March 2009 Volume 8 Issue 1

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 8(1) 2009 [14-20]

Studies on the interaction between garcigerin and DNA by spectroscopic method

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Received: 24th December, 2008 ; Accepted: 29th December, 2008

ABSTRACT

The interaction of 12b-hydroxy-des-D-Garcigerin (GA) with DNA was studied by using acridine orange (AO) as a probe. The results indicated that there was a complex of GA and DNA, which has been confirmed by absorption and fluorescence spectra. The influence of salt effect and temperature on the interaction of GA with DNA was studied. The results suggested that the intercalation and electrostatic binding should be the two major modes for interaction between GA and DNA.

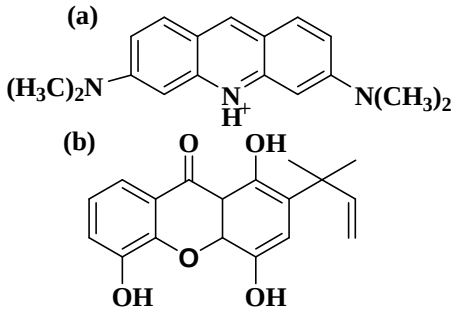
KEYWORDS

12b-Hydroxy-des-D-garcigerin;
Acridine orange;
DNA;
Action mode

INTRODUCTION

Deoxyribonucleic acid (DNA) is an important genetic substance in organism. The area of DNA involved in vital processes such as gene expression, gene transcription, mutagenesis, carcinogenesis and cell death, etc., are of particular interest as targets for a wide range of anticancer and antibiotic drugs[1] So, the study on the interaction of drug and DNA plays a key role in pharmacology and it is of great significance for designing and synthesizing the new drugs targeted to DNA and their effectiveness depends on the mode and affinity of the binding. The interaction of DNA with drugs has been studied by various techniques including fluorescence[2], UV[3], luminescence[4] electrophoresis[5], NMR[6], quartz crystal microgravimetry[7] and electroanalytical methods[8].

The fluorescence quantum yield of DNA is about 10^{-4} to 10^{-5} at room temperature[9] and the intrinsic fluorescence from DNA is of little practical usefulness. The utility of fluorescence probes can obtain the information of the structure and quantitative of DNA. Many organic dyes have already proven sensitive probes of DNA, such as ethidium bromide (EB)[10], acridine orange (AO)[11], oxazide yellow homodimers[12], nile blue[13], diphenylamine blue[14] and neutral red[15], and so on. In this study, AO (a in SCHEME 1) was selected as a probe to investigate the interactions of



SCHEME 1: The structure of acridine orange (a) and 2b-hydroxy-des-D-Garcigerin (b)

xanthones with DNA by spectrometric methods.

In principle, there are three modes for reversible binding of molecules with double-helical DNA: (i) electrostatic attractions with the anionic sugar-phosphate backbone of DNA, (ii) interactions with the DNA groove, (iii) intercalation between base pairs via the DNA groove. Depending on structural features of both the molecule and DNA, many molecules show more than a single interaction mode with DNA[16].

The natural xanthones from high plants have been obtained mainly from about 150 plants associated with four families: *Guttiferae*, *Gentianaceae*, *Moraceae* and *Polygalaceae*[17], having many pharmacological effects such as MAO inhibition, anti-tumor activity, cytotoxicity, antibacterial activity, antifungal activity, anti-inflammatory properties, antioxidant activity and tuberculoatatic activity[18]. Due to their strong bioactivities, some herbal medicines in *Polygalaceae* family containing xanthones have been used as anti-inflammatory, anti-bacterial and anti-rheumatism agents in clinic[19]. In the present work, 12b-hydroxy-des-D-Garcigerin ((b in Scheme 1)) was selected as a probe to investigate the interactions of xanthones with DNA by spectrometric methods. Studies of the effect of ionic strength, the measurements of viscosity, melting temperatures, etc., were carried out to probe the binding mechanism.

EXPERIMENTAL

Apparatus

All fluorescence measurements were made with a Hitachi F-2500 spectrofluorimeter (Tokyo, Japan) equipped with a 1 cm quartz cell and a thermostat bath. A UV-757CRT visible ultraviolet spectrophotometer (Shanghai Precision and Scientific Instrument Co.,Ltd., China) equipped with 1.0 cm quartz cells was used for scanning the UV spectrum. All pH measurements were made with a pHs-3 digital pH-meter (Shanghai Lei Ci Device Works, Shanghai, China) with a combined glass electrode. An electronic thermostat water-bath (Tianjin Taisite Instrument Company, Tianjin, China) was used for controlling the temperature. The viscosity determination was carried out using NDJ-79 viscosity meter (Yinhuan Flowmeter Co. Ltd., Zhejiang, China).

Reagents

All starting materials were analytical reagent grade and double distilled water was used for all the measurements. Calf Thymus dsDNA (Sigma) was directly dissolved in water to prepare stock solutions and then stored at 4^0C, and its concentration was determined to be 2.45×10^{-4} mol L^{-1} by absorption spectrometry, using the absorptivity $\varepsilon_{260} = 6600$ mol^{-1} cm^{-1}. Purity of DNA was checked by monitoring the ratio of the absorbance at 260 to that at 280 nm. The solution gave a ratio of $A_{260}/A_{280} > 1.8$, indicating that DNA was sufficiently free from protein[20]. AO is purchased from Chemical Regents Co (Shanghai). A stock solution of AO (5.00×10^{-3} mol L^{-1}) was prepared by dissolving an appropriate amount of AO in water and was stored in cool and dark place. GA was isolated from *Garcinia xanthochymus* and its spectroscopic data was in good agreement with literature values[21]. 1.22×10^{-3} mol L^{-1} stock solution of GA was directly prepared in methanol. A tris-HCl buffer (pH 7.4) was used to control the pH of the reaction system. NaCl was used to adjust the ionic strength of the solution.

Procedures

UV-Vis spectrometry

A 3.0mL solution in 1.0 cm quartz cells, containing appropriate concentration of GA, was titrated by successive additions of a 2.45×10^{-4} mol L^{-1} stock solution of DNA; and a 3.0mL solution in 1.0 cm quartz cells, containing appropriate concentration of DNA or GA-DNA, was titrated by successive additions of a 5.00×10^{-3} mol L^{-1} stock solution of AO. Titration was done manually by using micro-injector. The aliquot of each injection is 10μL to avoid the change of the volume. Appropriate blanks corresponding to the buffer were used as the reference. The UV-Vis spectra or the absorption were measured after shaking for 5 min.

Fluorescence spectrometry

A 3.0mL solution in 1.0 cm quartz cells, containing appropriate concentration of AO-DNA, was titrated by successive additions of a 1.22×10^{-3} mol L^{-1} stock solution of GA. Titration was done manually by using micro-injector. The aliquot of each injection is 10μL to avoid the change of the volume. The widths of both the excitation slit and the emission slit were set to 5.0 nm

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with a nominal resolution of 0.5 nm. The excite wavelength was set at 514 nm. Appropriate blanks corresponding to the buffer were subtracted to correct background of fluorescence. After 5 min the fluorescence spectra were measured.

Melting temperature of DNA, DNA-AO or DNA-GA was determined, by monitoring the maximum fluorescence of the system as a function of temperature ranged from 25 to 100^{0}C at interval of 5^{0}C.

In the study the Binding of GA with DNA in the presence of AO, the fluorescence measurements were carried out in 3 ml pH 7.4 tris-HCl buffers by adding an appropriate amount of DNA and GA to get four series of solutions with various concentrations of GA and a constant concentration of DNA. Then each series of solutions was titrated by five successive additions of a 5.00×10^{-3} mol L^{-1} stock solution of AO. Before measurements, the tubes were shaken up and placed into a thermostat water bath for 5 min. Then the assay solutions were transferred into a quartz cell and fluorescence measurements were performed. At above fluorescence measurements two groups with or without addition of NaCl were done as contrast to study the salt on the interaction of GA with DNA.

Viscosity measurements

A series of solutions containing a constant amount of DNA and various concentrations of GA or AO were made, and the viscosity measurement was performed at 25^{0}C.

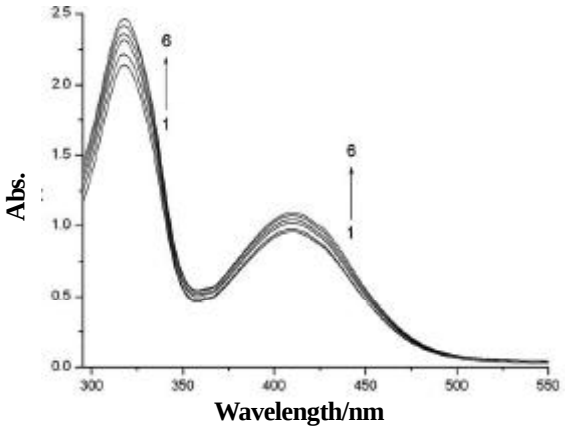

Figure 1: The absorption spectra of the DNA to GA in a tris-HCl buffer (pH = 7.4). $C_{GA}=1.62\times10^{-5}$ mol/L; $C_{DNA}=2.45\times10^{-4}$ mol/L (10L per scan), 1~6: 0~60 μL

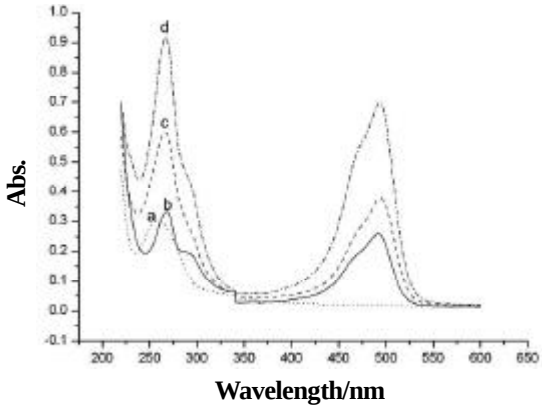

Figure 2: The absorption spectra of: (a) DNA ($C_{DNA}=6.00\times10^{-6}$ mol/L), (b) AO ($C_{AO}=2.10\times10^{-6}$mol/L), (c) AO-DNA ($C_{DNA}=6.00\times10^{-6}$ mol/L; $C_{AO}=2.10\times10^{-6}$ mol/L) and (d) AO-DNA ($C_{DNA}=6.00\times10^{-6}$ mol/L; $C_{AO}=4.20\times10^{-6}$ mol/L) in a tris-HCl buffer (pH = 7.4)

RESULTS AND DISCUSSION

UV-vis absorption spectra

Binding properties of GA with DNA

UV-vis absorption spectra were obtained by titration of a 1.62×10^{-5} mol L^{-1} GA solution with increasing concentration of DNA (Figure 1). In the absence of DNA, the GA (Curve a) has two peaks at 317nm and 412 nm. With the addition of DNA, the absorbance intensity of both 317nm and 412 nm increased slightly, and the maximum absorption peak is not changed (curve 2-6). Generally, bathochromic and hypochromic effects are observed in the absorption spectra of small molecules if they intercalate with DNA[22]. The experiment results show that there exist some interaction between GA and DNA.

Binding properties of DNA in presence of AO

Figure 2 is the absorption spectra of DNA in presence of AO. It can be seen that the absorption peaks of DNA are at 260 nm (curve a), and the absorption peaks of AO are at 269 and 492 nm. There is also a small absorption peak at about 287 nm for AO (curve b). With the addition of AO to the fixed DNA, the peak of DNA at 260 nm disappeared with a new peak at 265 nm appeared. It indicated that a new complex between DNA and AO is formed. Meanwhile, the peak at 492 nm of AO shifts to 495 nm (curve c and d). The change of the absorbance of both AO and DNA confirms that

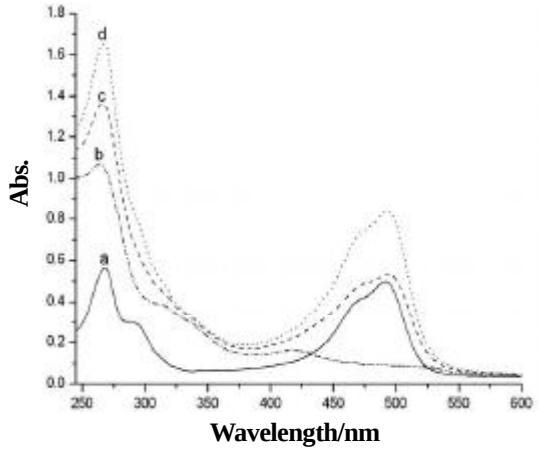

Figure 3: The absorption spectra of: (a) AO (C_{AO}=2.10×10^{-6}mol/L), (b) GA-DNA (C_{GA}=1.40×10^{-5} mol/L, C_{DNA}= 6.00×10^{-6} mol/L), (c) GA-DNA-AO (C_{GA}=1.40×10^{-5} mol/L, C_{DNA}= 6.00×10^{-6} mol/L, C_{AO}=2.10×10^{-6} mol/L) and (d) GA-DNA-AO (C_{GA}=1.40×10^{-5} mol/L, C_{DNA}=6.00×10^{-6} mol/L, C_{AO}=4.20×10^{-6} mol/L) in a tris-HCl buffer (pH = 7.4).

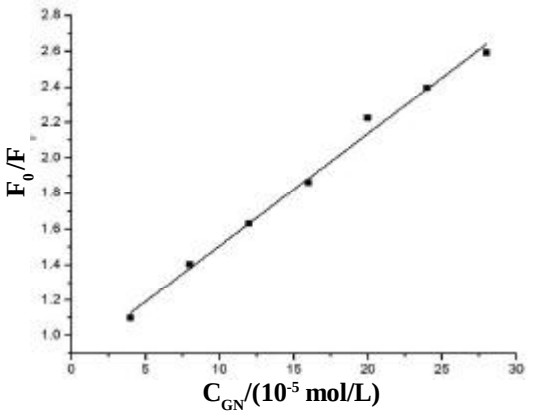

Figure 4: The plot of F_0 /F vs. C_{GA} for the quenching of GA with DNA-AO at 25^{0}C

AO interact with DNA and forms a DNA-GA complex. The π electrons of AO dye combine with the π electrons of DNA's bases, and the empty π* orbital of the AO dye couple with the π orbital of the bases. This facilitates a decrease in the energy of the π-π* electron transition, which is reflected in the observed red shift.

Binding properties of GA with DNA in presence of AO

Figure 3 is the absorption spectra of GA-DNA in presence of AO. It can seen that the two absorption peaks of GA-DNA are at 264 nm (λ_{max} (DNA)), 317nm (λ_{max} (GA)) and 412 nm (λ_{max} (GA)) (curve b), and the absorption peaks of AO are at 269 nm, 287 nm and 492 nm (curve a). With increasing AO concentration, peak intensity gradually increases and a slight red shift occurs from 264 nm ($_{max}$ (DNA)) to 266 nm. Meanwhile, the peak at 492 nm of AO shifts to 495 nm (curve c and d). The change of the absorption spectra of GA-DNA in presence of AO is similar as that of the absorption spectra of DNA in presence of AO, which indicated that GA intercalates into the DNA bases like as AO.

Fluorescence spectrometry

Fluorescence quenching

When GA is added into the solution of DNA-AO complex, the fluorescence intensity of DNA-AO complex decreased with the increasing concentration of GA. Quenching can occur by different mechanisms, which usually classified as dynamic quenching and static quenching. In order to confirm this point, the procedure was assumed to be dynamic quenching. The quenching equation is presented by

$$F_0/F = 1 + K_q\tau_0[Q] = 1+K_{SV}[Q] \quad (1)$$

where F and F_0 are the fluorescence intensities with and without quencher£¬K_q is the quenching rate constant of the biomolecule, K_{sv} is the Stern-Volmer quenching constant, τ_0 is the average lifetime of the biomolecule without quencher, [Q] is the concentration of quencher. Obviously,

$$Kq= K_{SV}/\tau_0 \quad (2)$$

The possible quenching mechanism can be interpreted by the fluorescence quenching spectra of DNA-AO and the F_0/FC (Stern-Volmer) curves of DNA-AO with GA at 303 K as shown in figure 4. The result of linear regressions of figure 4 is: F_0/F = 0.87786 + 0.06297 [GA]. Because the fluorescence lifetime of the biopolymer τ_0 is 10^{-8} s^{-1} [23], the corresponding Stern-Volmer quenching constant, K_q was obtained to be 6.297×10^{11} at 303 K. However, the maximum scatter collision quenching constant Kq of various quenchers with the biopolymer is 2×10^{10} L mol^{-1} s^{-1}[24]. Obviously, the rate constant of DNA-AO quenching procedure initiated by GA is greater than Kq of the scatter procedure. These results indicate that the probable quenching mechanism of fluorescence of DNA-AO by GA is not a dynamic quenching procedure but a static quenching procedure, and that confirms again GA interacts with DNA and forms a GA-DNA complex.

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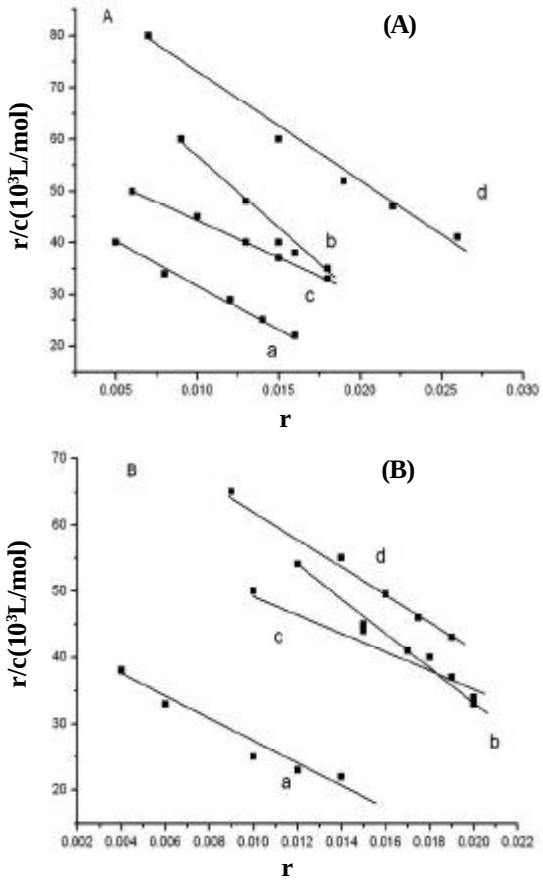

Figure 5: Influence of GA on the scatchard polt of DNA-AO system (A) with NaCl and (B) without NaCl. C_{GN}=6.75×10^{-5} mol/L; C_{DNA}=5.00×10^{-6} mol/L; C_{AO}= 4.00 ×10^{-4} mol/L (10μL per scan); R_t=C_{GN} / C_{DNA}; a, R_t=0.00; b. R_t=0.20; c. R_t=0.40; d. R_t=0.60

TABLE 1 : Binding parameters for the binding of GA to AO-DNA at PH 7.4 measured by scatchard procedure

Curve	C_{GN}/C_{DNA}	NaCl / %	Scatchard equation	K(×10^5 L/mol)	n
a	0	5.00	4.33×03-1.65×105 r	1.65	0.09
		0	4.76×103-1.60×105 r	1.60	0.12
b	0.20	5.00	8.36×103 -2.50×105 r	2.50	0.23
		0	8.55×103 -2.91×105 r	2.91	0.27
c	0.40	5.00	6.58×103 -1.51×105 r	1.51	0.34
		0	5.89×103 -1.44×105 r	1.44	0.29
d	0.60	5.00	8.54×103 -2.23×105 r	2.23	0.39
		0	9.28×103 -2.07×105 r	2.07	0.36

C_{GN} = 6.75×10^{-5} mol/L; C_{DNA} = 5.00×10^{-6} mol/L; C_{AO}= 4.00×10^{-4} mol/L (10μL per scan); R_t = C_{GN} / C_{DNA}; a, R_t = 0.00; b. R_t = 0.20; c. R_t = 0.40; d. R_t = 0.60.

Scatchard's procedure

The binding mode between small molecules with DNA can be determined using the Scatchard's procedure[25]. This method is based on the general equation:

r/c = Kn-rc (3)

where r is in 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. If GA interacts with DNA by intercalation mode, the value of n keeps constant and that of K changes in Scatchard plot. If GA interacts with DNA by intercalation mode or electrostatic interactions, both values of n and K change in Scatchard plot[2]. Collect the fluorescence value of fixed concentration of GA-DNA after interacted with different concentration of AO. In order to investigate the effects of ionic strength on the interaction between GA and DNA, two groups of buffers with or without adding NaCl used as a contrast. For NaCl, which is not an anionic quencher of DNA[27], its influence on the GA-AO-DNA comes only from ionic strength. As shown in figure 5, a calibration curve was prepared which related the value of r/C to the value of r. From the Scatachard plot, we can get the value of K and n. The results were shown in TABLE 1. From figure 5a it can be seen that both values of n and K change with the different concentration of AO. The result proves that GA interacts with DNA by intercalation mode or electrostatic interactions. Comparing figure 5b with figure 5a, the value of n become smaller with adding NaCl, which confirms that GA interacts with DNA by some electrostatic interactions.

Melting studies

Heat and alkali can destroy the double helix structure of DNA and change it into a single helix at the melting temperature (T_m). Interaction of small molecules with the dsDNA can influence T_m. Intercalation binding can stabilized the double helix structure and Tm increases by about 5-8^{0}C, but the non-intercalation binding causes no obvious increase in T_m [28]. The values of T_m for DNA, AO-DNA and GA-AO-DNA were determined, by monitoring the maximum fluorescence of the systems as a function of temperature ranged from 25 to 100 °C. For each monitored transition, the T_m of the assay solution was determined as the transition midpoint of the melting curve. The melting curves are shown in figure 6. The value of T_m for DNA is 75^{0}C under the experimental conditions. The observed melting temperatures of DNA-AO in the absence of and in presence of

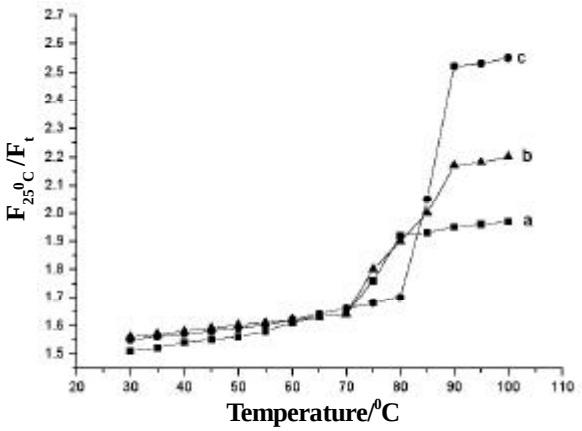

Figure 6: Melting curves of DNA (a), DNA-AO (b), and DNA-AO-GA (c). C_{GA}=1.40×10^{-5} mol/L, C_{DNA}= 6.00×10^{-6} mol/L, C_{AO}=2.10×10^{-6} mol/L

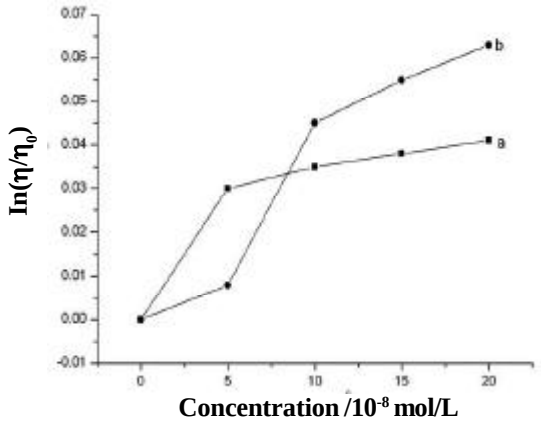

Figure 7: Effect of increasing amounts of AO (a) and GA (b) on the relative viscosities of DNA at 25^{0}C. C_{DNA}= 2.45×10^{-4} mol/L

GA are 85^{0}C and 90^{0}C, respectively. The changes in T_m of DNA-AO after the addition of GA reveal that the binding modes of GA with DNA are intercalated.

Viscosity measurements

The binding mode of GA with DNA was further investigated by viscosity measurements. A classical intercalation model results in lengthening the DNA helix, as base pairs ar1e separated to accommodate the binding ligand, leading to the increase of DNA viscosity. However, a partial and/or non-classical intercalation of ligand may bend (or kink) DNA helix, resulting in the decrease of its effective length and, concomitantly, its viscosity[29]. The effects of GA and AO on the viscosity of DNA were shown in figure 7. For GA or AO, as increasing its concentration, the viscosity of DNA increased steadily, and their trendline is similar. The experimental results suggested that GA and AO could bind DNA in similar mode - intercalative mode.

CONCLUSIONS

The binding of 12b-hydroxy-des-D-Garcigerin to Calf Thymus dsDNA in aqueous solution was studied by fluorescence and UV-Vis spectroscopic methods. By using AO as a DNA probe, the fluorescence quenching was observed in the system of GA-DNA-AO and the results show that GA has a strong ability to quench the DNA-AO fluorescence mainly through a static quench procedure. By taking account of the absorption spectra, ionic strength effects, the melting temperature determinations, and the viscosity measurements, the results revealed that the intercalation and electrostatic binding should be the two major modes for interaction between GA and DNA. The binding mode and binding constant of GA and DNA will provide necessary information on the mechanism of anti-tumor drugs binding with DNA, and they will benefit the designing of new drugs.

ACKNOWLEDGMENTS

This work was supported by National Natural Science Foundation of China (Nos.30670215 and 20777094) and the Natural Science Foundation of The South Central University for Nationalities (YZZ-06-008).

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