

## Benzo-15-crown-5 Fluoroionophore/ $\gamma$ -Cyclodextrin Complex with Remarkably High Potassium Ion Sensitivity and Selectivity in Water

Akiyo Yamauchi, Takashi Hayashita, Seiichi Nishizawa, Masatoshi Watanabe, and Norio Teramae\*

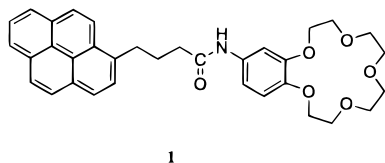
Department of Chemistry, Graduate School of Science  
Tohoku University, Aoba-ku, Sendai 980-8578, Japan

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We report herein a fluoroionophore/ $\gamma$ -cyclodextrin ( $\gamma$ -CyD) complex which shows remarkably high potassium ion ( $K^+$ ) sensitivity and selectivity in water.

Potassium ion maintains intracellular osmolarity in conjugation with sodium ion ( $Na^+$ ), and the concentration of  $K^+$  in a living cell is related to regulation of concentrations of other ions such as  $Ca^{2+}$  and  $Cl^-$  which transfer through the plasma membrane.<sup>1</sup> Although a number of ionophores such as biscrown ethers have been developed to determine  $K^+$ ,<sup>2</sup> most of them are limited to use in nonaqueous media.<sup>3</sup> As a means to visualize the concentration profile of  $K^+$  in living cells, new fluoroionophores such as "PBF1"<sup>4</sup> and "CD222"<sup>5</sup> for use in water have been developed. However, their complexation ability for  $K^+$  is strongly dependent on whether  $Na^+$  is present, and the sensitivity for  $K^+$  is not high enough in water.<sup>6</sup> Thus, more efficient fluoroionophores for  $K^+$  are still desired.

Although the benzo-15-crown-5 (B15C5) fluoroionophore **1**



**1**

can be classified as a photoinduced electron transfer (PET) sensor,<sup>7</sup> it shows dual fluorescence originating from monomer and intramolecular exciplex formation.<sup>8,9</sup> A moderate  $Na^+$  selectivity was obtained for **1** based on a 1:1 complex formation ability of the B15C5 moiety<sup>9</sup> in a nonaqueous solution. In contrast to the sensing ability of **1** in nonaqueous media, **1** was found to work as a novel ion probe in the presence of  $\gamma$ -CyD, and it achieved high sensitivity and selectivity for  $K^+$  even in the presence of  $Na^+$ .

\* To whom correspondence should be addressed. E-mail: tera@anal.chem.tohoku.ac.jp.

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Figure 1 shows fluorescence spectra of **1** ( $5.0 \times 10^{-7}$  M) in water. The bottom spectrum, a, was measured in the absence of  $\gamma$ -CyD, and no obvious fluorescence emission was observed. By contrast, significant fluorescence emission appears in the presence of 5.0 mM  $\gamma$ -CyD, as shown in spectrum b, where the solution contains 0.10 M tetramethylammonium chloride (TMACl) which does not form a complex with B15C5. This appearance of fluorescence indicates that **1** is solubilized in water by forming an inclusion complex with  $\gamma$ -CyD which also enhances the fluorescence quantum yield.<sup>10</sup> The fluorescence band with a vibronic structure appearing at 370–410 nm is assigned to the pyrene monomer emission. The **1**/ $\gamma$ -CyD complex does not show any obvious spectral change by adding 0.10 M NaCl instead of TMACl as shown in spectrum c. However, the broad featureless band with an emission maximum at 470 nm is strongly intensified by addition of 0.10 M  $K^+$ , and there is quenching of the monomer fluorescence in spectrum d, indicating high  $K^+$  sensitivity and selectivity over  $Na^+$ .

Figure 2 shows the dependence of the intensity ratio at 470 nm to that at 377 nm ( $I_{470}/I_{377}$ ) on the ionic radius. It can be easily recognized that high selectivity for  $K^+$  is obtained by the **1**/ $\gamma$ -CyD complex. On the basis of the selectivity for 1:1 complexations of B15C5 ( $Na^+ > K^+ > Rb^+ > Cs^+ \gg Li^+$ , in MeOH), preferential binding of  $K^+$  to **1** is unexpected.<sup>11</sup> It is well-known that B15C5 forms a 2:1 (ligand:metal) complex with  $K^+$ .<sup>12</sup> The selectivity shown in Figure 2 therefore suggests that **1** forms a 2:1 complex with  $K^+$ . In addition, in the presence of  $K^+$ , the absorption spectrum of the **1**/ $\gamma$ -CyD complex shows characteristic features<sup>13</sup> which are attributed to ground-state interactions between two pyrenyl moieties.<sup>14,15</sup> Thus, the structureless broad band observed at 470 nm in Figure 1d can be assigned to the emission of the pyrene dimer which was produced by incorporating two fluoroionophores in a  $\gamma$ -CyD. It is possible that two pyrenyl moieties of the 2:1 complex are included in one  $\gamma$ -CyD, since  $\gamma$ -CyD can form a 2:2 complex with pyrene.<sup>15,16</sup>

With an increase in  $K^+$  concentration, the intensity of the monomer fluorescence decreased, while that of the dimer fluorescence increased with a clear crossover point.<sup>13</sup> Figure 3 shows the dependence of the intensity ratio ( $I_{470}/I_{377}$ ) on the concentrations of  $K^+$  and  $Na^+$ . If it is assumed that the fluorescence change is only induced by the formation of a 2:1 complex between **1** (L) and metal ion ( $M^+$ ), the intensity ratio ( $I_{470}/I_{377}$ ) can be expressed by the following equations:<sup>17</sup>

$$\frac{I_{470}}{I_{377}} = \frac{4 \frac{\phi_{f1}}{\phi_{f2}} + \frac{\phi_{c1}}{\phi_{f2}} (-1 + \sqrt{1 + 8K[M^+][L]_0})}{4 + \frac{\phi_{c2}}{\phi_{f2}} (-1 + \sqrt{1 + 8K[M^+][L]_0})} \quad (1)$$

$$K = \frac{[ML_2^+]}{[M^+][L]^2} \quad (2)$$

where  $[L]_0$  is the initial concentration of **1**,  $\phi_{f1}$  and  $\phi_{f2}$  are the fluorescence quantum yields for **1** at 470 and 377 nm, respectively. Similarly,  $\phi_{c1}$  and  $\phi_{c2}$  are those for the 2:1 complex at

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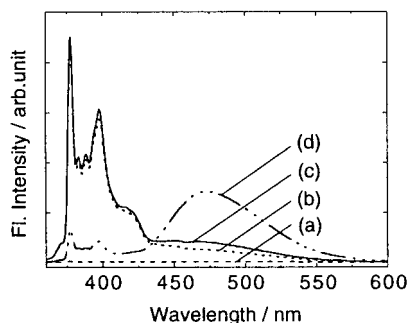
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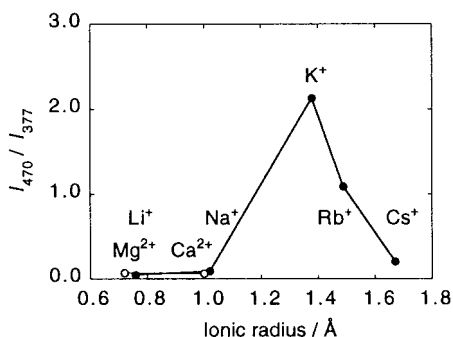
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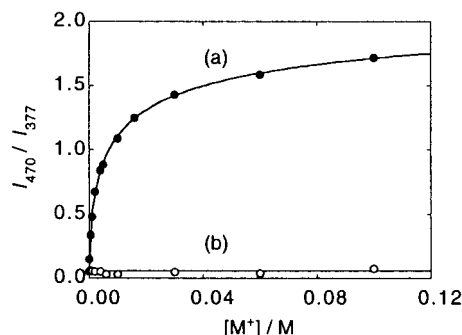
**Figure 1.** Fluorescence spectra of **1** ( $[1] = 5.0 \times 10^{-7}$  M (99% water–1% MeCN (v/v))) with added species. (a)  $[\gamma\text{-CyD}] = 0$  mM. (b)  $[\gamma\text{-CyD}] = 5.0$  mM and 0.10 M TMACl. (c)  $[\gamma\text{-CyD}] = 5.0$  mM and 0.10 M NaCl. (d)  $[\gamma\text{-CyD}] = 5.0$  mM and 0.10 M KCl. Excitation wavelength, 330.5 nm. Excitation bandwidth, 5 nm. Emission bandwidth, 1.5 nm.



**Figure 2.** Dependence of  $I_{470}/I_{377}$  on the ionic radius of (●) alkali and (○) alkaline-earth metal ions.  $[1] = 5.0 \times 10^{-7}$  M (99% water–1% MeCN (v/v)).  $[\gamma\text{-CyD}] = 5.0$  mM.  $[M^+] = [M^{2+}] = 0.10$  M ( $M^+ = \text{Li}^+, \text{Na}^+, \text{K}^+, \text{Rb}^+, \text{Cs}^+$ ;  $M^{2+} = \text{Mg}^{2+}, \text{Ca}^{2+}$ ).

470 and 377 nm, respectively. The value of  $\phi_{11}/\phi_{12}$  can be evaluated from the intensity ratio ( $I_{470}/I_{377}$ ) when  $[M^+] = 0$  M.  $K$  is the association constant of 2:1 complex. The points in Figure 3 are fitted well with eq 1 (solid line), and the association constant is calculated as  $(3.8 \pm 1.3) \times 10^9 \text{ M}^{-2}$ .

The 1:1 association constant of B15C5 for  $\text{K}^+$  in water is generally quite small (less than  $1.0 \text{ M}^{-1}$ ).<sup>11</sup> However, it should be noted that the  $K$  value of the **1**/ $\gamma$ -CyD complex obtained for  $\text{K}^+$  in water is much higher than that of B15C5 in MeOH ( $K = 1.4 \times 10^6 \text{ M}^{-2}$ ).<sup>11</sup> The remarkably high association constant for  $\text{K}^+$  obtained for the **1**/ $\gamma$ -CyD complex apparently suggests that the formation of 2:1 complex between **1** and  $\text{K}^+$  is promoted in the presence of  $\gamma$ -CyD. As to the interference by  $\text{Na}^+$ , the **1**/ $\gamma$ -



**Figure 3.** Dependence of  $I_{470}/I_{377}$  on the concentrations of (a)  $\text{K}^+$  and (b)  $\text{Na}^+$ . Concentrations of **1** and  $\gamma$ -CyD are the same as those in Figure 2.

CyD complex shows negligibly small changes in  $I_{470}/I_{377}$  for the concentration of  $\text{Na}^+$  from 0 to 0.10 M. In addition, the association constant of the **1**/ $\gamma$ -CyD complex for  $\text{K}^+$  is found to be  $(1.4 \pm 1.2) \times 10^9 \text{ M}^{-2}$  in solutions in which the concentration of  $\text{Na}^+$  varies from 0 to 0.10 M, where the total concentration of  $\text{Na}^+$  and  $\text{K}^+$  is kept as 0.10 M. This high association constant in the presence of  $\text{Na}^+$  is almost the same as that in the absence of  $\text{Na}^+$  ( $3.8 \times 10^9 \text{ M}^{-2}$ ). Although a stability of the hydrophobically assembled **1**/ $\gamma$ -CyD complex in a living cell should be further assessed, the high selectivity for  $\text{K}^+$  over  $\text{Na}^+$  and the high  $K$  value, achieved by the **1**/ $\gamma$ -CyD complex in water, are clearly superior to such fluoroionophores as “PBFI” and “CD222” which are used for  $\text{K}^+$  ion probes.<sup>4,5</sup>

In conclusion, the B15C5 fluoroionophore **1** bearing a pyrenyl moiety was found to form a 2:1 complex with high selectivity for  $\text{K}^+$  in the presence of  $\gamma$ -CyD, and it exhibited both monomer and dimer emission in water. The association constant for  $\text{K}^+$  was hardly affected by the presence of  $\text{Na}^+$ . These results demonstrated that the fluoroionophore **1**/ $\gamma$ -CyD complex is a novel ion probe exhibiting remarkably high sensitivity and selectivity for  $\text{K}^+$  in water.

The use of CyD with ionophores, as demonstrated in the present **1**/ $\gamma$ -CyD system, can be applied to various lipophilic chromo- and fluoroionophores to develop further superior sensing functions which differ from their originally intended functions.

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**Supporting Information Available:** The absorption spectra of **1**, and the fluorescence spectra of **1** as a function of 0–0.10 M  $\text{K}^+$  (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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