for the real-time monitoring and *in situ* detection of toxic Pb^{2+} in the environment.[3] In addition, we found that the indole ring of **PLT** as well as the formation of pyrene-dimer is crucially important for the selective recognition of Pb^{2+}. Due to the specific interactions between metal ions and a variety of amino acids, modification of the recognition site in other sensors is being actively undertaken in our laboratory to develop more advanced fluoroionophore sensors for metal ion recognition in water. These studies will provide a foundation for future investigations of metal ion–peptide or ion–protein interactions.

The authors are grateful to the projects of NSFC (No. 20373017 and 20473028), the Program for Changjiang Scholars and Innovative Research Team in University (IRT0422) and the Program of Introducing Talents of Discipline to Universities (B06009).

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10 $\Delta\delta_{H2}$, $\Delta\delta_{H4}$, $\Delta\delta_{H5}$, $\Delta\delta_{H6}$ and $\Delta\delta_{H7}$ are 0.033, 0.039, 0.064, 0.084 and 0.063 ppm, respectively; $\Delta\delta_{C2}$, $\Delta\delta_{C5}$, $\Delta\delta_{C6}$, $\Delta\delta_{C7}$, $\Delta\delta_{C7a}$ and $\Delta\delta_{C3}$, $\Delta\delta_{C3a}$ and $\Delta\delta_{C4}$ are 0.171, 0.203, 0.194, 0.154, 0.098 and −0.318, −0.3 (peak is part of overlap) and −0.162 ppm, respectively.

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