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Studies on Interaction of Safranine T with Herring Sperm DNA in γ−Cyclodextrin

[γ−Siklodekstrin'de Safranin T ve Ringa Balığı Sperm DNA'sının Etkileşimi*]

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ABSTRACT

Purpose: In order to understand the reaction mechanism and provide guidance for the application of organic dyes targeted to DNA, the interactions of Safranine T (ST) in γ−cyclodextrin (γ−CD) with herring sperm DNA were explored in physiological buffer (pH 7.40).

Method: The resultant inclusion compounds were characterized by fluorescence spectra, powder X−ray diffraction, and FT−IR spectroscopy.

Results: The results confirmed the existence of 1:1 inclusion complex of ST with γ−CD. Fluorescence spectra were employed to understand the binding of γ−cyclodextrin−safranine T (γ−CD−ST) with DNA. The binding ratio of γ−CD−ST with DNA is $n_{\gamma-CD-ST}$:n_{DNA} = 9:1. The thermodynamic parameter elucidated that the binding was exothermic, driven mainly by enthalpy. As acridine orange (AO) presumably binds initially to DNA by intercalation, AO was employed in the study. Competitive interaction of γ−CD−ST and AO with DNA suggested γ−CD−ST substituted for AO in the DNA–AO system, that means there exist intercalation binding. The influence of A, T, C, G to γ−CD−ST showed γ-CD-ST mainly acted on the A–T enrichment regions of the DNA. The relative viscosity of DNA further suggested there exist a partial non-classical intercalation binding. From Scatchard method, we can confirm that there are groove and intercalation binding between γ-CD-ST and DNA.

Conclusion: The results taken together suggested the interactions of γ−CD−ST with DNA are intercalation and groove binding

Key Words: Fluorescence spectra, DNA, γ−cyclodextrin, safranine T, supramolecular system

Conflict of interest: The authors declared that they have no conflicts of interest to this work.

ÖZET

Amaç: DNA'ya hedeflenmiş organik boyaların kullanımı ve reaksiyon mekanizmasını anlamak amacıyla, fizyolojik tamponda (pH 7.40) Safranin T'nin (ST) ringa balığı sperm DNA'sı ile γ−Siklodekstrin'de (γ−CD) etkileşimi incelenmiştir.

Yöntem: Oluşan bileşikler fluoresans spektrası, X-ışını toz kırınımı ve FT-IR spektroskopisi ile tanımlanmıştır.

Bulgular: Sonuçlar ST'nin γ−CD ile 1:1 oranında kompleks oluşturduğunu doğrulamaktadır. Fluoresans spektrası γ−siklodekstrin−safranin T (γ−CD−ST)'nin DNA ile bağlanmasını anlamak amacıyla kullanılmıştır. γ−CD−ST'nin DNA ile bağlanma oranı $n_{\gamma-CD-ST}$:n_{DNA} = 9:1'dir. Termodinamik parametreler bağlanmanın ekzotermik olduğunu ve esas olarak entalpi ile sağlandığını ortaya koymaktadır. Akridin turuncusu (AO) tahminen ilk olarak DNA'ya interkalasyon (araya ilave edilme) ile bağlandığı için çalışmaya dahil edilmiştir. γ−CD−ST ve AO'nın DNA ile yarışmalı etkileşimi, DNA-AO sisteminde γ−CD−ST'nin AO'nun yerini alabileceğini, bu da interkalasyon şeklinde bir bağlanmanın varlığını göstermektedir. A, T, C, G'nin γ−CD−ST'ye etkisi çoğunlukla DNA'nın A-T bakımından zengin bölgelerinde görülmektedir. DNA'nın göreli akışkanlığı, kısmi klasik olmayan interkalasyon bağlanmasının olduğunu öne sürmektedir. Scatchard metodu ile γ-CD-ST ile DNA arasında oluk ve interkalasyon bağlanmasının olduğunu doğrulayabiliriz.

Sonuç: Bulgular bir arada dikkate alındığında γ-CD-ST ve DNA arasındaki etkileşim interkalasyon ve oluk bağlanması şeklindedir.

Anahtar Kelimeler: Fluoresans spektrası DNA, γ−siklodekstrin, safranin T, supramoleküler sistem

Çıkar çatışması: Yazarlar bu çalışmada hiçbir çıkar çatışması bulu nmadığını beyan etti.

Introduction

Current studies of 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 imitate some biophysical process. In general, a variety of organic dyes reversibly have three distinct binding modes of non−covalent interaction with DNA [2]: intercalation into the base pairs, in the major or minor grooves and outside the helix by electrostatic interactions. These binding studies were driven partly by the need to understand the mechanism of organic dyes.

Organic dyes are stabilized on groove by binding and intercalation with DNA through a series of associative interactions such as hydrogen bond, attractive van der Waals, hydrophobic interaction and so on [3]. The intercalative binding is stronger than other two binding modes because the surface of intercalative molecule is inserted into base pairs of DNA.

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 [4–6]. As we know, the hydrophobic effect can impel the hydrophobic groups of the guest inside the cavity of CD and the hydrophilic groups are located outside [7]. Safranine T (ST) is an available biological dye, which features a planar phenazine ring and a positive charge. Therefore, the planar hydrophobic phenazinyl moiety of ST is expected to facilitate intercalation of ST into the nonpolar interior of the DNA helix [8]. So it can be used as a fluorescence probe to investigate the structure of DNA molecules and to construct a sensitive assay of DNA [9]. Its structure is shown in Figure 1.

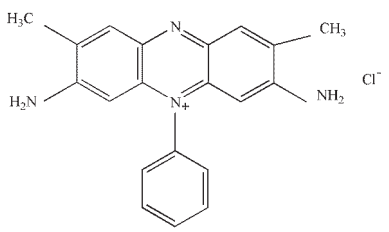

Figure 1. Molecular structure of Safranine T

In this study, the supramolecular interacting systems were used to change the microenvironment of ST molecule that interacts with DNA. The strong absorption and fluorescence characteristics of ST in γ−cyclodextrin provide sensitive spectroscopic changes to study its interaction with DNA. The changes in the intensities of these spectra can be used to explain the strength of the stacking interaction between ST and the DNA base pairs in γ−cyclodextrin.

Experiments and methods

Materials

Herring sperm DNA was purchased from Sigma Biological Co. (USA) and used as received. Purity of DNA was checked by monitoring the ratio of absorbance at 260−280 nm. The ratio was 1.89, indicating the DNA was free from protein. The DNA was dissolved in doubly distilled deionized water with 50 mM NaCl and dialyzed for 48 h against a buffer solution at 277K. The concentration of DNA stock solution was determined according to the absorbance at 260 nm by using the extinction coefficient of 6600 $(mol \cdot cm)^{-1}$.

All of the samples were dissolved in Tris−HCl buffer (pH 7.40, examined by acidometer). Tris and Safranine T were purchased from Tianjin Kemi'ou chemical reagents center (A.R.). Acridine orange (AO) was purchased from Shanghai China Medicine Chemical Plant (A.R.). γ−Cyclodextrin (γ−CD) were purchased from Sichuan Chengdu China Kelong chemical plant (A.R.). Other reagents were at least analytical grade, and were used without further purification.

Instruments

The fluorescence spectra were recorded on a PE LS55 spectrofluorophotometer, made by Perkin Elmer Instrument Co., USA. The X−ray diffraction patterns were collected on an X'Pert PRO diffractometer, made by PAN−alytical B.V. The infrared spectra were recorded on a Spectrum One spectrometry, made by PE Instrument Co., USA. The pH was recorded on a pHS−2C acidometer (made in China). All of the spectroscopic work was carried out at pH 7.40 maintained by a Tris–HCl buffer.

Procedures

Samples for fluorescence were prepared by mixing known amounts of stock solutions of ST, DNA and γ−CD in Tris–HCl buffer (pH 7.40) and diluted to the required concentrations. The fluorescence titrations were conducted by keeping the concentration of ST constant while varying the concentration of γ−CD, or keeping the concentration of γ−CD–ST inclusion complex constant while varying the concentration of DNA. In fluorescence mode, both excitation and emission bandwidths were set at 5 nm, λ_{ex} = 411 nm 1.0 cm path length quartz cuvettes were used for fluorescence measurements.

The powder X−ray diffraction operated at a voltage of 35 kV and a current of 50 mA. The samples were analyzed in the 2θ angle range of (5–90) ^{o} and the process parameters were set as: scan step size of 0.02^{o}, scan step time of 1.54 s.

The infrared spectra of the samples were mixed with KBr and compressed as disks. The selected wavenumber ranged between 400 and 4000 cm^{-1} being the spectra resolution of 4 cm^{-1} and 10 being the number of scans.

Viscosity measurements were performed using a viscometer, which was immersed in a thermostat water-bath at 25 ±0.1 °C. An appropriate amount of γ−CD−ST was added into the viscometer to give a certain r (r = [γ−CD−ST] / [DNA]) value while keeping the DNA concentration constant. After a thermal equilibrium was achieved, the flow times of the samples were repeatedly measured with an accuracy of ±0.2 s by using a digital stopwatch. The data were presented as $(h/h0)^{1/3}$ versus r, where h and $h0$ are the viscosity of DNA in the presence and absence of γ−CD−ST, respectively.

Results and Discussion

Confirmation of γ−CD−ST inclusion complexes

Usually, when organic dyes interact with DNA and form new complexes, changes in fluorescence should occur [10]. The changes of the fluorescence spectra of ST are inset in Figure 2. The fluorescence spectra of ST upon increasing the concentration of γ−CD showed the reduction in the fluorescence intensities. When the concentration of γ−CD reached to 2.00x10^{-5} mol.L^{-1}, the fluorescence intensity became slow and was gradually leveled off. It indicated that the ST was included completed by γ−CD. In this process, the absorption maximum exhibited red shifted by 2 nm.

In order to determine the stoichiometry of the formation of γ−CD−ST inclusion complex, the mole ratio [11] experiment was done at the peak 579 nm. The fluorescence spectra of ST upon increasing the concentration of γ−CD were recorded, and then the graph was plotted by mole ratio method. The mole ratio plots of ST with γ−CD are shown in Figure 2. The inclusion complex has a 1:1 stoichiometry. The inclusion formation constant (K) was a measure for complexing capacity of CDs, which can be determined by the double−reciprocal method using the following equation [12]:

$$1/(F_0 - F) = [1/(\alpha \cdot K_f)] \cdot (1/c_{g-CD}) + 1/\alpha$$

where F_0 and F are the fluorescence signals of ST in the absence and presence of γ−CD, respectively. K_f is the binding constant between γ−CD and ST. $c_{γ-CD}$ is the concentration of γ−CD. α is a constant. And the binding constants were calculated from the ratio of the intercept on the vertical: $K_f = 7.41 \times 10^3$ L.mol^{-1}.

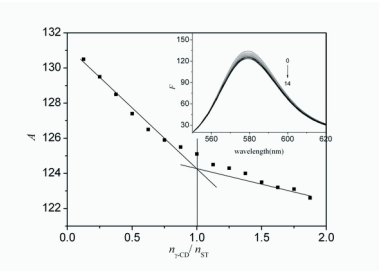

Figure 2. Mole ratio plots of ST with γ-CD in a Tris-HCl buffer (pH 7.40; λ = 579 nm);
c_{ST} = 2.00x10^{-5}mol·L^{-1}. Inset: Fluorescence spectra of ST in different concentrations of γ-CD (pH 7.40; λ_{ex} = 411 nm). From curve 0-14, c_{ST} = 2x10^{-5} mol·L^{-1}; $c_{γ-CD}$ = 0.00, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50, 2.75, 3.00, 3.25, 3.50×10^{-5} mol·L^{-1}, respectively.

X−ray diffraction studies allow the identification of true inclusion complexes of cyclodextrins, mainly based on the empirical evidence that the powder XRD patterns of these complexes should be clearly distinct from those obtained by the superimposition of the diffractograms of the individual components [13]. Figure 3 shows the patterns for the inclusion complex γ−CD−ST (a), ST (b), γ−CD (c) and physical mixture γ−CD and ST (d). The diffractogram for the inclusion complex γ−CD−ST is clearly different from the sum of the individual patterns of ST (b) and γ−CD (c). The pattern of the inclusion complex has two main peaks at 2θ = 17.6° and 22.8°. The sharp diffraction peaks appear in spectra of both γ−CD (c) and physical mixture (d), whereas the peaks disappear in the spectrum of the inclusion complex (a). This phenomenon verifies the inclusion formation between γ−CD and ST.

FT−IR is a useful tool to prove the presence of both guest and components in the inclusion complex [14]. Figure 4 shows the infrared spectra of wavenumber from 4000 to 400 cm^{-1} of γ−CD (a), ST (b), physical mixture γ−CD and ST (c) and the inclusion complex of γ−CD−ST (d). The IR absorption peak of the physical mixture (c) is simple superposition of the peak of γ−CD (a) and that of ST (b).The γ−CD (a) spectrum shows a band at 3420.0 cm^{-1} due to the symmetric and antisymmetric O−H stretching mode, a band at 2920.0 cm^{-1} due to the C−H stretching mode and other bands at low wave number.

Some bands of the host and guest are affected by the formation of the inclusion complex resulting in a change of position and relative intensities. The band at 3420.0 cm^{-1} of the γ−CD is shifted to high frequency at 3450.0 cm^{-1} in the inclusion complex. The absorption bands at 1420 and 1350 cm^{-1} of ST almost disappeared in the γ−CD−ST inclusion complex, indicating that ST was entrapped into the host cavities. The result is the same as that of X−ray diffraction mentioned above. This also testifies that the ST has entered into the cavity of γ−CD and formed inclusion complex.

Binding studies

The changes in the fluorescence of γ−CD−ST with increasing concentrations of DNA are inset in Figure 5. The fluorescence intensity of γ−CD−ST in the presence of increasing amounts of DNA showed a strong decrease and tended to constant at the high concentration of DNA, which indicated the interaction of γ−CD−ST with DNA.

In order to determine the stoichiometry of the formation of DNA−γ−CD−ST complex, the mole ratio experiment was also done at the peak 579 nm. The mole ratio plots of DNA with γ−CD−ST were shown in Figure 5. The binding ratio of the complex was: $n_{γ-CD-ST}$:n_{DNA} = 9:1.

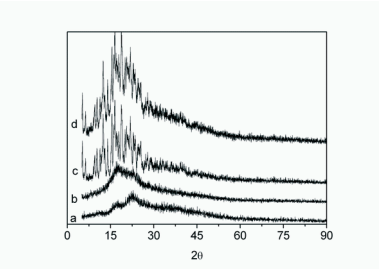

Figure 3. X-ray diffraction patterns corresponding to the following products: (a) γ-CD-ST; (b) ST; (c) γ-CD; (d) physical mixture.

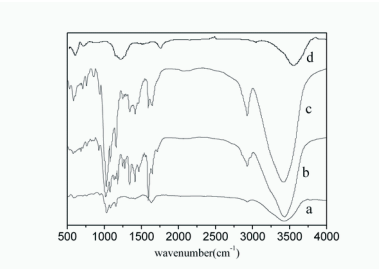

Figure 4. IR spectra of γ-CD-ST systems: (a) γ-CD; (b) ST; (c) physical mixture; (d) γ-CD-ST.

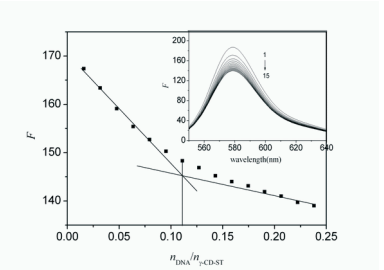

Figure 5. Mole ratio plots of γ-CD-ST with DNA in a Tris-HCl buffer (pH 7.40; λ = 579 nm); $c_{\gamma\text{-CD-ST}}$ = 2.1.00x10^{-5}mol·L^{-1}. Inset: Fluorescence spectra of γ-CD-ST in different concentrations of DNA (pH 7.40; λ_{ex} = 411 nm). From curve 1-15, $c_{\gamma\text{-CD-ST}}$ = 2.1x10^{-5} mol·L^{-1}; c_{DNA} = 0.00, 0.33, 0.67, 1.00, 1.33, 1.67, 2.00, 2.33, 2.67, 3.00, 3.33, 3.67, 4.00, 4.33, 4.67x10^{-6} mol·L^{-1}, respectively.

The fluorescence relationship between γ−CD−ST and DNA was expressed by double reciprocal equation:

$$1/(F_0-F) = [1/(\alpha \cdot K_f)] \cdot (1/c_{DNA}) + 1/\alpha \quad (1)$$

where F_0 and F are the fluorescence signals of γ−CD−ST in the absence and presence of DNA, respectively. K_f is the binding constant between DNA and γ−CD−ST. c_{DNA} is the concentration of DNA. α is a constant.

The double reciprocal plots of $1/(F_0-F)$ versus $1/c_{DNA}$ are linear (at 298.15K and 310.15K, respectively), and the binding constants are calculated from the ratio of the intercept on the vertical: $K^{\Theta}_{298.15K} = 2.84\times10^4$ L·mol^{-1}, $K^{\Theta}_{310.15K} = 1.24\times10^4$ L·mol^{-1}. Considering the dependence of binding constant on temperature, a thermodynamic process was considered to be responsible for the formation of the complex. Therefore, the thermodynamic parameters dependent on temperatures were analyzed in order to characterize the interaction forces between γ−CD−ST and DNA. The thermodynamic parameters of binding reaction are the main evidence for confirming the binding force. According to Thermodynamic equation of K^{Θ}, $\Delta_r H_m^{\Theta}$, $\Delta_r G_m^{\Theta}$, $\Delta_r S_m^{\Theta}$and T:

$$\ln K_2^{\Theta}/K_1^{\Theta} = -\Delta_r H_m^{\Theta}(1/T_2 - 1/T_1)/R \quad (2)$$

$$\Delta_r G_m^{\Theta} = -RT\ln K^{\Theta} \quad (3)$$

$$\Delta_r G_{m,T}^{\Theta} = \Delta_r H_m^{\Theta} - T\Delta_r S_m^{\Theta} \quad (4)$$

where K_1^{Θ} and K_2^{Θ} refer to standard binding constants of γ−CD−ST and DNA at 298.15K and 310.15K, respectively. T_1 is 298.15K, T_2 is 310.15K, $\Delta_r H_m^{\Theta}$ is standard molar reaction enthalpy. $\Delta_r G_m^{\Theta}$ refers to the standard molar reaction Gibbs free energy. $\Delta_r S_m^{\Theta}$ refers to the standard molar reaction entropy. Then $\Delta_r H_m^{\Theta} = -1.85\text{x}10^5$ J·mol^{-1}is deduced. The negative result showed it was an exothermic reaction. The $\Delta_r G_{m\ 298.15K}^{\Theta} = -2.56\text{x}10^4$ J·mol^{-1}. The negative result showed spontaneous interaction tide between γ−CD−ST and DNA. The $\Delta_r S_m^{\Theta} = -534.63$ J·mol^{-1}·K^{-1}. The results suggested that the process of interaction of γ−CD−ST and DNA were mainly driven by enthalpy [15−16].

Competitive interaction of γ−CD−ST inclusion complex and AO with DNA

Further support for the mode of binding between γ−CD−ST inclusion complex and DNA is given through the competitive experiment. Here AO has been employed in the examination of the reaction, as AO presumably binds initially to DNA by intercalation. Figure 6 shows the emission spectra of the DNA–AO system in the absence and presence of γ−CD−ST. When the concentration of γ−CD−ST inclusion complex was added, a remarkable fluorescence decrease of DNA–AO system was observed at the maximum of 529 nm and there is a new isosbestic point at 572 nm. This phenomenon suggested that γ−CD−ST substituted for AO in the DNA–AO system which led to a large decrease in the emission intensity of the DNA–AO system.

The influence of A, T, C, G to the inclusion complex system

In order to further confirm the action mode between γ-CD-ST and DNA, we also conducted a study on the influence of A, T, C, G to the inclusion complex system. If the fluorescence spectra of the inclusion complex has an obvious change by adding of these base pairs, then there are two possibilities: First, γ-CD-ST effects the base pairs of DNA through the major groove and minor groove of DNA molecule. Second, the inclusion complex can through the domain π system occurred π–π interaction and hydrophobic interaction with the π system of base pairs, which means that there are intercalation binding mode. Figure 7 shows the influence of these base pairs to the inclusion complex system, the fluorescence spectra of complex decreases steadily with increasing the amounts of base pairs. The rates of change of base pairs are as follow: C: 21.1%; G: 29.7%; A:

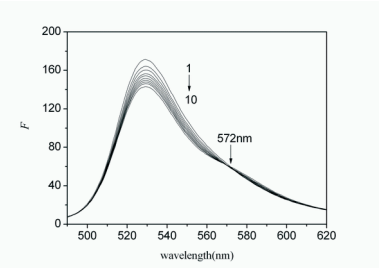

Figure 6. Emission spectra of DNA–AO mixture in different concentrations of γ-CD-ST (pH7.40; λ_{ex} = 411 nm). From curve 1-10, $c_{DNA–AO}$ = 1.00×10^{-5} mol·L^{-1}; $c_{γ\text{-CD-ST}}$ = 0.00, 0.67, 1.33, 2.00, 2.67, 3.33, 4.00, 4.67, 5.33, 6.00 ×10^{-5} mol·L^{-1}, respectively.

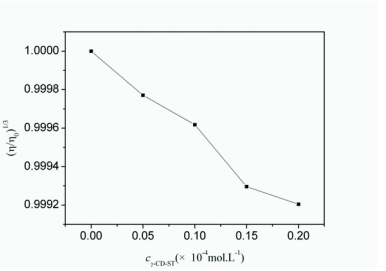

Figure 8. Influence on DNA viscosity with different concentrations of γ-CD-ST. c_{DNA} = 1.00x10^{-5} mol·L^{-1}.

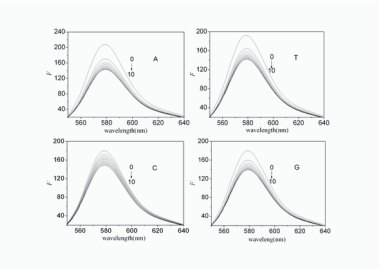

Figure 7. Fluorescence spectra of γ-CD-ST in different concentrations of A, T, C, G (pH 7.40). From curve 0-10, $c_{γ\text{-CD-ST}}$ = 2.00x10^{-5} mol·L^{-1}; c_A, c_T, c_C, c_G = 0.00, 0.20, 0.40, 0.60, 0.80, 1.00, 1.20, 1.40, 1.60, 1.80, 2.00×10^{-4} mol·L^{-1}, respectively.

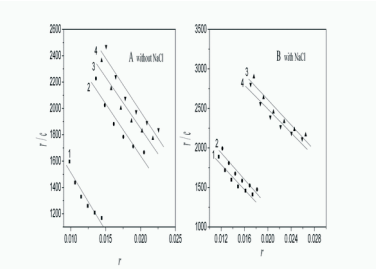

Figure 9. Scatchard plots of DNA-γ-CD-ST in different concentrations of AO (A:without NaCl; B: with NaCl. pH 7.40). c_{DNA} = 1.00x10^{-5} mol·L^{-1}; Rt = $c_{γ\text{-CD-ST}}$ /c_{DNA}; Rt = 1:0.00, 2:0.60, 3:1.20, 4:1.80.
en the inclusion complex γ-CD-ST and DNA.

39.4%; T: 35.5%. And as can be seen from these figures, in the vicinity of 579 nm, the binding force of the inclusion complex with A and T are greater than C and G, so that γ-CD-ST mainly acts on the A–T enrichment regions of the DNA. Therefore, we deduced that the interaction mode between γ-CD-ST inclusion complex and DNA might be groove binding and intercalation binding.

Viscosity measurements

Viscosity experiment is an effective tool to determine the binding mode of small molecules and DNA. A classical intercalation binding demands the space of adjacent base pairs to be large enough to accommodate the bound ligand and to elongate the double helix, resulting in an increase of DNA viscosity [17]. A partial non-classical intercalation of the complex would reduce the DNA viscosity. A series of solutions was made which contained a fixed concentration of DNA and various concentrations of γ-CD-ST. Then, the viscosity measurements were conducted at room temperature. The changes in relative viscosity of DNA with increasing concentrations of γ-CD-ST inclusion complex are shown in Figure 8. It can be seen that the relative viscosity of DNA decreased steadily with increasing amounts of γ-CD-ST. Such behavior further suggested that a partial non-classical intercalation binding should be the interaction mode of γ-CD-ST with DNA.

Scatchard method

The binding mode between small molecules and DNA can be determined using the Scatchard's procedure [18]. Scatchard equation expresses the binding of AO-DNA in the presence of γ-CD-ST:

$$r/c = K\,(n—r) \qquad (5)$$

where r is the moles of AO bound per mole of DNA, c is the molar concentration of free AO, n is binding site multiplicity per class of binding sites and K is the association binding constant of AO with DNA. Generally, it is regarded as an intercalation binding mode if the values of n are same in the presence and absence of the inclusion complex, and it is regarded as a nonintercalation binding mode if the values of K are same. And it is regarded as mix binding mode including nonintercalation and intercalation binding if both the values of n and K are changed. Two groups of buffers in presence of NaCl and absence of NaCl as a contrast were constructed in Figure 9. From the Scatchard plot, we can get the value of K and n. The results are shown in Table 1.

As shown in Table 1, it can be seen that both values of n and K changed with the different concentrations of γ-CD-ST. The variation of the parameter n and K suggests a mix interaction herein. Normally, the values of n in the presence of NaCl are lower than those in the absence of NaCl, indicating there is an electrostatic in-

Table 1. Data of Scatchard Equation of the interaction between g-CD-ST and DNA

Curve	$c_{g\text{-}CD\text{-}ST}$ /c_{DNA}	NaCl %	Scatchard	*K*/ (L·mol^{-1})	n
a	0	0.50	2.74 10^3—7.94 10^4x	7.94 10^4	0.035
		0	2.40 10^3—8.84 10^4x	8.84 10^4	0.027
b	0.60	0.50	2.95 10^3—8.44 10^4x	8.44 10^4	0.035
		0	3.22 10^3—7.91 10^4x	7.91 10^4	0.041
c	1.20	0.50	4.21 10^3—8.00 10^4x	8.00 10^4	0.053
		0	3.40 10^3—7.77 10^4x	7.77 10^4	0.044
d	1.80	0.50	4.00 10^3—7.54 10^4x	7.54 10^4	0.053
		0	3.60 10^3—8.11 10^4x	8.11 10^4	0.044

teraction between small molecules and DNA. While in Table 1, the values of *n* in the presence of NaCl are basically higher than those in the absence of NaCl, and combined with the results of base pairs, we can confirm that there are groove binding and intercalation binding between the inclusion complex γ-CD-ST and DNA.

Conclusions

The binding interactions of γ-CD-ST with DNA in physiological buffer were illustrated with fluorescence spectroscopic techniques. The binding constants of γ-CD-ST with DNA were measured at different temperatures and the thermodynamic parameters were calculated as well. The intercalative binding and groove binding of γ-CD-ST with DNA were deduced by taking account of relevant fluorescence spectra and viscosity measurements. We believe that the binding mode of γ-CD-ST with DNA studied here will provide useful information on the mechanism of small molecules binding to DNA.

Conflict of interest: The authors declared that they have no conflicts of interest to this work.

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