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Spectroscopic studies on the interaction of Safranine T with DNA in β-cyclodextrin and carboxymethyl-β-cyclodextrin

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Abstract

The interaction of Safranine T (ST) with cyclodextrins (CDs) including β-cyclodextrin (β-CD) and carboxymethyl-β-cyclodextrin (CM-β-CD) and DNA in aqueous solution is studied by UV–vis absorption and steady-state fluorescence technique. The interactive model of ST with double stranded DNA has been investigated by means of the inclusive action of β-CD and CM-β-CD. Based on the changes of absorption, fluorescence and resonance light scattering (RLS) spectra, the intrinsic binding constant (K_{ap}) and the binding site number (n) of ST with DNA and the inclusion complexes ST–CD with DNA are obtained in the case of 20 mmol/L Tris–HCl buffer solution (pH 7.2). According to the experimental results, it can be inferred that the interactive model of dimer ST with DNA is "electrostatic binding", while the monomer ST with DNA is "intercalative binding".

Keywords: DNA; Cyclodextrin; Safranine T; Supramolecular system

1. Introduction

Current studies on supramolecular interactions of organic dyes with biological molecules are significant to understand the structures and functions of bio-macromolecules [1], and can be used to simulate some biophysical process. Generally, the interactions of organic dyes with DNA involve three binding modes: namely, intercalative binding that dyes intercalate into the base pairs of nucleic acids [3], groove binding in which the dyes bound on nucleic acids are located in the major or minor groove [2], and long-range assembly on the molecular surfaces of nucleic acids so that the dye binding is not related to the groove structure of the nucleic acids [2]. The studies on the supramolecular interacting systems can be made by investigating the binding characteristics of organic dyes through UV–vis absorption, circular dichroism, and fluorescence and resonance light scattering (RLS) spectrum [4].

Cyclodextrins (CDs) are cyclic oligosaccharides containing six (α), seven (β), eight (γ) D-glucose units, and guest molecules can be included in their relatively hydrophobic cavities [5–10]. Because of the unique properties, CDs have been widely used as biomimetic microreactors, novel media for photophysical and photochemical studies, and building blocks for supramolecular structures and functional units as well as in various fields of industries [11,12] such as pharmacology [13–15], food [16], environmental protection analysis [17], and enzyme modeling [18]. As we know, the hydrophobic effect can impel the hydrophobic groups of the guest inside the cavity of CD and the hydrophilic groups outside. If several charged groups have been linked onto the edge of CD, such as carboxymethyl groups or sulfobutyl groups, the characteristics of parent CDs will be changed. Except for the normal inclusion interaction, these CDs will have a further charge attraction on those hydrophilic groups of the

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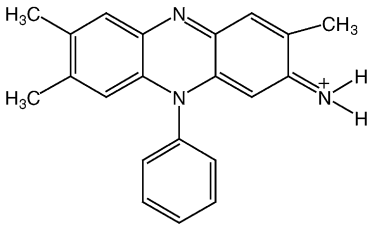

Scheme 1. The structure of Safranine T.

guest, which usually stay out of the cavity. If the hydrophilic groups are just those which interact with or intercalate into DNA, the inclusion action of neutral β-CD and negative CM-β-CD must affect the interaction in some degree.

Both DNA and CD are common in having hydrophilic coat/hydrophobic core structure. Thus, an aromatic ring staching between nucleobase pairs or incorporated in CD cavity is the main driving force for the binding of an intercalator into double stranded DNA and a guest molecule to CD, respectively. These stimulate used to investigate the complexing properties of CD and DNA as hosts for a guest. ST is an available biological dye, and it can be used as a fluorescence probe to investigate the structure of DNA molecules and to construct a sensitive assay of DNA [19]. Its structure is shown in Scheme 1.

In this study, CDs were used to change the microenvironment of ST molecule that interacts with DNA. Through comparing the difference, the conclusion is obtained that the dimer ST interacts with DNA by 'electrostatic binding' while the monomer ST with DNA by 'intercalative binding'.

2. Experimental

2.1. Apparatus

The absorption measurement was performed with a UV-265 spectrophotometer (Shimadzu). The fluorescence and resonance light scattering spectra were obtained with a F-4500 spectrofluorometer (Hitachi), equipped with a 150 W xenon lamp, a recorder, a dual monochromator, and a quartz cell (1 cm × 1 cm). The pH meter used (pH 2) was made in the 2th Instrument Factory of Shanghai. All experiments were carried out at 20 ± 1 °C.

2.2. Reagents

Calf thymus DNA (ctDNA, Baitai Biochemical Co., Chinese Academy of Sciences, Beijing, China) was purchased and used without further purification. The stock solutions were prepared by dissolving the solid DNA in double-distilled deionized water with occasionally gentle shaking, and stored at 4 °C and the useful life was not more than 5 days. The concentration was determined according to the absorbance at 260 nm after establishing that the absorbance ratio A_{260}/A_{280} was in the range 1.80–1.90, and the molarities of double stranded DNA solution were calculated based on $\varepsilon_{DNA} = 6600\ mol^{-1}\ cm^{-1}\ L$ [20].

The stock solution of 1.2×10^{-4} mol/L Safranine T (ST, the Third Reagent Factory of Shanghai, China) was prepared by directly dissolving its crystal into water. β-CD (95%, Yunnan Gourment Factory) was recrystallized twice from double-distilled water before use. Sodium salt of CM-β-CD was synthesized employing the paper written by Jacques Reuben [21] and has been used in our previous work [22] (D.S. = 4.8). Tris–HCl buffer solution (0.2 mmol/L; pH 7.2) was used to control the pH-value of the interacting system. All other reagents were analytical-reagent grade without further purification. Doubly distilled water was used throughout.

2.3. Procedure

The absorption, the fluorescence, and the resonance light scatting titrations were performed by keeping the concentration of ST constant while varying the concentrations of CDs or DNA and keeping the concentration of CD–ST constant while varying the concentration of DNA.

By scanning both the excitation and emission monochromators of a common spectrofluorometer with λ = 0 nm, a resonance light scattering spectrum can be developed [4], which has been proved to be able to investigate the aggregation of small molecules and the long-range assembly of organic dyes on biological templates [4,19].

3. Results and discussion

3.1. Formation of CD–ST inclusion complex

The changes of the absorption spectra of ST are shown in Fig. 1. The absorption spectra of ST upon increasing the concentration of CM-β-CD showed the strong enhancement in the absorption intensities. When the concentration of CM-β-CD reached to 7.0×10^{-3} mol/L, the absorption intensity became slow and was gradually leveled off at high concentration of CM-β-CD. It indicated that the ST was included completed by CM-β-CD. In this process, the absorption maximum exhibited red shifted by 3 nm. Similar phenomena were observed for β-CD.

As shown in Fig. 2, the fluorescence intensities of ST increase clearly in the presence of CDs. The maximum excitation and emission wavelength of ST itself were at 523 nm and 577 nm, respectively. Addition of different CDs (β-CD or CM-β-CD), the maxima emission wavelengths blue shifted to 573 nm or 571 nm, while the excitation wavelength red shifted to 525 nm or 527 nm, respectively. These changes were due to the interaction between the CDs and ST, again implying the formation of CD–ST inclusion complexes. It was noted that CM-β-CD resulted in greater fluorescence enhancement than β-CD, which suggested that the combination of negatively charged CM-β-CD with ST is superior to that of the neutral parent β-CD.

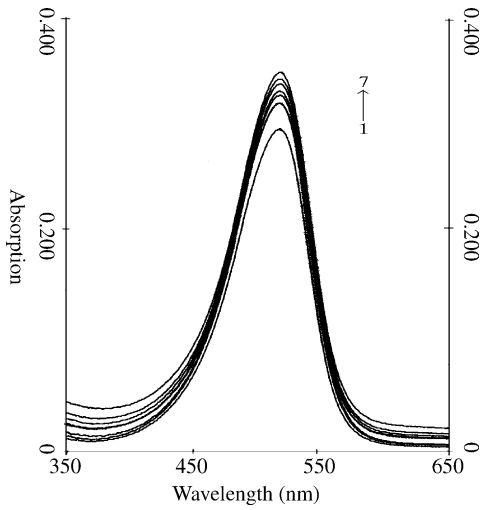

Fig. 1. The absorption spectra of 1.2×10^{-5} M ST in different concentrations of CM-β-CD media at pH 7.2. The concentration of CM-β-CD (M): (1) 0; (2) 5.0×10^{-4}; (3) 2.0×10^{-3}; (4) 3.0×10^{-3}; (5) 4.0×10^{-3}; (6) 5.0×10^{-3}; and (7) 7.0×10^{-3}.

The inclusion formation constant (K) was a measure for complexing capacity of CDs. The formation constants of ST with CD (β-CD or CM-β-CD) were estimated assuming a 1:1 (CD:ST) inclusion model. The inclusion equilibrium is as follows:

$$\mathrm{CD} + \mathrm{ST} \rightleftharpoons \mathrm{CD\text{–}ST}$$

where the symbols CD, ST, and CD–ST represent cyclodextrins (β-CD or CM-β-CD), Safranine T, and the inclusion complex, respectively. The formation constant can be obtained from fluorescence data by the modified Benesi–Hildebrand equation (double reciprocal plot) [23].

$$\frac{1}{F - F_0} = \frac{1}{(Kk[\mathrm{P}]_0[\mathrm{CD}]_0)} + \frac{1}{kQ[\mathrm{P}]_0} \quad (1)$$

where F and F_0 represent the fluorescence signals of ST in the presence and absence of CD; $[\mathrm{P}]_0$ and $[\mathrm{CD}]_0$ represent the initial concentration of ST and cyclodextrin; k is an instrumental constant; and Q is the fluorescence quantum yield of the inclusion complex. K is the formation constant of the complex, it was obtained from the ratio of the slope to the y-intercept.

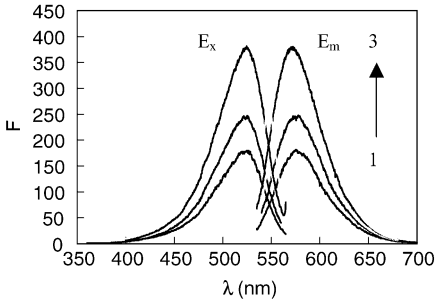

Fig. 2. Fluorescence spectra of 4.2×10^{-5} M ST in different media (pH 7.20): (1) H_2O; (2) β-CD; and (3) CM-β-CD.

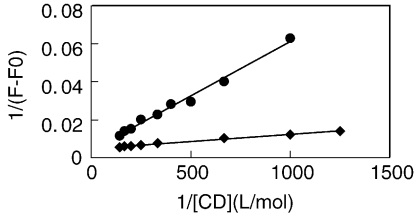

Fig. 3. Double reciprocal plots for ST complexed to (●) β-CD or (♦) CM-β-CD at pH 7.2.

Table 1
Formation constants K (M^{-1}) for CD–ST complexes at pH 7.2

	β-CD	CM-β-CD
f^a	1.5	2.1
Linear equation	$y = 3E{-}05x + 0.0048$	$y = 8E{-}06x + 0.0048$
r	0.996	0.995
K (M^{-1})	160	600

Fluorescence sensitive factor $f^a = (F - F_0)/F_0$, where F and F_0 represent the fluorescence intensity of ST in the presence and absence of CDs.

Fig. 3 shows the double reciprocal plots $1/(F - F_0)$ versus $1/[\mathrm{CD}]_0$ for ST to CDs at pH 7.2. The plots exhibit good linearity. This implies that the formation of inclusion complexes with a stoichiometry of 1:1 (β-CD:ST or CM-β-CD:ST). The formation constants for CD–ST complexes at pH 7.2 are shown in Table 1. It was noted that the β-CD derivative CM-β-CD exhibited stronger binding ability than the parent β-CD implying that the cavity of CM-β-CD provided a better protective microenvironment from the quenching that occurred in the bulk aqueous solution. Strong inclusion complexing ability can be understood from the enlarged cavity due to chemically modification and enhanced hydrophobicity. Moreover, CM-β-CD is negatively charged while the ST is positively charged under the experimental conditions. Thus, the additional electrostatic interactions of CM-β-CD led to stronger binding properties than that of β-CD.

3.2. The interaction of ST with DNA

The absorption spectra of ST in different concentrations of DNA are shown in Fig. 4. The absorption of ST–DNA complex decreased and red shifted to 510 nm with increasing concentration of DNA until the mole ratio of ST to DNA (m) approximate to 0.15. Considering that the absorption band of the dimer ST is located at 505 nm [24], it is possible that aggregation of ST occurs in the presence of DNA when $m > 0.15$. If, however, keeping on increasing the concentration of DNA, the maximum absorption of ST–DNA complex increases and bathochromically shifted to 540 nm, which is the absorption band of monomer ST with DNA. So, it can be obtained that the binding model is different from that of $m > 0.15$.

The dependence of the fluorescence spectra of ST on the concentration of DNA was shown in Fig. 5. At pH 7.2, ST has fluorescence emission at 577 nm if it is excited at 523 nm in

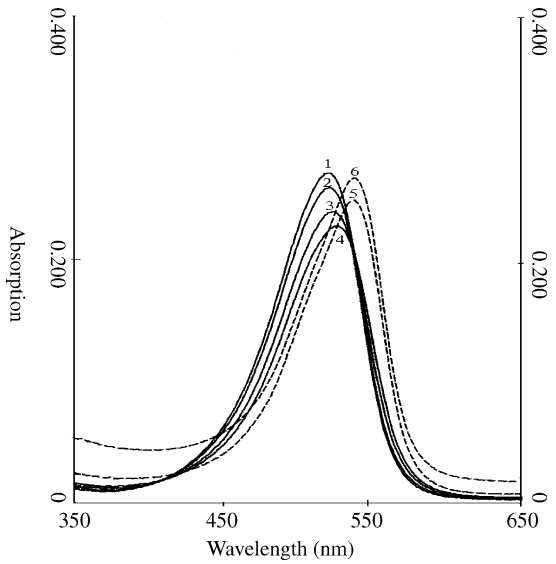

Fig. 4. The absorption spectra of 1.2×10^{-5} M ST in different concentrations of DNA at pH 7.20. The concentration of DNA (M): (1) 0; (2) 3.5×10^{-6}; (3) 1.2×10^{-5}; (4) 2.5×10^{-5}; (5) 2.6×10^{-4}; and (6) 7.8×10^{-4}.

aqueous solution. If DNA solution was pipetted into the ST solution at this time, the fluorescence maxima excitation and emission wavelengths showed a slight red and blue shifts, respectively. And the fluorescence emission was quenched linearly in the range $m > 0.15$.

In fluorescence quenching experiments, the data were plotted according to the Stern–Volmer Eq. (2):

$$\frac{I_0}{I} = I + K_{SV}[\text{DNA}] \tag{2}$$

where I_0 and I are the fluorescence intensities in the absence and presence of DNA. K_{SV} is the Stern–Volmer quenching constant, which is a measure of the efficiency of quenching by DNA [25]. According to the experimental data, it was obtained the quenching constant (K_{SV}) was obtained form the Stern–Volmer equation to be 11600 L/mol.

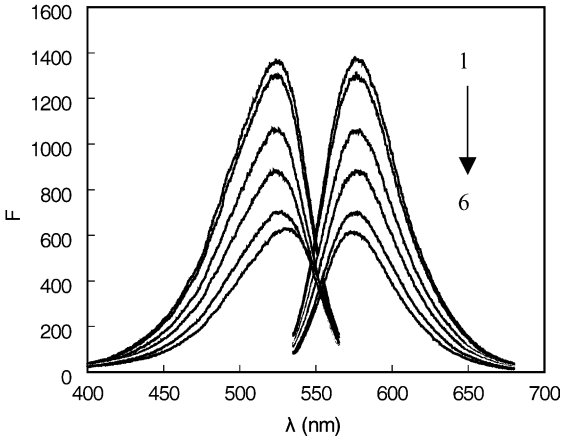

Fig. 5. Fluorescence spectra of 1.2×10^{-5} M ST in different concentrations of DNA at pH 7.2. The concentration of DNA (M): (1) 0; (2) 4.3×10^{-6}; (3) 1.1×10^{-5}; (4) 2.6×10^{-5}; (5) 3.5×10^{-5}; and (6) 4.8×10^{-5}.

Table 2
The intrinsic binding constant (K_{ap}) and binding number (n) of CDs–ST with DNA and the fluorescence quenching constant (K_{SV}) ($C_{ST} = 4.2 \times 10^{-5}$ mol/L, $C_{CM-\beta-CD} = 5.0 \times 10^{-3}$ mol/L) at pH 7.2

	K_{ap} (M^{-1})	n	K_{SV} (M^{-1})
In absence of CD	5.7×10^5	0.44	11600
β-CD	5.5×10^5	0.31	9200
CM-β-CD	1.3×10^5	0.15	8300

For the interaction of small molecules with macromolecules, Scatchard plot is commonly used to characterize the binding properties in terms of measuring the binding site number and binding constant. The data for Scatchard analysis are based on the measurements of absorbance or fluorescence of interacting system [19].

$$\frac{m}{[\text{ST}]} = nK - mK \tag{3}$$

where m is the molar ratio of bound ST to DNA base pair; n is the maximum value of m; K is the intrinsic binding constant of ST to DNA; [ST] is the equilibrium concentration of ST. From the experimental data, it can be obtained that the binding constant and binding site number of ST with DNA can be calculated, respectively (seen in Table 2).

Fig. 6 shows ST has two weak RLS peaks at 390 and 560 nm. We have proved that DNA has very weak RLS signals in this medium even if its concentration reaches 7.8×10^{-4} mol/L, but the RLS signals of ST depend on its concentration. In contrast, a strong broad RLS band in this range can be observed for the mixture of ST and DNA, indicating that the interaction between ST and DNA has occurred. These enhanced RLS signals increased with increasing DNA concentration when $m > 0.15$. Since the enhancement of RLS is always associated with the aggregation and depends sensitively on the electronic properties of the individual chromophores, so it is reasonable to establish the aggregation

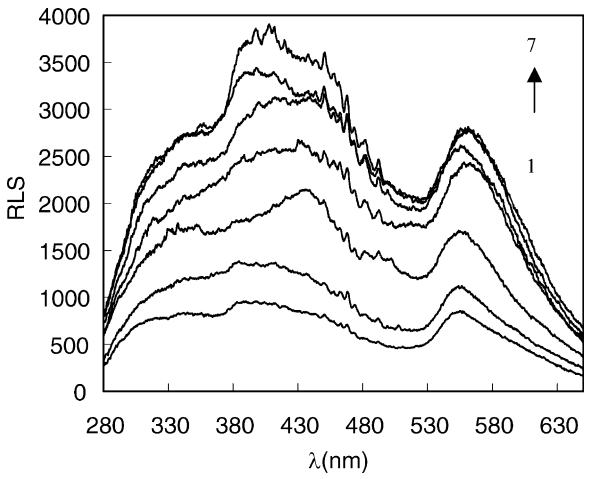

Fig. 6. The RLS spectra of 1.2×10^{-5} M ST in different concentrations of DNA at pH 7.2. The concentration of DNA (M): (1) 0; (2) 1.7×10^{-8}; (3) 5.2×10^{-8}; (4) 1.3×10^{-7}; (5) 1.6×10^{-7}; (6) 1.9×10^{-7}; and (7) 2.5×10^{-7}.

mechanism of the dimer ST in the presence of DNA when $m > 0.15$. When $m < 0.15$, the RLS signals decreased with increasing the concentration of DNA, which illustrated that the model of electrostatic binding had changed, the monomer ST intercalated into DNA.

As a positively charged molecule, ST can undoubtedly bind with a negatively charged DNA polyanion through electrostatic attraction. In the range $m > 0.15$, DNA can prompt the aggregation of ST to form the dimer of ST. The possible explanation is that DNA acts as a role of supplying negative charges to neutralize the positive charges of ST molecules, resulting in ST aggregation. So, the interaction of ST with DNA is ascribed to the electrostatic attraction between the positive ST and the negative DNA molecules. When $m < 0.15$, it was found that the absorption signals begin to increase while RLS signals begin to decrease with increasing DNA concentration, which means the binding mode of electrostatic attraction begins to change. The possible reason is that a large number of DNA molecules have been neutralized. In such case, there are not enough ST molecules for the binding of each nucleotide residue of DNA. Thus, the aggregate species of ST on the molecular surface of DNA was diluted to monomer ST by the presence of a large amount of DNA. Thus, the monomer ST intercalated into the double helix DNA.

3.3. The interaction of β-CD–ST and CM-β-CD–ST complexes with DNA

The effect of the addition of DNA to the complexed ST with CM-β-CD was investigated using absorption spectroscopy (Fig. 7). It was observed that, the absorbance of CM-β-CD–ST decreased with increasing of DNA while when the concentration of DNA was 2.6×10^{-4} mol/L, a new absorption peak obviously appeared which increased with increasing the concentration of DNA. This process was similar to that of ST alone to DNA. This suggested that the existence of β-CD or CM-β-CD did not affect the interactive model of ST with DNA and the inclusion complexes of CD–ST decomposed when it binds to DNA. Moreover, the interaction of ST with DNA is more favored and thus CD is replaced by DNA. This is quite reasonable due to the higher stability of ST–DNA complex than CD–ST complexes. However, the absorbance of turning point in the presence of CDs was still more than that in the absence of CDs. In addition, CM-β-CD concentration required for the turning point was much more than β-CD concentration. The reason is that CM-β-CD and DNA are negatively charged. The negatively groups of CM-β-CD compete with the phosphate groups of the DNA backbone, and have an attraction to the positive groups of ST. This competitive attraction supports the theory that the binding mode of ST with DNA is "electrostatic binding" when $m > 0.15$. Whereas monomer ST with DNA was "intercalative binding" when $m < 0.15$.

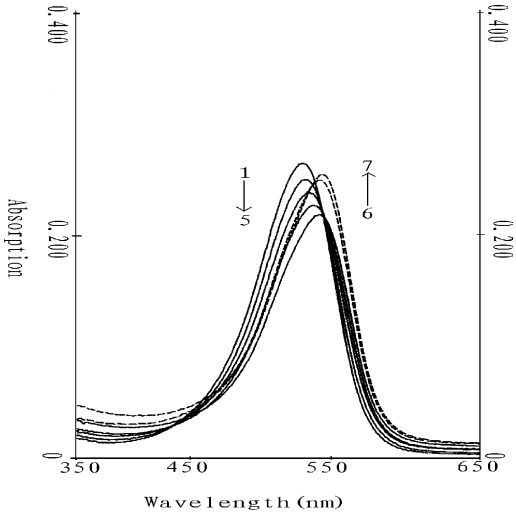

Fig. 7. The absorption spectra of ST with varying the concentrations of DNA in the presence of CM-β-CD at pH 7.2 ($C_{ST} = 1.2 \times 10^{-5}$ M and $C_{CM-\beta-CD} = 5.0 \times 10^{-3}$ M). The concentration of DNA (M): (1) 0; (2) 3.0×10^{-5}; (3) 6.5×10^{-5}; (4) 1.3×10^{-4}; (5) 2.4×10^{-4}; (6) 2.6×10^{-4}; and (7) 5.2×10^{-4}.

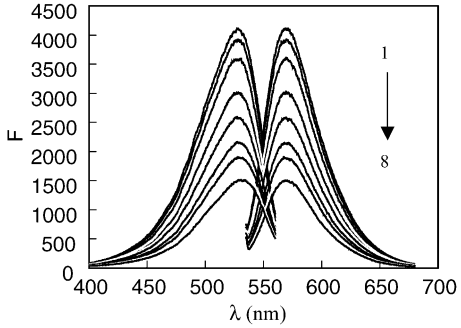

Fig. 8. Fluorescence spectra of ST with varying the concentrations of DNA in the presence of CM-β-CD at pH 7.2 ($C_{ST} = 1.2 \times 10^{-5}$ M and $C_{CM-\beta-CD} = 5.0 \times 10^{-3}$ M) at pH 7.2. The concentration of DNA (M): (1) 0; (2) 2.6×10^{-5}; (3) 6.5×10^{-5}; (4) 1.6×10^{-4}; (5) 2.2×10^{-4}; (6) 3.7×10^{-4}; (7) 4.4×10^{-4}; and (8) 5.2×10^{-4}.

Fig. 8 showed that the fluorescence intensity of CM-β-CD–ST decreased with increasing concentration of DNA. The intensity change and the shape of the fluorescence spectra of CM-β-CD–ST with the increase of DNA were similar to those of ST. The dependence of the fluorescence intensity on the concentration of DNA still corresponds to the Stern–Volmer's equation, the quenching constant and the intrinsic binding constant and binding number were listed in Table 2. It was clearly shown that the greater the inclusion formation constant, the smaller the intrinsic binding constant, binding number, and the quenching constant. These again implied that there existed competitive interaction between CDs and DNA with ST. In addition, negatively charged CM-β-CD had strong competitive interactive with DNA.

4. Conclusion

Based on the measurements of molecular absorption, fluorescence and resonance light scattering, the binding nature of

ST was characterized. Depending on the mole ratio of the two interacting components, the interaction of ST with DNA involves an aggregation of dimer ST on the molecular surfaces of DNA when $m > 0.15$ and an intercalation of monomer ST to double stranded DNA. A new idea was provided for further proving the interactive model of small molecules with DNA through the inclusion action of CD. Using a unique inclusion mechanism of the anion CD derivative, CM-β-CD, we conclude that the binding model of dimer and monomer ST with DNA are "electrostatic binding" and "intercalative binding", respectively. Furthermore, the existence of β-CD or CM-β-CD did not affect the interaction of ST with DNA and the inclusion complex decomposed when it binds to DNA. These investigations are of potential importance in understanding the mechanism of interaction and recognition of compounds in the living body.

Acknowledgements

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