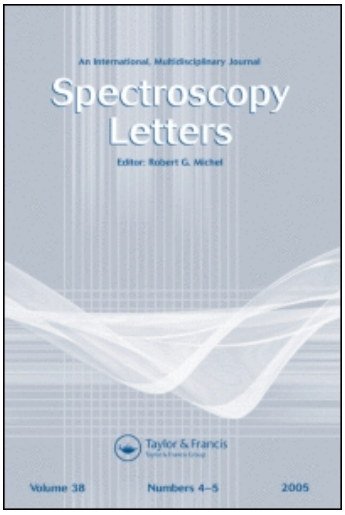

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A Spectral Study of the Interaction Between Chelerythrine Chloride and Adenosine

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A Spectral Study of the Interaction Between Chelerythrine Chloride and Adenosine

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Abstract: The possible anticancer mechanisms of chelerythrine (CHE) and its interactions with adenosine were investigated by UV-visible spectrophotometric and spectrofluorimetric measurements and by thermodynamic calculations. The binding of CHE to adenosine could be characterized by the hypochromic and bathochromic effects in the absorption bands and the quenching of fluorescence intensity. The spectral data were fitted by linear analysis, yielding a binding constant of 8.68×10^4 $L \cdot mol^{-1}$ at 25°C of CHE with adenosine, and a van't Hoff enthalpy of 92.8 kJ/mol for the endothermic interactions. In addition, with $\Delta G = -28.2$ kJ/mol and $\Delta S = 406$ J/mol · K, the interactions should be entropy-driven.

Keywords: Adenosine, chelerythrine, fluorescence quenching, thermodynamic calculations, ultraviolet

INTRODUCTION

Alkaloids are known to play important roles in medicinal chemistry due to their extensive biological activities. The study of the interactions of nucleosides with antivirus or anticancer agents has been done in an attempt to make clear that

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DNA probably is the target molecules of these alkaloids[1] and to further explore the anticancer mechanisms.[2] As one of the important alkaloids, chelerythrine (CHE), Fig. 1, shows anticancer activities.[3–5] Despite the arguments that the interactions of chelerythrine with DNA may interfere with DNA synthesis, there is still not a clear understanding of the anticancer mechanisms of CHE.[6] In particular, there are no interaction models between CHE and the bases of DNA that have been clearly defined in previous work.

To enrich the bank of data on anticancer mechanisms of CHE, the interactions of CHE with adenosine, one of four nucleosides that are the basic materials of DNA, have been closely examined by use of ultraviolet (UV) and fluorescence spectroscopies.

MATERIALS AND METHODS

Materials

CHE chloride with a high purity of $\geq$99% was purchased from Yongfeng Boyuan Industry Co. Ltd. (Beijing, Jiangxi Province, China) and was used without further purification. Adenosine with a high purity of $\geq$99% was purchased from the Sigma Chemical Co. (St. Louis, MO, USA) and was used without further purification. The binding experiments were performed in PBS buffer (0.2 M Na_2HPO_4, $12H_2O$, 0.2 M NaH_2PO_4, $2H_2O$, pH = 7.2 $\pm$ 0.05). Double-distilled water and analytical grade reagents were used throughout.

Methods

UV Absorption Spectra Measurements

The UV absorption spectra of CHE, adenosine and the different mixture of CHE with adenosine, were obtained using Varian CARY 1E spectrophotometer (Varian Corporation, USA) in matched quartz cuvettes of 1-cm pathlength. Spectrophotometric titrations were performed by keeping the constant concentrations of CHE while varying the adenosine concentrations.

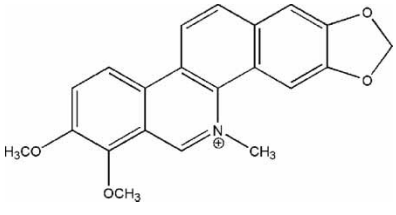

Figure 1. Chemical structure of CHE (chelerythrine).

Fluorescence Spectra Measurements

The fluorescence measurements were carried out on a Hitachi F4500 spectrofluorimeter (Hitachi Corporation, Japan) in matched quartz cuvette of 1-cm pathlength. All solutions were allowed to equilibrate for 5 min prior to measurements. The titrations were performed by keeping the constant concentrations of CHE while increasing the adenosine concentrations. Samples of CHE, adenosine and the different mixture of CHE with adenosine were excited at 267 nm, and the emission was monitored at 419 nm, but there was no detectable fluorescence for adenosine.

Fluorescence Lifetime Measurements

The emission lifetime measurements were performed on an Edinburgh FLS 920 spectrometer with special spectral processor software. The excitation wavelength was 267 nm, and the sample emission wavelength was 419 nm. The emission lifetime of CHE (2.50 μM) was measured in the absence and presence of adenosine (7.50 μM).

RESULTS AND DISCUSSION

UV Absorption Spectra

The Standard UV Absorbance of CHE

According to Fig. 2, the characteristic electronic absorption spectra of CHE can be found to have three peaks at 267.5 nm, 315.5 nm, and 338.5 nm, respectively, in the range of 220 nm to 400 nm. The concentrations (5.00 to 22.0 μM) of CHE and the corresponding absorbance values at 267 nm could be fitted to a straight line with a high correlation coefficient of 0.999 and selecting the concentration of 15 μM for absorption titration.

The Interactions of CHE with Adenosine

The interactions of CHE with adenosine have been characterized classically through absorption titration. The effects on the absorption of CHE by progressively increasing the concentration of adenosine are depicted in Fig. 2. CHE has three absorption bands at 267.5 nm (E absorbance), 315.5 nm (K absorbance), and 338.5 nm (B absorbance) in the range of 220 nm to 400 nm. [7]

The UV absorption spectra of adenosine are shown in Fig. 3. There is only one absorption band of adenosine at 259 nm in the range of 220 nm to 400 nm.

According to Fig. 2, an isosbestic point at 287 nm can be found, which is characteristic of the interactions of CHE with adenosine.[8] On interacting

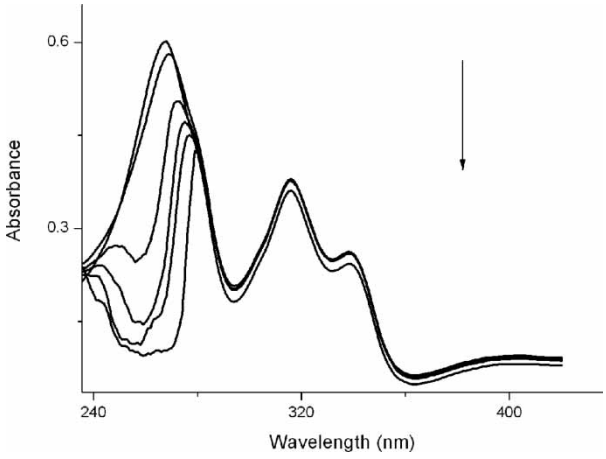

Figure 2. UV absorption spectra of CHE (15.0 μM) with increasing the concentrations of adenosine (top to bottom: 0 → 600 μM) (pH = 7.2, Na_2HPO_4-NaH_2PO_4 buffer).

with adenosine, a hypochromism can be found for the E absorbance of CHE, and the absorbance is reduced by 32% for the highest adenosine level employed (600.0 μM). Besides, a peak shift of 12.5 nm to lower energy is also found for the E absorbance. In contrast, minor but noticeable peak shift and hypochromism can be defined for the K and B absorbances from Fig. 2. In previous studies, the absorption reduction and red shift of the B absorbance with increasing the concentration of DNA has been regarded as an indicator of

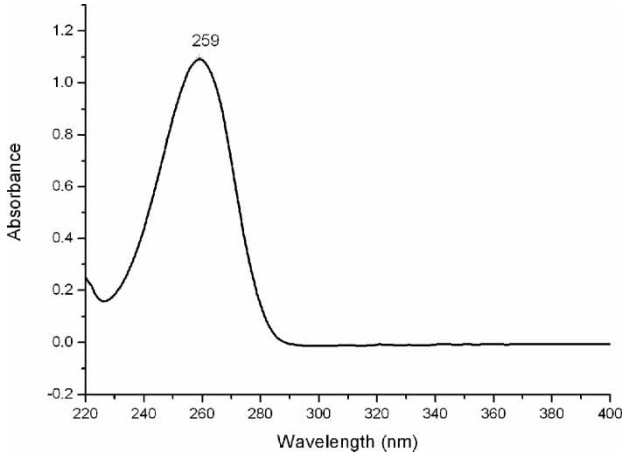

Figure 3. UV absorption spectra of adenosine (75.0 μM) (pH = 7.2, Na_2HPO_4-NaH_2PO_4 buffer).

the electronic stacking interaction of chlorobenzylidine with DNA.[9] In this work, all the results above indicate that CHE has interaction with adenosine.

Fluorescence Studies

The Standard Relative Intensity of CHE

According to the UV absorption, samples were excited at 267 nm, and the emission was monitored at 419 nm. The data of the concentrations (1.00 to 4.00 μM) of CHE and the corresponding fluorescence emission relative intensities at 419 nm can be fitted to a straight line with a high correlation coefficient of 0.995 and selecting the concentration of 2.50 μM for fluorescence titration.

The Interactions of CHE with Adenosine

Figure 4 shows the effects on the fluorescence spectra of CHE by gradually dropping adenosine. It can be seen that increasing the concentration of adenosine can result in a gradual decrease in fluorescence relative intensities of CHE, which is up to a maximum of approximately 95% for the highest level of adenosine (225 μM). The fluorescence quenching of CHE by adenosine addition indicates that CHE has interactions with adenosine.

In fluorescence quenching experiments, the data were plotted according to the Stern–Volmer equation,[10]

$$I_0/I = 1 + K_{SV}[Q_A], \tag{1}$$

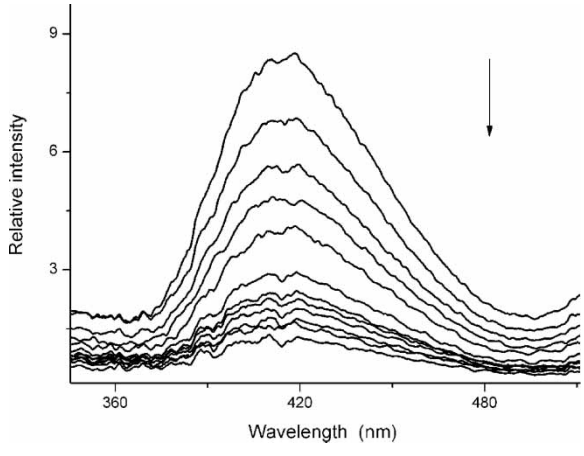

Figure 4. Effects on fluorescence emission of CHE (2.50 μM) in pH = 7.2 Na_2HPO_4-NaH_2PO_4 buffer of increasing the concentrations of adenosine (from top to bottom: 0 → 225 μM), $\lambda_{ex} = 267$ nm.

where I_0 and I are the fluorescence intensities in the absence and presence of adenosine, respectively. Q_A is the concentration of the adenosine. K_{SV} is the Stern–Volmer quenching constant, which is a measure of the efficiency of quenching by adenosine addition.

The Stern–Volmer quenching plot from the fluorescence titration data is shown in Fig. 5. The fluorescence quenching constant (K_{SV}) evaluated using the Stern–Volmer Equation is 1.50×10^4 L · mol $^{-1}$ at 25°C. The Stern–Volmer plot is linear, indicating that only one type of quenching process occurs, either static or dynamic quenching.[11]

In comparison, the measurement of the fluorescence lifetime is the most definitive method to distinguish between static and dynamic quenching. Table 1 shows the parameters of emission lifetime of CHE (2.5 μM) in the absence and presence of adenosine.

The fractional intensity (f_i) of each species is given by

$$f_i = (\alpha_i \tau_i) / \sum_i \alpha_i \tau_i \tag{2}$$

where α_i is a preexponential factor representing the fractional contribution to the time-resolved decay of the component with a lifetime τ_i. τ_1 and τ_2 are the fluorescence lifetime of CHE in Table 1. For CHE in the absence and presence of adenosine, the ratio of τ_1 is $3.46/3.43 \approx 1$, and that of τ_2 is $1.14/1.24 \approx 1$. Both of the two lifetime ratios are near 1, which shows that the fluorescence quenching is static.[9,12] A complex appearing nonfluorescent is formed between CHE and adenosine. Besides the measurement of the lifetime, one method to distinguish static and dynamic quenching is by careful examination of the absorption spectra of the fluorophore. Collisional quenching only affects

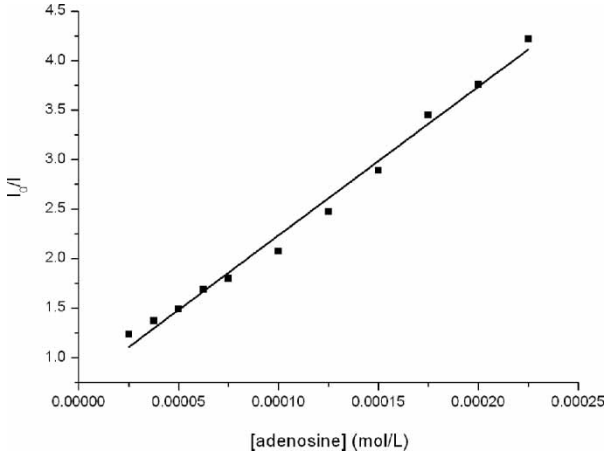

Figure 5. A Stern–Volmer quenching plot of CHE with increasing concentrations of adenosine. The data were fitted to a straight line with a correlation coefficient of 0.991.

Table 1. Emission lifetime of CHE measured in the absence and presence of adenosine

[Adenosine] (μM)	τ_1 (ns)	α_1	f_1 (%)	τ_2 (ns)	α_2	f_2 (%)	χ^2
0	3.46	0.058	86.27	1.14	0.028	13.73	1.17
7.5	3.43	0.053	85.24	1.24	0.025	14.76	1.12

χ^2 represents the emission maxima.

the excited states of the fluorophores, and thus no changes in the absorption spectra are predicted. In contrast, ground state complex formation will frequently result in perturbation of the absorption spectrum of fluorophore. [13] The fluorescence excitation spectra of fluorophore can be treated the same as the absorption spectra of the fluorophore. Figure 6 shows the fluorescence excitation spectra of CHE by gradually dropping adenosine at 25°C.

It indicates that complex appearing nonfluorescent is formed between CHE and adenosine, to increase the concentration of adenosine resulting in a gradual decrease in fluorescence relative intensities of CHE. This phenomenon also proves that the fluorescence quenching is static.

Generally, increase of temperature can result in the decrease of fluorescence relative intensities of the fluorophore, but the CHE shows an unusual increase of fluorescence relative intensities in the range of temperature between 25°C and 55°C. The fluorescence emission spectra of CHE at different temperatures are depicted in Fig. 7. So the fluorescence quenching

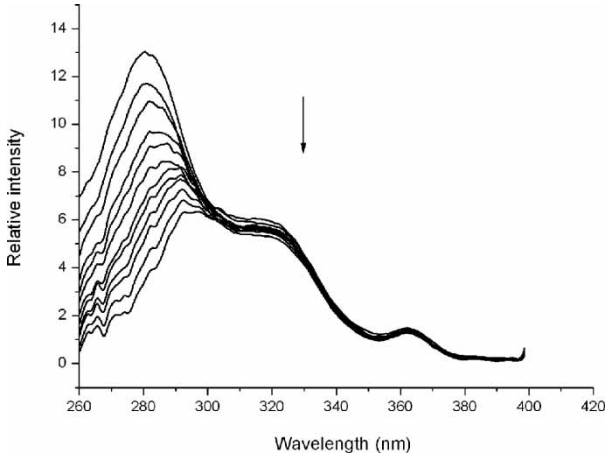

Figure 6. Fluorescence excitation spectra of CHE (2.50 μM) in pH = 7.2 Na_2HPO_4-NaH_2PO_4 buffer with increasing the concentrations of adenosine (from top to bottom: 0 → 225 μM), $\lambda_{em} = 419$ nm.

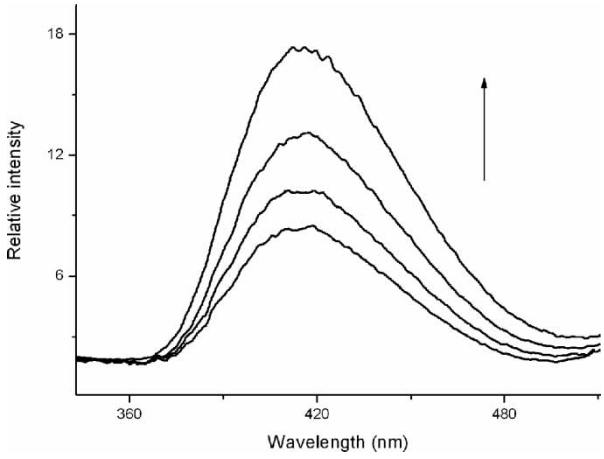

Figure 7. Fluorescence emission spectra of CHE (bottom to top: 25°C → 55°C) (2.5 μM) (pH = 7.2, Na_2HPO_4-NaH_2PO_4 buffer), $\lambda_{ex} = 267$ nm.

cannot be attributed to increase of temperature, for increasing the temperature resulted in a gradual increase of fluorescence relative intensities of CHE.

Thermodynamic Studies

Determination of Binding Constant

The binding constants of CHE with adenosine were calculated from the data of fluorescence titration. The binding constant K and the binding number n are calculated by the following equation:[14]

$$nQ + C \leftrightharpoons Q_nC, \tag{3}$$

where Q is the quencher known as adenosine, C is the CHE, and Q_nC is the complex.

$$K = [Q_nC]/[Q]^n \times [C], \tag{4}$$

where K is the intrinsic binding constant, and n is the binding number of adenosine per CHE. $[C]$ is the concentration of free CHE, $[Q]$ is the concentration of free quencher, and $[Q_nC]$ is the concentration of complex. If $[C_0]$ is the total concentration of the CHE,

$$[C_0] = [C] + [Q_nC]. \tag{5}$$

When $[Q] \gg [C_0]$, the concentration of free quencher is replaced by its initial concentration.

$$K = ([C_0] - [C])/[Q]^n \times [C]. \tag{6}$$

In static quenching,

$$[C]/[C_0] = I/I_0 \tag{7}$$

Substituting Eq. (7) into Eq. (6), the following relation can be obtained:

$$\lg((I_0 - I)/I) = \lg K + n \lg[Q] \tag{8}$$

A linear plot is shown in Fig. 8. By linear analysis from the data in Fig. 8, a binding constant K and a binding number n of adenosine per CHE are calculated out as 8.68×10^4 L · mol^{-1} and 1.22 at 25°C, respectively. Table 2 shows the binding parameters of CHE-adenosine complex at various temperatures in PBS buffer, pH = 7.2.

Determination of Thermodynamic Parameters

Temperature-dependent fluorescence measurements of CHE-adenosine binding were performed at 25°C, 35°C, 45°C, 55°C by titration method. Thermodynamic parameters were estimated by the analysis of ln$K$ versus $1/T$ plot (van't Hoff plot) obtained by the experimental data at the above temperatures. The gradient of this straight line of ln$K$ versus $1/T$ is equal to $-\Delta H/R$, which indicates the value of ΔH. ΔG and ΔS can be calculated from the following relationships:

$$\Delta G = -RT \ln K \tag{9}$$

$$\Delta G = \Delta H - T\Delta S. \tag{10}$$

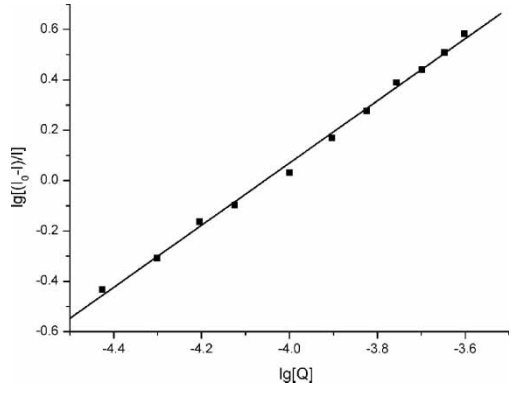

Figure 8. Plot of lg $((I_0 - I)/I)$ to lg $[Q]$ with the CHE concentration of 2.50 μM at 25°C. The data were fitted to a straight line with a correlation coefficient of 0.997.

Table 2. Stern–Volmer quenching constants and binding parameters for CHE with adenosine in PBS buffer

T (°C)	K_{SV} (L · mol^{-1})	lg [K] (L · mol^{-1})	n
25.0	$(1.50 \pm 0.01) \times 10^4$	4.94 ± 0.10	1.22 ± 0.02
35.0	$(1.48 \pm 0.05) \times 10^4$	5.33 ± 0.09	1.32 ± 0.02
45.0	$(1.55 \pm 0.10) \times 10^4$	5.82 ± 0.19	1.47 ± 0.05
55.0	$(1.86 \pm 0.10) \times 10^4$	6.41 ± 0.16	1.60 ± 0.04

The van't Hoff plot for the binding of CHE to adenosine is depicted in Fig. 9. The values of the thermodynamic parameters are given in Table 3. It can be seen that the binding of CHE to adenosine is characterized by both positive enthalpy and entropy changes. It is known that the occurrence of reaction requires a negative ΔG, and because enthalpy is positive, the interaction of CHE with adenosine should be entropy-driven. In addition, positive enthalpy of the reaction between CHE and adenosine hints at an endothermic process, therefore, the increase of temperature is in favor of the forward direction of the reaction [see Eq. (3)], resulting in bigger values of binding constant K (or quenching constant) with increasing temperature. At the same time, from the viewpoint of molecular stability, the reduced stability of compounds with increasing temperature can decrease the K value. In the case of CHE interacting with adenosine, the former seems dominant over the latter, and the fluorescent system thus presents an increasing K value with the increase of temperature.

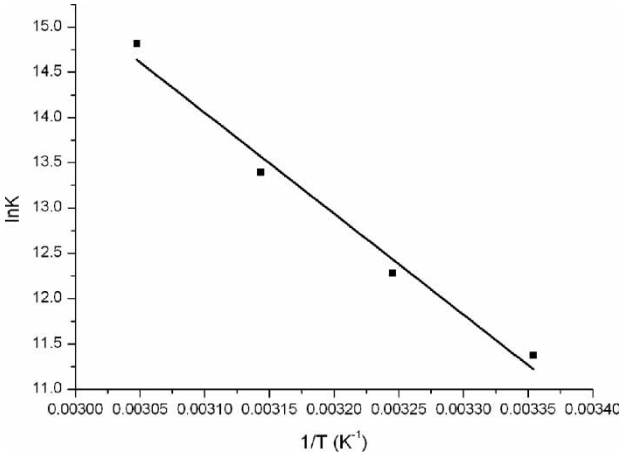

Figure 9. van't Hoff plots of the complex of CHE with adenosine. The data were fitted to a straight line with a correlation coefficient of 0.984.

Table 3. Thermodynamic function of CHE with adenosine in PBS buffer

Parameters (25°C)	Values
ΔG	-28.2 ± 0.57 KJ/mol
ΔH	92.8 ± 8.40 KJ/mol
ΔS	406 ± 30 J/mol · K

CONCLUSIONS

CHE binds to adenosine with a binding constant of 8.68×10^4 L · mol^{-1} at 25°C. The binding of CHE to adenosine results in hypochromism and red shift in UV absorption spectra and fluorescence quenching in fluorescence emission spectra. These spectral features strongly support the interactions of CHE with adenosine. In addition, the thermodynamic data for the binding of CHE to adenosine were also calculated and derived from experimental measurements, with the results of $\Delta H = 92.8$ KJ/mol, $\Delta G = -28.2$ KJ/mol, and $\Delta S = 406$ J/mol · K. These results show that the binding of CHE to adenosine is not only endothermic but also entropy-driven.

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