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Switchable fluorescence behaviors of pyronine Y at different pH values upon complexation with biquinolino-bridged bis(β-cyclodextrin)

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Abstract

An xanthene dye, pyronine Y, was found to exhibit opposite fluorescence behaviors at different pH values in the presence of *N*,*N*′-bis(2-aminoethyl)-2,2′-biquinoline-4,4′-dicarboxamide-bridged bis(β-cyclodextrin) (**2**). That is, pyronine Y showed the quenched fluorescence in an acidic (pH 2.0) or a neutral (pH 7.2) environment, while the enhanced fluorescence in a basic environment (pH 12.0), with the addition of **2**. Further studies by fluorescence titrations and 2D NMR indicated that different binding modes of positively charged and neutral PY molecules upon complexation with bis(β-cyclodextrin) **2** should be responsible for opposite fluorescence behaviors. This result may enable the biquinolino-bridged bis(β-cyclodextrin) as an efficient chemical sensor for the protonation and deprotonation of xanthene dyes.

Keywords: Spectrophotometric titration; Cyclodextrin; Pyronine Y; Fluorescence sensing; Photoinduced electron transfer

1. Introduction

Water-soluble xanthene dyes, such as pyronines, acridines and rhodamines, are very sensitive to the local microenvironment. Therefore, they have been widely applied in the labeling of proteins and cell organelles [1–4], the loading of gels [5], the doping of organic thin films [6], and depositing on a p-type silicon surface to construct diodes [7,8], etc. For all these applications, it is vital to understand their photophysical behavior in organized media [9,10] and to know the role of interactions between the substrate and the medium. On the other hand, as excellent model acceptors, cyclodextrins (CDs) can bind various substrates, including dye guests, to form host-guest supramolecular complexes due to the hydrophobicity of the CD cavity [11–15]. Recently, Novo et al. investigated the inclusion complexation of pyronine Y (PY) and pyronine B (PB) with native β-CD in different solvents, and characterized the photophysical properties of these included pyronines by using fluorescence measurements [16]. As compared with native CDs and simply modified CDs, bridged bis(CD)s generally exhibit higher binding abilities and molecular selectivities toward model substrates due to a multiple recognition mechanism [17–38]. In preliminary works, we have reported that biquinolino-bridged bis(β-CD)s can form more stable inclusion complexes with both fluorescent dyes [39] and steroids [40,41] than their monomeric analogues through the cooperative binding of one guest by two CD cavities in a single molecule. In this context, we wish to report pH-dependent fluorescence behaviors of PY upon complexation with *N*,*N*′-bis(2-aminoethyl)-2,2′-biquinoline-4,4′-dicarboxamide-bridged bis(β-CD) (**2**) (Plate 1). The aim of this work is to investigate how the nature of the media affects binding behaviors of bis(β-CD)s with model substrates. We are also particularly interested in examining the protonation/deprotonation of model substrates in the presence of bis(β-CD) through a fluorescence-sensing mechanism. This approach will serve our further understanding of this significant, but little investigated, area in the field of CD chemistry.

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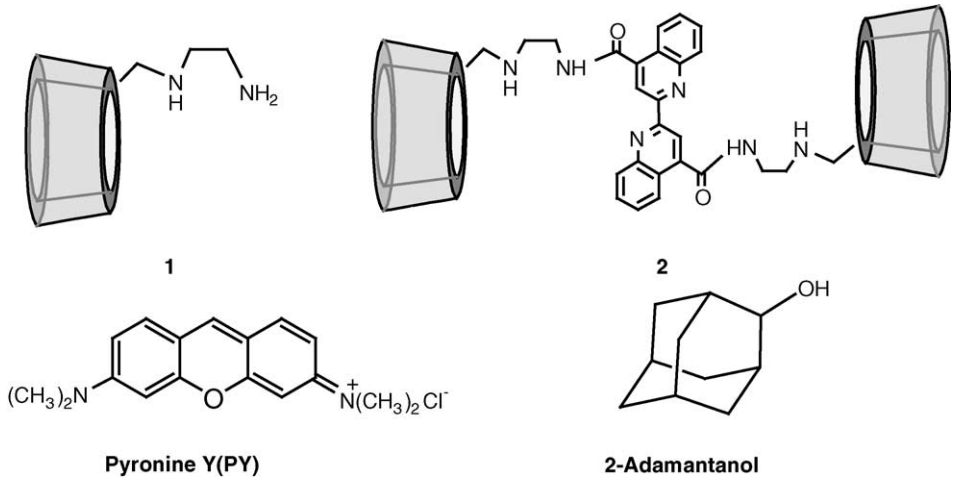

Plate 1. Structures of hosts and guests.

2. Experimental

2.1. Measurements

Fluorescence spectra were measured in a conventional rectangular quartz cell (10 mm × 10 mm × 45 mm) at 25 °C on a JASCO FP-750 spectrometer equipped with a constant-temperature water bath, with the excitation and emission slits width of 5 nm, and the excitation wavelength was 493 nm. A 2D NMR spectrum was obtained on a Varian INVOA 300 spectrometer at 25 ± 1 °C.

In spectral measurements, disodium hydrogen phosphate dodecahydrate (25.79 g) and sodium dihydrogen phosphate dihydrate (4.37 g) were dissolved in 1000 mL of deionized water to make a 0.10 M aqueous phosphate buffer solution of pH 7.2. Whereas 20 mM potassium chloride was adjusted to pH 2.0 with 1 M hydrochloric acid to give an acidic buffer solution or was adjusted to pH 12.0 with 1 M sodium hydroxide to give a basic buffer solution, which were used as solvent for all measurements.

2.2. Materials and sample preparation

6-(2-Aminoethylamino)-6-deoxy-β-CD (**1**) [42] and *N*,*N*′-bis(2-aminoethyl)-2,2′-biquinoline-4,4′-dicarboxamide-bridged bis(β-CD) (**2**) [40] were prepared according to our previous reports. 2-Adamentanol was purchased from Aldrich, and pyronine Y was purchased from Chroma. All other chemicals were commercially available and used without further purification, except noted otherwise.

Aqueous solutions of PY for measurements were freshly prepared by dissolving the commercial product in buffer solutions with concentrations in the range of 1.5 to 5.0 × 10^{-6} mol dm^{-3} for fluorescence titrations. These concentrations were low enough to avoid the aggregation of dyes in aqueous solution, while concentrations of **1** and **2** were varied from 0 to 100 times correspondingly in each titration experiment.

3. Results and discussion

3.1. Fluorescence titration

Fluorescence titrations of **1** and **2** with PY in different buffer solutions (pH 2.0, 7.2 and 12.0) were performed at 25 °C to quantitatively assess binding behaviors between hosts and guests. In spectral titration experiments, the concentration of PY was kept constant, while concentrations of **1** and **2** were varied from 0 to 100 times as high as that of PY correspondingly. The spectral changes depended critically on the formation of new species, i.e., host-guest inclusion complex, showing the spectral enhancement or quenching. As shown in Fig. 1, the fluorescence intensity of PY gradually decreased in buffer solutions of pH 2.0 and pH 7.2 upon the addition of bis(β-CD) **2**. In sharp contrast, in a basic buffer solution of pH 12.0, the fluorescence intensity of PY gradually increased with the addition of **2**.

Validating the 1:1 inclusion complexation stoichiometry between CD hosts and PY by Job's experiments, complex stability constants (K_S) could be calculated by analyzing sequential changes in fluorescence intensities (ΔI_f) of dyes that occurred with changes in host concentrations. This analysis was carried out by using a nonlinear least squares curve-fitting method [43]. For each dye guest examined, the plot of ΔI_f as a function of $[H]_0$ gave an excellent fit. In repeated measurements, K_S values were reproducible within an error of ±5%. Complex stability constants (K_S) obtained for all of host-guest combinations were listed in Table 1, along with free energy changes ($-\Delta G^\circ$) as well as fluorescence intensity changes of guest dye upon addition of hosts **1** and **2**.

3.2. Unique fluorescence behaviors of PY

As shown in Fig. 1, fluorescence behaviors of PY in the presence of bis(β-CD) **2** were largely dependent on the solution's pH value, i.e., the fluorescence intensity of PY gradully

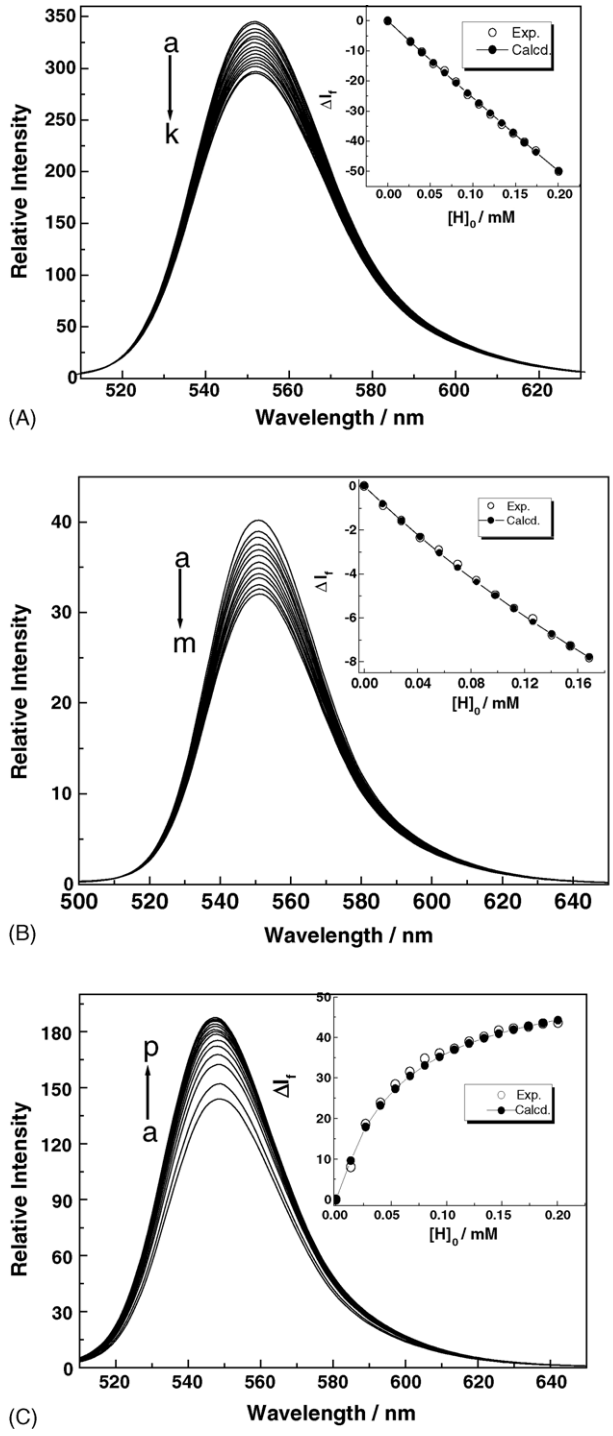

Fig. 1. Fluorescence spectral changes of PY upon addition of host **2** in buffer solutions of (A) pH = 2.0, (B) pH = 7.2 and (C) pH = 12.0; (inset) curve-fitting analyses; λ_{ex} = 493 nm.

decreased with increasing host **2** concentration in buffer solutions of pH 2.0 and pH 7.2, however, the opposite case happened in a buffer solution of pH 12.0. It is well known that the photoinduced electron transfer (PET) process is often responsible for the fluorescence quenching, so decreased fluorescence intensities of PY can be rationalized by the PET

Table 1
Fluorescence intensity changes, complex stability constants (K_S) and Gibbs free energy changes ($-\Delta G^\circ$) for 1:1 inclusion complexation of hosts **1** and **2** with PY in aqueous buffer solutions with different pH Value (pH 2.0, 7.2 and 12.0) at 25 °C; λ_{ex} = 493 nm and λ_{em} = 552 nm

Guest	Host	pH	K_S (M^{-1})	Fluorescence intensity	$-\Delta G^\circ$ (kJ mol^{-1})
PY	**2**	2.0	285	Quench	14.0
		7.2	1620	Quench	18.3
		12.0	17370	Enhance	24.2
PY	**1**	2.0		[a]	
		7.2		[a]	
		12.0	2510	Enhance	19.4

[a] Variations in the excimer emission of dye upon addition of hosts are too small to determine the stability constant.

process between PY and **2** in buffer solutions of pH 2.0 and pH 7.2. In aqueous solution, two electrical structures of PY are possible, one where the oxygen assumes a positive charge, and in the other the nitrogen assumes a positive charge. Because the nitrogen is less electronegative than oxygen, the former can support a positive charge more easily than the latter. Therefore the structure with positive nitrogen is the more likely one (Fig. 2). On the other hand, it is also well documented that a positively charged group is difficult to be included in the CD cavity [44]. Therefore, we can deduce that, at pH 2.0 and 7.2, PY is protonated and located outside the CD cavity. In these cases, the protonated PY molecule acts as an electron acceptor, whereas the biquinolino group in host **2**, possessing a big π-eletron system, acts as an electron donor, and the PET process between the biquinolino group and the protonated PY molecule results in the quenched fluorescence. In a control experiment, the fluorescence intensity change of PY upon the addition of 6-(2-aminoethylamino)-6-deoxy-β-CD (**1**) was negligible under identical conditions. These results jointly confirm that the decreased fluorescence of PY in the presence of **2** is attributed not to the inclusion complexation by CD cavity but mostly to the PET process.

To elucidate the binding mode between host **2** and PY molecule, the ^{1}H ROESY spectrum of an equimolar mixture of bis(β-CD) **2** and PY (0.5 mM each) was performed. As shown in Fig. 3, ethylamino protons of the tether in bis(β-CD) **2** gave clear NOE correlations with not only PY's methyl protons (cross peaks A) but also PY's aromatic protons (cross peaks B). In addition, NOE correlations between biquinoline protons and CD's H3/H5 protons (cross peaks C) indicated that the self-included biquinoline moiety in bis(β-CD) **2** [40] was not driven out of CD cavity by guest inclusion. Interestingly, we could not observe any NOE correlations between PY protons and CD's H3/H5 protons, but found clear NOE correlations between biquinoline protons and PY's aromatic protons (cross peaks D). These phenomena indicated that PY molecule was located outside the CD cavity but near the biquinoline group. This conformation will subsequently enable a PET process between the biquinoline group and PY.

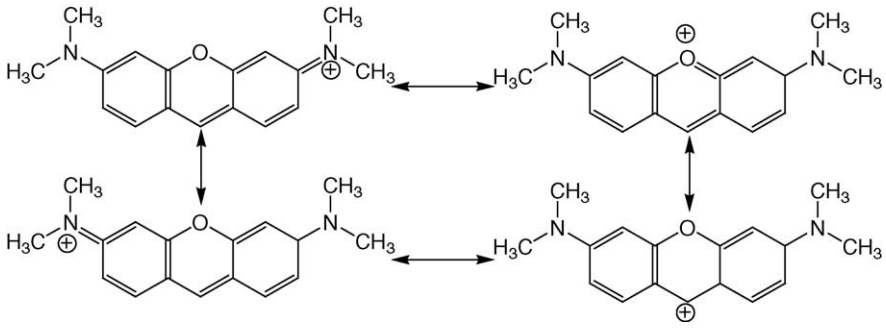

Fig. 2. Resonance structures of PY.

2-Adamentanol is reported to have very high binding ability with CD cavity [45]. Therefore, the guest molecule that is included in the CD cavity should be driven out when 2-adamentanol is added to the system. Generally, this process is accompanied by spectral changes if the included guest is luminescent. In a control experiment, the fluorescence intensity of an equimolar mixture of **2** and PY did not change upon the addition of 2-adamentanol. This result further verified that PY molecule was not included in the CD cavity.

Distinctly different from the cases at pH 2.0 and 7.2, in a basic buffer solution of pH 12.0, the fluorescence intensity of PY enhanced by increasing the bis(β-CD) **2** concentration. Such contrasting fluorescence behaviors indicated that the distinctly different binding modes were operative in the complexations of PY with bis(β-CD) **2**. Formerly, we have demonstrated that the protonated PY molecule was located outside the CD cavity at pH 2.0 and 7.2. Therefore, the PET process between biquinolino group and PY quenched the fluorescence. However, at pH 12.0, the fluorescent group of PY could be efficiently shielded from the PET process, which quenched fluorescence, by the inclusion complexation of the deprotonated PY with the CD cavity. Subsequently, the significantly enhanced microenvironmental hydrophobicity around the PY fluophore, which came from a cooperative contribution of two hydrophobic CD cavities, resulted in the fluorescence enhancement. Some control experiments further confirm the inclusion complexation of PY with CD cavities. For example, the fluorescence intensity of PY also enhanced with the gradual addition of **1** at pH 12.0, which indicates that the enhanced fluorescence of PY is mainly attributed to the inclusion complexation of CD cavity. In another control experiment, the fluorescence intensity of an equimolar mixture of **2** and PY significantly decreased with the addition of 2-adamentanol. These results indicate that the addition of 2-adamentanol, which has the stronger binding ability with CD, drives the originally included PY out of the CD cavity. To visualize these unique fluorescence behaviors of PY, a schematic version is shown in Fig. 4.

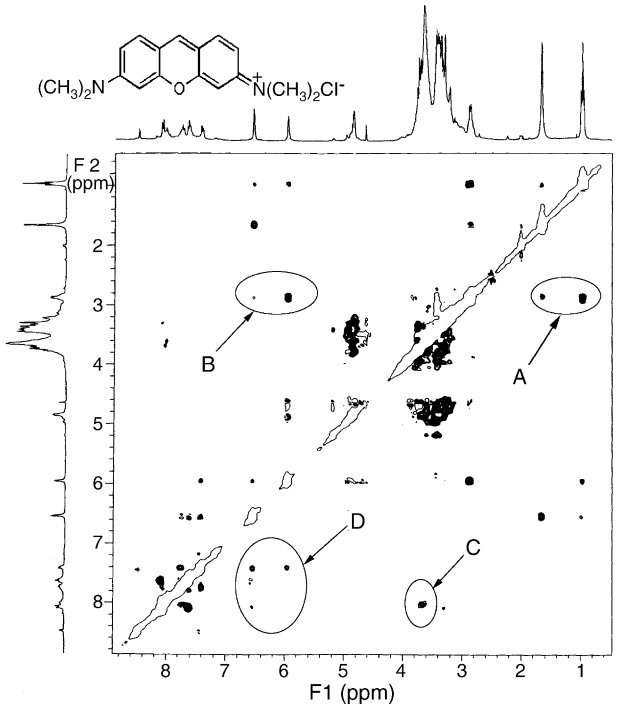

Fig. 3. The ^{1}H ROESY spectrum of **2** with PY ([2] = [PY] = 5×10^{-3} M) in a pD 7.20 buffer solution at 298.15 K with a mixing time of 600 ms.

3.3. Binding ability of bis(β-CD) *2* with PY

Complex stability constants also support this conclusion. Because the protonated PY cannot interact with the CD cavity and only interacts with the biquinolino group, PY gives the relatively low binding abilities with **2** at pH 2.0 (K_S = 285 M^{-1}) and 7.2 (K_S = 1620 M^{-1}), while mono-modified β-CD **1** exhibits no binding ability toward PY under comparable conditions. On the other hand, at pH 12.0, PY shows the quite high K_S value (17370 M^{-1}) upon complexation with **2**. This high binding ability may be attributed to the cooperative binding of a PY molecule by two CD cavities, which significantly strengthens the hydrophobic interactions between host and guest. In a control experiment, mono-modified β-CD **1** also gives the relatively high binding ability (K_S = 2510 M^{-1}) toward PY at pH 12.0, which unambiguously confirms the interactions between PY and the CD cavity.

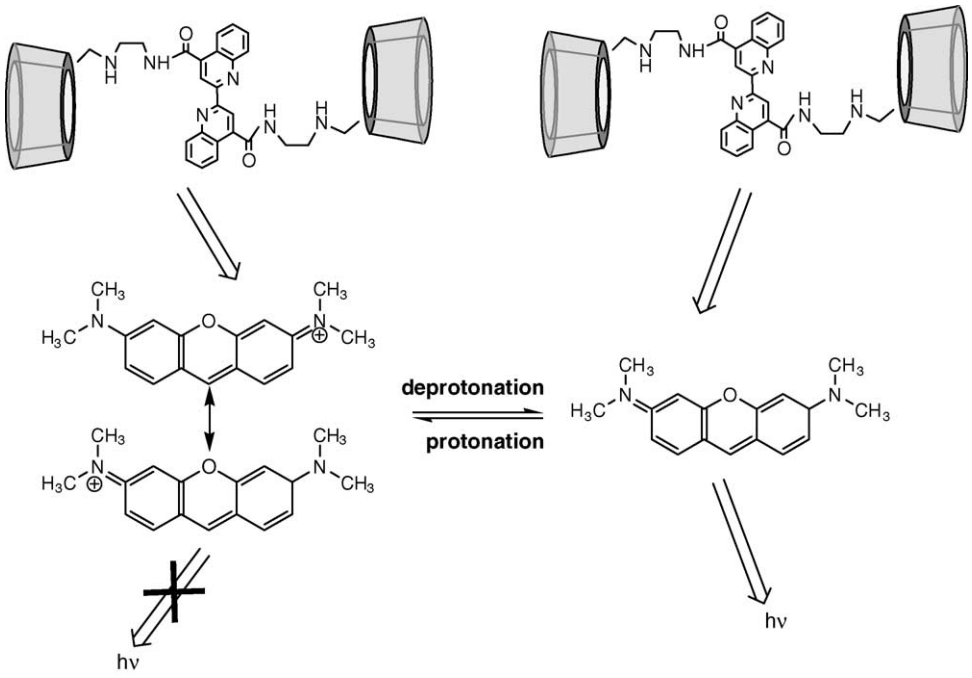

Fig. 4. The schematic fluorescence behaviors of PY in the presence of bis(β-CD) **2**.

Acknowledgments

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