# γ-CYCLODEXTRINS BEARING A PYRENYLAMIDE MOIETY. THE EFFECT OF PHOTOEXCITED-STATE ACID-BASE EQUILIBRIUM OF APPENDED CHROMOPHORE ON THEIR GUEST BINDING

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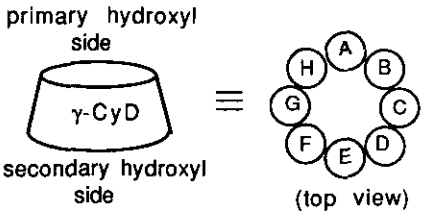
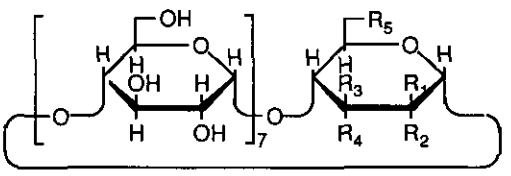
*Abstract*- γ-Cyclodextrins bearing a pyrenylamide moiety at primary or secondary hydroxyl side (**1** and **2**, respectively) showed red-shifted fluorescence as well as normal fluorescence in a solution of pH below 3. This red-shifted fluorescence was emitted from the pyrenylamide moiety protonated in the excited state. Guest binding ability of **2** was markedly affected by the protonation, while that of **1** was hardly affected.

Cyclodextrins (CyDs) are cyclic oligosaccharides composed of only α-*D*-glucopyranoside and have a cavity into which a guest compound is accommodated by hydrophobic interaction, forming a host-guest complex.[1,2] Chemical modification on CyDs can alter their guest accommodation ability. We have recently discovered[3] that modified CyDs had a potential for detecting organic substances spectroscopically. Since the guest accommodation of modified CyDs as well as native CyDs seems to be governed by hydrophobic interaction, an acid-base equilibrium of the modified residue would influence the complexation behavior.[4] In addition, an acid-base equilibrium of excited state should be taken into account if we determine the binding constants from

emission spectra because most compounds have different pKa values between the ground and the excited states.[5] In this connection, we investigated the influence of the acid-base equilibrium of the excited state of *N*-(1-pyrenecarbonyl)-6$^{A}$-amino-6$^{A}$-

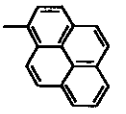



○: glucopyranose unit




| | 1 | 2 |
|---|---|---|
| $R_1$ | H | OH |
| $R_2$ | OH | H |
| $R_3$ | OH | H |
| $R_4$ | H | NHCOPy |
| $R_5$ | NHCOPy | OH |

Py=



deoxy-γ-CyD (**1**) and (2$^{A}$S,3$^{A}$S)-*N*-(1-pyrenecarbonyl)-3$^{A}$-amino-3$^{A}$-deoxy-γ-CyD (**2**), in which the sugar unit attaching the pyrenylamide moiety changed to altrose having a chair conformation, on the complexation behavior.[6]

Figure 1 shows the fluorescence spectra of **1** and **2** at various pH. Although each of **1** and **2** shows pyrene normal fluorescence at pH above ca. 3, the normal fluorescence decreased in its intensity at the lower pH region with appearance of the red-shifted fluorescence with a peak maximum around 460 nm. This red-shifted fluorescence can be attributable to the protonation to the nitrogen atom of pyrenylamide moiety at the first singlet excited state ($S_1$) because of the fact that no change in uv-visible absorption and circular dichroism spectra between neutral and acidic conditions were observed. This observation is consistent with the fact that the pKa of $S_1$ of the conjugate acid of naphthoamides was 2-3.[5] Although it seems to be difficult to determine the pKa values of $S_1$ in **1** and **2** accurately, they were estimated ca.2 and 1 for **1** and **2**, respectively, from their fluorescence life times.[5] Rising components (ca. 1 ns) in the red-shifted fluorescence of **1** and **2** were obtained in single photon counting measurements, indicating that the rate of protonation to the pyrenylamide moiety is relatively comparable to the fluorescence lifetimes of **1** and **2** (3-20 ns).

The addition of *l*-borneol as a guest compound into the acidic solution of **1** increased the fluorescence intensity of **1** in the whole wavelength region, while the red-shifted fluorescence intensity of **2** was decreased with the normal fluorescence intensity increasing. In neutral solution where the red-shifted fluorescence was absence for **1** and **2**,[6] the guest addition increased the normal fluorescence intensities of **1** and **2**. These observations indicate that for **1** the protonation to $S_1$ was not affected by guest accommodation and that the increase in the fluorescence intensity of **1** corresponded to locational change of the pyrenylamide moiety (Scheme 1a), while

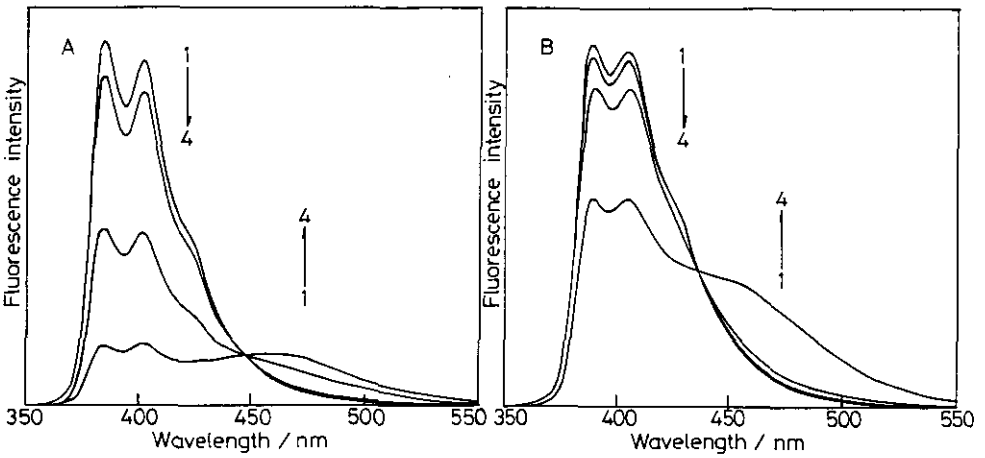
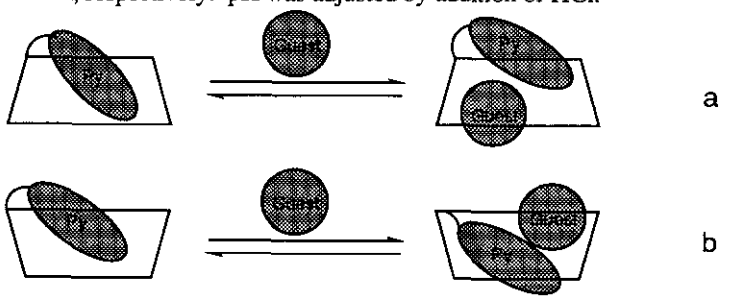



Figure 1. Fluorescence spectra of **1** ($3.08 \times 10^{-5}$ M, **A**: pH 6.32, 1; pH 3.24, 2, pH 1.88, 3; pH 1.07, 4) and **2** ($3.03 \times 10^{-7}$ M, **B**: pH 6.55, 1; pH 3.01, 2; pH 2.00, 3; pH 1.03, 4) in aqueous solutions at 25°C excited at 340 and 345 nm, respectively. pH was adjusted by addition of HCl.




Scheme 1

for **2** the protonation was restricted by deep penetration of the pyrenylamide moiety caused by the guest penetration from the secondary hydroxyl side of **2** (Scheme 1b).

The 1:1 host-guest binding constants under neutral and acidic conditions for several guests were determined[7] from the guest-induced fluorescence variations and are summarized in Table 1. Under the neutral conditions, **2** has larger binding affinities for the guests than **1** with factors of 17 - 154. However, under acidic solutions, the values of **1** were comparable to those under the neutral conditions while much smaller values with the factor of 0.02 - 0.08 were obtained for **2**.

*The marked depression in the guest accommodation ability of* **2** *in the acidic solution can be explained by* taking into account a polarity change of the host molecules associated with the acid-base equilibrium at $S_1$ of the pyrenylamide moiety. The fluorescence variations associated with the guest accommodation suggest that the guest penetrated into γ-CyD cavity of **1** and **2** from the secondary hydroxyl side. Under the acidic

Table 1. Host-Guest Binding Constants of **1** and **2** for Several Guests in Aqueous Solutions at 25°C [a,b]

| | **1** | | **2** | |
|---|---|---|---|---|
| Guest | pH 6.77 | pH 1.28 | pH 7.04 | pH 1.04 |
| Cyclohexanol | 85 | - | 2500 | 84 |
| *d*-Borneol | 1800 | 2480 | 278000 | 20600 |
| *l*-Borneol | 1750 | 1280 | 255000 | 18500 |
| *d*-Menthol | 540 | 451 | 9000 | 702 |
| *l*-Menthol | 430 | 355 | 21000 | 542 |

a) Unit in $M^{-1}$. Excited at 340 and 345 nm for **1** and **2**, respectively.

b) Determined from the guest-induced fluorescence variations of the normal fluorescence.

conditions, the protonation to the excited pyrenylamide moiety (the amide group might be protonated) was facilitated, changing the environment of primary and secondary hydroxyl side more hydrophilic for **1** and **2**, respectively. As a consequence, the positive charge formed in $S_1$ of **2** decreased the guest binding ability because the host-guest complexation of CyDs would be promoted by hydrophobic interaction. In contrast to **2**, the guest accommodation behavior of **1** was hardly affected by the protonation in $S_1$ because the positive charge formed after excitation exists at the opposite side to the guest inserting side.

In summary, excited state ($S_1$) acid-base equilibrium could affect the guest binding ability of **2** which possesses the pyrenylamide moiety on the secondary hydroxyl side. If one uses modified CyDs as sensing devices, the properties of $S_1$ which are drasitically different from those of ground state should be taken into account.

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