RESEARCH ARTICLE

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Spectroscopic and Viscositic Studies on the Interaction of Solifenacin Succinate with DNA

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Abstract

The interaction of native calf thymus DNA (ctDNA) with solifenacin succinate (SFS) was investigated under simulated physiological conditions by multi-spectroscopic techniques and viscometric measurements. It concluded that SFS could intercalate into the base pairs of ctDNA, and the fluorescence quenching by ctDNA was static quenching type. Thermodynamic parameters calculated suggested that the binding of SFS to ctDNA was mainly driven by hydrophobic interactions. Furthermore, the relative viscosity of ctDNA increased with the addition of SFS, which confirmed the intercalation mode.

Keywords: Solifenacin succinate, Calf thymus DNA, spectroscopic, viscositic studies.

INTRODUCTION

The biological activity of many small molecular drugs, have been known to play important roles in medicinal chemistry due to their interaction with DNA. The study of interaction mechanism between drug and DNA has promoted the developing of new drugs. The interaction between drugs and DNA is an active research area[1,2].

Solifenacin succinate (SFS) is a competitive muscarinic acetylcholine receptor antagonist. The binding of acetylcholine to these receptors, particularly the M_3 receptor sub type, plays a critical role in the contraction of the smooth muscle. By preventing the binding of acetylcholine to these receptors, solifenacin succinate reduces the smooth muscle tone in the bladder, allowing the bladder to retain larger volumes of urine and reducing the number of micturition, urgency and incontinence episodes[3,4]. A literature survey reveals one HPLC[5], one mass spectrometry[6] and one spectrophotometric method[7] for the assay of SFS.

But so far, there has not any report about the interaction study of SFS with DNA based on spectroscopic and viscometric behaviour. Among various analytical techniques, spectrofluorometry is one of the excellent methods to investigate the interaction of small molecular drugs with DNA for its convenience and high sensitivity. Thus, in this paper, we systematically investigated the interaction of SFS with calf thymus DNA (ctDNA) in combination with multi-spectroscopic and viscositic techniques under simulated physiological conditions (pH 7.4). The characteristics in spectroscopy measurements revealed that SFS could bind to ctDNA through intercalation binding modes. The results obtained from the viscosity experiments validated those conclusions. This work provides valuable information on the binding behavior of SFS with DNA and may be helpful for designing the alternative or even more active SFS.

EXPERIMENTAL

Materials and apparatus: All chemicals were of analytical or pharmaceutical grade and quartz processed high-purity water was used throughout. Pure SFS was obtained from Hetero Drugs limited, India. The stock solution (1×10^{-3} mol L^{-1}) of SFS was prepared in doubly distilled water. A solution of ctDNA (5×10^{-4} mol L^{-1}) was prepared by dissolving an appropriate amount of solid ctDNA (Sigma-Aldrich, India) and stored at 4^0C. Buffer solution was prepared by following the standard methods.

Absorption Measurements: The UV–vis spectra were recorded on a double beam Ellico UV-visible spectrophotometer (INDIA) in matched quartz cell of 1-cm path length by adding the increments of ctDNA stock solution into a fixed concentration of SFS. The solutions were allowed to incubate for 10 min before the absorption spectra were recorded.

Fluorescence Measurements: All fluorescence measurements were performed on a HITACHI F-4500 spectrofluorimeter equipped with a 150W Xenon lamp and a quartz cuvette of 1 cm path length. 2.0 mL pH 7.4 of Tris–HCl buffer solution, certain volume of drug and varying volume of ctDNA solution were transferred to a 10 mL volumetric flask, and diluted to the final volume with doubly distilled water. These solutions were allowed to stand for 8 min to equilibrate. The fluorescence emission spectra were measured at 287, 297 and 307 K in the wavelength range of 350–750 nm with an excitation wavelength at 256 nm.

Viscosity Measurements: Viscosity measurements were carried out by Ostwald viscometer, which was immersed in a thermostat water-bath at a constant temperature at 25 ± 0.1°C. Various concentrations of SFS were then added into the viscometer to give a certain r (r = [drug]/[DNA]) value while the ctDNA concentration was constant. The flow time of the samples were repeatedly measured by a digital stopwatch with an accuracy of $\pm$ 0.20 s after thermal equilibrium was achieved (15 min). The data were presented as η/η_0 versus r, where η and η_0 are the viscosity of ctDNA in presence and absence of SFS.

RESULTS AND DISCUSSION

Spectral characteristics of SFS binding to ctDNA: It is known that the intrinsic fluorescence of DNA is of little practical use[8], whereas the titled molecule displays luminescent property (λex/λem = 256/460 nm). Hence, the fluorescence emission spectra of SFS in the absence and presence of ctDNA were studied. As shown in Fig. 1, with the increasing amounts of ctDNA, the fluorescence intensity of SFS increases without apparent shift of λem, implying that the microenvironment around the chromophore of SFS is changed. The fluorescence intensity increases due to increase in the molecular planarity of the complex and decreases the collision frequency of solvent molecules with SFS, which indicated the binding of SFS to ctDNA indeed existed.
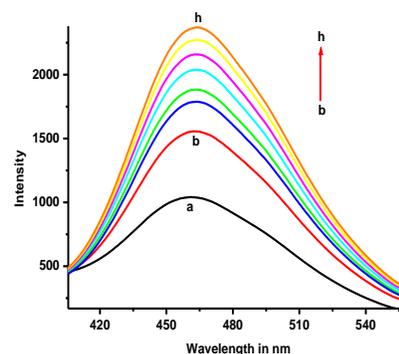
Fig. 1 Fluorescence spectra of SFS in the presence of ctDNA. C_{ctDNA} = 0, 5.0, 10.0, 15.0, 20.0, 25.0, 30.0 and 35.0 µML^{-1} for curves a – h; C_{SFS} = 1.5×10^{-4} M in pH-7.4

The formation of SFS-ctDNA complex was further confirmed by UV absorption spectra (Fig. 2). The UV absorption spectra of SFS showed an intense absorption band at 256 nm. It was apparent that as the concentration of ctDNA increased, the absorption peak at 256 nm increases.
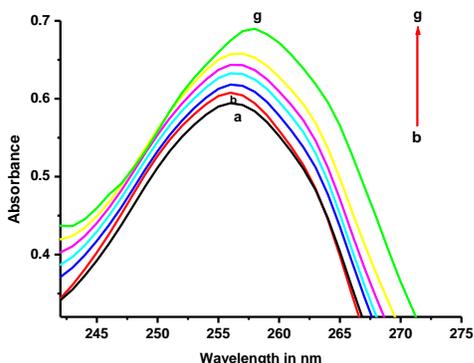
Fig. 2 Absorption spectra of SFS in presence of ctDNA at different concentrations (versus corresponding concentration of ctDNA) at pH = 7.4; Conditions: CSFS = 1 × 10−4 mol•L−1, from a to g: CctDNA = 0, 2.0, 4.0, 6.0, 8.0, 10.0 and 12.0 µML-1

The quenching mechanism of fluorescence of SFS by ctDNA :

The mechanisms of fluorescence quenching are usually classified as dynamic quenching and static quenching, which can be distinguished by examination of the temperature on the Stern-Volmer equation (Eq.(1):

$$\frac{F_0}{F} = 1 + K_q \tau_0 \ [DNA] = 1 + K_{SV} \ [DNA] \qquad 1$$

where, F_0 and F are the fluorescence intensities in the absence and presence of ctDNA, respectively, K_q is the quenching rate constant of biomolecular, τ_0 is the average lifetime of the fluorophore and K_{SV} is the Stern-Volmer quenching constant which can measure the fluorescence quenching efficiency. It was assumed that the interaction of SFS with ctDNA proceeded via a dynamic quenching. The K_{SV} value was obtained from the slope of the F_0/F versus [DNA] linear plot (Fig. 3A), and the values of K_{SV} and K_q at the three temperatures (287, 297 and 307 K) are listed in Table 1. As can be seen, the values of K_{SV} decreased with the increasing temperature, indicating that the fluorescence quenching of SFS by ctDNA was static[9].
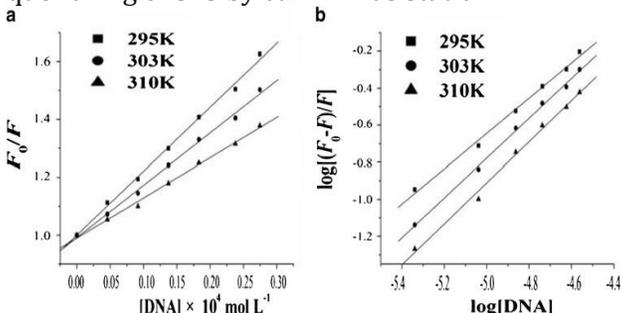
Fig. 3 (A) Stern-Volmer curves of ctDNA quenching the fluorescence of SFS. (B) Double logarithmic curves of ctDNA quenching the fluorescence of SFS

T (K)	Stern-Volmer equation	K_{SV}(L·mol−1)	K_q (L·mol−1·s−1)	R
287	Y = 1.0004 + 2.217 x 10⁴ [DNA]	7.282 x 10⁴	7.212 x 10¹²	0.9983
297	Y = 0.9913 + 1.801 x 10⁴ [DNA]	6.707 x 10⁴	6.815 x 10¹²	0.9981
307	Y = 0.9896 + 1.382 x 10⁴ [DNA]	6.361 x 10⁴	6.384 x 10¹²	0.9991

Table 1 Stern-Volmer quenching constants for the interaction of SFS with ctDNA at different temperatures

Binding constant and number of binding sites: For a static quenching interaction, it is often assumed that the binding capability of DNA at each binding site is equal[10]. The binding constant K and the number of binding site n can be calculated by using the double logarithm regression equation,

$$\log \left[\frac{(F_0 - F)}{F} \right] = \log K + n \log [DNA] \qquad 2$$

The values of K and n were obtained from the intercept and slope of the plot of log [(F₀-F)/F] versus log [DNA] (Fig. 3B). The corresponding results at different temperatures are summarized in Table 2. The value of n approximately equal to 1 indicated that there was just a single binding site between ctDNA and SFS. The increasing trend of K with temperature indicated that the capacity of SFS binding to DNA was enhanced with the temperature rising[11].

Thermodynamic parameters and nature of binding forces: To further characterize the interaction forces, the thermodynamic parameters which are the main evidence to determine the binding mode were analyzed. If the enthalpy changes (ΔH) does not vary significantly within the range of temperature, the thermodynamic parameters ΔH and ΔS can be estimated using van't Hoff plots[12]:

$$\ln K = - \frac{\Delta H}{RT} + \frac{\Delta S}{RT} \qquad 3$$

The free energy change (ΔG) was estimated from the Gibbs equation[13]

$$\Delta G = \Delta H - T\Delta S = - RT \ln K \qquad 4$$

The values of ΔH and ΔS were obtained from the slope (- ΔH/R, R is the gas constant) and intercept (ΔS/R). The values of ΔH, ΔS and ΔG at different temperature are given in Table 2. The negative values of ΔG revealed that the interaction processes was spontaneous, and the positive ΔH and ΔS values associated indicated that the hydrophobic interaction played a major role [14].

Effect of ionic strength on the spectrum of SFS-ctDNA : In order to prove the binding mode of SFS with ctDNA, the effect of different concentrations of NaCl (from 0 to 0.25 mol L-1) on the fluorescence of SFS-ctDNA system and free compound was investigated. It

represented that the fluorescence intensity was not dependent on ionic strength. When NaCl exists in the system, the electrostatic repulsion between the negatively charged phosphate skeletons on adjacent nucleotides is reduced with the increasing concentration of Na^+ [15]. Apparently, the groove bound molecules can be released from the helix by increasing the ionic strength, whereas it is difficult for the intercalation bound molecules to be released, owing that a small molecule binding in the groove of DNA duplex exposes much more to the solvent surrounding than it does for the intercalation[16]. As seen from Fig. 4, the results demonstrated that the effect of ionic strength on the SFS–ctDNA system was very limited, so the interaction between SFS and ctDNA was intercalative binding.

T (K)	K (L·mol⁻¹)	n	R	ΔG (kJ·mol⁻¹)	ΔH (kJ·mol⁻¹)	ΔS (J·mol⁻¹·K⁻¹)
287	2.227×10^5	0.956	0.9976	−23.646		133.8103
297	3.925×10^5	1.076	0.9985	−25.961	37.023	195.4603
307	4.379×10^5	1.125	0.9973	−27.975		170.2581

Table 2 The binding constants, number of binding sites and thermodynamic parameters of the interaction of SFS with ctDNA at different temperatures
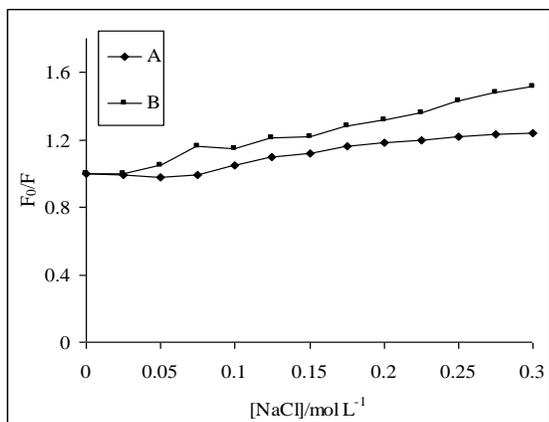
Fig. 4 Effect of ionic strength on the fluorescence intensity of SFS and SFS-ctDNA system. CSFS = 1x 10−4 mol•L−1; CctDNA = 9.1×10−6 mol•L−1; CNaCl = 0.01, 0.02, 0.03, 0.04, 0.05, 0.10, 0.15, 0.20, 0.25 mol•L−1

Iodide quenching studies : Fluorescence quenching is a reliable method to study the binding of small molecules to DNA. A highly negatively charged quencher such as I^- ion is expected to be repelled by the negatively charged phosphate backbone of DNA[17,18]. Therefore, the molecules buried by intercalating into the helix will be protected from being quenched by I^-, while the free aqueous complex and groove binding molecule should be quenched readily. Figure 5A depicts the Stern-Volmer plots of the free SFS and the SFS-ctDNA system in the presence of KI. It was obvious that the quenching effect for free SFS was stronger than

that of the bound SFS, suggesting that an intercalation binding should be the interaction mode of SFS with ctDNA[19].

Comparison of the interaction of SFS with dsDNA and ssDNA : The behaviors of native DNA and denatured DNA were compared. Double-strand DNA was converted into single strand DNA with the opening of its double helix by incubation at 100°C for 30 min followed by rapid cooling in ice water[20]. Generally, thermal denaturation involves the rupture of hydrogen bonds and no covalent bonds are expected to be broken. If the interaction between SFS and DNA belonged to a groove binding mode, the extent of the fluorescence quenching of SFS would be stronger by ssDNA than that by dsDNA[21]. The results of the comparison experiments are given in Fig. 5B. The fluorescence quenching of SFS by ssDNA was smaller than that by dsDNA, which also supported that SFS intercalated into the helix stack.
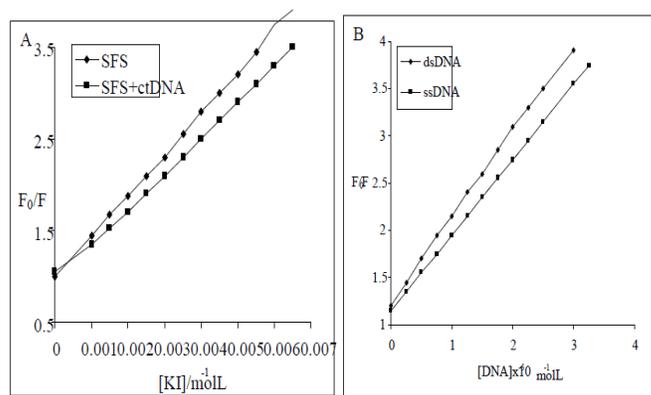
Fig. 5 A Fluorescence quenching plots of SFS by KI in the absence and presence of ctDNA. CctDNA = 9.2 x 10−6 mol.L−1, CSFS = 3.3 × 10−6 mol.L−1; B Effect of dsDNA and ssDNA on the SFS fluorescence intensity, CSFS = 3.3 x 10−6 mol•L−1

VISCOSITY MEASUREMENTS

Optical photophysical studies provide necessary but not sufficient clues to explain a binding between DNA and the complex, while hydrodynamic measurements that are sensitive to the length change are regarded as the least ambiguous tests of a binding model in solution[22]. Thus, viscosity measurements were carried out as an effective tool to further clarify the binding mode of SFS to ctDNA. An intercalator is generally known to cause a significant increase in the viscosity of a DNA solution due to lengthen the DNA helix as base pairs are separated to accommodate the binding ligand[23]. In contrast, a partial, non-classical ligand intercalation in grooves causes a bend in DNA helix reducing its effective length and thereby its viscosity[24]. As illustrated in Fig. 6, the relative viscosities of the ctDNA increased steadily upon the increasing concentrations of SFS. Such behavior further confirmed that SFS bound to DNA through an intercalative binding mode.
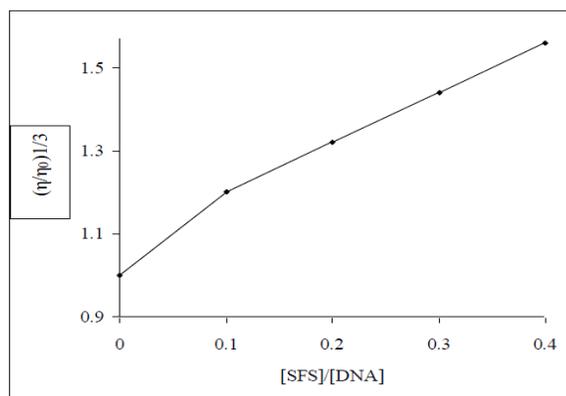
Fig. 6 Effect of increasing amount of SFS on the relatively viscosity of ctDNA

CONCLUSION

The interaction between SFS and ctDNA was studied by spectral analysis and viscosity methods. The spectral changes in UV and fluorescence spectroscopy showed that SFS could interact with ctDNA through static quenching mechanism. The binding constants and the number of binding sites of SFS binding to ctDNA were measured at different temperatures and the thermodynamic parameters were calculated as well. It was found that hydrophobic force played a significant role in the binding. The intercalative binding was much more reasonable by taking account of ionic strength effects, tests for the ability of SFS binding with dsDNA and ssDNA, KI quenching studies, and the viscosity measurements. These combinations of spectroscopic and viscometric methods were expected to provide important insight into the interactions of the physiologically important DNA with SFS congeners.

ACKNOWLEDGMENT

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