Original Article

SPECTRAL STUDIES OF THE INTERACTION BETWEEN SOLIFENACIN SUCCINATE AND HERRING SPERM DNA

BABU G GOWDA*, MALLAPPA M

**Department of Chemistry, Maharani's Science College for Women, Bangalore 560001, India.
Email: babgowda@gmail.com**

ABSTRACT

Objective: The interaction between solifenacin succinate (SFS) and herring sperm DNA (Hs-DNA) was investigated by using UV–vis and fluorescence spectra at pH 7.4.

Methods: The UV-vis and fluorimetric titrations were performed by keeping the concentration of SFS constant and varying the concentration of Hs-DNA in a buffer solution of pH 7.4. The absorption spectra shows an increase in absorbance at an absorption band of SFS with increasing concentration of Hs-DNA. A quenching of intrinsic fluorescence of SFS was observed with the increasing of Hs-DNA concentration.

Results: The fluorescence lifetime results, the varieties of the fluorophore absorption spectra and the decrease of the binding constant with the increasing temperature all indicate that the fluorescence quenching is static. The ratio and constant of the binding Hs-DNA to SFS are 1 and 6.76×10^5, respectively. The values of ΔH, ΔS and ΔG obtained for SFS-Hs-DNA were - 9.93 kJ/mol, 0.17 kJ/(mol K) and - 44.78 kJ/mol respectively at 298 K.

Conclusions: The absorption spectra and fluorescence quenching spectra of SFS in presence of Hs-DNA strongly supports the interaction of SFS with Hs-DNA. SFS binds to Hs-DNA with a binding constant of 6.76×10^5 at 298 K. In addition, thermodynamic data for the SFS binding to Hs-DNA showed that the binding of SFS to Hs-DNA is not only exothermic but entropy-driven.

Keywords: Solifenacin succinate, Herring sperm DNA, Spectral studies, Interaction.

INTRODUCTION

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

Solifenacin succinate is a competitive muscarinic acetylcholine receptor antagonist. The binding of acetylcholine to these receptors, particularly the M_3 receptor subtype, 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 spectrophotometeric method [7] for the assay of SFS. But so far, there has not any report about the interactive study of SFS with DNA based on spectroscopic behaviour.

A variety of analytical techniques have been employed to investigate the interaction of small molecular drug with DNA. Spectrofluorimetry is one of the excellent methods for its convenience and high sensitivity. Thus, in this work, efforts are being made to study the interaction of SFS with Hs-DNA at physiological conditions (pH 7.4) by using UV-vis and fluorescence spectra. The UV–vis spectra was recorded on a double beam Ellico UV-visible spectrophotometer (INDIA) in matched quartz cell of 1-cm path length. The fluorescence measurements were carried out on a HITACHI F-4500 spectrofluorimeter equipped with a 150W Xenon lamp and 1-cm quartz cell. The titrations were performed by keeping the concentration of SFS constant and varying the concentration of Hs-DNA. The pH measurements were made with Scott Gerate pH meter CG 804. An electronic thermostat water-bath was used for controlling the temperature.

Hs-DNA with a purity of > 98 % was purchased from Sigma Aldrich (India). SFS with a purity of > 99% was kindly supplied by Hetero Drugs limited, Hyderabad, India. They were used without further purification. The solutions were stored at 4℃ before being used. A buffer solution of pH 7.4 was prepared by following the standard methods. Analytical grade reagents and double distilled water were used in all experiments.

Interaction between SFS and Hs-DNA has been characterized classically by UV-vis absorption spectra. The effect of progressively increasing concentration of Hs-DNA on the absorption spectrum of SFS is shown in Fig. 1. The absorption spectra of SFS present one peak at 256 nm in the range of 200 - 400 nm. The absorption spectra show an increase of peak intensity about 26.4 % and a small red shift about 10 nm at an absorption band of SFS with increasing concentration of Hs-DNA. The hypochromicity and bathochromicity of an absorption band are due to the effective interaction between SFS with Hs-DNA. The varieties of absorption spectra are due to the effective interaction between SFS with Hs-DNA. A strong fluorescence emission spectrum of SFS at 460 nm was observed in the range of 350–550 nm after excitation at 256 nm. The fluorescence emission spectra of SFS increased with increasing concentration of Hs-DNA (Fig. 2). A quenching of intrinsic fluorescence of SFS was observed with the increasing concentration of Hs-DNA, but not altering the emission maximum and shape of the peak. These results show that there are the binding between SFS and Hs-DNA. The fluorescence intensity tends to be constant at a high concentration of Hs-DNA, which shows the binding of SFS to Hs-DNA reached saturation.
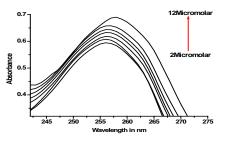

Fig. 1: Absorption spectral changes of SFS (1×10^{-4} mol/L) with increasing concentration of Hs-DNA (from down to up 2 – 12 μ mol/L) in the buffer of pH 7.4.
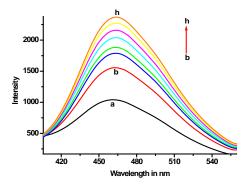
Fig. 2: Fluorescence emission spectra of SFS (1.5 x 10^{-5} mol/L) with increasing concentration of Hs-DNA (from down to up 5 - 35 µ mol/L) in pH 7.4 buffer.

The varieties of the fluorescence excitation spectra of SFS caused by gradually increasing the concentration of Hs-DNA were shown in Fig. 2. Fluorescence relative intensities of SFS has a gradual increase with the increasing concentration of Hs-DNA, which indicates that binding formed by SFS and Hs-DNA. This phenomenon also proves that the fluorescence quenching is static.

The binding constants of SFS with Hs-DNA were calculated from the data of fluorescence spectra. The binding constant K and the binding number n are calculated by the following equation (1) [8]:

$$M + nQ \leftrightarrow MQ_n \qquad \text{....(1)}$$

Where Q is the quencher known as Hs-DNA, M the SFS, and MQ_n is the binding of them.

$$K = \frac{[MQ_n]}{[M][Q]^n} \qquad \text{....(2)}$$

Where K is the intrinsic binding constant, and n is the binding number of Hs-DNA to SFS. [M] is the concentration of free SFS, and $[MQ_n]$ is the concentration of binding. If $[M]_0$ is the total concentration of SFS.

$$[M]_0 = [M] + [MQ_n] \qquad \text{....(3)}$$

When $[Q] [M]$, the concentration of free quencher is replaced by the initial concentration.

$$\frac{[M]_0 - [M]}{[M]} = \frac{[MQ_n]}{[M]} = K[Q]^n \qquad \text{..... (4)}$$

In static quenching, according to above equation, the following formula can be obtained:

$$\frac{F_0}{F} = 1 + K[Q]^n \qquad \text{.... (5)}$$

Stern-Volmer plot of F_0/F to [Q] is shown in Fig.3. By linear analysis from the data, a binding constant K is calculated out as 6.76 x 10^5 at 298 K. Binding parameters of SFS-Hs-DNA binding at different temperatures were shown in Table 1.
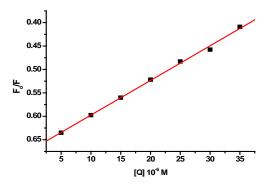

Fig. 3: Stern-Volmer plot of (F$_0$ / F) *vs* [Q] for SFS-Hs-DNA system
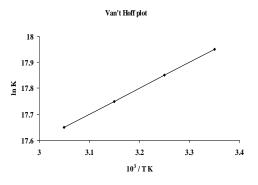

Fig. 4: Van't Hoff plot of ln *K* vs 1/T for SFS-Hs-DNA system

Fluorescence measurements of SFS-Hs-DNA binding were performed at different temperatures. Thermodynamic parameters were estimated by the analysis of ln *K vs* 1/T plot (Van't Hoff plot) obtained by the experimental data at different temperatures (Fig. 4). The gradient of this straight line of ln *K vs* 1/T is equal to ΔH/R which indicates the value of ΔH, ΔG and ΔS can be calculated from the following relationships:

$$\Delta G = - RT \ln K \qquad \text{....(6)}$$

$$\Delta G = - \Delta H - T\Delta S \qquad \text{....(7)}$$

The Van't Hoff plot for the binding of SFS to Hs-DNA is depicted in Fig. 4. The values of thermodynamic parameters are shown in Table 1. It can be seen that the SFS binding to Hs-DNA is characterized by negative enthalpy and positive entropy changes. It is known that the occurrence of reaction requires a negative ΔG, and since enthalpy is negative, the interaction of SFS with Hs-DNA should be entropy-driven. In addition, positive enthalpy of the reaction between SFS and Hs-DNA hints the increase temperature at an exothermic process, therefore, the increase of temperature is in favor of the reverse direction of the reaction (Eq. 2), resulting in smaller values of binding constant K (or quenching constant) with increasing temperature. Decreasing of the binding constant with increasing temperature also suggests that the fluorescence quenching is static. At the same time, from the viewpoint of molecular stability, the reduced stability of bindings with increasing temperature can also decrease the *K* value.

Table 1: Thermodynamic parameters for the SFS-Hs-DNA complexation obtained from fluorescence measurements in buffer solution of pH 7.4.

Temperature T in K	Binding Constant K (x 10^5)	ΔG (kJ/mol)	ΔH (kJ/mol)	ΔS (kJ/(molK))
288	1.44	- 43.57	- 9.93	0.17
298	6.76	- 44.78	- 9.93	0.17
308	4.03	- 45.90	- 9.93	0.17
318	2.21	- 48.83	- 9.93	0.17

CONCLUSION

The binding of SFS to Hs-DNA results in hypochromism and bathochromism in absorption spectra and fluorescence quenching in fluorescence emission spectra. These spectra features strongly support the interactions of SFS with Hs-DNA. SFS binds to Hs-DNA with a binding constant of 6.76×10^5 at 298 K. In addition, thermodynamic data for the SFS binding to Hs-DNA were also calculated and derived from experimental measurements, with the results of $\Delta H = -9.93$ kJ/mol, $\Delta G = -44.78$ kJ/mol and $\Delta S = 0.17$ kJ/(mol K) at 298 K. These results show that the binding of SFS to Hs-DNA is not only exothermic but entropy-driven.

CONFLICT OF INTERESTS

Declared None.

ACKNOWLEDGEMENTS

REFERENCES

1. Li SY, Ren YZ, Zhao FL. Chinese Chemical Letters 2006;17(8):1065-68.
2. Maiti M, Kumar GS. Molecular aspects on the interaction of protoberberine, benzophenanthridine, and aristolochia group of alkaloids with nucleic acid structures and biological perspectives. Medicinal Res Rev 2007;27(5):649-95.
3. Cardozo L, Lisec M, Millard R, *et al*. Randomized. Double-blind placebo controlled trial of the once daily antimuscarinic agent solifenacin succinate in patients with overactive bladder. J Urology 2004;172(5 Pt 1):1919-24.
4. Garely AD, Kaufman JM, Sand PK. Symptom bother and health-related quality of life outcomes following solifenacin treatment for overactive bladder: Clinical Therapeutic 2006;28:1935-46.
5. Yanagihara T, Aoki T, Soeishi Y, Iwatsubo T, Kamimura H. Determination of solifenacin succinate, a novel muscarinic receptor antagonist, and its major metabolite in rat plasma by semi-micro high performance liquid chromatography, J Chromatography B: Analytical Technologies in the Biomedical and Life Sciences 2007;859 (2):241-5.
6. Mistri HN, Jangid AG, Pudage A, Rathod DM, Shrivastav PS. Highly sensitive and rapid LC-ESI-MS/MS method for the simultaneous quantification of uroselective α1-blocker, alfuzosin and an antimuscarinic agent, solifenacin in human plasma. J Chromatography B: Analytical Technologies in the Biomedical and Life Sciences 2008;876(2):236-44.
7. Singh L, Nanda S. Spectrophotometric estimation of Solifenacin succinate in tablet formulations. Pharmaceutical Methods 2011;2(1):21-4.
8. Xu JG, Wang ZB. Fluorimetry, third edition. Science Press: Beijing; 2006.