Highly selective recognition of lead ion in water by a podand fluoroionophore/ γ -cyclodextrin complex sensor[†]

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We report herein a novel podand fluoroionophore/ γ -cyclodextrin (γ -CyD) complex sensor that shows markedly high selectivity for lead (Pb²⁺) ion in water.

Due to the toxicity of Pb²⁺, the real-time and *in situ* monitoring of Pb²⁺ in the environment as well as in biological systems remains an important issue.¹ Although ICP emission and atomic absorption spectroscopies are efficient tools for Pb2+ detection, they generally require a large amount of samples and are not suitable for real-time and in situ analysis.² Optical sensors involving fluoroionophores are becoming popular because of their ease of use in solution as well as their high sensitivity to and selectivity for trace analytes.3 Whereas many efficient fluoroionophores have been developed for alkali metals,4 alkaline earth metals⁵ and Zn²⁺,⁶ the number of Pb²⁺-selective fluoroionophores is still limited.7 The calix[4]arene fluoroionphore recently developed by Métivier et al. exhibits high sensitivity (detection limit of $0.02 \ \mu$ M) to and selectivity for Pb²⁺, but it requires a 60% CH₃CN/40% water (v/v) medium for analysis.8 The difficulty in designing Pb2+-selective fluoroionophores lies mainly in the lack of a design concept and problems in fluorescence quenching by heavy metal ions based on enhanced spin-orbital coupling9 and electron or energy transfer.10

Recently, we have developed supramolecular benzo-15-crown-5 fluoroionophore/y-CyD complex sensors for K+ ion recognition in water.¹¹ The dimerization of the fluoroionophore inside the γ -CyD was found to be selectively promoted by K⁺ ion binding, thereby resulting in a K+-selective pyrene dimer emission. This supramolecular function can be used in the design of Pb2+-selective fluoroionophores because the recognition site is efficiently separated from the photosignal transduction site by an alkyl chain spacer to avoid fluorescence quenching. For selective Pb²⁺ recognition, we have proposed a design concept based on 1) a hard binding site constructed from O-donor atoms to reduce the interaction with other relatively soft heavy metal ions, and 2) a podand structure possessing a flexible pseudo-crown ether cavity to stabilize chelate complexes with large metal ions such as Pb²⁺ while reducing the interaction with alkali metal cations.12

Based on the above design concept, we have designed a podand fluoroionophore (**PD-1**) that possesses a pseudo-18-crown-6 structure as the Pb²⁺ recognition site and a pyrene moiety as the photosignal transducer[†] and examined its response to heavy metal ions in the presence of γ -CyD in water (Fig. 1).

Fig. 2 shows the fluorescence spectra of **PD-1** (2.0×10^{-6} M) in 98% water/2% methanol (v/v) at pH 4.3. In the absence of γ -CyD, no obvious fluorescence was noted (spectrum a). By contrast, significant fluorescence emission appeared in the presence of 12.0 mM γ -CyD (spectrum b), indicating that **PD-1**

is solubilized in water by forming an inclusion complex with γ -CyD, which also enhances the fluorescence quantum yield.¹³ The fluorescence spectrum of **PD-1**/ γ -CyD complex in the presence of 1.0 mM Pb²⁺ is depicted in spectrum c. It is evident that the broad emission observed in the longer wavelength region (471 nm) is intensified whereas the pyrene monomer emission at 370–410 nm is decreased. In addition, the absorption spectrum of the **PD-1**/ γ -CyD complex in the presence of Pb²⁺ shows a reduction in resolution and intensity of the absorption compared with that in the absence of Pb²⁺. This characteristic feature is attributed to ground-state interactions between two pyrenyl moieties.¹⁴ The results strongly demonstrate that the dimer formation of **PD-1** inside γ -CyD is induced by Pb²⁺ binding, in accordance with our design concept (Fig. 1).



Fig. 1 Design concept of the PD-1/ $\gamma\text{-}CyD$ complex sensor for Pb^{2+} recognition in water.



Fig. 2 Fluorescence spectra of **PD-1** ([**PD-1**] = 2.0×10^{-6} M in 98% water/2% MeOH (v/v)) with added species. (a) [γ -CyD] = 0.0 mM, (b) [γ -CyD] = 12.0 mM, and (c) [γ -CyD] = 12.0 mM and [Pb²⁺] = 1.0 mM. Excitation wavelength, 328 nm. pH = 4.3 adjusted with 0.010 M acetate buffer.

 $[\]dagger$ Electronic supplementary information (ESI) available: synthesis of **PD-1**, effect of γ -CyD concentration, effect of pH, and metal ion selectivity. See http://www.rsc.org/suppdata/cc/b3/b305758e/

As we have previously reported, the fluorescence response of the **PD-1**/ γ -CyD complex must be dependent on γ -CyD concentration.¹⁵ Preliminary experiments revealed that selectivity for Pb²⁺/Cu²⁺ increased monotonically with an increase in γ -CyD concentration from 2.0 mM to 14.0 mM.† In this study, a γ -CyD concentration of 12.0 mM was selected for subsequent experiments. The pH of the sample solutions was adjusted to 4.3 by 0.010 M acetate buffer with an ionic strength of 0.10 by NaNO₃. No significant difference in the selectivity for Pb²⁺/ Cu²⁺ was noted in the pH range from 3.7 to 5.5.†

Fig. 3a shows changes in the fluorescence spectra of the **PD**- $1/\gamma$ -CyD complex with the addition of Pb²⁺. As the Pb²⁺ concentration was increased, the intensity of the monomer emission decreased whereas that of the dimer emission increased. Fig. 3b shows plots of the intensity ratio (I_{471}/I_{378}) as a function of Pb²⁺ concentration. When the change in fluorescence is induced only by the 2 : 1 complex formation between **PD-1** and the metal ion (M²⁺), the fluorescence ratio (I_{471}/I_{378}) can be expressed by the following equations:¹¹

$$\frac{I_{471}}{I_{378}} = \frac{4\frac{\phi_{11}}{\phi_{12}} + \frac{\phi_{c1}}{\phi_{12}} \left(-1 + \sqrt{1 + 8K_{21}[M^{2+}][PD-1]_0}\right)}{4 + \frac{\phi_{c2}}{\phi_{12}} \left(-1 + \sqrt{1 + 8K_{21}[M^{2+}][PD-1]_0}\right)}$$
(1)

$$K_{21} = \frac{[\mathbf{M}^{2+}(\mathbf{PD} - \mathbf{1})_2]}{[\mathbf{M}^{2+}][\mathbf{PD} - \mathbf{1}]^2}$$
(2)

where **[PD-1]**₀ is the initial concentration of the fluoroionophore, and ϕ_{f1} and ϕ_{f2} are the fluorescence quantum yields for **PD-1** at 471 and 378 nm, respectively. Similarly, ϕ_{c1} and ϕ_{c2} are those for the 2 : 1 complex at 471 and 378 nm, respectively. K_{21} is the apparent 2 : 1 binding constant of **PD-1** with M²⁺. As shown in Fig. 3b, the observed values are well fitted by eqn. (1) (solid line), and the binding constant is calculated as (1.17 ± 0.75) × 10⁹ M⁻². Also shown in Fig. 3b are the fluorescence responses of the **PD-1**/ γ -CyD complex in the presence of Zn²⁺ and Cu²⁺. The **PD-1**/ γ -CyD complex did not show any obvious spectral changes upon the addition of Zn²⁺ and Cu²⁺, indicating that fluorescence response upon the addition of 1.0 mM K⁺, Mg²⁺, Ca²⁺, Ni²⁺, Co²⁺, and Cd²⁺ was also confirmed.[†]



Fig. 3 (a) Fluorescence spectra of **PD-1**. [**PD-1**] = 2.0×10^{-6} M in 98% water/2% MeOH (v/v); [γ -CyD] = 12.0 mM. Excitation wavelength, 328 nm. pH = 4.3 adjusted with 0.010 M acetate buffer (I = 0.10 by NaNO₃). (b) Dependence of I_{471}/I_{378} on the concentration of Pb²⁺, Cu²⁺, and Zn²⁺.

Thus, the PD-1/ γ -CyD complex was found to exhibit high selectivity for Pb²⁺ ion in water.

In summary, a supramolecular **PD-1**/ γ -CyD complex sensor that exhibited the monomer/dimer emission ratio response with markedly high selectivity for Pb²⁺ in water was successfully designed. It should be noted that the observed detection limit of 10 μ M is evidently insufficient for practical Pb²⁺ ion analysis (0.02–1.0 μ M) in water.¹ Although we selected the pseudo-18-crown-6 structure as the Pb²⁺ binding site, the podand structure as well as the alkyl spacer design in the fluoro-ionophore is an important factor for enhancing the Pb²⁺ recognition efficiency of the γ -CyD complex sensors by several order of magnitude.¹⁵ Such modifications are being actively undertaken in our laboratory in order to develop more advanced fluoroionophore/ γ -CyD complex sensors for heavy metal ion recognition in water.

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