Interaction of cyclodextrins with pyrene-modified polyacrylamide in a mixed solvent of water and dimethyl sulfoxide as studied by steady-state fluorescence

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Abstract
The interaction of β- and γ-cyclodextrins (β-CD and γ-CD, respectively) with polyacrylamide modified with pyrenyl (Py) residues (pAAmPy) was investigated in a mixed solvent of water and dimethyl sulfoxide (DMSO) by steady-state fluorescence. In the absence of CD, the fluorescence spectra indicated that the formation of Py dimers became less favorable with increasing volume fraction of DMSO (x_DMSO). The fluorescence spectra at varying x_DMSO and CD concentrations indicated that β-CD and γ-CD included monomeric and dimeric Py residues, respectively. Using the fluorescence spectra, equilibrium constants of the formation of Py dimers and the complexation of β-CD and γ-CD with Py residues were roughly estimated based on simplified equilibrium schemes.

Introduction
Cyclodextrins (CDs) are cyclic oligomers composed of glucopyranose units linked through α-1,4-glycoside bonding. They bear a tapered structure with a narrower rim of primary hydroxy groups and a wider rim of secondary hydroxy groups. CDs of 6, 7, and 8 glucopyranose units are called α-CD, β-CD, and γ-CD, respectively. CDs have a hydrophilic exterior and a rather hydrophobic cavity, and thus, recognize guest compounds of a size and shape matching their cavity, to form inclusion complexes [1-5]. Since CDs are nontoxic, they have been utilized in a variety of fields, including food additives, cosmetics, and personal care items [6-12]. In the past decade, the formation of inclusion complexes of CDs with guest residues attached on water-soluble polymers has attracted increasing interest from a number of research groups because these systems are applicable to stimuli-responsive systems [13-18].
We have been working on the interaction of CDs with water-soluble polymers bearing various guest residues, including linear, branched, and cyclic aliphatics, as well as aromatics [19-21], and realized stimuli-responsive hydrogels [22-27] and macroscopic assemblies based on molecular recognition [28-31]. Aromatic residues absorb light to become excited, and subsequently they can transfer energy and electrons. The interaction of CDs with water-soluble polymers carrying aromatic residues may allow one to construct functional systems that convert photo energy based on molecular recognition. Among aromatic compounds, pyrene is the most examined as a fluorescence probe or label because it shows a relatively high fluorescence quantum yield and a relatively long fluorescence lifetime in both monomer and excimer states [32,33]. Since pyrene is very hydrophobic, it may tend to form aggregates, e.g., dimers, in aqueous solutions. It is also known that pyrene forms inclusion complexes with β-CD and γ-CD in different manners; β-CD includes monomeric pyrene whereas γ-CD includes dimeric pyrene [34-36]. Recently, we have demonstrated this selectivity switching on macroscopic molecular recognition for polyacrylamide-based gels carrying pyrenyl (Py) and CD residues, by changing the composition of a mixed solvent of water and dimethyl sulfoxide (DMSO) [37]. In the present study, the interaction of β-CD and γ-CD with Py-modified polyacrylamide (pAAmPy, Scheme 1) was investigated in the water/DMSO mixed solvent of varying composition by steady-state fluorescence to elucidate the mechanism of the selectivity switching.

Results

Figure 1a demonstrates the steady-state fluorescence spectra measured for 0.04 g L⁻¹ pAAmPy (5 μM in Py residue) at varying volume fractions of DMSO (x_DMSO) in the water/DMSO mixed solvent in the absence of CD. At x_DMSO = 0 (i.e., in water), the spectrum exhibits not only emission bands ascribable to monomeric Py in the region of 370–430 nm, but also a broad band assignable to a Py excimer around 480 nm, indicating that Py residues tend to form dimers because of the hydrophobicity. It is likely that Py residues associate intramolecu-
residues dissociate to the monomers. In the spectra of the γ-CD/pAAmPy system at \(x_{\text{DMSO}} = 0\), on the other hand, the intensity of the excimer fluorescence increases whereas that of the monomer fluorescence decreases with increasing \([\text{CD}]_0\), indicating that γ-CD forms inclusion complexes with dimeric Py residues, and monomeric Py residues further associate to form the dimers. Using the steady-state fluorescence spectra, \(I_{480}/I_{376}\) values were calculated. Figure 3 compares \(I_{480}/I_{376}\) as a function of \([\text{CD}]_0\) for the β-CD/pAAmPy system at \(x_{\text{DMSO}} = 0.1–0.6\) and for the γ-CD/pAAmPy system at \(x_{\text{DMSO}} = 0–0.2\). At other \(x_{\text{DMSO}}\), \(I_{480}/I_{376}\) was practically independent of \([\text{CD}]_0\), indicative of no significant interaction of β-CD or γ-CD with pAAmPy. For the β-CD/pAAmPy system (Figure 3a), \(I_{480}/I_{376}\) decreases with increasing \([\text{CD}]_0\) at \(x_{\text{DMSO}} = 0.1–0.6\). For the γ-CD/pAAmPy system (Figure 3b), on the other hand, \(I_{480}/I_{376}\) increases with \([\text{CD}]_0\) at \(x_{\text{DMSO}} = 0–0.2\).

**Discussion**

Detailed study of the equilibria of the inclusion complex formation of CDs with Py-modified water-soluble polymers, including the formation of the dynamic excimer, requires not only steady-state fluorescence measurements but also time-resolved fluorescence measurements [38-43]. In this study, however, equilibrium constants are roughly estimated by analyzing the steady-state fluorescence data, assuming that dynamic excimer formation is negligible. In the absence of CD, \(I_{480}/I_{376}\) for pAAmPy decreases from 0.125 to 0.025 with increasing \(x_{\text{DMSO}}\) from 0 to 1. It should be noted here that the fluorescence of the Py monomer is dominant compared to that of the Py excimer even at \(x_{\text{DMSO}} = 0\) (i.e., in water), implying that there are a significant fraction of Py residues (Py°) that cannot form Py dimers (Py₂). Since the steady-state fluorescence measurements were performed under dilute conditions in this study, most of the Py₂ were formed intramolecularly. Thus, Py residues in pAAmPy carrying a Py residue may not form Py₂. The fraction of Py° is defined as \(f\). Scheme 2a indicates a simplified equilibrium of the formation of Py₂ from two Py residues. On the basis of the derivation of equations in the Supporting Information File 1, the equilibrium constant for the Py₂ formation (\(K_{\text{Py}}\)) can be calculated as

\[
K_{\text{Py}} = \frac{x^2 - 1}{8(1-f)[\text{Py}]_0}
\]

where \([\text{Py}]_0\) is the total concentration of Py residue and \(x\) as given in Equation 2.
Here, $A_{1,376}$, $A_{1,480}$, $A_{2,376}$, and $A_{2,480}$ are constants corresponding to the products of the molar extinction coefficient and the fluorescence quantum yield (subscripts 1 and 2 indicate monomeric and dimeric Py residues, respectively, and subscripts 376 and 480 indicate the wavelengths), and $B_{376}$ and $B_{480}$ are constants corresponding to the background. If it is assumed that all the Py residues are in the monomer state at $x_{\text{DMSO}} = 1$ (i.e., in DMSO), $f = 0.5$, $A_{2,480}/A_{1,376} = 0.5$, and $B_{376} = B_{480} = 0$, $K_{\text{Py}}$ can be calculated as can be seen in Figure 4a. This figure indicates that $K_{\text{Py}}$ decreases monotonously from $6.2 \times 10^4$ to 0 M$^{-1}$ with increasing $x_{\text{DMSO}}$ from 0 to 1.

In the $\beta$-CD/pAAmPy system, $\beta$-CD forms inclusion complexes with both Py and Py° (Scheme 2b). On the basis of the derivation described in the Supporting Information File 1, the concentrations of all species can be calculated by using the equilibrium constant ($K_{\beta}$) for the inclusion complex formation, and $I_{480}/I_{376}$ can be also obtained as given in Equation 3.

Here [Py], [Py°], [CD], [CD·Py], and [CD·Py°] denote the concentrations of Py, Py°, free CD, and the complexes of CD with Py and with Py°, respectively, and $A'_{1,376}$ and $A'_{1,480}$ are constants. In this study, $A'_{1,480}/A'_{1,376}$ is fixed at 0.025 (Supporting Information File 1). It is also likely that $A_{2,376} = 0$. When $K_{\beta}$ and $A'_{1,480}/A'_{1,376}$ are chosen appropriately, the calculated $I_{480}/I_{376}$ values agree with the experimental data, as can be seen in Figure 3a. The $K_{\beta}$ values were plotted in Figure 4b against $x_{\text{DMSO}}$. As $x_{\text{DMSO}}$ increases from 0.1 to 0.6, $K_{\beta}$ decreases from $4 \times 10^2$ to $8 \times 10^1$ M$^{-1}$. This observation indicates that the formation of inclusion complexes becomes less favorable with increasing $x_{\text{DMSO}}$.

In the $\gamma$-CD/pAAmPy system, $\gamma$-CD forms inclusion complexes with Py$_2$, in which Py° is not involved. On the basis of the

\[
I_{480} / I_{376} = \frac{A_{1,480}([\text{Py}] + [\text{Py}^\circ] + A_{1,480}([\text{CD} \cdot \text{Py}] + [\text{CD} \cdot \text{Py}^\circ]) + A_{2,480}([\text{Py}_2] + B_{480})}{A_{1,376}([\text{Py}] + [\text{Py}^\circ] + A_{1,376}([\text{CD} \cdot \text{Py}] + [\text{CD} \cdot \text{Py}^\circ]) + A_{2,376}([\text{Py}_2] + B_{376})}
\]
methanol to give a precipitate. The polymer obtained was recovered by filtration and dried under vacuum. The molecular weight of the polymer was estimated to be $4 \times 10^3$ by size exclusion chromatography (SEC), and the Py content was determined to be ca. 1 mol % by $^1$H NMR.

Steady-state fluorescence spectra were obtained on a HITACHI F-2500 spectrophotometer with excitation at 335 nm by using a 1 cm quartz cuvette. The slit widths for both excitation and emission sides were kept at 2.5 nm during measurement. SEC analysis was carried out at 40 °C on a TOSOH CCP & 8020 system equipped with two TOSOH TSKgel α-M columns connected in series, using formamide as the eluent at a flow rate of 0.3 mL min$^{-1}$. TOSOH UV-8020 and TOSOH RI-8021 detectors were used. The molecular weights were calibrated by polystyrene sulfonate sodium-salt samples (American Polymer Standards). $^1$H NMR spectra were measured on a JEOL JNM-ECA500 spectrometer by using a mixed solvent of DMSO-$_d_6$ and D$_2$O (1/1, v/v) as a solvent, and chemical shifts were referenced to the solvent value (i.e., 2.49 ppm for DMSO).

### Supporting Information

**Supporting Information File 1**

Equilibria for the CDs/pAAmPy systems.

[http://www.beilstein-journals.org/bjoc/content/supplementary/1860-5397-8-150-S1.pdf](http://www.beilstein-journals.org/bjoc/content/supplementary/1860-5397-8-150-S1.pdf)

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### References
