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Thermodynamic and spectrographic studies on the interactions of ct-DNA with 5-fluorouracil and tegafur

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ABSTRACT

The interactions of calf thymus deoxyribonucleic acid (ct-DNA) with two antitumour drugs (5-fluorouracil and tegafur) in aqueous buffer solution (pH 7.40) have been investigated using nano-watt-scale isothermal titration calorimetry (ITC), circular dichroism (CD), ultraviolet absorption (UV) and fluorescence spectroscopy. Thermodynamic parameters, i.e., binding proportions and constants, standard changes of enthalpy (ΔH°), Gibbs free energy (ΔG°) and entropy (ΔS°) have been derived from the calorimetric data. The binding ratios of 5-fluorouracil and tegafur with base pairs in ct-DNA are 1:3 and 1:4, respectively. The thermodynamic parameters have been discussed according to the influence of drugs on molecular structure of the DNA shown spectrogram. The results indicate that molecule of 5-fluorouracil or tegafur can intercalate itself into the intra-molecular space formed by DNA double helix and cause some changes in the secondary structure of DNA molecule.

1. Introduction

Apoptosis of cell is a very important issue of the life science research domain [1–3]. The non-balance matching of the tumour cell multiplication and apoptosis as well as the control of the malignant transfer and diffuse is an important content of study on cell apoptosis intervention, which is a developing therapeutical method of disease [4,5]. Determination of thermodynamic properties and discovering relationship between the properties and interaction pattern of antitumour drugs with deoxyribonucleic acid (DNA) are an important approach of the study. To illustrate the influence on the copy and transfer of the nucleic acid induced by the small molecules, or understand the pathogenesis of disease on the gene level, especially found convenient filtrate method outside the antitumour drugs, the investigation of the interaction between antitumour drugs and DNA all have potential application value [6,7]. Many anticancer drugs are known to interact with DNA to exert their biological activities. Generally, DNA-acting anticancer drugs can be classified into three categories. Drugs of the first category form covalent linkages with DNA while drugs of the second category form noncovalent complexes with DNA by either intercalation or groove-binding. Drugs of the final category cause DNA backbone cleavages [8].

5-Fluorouracil (5FU) is a chemotherapy drug that interferes with the growth of cancer cells which can be used to treat many types of cancers, including cancer of the colon, rectum, breast, stomach, head, and neck [9–11]. It can also induce apoptosis of HCT-116 colorectal cancer cells. However, this drug has serious side effects including nausea, fatigue, and a decrease in the number of blood cells [12,13]. 5-Fluoro-1-(tetrahydro-2-furanyl)-2, 4 (1*H*, 3*H*)-pyrimidinedione (tegafur, FT) is a prodrug of 5FU, which will lead to further activation to the inhibition of thymidylate synthase or incorporation into RNA [14]. The molecular structures of the two drugs are given in Scheme 1.

In spite of large number of studies and a wealth of information on the interaction of 5FU with bio-macromolecules [15–18], the energetics of its interaction is not clearly delineated. Recently, isothermal titration calorimetry (ITC) has emerged as a powerful, high precision and sensitive technique that can quickly and directly elucidate the complete thermodynamic profiles of small molecules interacting with macromolecules in a single experiment [19–21]. So calorimetric techniques are far superior compared to several other techniques, it can provide a lot of useful information such as the energetics, binding affinity and so on. In the present work, ITC combining with circular dichroism (CD), ultraviolet absorption (UV) and fluorescence spectroscopy were used to research the interactions of ct-DNA with 5FU and FT. Thermodynamic parameters were calculated based on the calorimetric data and were discussed according to the supramolecular structure of the DNA–drug systems shown by the spectra.

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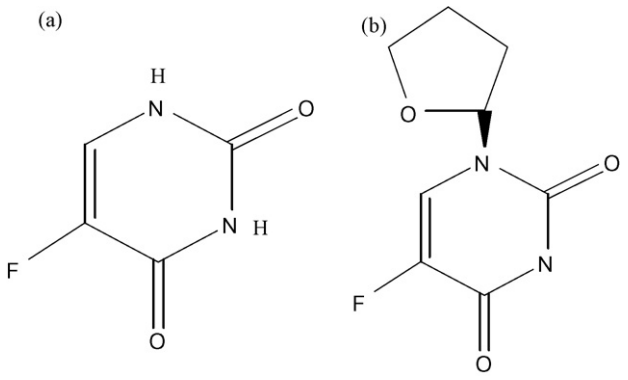

Scheme 1. Molecular structures of the two drugs: (a) 5FU and (b) FT.

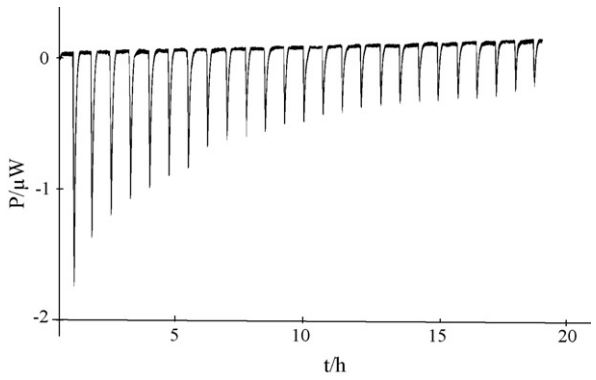

Fig. 1. Variation of heat-flow/electrical power P as a function of time t, titrant: FT (10 mM); titrand: DNA (37.6 μM).

2. Experimental

2.1. Materials

Calf thymus deoxyribonucleic acid (ct-DNA) was purchased from Sigma company of which the solution purity was scaled by $A_{260}/A_{280} > 1.8$. DNA concentration was determined by the absorbency of ultraviolet spectrum at the 260 nm using a molar extinction coefficient of 6600 L mol^{-1} cm^{-1} [22]. 5FU and FT were purchased from J&K Chemical Company (China) and the purity of each of the drugs was 99%. Berberine hemisulfate (BR, Alexis) whose purity was 98% was used as received. Tri-(hydroxymethyl) aminomethane (Tris), hydrochloric acid and sodium chloride were all of analytical grade, and the water used in the experiment was double distilled water, prepared in the presence of basic potassium permanganate. All other reagents were of analytical purity and were prepared with Tris–HCl buffer solution of which the concentration was 0.01 M (pH 7.40).

2.2. Isothermal titration calorimetry

The nano-watt isothermal titration microcalorimeter was supported by 2277 Thermal Activity Monitor (Thermometric, Sweden), possessing a heat power determination with an accuracy of ±10 nW, controlled by Digitam 4.1 software. The instrument had an electrical calibration with a precision better than ±1% and the accuracy was regularly verified by measuring the dilution enthalpy of a concentrated sucrose solution [23]. The 1 mL reaction cell and reference cell were initially loaded with 500 μL ct-DNA solution (the concentration was 37.6 μM) and 620 μL Tris–HCl buffer solution, respectively. A drug (5FU or FT) solution at the concentration 10 mM was injected into the under stirring reaction cell in 25 portions of 20 μL using a 500 μL Hamilton syringe controlled by a 612 Lund Pump. The interval between two injections was 40 min, which was sufficiently long for the signal to return to the baseline. The system was stirred at 30 rpm with a gold propeller. The experiment was startup after the base line became stable so that the heat produced by stirring can be automatically deducted. All experiments were performed at a fixed temperature of (298.15 ± 0.01) K. To deduct the dilution heat of drug and the DNA solutions, titration experiments were also performed for drug solution dropped into Tris–HCl buffer solution and Tris–HCl buffer solution into the DNA solution, respectively. The representative titration curve was given in Fig. 1.

2.3. Circular dichroism

Circular dichroism spectra were recorded on a Jasco J-810 spectropolarimeter (Japan Spectroscopic Ltd., Japan) attached with a Jasco temperature controller and thermal programmer in circular quartz cells of 1.0 cm path length. During the experiment, the lamphouse system is in protection of nitrogen atmosphere with the flow rate at 5 L/min. Spectra were measured as the average of three scans from 220 to 320 nm at a scan rate of 100 nm/min.

2.4. Ultraviolet absorption spectra

Ultraviolet absorption spectra were determined using UV–vis spectrophotometer (Hp 8453, USA) equipped with a xenon lamp and a 1.0 cm quartz cuvette of 10 mm light-path. The concentration of the drug (5FU or FT) and the DNA solutions in the cuvette were 0.1 mM and 42.3 μM, respectively.

2.5. Fluorescence spectroscopy

Fluorescence intensity of the DNA–BR–drug aqueous systems was measured using a fluorescence spectrophotometer (LS55, Perkin–Elmer, USA). The excitation (λex) and emission (λem) wavelengths were 370 and 520 nm, respectively. The corresponding emission spectra were recorded in the ranges of 450–650 nm. Various amounts of solution of drug, 5FU or FT, were added into the DNA–BR mixture in which the concentration of DNA and BR were 90 and 66 μM, respectively.

3. Results and discussion

3.1. Method of data process for calorimetric experiment

To calculate the standard combination enthalpy of the DNA with drug in the solution, the overall binding reaction and the cumulative equilibrium constant, β_i have been defined as follows:

$$\begin{aligned} &\mathrm{M} + i\mathrm{L} = \mathrm{ML}_i \quad (\Delta H^\circ) \\ &\beta_i = \frac{[\mathrm{ML}_i]}{[\mathrm{M}][\mathrm{L}]^i} \end{aligned} \tag{1}$$

Or the stepwise combination equilibrium

$$\begin{aligned} &\mathrm{ML}_{i-1} + \mathrm{L} = \mathrm{ML}_i \\ &K_i = \frac{[\mathrm{ML}_i]}{[\mathrm{ML}_{i-1}][\mathrm{L}]} \end{aligned} \tag{2}$$

where i = 1, 2, 3 and so on, and L represents the drug molecules, M is ordered to represent a group of base pairs (bps) in DNA molecule as it is much bigger than a drug molecule. In other words, our supposition is that all base pairs in the group interact with one drug molecule simultaneously and each DNA molecule may possess a number of such groups. The total concentrations of L and M can be

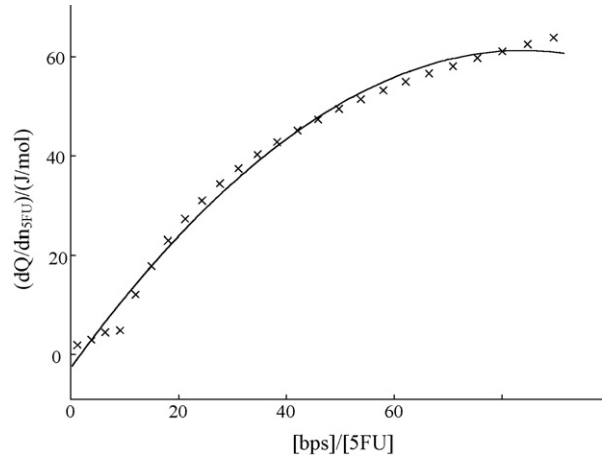

Fig. 2. Change rates of experimental thermal effect for combination process versus the molar ratio of [bps]/[5FU], where points were obtained from experiments and the line was the result of calculation.

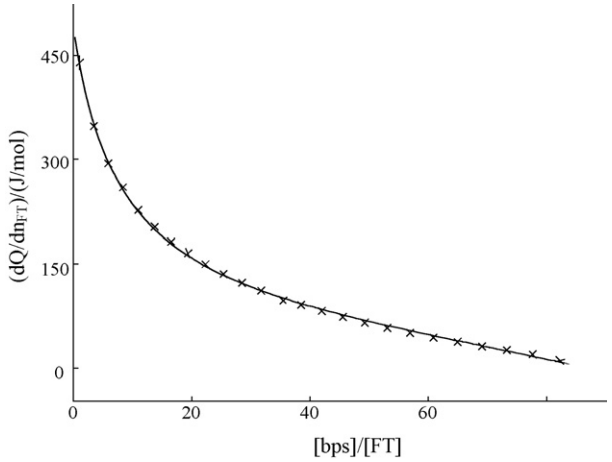

Fig. 3. Change rates of experimental thermal effect for combination process versus the molar ratio of [bps]/[FT], where points were obtained from experiments and the line was the result of calculation.

obtained from the expressions thereinafter

$$[\mathrm{M}]_\mathrm{o} = [\mathrm{M}](1 + \Sigma\beta_i[\mathrm{L}]^i) \quad (3)$$

$$[\mathrm{L}]_\mathrm{o} = [\mathrm{L}](1 + [\mathrm{M}]\Sigma i\beta_i[\mathrm{L}]^{(i-1)}) \quad (4)$$

The relationship between the overall equilibrium constants, β_i, and the stepwise equilibrium constants, K_i, is given by

$$\beta_i = \Pi K_i \quad (5)$$

ΔH° and β_i (or K_i) are uncertain parameter, and they can be obtained by the multi-nonlinear regression equation analysis (to avoid linear dependence of the parameter, the stepwise equilibrium constants, K_i was substituted by the overall equilibrium constants, β_i). This nonlinear simulation was accomplished by the Ligand Binding program of the Digitam 4.1 software. By comparing the congruousness between the simulation curve and the experiment point, the most reasonable result can be obtained. Furthermore, the standard changes of Gibbs free energy (ΔG°) and entropy effect ($T\Delta S^\circ$) of the combination can be derived using the thermodynamic formulas below:

$$\Delta G^\circ = -RT\ln K^\circ \quad (6)$$

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad (7)$$

The simulation curves were provided in Figs. 2 and 3, and the thermodynamic parameters were shown in Table 1.

3.2. Results of data process and analysis of the thermodynamic parameters

By applying the method of data process introduced in Section 3.1 to DNA–5FU system, it can be known that the binding model of this drug with the DNA most probably be

$$\mathrm{M} + \mathrm{L} = \mathrm{ML} \quad \beta$$

where M and L represent a group containing three bps and one drug molecule, respectively. In other words, three bps combined with one 5FU molecule at a binding site on DNA molecules. By using the same method of data process, the simulated results indicate that the binding model of FT with DNA may be

$$\mathrm{M} + \mathrm{L} = \mathrm{ML} \quad \beta_1$$

$$\mathrm{M} + 2\mathrm{L} = \mathrm{ML}_2 \quad \beta_2$$

where M and L stand for a group containing eight bps and one drug molecule, respectively. The calculated thermodynamic parameters of the two DNA–drug systems are gathered in Table 1.

According to the above-mentioned binding models, it can be deduced that 5FU combine with bps of the DNA in the ratio of 1:3. When 5FU is replaced by FT, it seems that there are possibly two binding reactions in the coordinate model. However, the binding model is mainly four bps associate with one FT molecule since β_2 is evidently larger than β_1. This discrepancy of stoichiometry can be attributed to the difference of molecular structure of the two drugs. The formation of FT molecule can be regarded as the substitution of the hydrogen atom (3-*H*) in 5FU molecule by a furan ring, which is evidently bigger than the replaced hydrogen atom and can attract more bps. Furthermore, its oxygen atom might arouse the hydrogen bond function that may be able to make the combination between FT and DNA easier. So the supramolecular structure formed by a FT molecule combined with four bps is more stable than that formed by a 5FU molecule with three bps. Hence β_2 is obviously larger than β.

It can be seen from the aforesaid analysis that primary binding model of FT with ct-DNA is four bps associating with one FT molecule, while that of 5FU with the DNA is three bps with one 5FU molecule. In other words, when a drug molecule approaches a binding site on the DNA molecule, it can simultaneously interact with a number of bps at the site, and the number may be different with change in molecular structure of the drug. The difference between binding sites for FT and 5FU and that between molecular

Table 1
Stability constants (β_i), standard changes of enthalpy (ΔH°), Gibbs free energy (ΔG°) and the entropy effect ($T\Delta S^\circ$) for the combination process.

Drugs with DNA	Reaction model	$10^{-6}\beta_i$ (L/mol)	ΔH° (J/mol)	ΔG° (kJ/mol)	$T\Delta S^\circ$ (kJ/mol)
5FU–DNA	M + L = ML[a]	12.70 ± 0.21	−2.89 ± 0.07	−40.55	40.54
FT–DNA	M + L = ML[b]	4.90 ± 0.13	493.94 ± 0.31	−38.19	38.68
	M + 2L = ML_2[b]	(2.29 ± 0.14) × 10^8	17.73 ± 0.14	−81.96	81.97

[a] M represents the group made up of three bps.
[b] M represents the group made up of eight bps.

structures of the drugs must cause differences between thermodynamic parameters of the two drug–DNA systems discussed in the following sections.

The standard changes of enthalpy (ΔH°) corresponding to the combining process are listed in Table 1. The data indicate that combination of 5FU or FT to the DNA molecule show very weak exothermic or endothermic heat effect, respectively. This can be explained by considering the interaction forces between the bio-macromolecules and the drug molecules, which may comprise hydrophobic, hydrogen bonds and electrostatic interactions [24]. The direct attraction caused by these interactions of the bps with the drug molecules lead to exothermic effect. On the other hand, the drug molecules must lose some water molecules in their hydration layers when they are approaching to the DNA molecules. Meanwhile the water molecules coordinated to the DNA molecules are extruded by drug molecules in the combining process. Both the dehydration processes are endothermic. The furan ring is much bigger than the replaced hydrogen atom, so FT molecule can extrude much more water molecules than 5FU molecule does. Therefore the substitution of hydrogen atom from the drug molecule by furan ring changes the combining process of drug with the DNA from exothermic process to endothermic one.

The entropy effect ($T\Delta S^\circ$) of the combination process are all positive and make evidently larger contribution to the negative changes of standard Gibbs free energy (ΔG°) than the heat effects do. Hence the combination of the DNA with each of the drugs is entropy driven process. According to literature [7], the binding manner might be that the drug molecules entered the double helix made up of bps of the DNA molecule. Because the drug molecules need to (at least partly) dehydrate themselves firstly and some water molecules in the grooves of the bio-macromolecules must be released while the drugs entered, the combination of a drug with DNA lead to quite large entropy increase although net interaction of DNA with the drug can cause some entropy decrease.

The negative changes of standard Gibbs free energy (ΔG°) can be attributed to the entropy changes in the researched system, indicating that the combination of the DNA with 5FU or FT is a spontaneous process in thermodynamics. The absolute value of change in Gibbs free energy of a 5FU molecule combined with three bps is less than that of a FT molecule combined with four bps. This also indicates that the interaction of FT with the DNA is stronger than that of 5FU with DNA.

3.3. Circular dichroism study

The CD spectra of DNA–5FU/FT systems are shown in Figs. 4 and 5, which can show the influence of ligand on the conformation of DNA molecule. The changes in CD signals of DNA observed on interaction with drugs may be assigned to the corresponding changes in DNA structure [25,26]. Since both 5FU and FT have no CD signal in the region of 220–320 nm, the CD signal in the systems must be aroused only by the DNA molecule. The CD spectrum of DNA is the representative B-conformation when no ligand/drug is in existence [27], which contains a positive band at 275 nm due to base-stacking and a negative band at 248 nm due to the right-handed helicity. Both the bands are quite sensitive to the interaction mode with small molecules [28,29].

As is shown in Figs. 4 and 5, both 5FU and FT can disturb on the DNA conformation. Compared with the CD spectra of free DNA, the negative Cotton effect [27] of 5FU–DNA system has a blue shift about 0.9 nm, while the positive Cotton effect has a red shift about 0.7 nm. The negative Cotton effect of FT–DNA system has a blue shift of 1.1 nm. However, the positive Cotton effect has no shift in the band positions. This reveals that the effect of intercalation of the drug into base-stacking and decreased right-handedness of ct-DNA as well [29]. The value of $\Delta\varepsilon$ between positive and negative peaks decreases with the increase in concentration of the drugs. These data suggest that the drugs may not have a more evident effect on base-stacking than on the polynucleotide helicity of the DNA, and the interaction of the drugs with DNA can cause some change in the secondary structure of the DNA [28]. Moreover, FT induces a more pronounced blue shift of the negative band than 5FU does, indicating that the furan ring bring stronger influence on the intercalation of FT into DNA. This can be used to explain the difference of thermodynamic parameters (ΔH°, ΔS° and ΔG°) between the two DNA–drug binding processes.

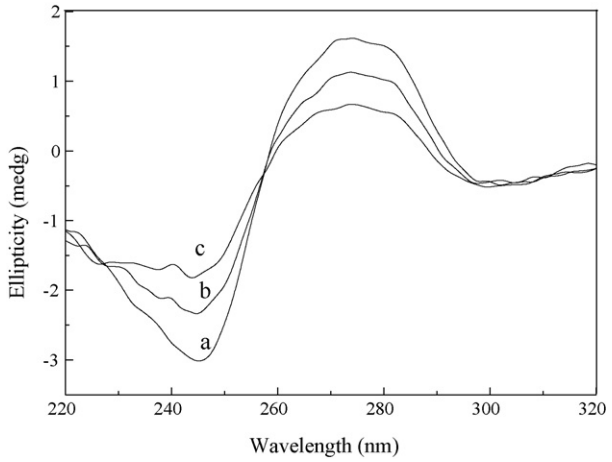

Fig. 4. The influence of 5FU of different concentration inflected the CD spectra of DNA (26.8 μM), n_{5FU}/n_{DNA} was 0 (a), 2 (b), 4 (c), respectively.

3.4. Ultraviolet absorption spectra

The UV spectrometry is also one of the most important methods to investigate the interaction between the small molecules and DNA [30]. The typical absorption peaks of 5FU, FT and DNA center at 267, 271 and 259 nm, respectively. The mixed solution of 5FU with DNA presents an absorption peak around 265 nm whereas the mixed solution of FT with DNA shows an absorption peak around 270 nm. The absorption peak of the mixed solution is not the simple superposition of the characteristic absorption peaks corresponding to the drugs and DNA. The addition of increasingly higher concentrations of drugs led to bathochromic effect and

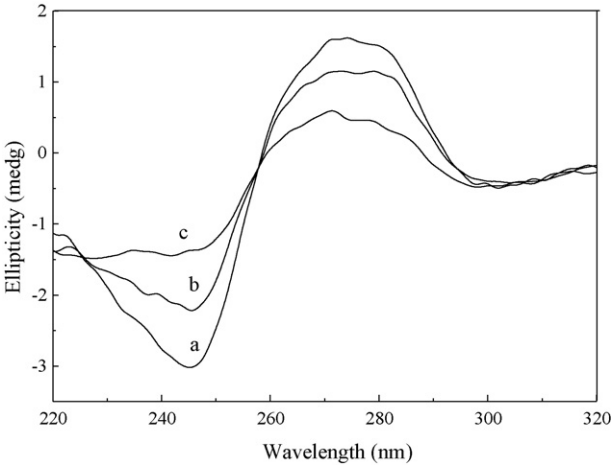

Fig. 5. The influence of FT of different concentration inflected the CD spectra of DNA (26.8 μM), n_{FT}/n_{DNA} was 0 (a), 3 (b), 6 (c), respectively.

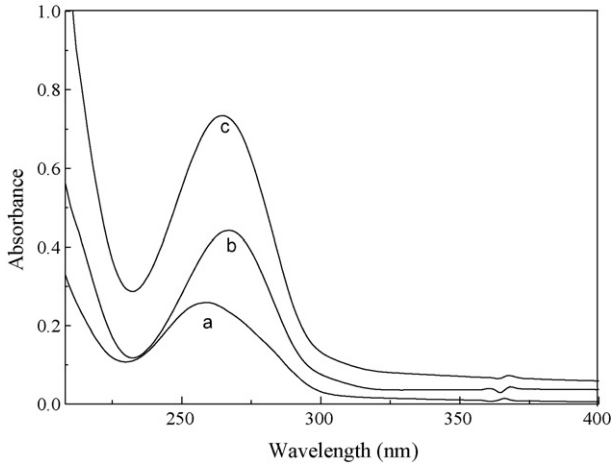

Fig. 6. The UV absorption spectra of the interaction between 5FU and DNA in the buffer solution (pH 7.40) [DNA] = 42.3 μM, [5FU] = 0.1 mM, (a) DNA, (b) 5FU, (c) 5FU + DNA (n_{5FU}/n_{DNA} = 2).

hyperchromicity changes, as seen in Figs. 6 and 7, i.e., the interaction between a drug and DNA result in a strong increase of the absorption intensity at maximal peaks, accompanied by a slight red shift.

Hyperchromism and hypochromism are the proper spectrum property, corresponding to the helix structure and the steric configuration of DNA. Hyperchromism may be attributed to the interactions between small molecules and DNA, and the change of DNA helix structure. Hypochromism was assigned to a strong interaction between the electronic states of the intercalating chromophore and that of the DNA base [31]. According to the Long theory [32], the hyperchromism and the bathochromic effect are the signs of drugs interact with DNA by the insert function. When the small molecules interact with DNA by the insert function, the π electron clouds of the DNA and drug molecules participate in the interaction, the maximal absorbing peaks shift toward higher wavelengths and the absorbency increased. When the small molecules interacted with DNA by the electrostatic function, the peak position is fixed but the intensity of the peak changed. Consequently, the UV spectra prove that the interactions of drug–DNA systems are mainly the insert function [32]. The discrepancy between 5FU–DNA and FT–DNA systems is the different

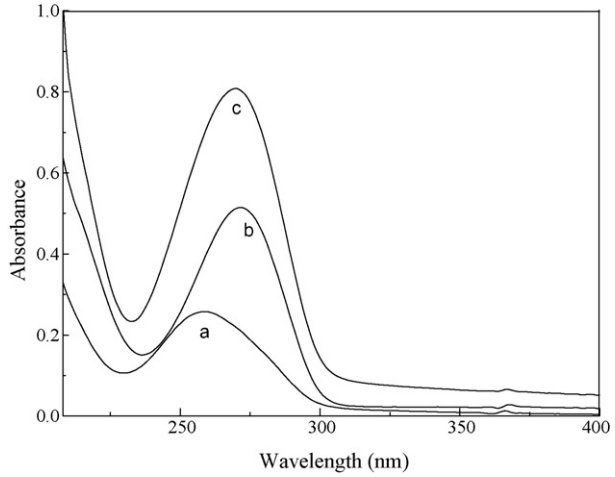

Fig. 7. The UV absorption spectra of the interaction between FT and DNA in the buffer solution (pH 7.40) [DNA] = 42.3 μM, [FT] = 0.1 mM, (a) DNA, (b) FT, (c) FT + DNA (n_{FT}/n_{DNA} = 2).

extent of the red shift and the absorbency. The maximal peaks of 5FU–DNA and FT–DNA systems red shift for 6 and 11 nm, respectively. These results indicate that the interaction between FT and DNA is stronger than that of 5FU with DNA. This agrees well with the data from CD spectra and can be used to explain the thermodynamic parameters.

3.5. Fluorescence analysis

No luminescence is observed for both 5FU and FT in any solvent or even in the presence of DNA. Berberine is a natural plant product obtained from various species of Berberis, belonging to the camptothecin family of drugs. It has been traditionally used in Chinese and Native American medicine to fight a number of infectious organisms, and its sulfate, hydrochloride, and chloride forms are used in Western pharmaceutical medicine as antibacterial agents [33–35]. Being different from highly toxic fluorescent probe such as ethidium bromide [36–38], BR can be widely applied as a non-toxic fluorescent probe [8]. It has an extremely weak intrinsic fluorescence emission spectrum with a peak wave length (λ_{max}) at 550 nm in aqueous solution, but it can emit quite intense fluorescence in the presence of DNA due to its strong intercalation between the bps of double-stranded DNA and it has been demonstrated that BR being an perfect intercalator [8,39]. Our experiments show that there is almost no change in the fluorescence intensity of free BR (in the absence of DNA) but has obvious quenching in the presence of DNA, on increasing concentration of 5FU and FT. It has been reported that the enhanced fluorescence can be quenched, at least partially by the addition of molecules of a second drug [40]. The extent of quenching fluorescence of DNA-bound BR can be used to determine the extent of binding between the new drug molecules and DNA molecules. So steady-state competitive binding experiments using 5FU and FT as quenchers may provide further information for study on the binding of the antitumour drugs to DNA. The emission spectra of DNA bound to BR in the absence and the presence of drugs are shown in Fig. 8. From the curves in Fig. 8, a quite evident decrease of the emission intensity is observed on the addition of the drugs to the DNA-bound BR solutions.

In order to understanding the mechanisms of the DNA–drug interactions, the Scatchard plots belonging to the inhibitory effect of BR binding with DNA in the absence and in the presence of drugs (5FU, FT) were obtained according to literature [38] (see Fig. 9). The emission of BR–DNA complex is quenched by the drugs and the Scatchard plots show competitive and noncompetitive behavior coexists for such quenching. This result shows that the binding sites of drugs and BR on DNA molecule are not exactly the same. When drugs interact with DNA they can compete with BR and insert themselves into the double helix made up of bps by the insert function, as well as binding with the phosphate groups of DNA by the electrostatic interaction. 5FU is a planar molecule which can insert itself into the double helix made up of bps so the insert function is the predominant interaction of 5FU–DNA system. Besides the insert function, the hydrophobic moiety of the furan ring in FT molecule is expected to facilitate intercalation into the relatively non-polar interior of the DNA helix, which strengthens the macromolecule–drug interaction. The extent of the quenching is increasing by the enhancement of the concentration of 5FU and FT, but to different extent at the same concentration, and the quenching ability of FT is stronger than that of 5FU. This is in good agreement with the fact that the complex of the DNA with FT is more stable than that of the DNA with 5FU. This is also in accordance with the results of ITC, CD and UV experiments.

The comprehensive analysis according to thermodynamic and spectra data indicate that interaction of ct-DNA with FT is stronger than that of the DNA with 5FU. The difference might have some-

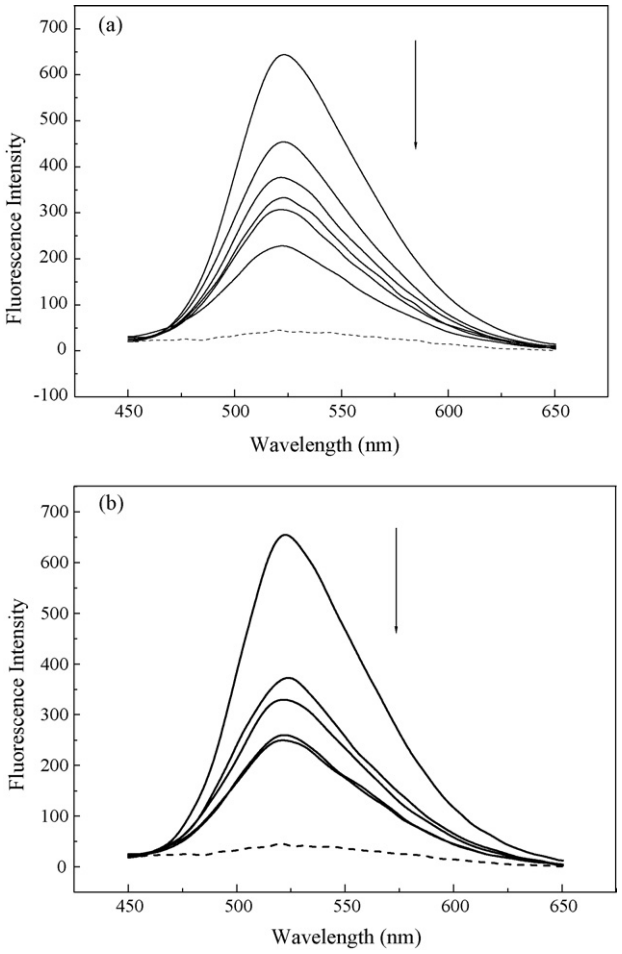

Fig. 8. Changes in the emission spectra of DNA-bound BR in buffer solution ([BR] = 66 μM, [DNA] = 90 μM) with increasing concentration of drugs. The emission spectrum of BR (in the absence of DNA) was shown as the dashed line and the arrow shows the intensity changes upon increasing the concentration of the drugs. (a) The concentration of 5FU, 10^5 [5FU]/M: 0, 3.77, 7.55, 9.44, 11.80, 18.55. (b) The concentration of FT, 10^5 [FT]/M: 0, 1.89, 3.77, 7.55, 11.80.

thing to do with the different cytotoxicity of the two drugs. For instance, Yuan and Engel et al. have evaluated cytotoxicity of 5FU and FT using a kind of tumour cells (CT-26), respectively. The 5FU and FT concentration required to cause 50% growth inhibition (IC50) in CT-26 cells are 4.30 μg/mL (33.1 μM) and 136 μM, respectively [17,41]. It is of importance that we know the difference, because FT and 5FU are in coexistence in cells when the former is used as prodrug.

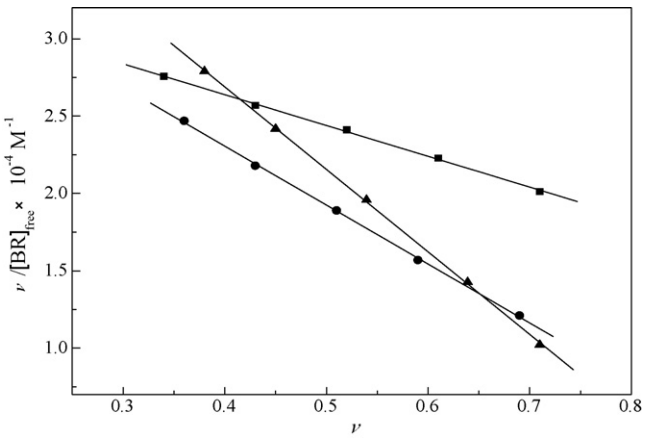

Fig. 9. The Scatchard plots of the binding of BR to DNA in the absence of drugs (■), and in the presence of drugs: 33.6 μM 5FU (●), 33.6 μM FT (▼). The DNA concentration was 70 μM and BR concentration varied from 40 to 100 μM.

4. Conclusion

The interactions of DNA with two antitumour drugs (5FU, FT) have been investigated by isothermal titration calorimetry and spectroscopy. The analysis of the thermodynamic data indicates that each 5FU molecule combined with three bps while each FT molecule combined with four bps when binding to the DNA molecules. The binding processes are all predominantly driven by entropy. The CD spectra indicate that the molecular structure of the DNA has been slightly changed, but the base group stacking which makes the DNA structure stable is not evidently changed. The UV and fluorescence spectra show that the drugs interact with DNA mainly by insert function, and meanwhile, electrostatic interaction may also be in existence.

Acknowledgement

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References

[1] J. Monaselidze, M. Abuladze, N. Asatiani, E. Kiziria, S. Barbakadze, G. Majagaladze, M. Iobadze, L. Tabatadze, H.Y. Holmanc, N. Sapojnikova, Thermochim. Acta 441 (2006) 8–15.
[2] Z. Nikolovska Coleska, J.L. Meagher, S. Jiang, C. Yang, S. Qiu, P.P. Roller, J.A. Stuckey, S. Wang, Biochemistry 47 (2008) 9811–9824.
[3] H. Marcal, N.S. Wanandy, V. Sanguanchaipaiwong, C.E. Woolnough, A. Lauto, S.M. Mahler, L.J.R. Foster, Biomacromolecules 9 (2008) 2719–2726.
[4] N.A. Thornberry, Y. Lazebnik, Science 281 (1998) 1312–1316.
[5] R. Minami, M. Shimada, H. Yokosawa, H. Kawahara, Biochem. J. 405 (2007) 495–501.
[6] M.E. Gorre, M. Mohammed, K. Ellwood, N. Hsu, R. Paquette, P.N. Rao, C.L. Sawyers, Science 293 (2001) 876–880.
[7] K. Nazari, A.R. Golchin, A.A. Moosavi Movahedi, A.A. Saboury, G.H. Hakimelahi, A. Shockravi, S. Tangestani-nejad, Thermochim. Acta 428 (2005) 157–163.
[8] A. Xia, H. Wu, S. Li, S. Zhu, Y. Zhang, Q. Han, R. Yu, Talanta 73 (2007) 606–612.
[9] S. Marsh, Invest. New Drug 23 (2005) 533–537.
[10] M.D. Kaeser, S. Pebernard, R.D. Iggo, J. Biochem. 279 (2004) 7598–7605.
[11] S. Chen, X. Zhang, S. Cheng, R. Zhuo, Z. Gu, Biomacromolecules 9 (2008) 2578–2585.
[12] L. Delval, J. Klastersky, J. Neuro-Oncology 60 (2002) 165–169.
[13] C. Wang, X. Luo, B. Zhang, W. Song, M. Ni, S. Mehendale, J. Xie, H.H. Aung, T. He, C. Yuan, Cancer Chemoth. Pharm. 60 (2007) 69–79.
[14] T. Komatsu, H. Yamazaki, N. Shimada, M. Nakajima, T. Yokoi, J. Drug Metabol. Dispos. 28 (2000) 1457–1463.
[15] Y.A. Woo, G.H. Kim, E.J. Jeong, C.Y. Kim, J. Chromatogr. B 875 (2008) 487–492.
[16] H. Wu, W. Li, T. Wang, Y. Shu, P. Liu, Biomed. Pharmacother. 62 (2008) 659–666.
[17] F. Yuan, X. Qin, D. Zhou, Q. Xiang, M. Wang, Z. Zhang, Y. Huang, Eur. J. Pharmaceut. Biopharmaceut. 70 (2008) 770–776.
[18] L. Yao, Y. Li, Y. Wu, A. Liu, H. Yan, Biochemistry 15 (2005) 5940–5947.
[19] A.A. Saboury, M.S. Atri, M.H. Sanati, M. Sadeghi, J. Therm. Anal. Calorim. 83 (2006) 175–179.
[20] K.R. Wolthers, X. Lou, H.S. Toogood, D. Leys, N.S. Scrutton, Biochemistry 42 (2007) 11833–11844.
[21] J. Tellinghuisen, J. Phys. Chem. B 39 (2007) 11531–11537.
[22] C.V. Kumar, E.H. Asuncion, J. Am. Chem. Soc. 115 (1993) 8547–8553.
[23] G. Bai, Y. Wang, H. Yan, J. Phys. Chem. B 106 (2002) 2153–2159.
[24] M. Gharagozlou, D.M. Boghaei, Spectrochim. Acta A 71 (2008) 1617–1622.
[25] B.K. Sahoo, K.S. Ghosh, R. Bera, D. Swagata, Chem. Phys. 351 (2008) 163–169.
[26] Y. Liu, X. Wei, F. Wu, W. Mei, L. He, Spectrochim. Acta A 70 (2008) 171–176.
[27] F. Picard Jean, I. Bougie, M. Bisaillon, Biochem. J. 407 (2007) 331–341.
[28] P. Shi, Q. Jiang, Y. Zhao, Y. Zhang, J. Lin, L. Lin, J. Ding, Z. Guo, J. Biol. Inorg. Chem. 11 (2006) 745–752.
[29] P.U. Maheswari, M. Palaniandavar, J. Inorg. Biochem. 98 (2004) 219–230.
[30] L. Chen, J. Liu, J. Chen, C. Tan, S. Shi, K. Zheng, L. Ji, J. Inorg. Biochem. 102 (2008) 330–341.
[31] K. Sakurai, M. Mizu, S. Shinkai, Biomacromolecules 2 (2001) 641–650.
[32] E.C. Long, J.K. Barton, Accounts Chem. Res. 23 (1990) 271–273.

[33] P.N. Domadia, A. Bhunia, J. Sivaraman, S. Swarup, D. Dasgupta, Biochemistry 47 (2008) 3225–3234.
[34] M. Megyesi, L. Biczók, Chem. Phys. Lett. 447 (2007) 247–251.
[35] X. Tian, Y. Song, H. Dong, B. Ye, Bioelectrochemistry 73 (2008) 18–22.
[36] J. Ren, J.B. Chaires, Biochemistry 38 (1999) 16067–16075.
[37] V. Jadhav, S. Maiti, Biomacromolecules 9 (2008) 1852–1859.
[38] A.A. Moosavi-Movahedi, A.R. Golchin, K. Nazari, J. Chamani, A.A. Saboury, S.Z. Bathaie, S. Tangestani-Nejad, Thermochim. Acta 414 (2004) 233–241.
[39] M.M. Islam, G.S. Kumar, J. Mol. Struct. 875 (2008) 382–391.
[40] B.C. Baguley, M.L. Bret, Biochemistry 23 (1984) 937–943.
[41] D. Engel, A. Nudelman, N. Tarasenko, I. Levovich, I. Makarovsky, S. Sochotnikov, I. Tarasenko, A. Rephaeli, J. Med. Chem. 51 (2008) 314–323.