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Yuling Zhang[a]; Rezik A. Agbaria[ab]; Isiah M. Warner[a]
[a] Department of Chemistry, Louisiana State University, Baton Rouge, LA [b] School of Medicine, Biological Chemistry, Center for Fluorescence Spectroscopy, University of Maryland at Baltimore, Baltimore, MD

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Complexation Studies of Water-soluble Calixarenes and Auramine O Dye

YULING ZHANG, REZIK A. AGBARIA#, and ISIAH M. WARNER*

**Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803*

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The dye, Auramine O, whose fluorescence is sensitive to its microenvironment, is used to study the complexation properties of the host molecules, calix[6]arene sulfonates. Complexation between the dye and the sulfonated calixarenes has been characterized by use of absorption and steady state fluorescence measurements. A comparison study of the calixarenes and the monomers suggests that the observed shifts of the absorption and the fluorescence of Auramine O are due to complex formation. In addition, the fluorescence of Auramine O is enhanced as a result of its association with calixarenes. Fluorescence intensities in the absence and presence of calixarenes are employed to calculate formation constants of the inclusion complexes. The stoichiometric ratio for both SCX6/AuO and SCX6–C_5H_{11}/AuO is 1:1. The formation constants for these complexes are estimated to be 1.24×10^4 M^{-1} and 1.53×10^4 M^{-1}, respectively.

INTRODUCTION

Calixarenes [1,2] are cyclic phenol oligomers linked by ethylene groups. These compounds are cylinder–shaped with various cavity sizes and can form a variety of host–guest type inclusion complexes, similar to cyclodextrins. For many years, cyclodextrins as host molecules have been a focus for chemical research. The α–, β–, and γ–cyclodextrins have inner cavity diameters of 5.7, 7.8, and 9.5 Å, respectively [3] These compounds are known to form non–covalent inclusion complexes with various molecules of appropriate size and polarity. In contrast, the inner cavity diameters of calix[4]arene, calix[6]arene and calix[8]arene are 3.0, 7.6, and 11.7 Å respectively.[1]

*Corresponding author
#Present address: School of Medicine, Biological Chemistry, Center for Fluorescence Spectroscopy, University of Maryland at Baltimore, Baltimore, MD 21201

Thus, the inner cavity diameter of calix(6)arene is comparable to that of β–cyclodextrin. However, there is a significant difference between the oligosaccharide units of cyclodextrin and the phenols of calixarene. These latter molecules are known to absorb ultraviolet light and to fluoresce. Therefore, the photophysics of calixarenes have been investigated.[4,5] In addition, the acid base properties with and without protonation of calixarenes have been studied in detail.[6,7]

There are many advantages to using calixarenes as host molecules because of their unique properties. The weak forces which play a major role in complex formation include hydrogen bonding, π–π interactions, electrostatic interactions, and dipole–dipole moments.[8] Calixarenes provide all of those characteristics. Although *p–tert*–butylcalixarenes are insoluble in aqueous solution, derivitization of these calixarenes produces water–soluble compounds.[1,2] The result is that a variety of calixarenes with various solubilities can be obtained by organic synthesis. Several water–soluble derivatives which have been synthe-sized are sulfonato–[4–7, 9–11] , amino–[12], nitro–[13], carboxyl–[14] and phosphonato–calixarenes.[15] Among those calixarenes, the sulfonated calixarene derivatives are highly soluble in water and can be potentially useful as mimics of enzyme–substrate complexes.[16]

Recognition of metals, such as alkali, alkaline earth metal ions, and lanthanides is one of the most interesting topics in host–guest calixarene chemistry. Although most of the reported metal binding ligands of calixarenes are non water–soluble calixarenes,[17,18], studies on the recognition of UO_2^{2+} and lanthanides by use of water–soluble sulfonated calixarene derivatives have also been reported. Shinkai, *et al.* examined the complexation of UO_2^{2+} by *p*-carboxymethoxy sulfonated calix[6]arenes[19] and a polymer resin immobilizing calixarene–based uranophiles.[20] It was revealed that the specific affinity of the calixarene is selective to UO_2^{2+} due to size compatibility. Atwood, *et al.* have investigated *p*–sulfonated calix[5]arene by use of X–ray crystallography[11] with lanthanides as guests.

There have also been several studies reported on complexation of calixarenes and organic compounds. Since water–soluble calixarenes possess hydrophobic and hydrophilic properties, they are capable of encapsulating neutral organic as well as ionic species. This was reported by Shinkai, *et al.*[21] who investigated the complexation of a polarity fluorescence probe: phenol blue. The observed large wavelength shifts in the fluorescence spectrum of phenol blue when complexed with hexasulfonated calixarene was attributed to stabilization of the included cationic species by the six anionic groups on the upper rim of the calixarene cavities. In contrast to the more accepted hydrophobicity of the calixarene cavities, the authors concluded that the cavities of these calix[6]arene derivatives are more polar than water.[22] In addition, the stoichiometry of the complex was determined to be 1:1. Additional calixarenes with different sizes [calixarenes [4], [6], and [8] have been examined. The binding constant of each host molecule has been systematically estimated by applying two different guest molecules, i.e. phenol blue (PB, a slim long molecule) and anthrol blue (AB, three times bigger than PB). The estimated binding constants for the small guest molecule (PB) were found to be CX6> CX4 > CX8, whereas the constants for the larger guest molecule (AB) were on the order of CX8 > CX6 > CX4. Therefore, calixarenes were capable of size–selectivity similar to cyclodextrins.[22] This observation further supports the idea of designing calixarenes as synthetic enzyme mimics. Shinkai, *et al.*[23-24] also employed other guests such as trimethylanilinium chloride and 1–adamantyltrimethyl ammonium chloride to bind with sulfonated calixarenes [4], [6], [8]. The observed binding constants as determined by use of NMR were in the range of (0.5–2.0) × $10^4 M^{-1}$.

Auramine O is a cationic dye and a derivative of diphenylmethane. The fluorescence of this dye is significantly altered upon changes in its

microenvironment. It has been reported that the fluorescence of this compound in water is very weak. However, the fluorescence is significantly enhanced in a viscous solution,[25] or upon binding to a biological membrane. [26] Factors which could account for the observed fluorescence enhancement include the rigidity and the hydrophobicity of the microenvironment. When Oster reported an increase of AuO fluorescence in the presence of DNA, they concluded that it was the rigidity of the environment provided by the DNA which prevented internal rotation of the N,N–dimethylaniline groups, and consequently, prevented quenching due to dissipation of electronic energy. Therefore, the fluorescence intensity of the dye reflects the effective microviscosity of its microenvironment. More studies supporting this theory include binding studies of AuO with DNA and HLADH,[26–27] membranes,[28] micelles,[29–33] polymers,[34–36] and cyclodextrins.[37] An alternative explanation to rigidity is that the hydrophobicity of the microenvironment of the binding substrate is responsible for the increase in the AuO quantum yield.[38,39]

The *p*–sulfonated calix[6]arenes provide not only a hydrophobic environment (benzene rings or alkyl chains), but also hydrophilic heads (SO_3^-). In other words, these calixarenes possess properties of both cyclodextrins and micelles. The literature on studies of calixarene complexes is limited. Therefore, it is very valuable to use calixarenes as host molecules and AuO as a fluorescence probe to study the photophysics of the binding calixarenes with AuO as reported here. Four compounds including *p*–sulfonated calix[6]arene (SCX6), *p*–sulfonated phenol (MH), *p*–sulfonated pentyloxycalixarene (SCX6–C_5H_{11}), and *p*–sulfonated pentyloxyphenol (M–C_5H_{11}) were employed to conduct these binding studies. The structures of these chemicals and AuO dye are shown in Figure 1. The experimental results allow us to compare the effects of the cavities and the hydrophobicities of the binding substrates on the fluorescence of AuO. Absorption and steady state fluorescence were employed.

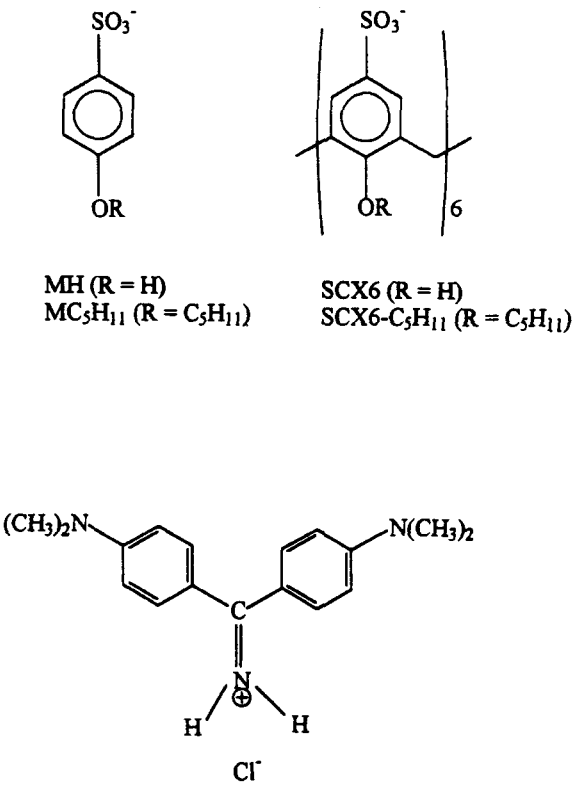


FIGURE 1 Chemical structures of calixarenes, monomers and fluorescence probe (AuO).

RESULTS AND DISCUSSION

The absorption and emission spectra of AuO in pure water at pH = 7.0 were measured (Figure 2). Two absorption bands were observed at 368 nm and 430 nm, which correspond to the transitions from the ground state to the second and the first excited electronic states, respectively.[25–27] The absorption spectra of AuO at 5.0×10^5 M^{-1} in aqueous solution follow Beer's law.[37] Therefore, this concentration was used in all experiments. The fluorescence intensity of AuO in water is very weak, and the fluorescence maximum is at 495 nm.

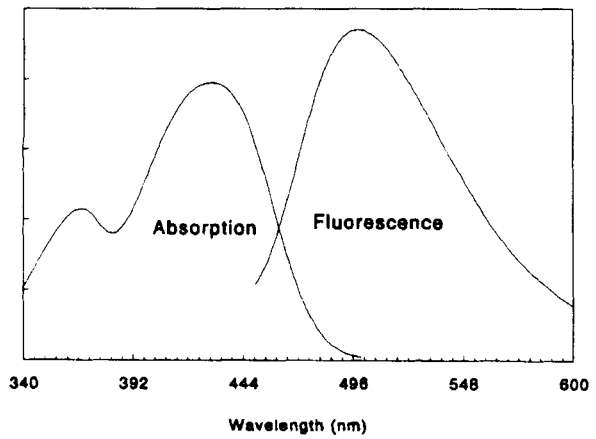


FIGURE 2 a. Absorption spectrum of AuO (5.0×10^{-5} M) at pH = 7.0. b. Emission spectrum of AuO.

Absorption Studies

Figure 2 shows the absorption spectra of AuO in aqueous solution at various concentrations of SCX6. At a concentration of 1.0×10^{-5} M of SCX6, the absorption slightly decreases and there is a 6 nm red shift from 430 nm. By further addition of SCX6, the spectrum shifts further down to the red region (445 nm) and a slight increase in the intensity is observed. The addition of SCX6–C_5H_{11} produces a similar effect on AuO absorption spectra to the addition of SCX6, whereas, addition of the monomers has no obvious affects. The shifts of the spectra in the presence and absence of SCX6 are larger than results which have been previously reported in other systems.[37] Apparently, the interactions between AuO and SCX6 are stronger than these other systems. The formation of a complex between SCX6 and AuO results in shifts and a small increase in absorption. Similar phenomena were reported by Mwalupindi *et al.* in the system involving AuO and β-CD[37] and surfactants. [34–37]

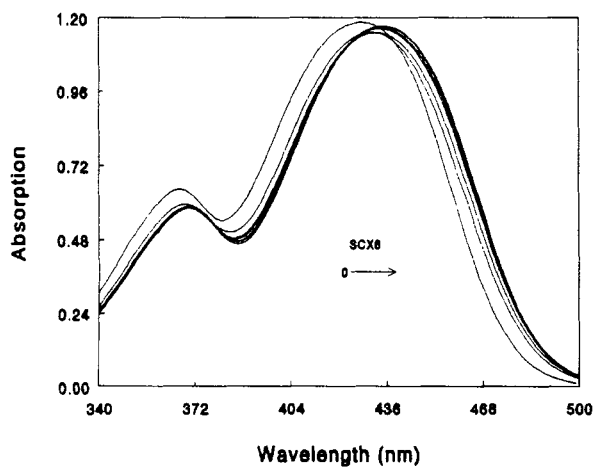
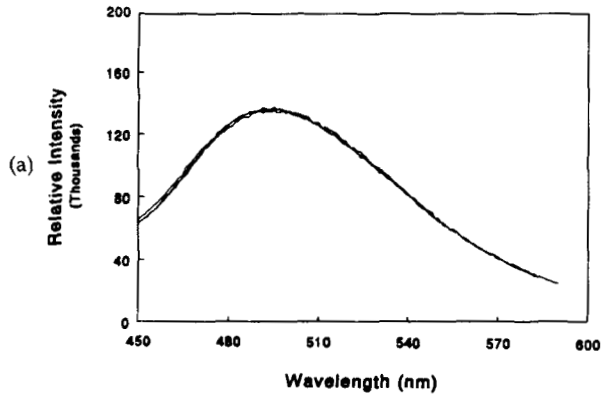


FIGURE 3 Absorption spectra of AuO at various concentrations of SCX6 in the range from 0.0 mM to 0.5 mM.

Fluorescence Studies

The fluorescence spectra of AuO in the presence of SCX6 and MH are shown in Figure 4a and b. The addition of MH does not affect the fluorescence of AuO, whereas the fluorescence of AuO in the presence of SCX6 is slightly enhanced. The enhanced fluorescence of the dye also shows a 12 nm red shift. One of the theories is that the internal rotation of the diphenylaniline is responsible for lowering the quantum yield of this probe in water. In the presence of β–cyclodextrin, the fluorescence intensity of AuO was much more enhanced than in the presence of SCX6. If the sole reason for the increase in AuO fluorescence is due to the restricted internal rotation, then one should expect much more enhancement when SCX6 is used as a host molecule since the sizes of both host molecules are comparable to each other. However, the experimental results do not support this conclusion. From Corey–Pauling–Kolton (CPK) space filling model, the AuO molecule could be totally included into the SCX6 cavity such that it would reduce the rotational freedom of the dye molecule. Hence, there must be some other reasons accounting for the observed increase. It is known that β–cyclodextrin provides a hydrophobic environment. Many studies involving cyclodextrins and pyrene have verified the hydrophobicity of cyclodextrins. [40–41] In contrast, the SCX6 cavity does not provide much of a hydrophobic environment since there are sulfonate



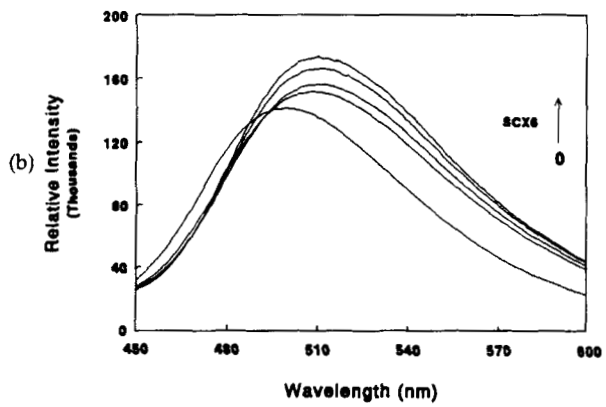
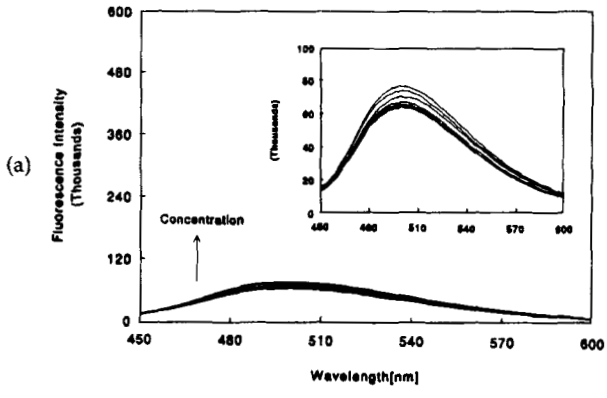


FIGURE 4 a. Fluorescence spectra of AuO (5.0×10^{-5} M) in the presence of monomer (MH) at concentrations from 0.0 mM to 3.0mM. b. Fluorescence spectra of AuO (5.0×10^{-5} M) in the presence of calixarene (SCX6) at concentrations from 0.0 mM to 0.5 mM.



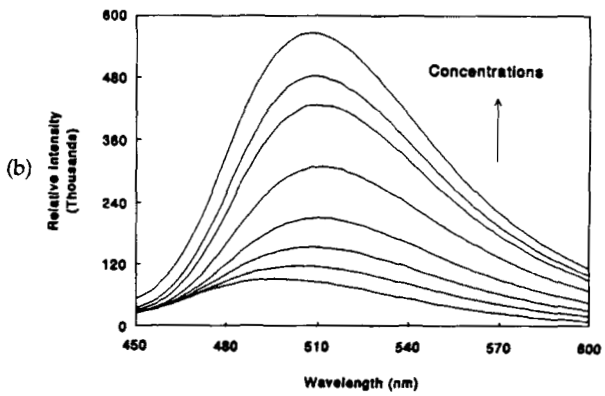


FIGURE 5 a. Fluorescence spectra of AuO (5.0×10^{-5} M) in the presence of monomer (M-C_5H_{11}) at concentrations from 0.0 mM to 3.0 mM. The insert figure is the same spectra in a large scale. b. Fluorescence spectra of AuO (5.0×10^{-5} M) in the presence of calixarene (SCX6-C_5H_{11}) at concentrations from 0.0 mM to 0.5 mM.

groups occupying the upper-rim while hydroxyl groups occupy the lower-rim. Water molecules may also be bound to the dye molecule. Therefore, the dye fluorescence does not increase much even though the diphenylaniline rotation is restricted by the calixarene cavity.

More studies have been undertaken in order to distinguish the mechanisms which produce the enhancement of fluorescence. We have synthesized alkyl derivatives for the monomer and SCX6. We have employed these two compounds to conduct a comparison study. Since the alkyl chains substitute for the protons on the hydroxyl groups, the microenvironment of the calixarene cavity is more hydrophobic. The fluorescence spectra of AuO in the presence of M–C_5H_{11} and SCX6–C_5H_{11} are collected and shown in Figure 5a and b. It is evident that the AuO fluorescence is significantly enhanced in the presence of the host molecule SCX6–C_5H_{11}, whereas this is only slightly affected by the addition of M–C_5H_{11}. There are some similarities between the Figure 4b and 5b, in which the spectra of AuO are shifted about 12 nm to the red in the presence of both SCX6 and SCX6–C_5H_{11}, but not with the monomers. The shifts suggest complex formation between the calixarenes and the dye.

Estimation of the Formation Constants

Mwalupindi *et al.* reported that the stoichiometric ratio for β–CD and AuO was 1:1 based on the enhancement of fluorescence intensity.[37] As described earlier, calix[6]arene also has a similar diameter to β–CD. Therefore, we assume the association stoichiometric ratio to be 1:1 for the complex between calix[6]arenes and AuO at this point. This assumption will be tested by use of a Benesi–Hilderbrand plot.[42] First, we assume that the equilibrium for complex formation is as follows:

$$SCX6 + AuO \leftrightharpoons SCX6 * AuO \quad (1)$$

The equilibrium constant (K_1) for a 1:1 association between SCX6 and AuO is given by:

$$K_1 = \frac{[SCX6 * AuO]}{[SCX6]\,[AuO]} \quad (2)$$

where [SCX6] and [AuO] are the concentrations of SCX6 and AuO at the equilibrium moment, respectively. The [SCX6*AuO] is the equilibrium concentration of the inclusion complex for a given SCX6 concentration.

Based on experimental results, the fluorescence of AuO is enhanced due to inclusion formation. In other words, the enhanced fluorescence is from the complexed AuO. Therefore, the equilibrium concentrations of SCX6 and AuO correspond to their contributions to the fluorescence intensities. A reasonable expression between the equilibrium concentrations and the fluorescence peak areas is as follow:

$$\frac{[AuO]_0}{[SCX6*AuO]} = \frac{(A_1 - A_0)}{(A - A_0)} \quad (3)$$

where $[AuO]_0$ is the initial analytical concentrations of AuO and [SCX6*AuO] is complexed AuO concentration. The parameters A_0 and A_1 denote the fluorescence peak areas for AuO dye in pure aqueous solution and in complexed AuO, respectively, A is the peak area at a given SCX6 concentration. In general, the initial concentration of the host molecule (SCX6) needs to be 20 times larger than the guest molecule (AuO) (The concentration of SCX6 is much larger than that of the complex, i.e. [SCX6]>>[SCX6*AuO]). The classical method for determination of K_1 is the preparation of a double–reciprocal plot as:

$$\frac{1}{A - A_0} = \frac{1}{k_1(A_1 - A_0)[SCX6]_0} + \frac{1}{A_1 - A_0} \quad (4)$$

and a plot of $1/(A–A_0)$ versus $1/[SCX6]_0$ should provide a straight line.

Similarly, if we assume a 2:1 complex, then:

$$2\ SCX6 + AuO = (SCX6)_2 * AuO \quad (5)$$

The overall equilibrium constant (K_2) is expressed as:

$$K_2 = \frac{[(SCX6)_2 * AuO]}{[AuO][SCX6]^2} \quad (6)$$

with the assumptions $[SCX6]_0$>> [$(SCX6)_2$*AuO] >>[SCX6*AuO], thus:

$$\frac{1}{A - A_0} = \frac{1}{K_2(A_2 - A_0)\,[SCX6]_0^2} + \frac{1}{A_2 - A_0} \quad (7)$$

and a plot of $1/(A–A_0)$ versus $1/[SCX6]_0^2$ should give a straight line.

Use of this approach allows us to graphically determine the stoichiometry of the complexes used in our studies. Benesi–Hildebrand plots [42] (double–reciprocal plots based on equations 7 and 10) of the fluorescence data are displayed in Figure 6a and b. A linear relationship should be observed when $1/(A–A_0)$ is plotted against 1/[SCX6] (Figure 6a) and a convex curvative is obtained when $1/(A–A_0)$ is plotted against $1/[SCX6]_0^2$ according to equation 10. The curvilinear nature of the 2:1 plot suggests that the most appropriate stoichiometry for the SCX6 and AuO complex

TABLE I Formation Constants calculated from Benesi-Hildebrand 1:1 Stoichiometry Plot and from Nonlinear Regression

Formation Constants/(k)	From Double Reciprocal	From Nonlinear Regression
SCX6/AuO	7.38×10^3	1.24×10^4
SCX6-C_5H_{11}/AuO	1.24×10^4	1.53×10^4

is 1:1. Similar methods were performed with SCX6–C_5H_{11}. The association constants estimated from Figure 5 and 6 are summarized in Table I.

It is important to remember that the concentration of the host should be twenty times higher than that of the guest. The values estimated from the double reciprocal plots are trustworthy only if the above assumption is valid. This question may be raised in regards to the fact that Benesi-Hildebrand plots tend to place more emphasis on lower concentration values than on higher values. In other words, the slope of the straight line is more sensitive to the ordinate value of the point having the smallest concentration. However, the K values calculated from the Benesi–Hildebrand plots can be used as initial estimations for parameters in the nonlinear regression (NLR) analysis subroutine of the SAS/STAT program.[43] By use of this program, in which a 1:1 complex is suggested by the Benesi–Hildebrand plots, the fluorescence data and the calixarene concentrations may be fit to the following equation:

$$A = \frac{A_0 + A_1 K_1 [SCX6]_0}{1 + K_1 [SCX6]_0} \quad (8)$$

The calculated values from this non–linear program are summarized in Table I. Since the host calixarene concentrations are in the same range as the guest molecule, the values obtained from the non–linear regression are used for discussion. It is noted that the association constants of the complexes of SCX6–C_5H_{11}/AuO and SCX6/AuO are on the same order, although the fluorescence intensity in the presence of SCX6–C_5H_{11} is more enhanced than that of SCX6/AuO.

The magnitudes of the binding constants are in the same range as the values reported by Shinkai, *et al.* for the system of trimethylanilinium chloride and 1–adamantyltrimethyl ammonium chloride and sulfonated calix[6]arenes determined by NMR.[23–24] It is reasonable to believe that the strengths of the complexes between these two calixarenes and AuO are similar. It should also be

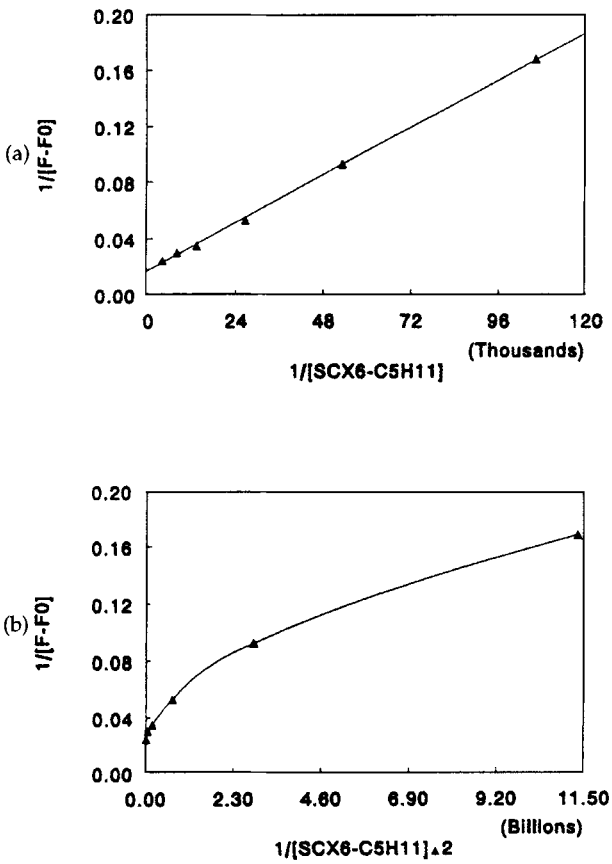


FIGURE 6 a. Benesi-Hildebrand plot assuming a 1:1 stoichiometry between SCX6 and AuO. b. Benesi-Hildebrand plot assuming a 2:1 stoichiometry between SCX6 and AuO.

noted for our data that AuO dye fluoresces much more when included in the cavity of SCX6–C_5H_{11} which possesses a more hydrophobic environment as well as a more restraining cavity than that of SCX6. It is therefore reasonable to conclude that such an enhancement is caused by the hydrophobic nature of the cavity, as well as more restrains on AuO rotations, provided by SCX6–C_5H_{11}.

EXPERIMENTAL SECTION

Materials

Synthesis of p–sulfonated–calix[6]arene.

The *p*–sulfonated–calix[6]arene was synthesized by incorporating a combination of previously developed procedures of Gutsche, *et al.* and Shinkai, *et al.*[4, 5, 44–46]

Synthesis of p–pentyloxysulfonated calix[6]arene[4]

To a three necked round bottom flask, 1.50 g of sulfonated calixarene (6) and 1.25 g of NaOH were dissolved in 7.5 mL H_2O, and 4.0 ml of bromopentane in 30.0 mL DMSO were added, stirred, and heated to 50°C. The reaction takes about two days to complete, which is much longer than the monomer reaction. The precipitate (PPT) was isolated from the reaction by filtration. The PPT was washed with EtOH to obtain a crude product. The filtrate can be diluted with MeOH to generate a PPT which is also a crude product. The crude product can be recrystallized by use of water–EtOH to obtain a white powder. This method is similar to the method of Shinkai, *et al.*[4] Chemical analysis: $C_{72}H_{90}O_{24}S_6Na_6 \cdot 3H_2O$. Theoretical values (%) are: C 49.65, H 5.67, S 11.04, Na 7.92 and experimental values are: C 49.40 (0.5%), H 5.59 (1.4%), S 10.90 (1.2%), Na 7.73 (2.4%).

Synthesis of p–pentyloxyphenol sulfonate[4]

To a three necked round bottom flask, 3.00 g of 4–hydroxybenzenesulfonic acid, sodium salt and 2.35 g of NaOH were dissolved in 15.0 mL H_2O, and 8.0 mL of bromopentane in 60.0 mL DMSO were added, stirred, and heated to 50°C. After ten hours, the precipitate (PPT) was isolated from the reaction by filtration. The PPT was washed with EtOH to obtain a crude product. The filtrate can be diluted with MeOH to generate a PPT which is also a crude product. This crude product can be recrystallized by use of water–EtOH to obtain a white powder. This method is similar to that reported by Shinkai *et al.*[4] Chemical analysis: $C_{11}H_{15}O_4SNa$. Theoretical values (%), without assuming any waters of hydrogen, are: C 49.61, H 5.68, S 12.02, Na 8.64 and experimental values are: C 48.62 (2.0%), H 5.58 (1.7%), S11.87 (1.2%), Na 8.30 (3.9%).

Auramine O and 4–hydroxybenzenesulfonic acid, sodium salt was purchased from Aldrich Chemical Company (Milwaukee, WI) and used without alteration. A 5.0×10^{-4} M stock solution of Auramine O was prepared in deionized water. To prepare Auramine O solution in aqueous SCX6, a 1.0 ml aliquot of AuO aqueous solution was transferred into a 10–ml volummetric flask. The Auramine O concentration was held constant at 5.0×10^{-5} M and the pH at 7.0 in all experiments. The fluorescence emission spectra were taken at an excitation wavelength of 365 nm. The buffers were prepared using Na_2HPO_4 and NaOH which were purchased from Fisher Scientific Company (Fair Lawn, NJ).

Apparatus

Steady–state fluorescence measurements were acquired with a SPEX–Fluorolog, Model F2T21I spectrofluorometer equipped with a cell compartment, thermostated by use of a VWR Model–1160 constant temperature circulator. The excitation and emission bandwidths were both set at 5 nm. Absorption spectra were recorded by use of a Shimadzu UV–3101PC scanning spectrophotometer. All absorption spectroscopic analyses were conducted at room temperature.

CONCLUSION

The SCX6 and SCX6–C_5H_{11} which have hydrophobic cavities and hydrophilic groups provide the advantages of cyclodextrins and micelles. Complexation between AuO and SCX6 or SCX6–C_5H_{11} is responsible for the spectroscopic shifts of the absorption spectra and the emission spectra. The complexes with AuO appear to suppress the rotation of the diphenylanline groups of AuO. However, restriction of the rotation of AuO seems not to be complete in SCX6 as compared with higher degree of restraints in the SCX6–C_5H_{11}. In addition, the hydrophobicities of the calixarene cavities aid in the production of more intense fluorescence of AuO. The fluorescence intensities in the absence and presence of calixarenes are employed to calculate formation constants of the inclusion complexes. The stoichiometric ratio for both SCX6/AuO and SCX6–C_5H_{11} /AuO is 1:1. The formation constants of these complexes are estimated to be 1.24×10^4 M^{-1} and 1.53×10^4 M^{-1}, respectively.

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References

[1] Gutsche C.D. (1989). *Calixarenes*, The Royal Society of Chemistry, Cambridge.
[2] Perrin R., Harris S. (1991). *Topics in Inclusion Science: Calixarenes, a Versatile Class of Macrocyclic Compounds*, eds by Vicens, J., Böhmer, V. Kluwer Academic Publishers, Dordrecht.
[3] Szejtli J. (1982). *Cyclodextrins and their Inclusion Complexes*, Akademia Kiadio, Budapest.
[4] Shinkai S., Mori S., Koreishi H., Tsubaki T., Manabe O. (1986). *J. Am. Chem. Soc.*, **108**, 2409.
[5] Zhang Y., Agbaria R.A., Mukundan N.E., Warner I.M. (1996). *J. Inclusion Phenom.*, in press.
[6] Arena G., Cali R., Lombardo G.G., Rizzarelli E., Sciotto D., Ungaro R., and Casnati A. (1992). *Supramol. Chem.*, **1**, 19.
[7] Scharff J., Mahjoubi M. (1991). *New J. Chem.*, **15**, 883.
[8] Stryer L. *Biochemistry*, W. H. Freeman and Company, New York, 1995.
[9] Atwood J.L., Orr G.W., Hamada F., Vincent R.L., Bott S.G., Robinson K.D. (1991). *J. Am. Chem. Soc.*, **113**, 2760.
[10] Atwood J.L., Clark D.L., Juneja R.K., Orr G.W., Robinson K.D., Vincent R.L. (1992). *J. Am. Chem. Soc.* **114**, 7558.
[11] Steed J.W., Johnson C.P., Barnes C.L., Juneja R.K., Atwood J.L., Reilly S., Hollis R.L. Smith P.H., Clark D.L. (1995). *J. Am. Chem. Soc.*, **117**, 11426.
[12] Gutsche C.D., Jevine J.A., Sujeeth P.K. ***(1985)***. *J. Org. Chem.*, **50**, 5802.
[13] Shinkai S., Araki, K. Tsubaki, T. , Arimura T., Manabe O. (1987). *J. Chem. Soc. Perkin Trans. I.*, 2297.
[14] Arduini A., Pochini A., Reverberi S., Ungaro R. (1984). *J. Chem. Soc., Chem. Commun.*, 981.
[15] Arimura T., Nagasadi T., Shinkai S., Matsuda T. (1989). *J. Org. Chem.* **54**, 3766.
[16] Shinkai, S., Kawabata, H., Arimura, T., Matsuda, T., Satoh, H., Manabe, O. (1989). *J. Chem. Soc., Perkin Trans. I.*, 1073.
[17] Goldman, H., Vogt, W., Paulus E., Böhmer, V. (1988). *J. Am. Chem. Soc.* **110**, 6811.
[18] Bünzli, J.G., Foidevaux, P., Harrowfield, J.M. (1993). *Inorg. Chem.*, **32**, 3306.
[19] Shinkai, S., Koreishi, H., Ueda, K., Arimura, T., Manabe, O. (1987). *J. Am. Chem. Soc.*, **109**, 6371.
[20] Shinkai, S., Kawaguchi, H., Manabe, O. (1988). *J. of Polym. Science: Part C: Polym. Lett.*, **26**, 391.
[21] Shinkai, S., Mori, S., Araki, K., Manabe, O. (1987). *Bull. Chem. Soc. Jpn.*, **60**, 3679.
[22] Shinkai, S., Araki K., Manabe, O. (1988). *J. Chem. Soc. Chem. Commun.*, 187.
[23] Arimura, T., Kubota, M, Araki, K., Shinkai, S., Matsuda, T. (1989). *Tetrahedron Lett.*, **30**, 2563.
[24] Shinkai, S., Araki, K., Matsuda, T., Manabe, O. (1989). *Bull. Chem. Soc. Jpn.*, **62**, 3856.
[25] Oster, G., Nishijima, Y. (1956). *J. Am. Chem. Soc.*, **79**, 1581.
[26] Heitz, J.R., Brand, L. (1971). *Archives of Biochemistry and Biophysics*, **144**, 286.
[27] Gautam, P. , Harriman, A. (1994). *J. Chem. Soc. Faraday Trans.*, **90**, 667.
[28] Kobayashi, Y., Nishimura, M. (1972). *J. Biochem.*, **71**, 275.
[29] Hasegawa, M., Sugimura, T., Kuraishi, K, Shindo, Y., Kitahara, A. (1992). *Chem. Lett.*, 1373.
[30] Miyagishi, S., Asakawa, T., Nishida M. (1987). *J. Collosid and Interface Sci.*, **115**, 199.
[31] Miyagishi, S., Kurimoto, H., Ishihara, Y., Asakawa, T. (1994). *Bull. Chem. Soc. Jpn.*, **67**, 2398.
[32] Choi, L., Kim, O. (1994). *Langmuir*, **10**, 57.
[33] Hasegawa, M., Sugimura, T., Suzaki, Y., Shindo, Y. (1994). *J. Phys. Chem.*, 98, 2120.
[34] Fenyo, J., Braud, C., Beaumais, J., Muller, G. (1975). *J. Polymer Letters Edition*, **13**, 669.
[35] Wang, Y., Morawetz, H. (1986). *Macromolecules*, **19**, 1925.
[36] Meyer, E.F., Jamieson, A.M., Simha, R., Palmen, J.H.M., Booij, H.C., Maurer, F.H.J. (1990). *Polymer*, **31**, 243.
[37] Mwalupindi, A.G. , Rideau, A., Agbaria, R.A., Warner, I.M. (1994). *Talanta*, **41**, 599.
[38] Erny, B., Muller, G. (1979). *J. Polym. Sci., Polym. Chem. Ed.*, 17, 4011.

[39] Kenner, R.A., Aboderin, A.A. (1971). *Biochemistry*, 10, 4433.
[40] Muñoz De La Peña A., Ndou, T., Zung, J.B., Warner, I.M. (1991). *J. Phys. Chem.*, **95**, 3330.
[41] Will, A.Y., Muñoz De La Peña A., Ndou, T., Warner, I.M. (1993). *Applied Spectroscopy*, 47.
[42] Benesi, H.A, Hildebrand, J.H. (1949). *J. Am. Chem. Soc.*, **71**, 2703.
[43] SAS/STAT, Release 6.03, (1988). SAS Institute Inc.: Cary, NC.
[44] Gutsche, C.D., Dhawan, B., Leonis, M., Stewart, D. (1990) in *Organic Syntheses*, White, J.D. (Editor), **vol. 68**, Wiley, New York, pp 238–242.
[45] Gutsche, C.D., Lin, L.G. (1986). *Tetrahedron*, **42**, 1633.
[46] Zhang, Y., Warner, I.M. (1994). *J. Chromatogr. A.*, 688, 293.