Interaction of Sulfonated Calix[*n*]arenes with Rhodamine B and Its Application to Determine Acetylcholine in a Real Neutral Aqueous Medium

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Complexation between Rhodamine B (RB) and sulfonated calix[*n*]arenes (SC*n*A) were studied by means of UV-vis spectroscopy and fluorescence spectroscopy. In the presence of sulfonated calix[*n*]arenes, the absorption band of Rhodamine B shifts to longer wavelength and its intensity decreases. The formation of a host-guest type complex also results in the fluorescence quenching of Rhodamine B. The association constants for the RB/SC*n*A complexes increase in the order of SC4A < SC6A < SC8A and show dependence on the size of the cavities of the calixarenes. The fluorescence is selectively regenerated by adding acetylcholine. Based on this observation, a method to determine acetylcholine in a real neutral aqueous medium was developed.

Keywords sulfonated calix[*n*]arene, Rhodamine B, host-guest complex, artificial-signaling receptor

Introduction

Calixarenes have received increasing attention during the last decades due to their ability to form host-guest type complexes with a variety of organic or inorganic compounds and their ready accessibility as well.[1-3] Sulfonated calix[*n*]arenes (SC*n*A, $n = 4, 6, 8$) can form inclusion complexes with guest compounds such as quaternary ammoniums,[4-7] cationic dyes[8,9] and neutral dyes[10] in aqueous solution or in solid state. It is usually considered that the hydrophobic attraction,[4] electrostatic force and the so called "cation-π" interaction[9] are responsible for the complex formation.

Acetylcholine [AC, $CH_3COCH_2CH_2N(CH_3)_3^+X^-$, X = Cl, Br or I] is one of the most abundant neurotransmitters in nerve cells but no reliable methods are currently available for the chemical transformation of AC to its fluorescent derivatives in the presence of other neurotransmitters. Inouye *et al.*[11] first designed an elegant artificial-signaling AC receptor system in which resorcin[4]arene (R4A) includes a cationic reporter molecule (a pyrene-substituted pyrydium salt, PSP) in alkali protic media. The PSP fluorescence was quenched when it entered the cavity of R4A but was regenerated by adding AC. The substitution of PSP by AC and the regeneration of the fluorescence of PSP is illustrated in Scheme 1.

Koh *et al.*[12] pointed out this system has some disadvantages, because both AC and PSP are susceptible to nucleophiles and decompose easily under alkali conditions. They employed sulfonated calix[6]arene (SC6A) instead of R4A to examine the transformation of PSP by AC and confirmed that it meets the requirement for the time-dependent monitoring of AC. But Koh's system still has some disadvantages. The reporter molecule PSP is not commercially available. In order to suppress its aggregation in an aqueous solution, a water/methanol ($V/V = 1:1$) medium at pH = 8 was employed.

Rhodamine B (RB) is a well-known fluorescent cationic dye. It is expected that RB can also be included in the cavity of the sulfonated calix[*n*]arenes with the quenching of its fluorescence. It would be interesting if RB can be used as a reporter molecule in the detection of

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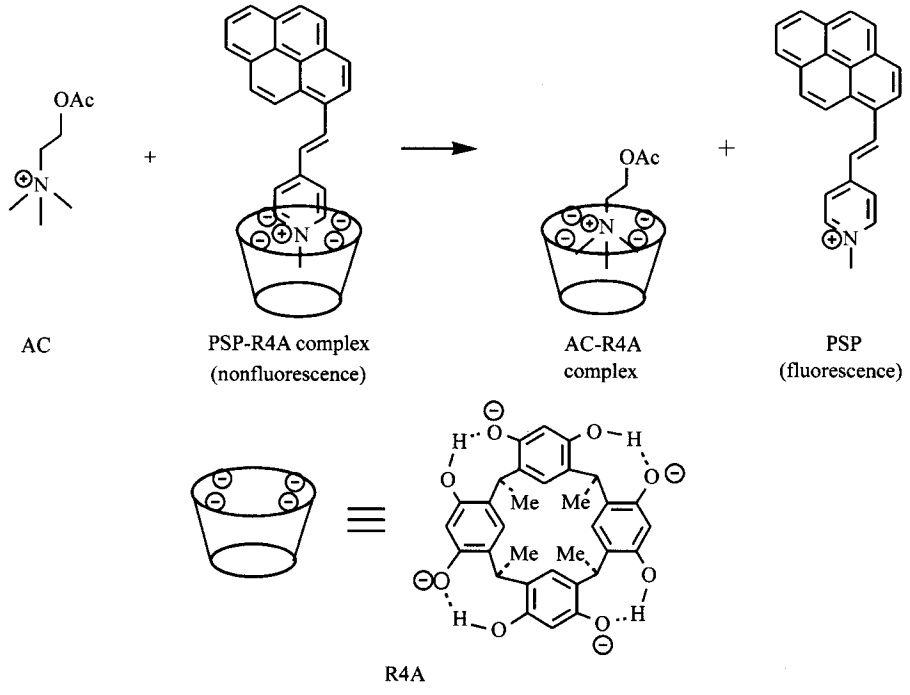
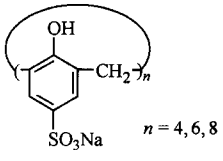
Scheme 1 Schematic illustration of Inouye's design

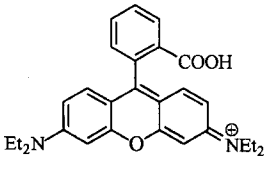


AC because it is commercially available, cheap, and does not aggregate in neutral aqueous solution.

In this article we report that RB fluorescence is quenched by SC*n*A as a result of the inclusion complex formation but re-generates when AC is added. The structural formulae of SC*n*A and RB are illustrated as follows:



SC*n*A



RB

Experimental

p-Tert-butylcalix[*n*]arene (*n* = 4, 6, 8) were prepared according to the method reported by Gutsche *et al*.[13] Then they were sulfonated directly in a method similar to that of Shinkai *et al*.[14] The resulting *p*-sulfonated calix[*n*]arenes were identified spectroscopically. Sodium *p*-hydroxybenzenesulfonate (HBS), Rhodamine B and acetylcholine chloride(Acros)were used as received.

UV-vis absorption spectra were recorded on a Shimadzu UV2100 spectrophotometer. Fluorescence spectra were recorded on a Hitachi F-4500 fluorescence spectrophotometer. Before the florescence measurement, the samples were deoxygenated by bubbling nitrogen for 10 minutes. All titration experiments are repeated for at least 3 times.

Results and discussion

The UV-vis spectra of RB in different concentrations of SC*n*A are shown in Fig. 1. The experiment was repeated 3 times, and the same result was obtained each time. In Fig. 1 a somewhat red shift of λ_{max} and decrease in the absorption intensity were observed. The red shift of the λ_{max} can be ascribed to the alteration in the energy difference between the ground and excited states of RB resulting from the formation of an inclusion complex. A clear isosbestic point observed in all cases indicates that only one complex was formed.

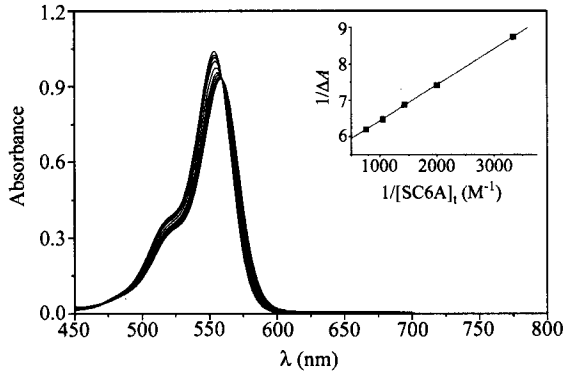


Fig. 1 UV-vis spectra of RB with different concentration of SC6A (0 to 7.2×10^{-4} M, from top to bottom). The inserted one is a double reciprocal plot. [RB] = 1×10^{-5} M in aqueous solution (pH = 6.6, 0.02 M phosphate buffer).

Based on the assumption of forming a 1:1 inclusion complex, according to Benesi-Hildebrand equation,[15] when $[SCnA]_t \gg [RB]_t$ (where t refers to total concentration), the change in absorbance (ΔA) is a function of $[SCnA]_t$:

$$\frac{1}{\Delta A} = \frac{1}{K_a \cdot \Delta\varepsilon \cdot [RB]_t \cdot [SCnA]_t} + \frac{1}{\Delta\varepsilon \cdot [RB]_t}$$

where K_a represents the association equilibrium constant and $\Delta\varepsilon$ the difference in the molar extinction coefficients between the complexed and free RB. The double reciprocal plots are satisfactorily linear, and K_a values can be obtained by dividing the resulting intercept by the slope. As shown in Table 1, K_a values increase in the order of SC4A < SC6A < SC8A, which indicates that for a large guest molecule, sulfonated calix[n]arene with larger cavity can accommodate the guest better and also possesses larger association constants.

The fluorescence of RB can be quenched by sulfonated calix[n]arene. As a control experiment, HBS, the monomeric unit of SCnA, has only a limited effect on the fluorescence of RB (Fig. 2). The result clearly indicates that the quenching effect of SCnA on RB is mainly originated from their inclusion complexation of RB, not just the simple effect of sulfonate groups. As shown in Fig. 2, the plot of I/I_0 (where I_0 and I represent the fluorescence intensity of RB without and in the presence of SCnA respectively) against $[SCnA]/[RB]$ is a typical saturation curve, indicating that the excited state of RB is quenched in a pseudo-intramolecular manner.[12] Liu *et al.* pointed out the polarity or hydrophilicity around the dye molecule that resulted from the hydrogen bonding and electrostatic interaction afforded the quenching effect.[8] The fluorescence intensity I is proportional to the concentration C of the fluorophore:[8]

$$I = f \cdot C$$

where f is the molar fluorescence intensity. Assuming the formation of a 1:1 inclusion complex, under the condition of $[SCnA]_t \gg [RB]_t$, Benesi-Hilderbrand type relationship between the change in fluorescence intensity ΔI and the total concentration of SCnA ($[SCnA]_t$) can be drawn:

$$\frac{1}{\Delta(I/I_0)} = \frac{1}{K_a \cdot \Delta f \cdot [RB]_t \cdot [SCnA]_t} + \frac{1}{\Delta f \cdot [RB]_t}$$

where $\Delta(I/I_0)$ and Δf represent the changes in relative fluorescence intensity and molar fluorescence intensity, respectively. As shown in Fig. 3, satisfactorily linear double reciprocal plots are also received, and the association constant K_a can be obtained.

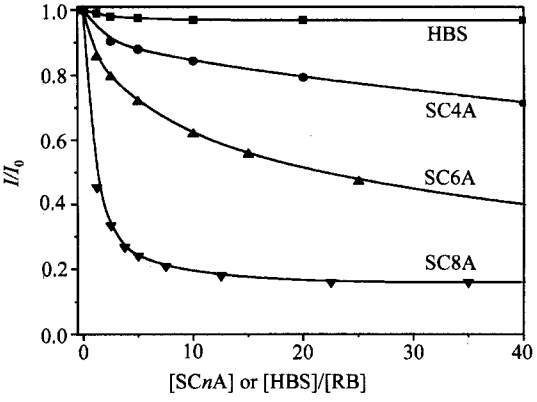


Fig. 2 Relative fluorescence intensity of RB in the presence of various concentration of SCnA or HBS. [RB] = 1×10^{-5} M (in 0.02 M pH = 6.6 phosphate buffer), λ_{ex}: 400 nm, λ_{em}: 581 nm.

K_a values obtained from the two methods were summarized in Table 1. The K_a values obtained from the fluorescence and UV-vis method are in good agreement for SC6A and SC8A. But for SC4A, the K_a value from the fluorescence method is 3 times larger than that from UV-vis method. The reason is not clear yet. A reasonable explanation may be that RB does not form an inclusion in

the SC4A cavity as deep as it does in the larger cavities of SC6A and SC8A, and the UV-vis absorption is not as sensitive toward the inclusion depth as compared with the fluorescence.

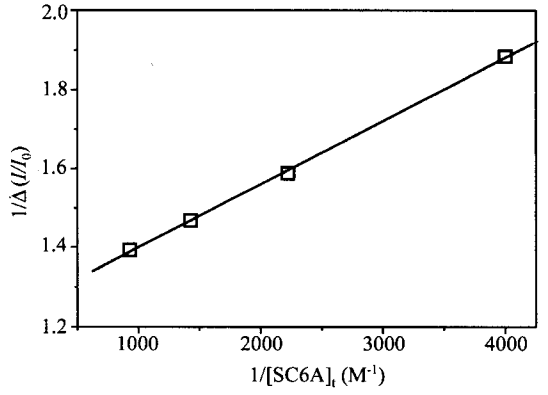


Fig. 3 Double reciprocal plot of $1/[\Delta(I/I_0)]$ against $1/[SC6A]_t$.

Table 1 The association constants K_a (M^{-1}) of RB with SC*n*A measured by UV and fluorescence methods

	SC4A	SC6A	SC8A
By UV method	0.55×10^3	5.57×10^3	1.75×10^5
By fluorescence method	2.01×10^3	7.74×10^3	1.41×10^5

It is known that quaternary ammoniums, such as acetylcholine, can be included in the cavities of sulfonated calix[*n*]arene in the aqueous solution owing to the "cation-π" interaction with a large association constant.[12] If acetylcholine is added to the RB-SC*n*A solution, RB may be substituted and as a result its fluorescence will be regenerated. SC8A was chosen for this purpose because it quenches RB fluorescence most effectively. Fig. 4 shows that the fluorescence intensity of the system increases with increasing amounts of acetylcholine in the solution. In contrast, adding amino acids has no effect on the fluorescence intensity (Fig. 4), which indicates that amino acids cannot compete with RB for inclusion within the cavity of SC8A.

Conclusion

In conclusion, RB can be included in the cavities of sulfonated calix[*n*]arene (SC*n*A, $n = 4, 6, 8$) resulting in a bathochromic shift and a decrease in the intensity of its absorption spectrum. The fluorescence of RB is quenched by adding SC*n*A due to the formation of an inclusion complex between RB and SC*n*A. The association constant of the RB/SC*n*A complexes increases in the order of SC4A < SC6A < SC8A, and shows dependence on the size of the cavity of the calix[*n*]arenes. RB acts as an artificial-signaling acetylcholine reporter due to its selective fluorescence regeneration following the addition of acetylcholine to an SC8A-RB solution. The advantages of this system are that (1) a real neutral aqueous medium is employed and (2) RB is a commonly available reagent.

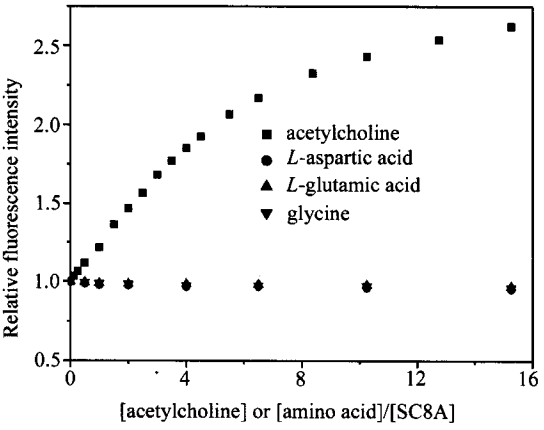


Fig. 4 The fluorescence intensity of RB changes with the molar ratio of [AC]/[SC8A] or [amino acid]/[AC8A]: [RB] $= 1.0\times10^{-5}$ M, [SC8A] $= 1.0\times10^{-4}$ M. Other conditions are the same as those in Fig. 2.

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