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Binding Interactions of 4-phenyl butan-2-amine and Bovine Serum Albumin Probed by Spectroscopic Techniques

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ABSTRACT

The binding ability between 4-phenylbutan-2-amine (PBA) and Bovine Serum Albumin (BSA) was mainly investigated by UV-Visible and fluorescence spectroscopic methods. From the intensity of fluorescence spectra, it was observed that the 4-phenylbutan-2-amine has a strong ability to quench the intrinsic fluorescence of BSA through a static quenching mechanism. The binding constant and number of binding sites were investigated using Stern-Volmer equation. Conformation changes of BSA were observed from synchronous and Circular Dichroism (CD) spectra. The results of UV-Vis, CD, synchronous fluorescence spectral revealed the changes in the secondary structure of BSA upon interaction with 4-phenylbutan 2-amine thereby confirming their binding nature.

Keywords: 4-phenylbutan 2-amine, Bovine serum albumin, Fluorescence studies, Stern-Volmer equations, Circular dichroism, Synchronous spectral studies

INTROUCTION

It is known that the free concentration, metabolism and distribution of various drugs are immensely affected by drug-protein interactions in the blood stream [1]. Interactions between drug and protein are important, as most of the drugs that are administered are widely and reversibly bound to the protein serum albumin. They are carried mainly to the target cells as a complex with proteins. The nature and extent of drug-protein binding interactions extensively influence the biological activity of the drug [2-4]. Reductive amination of carbonyl compounds is an important and powerful tool for chemists to synthesis the structurally diverse primary, secondary and tertiary amines, and related compounds. These compounds have shown diverse biological activities such as antioxidant, anti-inflammatory, antimalarial, antimicrobial, antituberculosis and anticancer activities [5-7].

Serum albumins owing to their availability, low cost, stability, and unusual ligand binding properties, are the most extensively studied and applied proteins. Albumin is the most abundant protein in blood plasma that serves as storage and carrier protein for numerous exogenous and endogenous compounds [8]. Our recent evidence on the binding interactions of benzylidene piperidone and Bovine Serum Albumin (BSA) using spectroscopic methods [9] further made us keen to investigate whether similar interactions exists between BSA and 4-phenylbutan-2-amine (PBA). In order to understand the binding mechanism of BSA with 4-phenylbutan-2-amine under physiological conditions, we have carried out detailed investigation using fluorescence spectroscopy. In addition conformational changes of BSA were discussed on the basis of UV-Vis and CD-spectroscopy.

MATERIALS AND METHODS

Reagents and chemicals

Bovine Serum Albumin (BSA), and all the chemicals were purchased from Sigma Aldrich, 4-phenylbutan 2-amine was prepared according to the experimental procedure. All other materials were of analytical reagent grade and millipore water was used throughout the work.

Spectral measurements

Fluorescence spectra were recorded using a Model F-7000 FL Spectrophotometer Hitachi Serial Number, 2573-001 with a 150 W Xenon lamp, 1 cm quartz cell and a thermostatic cuvette holder. The excitation and emission bandwidths were both kept at 5 nm. The BSA solution was prepared using phosphate buffer with millipore water and maintained at 4°C. The absorption spectra were recorded using a double beam U-2900 Spectrophotometer in the range of 200-400 nm, Circular Dichroism (CD) was recorded in JASCO Corp., J-715 model in the range of 200-250 nm. pH measurements were carried out using Elico LI120 pH meter (Elico Ltd., India).

General procedure for Synthesis of 4-Phenyl-butan-2-amine

Synthesis of 4-Phenyl-butan-2-amine

The synthesis of PBA was carried out following our previous method using ultrasound irradiations [10]. A mixture of the ketone (1.0 eq), titanium (IV) isopropoxide (1.5 eqv.) and ammonia in ethanol (5 M, 10 vol) under ultrasound irradiation at 30°C for 5 min. Sodium borohydride (1.5 eqv.) was added pinch wise over a period for 5 min. After completion of the reaction, excess ethanol was concentrated under reduced pressure to get the residue. The residue was diluted with water and extracted with ethyl acetate, dried with Na_2SO_4 and concentrated under reduced pressure to obtain the corresponding primary amine (Figure 1).

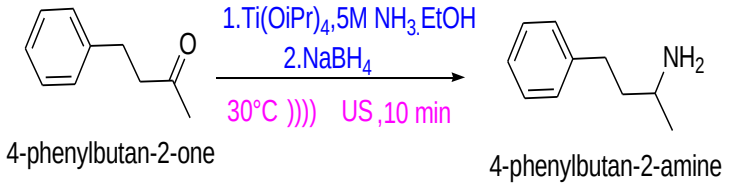

Figure 1: Synthesis of the 4-phenyl butan-2-amine

PBA-BSA interaction study

Based on the initial experiments, 7 samples were made keeping BSA concentration fixed at 12 µM and drug concentration being varied from 20-140 µM with an interval of 20 µM. Fluorescence spectra for each sample were recorded at three different temperatures 295, 305 and 315 K, in the wavelength range of 300-500 nm upon excitation at 286 nm. UV measurements were also carried on the same samples using phosphate buffer at room temperature. The CD spectra of BSA which is dissolved in phosphate buffer with and without PBA were recorded using 20, 40 and 60 µM concentration of PBA. Synchronous fluorescence spectra were recorded with scan ranges, $\Delta\lambda$=15 nm and 60 nm ($\Delta\lambda=\lambda_{em}-\lambda_{ex}$) in the absence and presence of PBA and the spectra were recorded in the range of 250-400 nm.

RESULTS AND DISCUSSION

Fluorescence quenching of BSA by 4-phenyl butan-2-amine

The fluorescence spectroscopy was used to determine the nature of interaction between PBA and BSA. The fluorescence intensity of a compound is quenched by a variety of molecular reactions viz., energy transfer, ground state complex formation, excited state reactions, collisional quenching and molecular rearrangements [11,12]. In order to know the binding interactions of PBA and BSA, the fluorescence spectra given in Figure 2, were recorded up on excitation with 280 nm wavelength which is due to contribution from tryptophan and tyrosine residues of BSA. The quantitative analysis of the binding of PBA with BSA was carried out using the fluorescence quenching studies at 343 nm. From Figure 2 it is clear that initially with increasing concentration of PBA, the fluorescence intensity of BSA is found to decrease gradually and with further addition of PBA the intensity of BSA decreases indicating the beginning of saturation of the BSA binding site.

In order to predict the possible quenching mechanism, the fluorescence quenching data were subjected to Stern-Volmer analysis using the following equation:

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

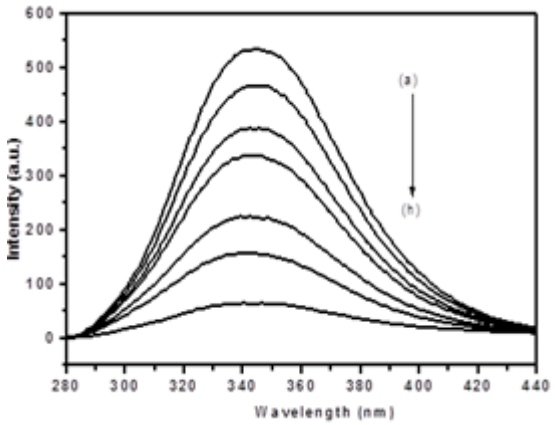

Figure 2: Fluorescence spectra of BSA (a) 12 µM in the presence of PBA: (b) 20 µM, (c) 40 µM, (d) 60 µM, (e) 80 µM, (f) 100 µM, (g) 120 µM, (h) 140 µM (a-h)

Where, F and F_0 are the fluorescence intensities of BSA with and without quencher (PBA) respectively. K_q is the quenching rate constant of the bio molecule, K_{sv} is the Stern-Volmer quenching constant, τ_0 is the average life time of bio molecule without the quencher and [Q] is the concentration of the quencher [13].

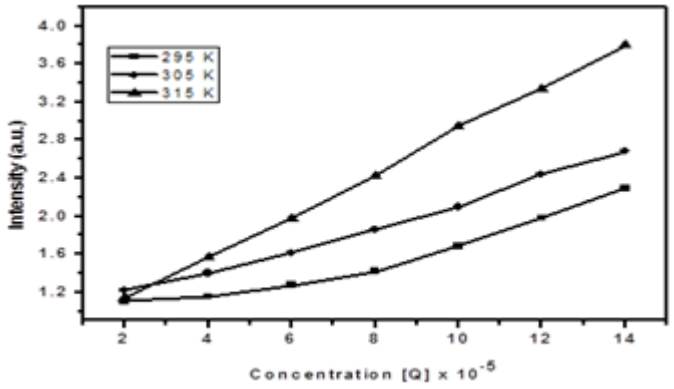

Figure 3: The Stern-Volmer curves showing quenching of BSA, [BSA]=12 µM

The Stern-Volmer plots for the result of interactions carried out at different concentrations were observed to be linear with slopes (K_{sv} values) decreasing with increasing temperature as shown in Figure 3 indicating the presence of static quenching mechanism. The values of K_{SV} for PBA-BSA system at different temperatures were calculated to be 1.0059×10^4, 1.2399×10^4, 2.2287×10^4 l/mol^{-1} respectively. Further, the values of Kq were evaluated using the Eqn. (2):

$$K_q = \frac{K_{SV}}{\tau_0} \qquad (2)$$

Since the fluorescence life time of the biopolymer is 10^{-8} s [14], the value Kq for PBA–BSA system was observed to be 1.0059×10^{12}, 1.2399×10^{12}, 2.2287×10^{12} l/mol^{-1}.s^{-1} at 295, 305 and 315 K respectively.

Binding constant and the number of binding sites

Fluorescence intensity data can be used to acquire the binding constant K and the number of binding sites n, by using the following Eqn. (3):

$$\log \frac{F_0 - F}{F} = \log K_a + n \log[Q] \qquad (3)$$

The values of K and n were calculated from the intercept and slope of the straight line obtained from the plot of log [(F_0-F)/F] versus log [Q] given in Figure 4. It is clear from the values of K and n given in Table 1 that the binding constant increased with increasing temperature suggesting that the interactions involved in the binding of PBA to BSA are primarily non-ionic in nature. The number of binding sites (n) obtained are close to the reported values [15] indicating the existence of only one independent class of binding site for PBA on BSA. The value of correlation coefficients obtained are closer to 0.9985, demonstrating that the PBA-BSA interactions agree well with the site binding model given by the Eqn. 3:

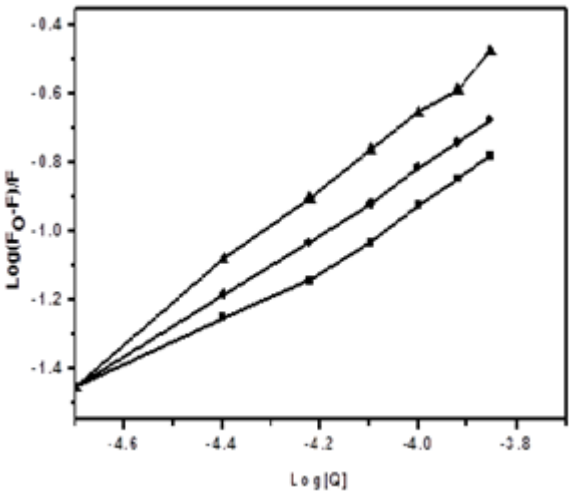

Figure 4: The Stern-Volmer curves showing quenching of BSA, [PBA]=20-140 µM, [BSA]=12 µM

Since the values of Kq obtained are higher than Kq of scatter procedure, which is 2×10^{10} l/mol^{-1}.S^{-1}, it is clear that the quenching was not initiated by dynamic collision to the development of a complex. From the above information, static quenching mechanism is effective in the PBA–BSA interactions.

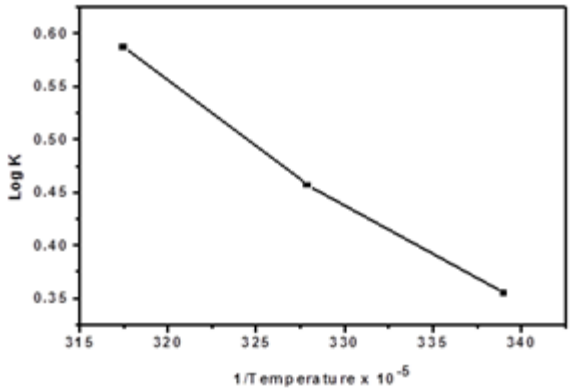

Figure 5: The temperature dependence of binding constant of PBA-BSA

Table 1: Parameters of PBA-BSA interactions at various temperatures

Temp (K)	Binding constant K × 10^{-5} L mol^{-1}	R^2	No. of binding sites, *n*	ΔH^0 (k J mol^{-1})	ΔS^0 (J K^{-1} mol^{-1})	ΔG^0 (k J mol^{-1})
295	2.26	0.9845	0.79	20.59	76.47	-22.51
305	2.86	0.9985	0.92			
315	3.86	0.9974	1.13			

Binding mode between PBA and BSA

The binding studies were carried out at different temperatures 295, 305 and 315 K. The acting forces between a small molecule and macro molecule include hydrogen bond, Vander Waals force, electrostatic force, hydrophobic interaction force and so on [16-18]. The thermodynamic parameters were evaluated using the following Eqns. 4 and 5:

$$LogK = -\Delta H^0/2.303\ RT + \Delta S^0/2.303R \qquad (4)$$

The log K versus 1/T plot shown in Figure 5 enabled the determination of ΔH^0 and ΔS^0 for the binding process and the values are given in Table 1. The value of ΔG^0 was calculated from the equation (5).

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \qquad (5)$$

Where ΔH^0, ΔG^0 and ΔS^0 are enthalpy change, free energy change and entropy change respectively. The negative value of ΔG^0 reveals that the interaction process is spontaneous [19,20]. The positive ΔH^0 and ΔS^0 values of the interaction of PBA and BSA indicate that the binding is mainly entropy driven and the enthalpy is unfavorable for it, the hydrophobic forces thus playing a major role in the binding process [21].

Absorption spectroscopic studies

UV-visible absorption spectroscopy is employed to explore the structural changes [22,23] and to know the formation of complex between the drug and protein. From the UV-Vis spectra given in Figure 6a, the λ_{max} of BSA observed at around 280nm was mainly due to the presence of tryptophan and tyrosine residues in BSA. It was evident from the UV spectra given in Figure 6b that the absorption intensity of BSA increased regularly with increasing concentration of PBA.

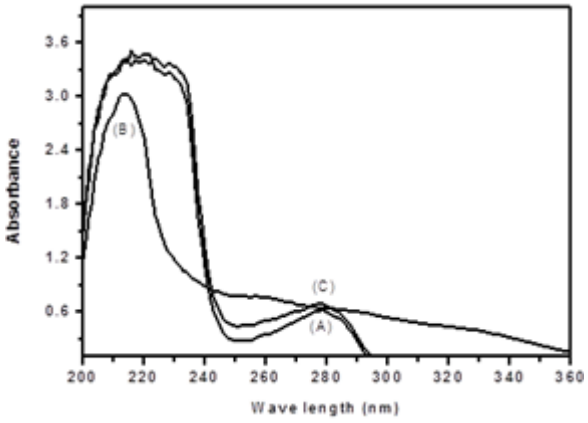

Figure 6a: UV-vis absorption spectra of (a) BSA, (b) PBA and (c) BSA+PBA

The shift in λ_{max} is also evidence of the ground state complex formation in the interaction study. Further, the blue shift in absorption maximum indicated the change in polarity around tryptophan residue and changes in the peptide strand of BSA molecule and hence the change in hydrophobicity [24].

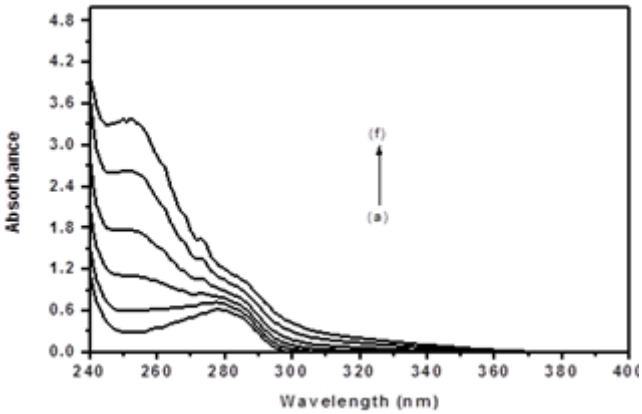

Figure 6b: Absorption spectra of BSA (a) and PBA-PBA complex (b-f), BSA: 12 µM; PBA: 20-100 µM (b-f)

CD studies

Further evidence for conformational changes in BSA up on the addition of PBA was obtained by CD spectroscopic studies. CD spectra of BSA in presence and absence of various amounts of PBA are shown in Figure 7. CD spectrum of BSA exhibited two bands at 208 and 222 nm, characteristic of a predominantly α-helical structure of BSA [25-28]. These bands are caused by a negative cotton effect of helical structure without any significant shift of the peaks, indicating that the addition of PBA changes the secondary structure of BSA. Moreover, the decrease in negative elasticity meant that the peptide strand extended even more, while the hydrophobicity was decreased.

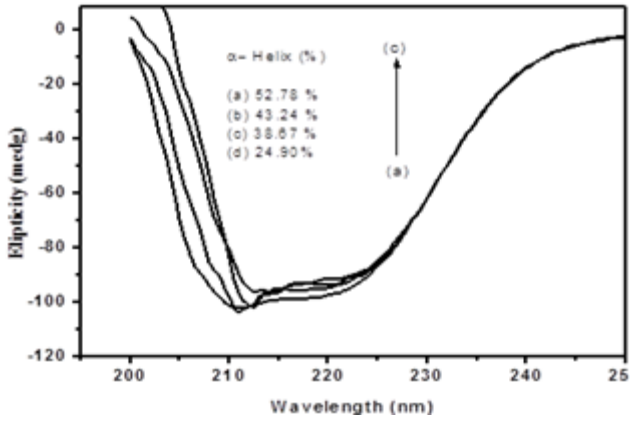

Figure 7: CD spectra of BSA–PBA system: (a) BSA; (b) BSA+PBA; 20, 40 and 60 µM

The CD results were expressed in terms of mean residue elasticity (MRE) in deg cm^2 $dmol^{-1}$ according to the following equation [29]:

$$\text{MRE} = \frac{\text{Observed CD (m deg)}}{C_p \, nl \times 10} \qquad (6)$$

Where, C_p is the molar concentration of protein, in the number of amino acid residues and *l* is the path length. The α-helical contents of free and combined BSA were calculated from MRE values at 208 nm using the equation [30]:

$$\alpha - \text{helix}(\%) = \frac{\text{MRE}_{208} - 400}{33{,}000 - 4000} \times 100 \qquad (7)$$

Where, MRE_{208} is the observed MRE value at 208 nm, 4000 is the MRE of the β- form and random coil conformations cross at 208nm and 33,000 is the MRE value of a pure α-helix at 208nm. From the Eqns. 6 and 7, the α-helicity in the secondary structure of BSA can be determined. The α-helicity decreased from 52.2% in free BSA to 24.9% in PBA–BSA complex, which was indicative of the loss of α-helicity upon interaction. The decrease in α-helix structure indicated that the drug bound with the amino acid residues of the main polypeptide chain of protein and destroyed their hydrogen bonding networks [31-33]. The CD spectra of BSA in presence and absence of PBA were observed to be similar in shape indicating that the structure of BSA was also predominantly α-helical even after binding to PBA.

Synchronous fluorescence spectra

Synchronous fluorescence was recorded for BSA with and without PBA, for evaluating the conformational changes of BSA as shown in Figure 8. The environment of amino acid residues can be deliberated by measuring the emission wavelength (λ_{max}) shift, which is due to the change in the polarity around the chromophore molecule. When the Δλ between the excitation wavelength and the emission wavelength was fixed at 15 nm or 60 nm, the synchronous fluorescence spectra of BSA can involve a change in the polarity of the micro-environment around tyrosine or tryptophan residues, respectively [34].

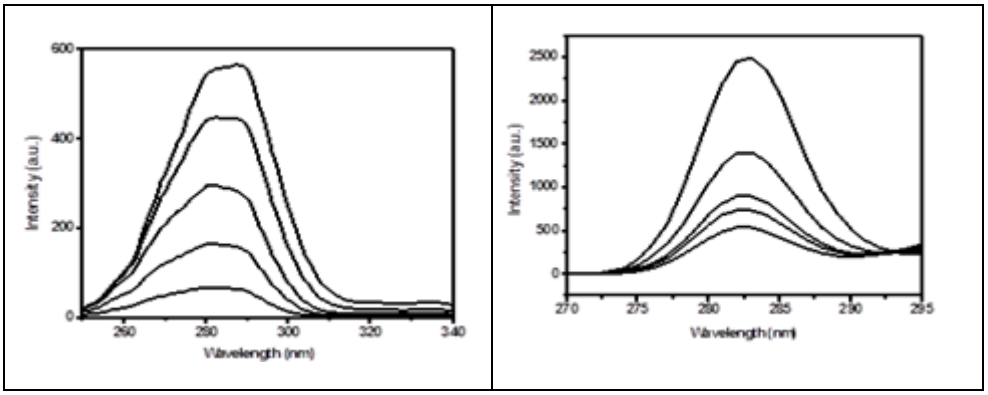

Figure 8: Synchronous fluorescence spectra of BSA–PBA: (A) For Δλ=15 nm while for (B) Δλ=60 nm, the Concentration of BSA was 12 µM, the concentration of PBA: (a) 20-80 µM

The maximum emission wavelength of Tyr residues remained principally unchanged, indicating that no discernible change occurred in the micro environment around Tyr residues during the binding process. On the other hand, the maximum emission wavelength of Trp residues has a slight blue-shift, indicating that the polarity around Trp residues micro-regions decreased and the hydrophobicity increased upon interaction with PBA. Also the field of quenching at Δλ=60 nm was higher than that of Δλ=15 nm, implying that PBA was more rapidly bound to Trp residues compared to Tyr residues. Hence it is proven that the binding sites are mainly focused on tryptophan moiety [35,36] that is responsible for the conformational changes in the secondary structure of BSA.

CONCLUSION

This paper provided an approach for studying the interactions of protein with PBA using absorption, fluorescence and CD. The results showed that BSA fluorescence quenched by PBA through static quenching. CD results revealed the change in secondary structure of BSA upon interaction with the drug. The biological significance of this work is evident since albumin serve as a carrier molecule for multiple drugs and the interaction of PBA with albumin is not reported so far, this report has a great significance in pharmacology and clinical medicine as well as methodology.

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