The investigation of inclusion behavior of Solvent Violet 9 with 4-sulfonatocalix[n]arenes and its recognition to DNA

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ABSTRACT

The inclusion behavior of Solvent Violet 9 (SV9) with 4-sulfonatocalix[n]arenes (SCXₙ) (n = 4, 6, 8) was investigated at various pH values by ultraviolet–visible spectroscopy. SV9 is able to form an inclusion complex with calixarenes. Different absorption behaviors were observed for the dye with the various host calixarenes. The molecular binding abilities were affected by the configuration of the calixarene cavities and the solution pH. Various experimental conditions, including calixarenes concentrations, were investigated and the results suggested that the three calixarene were most suitable for inclusion of the dye at pH = 3.05. The formation constant could be calculated. The inclusion behavior of the complexes was studied in detail using nuclear magnetic resonance spectroscopy. Finally, the interactions of SV9 with Salmon testes DNA in SCXₙ supramolecular system were studied by UV–Vis absorption spectroscopy. The UV–Vis absorption show that the interaction of SV9 with DNA depends on the concentration ratio of SV9 to DNA and the pH values. The binding constants of inclusion complexes with DNA are calculated. It was observed that SCXₙ can affect the interactive mode of SV9 with DNA.

Keywords:
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4-Sulfonatocalix[n]arene
UV–Vis absorption
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DNA

Introduction

Calix[n]arenes are a family of macrocyclic compounds that can be synthesized by base-induced reactions of certain p-substituted phenols with formaldehyde [1–3]. The calixarene incorporates a cavity [4], which can be used to form inclusion compounds in a similar manner to crown-ethers and cyclodextrins [5–7]. Compared with cyclodextrins, calixarenes have large spatial flexibility. Accordingly, they are used in a broad range of applications [8,9] and have recently been the focus of many investigations [10–42].

In recent years, some calixarenes have been found to have antimicrobial activity [11,12]. Calixarenes have also been used for separation of complexes [13,14], and for inclusion of metals or metal ions [15–18]. They have been applied in chemosensor design [19–21], environmental protection [22–24], for inclusion of...
pharmaceuticals [25–27], and can function as enzymes in some complex organisms [28–30]. Recent research has focused on the interaction of calixarenes and their derivatives with dyes. Among the calixarenes, water-soluble calixarenes are of particular interest. Because of the potential applications of p-sulfonatocalix[n]arenes (SCXn) in aqueous systems, many studies have investigated their inclusion of dye guest molecules through ionic/molecular recognition [31–34]. Chen and Diao [4] investigated inclusion complexation of neutral red and water-soluble sodium salts of SCXn (n = 4, 6, 8) by UV–visible (UV–Vis) and fluorescence spectroscopy. They found that electrostatic interactions and hydrogen bonding were important in the formation of inclusion complexes. Zhang et al. [35] used fluorescence, UV–Vis, and nuclear magnetic resonance (NMR) spectroscopy to study inclusion complexation of C.I. Basic Red 5 and SCX4 or carboxymethyl-β-cyclodextrin. After inclusion in the hosts, the physical and chemical properties of C.I. Basic Red 5 were altered. There are many organic dyes are included with sulfonated calixarenes have been studied. For example, azo dyes [36–38], hetarylazo disperse dyes [39] and methylene blue [40]. At the same time, because of their good DNA binding affinity [43,44], investigation on the interaction of organic dyes with DNA have attracted much attention over the past years [45,46].

Solvent Violet 9 (SV9, C25H31N3O) is an organic dye that has been widely used in textile dyes, printing inks, and stationery. However, there is little information available in literature about SV9. In this paper, complexation of SV9 with three SCXn (n = 4, 6, 8) was explored. SV9 is non-toxic and could be used in enzyme mimetics and biological probes. In this paper, SV9 be used with DNA. Because fluorescence cannot be detected from SV9, its interaction with the hosts was studied by UV–Vis and NMR spectroscopy, and the complexes with DNA only were by UV–Vis. A novel method was developed to prepare and characterize the inclusion complexes.

Materials and methods

Apparatus

UV–Vis spectra were collected using a Shimadzu UV-2450 absorption spectrophotometer (Tokyo, Japan). The pH was measured using a pHS-2 pH meter (The 2nd Instrument Factory of Shanghai, China). 1H NMR and 13C NMR spectra were recorded in a mixed solution of DMSO-d6 and water on a DRX-300MHZ NMR spectrometer (Bruker, Billerica, MA).

Reagents

SV9 and one of the 4-sulfonatocalix[n]arenes (n = 4, SCX4; n = 6, SCX6; and n = 8, SCX8) were mixed ultrasonically in distilled water for 1 h. The final concentrations of SV9 and the calixarene in solution were 0.0001 mol L–1 and 0.01 mol L–1, respectively. Salmon testes DNA was purchased from Beijing Solarbio Life Sciences (Beijing, China). Stock solutions of DNA were prepared by dissolving the solid DNA in 0.10 M NaCl solution and incubated for no more than 1 h. The final concentrations of SV9 and the calixarene in solution were 0.0001 mol L–1/C01 and 0.01 mol L–1/C01, respectively. Other all general solvents were used as provided and were of analytical grade. DDW was used in all experiments.

Procedures

Absorption spectroscopy

Five milliliter aliquots of the SV9 solution (0.0001 mol L–1) were transferred into 10.0 mL volumetric flasks. Phosphate buffer (2 mL) was used to adjust the pH to 3.05, 6.50, or 8.40, and then the solutions were diluted to the final volume with distilled water and shaken thoroughly. The absorption titrations were fulfilled by keeping the concentration of SV9 constant while varying the concentration of SCXn or DNA, or keeping the concentration of SV9–SCXn inclusion complex while varying the concentration of DNA.

1H NMR and 13C NMR spectroscopy

Each of the 4-sulfonatocalix[n]arenes was added to a solution of SV9 in an NMR tube. The samples were then analyzed by 1H NMR and 13C NMR spectroscopy, and the chemical shifts, Δδ, caused by addition of the 4-sulfonatocalix[n]arenes were evaluated.

Results and discussion

UV–Vis spectroscopy of the inclusion complexes of SCX4, SCX6 and SCX8 with SV9 at different pH values

The complexation of SV9 (5 × 10–5 mol L–1) with the sulfonated calixarenes was studied at different pH values. The spectral changes that occurred during the titration indicated that the inclusion complexes of SV9 with SCX4, SCX6 and SCX8 were distinctly different.

Fig. 1a shows the UV–Vis spectra of 5 × 10–5 mol L–1 SV9 with various concentrations of SCX4 at pH 3.05. The peak for SV9 was at 589 nm. As the concentration of SCX4 increased, the intensity of this peak decreased and a very noticeable redshift occurred from 589 nm to 627 nm. At pH 6.50, the SV9 peak was at 588 nm, and it again decreased in intensity and redshifted to 593 nm as the concentration of SCX4 increased (Fig. 1a). At pH 8.40, the SV9 peak was at 587 nm, and it decreased in intensity and redshifted to 592 nm as the concentration of SCX4 increased (Fig. 1b). The spectra at all pH values showed obvious isoabsorptive points, which were located at 612, 611, and 613 nm at pH 3.05, 6.50, and 8.40, respectively. The spectra for SV9 (5 × 10–5 mol L–1) in the absence and presence of SCX6 at pH 3.05, 6.50 and 8.40 are shown in Fig. 2. At pH 3.05, the peak for SV9 was at 588 nm, and addition of SCX6, decreased the intensity of this peak (Fig. 1b). Initially, a blueshift from 588 nm to 534 nm was observed and then the peak gradually redshifted from 534 nm to 636 nm. At pH 6.50, the peak for SV9 was at 587 nm (Fig. 2a). When the concentration of SCX6 increased, another two peaks appeared at 552 nm, 588 nm and the intensities of these peaks first decreased and then increased, and both peaks redshifted slightly. Similar results were obtained at pH 8.40 (Fig. 2b), but the peak intensity increased more rapidly than that at pH 6.50 as the concentration of SCX6 increased.

The spectra for SV9 (5 × 10–5 mol L–1) in the absence and presence of SCX8 at pH 3.05 are shown in Fig. 1c. The absorption peak took place red shift from 589 nm to 636 nm and the intensity increased when SCX8 was added. There was an isoabsorptive point at 609 nm. At pH 6.50, the peak also shifted redly (587–596 nm) and increased in intensity as the concentration of SCX8 increased (Fig. 3a). At pH 8.40, the results were similar (Fig. 3b), but the peak intensity increased more rapidly than that in Fig. 3b.

Non-linear curve fitting of SCX4, SCX6, SCX8 with SV9 at different pH values

At the different pH values the absorbance phenomena for the inclusion complexes of SV9 with the 4-sulfonatocalix[n]arenes (n = 4,6,8) were obviously different. This indicates that the extent of inclusion and structures of the inclusion complexes are different. The inclusion formation constant (K) is a measure of the complexation capacity of a host compound (H) with a guest molecule (G) [35]. The inclusion formation constants of SV9 with the
4-sulfonatocalix[n]arenes (n = 4, 6, 8) were evaluated at different pH values. The inclusion equilibrium reaction (1) and formation constant (2) are shown below, where $[H]$, $[G]$ and $[HG]$ represent the equilibrium concentrations of the host, guest, and host–guest complex, respectively.

$$H + G \rightleftharpoons HG$$

(1)

$$K_s = \frac{[HG]}{[H][G]}$$

(2)

The inclusion formation constant ($K_s$) can be obtained from the absorbance data using a non-linear curve fitting approach [34] and the following equation:
The activity factor for the structural changes that occur on complexation at different pHs. Non-linear curve-fitting was performed for SV9 as a function of the concentration of 4-sulfonatocalix[n]arene (n = 4, 6, 8) at pH 3.05, pH 6.50, and pH 8.40 (Figs. 2, S4 and S5). Different inclusion complexes were formed at each of the pH values, and with the various sulfonated calixarenes at the same pH value. The correlation coefficient ($R^2$) and inclusion formation constant ($K$) were obtained from the non-linear curve fitting. Larger correlation coefficients indicate that combination of the host–guest molecules is more regular, and larger $K$ values indicate that integration of the guest within the host is tighter.

The K for SV9 with SCX4 increased as the pH value increased (Table 1). At pH 6.50 and 8.40, the $R^2$ were only 0.69679 and 0.74170, respectively. The $K$ values at pH 6.50 and 8.40 were 0.68 and 0.64, respectively. These data suggest that there is almost no inclusion of SCX6 in SV9 at pH 6.50 and 8.40. By comparison, the $K$ at all pH values for SV9 and SCX8 indicated that inclusion was tight.

$^{1}H$ NMR and $^{13}C$ NMR of the complexes of SCX4, SCX6, SCX8 with SV9

NMR spectroscopy is a powerful tool for studying the formation of inclusion complex between host and the guest molecules, especially their interaction mechanisms [17]. In the present research, $^{1}H$ NMR and $^{13}C$ NMR spectroscopies were used to study the inclusion complexes.

For the $^{1}H$ NMR experiment, the chemical shift of the guest molecule was obtained first and then compared to those in the host–guest complexes. The $^{1}H$ NMR signals were relatively broad in the $^{1}H$ NMR spectra of SV9 (Fig. 3a), SV9 with SCX4 (Fig. 3b), SV9 with SCX6 (Fig. 3c), and SV9 with SCX8 (Fig. 3d). When the spectra were compared to those of the original guest molecules, a low field shift of the H was observed. The largest $\Delta \delta$ was for H-8 and H-9, followed by H-2 and H-6, and then H-3 and H-5 (Table S1).

$^{13}C$ NMR was used to clarify the inclusion sites of the 4-sulfonatocalix[n]arenes and SV9. The $^{13}C$ NMR spectra of SV9, SV9 with SCX4, SV9 with SCX6, and SV9 with SCX8, are presented in Fig. 4a–d, respectively. The chemical shift of the guest was compared to those of the host–guest complexes, and low field shifts were observed after inclusion. The largest $\Delta \delta$ was for C-1, followed by C-2 and C-6, and then C-3 and C-4 (Table S2).

Based on the spectral data, we can conclude that the binding sites between SV9 and sulfonated calixarenes may be in the ring-shaped arenes. SV9 probably did not enter the cavity of the sulfonated calixarene but formed a cap as shown in Scheme S1.

UV–Vis absorption studies of the interaction of SV9 with DNA at different pH values

Addition of DNA to SV9 ($5 \times 10^{-5}$ mol L$^{-1}$) solution at pH 3.05, 6.50 or 8.04 resulted in absorption spectral changes of SV9, respectively. It is commonly observed that were decreased of the absorption peak of dyes in the presence of DNA are good indication of the binding of these small molecules to DNA at pH 6.50 and 8.40. However, when the DNA was added, SV9 the absorbance increases at pH 3.05. With increasing DNA concentration, the absorbance did not change significantly. Fig. 5 displays the UV–Vis absorption spectra of SV9 containing various concentrations of DNA at different pH values. All of the absorption was at 586 nm apparently. It is obvious that the pHs have an effect on the binding of SV9 to DNA.

UV–Vis absorption studies of the interaction of SV9 with DNA in the presence of SCXn

When the pH was 6.50, Fig. 6 depicts UV–Vis absorption spectra of $5 \times 10^{-5}$ mol L$^{-1}$ SV9 and $1 \times 10^{-2}$ mol L$^{-1}$ SCXn at various concentrations of DNA. With the addition of DNA, the absorption of SV9-SCX4 at 590 nm increases. The Fig. 6a shows the binding of

![Fig. 3. The $^{1}H$ NMR spectra of Solvent Violet 9 and Solvent Violet 9 with sulfonatocalix[4, 6, 8]arene. The Solvent Violet 9 (a); Solvent Violet 9 with SCX4 (b); Solvent Violet 9 with SCX6 (c) and Solvent Violet 9 with SCX8 (d).](image)

![Fig. 4. The $^{13}C$ NMR spectra of Solvent Violet 9 and Solvent Violet 9 with sulfonatocalix[4, 6, 8]arenes. The Solvent Violet 9 (a); Solvent Violet 9 with SCX4 (b); Solvent Violet 9 with SCX6 (c) and Solvent Violet 9 with SCX8 (d).](image)
SV9 to DNA was affected after SV9 inclusion with SCX4. Fig. 6b exhibits the two spectral peaks of SV9-SCX6 appeared at 546 nm, 588 nm. And both of the peaks start to decrease with the increase in DNA concentration. There was nearly no binding of SV9 to DNA in the presence of SCX8. And in Fig. 6c, the absorption was at 596 nm. It is well known that the spectral changes at the occurrence of SCXn, indicating interaction of SCXn–SV9 with DNA. The intercalative binding of SCXn–SV9 to a DNA helix has been characterized by large changes in the absorbance than the pure SV9 and DNA. These remarkable changes were attributed to the hydrogen bonding and electrostatic interaction between SV9 and SCXn which affected distribution of the electron cloud around of the SV9 molecular and the binding of SV9 with DNA. The results leded to a change in the molar absorbance coefficients, the absorbance are also changed. There were different behaviors when the SCXn was difference. It can be designed to perform recognition with the DNA and demonstrated a low range of recognition \((0.32–16) \times 10^{-7} \text{ mol L}^{-1}\).

Fig. 5. UV–Vis absorption spectra of \(5 \times 10^{-3} \text{ mol L}^{-1}\) SV9 in the presence various concentrations of DNA at pH 3.05 (a), pH 6.50 (b) and pH 8.40 (c), respectively.

Fig. 6. UV–Vis absorption spectra of \(5 \times 10^{-3} \text{ mol L}^{-1}\) SV9 and \(1.0 \times 10^{-2} \text{ mol L}^{-1}\) SCXn in the presence of various concentrations of DNA at pH 6.50. (a) \(n = 4\); (b) \(n = 6\); and (c) \(n = 8\).
Conclusion

Inclusion complexes of SV9 with SCX4, SCX6 and SCX8 were investigated by UV–Vis, 1H NMR, and 13C NMR spectroscopy. The inclusion of SV9 with SCX4 was relatively tight under basic conditions, while the inclusions with SCX6 and SCX8 were tighter under acidic conditions than under basic conditions. The NMR spectra suggested that the host formed a cap on the guest during inclusion. Throughout the experiment, SV9 was more soluble under acidic conditions than under basic conditions. Subsequently, UV–Vis absorption has been used to investigate the binding nature of SV9 to DNA in the presence of SCXn. The results show that the interaction of SV9 with DNA depends on the pH values. Furthermore, there were differences affected by difference SCXn. These conclusions strongly support the idea that SCXn–SV9 inclusion complex has important theoretical and practical value for the recognition mechanisms. The potential applications of the complexes of SV9 with sulfonated calixarenes require further study.

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Appendix A. Supplementary material

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References