High Sensitive Molecular Recognition by Monomer and 
Exciplex Emissions for Endocrine-Disrupting Chemicals and 
Their Analogs Based on Fluorescent Hetero-Modified Cyclodextrins

Miyuki NARITA, Akira MAKABE, Kentaro KINOSHITA, Ken ENDO and Fumio HAMADA

Department of Materials-process Engineering and Applied Chemistry for Environments, 
Faculty of Engineering and Resource Science, Akita University, Tegata, Akita 010-8502, Japan 
E-mail : hamada@ipc.akita-u.ac.jp

A fluorescent molecular sensing for endocrine-disrupting chemicals and their analogs has been investigated by fluorescence spectral change of regio-selective 1-pyrene-p-cyanobenzene-modified γ-cyclodextrins on the addition of a guest. These host compounds showed both monomer and exciplex fluorescence at around 378 and 397 nm, and 468 nm, respectively. On accommodation of the guests such as dioxin analogs, p-nonylphenol, 2,4-dichlorophenoxyacetic acid, bisphenol A, and diethylphthalate, the intensities of the guest-induced monomer fluorescence increased or decreased, and those of the guest-induced exciplex emission decreased. The extent of the fluorescence variations with the guest was employed to display the sensing abilities of these hosts. The sensing parameters, \( \frac{I_{m} - I_{0m}}{I_{m} + I_{0m}} \) and \( \frac{I_{ex} - I_{0ex}}{I_{ex} + I_{0ex}} \), were used to describe the sensing ability of the hosts, where \( I_{m} \) and \( I_{0m} \), and \( I_{ex} \) and \( I_{0ex} \) were fluorescence intensities of monomer and exciplex emission, respectively, in the presence and absence of the guest, and \( I_{m} = I_{0m} \) and \( I_{ex} = I_{0ex} \). These hosts particularly exhibited high sensitive and selective molecular recognition ability for 2,4,6-trichlorophenol, p-nonylphenol and bisphenol A.

Key Word: Endocrine-disrupting chemicals, Pyrene, Cyanobenzene, Exciplex, Cyclodextrin, Fluorescent recognition

1. Introduction

Cyclodextrins, which are torus-shaped cyclic oligomers of D-glucopyranose and are named α, β and γ-for the hexamer, heptamer and octamer, respectively, can include a variety of organic compounds in their cavity in an aqueous solution. For at least a decade, we have studied fluorescent molecular sensing for bile acids and terpenes by cyclodextrins modified with chromophores. In these papers, fluorescent cyclodextrin derivatives showed high sensing abilities for bile acids such as lithocholic acid, chenodeoxycholic acid and ursodeoxycholic acid. Since, we have tried to examine fluorescent molecular sensing based on such modified cyclodextrins for endocrine-disrupting chemicals. The damaging of endocrine-disrupting chemicals for humans, animals and environments have attracted a great deal of public attention. However, the detection of these chemicals is needed high technology such as analysis by GC/MS which is very costly and analytical results are needed many days. Therefore, it is urgent to quickly and conveniently confirm existence of these chemicals in rivers and lakes. Accordingly, it is desirable to detect endocrine-disrupting chemicals directly by a fluorescent chemo-sensor using cyclodextrin derivatives. Previously, we have discussed the first example of fluorescent molecular sensing for endocrine-disrupting chemicals and dioxin analogs by homo fluorescent β- and γ-cyclodextrins which were modified with bis fluorescent active units such as dansyl or anthranilate and fluorescent hetero-modified β- and γ-cyclodextrins, of which appended moieties are dansyl and tosyl. In these reports, these chemicals including p-nonylphenol and bisphenol A and dioxin analog such as 2,4,6-trichlorophenol were detected conveniently and directly by these fluorescent cyclodextrins with high sensitivity and selectivity; pattern recognition of endocrine-disrupting chemicals would be expected to be possible. Furthermore, it was obvious that bis anthranilate-modified β-cyclodextrins could detect 10^-6 M of p-nonylphenol, and some dansyl-tosyl-modified γ-cyclodextrins recognized particularly 2,4,6-trichlorophenol. Recently, we have reported synthesis of regioselectively fluorescent hetero-substituted cyclodextrins, which are 6'-pyrene-6'-cyanobenzene-modified γ-analogs (X = B or H, C or G, D or F, and E for γ-1, γ-2, γ-3, and γ-4, respectively), and examination of their fluorescent molecular-sensing for organic compounds such as bile acids and cyclic alcohols. In this system, these hosts showed exciplex fluorescence spectra and exhibited high sensitivity for bile acids such as ursodeoxycholic acid, chenodeoxycholic acid and deoxycholic acid and cyclic alcohol such as (-)-borneol by indicating the exciplex fluorescence spectral changes. As a further extension of our work, we investigated the guest-induced variations of monomer and exciplex emissions of titled hosts when
endocrine-disrupting chemicals was used as the guests. In the present study, we would like to describe the fluorescent sensing abilities of these host compounds (γ-1, γ-2 and γ-3) for the endocrine-disrupting chemicals. These hetero-modified hosts detect 2,4,6-trichlorophenol, p-nonylphenol and bisphenol A with high sensitivity and selectivity, indicating exciplex fluorescence spectral changes.

2. Experimental

2.1 Preparations of γ-1, γ-2 and γ-3

The host compounds (γ-1, γ-2 and γ-3) were prepared according to previously reported procedures. The fluorescence spectra were measured at 25°C with a Perkin Elmer LS 40B fluorescence spectrometer. In the fluorescence measurements, the excitation wavelength of the fluorescence spectra was 360 nm and the excitation and emission slits were 4 nm. An ethylene glycol aqueous solution (10 vol.%) was used as the solvent for hosts for the spectroscopic measurements, because their solubility in pure water is poor. Five microliters of guest species (500, 50 and 5 mM) in dimethyl sulfoxide (DMSO) or MeOH were injected into a 10 vol.% ethylene glycol aqueous solution of the host (2.5 mL) to make a sample solution with a host concentration of 2.0x10^-6 M and guest concentrations of 0.01, 0.1 and 1.0 mM, respectively.

3. Results and Discussion

3.1 Fluorescence spectra

Figure 1 shows the fluorescence spectra of γ-2 in a 10 vol.% ethylene glycol aqueous solution in the absence and presence of 2,4,6-trichlorophenol. The fluorescence spectra of γ-2 are composed of both monomer and exciplex emissions with peaks at around 378 and 397 nm, and 468 nm, respectively; the intensities of monomer and exciplex emissions decrease with increasing the guest concentrations. On the other hand, the intensities of monomer and exciplex emissions of γ-2 with peaks at around 378 and 397 nm, and 468 nm, respectively, in a 10 vol.% ethylene glycol aqueous solution in the presence of p-nonylphenol increase and decrease, respectively, with increasing the guest concentrations, as shown in Figure 2. 6A-Pyrene-6E-cyanobenzene-modified γ-analog (γ-4) showed only monomer fluorescence, of which intensities were negligible, and exhibited no guest-induced fluorescent variations. It is reported that the exciplex intensity of β-(1-pyrenyl) ethyl-p-cyanobenzoate system was observed in less medium polarity such as the Cyclodextrin cavity. The exciplex emission observed in these hosts, γ-1, γ-2 and γ-3, indicates that hetero...
moieties, pyrene and cyanobenzene, place the face to face orientation, which is needed for exciplex formation which are folded conformer. Since, it is seemed that the two moieties of 6'-pyrene-6'-cyanobenzene-modified γ-analog are too long distant position enough to form folded conformer. To clarify whether the exciplex emission is derived from intramolecular exciplex formation or not, dependence on the concentration of the 7-2 for the exciplex emission is studied, as shown in Figure 3. The exciplex emission appears even at low concentration such as 2x10⁻⁷ M which is limit concentration of fluorescence spectra measurement at 4 nm of excitation and emission slits. It means that there is no intermolecular complex, and the exciplex emission is derived from intramolecular exciplex formation. The results obtained from fluorescence spectral changes suggest that pyrene moieties of γ-1, γ-2 and γ-3 are moving far from the chiral environment of the cyclodextrin cavity to work as a hydrophobic cap, simultaneously a guest is included in the cyclodextrin cavity. Furthermore, it is estimated that cyanobenzene moieties of γ-1, γ-2 and γ-3 are excluded out from the cyclodextrin cavity upon the addition of the guest, quenching exciplex emission produced by interaction between pyrene and cyanobenzene moieties.

The extent of the variation on the fluorescence intensity of γ-1, γ-2 and γ-3 is affected by the presence of guest molecules, even at low concentration, these hosts can be used as fluorescent sensors of molecule. To evaluate the sensing ability of these hosts, the \( \Delta I_m/I_m, \Delta I_e/I_e \) and \( \Delta I_m/I_m \) values were used as sensitivity parameters. Here, \( \Delta I_m, \Delta I_e \) and \( \Delta I_m \) are \( I_m-I_m, I_e-I_e \) and \( I_m-I_m \), respectively, where \( I_m \) and \( I_e \) are monomer fluorescence intensities at 378 and 397 nm, respectively, and \( I_m \) is exciplex fluorescence intensity at 468 nm for the host alone and \( I_m, I_e \) and \( I_m \) are that for a complex. Figure 4 shows the parameter values of

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**Figure 2** Fluorescence spectra γ-2 in a 10 vol.% ethylene glycol aqueous solution (2.0x10⁻⁶M, 25°C) at various concentration of p-nonylphenol (1:0, 2:4.0x10⁻⁶, 3:1.2x10⁻⁵, 4:2.4x10⁻⁵, 5:4.0x10⁻⁵, 6:6.0x10⁻⁵, 7:8.3x10⁻⁵, 8:1.1x10⁻⁴M)

**Figure 3** Monomer and exciplex emissions depending on concentration of γ-2 in a 10 vol.% ethylene glycol aqueous solution (1:0,2:2.0x10⁻⁷, 3:4.0x10⁻⁷, 4:6.0x10⁻⁷, 5:8.0x10⁻⁷, 6:1.0x10⁻⁶, 7:2.0x10⁻⁶, 8:3.0x10⁻⁶, 9:4.0x10⁻⁵M)

**Figure 4** Sensitivity factors of γ-1, γ-2 and γ-3 in a 10 vol.% ethylene glycol aqueous solution (2.0x10⁻⁶M, 25°C) at 378, 397 and 468 nm for all guests examined

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7-1, 7-2 and 7-3 with 1,2-dichlorobenzene (1), 3,4-dichlorophenol (2), 2,4,6-trichlorophenol (3), p-nyloniphenol (7), bisphenol A (9), and diethyl phthalate (10) at 1.0 mM, 3,4-dichlorobenzoeic acid (4) and 2,4-dichlorophenoxyacetic acid (8) at 0.1 mM, and 4-chlorodiphenyl (5) and dibenzofuran (6) at 0.01 mM. Guest 9, which bears three chlorines, was detected with the highest sensitivity by the hosts, exhibiting the values $\Delta \omega /I_\omega$ of 0.332, 0.322 and 0.284 for $\gamma$-1, $\gamma$-3 and $\gamma$-2, the values $\Delta \omega /I_{\omega}$ of 0.307, 0.301 and 0.260 for $\gamma$-3, $\gamma$-1 and $\gamma$-2, and the values $\Delta \omega /I',_{\omega}$ of 0.687, 0.680 and 0.665 for $\gamma$-1, $\gamma$-3 and $\gamma$-2, respectively, in the order $\gamma$-1 > $\gamma$-3 > $\gamma$-2, roughly. The sensing parameters obtained through exciplex emission of the hosts for the guest 3 were twice those obtained through monomer emission of the hosts for the guest 3. Guest 9, which bears two benzene rings and hydroxyl groups, was detected with the next highest sensitivity, exhibiting the values $\Delta \omega /I_{\omega}$ of 0.238, 0.216 and 0.208, the values $\Delta \omega /I_{\omega}$ of 0.224, 0.214 and 0.203 for $\gamma$-2, $\gamma$-3 and $\gamma$-1, respectively, and the values $\Delta \omega /I',_{\omega}$ of 0.600, 0.593 and 0.569 for $\gamma$-3, $\gamma$-2 and $\gamma$-1, respectively, in the order $\gamma$-2 > $\gamma$-3 > $\gamma$-1, roughly. The sensing parameters obtained through exciplex emission of the hosts for the guest 9 were three times as high as those obtained through monomer emission of the hosts for the guest 9.

On the other hand, guest 7, which bears one hydroxyl group and long alkyl chain, was detected with higher sensitivity, exhibiting the values $\Delta \omega /I_{\omega}$ of 0.579, 0.493 and 0.419 for $\gamma$-1, $\gamma$-3 and $\gamma$-2, respectively, in the order $\gamma$-1 > $\gamma$-3 > $\gamma$-2, whereas the values $\Delta \omega /I_{\omega}$ and $\Delta \omega /I_{\omega}$ were negligible. The relationship of sensing parameters obtained through guest-induced monomer and exciplex emission of the hosts for guest 7 is not similar to those for guests 3 and 9. Guest 1, which bears two chlorines, was detected with high sensitivity, exhibiting the values $\Delta \omega /I_{\omega}$ of -0.129, -0.052 and -0.008, the values $\Delta \omega /I_{\omega}$ of 0.106, -0.057 and -0.008, and the values $\Delta \omega /I_{\omega}$ of 0.338, 0.222 and 0.181 for $\gamma$-1, $\gamma$-2 and $\gamma$-3, respectively, in the order $\gamma$-1 > $\gamma$-2 > $\gamma$-3. The sensing parameters obtained through monomer and exciplex emissions of the hosts for guest 1 were opposite, indicating that $\Delta \omega /I_{\omega}$ and $\Delta \omega /I_{\omega}$ are minus values and $\Delta \omega /I_{\omega}$ is plus values. Guest 10, which is phthalate diester, was detected with high sensitivity, exhibiting the values $\Delta \omega /I_{\omega}$ of 0.228, 0.186 and 0.168, the values $\Delta \omega /I_{\omega}$ of 0.226, 0.186 and 0.171 for $\gamma$-1, $\gamma$-3 and $\gamma$-2, respectively, and the values $\Delta \omega /I_{\omega}$ of 0.171, 0.151 and 0.148 for $\gamma$-3, $\gamma$-2 and $\gamma$-1, respectively, in the orders $\gamma$-1 > $\gamma$-3 > $\gamma$-2 for the values $\Delta \omega /I_{\omega}$ and $\Delta \omega /I_{\omega}$, and $\gamma$-3 > $\gamma$-2 > $\gamma$-1 for the values $\Delta \omega /I_{\omega}$. The sequence of each sensing parameter obtained through guest-induced monomer and exciplex emission of the host for guest 10 was different, whereas the sequences of the sensing values obtained through guest-induced monomer and exciplex emission of the hosts for other guests such as 1, 3, 7, and 9 were almost same. Furthermore, the sensing values obtained through guest-induced exciplex emission were lower than those obtained from guest-induced monomer emission. Guests 2, which bears one more hydroxyl group than 1, was detected with high sensitivity, exhibiting the values $\Delta \omega /I_{\omega}$ of 0.058, 0.047 and 0.045, the values $\Delta \omega /I_{\omega}$ of 0.049, 0.035 and 0.034 for $\gamma$-2, $\gamma$-3 and $\gamma$-1, respectively, and the values $\Delta \omega /I_{\omega}$ of 0.285, 0.263 and 0.238 for $\gamma$-3, $\gamma$-1 and $\gamma$-2, respectively, in the orders $\gamma$-2 > $\gamma$-3 = $\gamma$-1 for the values $\Delta \omega /I_{\omega}$ and $\Delta \omega /I_{\omega}$, and $\gamma$-3 > $\gamma$-1 > $\gamma$-2 for the values $\Delta \omega /I_{\omega}$. Guest 8, which bears two chlorines, was detected with selective sensitivity, exhibiting the values $\Delta \omega /I_{\omega}$ of 0.222, 0.126 and 0.054 for $\gamma$-3, $\gamma$-2 and $\gamma$-1, respectively, in the order $\gamma$-3 > $\gamma$-2 > $\gamma$-1, and the values $\Delta \omega /I_{\omega}$ and $\Delta \omega /I_{\omega}$ were negligible. Guests 4, 5 and 6, which are dioxygen analogs, were detected with low sensitivity, exhibiting the values $\Delta \omega /I_{\omega}$ of 0.056-0.118, and the values $\Delta \omega /I_{\omega}$ and $\Delta \omega /I_{\omega}$ were negligible. The results obtained from the sensing parameters of $\gamma$-analogos were clarified that $\gamma$-analogos detected the guests with higher sensitive and selective exciplex and monomer fluorescence spectral variations. It means that regio-selective modification with hetero molecules, pyrene and cyanobenzene, on the $\gamma$-cyclodextrin rim can give sensitivity and selectivity to the sensing ability of the hosts. Furthermore, it suggests that a combination of guest-induced exciplex and monomer emissions can be a new indicator as a sensor of endocrine-disrupting chemicals.

### 3.2 Binding constants

The guest-induced monomer and excimer fluorescence variations at 378 and 397 nm, and 468 nm, respectively, were employed to calculate the binding constants of the host. The binding constants ($K$) are derived from the following equilibrium state (1).

$$
\frac{1}{I_\omega - I_{ex}} = \frac{1}{a[CD]} + \frac{1}{a[CD]K} \times \frac{1}{[G]} \quad A = m1, m2 \text{ and } ex
$$

Here, $I_\omega$ is the fluorescence intensity at 378, 397 and 468 nm ($I_{\omega}$, $I_{\omega}$ and $I_{\omega}$ for complex, $I_{\omega}$, $I_{\omega}$ and $I_{\omega}$ for the host alone, respectively), $[CD]$ is the total host concentration, $[G]$ is the total guest concentration, $A$ is a constant. The binding constants of the host were obtained in the order to examine the correlation between the fluorescence variations and the binding of the host. The results are listed in Table 1. The binding constants at exciplex emission for 1,2-dichlorobenzene are in the order $\gamma$-3 > $\gamma$-1 > $\gamma$-2, for 3,4-dichlorophenol and 2,4,6-trichlorophenol are in the order $\gamma$-3 > $\gamma$-2 > $\gamma$-1, for p-nyloniphenol and diethyl phthalate are in the order $\gamma$-3 > $\gamma$-2 > $\gamma$-1, for 2,4-dichlorophenoxyacetic acid are in the order $\gamma$-1 > $\gamma$-2 > $\gamma$-3, and for bisphenol A are in the order $\gamma$-2 > $\gamma$-1 > $\gamma$-3. The binding constants obtained from monomer emission change for 2,4,6-trichlorophenol, bisphenol A and diethyl phthalate are lower that those at exciplex emission and are roughly the same in the hosts. These differences are seemed to be caused by the different guest-induced fluorescence spectra such as monomer and exciplex emissions. Because guest-induced mechanisms obtained from both of monomer and exciplex fluorescence spectral changes are not similar as mentioned in section 3.1. The order of binding constants at exciplex emission of the host for the guests is roughly parallel with that of the sensing factors. This means that the sensitivity value at exciplex emission gives absolute sensing ability.

### 3.3 Response ranges

It is very important to examine the lower detection limits for
endocrine disruptors, because endocrine disruptors exist in the rivers and the lakes at low concentrations of $10^{-12} - 10^{-18}$ g/L. Accordingly, to investigate response range of the host for the guest obtained from guest-induced fluorescence spectral changes, it is clarified the lower detection limits for endocrine disruptors as a quantitative limit. Figures 5, 6 and 7 show the response curves of the host compounds for a couple of guests such as 2,4,6-trichlorophenol, p-nonylphenol and bisphenol A. 

**Table 1 Binding constants (K/mol dm$^3$) of pyrene-cyanobenzene-modified $\gamma$-cyclodextrins in a 10 vol.% ethylene glycol aqueous solution (2.0x10$^{-6}$ M, 25°C) at monomer (378 and 397 nm) and exciplex (468 nm) emission**

<table>
<thead>
<tr>
<th></th>
<th>$\gamma$-1</th>
<th>$\gamma$-2</th>
<th>$\gamma$-3</th>
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<tbody>
<tr>
<td></td>
<td>378 nm</td>
<td>397 nm</td>
<td>468 nm</td>
</tr>
<tr>
<td>1,2-Dichlorobenzene (1)</td>
<td>—</td>
<td>—</td>
<td>690±55</td>
</tr>
<tr>
<td>3,4-Dichlorobenzophen (2)</td>
<td>—</td>
<td>130±10</td>
<td>—</td>
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<tr>
<td>2,4,6-Trichlorophenol (3)</td>
<td>1500±60</td>
<td>1230±60</td>
<td>1680±110</td>
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<tr>
<td>p-Nonylphenol (7)</td>
<td>—</td>
<td>27200±660</td>
<td>—</td>
</tr>
<tr>
<td>2,4-Dichlorophenoxyacetic acid (8)</td>
<td>—</td>
<td>1180±110</td>
<td>—</td>
</tr>
<tr>
<td>Bisphenol A (9)</td>
<td>290±20</td>
<td>300±20</td>
<td>1070±25</td>
</tr>
<tr>
<td>Diethyl phthalate (10)</td>
<td>110±20</td>
<td>140±10</td>
<td>730±90</td>
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Figure 5 Flusitivity variations of $\gamma$-1 in a 10 vol.% ethylene glycol aqueous solution (2.0x10$^{-6}$ M, 25°C) 2,4,6-trichlorophenol, p-nonylphenol and bisphenol A as a function of th guest concentration at 378 (○), 397 (△) and 468 (□) nm.
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Figure 6 Flusitivity variations of $\gamma$-2 in a 10 vol.% ethylene glycol aqueous solution (2.0x10^{-6}M, 25°C) 2, 4, 6-trichlorophenol, $p$-nonylphenol and bisphenol A as a function of guest concentration at 378 ($\mathbf{\odot}$), 397 ($\mathbf{\triangle}$) and 468 ($\square$) nm.

Figure 7 Flusitivity variations of $\gamma$-1 in a 10 vol.% ethylene glycol aqueous solution (2.0x10^{-6}M, 25°C) 2, 4, 6-trichlorophenol, $p$-nonylphenol and bisphenol A as a function of guest concentration at 378 ($\mathbf{\odot}$), 397 ($\mathbf{\triangle}$) and 468 ($\square$) nm.
exciplex emission of these hosts for  \( p \)-nonylphenol are lower than those at monomer emission of fluorescent dansyl-tosyl-modified  \( \beta \)- and  \( \gamma \)-cyclodextrins reported previously.\[8\]

4. Conclusion

Regioselectively hetero-modified, pyrene and cyanobenzene,  \( \gamma \)-cyclodextrins have been investigated concerning their sensing ability for endocrine-disrupting chemicals. The variation of monomer and exciplex fluorescence intensities could be used as a parameter to describe the sensing ability in which these host compounds recognize endocrine-disrupting chemicals such as 2,4,6-trichlorophenol,  \( p \)-nonylphenol and bisphenol A with high sensitivity at exciplex emission. Particularly,  \( 6^* \)-pyrene-6\( ^\text{car} \)-cyanobenzene-modified  \( \gamma \)-cyclodextrin can detect  \( 10^{-14} \) M of  \( p \)-nonylphenol at exciplex emission. Introduction of hetro functional groups such as pyrene and cyanobenzene gives new sensing factor which is obtained from guest-induced exciplex emission, indicating high sensitivity and selectivity to these hosts. This study will be contributed to establish high sensitive fluorescent molecular recognition for endocrine-disrupting chemicals using guest-induced exciplex emission.

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