Synthesis of novel indolyl modified β-cyclodextrins and their molecular recognition behavior controlled by the solution's pH value

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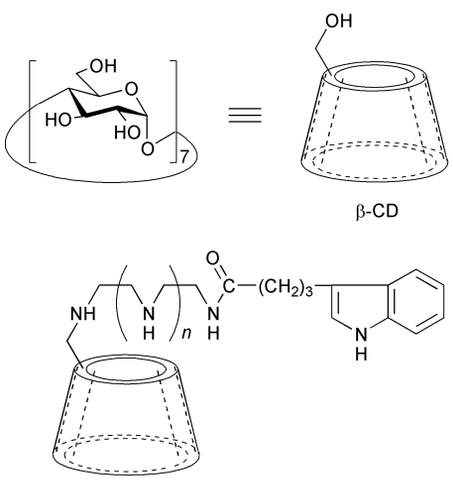
In order to investigate the effects of substituent and tether length in molecular recognition, three novel indolyl-contained β-cyclodextrin derivatives were synthesized by the condensation of indol-3-ylbutyric acid with the corresponding oligo(aminoethylamino)-β-cyclodextrin in the presence of DCC. Their molecular recognition behavior with some representative dye guests, *i.e.* Acridine Red, Rhodamine B, Neutral Red, Brilliant Green and Methyl Orange, was studied by using absorption, fluorescence and circular dichroism spectrometry. From the results of induced circular dichroism spectra and two-dimensional NMR spectroscopy, it was found that the initial conformations of these compounds are dramatically different in aqueous buffers of pH 2.0 and 7.2, which intrinsically determine the molecular binding ability of the host. It was also revealed that both the guest structure and the host tether length were responsible for the inclusion complexation stability. Therefore, on the one hand the hydrophobicity and substituent effect of the guest simultaneously determine the stability of host–guest complex through hydrophobic, van der Waals, and electrostatic interactions. On the other hand, the size/shape-matching relationship and induced-fit concept working between host and guest also play crucial roles in the selective molecular binding process of cyclodextrin hosts.

Introduction

As a type of seminatural macrocycle, cyclodextrins are composed of six or more D-glucoses to form truncated cone-shaped molecules with an hydrophobic interior and hydrophilic surface and may encapsulate various organic molecules to afford host–guest complexes or supramolecular species in aqueous solution.[1–4] Therefore, cyclodextrins and their derivatives have received much attention during the past three decades, accompanying the progress of supramolecular chemistry. Until now, cyclodextrins have been extensively applied in diverse fields of science and technology, for example as drug carriers,[5,6] chemical sensors,[7–9] artificial enzymes,[10,11] and so on. Cyclodextrins are, however, not preorganized or flexible enough to allow allosteric conformational changes that are permissible in biological supramolecular systems,[12] and the rigid cavity of native cyclodextrins do not always accommodate guest molecules according to a cooperative recognition mechanism. Therefore, much effort has been devoted to design and synthesize novel cyclodextrin derivatives and various mono-substituted, multiplesubstituted and dimeric cyclodextrins have been obtained.[13,14] Among them, a chromophoric derivative belongs to one of the most important categories, and may undergo spectral change upon guest binding and be detected expediently by conventional spectrometric studies. A self-inclusion–exclusion procedure of substituents was observed and an induced-fit mechanism has been proposed for the inclusion complexation of this type of cyclodextrin host.

In previous reports,[15–18] we prepared a series of chromophoric cyclodextrins and examined their molecular binding ability with aliphatic guests. The results obtained indicate that the electronic density of the substituent influences the stability of intramolecular complexes and consequently their molecular recognition ability. In the present study, we have prepared three novel indolyl-contained β-cyclodextrins (**1**–**3**, Chart 1) tethered by an oligo(ethylenediamine) moiety and examined the effect of tether length on recognizing some structurally related organic dye guests by using spectrofluorometric or spectrophotometric titrations. From the results obtained, we discuss the crucial role of tether length, especially the initial conformation of hosts in guest binding. It was also found that the stability of host–guest complex significantly depends upon the pH value of the solvent used.



1: $n = 0$
2: $n = 1$
3: $n = 2$

Chart 1

Experimental

Materials

All guest dyes, *i.e.*, Acridine Red (AR), Rhodamine B (RhB), Neutral Red (NR), Brilliant Green (BG), and Methyl Orange

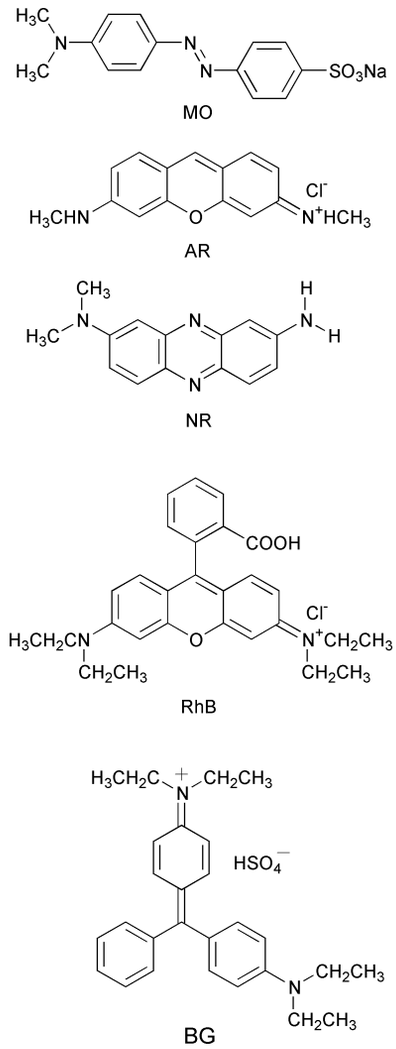


Chart 2

(MO) (Chart 2), were commercially available and used without further purification. β-Cyclodextrin of reagent grade (Shanghai Reagent Factory) was recrystallized twice from water and dried *in vacuo* at 95 °C for 24 h prior to use. *N*,*N*-Dimethylformamide (DMF) was dried over calcium hydride for two days and then distilled under a reduced pressure prior to use. Dicyclohexylcarbodiimide (DCC) was commercially available and used without further purification.

Synthesis of indolyl-β-cyclodextrin (1)

Mono[6-*O*-(toluene-*p*-sulfonyl)]-β-cyclodextrin (6-OTs-β-CD) was prepared by the reaction of tosyl chloride with β-cyclodextrin in alkaline aqueous solution according to literature reports.[19] Then, 6-OTs-β-CD was converted to mono(6-aminoethylamino-6-deoxy)-β-cyclodextrin in 70% yield on heating in excess ethylenediamine at 70 °C for 7 h.[20] To a solution of DMF (50 ml) containing 1.3 g of mono(6-aminoethylamino-6-deoxy)-β-cyclodextrin and 0.62 g of dicyclohexylcarbodiimide (DCC) was added 0.31 g of indol-3-ylbutyric acid in the presence of a small amount of 4 Å molecular sieves. The reaction mixture was stirred for 2 d in an ice bath and another 3 d at room temperature, and then allowed to stand for 1 h. The precipitate was removed by filtration and the filtrate was poured into 300 ml of acetone. The white precipitate was collected and subsequently purified on a Sephadex G-25 column with water as eluent. After the residue was dried *in vacuo*, a pure sample was obtained in 29% yield. MALDI–TOF MS *m*/*z* 1362.2 ($M + H^+ - 5H_2O$), 1383.3 ($M + Na^+ - 5H_2O$); UV/Vis λ_{max} (H_2O)/nm (log ε) 221.6 (4.33), 280.8 (3.57); ^{1}H NMR (D_2O, TMS, ppm) δ 1.8–2.2 (m, 4H), 2.6–3.1 (m, 6H), 3.**1**–**3**.9 (m, 42H), 5.1 (m, 7H), 6.9–7.2 (m, 3H), 7.3–7.6 (m, 2H); ^{13}C NMR (D_2O, TMS, ppm) δ 182.4, 136.7, 127.0, 122.1, 121.9, 119.0, 118.2, 115.1, 111.7, 102.0, 83.7, 81.1, 73.2, 72.2, 70.3, 60.3, 48.9, 46.5, 45.0, 38.1, 37.0, 27.0, 24.8; FT–IR (KBr) ν/cm^{-1} 3312.0, 2928.6, 1667.6, 1558.0, 1497.1, 1455.1, 1404.2, 1364.8, 1336.5, 1299.1, 1244.3, 1203.0, 1153.4, 1079.1, 1032.1, 943.8, 846.4, 754.5, 705.6, 577.8. Anal. Calcd for $C_{56}H_{87}O_{35}N_3 \cdot 5H_2O$: C, 46.31; H, 6.73; N, 2.89. Found: C, 46.17; H, 6.54; N, 3.17.

Synthesis of indolyl-β-cyclodextrin (2)

Compound **2** was prepared in 27% yield from indol-3-ylbutyric acid and mono[6-2-(2-aminoethylamino)ethylamino-6-deoxy]-β-cyclodextrin, according to similar procedures described above. MALDI–TOF MS *m*/*z* 1426.4 ($M + Na^+ - 5H_2O$); UV/Vis λ_{max} (H_2O)/nm (log ε) 221.8 (4.27), 281.0 (3.51); ^{1}H NMR (D_2O, TMS, ppm) δ 1.7–2.1 (m, 4H), 2.6–3.1 (m, 10H), 3.**1**–**3**.9 (m, 42H), 4.9 (m, 7H), 6.9–7.2 (m, 3H), 7.3–7.6 (m, 2H); ^{13}C NMR (D_2O, TMS, ppm) δ 182.2, 136.6, 125.7, 125.1, 121.9, 119.1, 111.7, 102.0, 83.8, 81.2, 73.2, 72.2, 68.4, 60.3, 47.4, 45.5, 38.6, 36.7, 26.8, 24.7; FT–IR (KBr) ν/cm^{-1} 3316.4, 2928.3, 1654.0, 1560.4, 1455.2, 1402.6, 1366.1, 1336.8, 1300.6, 1242.0, 1202.7, 1153.8, 1080.0, 1032.4, 943.8, 848.1, 753.7, 705.4, 577.9. Anal. Calcd for $C_{58}H_{92}O_{35}N_4 \cdot 5H_2O$: C, 46.58; H, 6.87; N, 3.75. Found: C, 46.53; H, 6.75; N, 3.75.

Synthesis of indolyl-β-cyclodextrin (3)

Compound **3** was prepared in 21% yield from indol-3-ylbutyric acid and mono-{6-2-[2-(2-aminoethylamino)ethylamino]ethylamino-6-deoxy}-β-cyclodextrin, according to similar procedures as described above. MALDI–TOF MS *m*/*z* 1469.7 ($M + Na^+ - 5H_2O$); UV/Vis λ_{max} (H_2O)/nm (log ε) 221.8 (4.28), 281.0 (3.56); ^{1}H NMR (D_2O, TMS, ppm) δ 1.7–2.1 (m, 4H), 2.5–3.0 (m, 14H), 3.1–4.0 (m, 42H), 4.8 (m, 7H), 6.9–7.6 (m, 5H); ^{13}C NMR (D_2O, TMS, ppm) δ 182.6, 136.7, 127.1, 122.2, 121.8, 118.9, 118.3, 115.2, 111.7, 102.0, 83.5, 81.2, 73.2, 72.1, 70.3, 60.3, 52.1, 49.2, 47.4, 38.9, 37.4, 27.0, 24.8; FT–IR (KBr) ν/cm^{-1} 3313.9, 2928.4, 1650.0, 1559.5, 1455.6, 1404.2, 1364.4, 1300.5, 1242.8, 1203.0, 1154.2, 1080.3, 1034.1, 943.5, 855.5, 754.4, 705.1, 579.2. Anal. Calcd for $C_{60}H_{97}O_{35}N_5 \cdot 5H_2O$: C, 46.84; H, 7.01; N, 4.55. Found: C, 47.00; H, 6.99; N, 4.59.

Measurements

CD and UV/Vis spectra were recorded in a conventional quartz cell (light path 10 mm) on a JASCO J-715S spectropolarimeter or a Shimadzu UV-2401PC spectrophotometer equipped with a PTC-348WI temperature controller to keep the temperature at 25 °C. Fluorescence spectra were measured in a conventional rectangular quartz cell (10 × 10 × 45 mm) at 25 °C on a JASCO FP-750 fluorescence spectrometer with excitation and emission slits of 5 nm. In the spectral measurements, sodium dihydrogen phosphate and disodium hydrogen phosphate were dissolved in deionized water to make a buffer solution of pH 7.2, whereas hydrochloric acid and potassium chloride were dissolved in deionized water to make a buffer solution of pH 2.0, which were used as solvent for all measurements.

Results and discussion

ICD spectra

Circular dichroism spectrometry has become a convenient and widely employed method for the elucidation of the absolute conformation of chiral organic compounds in the past three decades.[21] Achiral organic compounds can also show an induced circular dichroism (ICD) signal in the corresponding transition band in cases where there is a chiral microenvironment. Cyclodextrins, which possess inherent chiral cavities, may provide such a microenvironment for the included achiral chromophore. Some empirical rules have been proposed for the

inclusion complexation of cyclodextrins with various guest molecules.[22] In this context, we have measured the ICD spectra of β-cyclodextrin derivatives **1**–**3**, which are shown in Fig. 1 and 2.

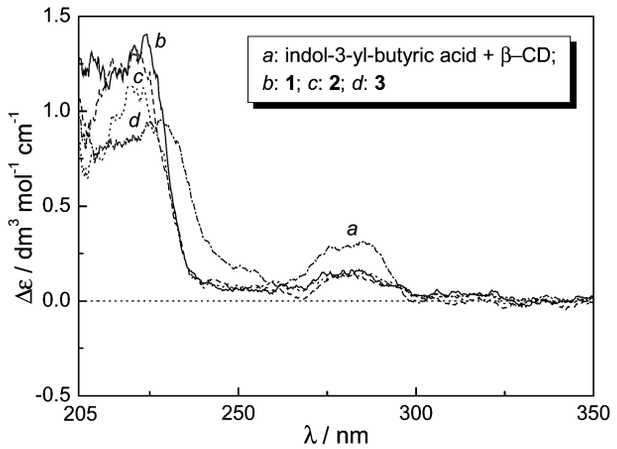


Fig. 1 Circular dichroism spectra of compounds **1**–**3** (0.1 mM) in pH 7.2 aqueous buffer solution.

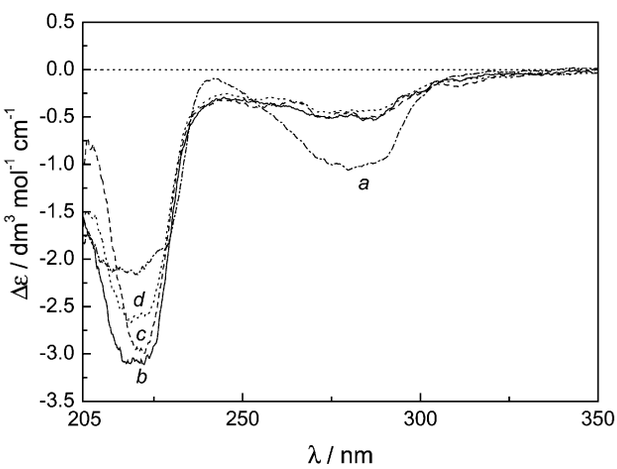


Fig. 2 Circular dichroism spectra of compounds **1**–**3** (0.1 mM) in pH 2.0 aqueous buffer solution.

As can be seen from Fig. 1, in a buffer of pH 7.2 compounds **1**–**3** afford quite similar shaped ICD spectra. Thus, these three indolyl-containing β-cyclodextrins show a weak positive Cotton effect peak around 280 nm and a moderate positive Cotton effect peak around 220 nm, respectively. As an excellent reference system, the circular dichroism spectrum of indol-3-ylbutyric acid (0.1 mM) in the presence of excess amount of β-cyclodextrin (10 mM) was also measured. Indol-3-ylbutyric acid is an achiral molecule and shows no circular dichroism signal itself. In the presence of β-cyclodextrin, however, indol-3-ylbutyric acid shows two positive Cotton effect peak around the corresponding transition bands, which indicates that there exists the interaction between the chiral cavity of β-cyclodextrin and indol-3-ylbutyric acid guest. From the above phenomena, we may conclude that the indolyl substituent in compounds **1**–**3** penetrate into the cavity to form a self-inclusion complex. The extent of self-inclusion, however, seems not very strong, estimated from the intensity of their circular dichroism signals.

Interestingly, it may be noted from Fig. 2 that in a buffer solution of pH 2.0 compounds **1**–**3** give distinctly different shapes of circular dichroism spectra as compared with those in a buffer solution of pH 7.2. As illustrated in Fig. 2, compounds **1**–**3** show a weak negative Cotton effect peaks around 280 nm and a moderate negative Cotton effect peak around 220 nm, respectively. Meanwhile, it can also be seen that in a buffer solution of pH 2.0 indol-3-ylbutyric acid shows two negative Cotton effect peak around its transition bands in the presence of β-cyclodextrin. Kodaka[23] has proposed that the induced circular dichroism signal is inversed when the position of a guest molecule is changed from the inside of the cavity to the outside and the direction of the transition moment is fixed, on the basis of a Kirkwood–Tinoco theoretical calculation. Therefore, we deduce that the chromophoric group of indol-3-ylbutyric acid is located in different positions of the cyclodextrin cavity in buffers of pH 7.2 and 2.0.

In this context, it would be essential to elucidate the conformations of compounds **1**–**3** in buffer solution at different pH values. De Rossi and co-workers[24] have systematically studied the inclusion complexation of β-cyclodextrin with several 3-substituted indole derivatives. Their results revealed that at pH 2.0 the free energy change increased linearly with the number of methylene groups for the derivatives with $(CH_2)_n$-COOH as substituent, which indicated that the alkyl chain was encapsulated in the cavity. Thus, we would propose that at pH 2.0 the alkyl chain of indol-3-ylbutyric acid was included in the cavity while the indolyl group was exposed outside; on the other hand, at pH 7.2 the aromatic moiety of indol-3-ylbutyric acid was included in the cavity, just as illustrated in Fig. 3(a).

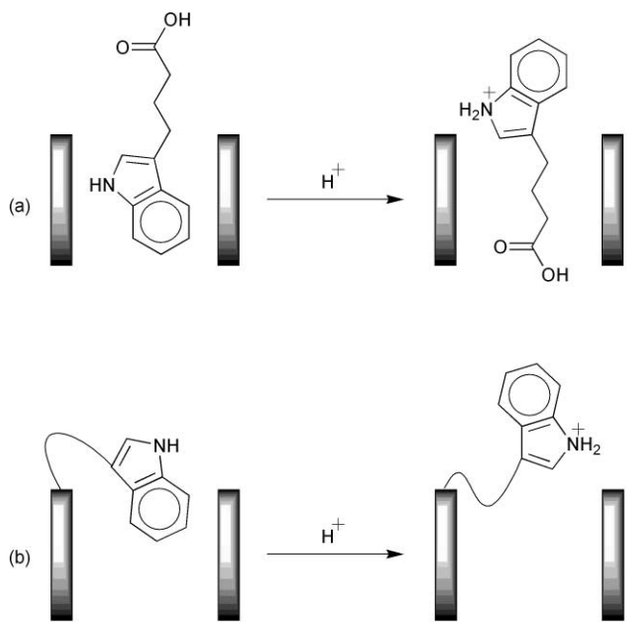


Fig. 3 Presumed inclusion modes of (a) indol-3-ylbutyric acid and β-cyclodextrin and (b) indolyl modified β-cyclodextrin in acidic and basic microenvironment.

When the indolyl group was introduced onto the rim of β-cyclodextrin through covalent bonding, the above inclusion mechanism should also be operative. As shown in Fig 3(b), the indolyl group was partially self-included in the cavity to form an intramolecular complex under slightly basic conditions, and then the aromatic moiety was exposed outside and the alkyl chain was partially self-included when the nitrogen atom in the indolyl group was protonated.

From the above speculation, we may rationally interpret the opposite ICD signals of compounds **1**–**3** in buffers of pH 7.2 and 2.0. On the basis of Kodaka's proposal, the opposite ICD spectra should be ascribed to the different conformations of host compounds, just as illustrated in Fig. 3(b). Therefore, the indolyl substituent should switch between the inside form and the outside form along with the change of the pH value of the system, in which the protonation/deprotonation of the indolyl moiety plays a key role. In fact, we have also measured the ICD spectra of compounds **1**–**3** in pH 11, and these are consistent with those measured at pH 7.2. The determination of the initial conformations of compounds **1**–**3** is essential for the further understanding of their binding ability.

NMR Spectroscopy

Two-dimensional NMR spectroscopy has recently become an important method for the investigation of not only the interaction between host cyclodextrins and guest molecules, but also the self-included mode between the cyclodextrin cavity and its substituting groups, since two protons located closely in space can produce an NOE cross-peak between the relevant protons in the NOESY or ROESY spectra. To obtain further evidence about the initial geometry of the self-included mode of cyclodextrin derivatives **1**–**3** at different pH values, ^{1}H NOESY experiments have been performed on a Varian INVOA 300 spectrometer. Fig. 4 shows a couple of representative ^{1}H NOESY spectra of **2** in buffers of pH 7.2 and 2.0, respectively. As illustrated in Fig. 4a, the NOESY spectrum of **2** displays clear NOE cross-peaks between the 5-H of cyclodextrin and aromatic protons of the indolyl group in compound **2** (peaks A), which indicate distinctly that the indolyl group in **2** is shallowly self-included in the cavity from the primary side of cyclodextrin. In contrast, no cross-peaks were observed between aromatic protons of the indolyl group and the 5-H and 3-H protons in the NOESY spectrum of **2** in a buffer of pH 2. However, as can be seen from Fig. 4b, an NOE cross-peak between the 5-H of cyclodextrin and the β-proton in the alkyl chain of the indol-3-ylbutyric substituent signified that the alkyl chain in **2** was partially included in the cavity while the indolyl group was exposed outside. Hence, the results of the NOESY experiments not only nicely coincide with that of the ICD spectra, but they also strongly support the presumed inclusion modes at different pH values as illustrated in Fig. 3b.

Spectral titration

As elucidated above, the chromophoric moiety of cyclodextrin derivatives **1**–**3** was only shallowly self-included in the cavity, therefore the ultraviolet-visible or fluorescence spectra of compounds **1**–**3** showed only a little change upon guest binding. In this context, some structurally related dye molecules were used as guests to investigate the binding ability and recognition ability of these indole-based β-cyclodextrins. As can be seen from Fig. 5 and 6, the relative fluorescence intensity of Acridine Red increased upon addition of host **2**, while that of Rhodamine B decreased upon addition of host **2**, indicating that there is an interaction between the host and guest. From Fig. 7, it can also be noted that the absorption spectra of Methyl Orange decreased when cyclodextrin hosts were added. Thus, the complex stability constants were determined by using spectrofluorometric or spectrophotometric titration methods.

Assuming the conventional 1 : 1 host : guest stoichiometry, the complexation of guest dye (Dye) with host cyclodextrin (CD) may be expressed by eqn. 1.

$$\mathrm{CD} + \mathrm{Dye} \overset{K_S}{\rightleftharpoons} \mathrm{CD \cdot Dye} \qquad (1)$$

The fluorescence spectral change (ΔI) upon addition of host, where $\Delta I = \Delta I$ (with host) $- \Delta I$ (without host), is assumed to be proportional to the concentration of inclusion complex produced, *i.e.* $\Delta I = a$[CD·Dye]. The proportionality coefficient a is taken as a sensitivity factor for the fluorescence change induced by the addition of one molar host, or a quantitative measure of the complex formation. Then, the complexation stability constant (K_S) may be obtained by using eqn. (2).[25]

$$\Delta I = \{([\mathrm{CD}]_0 + [\mathrm{Dye}]_0 + 1/K_S) - \sqrt{\alpha^2([\mathrm{CD}]_0 + [\mathrm{Dye}]_0 + 1/K_S)^2 - 4\alpha^2[\mathrm{CD}]_0[\mathrm{Dye}]_0}\}/2 \qquad (2)$$

Where $[\mathrm{CD}]_0$ and $[\mathrm{Dye}]_0$ denote the initial concentrations of host cyclodextrin and guest dye, respectively. In the case of spectrophotometric titration, eqn. (3) may be deduced.[26]

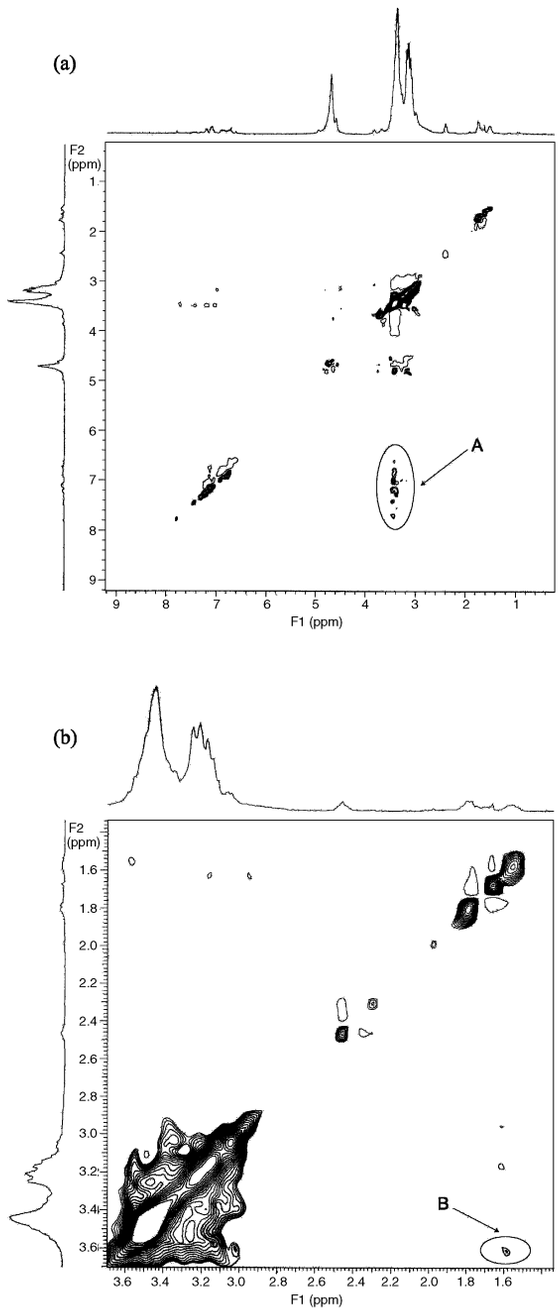


Fig. 4 ^{1}H NOESY spectra (300 MHz) of **2** in (a) pH = 7.2 and (b) pH = 2.0 buffers in D_2O at 298 K with a mixing time of 800 ms.

$$\Delta A = \{([\mathrm{CD}]_0 + [\mathrm{Dye}]_0 + 1/K_S) - \sqrt{\alpha^2([\mathrm{CD}]_0 + [\mathrm{Dye}]_0 + 1/K_S)^2 - 4\alpha^2[\mathrm{CD}]_0[\mathrm{Dye}]_0}\}/2 \qquad (3)$$

Using the nonlinear least squares curve-fitting method, we obtained the complexation stability constant for each host–guest combination from eqn. 2 or 3. Fig. 8 illustrates some representative plots of experimental and calculated data obtained by using eqn. 2—no serious deviations are observed. The excellent curve fits indicate not only that the stability constants obtained are reliable but also that the host–guest complexation by the cyclodextrin derivatives proceeds through the 1 : 1 stoichiometry. The isosbestic point observed in spectrophotometric titration further confirms the simple one-step transformation from free guest to the final 1 : 1 complex.

Complex stability constant

The stability constant (K_S) and Gibbs free energy change

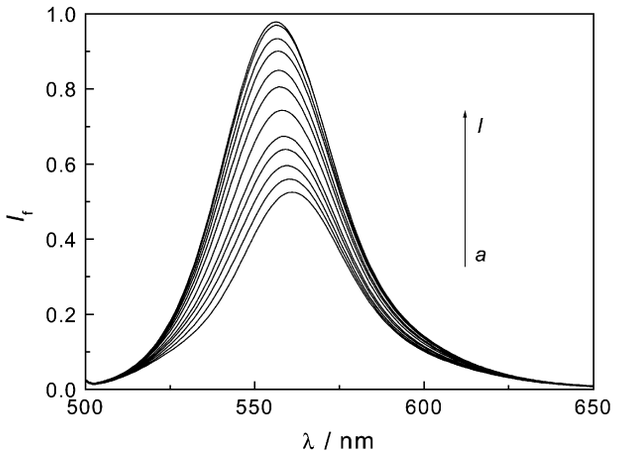


Fig. 5 Fluorescence spectral changes of phosphate buffer solution (pH 7.2) of Acridine Red (8.7 μM) in the absence and presence of host **2**. The concentration of **2** was 0–0.45 mM from *a* to *l*. The excitation wavelength was 490 nm.

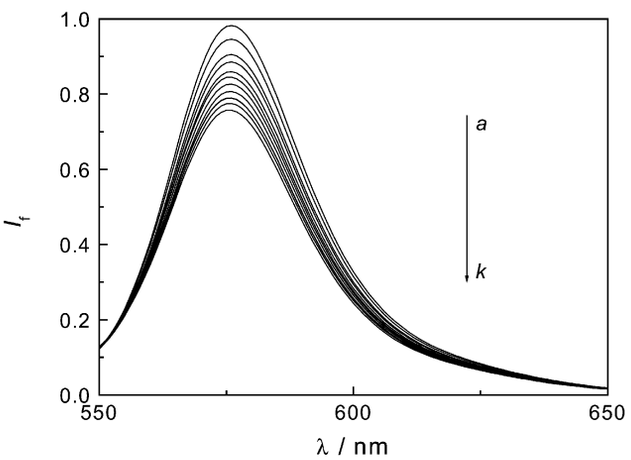


Fig. 6 Fluorescence spectral changes of phosphate buffer solution (pH 7.2) of Rhodamine B (9.3 μM) in the absence and presence of host **2**. The concentration of **2** was 0–0.45 mM from *a* to *k*. The excitation wavelength was 520 nm.

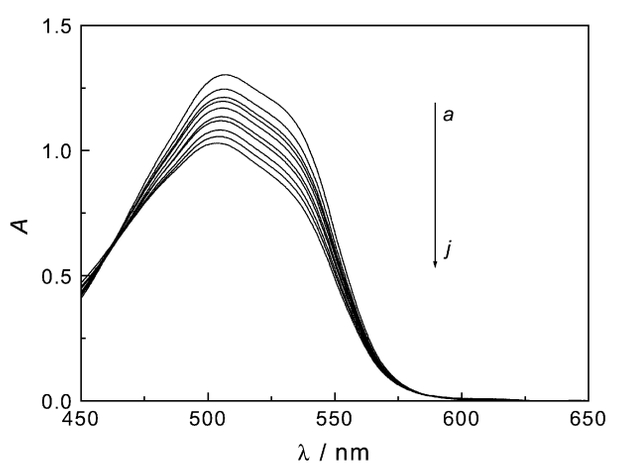


Fig. 7 Absorption spectral changes of hydrochloric buffer solution (pH 2.0) of Methyl Orange (30 μM) in the presence of modified β-cyclodextrin **1**. The concentration of compound **1** was 0–3 mM from *a* to *j*.

($-\Delta G°$) for the inclusion complexation of native β-cyclodextrin and hosts **1**–**3** with a series of dye guests are listed in Table 1. In order to visualize the inclusion complexation behavior of cyclodextrin hosts with dye guests, the changing profiles of free energy change ($-\Delta G°$) upon complexation with **1**–**3** are shown in Fig. 9 and 10, respectively.

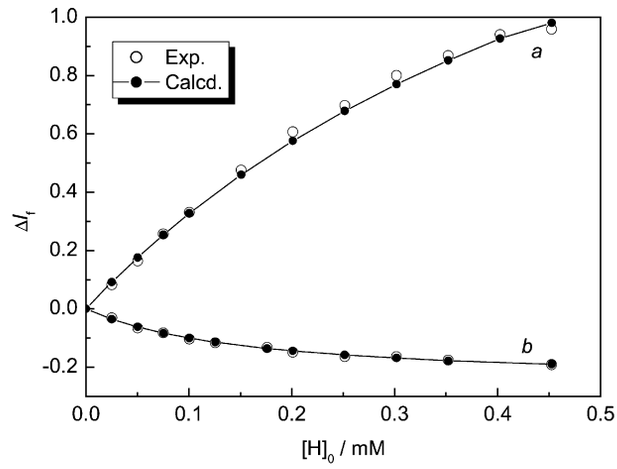


Fig. 8 Curve-fitting analyses for the complexation of host **2** with (*a*) Acridine Red and (*b*) Rhodamine B, respectively, in phosphate buffer solution (pH 7.2).

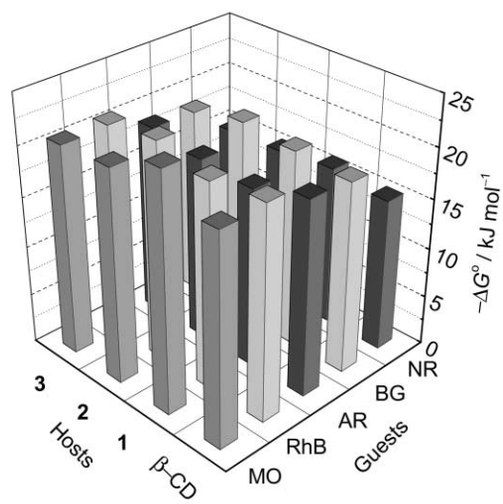


Fig. 9 Gibbs free energy changes ($-\Delta G°$) for the inclusion complexation of β-cyclodextrin and its derivatives **1**–**3** with some guest dyes at pH 7.2.

Guest structure and tether length

As can be seen from Table 1, the complexation stability constants for a certain host with dye guests are variable according to the guest structure. Therefore, it is interesting to investigate the relationship between the complex stability and guest structure. Acridine Red and Rhodamine B, both constructed by xanthene skeleton, afford significantly different binding affinity toward cyclodextrin host. We may note from the fluorescence titration spectra (Fig. 5 and 6) that Acridine Red and Rhodamine B show dramatically different fluorescence behavior upon addition of cyclodextrin host. We have previously proposed that only the lactonic form of Rhodamine B, which is non-fluorescent, participates in the complexation process.[27] Therefore, it is not surprising to note that the binding affinity of most hosts toward Acridine Red is less than that toward Rhodamine B, since the later has a stucture that is neutral and more hydrophobic.

From Table 1 and Fig. 9, we may also see that at pH 7.2 the hosts show different selectivity profiles for Acridine Red and Rhodamine B, *i.e.* **1** < **2** < **3** < β-CD for Acridine Red, and **1** < **3** ≈ β-CD < **2** for Rhodamine B. It is well known that the linear Acridine Red may be incorporated longitudinally in the cavity of β-cyclodextrin in a perfect manner. On the other hand, the extent of self-inclusion of the substituent decreases in the order of **1** > **2** > **3**, as proposed previously. Thus, the

Table 1 Complex stability constant (K_S) and Gibbs free energy change ($-\Delta G°$) for 1 : 1 inclusion complexation of various guest dyes with β-cyclodextrin and indolyl modified β-cyclodextrins (**1**–**3**) in aqueous buffer solution (pH 7.20) at 25 °C

Host	pH	Guest	K_S/M^{-1}	log K_S	$-\Delta G°$/kJ mol^{-1}
β–CD	2.0	AR	523	2.72	15.5
	7.2	AR	2630	3.42	19.5
	2.0	RhB	16700	4.22	24.1
	7.2	RhB	5100	3.71	21.2
	7.2	NR	480	2.68	15.3
	7.2	BG	2187	3.34	19.1
	2.0	MO	292	2.47	14.1
	7.2	MO	4550	3.66	20.9
1	2.0	AR	804	2.90	16.6
	7.2	AR	1390	3.14	17.9
	2.0	RhB	2270	3.36	19.2
	7.2	RhB	4120	3.61	20.6
	7.2	NR	636	2.80	16.0
	7.2	BG	2670	3.43	19.6
	2.0	MO	645	2.81	16.0
	7.2	MO	13700	4.14	23.6
2	2.0	AR	800	2.90	16.6
	7.2	AR	1630	3.21	18.3
	2.0	RhB	5800	3.76	21.5
	7.2	RhB	6780	3.83	21.9
	7.2	NR	499	2.70	15.4
	7.2	BG	3340	3.52	20.1
	2.0	MO	241	2.38	13.6
	7.2	MO	5320	3.73	21.3
3	2.0	AR	1130	3.05	17.4
	7.2	AR	2190	3.34	19.1
	2.0	RhB	4910	3.69	21.1
	7.2	RhB	5010	3.70	21.1
	7.2	NR	461	2.66	15.2
	7.2	BG	2040	3.31	18.9
	2.0	MO	277	2.44	13.9
	7.2	MO	4810	3.68	21.0

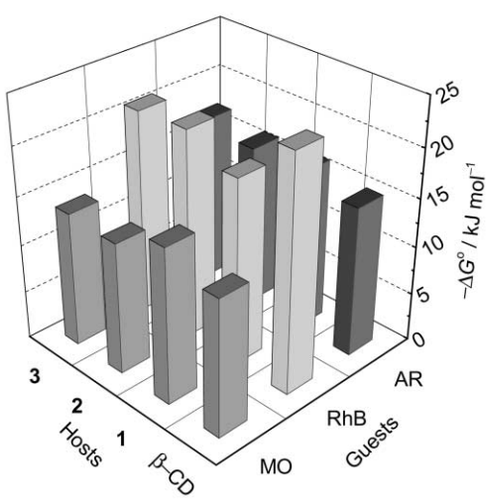


Fig. 10 Gibbs free energy changes ($-\Delta G°$) for the inclusion complexation of β-cyclodextrin and its derivatives **1**–**3** with some guest dyes at pH 2.0.

complexation stability constants of cyclodextrin hosts **1**–**3** with Acridine Red decrease in a consistent order, due to the competitive inclusion with the substituent. At the same time, native β-cyclodextrin affords the strongest complex among the four hosts, since there is no appendant on its side arm which could potentially interfere with the inclusion complexation. In the case of Rhodamine B, however, its lactonic form does not match the cavity of β-cyclodextrin perfectly and obviously size/shape-matching plays a crucial role here. As a result, the selectivity profile for Rhodamine B is fairly different from that for Acridine Red.

As can be seen from Table 1 and Fig. 9, the host selectivity profile for Brilliant Green is in the order of **3** ≈ β-CD < **1** < **2**. As a triangular molecule, Brilliant Green is very similar to Rhodamine B in structure. Topologically, only one benzene ring can penetrate into β-cyclodextrin cavity in the inclusion complexation process. It is well known that a single benzene ring could not fill the space of the β-cyclodextrin cavity. Hence, the inclusion complexation of hosts **1** and **2** with Brilliant Green is stronger than that of host **3** and native β-cyclodextrin, since an induced-fitting mechanism operates. These results indicated that the substituent could not only interrupt the inclusion complexation, but also adjust the cavity size.

The above proposal may be further verified by molecular recognition with Methyl Orange. As shown in Table 1 and Fig. 9, at pH 7.2 the host selectivity profile for Methyl Orange is in the order β-CD ≈ **3** ≈ **2** ⋘ **1**. This result is reasonable, because the cavity of β-cyclodextrin could not encapsulate the Methyl Orange molecule tightly and native β-cyclodextrin could slip onto the guest molecular chain like a bead.[28] The introduction of self-included substituents may reduce the effective cavity of β-cyclodextrin and then hold Methyl Orange much more tightly. The molecular binding ability of **2** and **3** toward Methyl Orange is similar to that of native β-cyclodextrin, probably due to the weak self-inclusion of substituents.

The selectivity profile of β-cyclodextrin and hosts **1**–**3** for Neutral Red is similar to the behavior of Methyl Orange, *i.e.* **3** ≈ β-CD ≈ **2** < **1**. Structurally, both Methyl Orange and Neutral Red possess *N*,*N*-dimethylaniline subunit. Thus, it is reasonable to believe that only this moiety participates in the inclusion complexation process, though the structures of these two guest molecules are dramatically different from each other.

Solvent effect (pH value)

Many other studies have revealed that the solvent would affect the complex stability[29,30] and even the inclusion mode[31,32] in the complexation of cyclodextrin. Thus, the polarity, the acidity and the ionic strength of the solvent must be taken into account in the molecular recognition studies with cyclodextrins as hosts. In the previous section, it has been demonstrated that indole-containing β-cyclodextrins **1**–**3** show different conformations in aqueous solution at different pH values. Therefore, it is interesting to compare the inclusion complexation stability of cyclodextrin hosts at different pH values and to reveal how the conformation influences the host binding ability.

From Table 1 and Fig. 9 and 10, it may be clearly noted that the selecitivity sequence of host compounds **1**–**3** for a certain guest molecule is basically coincident. This result should be ascribed to the fact that the tether length of **1**–**3** increases gradually, which directly determines the spatial position of the indolyl moiety relative to the β-cyclodextrin cavity. Furthermore, the Gibbs free energy changes ($-\Delta G$) at pH 7.2 for the complexation of the hosts with Acridine Red and Rhodamine B are somewhat larger than those at pH 2.0. These phenomena seem reasonable, because the amino group in the guest would be protonated in a buffer solution of pH 2.0, which may reduce the hydrophobicity of the guest itself. In the case of Methyl Orange, however, the solvent effect was remarkable, *i.e.* the differential Gibbs energy changes ($\Delta\Delta G = \Delta G_{pH2.0} - \Delta G_{pH7.2}$) are around 7 kJ mol^{-1}. As is well known, Methyl Orange would be converted to a positively charged quinoid form in acidic environment, which is much more hydrophilic than the neutral format existing in basic environment. Therefore, it may be anticipated that cyclodextrin hosts favor to complex with Methyl Orange in a basic environment. On the other hand, we may also note that compound **1** formed the most stable complex with Methyl Orange in aqueous buffer of pH 2.0, among the four β-cyclodextrin hosts. Clearly, the partially self-included substituent enhanced the interaction between host and guest and size/shape-matching played a key role.

In aqueous buffers with different pH values, a host showed substantially different molecular recognition abilities toward

Acridine Red, Rhodamine B, and Methyl Orange. In an aqueous buffer of pH 7.2, the molecular selectivity profile of host **1** is in the order MO > RhB > AR, while hosts **2** and **3** showed a selectivity profile of RhB > MO > AR. In aqueous buffer of pH 2.0, however, hosts **1**–**3** afforded the same molecular selectivity sequence of RhB > AR > MO.

From the above comparison, it can be noted that hosts **1**–**3** gave different molecular selectivity sequences in different pH systems. That is, the molecular selectivity sequence for Acridine Red and Methyl Orange was inverted when the pH was changed from 7.2 to 2.0. This result depends clearly on the transformation of the guest molecule with change of buffer. Whether in buffer of pH 7.2 or 2.0, of the three hosts the indolyl substituent of compound **1** is closest to the cavity and would interfere with the inclusion complexation most effectively. Thus, it gives a unique molecular binding ability and hence a unique molecular selectivity sequence.

Conclusions

In summary, three novel indolyl-contained β-cyclodextrin derivatives were synthesized and their molecular recognition behavior was studied using spectroscopic methods. Because the indolyl moiety may be protonated in acidic conditions, the spatial position of this substituent is different in solutions with different pH. This idea may also be extended to other conformation-changeable modified cyclodextrin systems, which would potentially provide molecular switches depending upon the acidity of microenvironment. On the other hand, the molecular recognition studies revealed that several factors greatly affect the inclusion complexation of guest dyes with cyclodextrin derivatives, including not only the shape/charge/substituent of the guest and the tether of the host but also the critical changes in host–guest interactions such as hydrophobic, van der Waals and electrostatic interactions as well as the size/shape-matching and induced-fit mechanisms. Because the pH of the system would affect both the host conformation and the guest structure, the host–guest inclusion complexation behavior depends on the acidity of the aqueous buffer. Thus, this method is a convenient and powerful tool for controlling the molecular binding ability and relative molecular selectivity of cyclodextrins—altering the conformation/structure of the modified cyclodextrins or guest molecules upon inclusion complexation—by simply changing the pH value of the solution. Further studies should consider the solvent itself when discussing the molecular recognition process of cyclodextrin hosts.

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