Studies on the Binding of DNA with the Inclusion of Brilliant Green Inside the Cavity of γ-Cyclodextrin

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ABSTRACT: *The interaction of brilliant green with herring sperm DNA was investigated in detail by spectrometric methods in γ-cyclodextrin systems. On the condition of physiological pH, brilliant green prefers to form the 1:1 inclusion complex with γ-cyclodextrin. All the evidences indicated that the binding modes between γ-cyclodextrin-brilliant green and DNA were grooving binding and partial non-classical intercalative binding. The binding ratio of the inclusion complex with DNA is 6:1. The calculated thermodynamic parameters suggested that the binding of the inclusion complex to DNA was driven mainly by entropy.*

KEY WORDS: *Brilliant green, γ-cyclodextrin, Spectrometric, Interaction.*

INTRODUCTION

Investigations on the interaction of small molecules with DNA have attracted many scientists over the past years because it is fundamental for biotechnological relevance [1]. The results of interaction modes studies have been used in designing new and efficient drugs, studying the structure of DNA and protein-nucleic acid recognition [2–4]. For the agents which bind to DNA, intercalation, groove binding and electrostatic binding are the three mainly binding modes [5-6]. As one of the commonly known cationic dyes, Brilliant Green (BG, as shown in Fig. 1) has been used for biological staining and diagnosis of disease [7].

In recent years, CycloDextrins (CDs, as shown in Fig. 2) were employed as a highly organized host media to investigate the interaction of organic dyes with DNA [8–10]. CD is found to be of great importance because of the amphiphilicity of combining the hydrophobic cavity with hydrophilic periphery. The hydrophobic cavity can serve as a selective container for the molecules of proper size, and the hydrophilicity is really very convenient for carrying out experiments in aqueous solution. In this paper, we have confirmed that BG could enter the cavity of γ-CD to form the inclusion complex. Unlike the interactive mode of BG with DNA [11], through the effect of γ-CD medium, the interaction of γ-CD-BG with DNA has been investigated by different interactive modes using absorption and fluorescence spectra.

EXPERIMENTAL SECTION

Materials

Herring sperm DNA (Sigma biological Co.) was purchased and used as received. 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 after establishing that the absorbance ratio A_{260}/A_{280} was in the range 1.80-1.90, by using the extinction coefficients of 6600 $(mol\ cm)^{-1}$.

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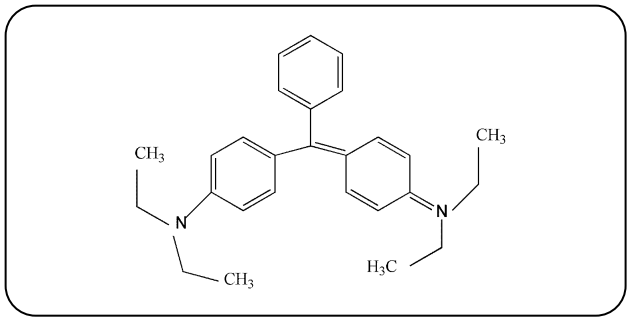

Fig. 1: Molecule structure of Brilliant green.

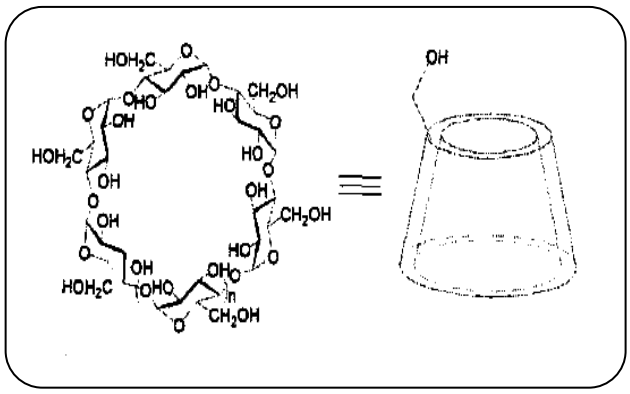

Fig. 2: Molecule structure of Cyclodextrin (n=1: α-cyclodextrin, n=2: β-cyclodextrin, n=3:γ-cyclodextrin).

Tris and brilliant green were purchased from Tianjin Kemi'ou chemical reagents center (China). Acridine Orange (AO) was purchased from Shanghai medicine chemical plant (A.R., China). γ-CD was purchased from Sichuan Chengdu Kelong chemical plant (A.R., China). Other reagents were at least analytical grade, and were used without further purification.

Methods

Sample preparation

Samples for absorption and emission experiments were prepared by mixing certain amounts of stock solutions of BG, hsDNA and CD, diluting the mixture with Tris-HCl buffer (pH=7.40, examined by acidometer) to the appropriate concentrations.

Absorption studies

Absorbance spectra were recorded on an UV-3150 spectrophotometer, made by Japan Shimadzu using a 1.0 cm path length cell. The absorption titrations were performed by keeping the concentration of BG constant while varying the concentration of γ-CD, or keeping the concentration of γ-CD-BG inclusion complex constant while varying the concentration of DNA. All the absorption measurements were made against the blank solution treated in the same way.

Fluorescence studies

The fluorescence spectra were measured on a PE LS55 spectrofluorophotometer, made by PerkinElmer Insrtument Co. USA. Both excitation and emission bandwidths were set at 5 nm, λ_{ex}= 411 nm. 1.0cm path-length quartz cuvettes were used for fluorescence measurements.

X-ray diffraction

The X-ray diffraction patterns were collected on X'Pert PRO diffractometer, made by PANalytical B.V. The powder X–ray diffraction operated at a voltage of 35kV and a current of 50mA. The samples were analyzed in the 2θ angle range of (5–90)◦ and the process parameters were set as: scan step size of 0.02◦, scan step time of 1.54s.

IR spectra

The infrared spectra were recorded on a Spectrum One spectrometry, made by PE Insrtument Co. USA. The infrared spectra of the samples were mixed with KBr and compressed as disks. The selected wave number ranged between 400 and 4000cm^{-1} being the spectra resolution of 4cm^{-1} and 10 being the number of scans.

Viscosity measurements

Viscosity measurements were performed using a viscometer, which was immersed in a thermostat water bath at room temperature. Different amounts of γ-CD-BG were added into the viscometer while keeping the DNA concentration constant. The flow times of each sample were measured three times with an accuracy of ±0.20 s and above 250 s. The data were presented as $(\eta/\eta_0)^{1/3}$ versus $c_{\gamma\text{-CD-BG}}$, where η and η_0 are the viscosity of DNA in the presence and absence of the inclusion complex, respectively.

RESULTS AND DISCUSSION

Study on the inclusion complex of BG with γ-CD

Absorption spectra of γ-CD adding in BG were put in the inset of Fig. 3. Added γ-CD to BG solution at pH of 7.40

resulted in absorption spectral change, indicating the formation of an inclusion complex between γ-CD and BG. The absorption of BG decreased at 320nm and 427nm and increased at 350nm by increasing concentrations of γ-CD. The appearance of isosbestic points at 333nm and 379nm also confirm that a new inclusion complex of γ-CD-BG is formed [12].

Fig. 3 shows the mole ratio plots of BG with γ-CD, similar to that explained elsewhere [13]. The absorption spectra of BG upon increasing the concentration of γ-CD were recorded at 427nm, and then the graph was plotted by mole ratio method to determine the stoichiometry of the formation of γ-CD-BG inclusion complex. As can be seen in Fig. 3, the inclusion complex has a 1:1 stoichiometry. The inclusion constant (K) is an important parameter to represent the inclusion capacity, which can be determined by the double-reciprocal method using the following equation [14]:

$$1/\Delta A = 1/(A_0 - A) = [1/(\alpha \cdot K_f)] \cdot (1/c_{\gamma\text{-CD}}) + (1/\alpha) \qquad (1)$$

Where A_0 and A are the absorbencies of BG in the absence and presence of γ-CD. α is the constant. K_f is the binding constant between γ-CD and BG, $c_{\gamma\text{-CD}}$ is the concentration of γ-CD. The binding constants were calculated from the ratio of the intercept on the vertical axis: K_f=3537.56 L/mol, similar to the experiments presented elsewhere [15].

X-ray powder diffraction patterns of BG (**a**), γ-CD (**b**), the inclusion complex γ-CD-BG (**c**) and physical mixture γ-CD and BG (**d**) were collected in Fig. 4. The host molecule (γ-CD) reacts with guest molecule (BG) to form a 1:1 host–guest complex. The spectra of **c** is clearly different from those of **a**, **b** and **d**. The X-ray diffraction peaks in the 2θ region of 16. 74°, 18.40° and 24.22° for **a** are absent in those for **c**. However, they are all present in the physical mixture **d**. We have reason to believe that signals in the physical mixture are simply the signal superposition of the two components, **a** and **b**. Those differences and new peaks in **c** provide an indication of the formation of the inclusion complex.

Fig. 5 shows the infrared spectra of BG (**a**), γ-CD (**b**), the physical mixture of γ-CD and BG (**c**) and the inclusion complex of γ-CD-BG (**d**). If γ-CD and BG form an inclusion complex, the non-covalent interactions between them such as hydrophobic interactions,

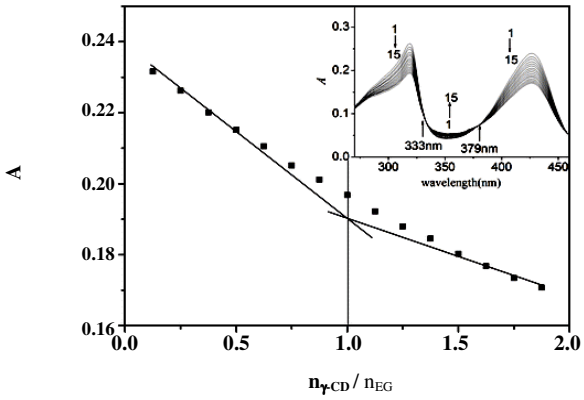

Fig. 3: Mole ratio plots of BG with γ-CD in a Tris-HCl buffer (pH 7.40; λ= 427 nm); c_{BG} =2.00×10^{-5}mol/L. Inset: Absorption spectra of BG in different concentrations of γ-CD (pH 7.40). From curve 1-15, c_{BG}=2×10^{-5} mol/L; $c_{\gamma\text{-CD}}$ =0.00, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50, 2.75, 3.00, 3.25, 3.50, 3.75×10^{-5} mol/L, respectively.

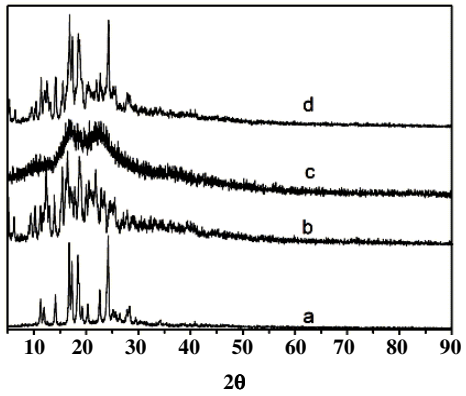

Fig. 4: X-ray diffraction patterns corresponding to the following products: (a)BG; (c) γCD-BG;(b) γCD; (d) physical mixture.

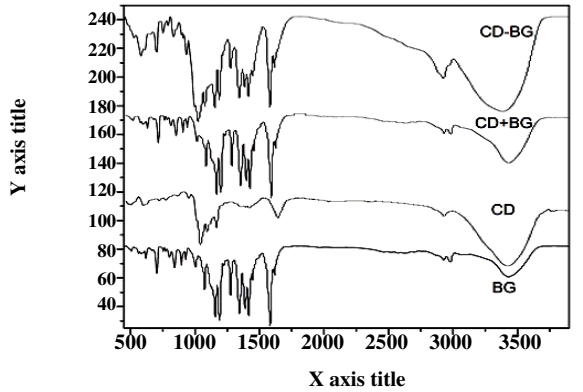

Fig. 5: IR spectra of γCD-BG systems: (a) BG; (b) γCD; (c) γCD-BG; (d) physical mixture.

van der Waals interactions and hydrogen bonds will lower the energy of the included part of BG, reduce the absorption intensities of the corresponding bonds [16]. We can see that there are apparent differences between the spectra of **a**, **c,** and **d** and that some characteristic peaks of **a** change obviously by comparison to the spectrograms of **d**. **d** does not show any new peaks, indicating no chemical bonds were created in the formed complexes.

Study on the binding mode of BG with DNA in γ-CD supramolecular systems

Absorption studies

Generally, red shift and hypochromic effect are observed in the absorption spectra of small molecules if they intercalate with DNA, red shift and hypochromic effect are unremarkable when groove binding or electrostatic interaction takes place [17]. The UV–vis absorption spectra of γ-CD–BG inclusion complex at various concentrations of DNA were shown in Fig. 6. The absorption decreased at 260nm and increasesed at 626nm by increasing DNA concentration. The appearance of an isosbestic point at 304nm confirms that a new complex of DNA–γ-CD–BG is formed. The result means that there is an interaction between γ-CD–BG and DNA.

In order to determine the stoichiometry of the formation of DNA-γ-CD-BG inclusion complex, the mole ratio was also done at the peak 626 nm. The mole ratio plots of DNA with γ-CD–BG were shown in Fig. 7. The binding ratio of the inclusion complex was: $n_{\gamma\text{-CD–BG}}:n_{DNA}$ = 6:1. According to Lambert–Beer law:

$$A = \varepsilon bc \quad (2)$$

Where A is the absorbance of the DNA-γ-CD-BG; ε is the molar absorptivity of DNA-γ-CD-BG; *c* is the concentration of DNA-γ-CD-BG. The apparent mol absorption coefficient of DNA-γ-CD-BG was counted: $\varepsilon = 4.48\times10^4$ L/(mol cm).

The absorption relationship between the inclusion complex and DNA was expressed by double reciprocal equation:

$$1/(A_0-A)=1/A_0+1/(K\times A_0\times c_{DNA}) \quad (3)$$

Where A_0 and A are the absorbance of γ-CD-BG in the absence and in the presence of DNA. K is the binding constant between γ-CD-BG and DNA, c_{DNA} is the concentration of DNA.

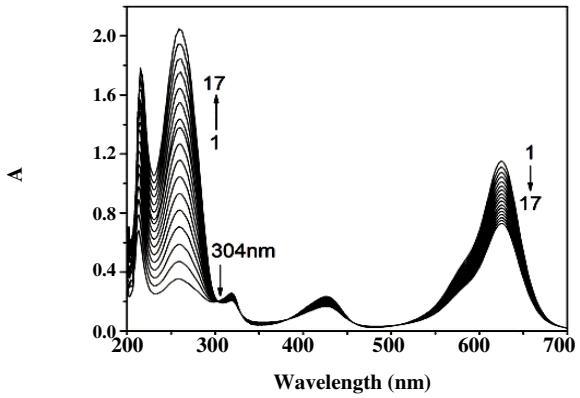

Fig. 6: UV-vis absorption spectra of γ-CD-BG in different concentrations of DNA (pH 7.40). From curve 1-17. $c_{\gamma\text{-CD-BG}}$ =2.00×10^{-5} mol/L, c_{DNA} =0.00, 0.33, 0.67, 1.00, 1.33, 1.67, 2.00, 2.33, 2.67, 3.00, 3.33, 3.67, 4.00, 4.33, 4.67, 5.00, 5.33×10^{-6} mol/L, respectively.

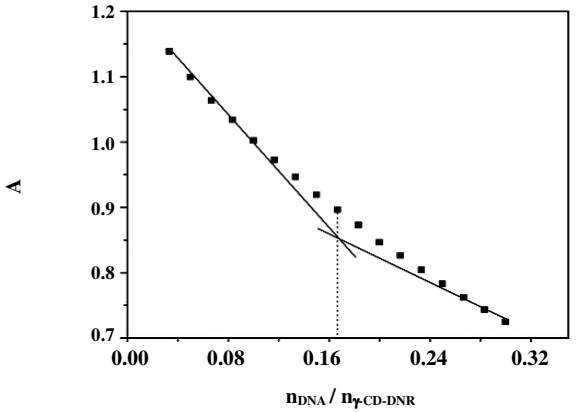

Fig. 7: Mole ratio plots of γ-CD-BG with DNA in a Tris-HCl buffer (pH 7.40, λ= 626nm); $c_{\gamma\text{-CD-BG}}$ =2.00×10^{-5}mol/L.

The double reciprocal plots of $1/(A_0-A)$ versus $1/c_{DNA}$ were linear (at 298.15K and 310.15K respectively.), and the binding constants were calculated from the ratio of the intercept on the vertical: $K^{\Theta}_{298.15K}=2.67\times10^5$ L/mol, $K^{\Theta}_{310.15K}=4.48\times10^5$ L/mol. According to thermodynamic equation:

$$\ln K_2^{\Theta}/K_1^{\Theta}=-\Delta_r H_m^{\Theta}(1/T_2-1/T_1)/R \quad (4)$$

$$\Delta_r G_m^{\Theta}=-RT\ln K^{\Theta} \quad (5)$$

$$\Delta_r G_{m,T}^{\Theta}=\Delta_r H_m^{\Theta}-T\Delta_r S_m^{\Theta} \quad (6)$$

Where K_1^{Θ} refers to standard binding constant of γ-CD-BG with DNA at 298.15K, K_2^{Θ} refers to standard binding constant of γ-CD-BG with DNA at 310.15K.

Table 1: Data of Scatchard Equation of the interaction between γ-CD-BG and DNA.

Curve	$c_{\gamma\text{-CD-BG}}/c_{DNA}$	NaCl %	Scatchard Equation	K/ (Lmol)	n
a.	0.00	0.50 0	$r/c=6.05\times10^3-1.50\times10^5 r$ $r/c=5.04\times10^3-1.57\times10^5 r$	1.50×10^5 1.57×10^5	0.04 0.03
b.	0.60	0.50 0	$r/c=8.96\times10^3-1.64\times10^5 r$ $r/c=7.99\times10^3-1.98\times10^5 r$	1.64×10^5 1.98×10^5	0.05 0.04
c.	1.20	0.50 0	$r/c=1.27\times10^4-2.55\times10^5 r$ $r/c=1.30\times10^4-2.54\times10^5 r$	2.55×10^5 2.54×10^5	0.05 0.05
d.	1.80	0.50 0	$r/c=1.91\times10^4-3.64\times10^5 r$ $r/c=1.65\times10^4-3.45\times10^5 r$	3.64×10^5 3.45×10^5	0.05 0.04

T_1 is 298.15 K, T_2 is 310.15 K, $\Delta_r H_m^\Theta$ 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 $\Delta_r H_m^\Theta = 3.33\times10^4$ $J\cdot mol^{-1}$ is deduced. The positive result shows that it is an endothermic reaction, temperature enhancement redounded to reaction processes. The $\Delta_r G_m^\Theta{}_{298.15K} = -3.10\times10^4$ J/mol. $\Delta_r G_m^\Theta$ shows spontaneous interaction between γ-CD-BG and DNA. The $\Delta_r S_m^\Theta$ is 215.66 J/(mol K). As explained previously [18-19], the results suggest that the process of interaction of γ-CD-BG with DNA is driven by entropy.

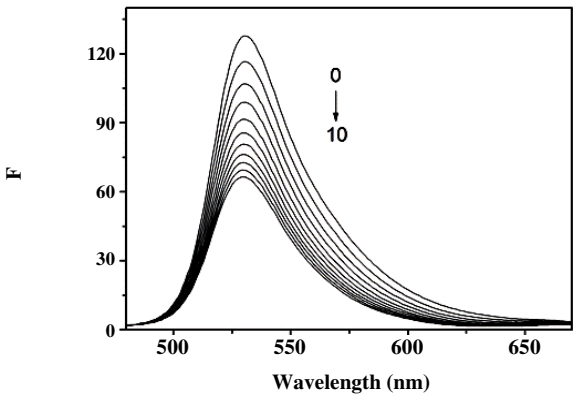

Fig. 8: Emission spectra of DNA–AO mixture in different concentrations of γ-CD-BG (pH7.40; λ_{ex}=411 nm). From curve 0-10, $c_{DNA-AO} = 2.00\times10^{-5}$ mol/L; $c_{\gamma\text{-CD-BG}}$ =0.00, 0.33, 0.67, 1.00, 1.33, 1.67, 2.00, 2.33, 2.67, 3.00, 3.33×10^{-5} mol/L, respectively.

Fluorescence studies

Fluorescence Measurements

The fluorescence measurements were carried out with Acridine Orange (AO) as probe. As we all known, AO has conjugated planar structure and can insert between two adjacent base pairs in a DNA helix to make the fluorescence intensity remarkably increased. Fig. 8 shows the influence of emission spectra of γ-CD-BG to DNA-AO. It can be seen that the fluorescence of DNA-AO was efficiently quenched by γ-CD-BG at 531nm. It means that the competition between AO and γ-CD-BG to bind DNA is remarkable. According to the intercalation binding mode between AO and DNA, we basically confirmed that there existed intercalation binding between γ-CD-BG and DNA.

Scatchard method

The binding mode between γ-CD-BG and DNA can also be determined by the Scatchard's procedure [20]. Scatchard equation expresses the binding of AO-DNA in the presence of γ-CD-BG:

$$r/c = K(n - r) \quad (7)$$

Where r is the number of moles of AO bound per mole of DNA, c is the molar concentration of free AO, K is the association binding constant of AO with DNA and n is binding site multiplicity per class of binding sites. From the Scatachard plot, we can get the values 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 at the different concentrations of γ-CD-BG. Generally, the variation of the parameter n and K suggest a mix interaction herein. As a contrast, two groups of buffers in presence of NaCl and absence of NaCl were constructed in Fig. 9 and Fig. 10. Normally, the values of n in the present of NaCl are lower than those in the absence of NaCl, indicating there is an electrostatic interaction between small molecules and DNA. While in Table 1, the values of n in the present of NaCl are basically higher than those in the absence of NaCl. That means there is no electrostatic interaction. From Table 1, the variation of n and K suggests a mix interaction herein. So we can confirm that there are

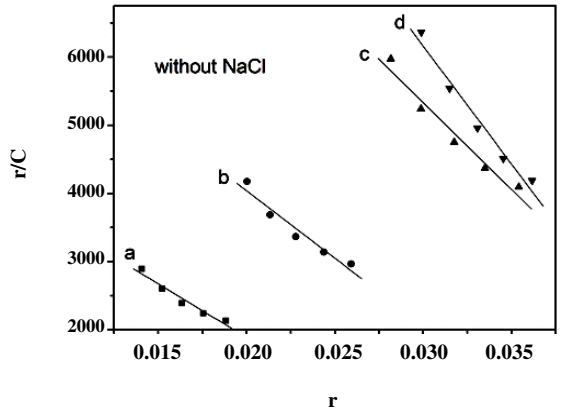

Fig. 9: Scatchard plots of DNA-γCD-BG in different concentrations of AO (without NaCl; pH 7.40). c_{DNA}=1.00×10^{-5} mol/L; Rt=$c_{\gamma CD\text{-}BG}$ / c_{DNA} ; Rt =a:0.00, b:0.60, c:1.20, d:1.80.

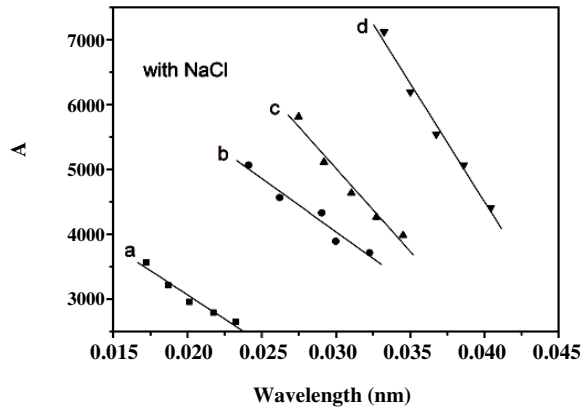

Fig. 10: Scatchard plots of DNA-γCD-BG in different concentrations of AO (with NaCl; pH 7.40). c_{DNA}=1.00×10^{-5} mol/L;Rt= $c_{\gamma CD\text{-}BG}$ / c_{DNA}; Rt =a:0.00, b:0.60, c:1.20, d:1.80.

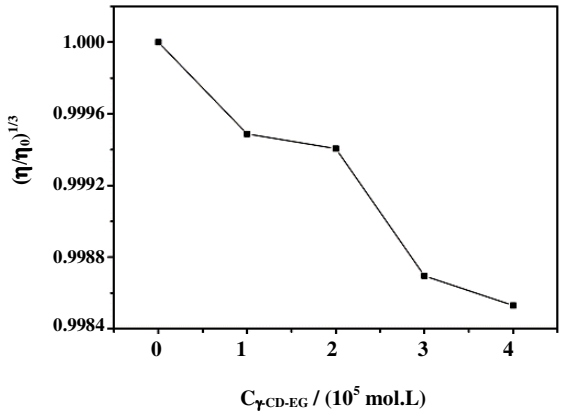

Fig. 11: Influence on DNA viscosity with different concentrations of γCD-BG. c_{DNA} = 1.00×10^{-5} mol/L.

groove binding and intercalation binding between the inclusion complex γ-CD-BG and DNA.

VISCOSITY MEASUREMENTS

A classical intercalation binding demands the space of adjacent base pairs to accommodate the ligand, leading to an increase of DNA viscosity [21]. A partial non-classical intercalation would make DNA chain possible cause distortion and tangles, leading to DNA viscosity decrease. The changes in relative viscosity of DNA with increasing concentrations of γ-CD-BG are shown in Fig. 11. It can be seen that the relative viscosity of DNA decreased steadily with increasing the amounts of γ-CD-BG. The interaction between γ-CD-BG and DNA makes DNA chain distortion and tangle. Such behavior further suggested that a partial non-classical intercalation binding should be the interaction mode of γ-CD-BG with DNA. We concluded that the partial non-classical intercalation is because the three benzene ring of BG (As shown in Fig.1) form plane aromatic nucleus. According to previous studies, we can confirm that the interaction between γ-CD-BG and DNA is groove binding and intercalation.

CONCLUSIONS

The interactive mode of BG with DNA has been studied by γ-CD supramolecular system. Brilliant green prefers to form the 1:1 inclusion complex with γ-cyclodextrin. The binding ratio of the inclusion complex with DNA was: $n_{□\text{-CD–BG}}$:n_{DNA} = 6:1. Our results indicate that γ-CD-BG can bind to DNA by groove binding and partial non-classical intercalation.

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