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Fluorescence enhancement of guests by the formation of inclusion complexes with *p*-tert-butylcalix[8]arene bearing polyoxyethylene chains in aqueous solution

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Abstract

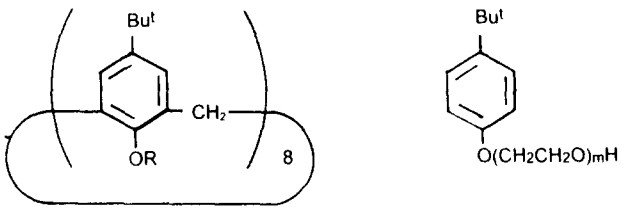
The reaction of *p*-tert-butylcalix[8]arene with ethylene oxide gives a water-soluble host compound bearing polyoxyethylene chains (C3). The polarity of the *p*-tert-butylcalix[8]arene cavity was determined by fluorescence measurements using pyrene (Py) as a probe. It was found that C3 has a hydrophobic cavity, with a polarity similar to that of 1-butanol, and can form host–guest complexes with Py, 1-anilino-8-naphthalenesulphonate (ANS) and *N*-phenyl-naphthylamine (NPN) in aqueous solution. A pronounced fluorescence enhancement of the guests on inclusion was observed. The results reveal that C3 can shield the guests from quenchers or the bulk solvent and can protect against the quenching of their excited states.

Keywords: Fluorescence enhancement; Guests; Host–guest complexes

1. Introduction

Considerable interest has recently been shown in calixarenes [1,2], which exhibit the ability to form inclusion complexes with organic molecules and ions. Water-soluble calixarenes are of great interest because of their potential to act as enzyme mimics. Several calixarene derivatives, such as *p*-tert-butylcalix[4]arene tetracarboxylic acid [3], sulphonated calixarenes [4–6], *p*-(diallylaminomethyl)-calixarenes and *p*-(2-carboxyethyl)calixarene [7,8] have been reported to dissolve in water. However, they are all ionic compounds and can only dissolve in potassium carbonate or hydrochloric acid aqueous solutions. Some time ago, Cornforth et al. [9,10] synthesized polyoxyethylated *p*-tert-octylcalix[4]arenes and investigated their anti-tuberculosis properties. Recently, we have synthesized a new water-soluble host, *p*-tert-butylcalix[8]arene bearing polyoxyethylene chains (C3), consisting of a hydrophobic calixarene cavity and flexible hydrophilic chains; this compound can provide a hydrophobic microenvironment and can accept organic molecules and ions in aqueous solution [11,12].

In this study, the absorption and fluorescence emission spectra of pyrene (Py), 1-anilino-8-naphthalenesulphonate (ANS) and *N*-phenyl-naphthylamine (NPN) in aqueous solutions of calixarenes bearing polyoxyethylene chains (see Scheme 1) were measured in order to determine the behaviour of the guest molecules in their ground and excited states on inclusion.



C1: R = H
C2: R = K
C3: R = $(CH_2CH_2O)_nH$

C4

Scheme 1.

2. Experimental details

2.1. Materials

Py (Fluka, A.R.) was purified by vacuum sublimation and then recrystallized from ethanol. ANS was kindly provided

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by Professor C. Tung. NPN was of analytical grade (Beijing Chemicals) and was recrystallized from ethanol. Potassium iodide (KI, AR) was used as supplied. The water used was doubly distilled over potassium permanganate.

Compound C1, obtained according to the method of Gutsche et al. [1], was treated with eight equivalents of potassium tert-butoxide in dry toluene and refluxed for 8 h to afford C2 after removal of the solvent. C2 was placed in a 250 ml stainless steel autoclave and purged with dry nitrogen; ethylene oxide was added, and the autoclave was heated at 170–180 °C in an oil bath until the pressure fell to zero. Potassium ions in the product (C3) were removed by the addition of ion exchange resins.

For comparison, Triton X-100 and polyoxyethylene- *p*-tert-butylphenol ether (C4) were used as reference compounds.

2.2. Measurements

IR spectra were determined on a Perkin–Elmer 983G spectrometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker WH-500 spectrometer. The absorption spectra and difference spectra were recorded using 1 cm path length cuvettes on a Hitachi UV-557 spectrophotometer at ambient temperature. The fluorescence spectra were measured on a Hitachi MPF-4 fluorescence–phosphorescence spectrophotometer using 1 cm × 1 cm quartz cuvettes at ambient temperature. Fluorescence yields were obtained by comparison of the emission spectral areas observed on a Perkin–Elmer LS-5 fluorescence spectrophotometer with data processing using a Perkin–Elmer 3600 data station. Samples were not deoxygenated.

3. Results and discussion

3.1. Characterization of C3

C3 is soluble in water and its molecular structure was confirmed by IR and ^{1}H NMR spectrometry. IR (KBr): 3456 cm^{-1} (ν_{OH}); 1604 cm^{-1} ($\nu_{C=C}$ of Ar); 1112 cm^{-1} ($\nu_{as(C-O-C)}$). ^{1}H NMR ($CDCl_3$): δ 6.85 (b s, 16, ArH); 4.01 (s, 16, $ArCH_2Ar$); 3.75 (s, 8, OH); 3.63 (s, 320, OCH_2CH_2O); 0.95–1.30 (m, 72, $C(CH_3)_3$). The average length of the polyoxyethylene ether chain is about 10 segments. It can be detected by UV spectroscopy whether or not the free phenolic groups of C2 have reacted with ethylene oxide completely. In potassium hydroxide alcohol, any free phenolic group gives phenoxide ions absorbing strongly at 300 nm, whereas the phenolic ethers show a characteristic double peak around 270–280 nm in neutral or alkaline solution [9]. The absorption spectra of C3 show that there are no free phenolic groups (Fig. 1).

The fluorescence emission and excitation spectra of C3 in aqueous solution are shown in Fig. 2. C3 has a fluorescence emission peak at 310 nm and an excitation peak at 280 nm.

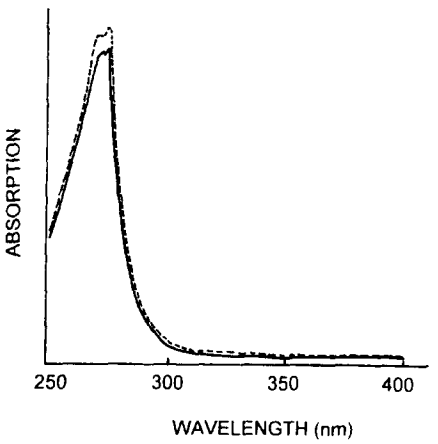

Fig. 1. Absorption spectra of C3 in water (full line) and potassium hydroxide alcohol (broken line); [C3] = 1.0×10^{-4} mol dm^{-3}; [KOH] = 1.0 mol dm^{-3}.

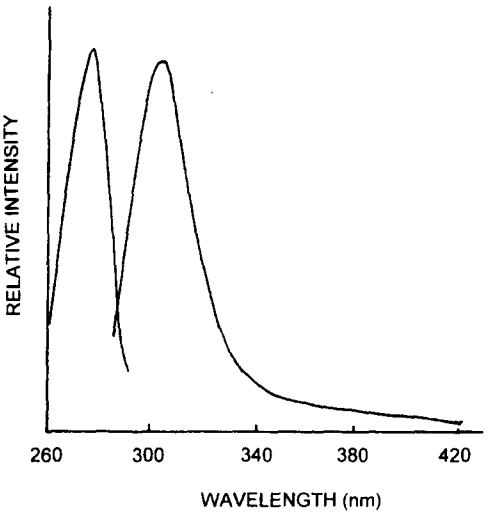

Fig. 2. Fluorescence emission and excitation spectra of C3 in aqueous solution: (a) excitation, λ_{em} = 310 nm; (b) emission, λ_{ex} = 280 nm.

3.2. Polarity of calixarene cavity

The polarity of the calixarene cavity may have a specific influence on complex formation. It is therefore important to determine the nature of the environment inside the calixarene cavity. Py monomer emission shows a strong solvent dependence and is widely used as a polarity probe in microheterogeneous media: a marked change in the ratio between the first (0–0) and third emission bands (I_{III}/I_I ratio) is observed [13]. The peak ratio (I_{III}/I_I) increases with decreasing dipole moment of the solvent. It is small in highly polar solvents (0.63 in water) and becomes larger in low polarity or nonpolar solvents (1.65 in *n*-hexane). Py was employed as a probe of the polarity of the cavity of C3. It can be seen from Fig. 3 that the I_{III}/I_I ratio of Py in aqueous solution increases with increasing C3 concentration and reaches 1.0–1.02 at a C3 concentration of approximately 1.5×10^{-4} mol dm^{-3};

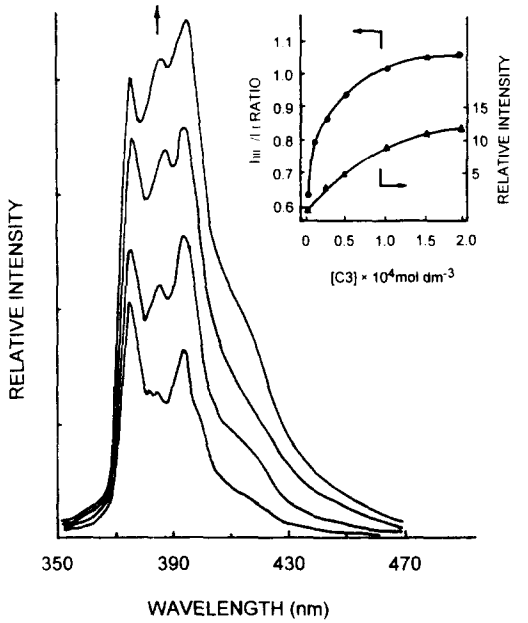

Fig. 3. Fluorescence emission, intensity ratio I_{III}/I_I (●) and relative intensity (▲) for Py with C3; $[Py] = 5 \times 10^{-7}$ mol dm^{-3}, $\lambda_{ex} = 336$ nm.

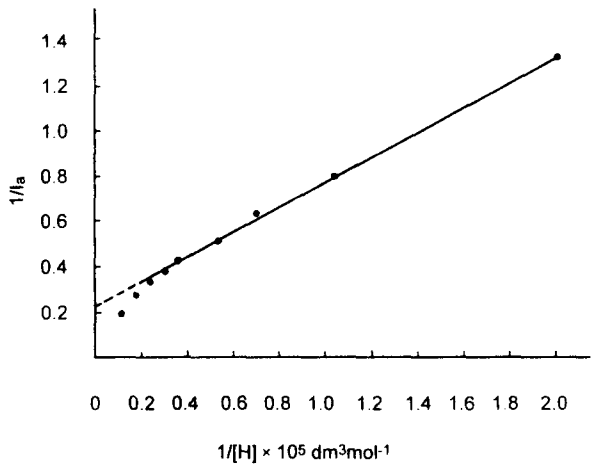

Fig. 4. Plot of $1/I_a$ vs. $1/[H]$ for the Py–C3 inclusion complex in aqueous solution from the Benesi–Hildebrand equation; $\lambda_{ex} = 336$ nm; $\lambda_{em} = 392$ nm.

the I_{III}/I_I ratio is only 0.76 in Triton X-100. These results indicate that the hydrophobicity of the *p*-tert-butylcalix[8]arene cavity is close to those of 1-butanol (0.98) and *p*-xylene (1.00). The Py monomer fluorescence intensity also increases markedly on addition of C3 in aqueous solution. This suggests that the host eliminates quenchers, such as water and oxygen, from the surroundings of Py as a result of encapsulation.

The stoichiometry and binding constant of the calixarene–Py complex were obtained according to the Benesi–Hildebrand equation assuming the formation of a 1 : 1 host–guest complex [14]

$$\frac{1}{I_a} = \frac{1}{I_c[G]} + \frac{1}{I_c[G]}\frac{1}{K}\frac{1}{[H]}$$

where I_a is the extent of fluorescence intensity change on addition of the host, I_c is the difference in fluorescence intensity between the complex and guest and [G] and [H] are the total concentrations of guest and host molecules respectively.

If the stoichiometry is 1:1, a linear plot should be obtained. Fig. 4 depicts a plot of $1/I_a$ vs. $1/[H]$. Good linear correlations of $1/I_a$ vs. $1/[H]$ were obtained from the fluorescence spectra of Py with a change in C3 concentration from 5×10^{-6} to 3.0×10^{-5} mol dm^{-3}. This shows that a 1:1 host–guest inclusion complex is formed between C3 and Py. The binding constant is about 5.1×10^4 dm^3 mol^{-1} (correlation coefficient $r = 0.99$) calculated from the plot using the least-squares method. When the C3 concentration is increased, the plot of $1/I_a$ vs. $1/[H]$ deviates from linearity, suggesting that C3 and Py tend to form complexes with higher stoichiometric ratios than 1:1.

3.3. ANS interaction with C3

The addition of progressively increasing amounts of C3 to ANS in water results in a red-shifted absorption (Fig. 5). The absorption peak at 265 nm shifts to 272 nm, and the extinction coefficient decreases slightly. In addition, the broad absorption maximum at 350 nm is red shifted to 370 nm. The appearance of two isosbestic points at 305 and 355 nm indicates that a C3–ANS inclusion complex has been formed in the ground state and the stoichiometry is 1:1.

ANS shows very weak fluorescence in water with a quantum yield as low as 0.004 and an emission maximum (λ_{max}) at 515 nm; however, in organic solvents, such as ethanol, ANS exhibits very strong fluorescence with an emission maximum at 468 nm [15]. Fig. 6 shows that the fluorescence of ANS increases markedly on addition of C3. The quantum yield increases over 40-fold and λ_{max} shifts from 515 nm to 460 nm. In the case of C4 (concentration, approximately 10^{-4} mol dm^{-3}), the quantum yield only increases twofold

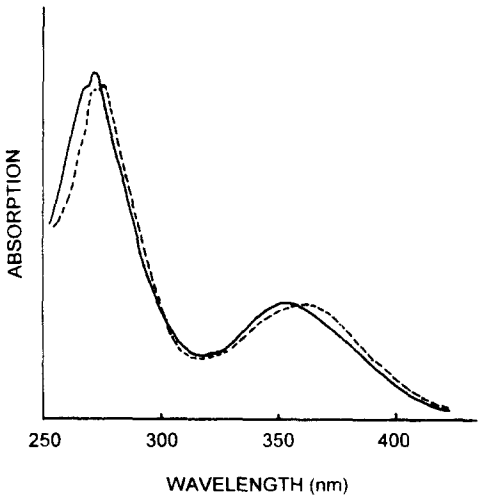

Fig. 5. Absorption spectra of 1.0×10^{-5} mol dm^{-3} of ANS (full line) and ANS complexed by C3 (broken line). The absorption spectrum of ANS complexed with C3 was obtained as a difference spectrum (ANS–C3 complex vs. C3); $[C3] = 1.0 \times 10^{-5}$ mol dm^{-3}.

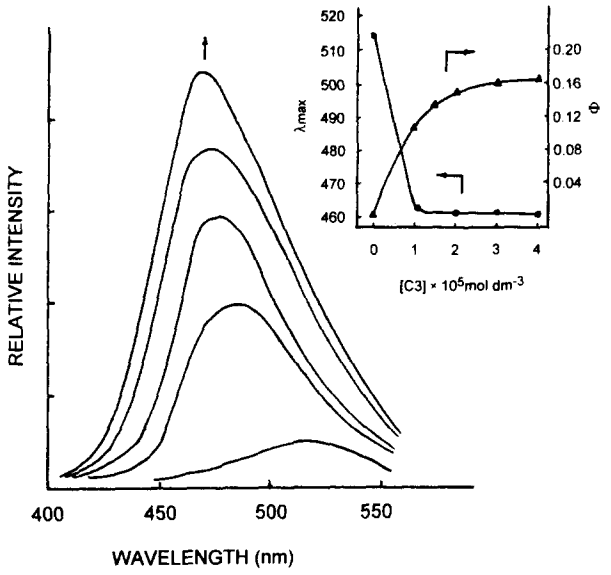

Fig. 6. Fluorescence emission maxima (●) and quantum yields (▲) of ANS vs. C3 concentration in aqueous phosphate buffer solution (pH 7.0); [ANS] = 1.0×10^{-5} mol dm^{-3}, λ_{ex} = 280 nm.

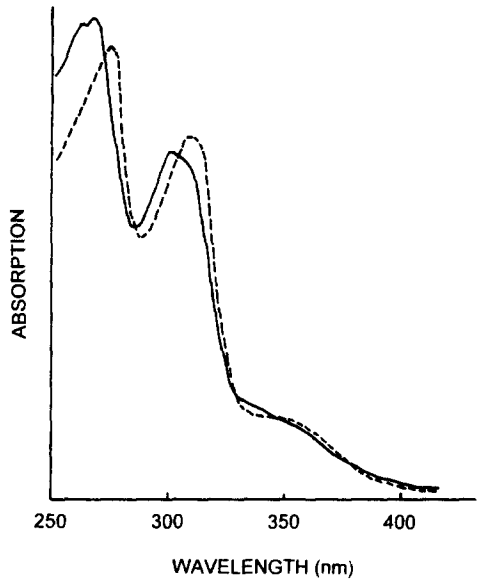

Fig. 7. Absorption spectra of 1.0×10^{-5} mol dm^{-3} of NPN (full line) and NPN complexed by C3 (broken line). The absorption spectrum of NPN complexed with C3 was obtained as a difference spectrum (NPN–C3 complex vs. C3); [C3] = 1.0×10^{-5} mol dm^{-3}.

and λ_{max} is slightly blue shifted. This suggests that ANS is located in the hydrophobic *p*-tert-butylcalix[8]arene cavity and forms an inclusion complex. The excited states of ANS decay by rotation of the anilino group around the *N*-naphthyl bond towards an emissive "twisted intramolecular charge transfer" state. Due to the polar nature of the excited state, the λ_{max} value depends strongly on the polarity of the medium. Since the microenvironment of the *p*-tert-butylcalix[8]arene cavity is similar to that of 1-butanol, when ANS is included in the cavity the rotational freedom of the guest is restricted and the energy loss caused by molecular motion decreases; the radiative rate increases and the quenching by water of the excited guest molecule decreases; the fluorescence efficiency of ANS increases. The equilibrium constant of ANS with C3 according to the Benesi–Hildebrand equation is about 3.5×10^4 dm^3 mol^{-1}.

3.4. Fluorescence enhancement of NPN by inclusion complexation with C3

NPN shows strong fluorescence enhancement and a blue shift in hydrophobic environments and is widely used in fluorescence polarization studies [16]. There is a substantial change in the absorption spectrum of NPN on inclusion (Fig. 7). The absorption peaks at 266 and 304 nm exhibit a red shift of about 10 nm in aqueous solution on addition of C3. The isosbestic points at 300 and 332 nm indicate a 1:1 equilibrium between NPN and C3 and inclusion complexation in the ground state.

It can be seen from Fig. 8 that the emission maximum of NPN in aqueous solution shifts from 446 to 410 nm on addition of C3, and the fluorescence increases by 15-fold at 4.0×10^{-5} mol dm^{-3} of C3. However, the emission maximum of NPN shows a smaller shift (about 10 nm) and the intensity shows no detectable change on addition of C4 (concentration, approximately 1.0×10^{-3} mol dm^{-3}). These data imply that NPN is well shielded from the surrounding water molecules and thus must be included in the hydrophobic cavity of C3. In view of the concentration ratio of the host and guest at the break point in the curve, it is assumed that one host molecule includes one NPN molecule. A Benesi–Hildebrand plot based on 1:1 host–guest complexation is linear, confirming the 1:1 stoichiometry, and the formation constant for inclusion of NPN by C3 is about 1.4×10^5 dm^3 mol^{-1}.

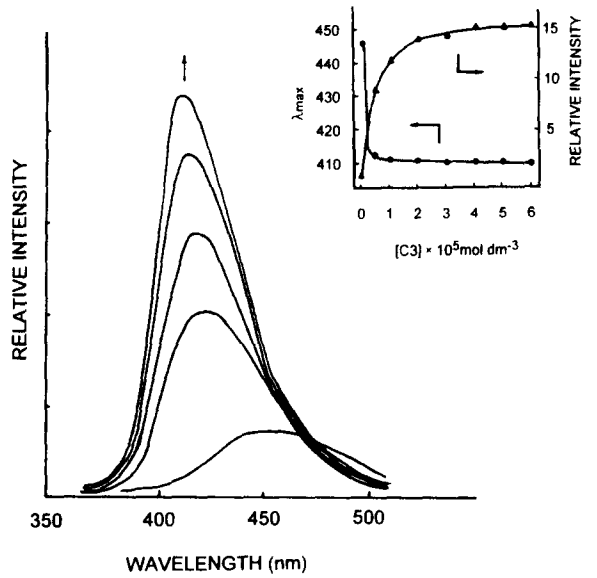

Fig. 8. Fluorescence emission maxima (●) and relative intensity (▲) of NPN at different concentrations of C3 in aqueous solution (phosphate buffer solution, pH 7.0); [NPN] = 5.0×10^{-6} mol dm^{-3}; λ_{ex} = 310 nm; λ_{em} = 410 nm.

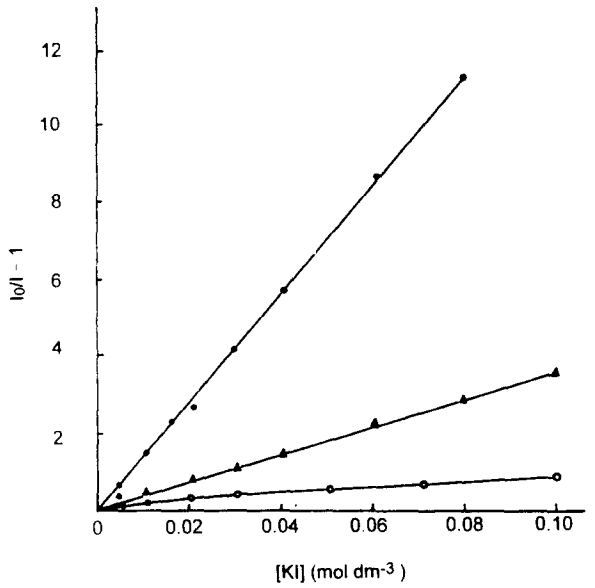

Fig. 9. Stern–Volmer plots of I_0/I vs. [KI] in the absence (●) and presence of C3 (○) and C4 (▲) in aqueous solution; [Py] = 5×10^{-7} mol dm^{-3}; λ_{ex} = 336 nm; λ_{em} = 492 nm; [C3] = 4.0×10^{-5} mol dm^{-3}; [C4] = 3.0×10^{-4} mol dm^{-3}.

ANS and NPN have a similar molecular structure and size, but NPN is more hydrophobic than ANS. The binding constant for the inclusion complex of C3 with NPN is one order of magnitude larger than that for ANS with the same host. This demonstrates that C3 binds more effectively with hydrophobic molecules than hydrophilic molecules in aqueous solution.

3.5. Quenching of Py fluorescence by KI

In order to obtain a better insight into host–guest complexation, the quenching of Py fluorescence by KI was studied in the presence and absence of host. Fig. 9 shows the Stern–Volmer plots of I_0/I vs. the concentration of KI in the absence and presence of C3 and C4. It can be calculated from the plots that the Stern–Volmer constant in the presence of C3 is much smaller than that obtained in the presence of C4 or in the absence of C3. The fluorescence of Py quenched by KI produces a structureless spectrum with a λ_{max} value around 400 nm due to charge transfer interaction. The large decrease in the quenching efficiency observed in C3 aqueous solution may be caused by the complexation of Py with C3.

4. Conclusions

p-tert-Butylcalix[8]arene bearing polyoxyethylene chains can provide a hydrophobic microenvironment and forms host–guest inclusion complexes with organic molecules and ions in aqueous solution. The guest fluorescence of Py, ANS and NPN increases markedly on inclusion. This host molecule has potential application for the enhancement of fluorescence analysis sensitivity in aqueous solution.

Acknowledgement

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