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Research Article

THERMODYNAMIC STUDIES ON THE INTERACTION OF 5-FLUOROURACIL WITH HUMAN SERUM ALBUMIN

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ABSTRACT

The interaction of 5-Fluorouracil with human serum albumin have been studied. The mechanism of binding of 5-Fluorouracil (5-FU) with Human serum albumin (HSA) was investigated by fluorescence, absorption and lifetime measurements. The analysis of fluorescence data indicated the presence of static quenching mechanism in the binding. Various binding parameters have been evaluated. The thermodynamic parameters, enthalpy change (ΔH), and entropy change (ΔS) were also calculated according to fluorescence data. The results show that hydrophobic interaction is a predominant intermolecular force for stabilizing the complex.

INTRODUCTION

Protein-drug interaction plays an important role in pharmacokinetics of the drugs. This interaction can take place with a variety of agents in blood, including proteins such as human serum albumin (HSA). HSA has been used as a model protein for many years. It is the most abundant protein in blood plasma, which constitutes up to 60% of the total protein corresponding to a concentration of 42 gL^{-1} [1], and provides about 80% of the osmotic pressure of blood [2]. HSA plays an important role in the transport and deposition of a wide range of endogenous and exogenous substances, such as fatty acids, amino acids, hormones, dyes and many diverse drugs [3,4]. Because of clinical and pharmaceutical importance, a great deal of attention has been paid to the interaction of HSA with natural or synthetic drugs, including the study of binding constants and binding sites [5-7]. The measurement of the binding parameters of a drug bound to HSA is of utmost importance for drug discovery and preclinical studies of drug candidates in pharmaceutical research [8].

5-Fluorouracil (5-FU) (first introduced into medicine in 1957) is widely recognized today as effective treatment modalities, especially with tumours of the head, neck and breast, and in combination drug regimens for cancer chemotherapy. It has been widely used in the treatment of cancer and it exhibits also an antibacterial activity[9], and augments the bactericidal effect of antibiotics [10,11]. The molecular structure of 5-FU is shown in Fig. 1.

In this paper, the interaction between 5-FU and HSA at two different temperatures was studied under physiological conditions by steady-state fluorescence, UV/vis, and time resolved fluorescence spectroscopies. The binding constants, the type of interaction force, and the specific binding site were investigated in this paper.

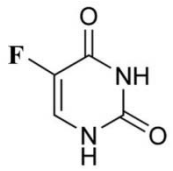

Fig. 1: Molecular structure of 5-Fluorouracil

MATERIALS AND METHODS

Materials

HSA and 5FU were obtained from Sigma Aldrich, Bangalore and used directly. Double –distilled water was used in all the experiments.

Methods

The absorption spectra were recorded on **Perkin Elmer lamda 25 uv-visible spectrometer.** Steady state fluorescence quenching measurements were carried out in a **Varian Carry Eclipse Fluorescence Spectrophotometer.** The excitation wavelength was 280nm and the emission wavelength was monitored at 335 nm. The excitation and emission slit widths (5 nm) were maintained constant for all the experiments. Fluorescence lifetime measurements were carried out in a **Horiba-Jobin Yvon [Spex-Sf13-11] Spectrofluorimeter.** The fluorescence decay of BSA was measured at 335 nm with a monochromator photomultiplier setup.

RESULTS AND DISCUSSION

Fluorescence Quenching Study

The intensity of fluorescence can be decreased by a wide variety of processes. Such decreases in intensity are called quenching. In order to investigate the binding of 5FU to HSA, the fluorescence emission spectra of HSA in the presence of various concentrations of 5FU were recorded upon excitation at 280 nm[12]. It can be seen from Fig. 2 and 3 that the fluorescence intensity of HSA decreased regularly with the increasing concentrations of 5FU without changing the emission wavelength and shape of the peaks at two different temperatures (25 and 30^{0}C). Under the same condition, no fluorescence of 5FU was observed. These results indicated that the binding of 5FU to HSA quenches the intrinsic fluorescence of the single tryptophan in HSA (Trp-214).

Fluorescence quenching can occur by different mechanisms, usually classified as either dynamic or static quenching, which can be distinguished by their differing dependence on temperature and viscosity [13]. Since higher temperatures result in large diffusion coefficients for dynamic quenching, and the quenching constants is expected to increase with increasing temperature. In contrast, a higher temperature may bring about the decrease in the stability of the complexes, resulting in lower quenching constant for the static quenching. The Stern-Volmer equations is often applied to elucidate the quenching mechanism [14].

$$F_0/F = 1 + K_{sv}[Q] = 1 + K_q\tau_0[Q] \quad ...(1)$$

Where, F_0 and F are the fluorescence intensities in the absence and presence of quencher, respectively. K_q is the bimolecular quenching constant τ_0 is the lifetime of the fluorophore in the absence of quencher and the fluorescence lifetime of the biopolymer is 10^{-8}s [15]; K_{sv} is the Stern-Volmer quenching constant, and [Q] is the concentration of quencher. The Stern-Volmer plots of F_0/F versus [Q] at different temperatures were presented in Fig. 4 Shows that K_{sv} values were inversely correlated with temperatures, which suggested that the fluorescence quenching of HSA was initiated by the formation of ground-state complex. Furthermore, K_q values were much greater than the maximum scatter collision quenching constant (2.0×10^{10} $M^{-1}s^{-1}$) for various quenchers with biomolecule[16], so it was implied that the static quenching was dominant in drug-HSA interaction.

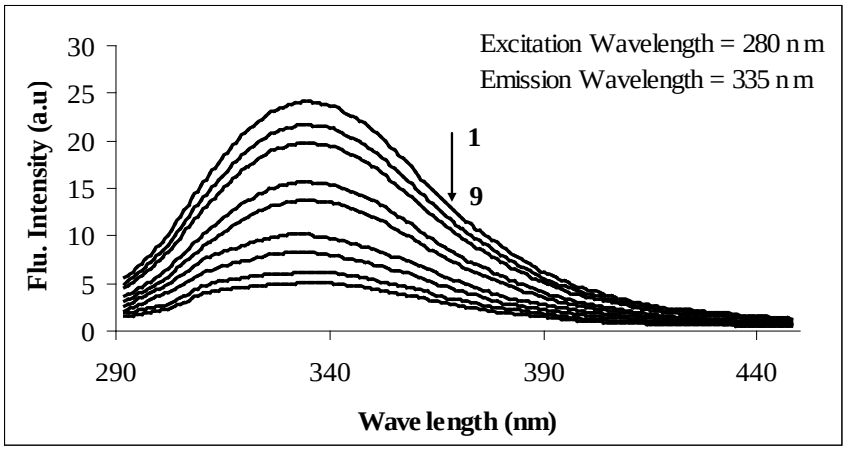

Fig. 2: Steady-state fluorescence spectra of HSA in different concentrations of 5-FU (mol dm^{-3}) in water at 25^{0}C (1) 0, (2) 1, (3) 2, (4) 3, (5) 4, (6) 5, (7) 6, (8) 7, 9) 8

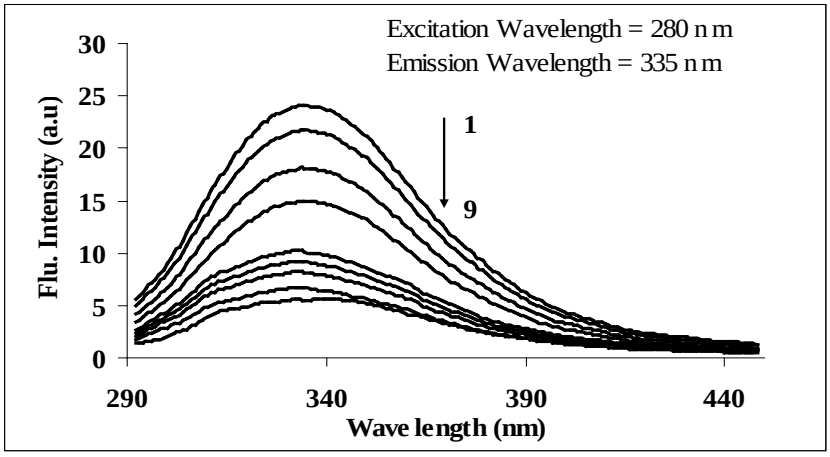

Fig. 3: Steady-state fluorescence spectra of HSA in different concentrations of 5-FU (mol dm^{-3}) in water at 30^{0}C (1) 0, (2) 1, (3) 2, (4) 3, (5) 4, (6) 5, (7) 6, (8) 7, 9) 8

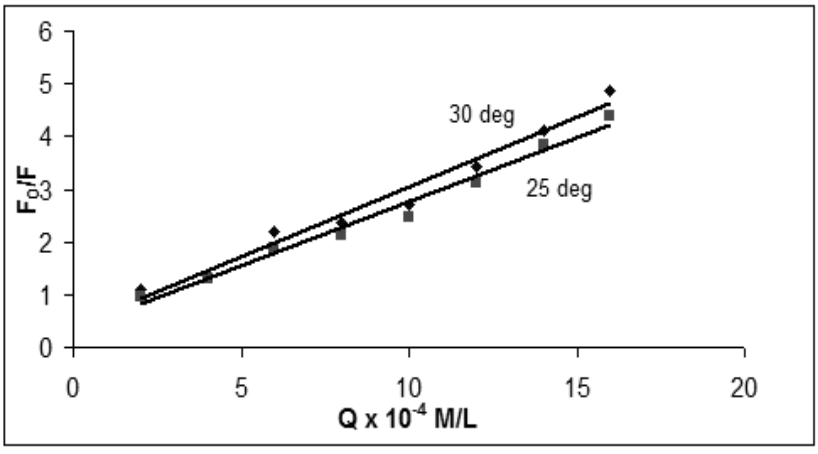

Fig. 4: Stern-Volmer plot for HSA with 5-FU at different temperatues

Binding Parameters

The binding constant (K_a) and the number of binding sites (n) can be calculated using the equation shown below:

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

A plot of log $[(F_0-F)/F]$ *versus* log [Q] gives a straight line (Fig. 5), whose slope equals to log K_a. The values of K_a and n at 25 and 30^{0}C are listed in the Table 1. The results indicate that there is one class of binding site for drug in HSA. The fact that the binding constant between drugs and HSA increased with increasing temperature suggested that there was strong interaction between drugs and HSA[17]. This clearly implied that drug (5-FU) should be bound, stored and transported by HSA in the body.

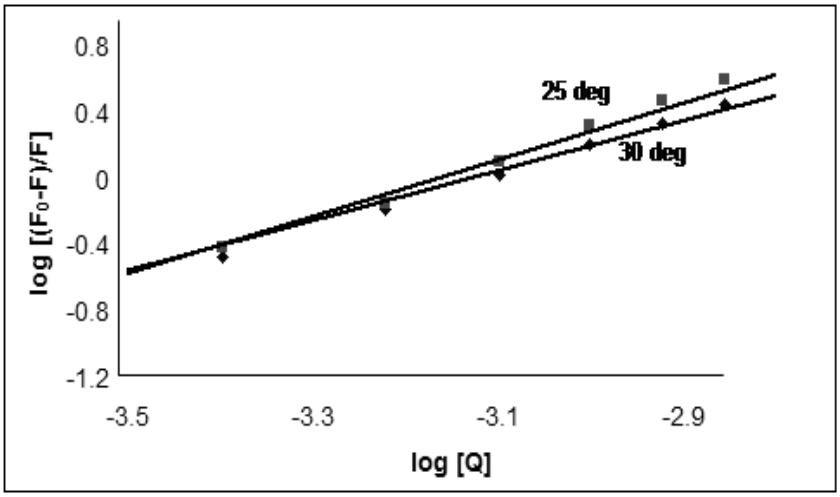

Fig. 5: Double log plot for HSA with 5-FU at different temperatures

Table 1: Stern-Vomer constant(K_{sv}), Binding constant(K_a) and Bimolecular quecking constant(K_q) of HSA at two different temperatures

T(^{0}C)	K_{sv}(x10^{3}M^{-1})	K_q(x10^{11}M^{-1}s^{-1})	K_a(x10^{3}M^{-1})	R^{2a}	SD^b	n
25	2.632	9.552	0.630	0.974	1.306	1.0
30	1.313	4.765	0.794	0.979	1.433	1.0

[a] → Regression co-efficient

[b] → Standard Deviation

Time-resolved Fluorescence study

We also used the lifetime measurements for the further support to prove the quenching mechanism is static. Fig. 6 shows the fluorescence decay of human serum albumin in the presence and absence of 5-FU. HSA exhibit double exponential decay even in the presence of 5-FU.

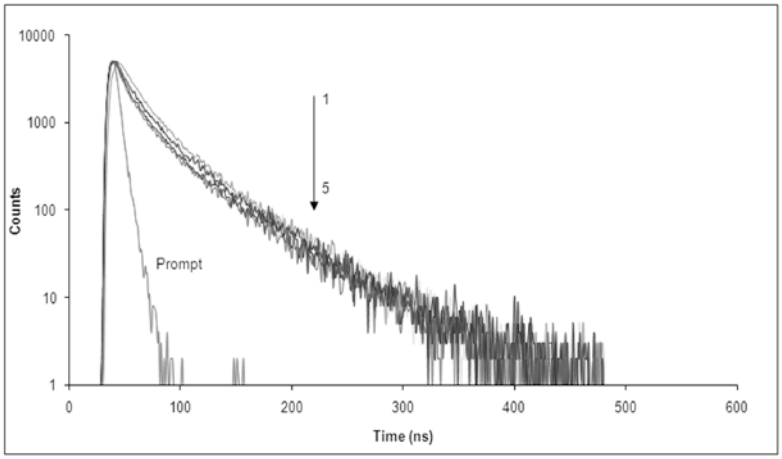

Fig. 6: Time-resolved fluorescence spectra of HSA in different concentrations of 5-FU (mol dm^{-3}) in water (1) 0, (2) 2, (3) 4, (4) 6, (5) 8

The average lifetime < τ > was calculated by following relation

$$< \tau > = \frac{\alpha_1 \tau_1^2 + \alpha_2 \tau_2^2}{\alpha_1 \tau_1 + \alpha_2 \tau_2} \quad ...(3)$$

Where, τ_1 and τ_2 are the decay times and α_1 and α_2 are the pre exponential factor. The time resolved fluorescence spectral data is shown in Table 2.

Table 2: Time-resolved fluorescence spectral data

Concentration of 5FU (M)	Life time		Relative amplitudes		χ^2	Average Life time
	τ_1(ns)	τ_2(ns)	B_1	B_2		< τ >(ns)
0	1.37	4.27	52.22	47.78	1.27	2.76
4x10^{-4}	1.40	4.38	58.72	41.28	1.07	2.62
8x10^{-4}	1.31	4.25	66.73	33.27	1.23	2.29
1.2x10^{-5}	1.67	4.02	67.57	32.43	1.08	2.09
1.6x10^{-5}	0.98	0.04	63.44	36.56	1.22	2.03

Thermodynamic parameter and nature of the binding forces

The interaction forces between drug and bio molecule may involve hydrophobic force, electrostatic interactions, Van der Waals interactions, hydrogen bonds, etc. In order to elucidate the interaction of 5FU with HSA, we the thermodynamic parameters were from Eqs. calculated (4) – (6). If the temperature does not vary significantly, the enthalpy change ΔH can be regarded as a constant. The free energy change ΔG can be estimated from the following equation based on the binding constants at different temperatures.

$\Delta G = - RT \ln K$ ...(4)

Where, R is the gas constant, T is the experimental temperature, and K is the binding constant at the corresponding T. Then, the enthalpy change (ΔH) and entropy change (ΔS) can be calculated from the following equations.

$\ln K_2/K_1 = [1/T_1 - 1/T_2]\Delta H/R$ ...(5)

$\Delta G = \Delta H - T\Delta S$ ...(6)

Table 3: The thermodynamic parameters for the interaction of 5FU with HSA

T(^{0}C)	ΔH(KJ mol^{-1})	ΔG(KJ mol^{-1})	ΔS(J mol^{-1}K^{-1})
25	34.742	-15.969	170.172
30		-16.821	

The negative sign for ΔG means that the interaction process is spontaneous. The positive ΔH and ΔS values indicate that the binding between 5FU and HSA is mainly ΔS -driven, with little contribution from the enthalpy factor, and hydrophobic force may play a major role in the reaction.

CONCLUSION

The interaction of 5-FU with HSA under the physiological condition was studied by fluorescence UV-vis and fluorescence lifetime spectra. Experimental results showed that 5-FU quenched the fluorescence of HSA through static quenching mechanism. The enthalpy change (ΔH) and entropy change (ΔS) were found to be 34.742 KJ mol^{-1} and 170.172 J mol^{-1}K^{-1}, indicating that hydrophobic and electrostatic interactions played major roles in stabilizing the complex. Determination of binding constants and binding sites can reveal the molecular basis of this versatile transporter protein, and is helpful for clarifying the structural and functional relationship of HSA.

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