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Fluorescence Spectroscopic Study of Interaction between Olanzapine and Bovine Serum Albumin

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Abstract

The binding capacity of an antipsychotic drug, olanzapine with bovine serum albumin (BSA) was studied. The experiment was designed to investigate the interaction between olanzapine and BSA using fluorescence spectroscopy at different temperatures (298 K and 308 K). Fluorescence quenching constant was determined from Stern-Volmer equation. Van't Hoff equation was used to determine the thermodynamic parameters such as free energy (ΔG), enthalpy (ΔH) and entropy (ΔS). A strong quenching was observed in the fluorescence spectrum. The quantitative analysis revealed that olanzapine bound with BSA via a dynamic quenching through hydrophobic interactions, where binding constant K_b at 280 nm was $10.28x10^4$ μM^{-1} and $10.739x10^4$ μM^{-1} at 298 and 308 K, whereas it was $19.31x10^4$ μM^{-1} and $18.923x10^4$ μM^{-1} when the study was conducted at 293 nm, respectively. The number of bound olanzapine molecules per BSA protein was ~0.5 at both the temperatures. The K_b value in different temperatures suggested that the stability of BSA-olanzapine complex increased with the increase of temperature at 280 nm but reversed effect was observed in excitation wavelength of 293 nm. Positive ΔH_o and ΔS_o were the distinctive characteristics that allowed us to suggest that the interaction was mostly hydrophobic in nature.

Keywords: Olanzapine; Bovine serum albumin; Interaction; Fluorescence spectroscopy; Fluorescence quenching

Introduction

Plasma protein binding is an important factor to understand the pharmacokinetics and pharmacodynamics properties of drugs, as it strongly influences drug distribution and determines the free fraction, which is available to the target [1]. Much of the clinical and pharmaceutical interests on protein binding is due to its effects for drug pharmacokinetics [2]. Human Serum albumin (HSA) is the most abundant protein in the circulatory system, comprising 60% of plasma [3]. It is synthesized in the liver, exported as a non-glycosylated protein and is present in the blood at around 40 $mgmL^{-1}$ (~0.6mM) [3]. Bovine serum albumin (BSA) and human serum albumin (HSA) display approximately 76% sequence homology and the 3D structure of BSA is believed to be similar to that of HAS [4]. Serum albumin binds and transports many ligands including fatty acids, amino acids, hormones, cations, anions and variety of pharmaceuticals. It is suggested that the principal regions of ligand binding to HSA are located in hydrophobic cavities in the sub domains IIA and IIIA, which are consistent with sites I and II, respectively and single tryptophan residue of HSA is in sub domain IIA [5,6]. Protein–drug binding greatly influences absorption, distribution, metabolism and excretion properties of typical drugs [7,8]. Thus, it is important and necessary to study the interaction of drug with serum albumins at molecular level.

Antipsychotic drugs are the cornerstone of treatment for schizophrenia but have limited effectiveness [9]. Olanzapine is an atypical antipsychotic, antimanic and mood stabilizing agent that demonstrates a broad pharmacological profile across a number of receptor system (Figure 1). It has been proved that in the treatment of schizophrenia olanzapine is more effective than quetiapine and ziprasidone and treatment of schizophrenia also encourage the use of atypical antipsychotic medications [9].

In this paper, we described the study of interaction of olanzapine with bovine serum albumin (BSA). The details of the binding modes of olanzapine to BSA were studied through the binding strength, position and mode of binding of protein using fluorescence spectroscopy.

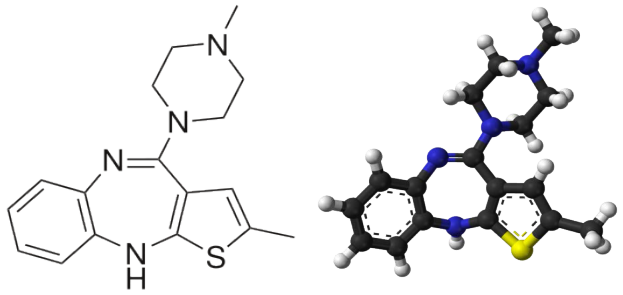

Figure 1: A. Structure of olanzapine, **B.** 3D structure of olanzapine.

Materials and Methods

Chemicals and reagents

All starting materials were of reagent grade and double distilled water was used throughout the study. BSA was purchased from Sigma Chemical Company; St Louis, USA, with molecular weight of

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68,000 and used without further purification. BSA solution ($2x10^{-5}$ M) was prepared in 0.1M phosphate buffer (pH 7.40) and kept in 4°C. Olanzapine standard was (potency: > 99.98 %) obtained from Incepta Pharmaceutical Ltd., Bangladesh. Stock solution ($1.0x10^{-3}$ M) of olanzapine was prepared in pH 7.40 buffer. Due to lack of complete solubility, olanzapine was first dissolved in DMSO and the volume adjusted by phosphate buffer (pH 7.40). Buffer solution was prepared by mixing Na_2HPO_4 (20 mM) and NaH_2PO_4 (30 mM).

Apparatus

Fluorescence emission spectra were recorded with a Hitachi FL-7000 (Tokyo, Japan) fluorescence spectrometer equipped with 1 cm quartz cell. Observations was taken at two different temperatures (298- and 308 K) using 5/5 nm slit width. For adjusting different temperatures a thermostatic bath (Unitronic Orbital, Spain) was used. The temperatures of the samples were maintained by recycling water throughout the experiment. The pH was measured with Hanna HI 2210 pH meter, USA.

Data measurement and analysis

Statistical analysis and all other measurement were calculated by Microsoft Office Excel 2007 program. SD (Standard deviation) value was calculated statistically from three replicate runs for each sample.

Sample preparation and observation

Stock solutions of 1000 μM of olanzapine and BSA were prepared by phosphate buffer at pH 7.40. Appropriate volumes of BSA stock solution were diluted to get 20 μM, 40 μM, 80 μM, 120 μM, 180 μM, 220 μM and 320 μM test concentrations. Fluorescence spectra were recorded at 298 and 308 K in the range 250-600 nm upon excitation at 280 and 293 nm for BSA molecule considering tryptophan and tyrosine residue [10]. The solutions of BSA and olanzapine were mixed well by sonication and incubated at 298- and 308 K for 30 minutes before the analysis. Phosphate buffer (pH 7.40) was used as blank for pre-scan purpose.

Results and Discussion

Fluorescence quenching is the decrease of the quantum yield of fluorescence from a fluorophore induced by a variety of molecular interaction with quencher molecule [11].Generally, the fluorescence of bovine serum albumin comes from tryptophan, tyrosine and phenylalanine residues. During data recording at excitation wavelength of 280 nm, fluorescence of albumin came from both tryptophan and tyrosine residues, whereas 293 nm wavelength excited the tryptophan residues only [7].

Analysis of fluorescence quenching of BSA by olanzapine

The fluorescence measurement can give some information on the binding of small molecules substance to protein, such as the binding mechanism, binding mode, binding constants, binding sites and the intermolecular distance.

Figure 2 illustrates the emission spectra of BSA in the presence of olanzapine at various concentrations. The fluorescence emission wavelength of BSA was about 350 nm, which was characteristic of partial shielding of the tryptophan residues from aqueous solvent [12]. It was apparent that the fluorescence intensity of BSA decreased regularly with the increase in olanzapine concentration of microenvironment, implying that the binding of olanzapine to BSA occurred and the around fluorophore of BSA has been changed or blocked by available amount of drugs.

From figure 2, the fluorescence emission wavelengths of BSA showed obviously blue shifts after the addition of the drug, which indicated that the tryptophan and tyrosine residues in protein are located in a more hydrophobic environment [13].

The fluorescence quenching data were analyzed by the well-known Stern-Volmer equation from equation 1.

$$\frac{Fo}{F} = 1+Kq\tau_o[Q] = 1 + K_{SV}[Q] \quad (1)$$

Here, where F_o and F represent the fluorescence intensities in the absence and presence of the quencher, respectively. [Q] is the concentration of the quencher (olanzapine), K_q is the quenching rate constant of the bio-molecule, K_{SV} is the Stern-Volmer quenching constant, and τ_o (10^{-8} s) is the average lifetime of the fluorescent substance without any quencher [14].

Hence, equation (1) can be used to determine K_{SV} by linear regression of a plot of F_o/F against [Q] and Kq ($Kq = K_{SV}/\tau_o$) (Figure 3).

The concentration of quencher [Q] olanzapine. τ_o and τ are the average lifetime of the bimolecular without and with the quencher. Figure 3 shows the Stern-Volmer plots at different temperatures. It can be found that the Stern-Volmer plots are linear and the slopes increase with increase in temperature. The value for K_{SV}, K_q and R^2 at different temperatures are given in Table 1.

As Stern-Volmer quenching constant (K_{SV}) decrease with the increase in temperature for static quenching, while for the dynamic quenching reverse effect was observed [14]. The results showed that K_{SV} was proportionally co-related with temperature increase, suggested that the fluorescence quenching process may be mainly controlled by a dynamic quenching mechanism rather than a static quenching mechanism.

Analysis of binding parameter

When small molecules bind independently to a set of equivalent sites on a macromolecule, the equilibrium between free and bound molecules is given by the equation from equation 2 [15].

$$Log[(F_o-F)/F] = logK_b + nlog[Q] \quad (2)$$

Where, K_b and n are the binding constant and number of binding sites, respectively. Table 2 shows that the values of K_b and n increase with increasing in temperature at 280 nm excitation wavelength (Figure 4), which may indicate that there is molecular binding of olanzapine

λ_{ex}	pH	T (K)	K_{SV} (x 10^3 $Lmol^{-1}$)	K_q (x 10^{11} $Lmol^{-1}$ S^{-1})	SD[a]	R^{2}[b]
280 nm	7.40	298	9.10	9.10	0.0055	0.995
		308	11.20	11.20	0.0097	0.993
293 nm	7.40	298	9.13	9.13	0.0057	0.995
		308	10.21	10.21	0.0068	0.992

(a) SD is the standard deviation; (b) R^2 is the correlation co-efficient.

Table 1: Stern-Volmer quenching constants of BSA-olanzapine system at different temperatures (pH=7.40).

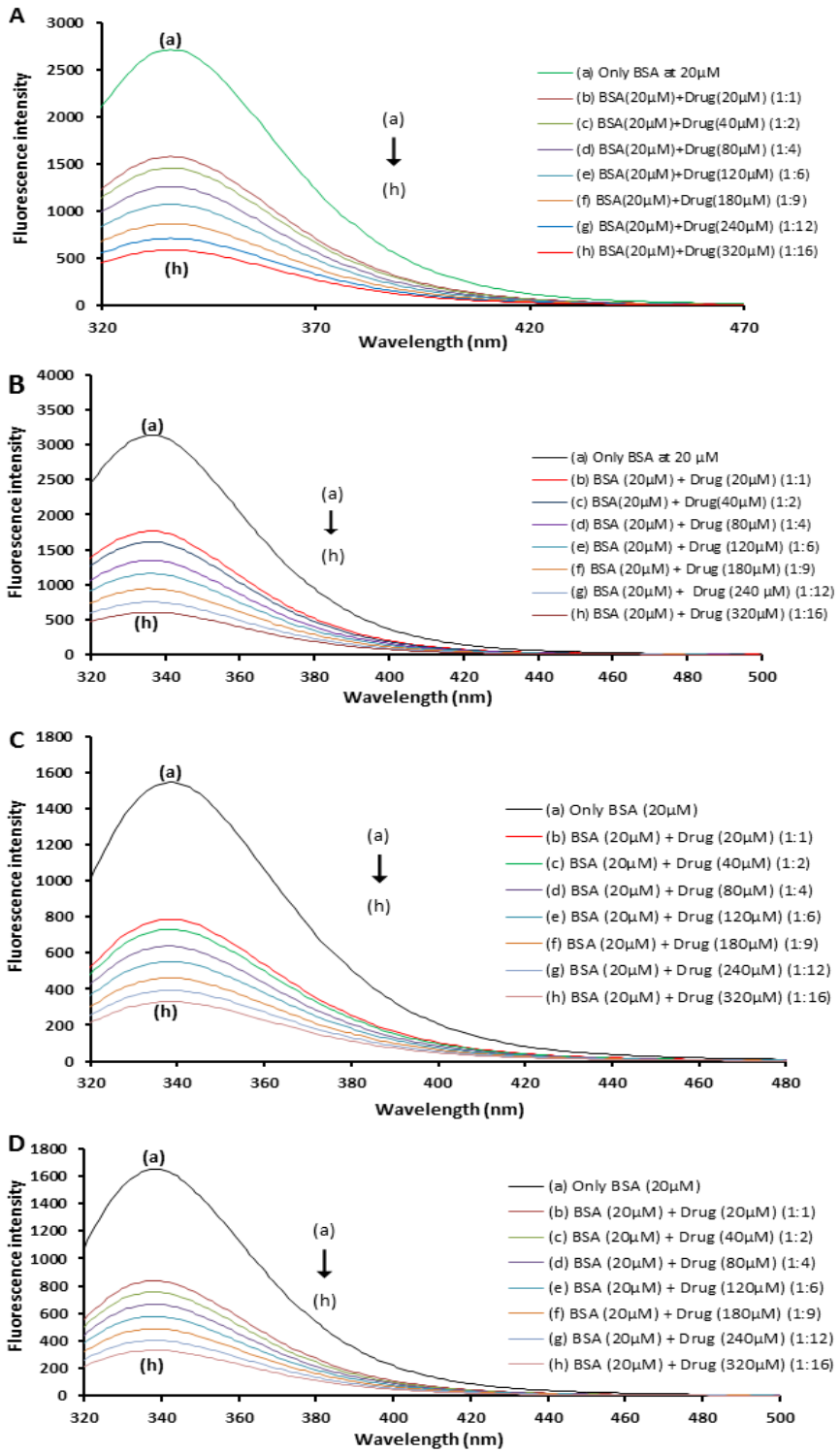

Figure 2: A. BSA fluorescence spectra in the presence of olanzapine, λ_{ex}= 280 nm, C_{BSA}= 2x10^{-5} mol L^{-1} ; molar ratio of drug (olan) to protein (BSA) is 0, 1, 2, 4, 6, 9, 12, 16 μM [from (a to h)] at 298 K , pH=7.40 .
B. BSA fluorescenc spectra in the presence of olanzapine, λ_{ex}= 280 nm, C_{BSA}= 2x10^{-5}mol L^{-1} ; molar ratio of drug to protein is 0, 1, 2, 4, 6, 9, 12, 16 μM [from (a to h)] at 308 K , pH=7.40.
C. BSA fluorescenc spectra in the presence of olanzapine, λ_{ex}= 293 nm, C_{BSA}= 2x10^{-5}mol L^{-1} ; molar ratio of drug to protein is 0, 1, 2, 4, 6, 9, 12, 16 μM [from (a to h)] at 298 K , pH=7.40.
D. BSA fluorescenc spectra in the presence of olanzapine, λ_{ex}= 293 nm, C_{BSA}= 2x10^{-5}mol L^{-1} ; molar ratio of drug to protein is 0, 1, 2, 4, 6, 9, 12, 16 μM [from (a to h)] at 308 K , pH=7.40.

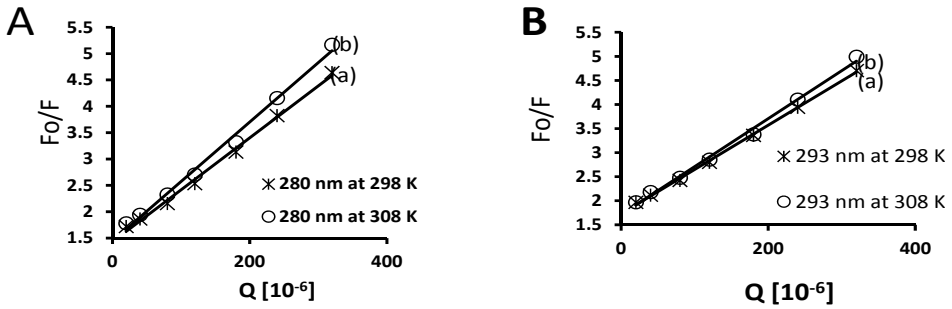

Figure 3: A. The Stern–Volmer plots of BSA quenched by olanzapine in λ_{ex} 280 nm at pH 7.40 at different temperatures: (a) 298 K; (b) 308 K. **B.** The Stern–Volmer plots of BSA quenched by olanzapine in λ_{ex} 293 nm at pH 7.40 at different temperatures: (a) 298 K; (b) 308 K.

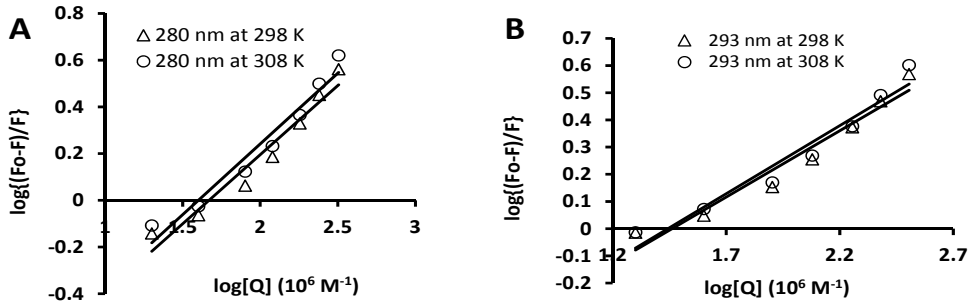

Figure 4: A. Logarithmic plot of the fluorescence quenching of BSA with various amounts of olanzapine in excited wavelength 280 nm at 298 K (Δ) and 308 K (○). **B.** Logarithmic plot of the fluorescence quenching of BSA with various amounts of olanzapine in excited wavelength 293 nm at 298 K (Δ) and 308 K (○).

λ_{ex} nm	T(K)	$\ln K_{sv}$	K_b(x 10^4 $Lmol^{-1}$)	n	ΔG_o (kJ mol^{-1})	ΔH_o (kJ mol^{-1})	ΔS_o (J mol^{-1} K^{-1})
280	298	9.1049	10.28	0.591	-22.5566	15.3097	127.0685
	308	9.3056	10.739	0.605	-23.8273		
293	298	9.1049	19.31	0.488	-22.5638	8.0415	102.7026
	308	9.2103	18.923	0.501	-23.5908		

Table 2 Modified Stern-Volmer association constants K_b, number of binding site n, and thermodynamic parameters of the interaction of olanzapine with BSA at different temperature (pH=7.40).

with BSA forming an stable complex with a molar ratio of about ~0.5:1 in between olanzapine and BSA molecules. Assume that one mole of BSA binds with 0.5 moles of olanzapine molecules. The complex begins to decompose when the temperature is increased in case of excitation wavelength of 293 nm.

Thermodynamic parameters and the nature of the binding forces

The acting forces between a small molecule and macromolecule include hydrogen bond, van der Waals force, electrostatic force and hydrophobic interaction. The thermodynamic parameters were determined using the Van't Hoff equation from equation 3. [16,17]

$$\ln K_b = - (\Delta H_o/RT) + (\Delta S_o/R) \quad (3)$$

Where, ΔS_o= Entropy change, ΔH_o= Enthalpy change, R= Universal gas constant and K_b= Analogous to the Stern-Volmer quenching constants K_{SV} at the corresponding temperature [14]. In equation (3), K_b corresponds to the modified Stern-Volmer association constant at specific temperatures and R is the gas constant. The plot of lnk Vs 1/T enabled the determination of the values of ΔH_o and ΔS_o (Figure 5) .The free energy change ΔG_o of the binding reaction at different temperature was estimated from the relation: from equation 4.

$$\Delta G_o = \Delta H_o - T\Delta S_o \quad (4)$$

Value of ΔG_o, ΔH_o and ΔS_o are summarized and listed in Table 2.

For typical hydrophobic interactions, both ΔH_o and ΔS_o are positive, while these are negative for van-der waals forces and hydrogen-bond formation in low dielectric media [16,17]. Moreover, the specific electrostatic interaction between ionic species in an aqueous solution is characterized by positive ΔS_o value and negative ΔHo value (small).

A positive value of ΔSo was evidence of hydrophobic interaction, the positive ΔH_0 values nullify the evidence of hydrogen bonding in binding process [16,17]. Furthermore, the main source of ΔGo was derived from a large contribution of the ΔSo term with a small contribution from the ΔH0. So the main interaction was hydrophobic in nature and evidence of the hydrogen bonding could be excluded. Negative (energy change) ΔGo postulates the spontaneous binding process throughout the interactions [16].

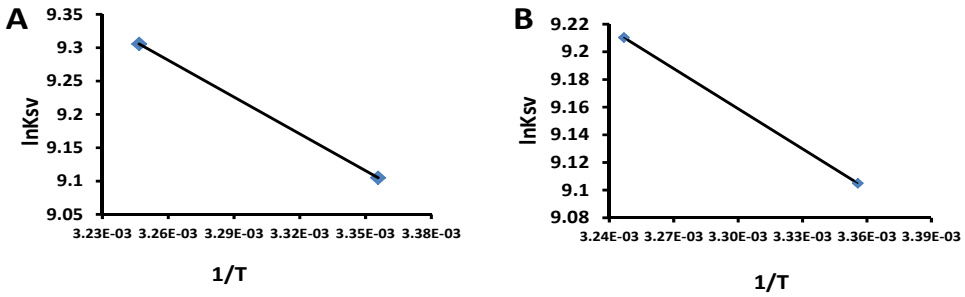

Figure 5: A. Van't Hoff plot for the interaction of olanzapine with BSA at pH 7.40 in λ_{ex}= 280 nm. **B.** Van't Hoff plot for the interaction of olanzapine with BSA at pH 7.40 in λ_{ex}= 293 nm.

Conclusions

Fluorescence spectroscopic method was used to study the interactions and nature of binding of olanzapine with BSA. The experimental results indicated that quenching of the fluorescence of BSA by olanzapine was probably a dynamic process and the binding reaction was mainly enthalpy driven, where hydrophobic interaction could play a major role. The binding nature assumed that the maximum binding occur at principal regions of ligand binding domain, located in hydrophobic cavities in the sub domains IIA and IIIA [5,6]. Binding constant $K_{b,}$ suggested that the stability of binding increased with the increase of temperature in most cases. Increase of stability of the complex was observed at 280 nm whereas reversed effect was noticed at 293 nm with increase of temperature. Molar ratio of BSA-olanzapine indicated that 1 mole of BSA bound with ~0.5 mole of olanzapine. Pharmacokinetic view point suggested that drug with higher affinity with plasma protein has a low therapeutic index. This indicates that there is a high risk of toxicity when using olanzapine [18]. Since olanzapine is an antipsychotic drug, it needs to take as prolong therapy to improve the diseased state of any of the patients. So, study of such type drug-protein interaction can be helpful for safe dosing regimen for better therapeutic outcome, and to minimize the toxicity of the drug [19,20].

Conflict of Interest

The authors declare that they have no conflict of interests to disclose.

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