

Investigation of the Interaction of Sertraline with Calf Thymus DNA by Spectroscopic Methods

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A interação entre a droga anti-depressiva, sertralina, com o DNA de fita dupla (dsDNA) de timo de vitelo em tampão fisiológico (pH 7,4) foi determinada por espectrofotometria de UV-Vis, espectrofluorimetria, dicroísmo circular, espectroscopia no infravermelho com transformada de Fourier (FTIR), medições da viscosidade e estudos de fusão de DNA. Os espectros de absorção da droga com o DNA mostraram um efeito hipercrômico. Ao usar o reagente Hoechst como uma sonda de fluorescência ocorreu a extinção do pico de emissão na mistura DNA-Hoechst após a adição da sertralina. Os espectros de FTIR revelaram o modo de ligação de menor sulco entre o fármaco e o dsDNA. A constante de ligação da sertralina ao DNA foi calculada utilizando os dados espectroscópicos. Os parâmetros termodinâmicos calculados sugeriram que as interações eletrostáticas são forças importantes na formação do complexo DNA-sertralina.

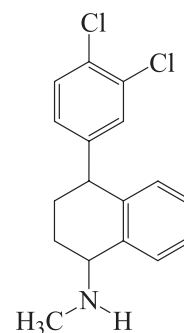
The interaction of the antidepressant drug, sertraline, with calf thymus double stranded DNA (dsDNA) in physiological buffer (pH 7.4) was investigated by UV-Vis spectrophotometry, spectrofluorimetry, circular dichroism, Fourier transform infrared spectroscopy (FTIR), viscosity measurements and DNA melting studies. The absorption spectra of the drug with DNA showed a hyperchromic effect. Using Hoechst reagent as a fluorescence probe, quenching of the emission peak occurred in the DNA-Hoechst mixture when sertraline was added. The FTIR spectra revealed minor groove binding mode between the drug and the dsDNA. The binding constant of sertraline to DNA was calculated using spectroscopic data. The calculated thermodynamic parameters suggested that electrostatic interactions are important forces in the formation of sertraline-DNA complex.

Keywords: DNA, sertraline, interaction, spectroscopy

Introduction

Selective serotonin-reuptake inhibitors (SSRIs) are used in the treatment of various forms of psychiatric disorders including depression, obsessive-compulsive disorder, panic attacks, and social phobias. Sertraline (SER), [(1S,4S)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-N-methyl-1-naphthylamine] (Scheme 1), is a SSRI.^{1,2} It is considered to be safe, effective within a wide therapeutic range, and with minimal and well-tolerated adverse effects.³⁻⁵ SER is mainly used therapeutically to treat the symptoms of depression and anxiety.^{2,4}

The study of the interaction of small molecules (often drugs or ligands) with DNA has been the focus of some recent research works in the scope of life science, chemistry and clinical medicine.⁶⁻⁸ DNA is quite often the main molecular target of the chemical substances in



Scheme 1. Chemical structure of sertraline.

the environment, thereby people are facing an increase of diseases and many types of cancer can be partly attributed to dramatic changes of the DNA physiological functions.^{9,10} Intercalation and groove binding are the two major binding modes of small molecules to DNA.

In this work, the interaction between SER and calf thymus DNA was investigated by UV-Vis absorption and

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fluorescence spectroscopy, Fourier transform infrared spectroscopy (FTIR), viscosity measurements and DNA melting technique. This study may be helpful to further understand the mechanism of sertraline action in body and valuable for the improvement of new drug design.

Experimental

Apparatus

UV-Vis absorption spectra were measured on an Agilent 8453 spectrophotometer (Waldborn, Germany) using a quartz cell (light path 1.0 cm). Fluorescence measurements were performed with a JASCO (FP6200) spectrofluorimeter (Tokyo, Japan) equipped with a 150W Xenon lamp and a thermostat bath, using a 1.0 cm quartz cell. The widths of the excitation and emission slits were set at 5.0 and 10 nm, respectively, and the scan rate was 1200 nm min⁻¹. Circular dichroism (CD) measurements were recorded on a JASCO (J-810) spectropolarimeter (Tokyo, Japan). The viscosity measurements were carried out using a SCHOT AVS 450 type viscometer. An electronic thermostat water-bath was used for controlling the temperature.

Chemicals

A stock solution (1.0 × 10⁻² mol L⁻¹) of sertraline hydrochloride (Osvah Pharmaceutical Co, Tehran, Iran) was prepared by dissolving its powder in absolute ethanol. Calf thymus double stranded DNA (dsDNA) (Sigma Chem. Co., USA) was used without further purification, and its stock solution was prepared by dissolving an appropriate amount of DNA in Tris-HCl buffer solution overnight and stored at 4 °C. The concentration of DNA in stock solution was determined by measuring its UV absorption at 260 nm using a molar absorption coefficient of 6600 L mol⁻¹ cm⁻¹.¹¹ Purity of DNA was checked by monitoring the ratio of the absorbance at 260 nm 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.¹² Hoechst 33258 (Sigma Chem. Co., USA) stock solution (1.0 × 10⁻⁴ mol L⁻¹) was prepared by dissolving in Tris-HCl buffer solution and stored in a cool and dark place. All other chemicals used were of analytical reagent grade. Doubly distilled deionized water was used throughout.

Procedures

UV-Vis absorption measurement

All measurements were carried out in pH 7.4 using a Tris-HCl buffer. The UV-Vis absorption spectra of DNA

and the mixture of DNA and SER solutions were measured on the spectrophotometer at room temperature in the range of 190 to 600 nm.

Fluorescence experiment

The competitive experiments were conducted by adding small aliquots of SER stock solution to DNA-Hoechst mixture and the change in fluorescence intensity was recorded. The excitation wavelength was 340 nm and the emission spectra were recorded from 340 to 600 nm.

DNA melting studies

DNA melting experiments were carried out by monitoring the maximum fluorescence intensities of the complex of DNA with methylene blue (DNA-MB) in the absence and presence of SER at different temperatures. The temperature of the sample was continuously monitored with a thermocouple attached to the sample holder. The fluorescence intensities were then plotted against temperature ranging from 40 to 90 °C. The melting temperature (T_m) was determined as the transition midpoint.

Viscosity measurements

The viscometer was immersed in a thermostatic bath at 25 ± 0.1 °C. Typically, 15.0 mL of buffer and 15.0 mL of DNA solution were transferred into the viscometer separately. Various concentrations of SER were added into the viscometer to give a specific mole-ratio r ($r = [\text{SER}] / [\text{DNA}]$) while keeping the DNA concentration at 5.0 × 10⁻⁵ mol L⁻¹. After thermal equilibration, the time of the solution's flowing through the capillary was determined to ±0.2 s by a digital stop-watch. At least three measurements were tested to calculate the average relative viscosity of the DNA solution. The data were presented as $(\eta/\eta_0)^{1/3}$ versus the mole-ratio values, where η and η_0 are the viscosity of DNA in the presence and absence of SER, respectively. Viscosity values were calculated from the observed flow time of DNA-containing solutions (t) and corrected for buffer solution (t_0), $\eta = (t - t_0)/t_0$.

FTIR measurements

FTIR spectra of DNA and DNA-SER solutions were recorded. The samples were prepared as thin films by volatilizing the solvent to appropriate concentrated solutions on transparent slides.

Circular dichroism (CD) measurements

CD measurements were recorded keeping the concentration of DNA constant (5 × 10⁻⁵ mol L⁻¹) while varying the concentration of SER ($r = c_{\text{SER}} / c_{\text{DNA}} = 0.0, 0.4, 0.6$).

Results and Discussion

Fluorescence measurements

In order to investigate the interaction mode between SER and DNA, a competitive fluorescence experiment was performed by using the reagent Hoechst 33258, SER has a negligible emission. Hoechst 33258 is a well known minor-groove binder, which is often used as a spectral probe to establish the mode of binding of small molecules to dsDNA.¹³ The fluorescence of Hoechst increases in the presence of DNA due to its higher planarity in the grooves of the host macromolecule as well as its protection from collisions with solvent.¹⁴ Upon addition of SER, if it competes for the groove sites in DNA, a significant decrease in the fluorescence intensity of the DNA-Hoechst complex would result.

As is shown in Figure 1a, the fluorescence of the Hoechst solution ($5.0 \times 10^{-6} \text{ mol L}^{-1}$) increases in the presence of DNA, which is due to the insertion of the probe in the grooves of the DNA helix. Based on the results shown in Figure 1a, DNA concentration in this study was selected as $1.77 \times 10^{-4} \text{ mol L}^{-1}$, *i.e.*, the ratio of DNA to Hoechst was 35.4:1.

The effect of addition of SER to the above-mentioned solution is shown in Figure 1b. As is obvious, the fluorescence of DNA-Hoechst complex is efficiently quenched by SER, with a little shift in the emission wavelength. In the absence of DNA, the fluorescence intensity of Hoechst showed no change upon addition of SER, therefore, direct quenching of Hoechst emission by SER is canceled out and the drug competes with the probe for DNA grooves.

In a similar spectrofluorimetric experiment, methylene blue (MB) was added to DNA solution. MB is a fluorescence probe that slips between adjacent base pairs and intercalates with DNA helix.^{15,16} As is shown in Figure 2, no obvious change was observed upon addition of SER to a mixture of MB and DNA which confirms the non-intercalative mode of binding of SER to DNA. Interestingly, the emission intensity of MB is quenched upon addition of DNA which reflects the changes in the excited state structure of MB surrounded by base pairs of DNA.¹⁷

Melting studies

Further support for the groove binding of SER to DNA was obtained through DNA melting experiments. Heat and alkali can destroy the double helix structure of DNA and change it into single helix at the melting temperature (T_m). Interaction of small molecules with DNA can influence T_m , so that it may be increased by about 5-8 °C as a result of intercalation binding which brings additional stability to

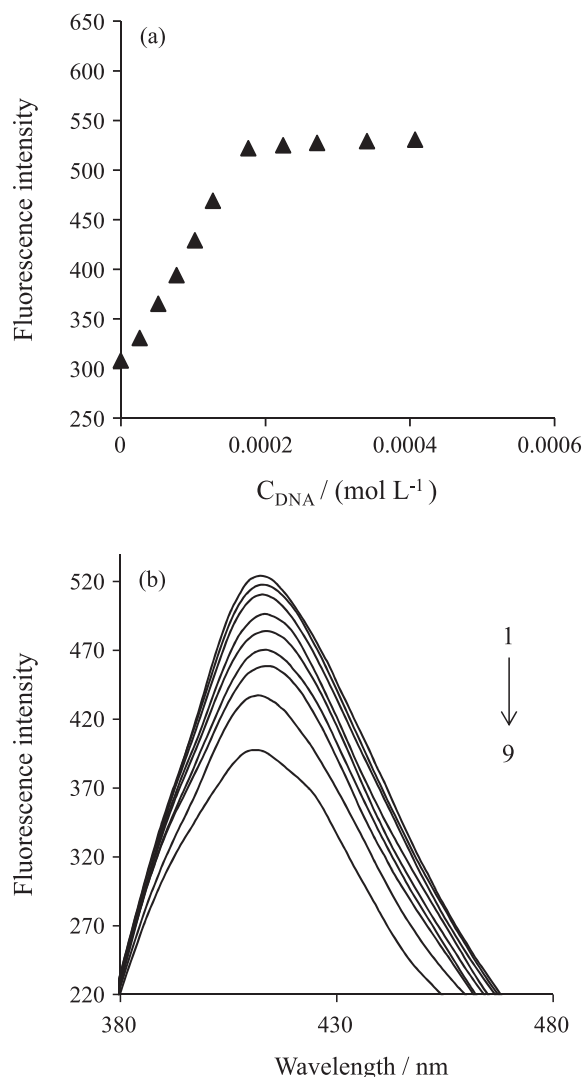


Figure 1. Fluorescence against the concentration of DNA (a). $C_{\text{Hoechst}} = 5.0 \times 10^{-6} \text{ mol L}^{-1}$. Fluorescence spectra of DNA-Hoechst in the presence of sertraline (b). Total concentration of sertraline: (1) 0, (2) 0.13, (3) 0.28, (4) 0.48, (5) 0.90, (6) 1.28, (7) 1.78, (8) 2.40 and (9) $3.44 \times 10^{-4} \text{ mol L}^{-1}$. The concentration ratio of DNA to Hoechst was 35.4:1.

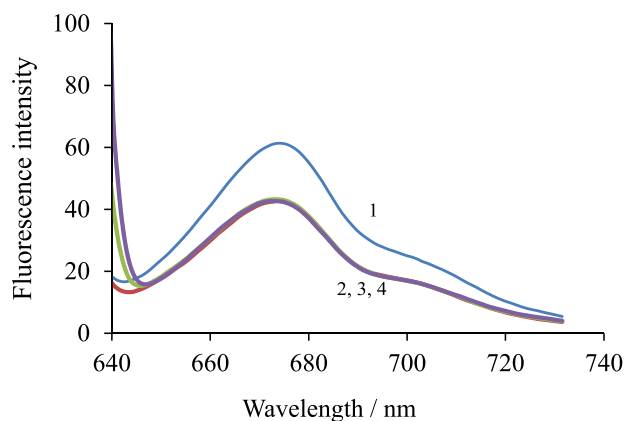


Figure 2. Emission spectra of (1) MB, (2) 1 + DNA, (3) 2 + $2.9 \times 10^{-5} \text{ mol L}^{-1}$ SER, (4) 2 + $9.1 \times 10^{-5} \text{ mol L}^{-1}$ SER. Tris-HCl buffer, pH 7.4.

DNA helix, but non-intercalative bindings cause no obvious increase in T_m .^{18, 19}

UV-Vis absorption spectroscopy is a general method for determining the melting temperature.²⁰ Considering the higher sensitivity, spectrofluorimetry was used in this study to determine T_m . The values of T_m for the mixtures DNA-MB and SER-DNA-MB were determined, by monitoring the maximum fluorescence intensities of the solutions as a function of temperature (40-90 °C). The transition midpoint of the melting curve (Figure S1) was assigned as T_m . The value of T_m was not changed significantly for DNA-MB (87.2 °C) in the presence of SER (86.5 °C) under the experimental conditions. Therefore, the interaction between DNA and SER may not be intercalative. The small decrease in T_m is presumably due to the groove binding of SER with DNA, which changes the conformation of DNA to some degree and influences the extent of interaction of DNA with MB.

Viscosity measurements

Viscosity experiment is an effective tool to study the binding mode of small molecules to DNA. A classical intercalation binding demands the space of adjacent base pairs to be large enough to accommodate the bound ligand and elongate the double helix, resulting in an increase of DNA viscosity.²¹ However, a partial non-classical intercalation of ligands can bend the DNA helix and reduce its effective length and its viscosity.²²⁻²⁴ There is a little effect on the viscosity of DNA if the groove binding occurs in the binding process.^{24,25} A series of solutions were prepared which contained a constant concentration of DNA and various concentrations of SER. Then, the viscosity measurements were conducted at room temperature. As is shown (Figure S2), there is no appreciable change in relative viscosity of DNA in the presence of SER. Such behavior suggests a non-intercalative mode of interaction, and possibly a groove binding should be the interaction mode of the drug with DNA.

Binding constant of SER with DNA

As was mentioned, the fluorescence intensity of Hoechst-DNA complex is quenched upon addition of SER. It is well known that there are two mechanisms involved in the quenching process: static and dynamic (collisional) quenching. The quenching nature of DNA-Hoechst complex in the presence of SER was analyzed using Stern-Volmer equation,²⁶

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

In this equation, F_0 and F are the fluorescence intensities of DNA-Hoechst complex in the absence and presence of SER, respectively; $[Q]$ is the SER concentration, K_q is the quenching rate constant, τ_0 is the average excited-state lifetime of DNA-Hoechst in the absence of SER (10^{-9} to 10^{-7} s)²⁷ and K_{SV} is the Stern-Volmer quenching constant.

The Stern-Volmer plot at 25 °C is shown in Figure 3. The maximum rate constant of collisional quenching of various quenchers with biopolymers is about $2.0 \times 10^{10} \text{ L s}^{-1} \text{ mol}^{-1}$.²⁸ From the slope of the line in Figure 3, a greater value for K_q is obtained ($9.09 \times 10^{10} \text{ L s}^{-1} \text{ mol}^{-1}$), which confirms a static quenching mechanism.

On the other hand, the values of K_{SV} decreased with the increasing temperature (Table 1), which indicated that the fluorescence quenching by SER was static, i.e., in the competition of SER for DNA grooves, Hoechst molecules were liberated to the solution.²⁹

The binding constant (K) and the numbers of binding sites (n) for DNA-SER system can be determined by the following equation,³⁰

$$\log (F_0 - F)/F = \log K_f + n \log [Q] \quad (2)$$

where K_f and n are the binding constant and the number of binding sites in base pairs unit, respectively. Thus, a plot of $\log (F_0 - F)/F$ versus $\log [Q]$ yields the K_f and n values which are summarized in Table 1 and Figure 4. An average value of one binding site on DNA for interaction with SER was obtained from the slope of the regression line in Figure 4.

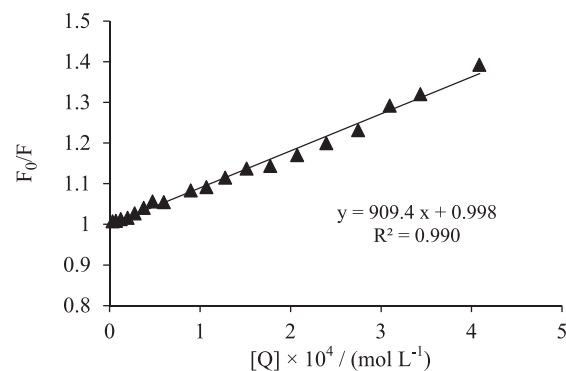


Figure 3. Stern-Volmer plot for the fluorescence quenching of DNA-Hoechst complex by SER.

Thermodynamic parameters and the nature of binding forces

There are several acting forces between small molecules and a biomacromolecule such as hydrophobic forces, hydrogen bonds, van der Waals, and electrostatic interactions. When the change in temperature is small, the

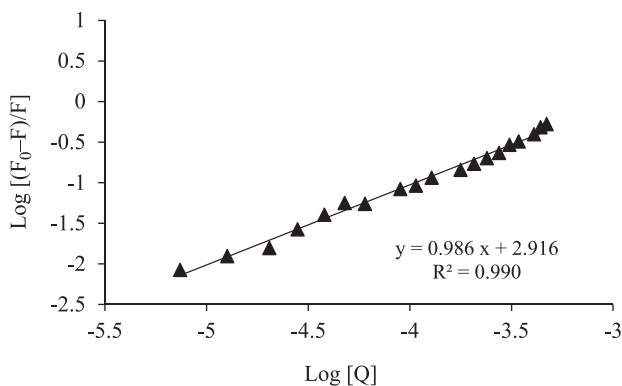


Figure 4. Plot of $\log (F_0 - F)/F$ against $\log [Q]$.

enthalpy change (ΔH^0) can be assumed as a constant, then its value and that of entropy change (ΔS^0) can be determined from the van't Hoff equation,

$$\ln K_f = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} \quad (3)$$

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 = -RT \ln K_f \quad (4)$$

where K_f is the binding constant at the corresponding temperature and R is the universal gas constant. The temperatures used were 278, 298 and 310 K. The values of ΔH^0 and ΔS^0 were obtained from the slope and intercept of the linear plot of $\ln K_f$ versus $1/T$ (Figure S3). The free energy change (ΔG^0) was estimated from Equation 4. The values of ΔH^0 , ΔS^0 and ΔG^0 are listed in Table 1. From Table 1, the negative value of ΔG^0 reveals that the interaction process between SER and DNA is spontaneous, while the negative sign of ΔH^0 and positive sign of ΔS^0 values indicates the reaction is exergonic and enthalpy and entropy favored. The positive entropy value confirms non-intercalative binding mode of SER to DNA.

When a small molecule interacts with DNA and forms a new complex, a shift in the absorbance wavelength of DNA and/or a change in the molar absorptivity may occur.³¹

UV-Vis absorption spectra of DNA with various amounts of SER (Figure 5a) were recorded by subtracting the spectrum of SER from that of drug-DNA complex. It is clear that the absorbance peak around 260 nm increased with the addition of the drug, which indicates the interaction between the two species.

The value of the binding constant (K) was obtained from the DNA absorption at 260 nm according to Equation 5 for weak binding affinities,³²

$$\frac{1}{A - A_0} = \frac{1}{A_\infty - A_0} + \frac{1}{K(A_\infty - A_0)} \times \frac{1}{[SER]} \quad (5)$$

where A_0 is the absorbance of DNA at 260 nm in the absence of SER, A_∞ is the final absorbance of SER-DNA and A is the recorded absorbance at different SER concentrations. Equation (5) is written on the assumption that the stoichiometry is 1:1. The linearity of the double reciprocal

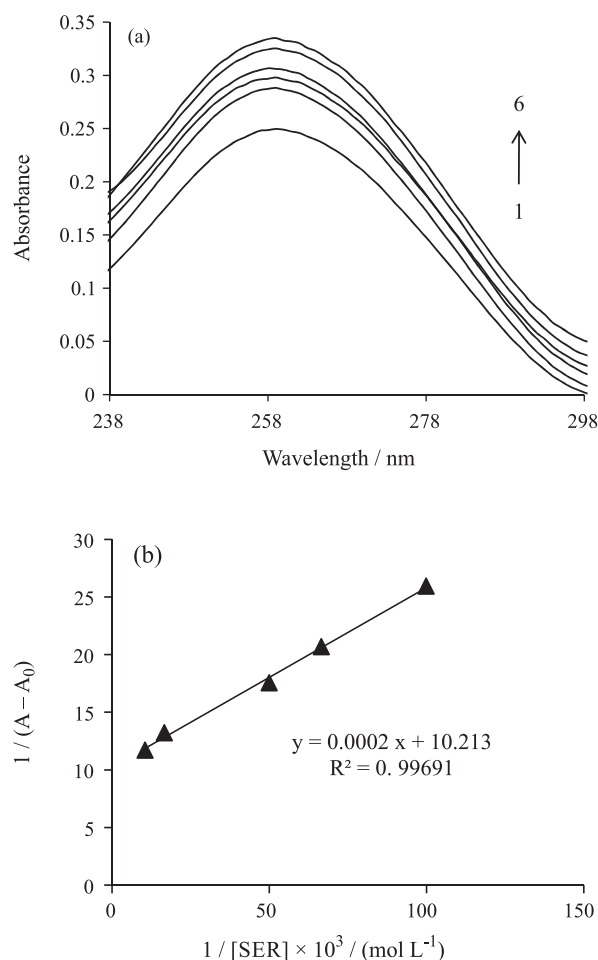


Figure 5. (a) Absorption spectra of DNA ($5.0 \times 10^{-5} \text{ mol L}^{-1}$) in the absence and presence of increasing amounts of SER ($r_1 = [\text{SER}] / [\text{DNA}] = 0, 0.2, 0.3, 0.4, 1.2, 1.9$). (b) Plot of $1/(A - A_0)$ against $1/[\text{SER}]$.

Table 1. Stern-Volmer (K_{SV}) and binding (K_f) constants, stoichiometry (n) and thermodynamic characteristics of the interaction between SER and DNA

T / °C	$K_{SV} / (\text{L mol}^{-1})$	$K_f / (\text{L mol}^{-1})$	n	$\Delta G / (\text{kJ mol}^{-1})$	$\Delta H^0 / (\text{kJ mol}^{-1})$	$\Delta S^0 / (\text{J mol}^{-1} \text{K}^{-1})$
5	1204	469.89	0.884	-16.42		
25	909.48	824.1	0.986	-16.85	-0.44	21.51
37	752.1	7907	1.31	-17.10		

plot of $1/(A - A_0)$ versus $1/[SER]$ (Figure 5b) confirms the 1:1 stoichiometry (as was obtained in fluorescence experiments) and the binding constant (K) can be estimated from the ratio of intercept to the slope³³. The binding constant for SER–DNA complex was calculated as 5.11×10^4 at 25 °C, which is different from the value obtained by fluorimetry (Table 1) due to the difference in the methods used.

The calculated binding constant is lower than the values reported for typical intercalators (for ethidiumbromide and $[Ru(phen)dppz]^{2+}$, binding constants have been reported to be in the order of 10^6 – 10^7 M^{-1}).^{34,35} This K value compare well with that of the well-established groove binding agent, spermine.³⁶

Circular dichroism spectroscopy (CD)

The changes in CD spectra of DNA on interaction with drugs may often be assigned to the corresponding changes in DNA structure. CD spectrum of calf-thymus dsDNA consists of a positive band centered at 275 nm due to base stacking and a negative band at 245 nm due to its helicity, which is characteristic of DNA in right-handed B form.³⁷

The results of CD studies of DNA in the presence of SER revealed that the conformation of DNA can be affected by the drug (Figure 6). A decrease in negative peak (shifting to zero level) is observed with an increase in the positive peak at the same time. Some investigators believe that this is a characteristic of $B \rightarrow A$ conformational transition of DNA³⁸, which is observed in groove binding mode.

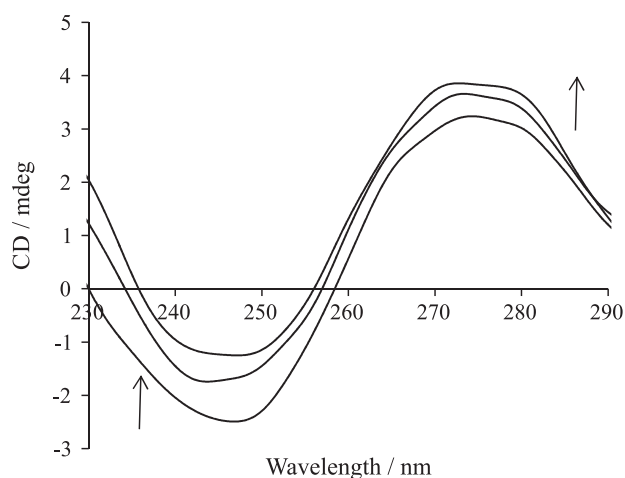


Figure 6. The changes of CD spectra of DNA (5×10^{-5} mol L^{-1}) in the presence of increasing amounts of SER ($r_1 = [SER]/[DNA] = 0, 0.4, 0.6$).

FTIR measurements

FTIR is often used to monitor the effects of various drugs on DNA structure. In this study, FTIR was used to compare the structural characteristics of DNA with

SER–DNA complex in aqueous solution. The IR spectral features for both DNA and SER–DNA are presented in Figure S4. Most important IR peaks for DNA are confined in the spectral region 1000–1800 cm^{-1} . The vibrational bands of DNA at 1716.5, 1681.8, 1625.8 and 1506.2 cm^{-1} are assigned to guanine (G), thymine (T), adenine (A) and cytosine (C) bases, respectively. Bands at 1234 and 1085.8 cm^{-1} denote phosphate ($-PO_2$) asymmetric and symmetric vibrations, respectively. The band at 1041.4 cm^{-1} is assigned to the sugar vibration.

The change of intensity and shifting of the $-PO_2$ bands is an indication of the interaction with SER. The large shifting of T and A bands accompanied by a major decrease in their intensities can be attributed to direct SER binding to these bases in the minor grooves of DNA.^{39,40} Small decrease in the intensities of G and C bands are indicative of some degrees of SER interactions with G–C bases. Therefore, an electrostatic interaction of SER with backbone phosphates and a strong minor groove binding can be concluded.

Conclusions

Detailed analysis of the interaction of SER with DNA in physiological buffer (pH 7.4) was carried out in this work by fluorescence, UV-Vis and FTIR spectroscopic techniques. The binding constants and number of binding sites of DNA with SER were measured at different temperatures, and the thermodynamic parameters were calculated. The groove binding of SER with DNA was deduced by taking into account the changes in fluorescence spectra, melting temperature and viscosity measurements. The study of interaction of SER with DNA may provide useful information on the mechanism of antidepressant drug binding to DNA and thus will be helpful to the design of new drugs with lower side effects.

Supplementary Information

Supplementary information is available free of charge at <http://jbc.ssbq.org.br> as PDF file.

Acknowledgements

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Supplementary Information

Investigation of the Interaction of Sertraline with Calf Thymus DNA by Spectroscopic Methods

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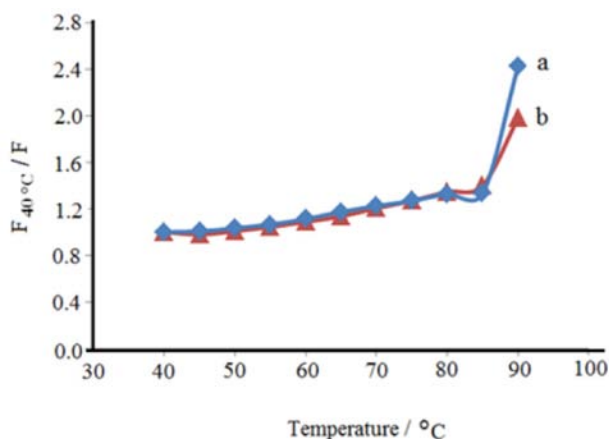


Figure S1. Melting curves of DNA-MB in the absence (a) and presence of (b) SER. $C_{MB} = 5.0 \times 10^{-6} \text{ mol L}^{-1}$, $C_{DNA} = 1.10 \times 10^{-4} \text{ mol L}^{-1}$, $C_{SER} = 1.04 \times 10^{-4} \text{ mol L}^{-1}$, pH 7.4.

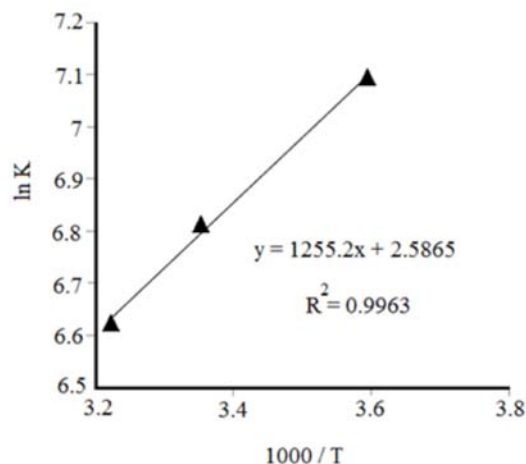


Figure S3. Van't Hoff plot for the binding of SER to DNA.

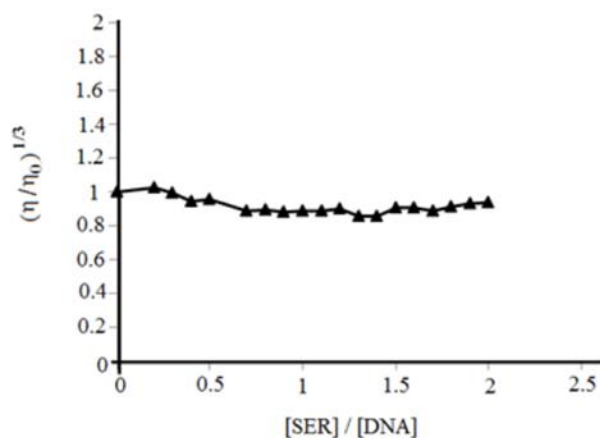


Figure S2. Effect of increasing amounts of sertraline on the viscosity of DNA ($5 \times 10^{-5} \text{ mol L}^{-1}$).

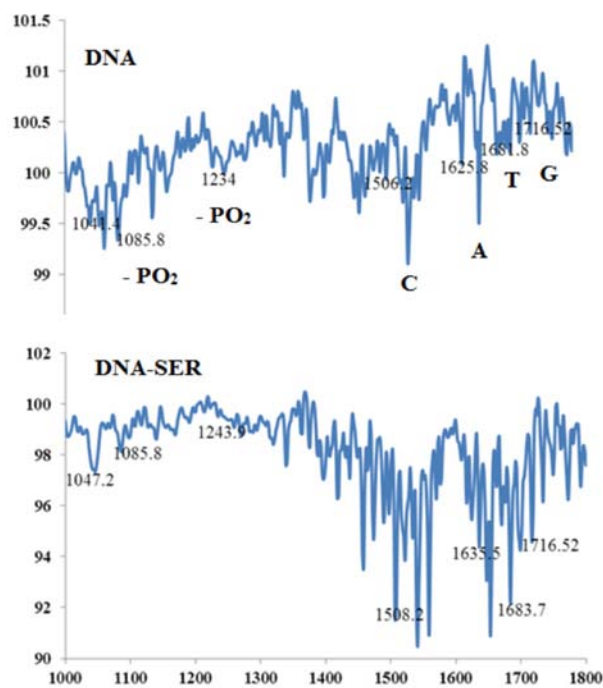


Figure S4. FTIR spectra in the region of $1800\text{-}1000 \text{ cm}^{-1}$ for the free calf-thymus DNA ($1.0 \times 10^{-2} \text{ mol L}^{-1}$) and its complex in aqueous solution at pH 7.4 with [SER]/[DNA] ratio (1/40).

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