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Synergic Effects of Anticancer Drugs to Bovine Serum Albumin: A Spectroscopic Investigation

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Abstract

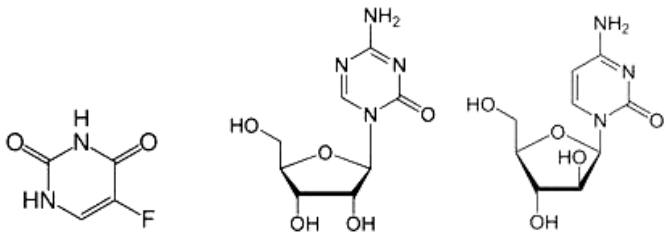
Anticancer drugs (i) 5-fluorouracil (5-FU), (ii) azacitidine (AZ) and (iii) cytarabine (CY) (pyramidine analogues) have the ability to quench bovine serum albumin (BSA).The synergic effect between the drugs and BSA were studied using fluorescence spectrophotometer and ultraviolet spectroscopic techniques under imitated physiological conditions. The results indicate that static quenching and non radiative energy transfer are the main reason of fluorescence quenching. The synergism results in both the reduction of the binding stability between drugs and BSA and an increase of the free drug concentration, which will increase the efficacy of drugs. The binding distances (r) between the drugs and BSA were obtained based on Försters theory of non-radiation energy transfer. The results indicated that the effect of synergism affected the conformation of BSA.

Keywords: BSA, anticancer drugs, spectroscopic investigation, FRET.

Introduction

Pharmaceutical drugs provide interaction with serum constituents are important issue in drug delivery. The number of docking sites on transport of proteins and specific interactions can significantly influence the behavior of drug protein interaction. The important serum albumins are human serum albumin (HSA) and bovine serum albumin (BSA)[1,2].

In pharmacological field the interaction between drugs to protein give wide variety of applications. in pyramidine analogues like 5-fluorouracil, azacitidine and cytarabine which possess antitumor and anti viral properties[3, 4]. Solid tumors seen in the breast which is resisted by using 5-FU as antimetabolite[5, 6]. Generally pyramidine analogues which resist the tumors and which are passed through cell membranes. These anticancer drugs structures are shown in figure.1.Serum albumins are abundant in blood plasma and provide 80% of the osmotic pressure[7-9].



(5-FU) **(AZ)** **(CY)**

Figure-1
Chemical structures of 5-Fluoro uracil, Azacitidine and Cytarabine

The biophysical and biochemical property of bovine serum albumin is well known in the past years. The structure shown in the figure-2. In BSA three fluorophore residues are there ie, tryptophan (Trp), tyrosine (Tyr) and phenylalanine (Phe). It has two tryptophan residues[10,11].Trp 134 and Trp 212. Trp 134 is located the periphery of the protein molecule and Trp 212 is within the hydrophobic pocket. In addition, drug-albumin complex may be considered a model for gaining general fundamental insights into drug-protein binding [12-14]. The aim of this work is to study the interaction of anticancer drugs with BSA through thermodynamic considerations using various spectroscopic tools.

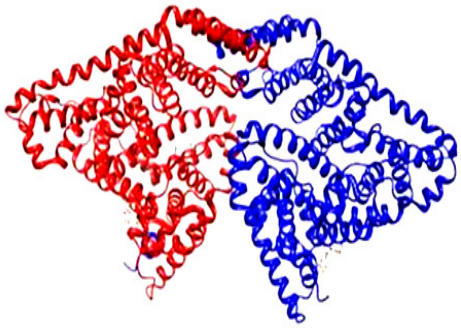


Figure-2
Structure of BSA

Methodology

Materials: BSA was directly dissolved in aqueous solution (10^{-5}molL^{-1}), and the stock solution was kept throughout in room temperature. 5-Fluoro uracil and the remaining pyramidine

analogues solutions (0.12 x 10^{-3} molL^{-1}) were prepared as stock solutions. For quenching studies, aliquots from each of the stock solutions were taken to get the desired quencher concentration. BSA and anticancer drugs, 5-fluorouracil and azacitidine and cytarabine, were purchased from Sigma Aldrich. Millipore water was used for preparing solutions throughout the course of investigations.

Methods: Fluorescence spectra were recorded on a Shimadzu RF-5301 fluorescence spectrophotometer. The emission and excitation slits were 5 nm. Fluorescence quenching spectra were measured in the range of 290-600 nm. The absorption spectra were obtained from a Shimadzu UV-2450 spectrophotometer. Fourier transform infrared (FTIR) spectroscopic measurements were performed on a Perkin Elmer-400 Fourier Transform Spectrometer.

Results and Discussion

Fourier transform infrared spectroscopy (FT-IR): The simple and powerful technique used to investigate secondary structure of protein is fourier transform infrared spectroscopy. In secondary structure of proteins mainly two bands are observed amide I and amide II. The C=O stretching vibration in amide I band in between 1600cm^{-1} and 1700cm^{-1} . The C-N stretching band coupled with the N-H bending mode of amide II band at 1550cm^{-1}. In the case of BSA + drugs solution the amide I band changes to 1656 cm^{-1} to 1661 cm^{-1} in all the three cases. These results indicate that the secondary structure of the protein changes due to the interaction with these drugs.

Fluorescence spectra: By varying the concentration of 5-FU with bovine serum albumin excitation at 290nm were recorded. the intensity decreased on the successive addition of 5-FU. The result suggests that binding between 5-FU and BSA take place. The binding also confirmed by using Stern Volmer equation

$$F_o / F = 1 + k_q \tau_o [Q] \quad (1)$$

where F_o and F are the steady state fluorescence intensities in the absence and presence of quencher. The Stern - Volmer quenching constant is given by k_q, τ_o and [Q] is the concentration of quencher (5-FU). There are two type of quenching dynamic and static. in the review suggest that the value of quenching constant k_q is calculated by the following equation and it is listed in the table 1.

$$k_q = k_{sv} / \tau_o \quad (2)$$

where τ_o is the average lifetime of protein without the quencher. Various value of fluorescence lifetime usually used is about 10^{-8}. The quenching rate constants k_q calculated in this is $k_q = k_{sv}/\tau_o$. In dynamic quenching the value of kq is limited to (k_q = 2.0×10^{10} M^{-1} S^{-1}). Here the result obtained greater that of value suggested in dynamic quenching. So the result confirmed that there is a non fluorescent complex is formed between the fluorophore and the drug, i.e., static quenching.

The remaining two drugs (azacitidine, cytarabine) also show these quenching properties similar to 5-FU (figure 4-5).

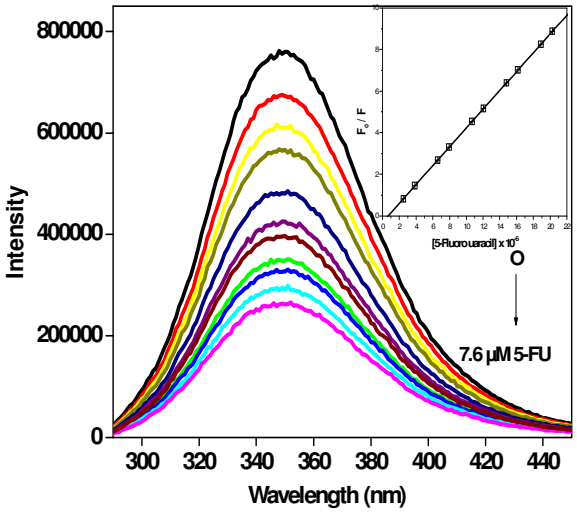


Figure-3
Effect of 5-fluoro uracil on the fluorescence spectrum of BSA [10^{-5} molL^{-1}] (298K) Inset: Stern-Volmer plot for quenching of BSA fluorescence by 5- fluoro uracil

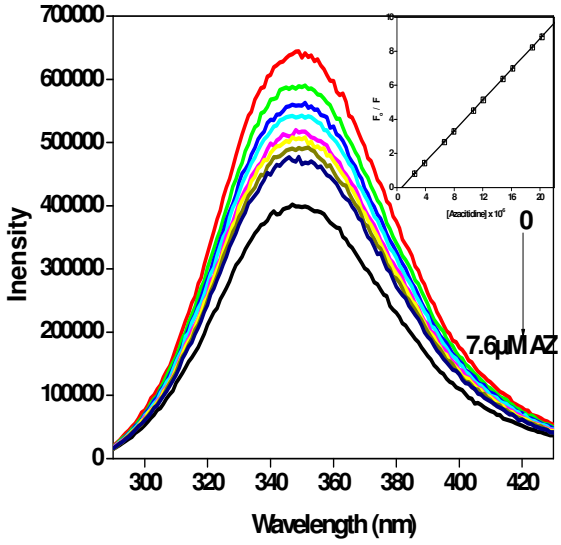


Figure-4
Effect of azacitidine on the fluorescence spectrum of BSA [10^{-5} molL^{-1}] (298K) Inset: Stern-Volmer plot for quenching of BSA fluorescence by azacitidine

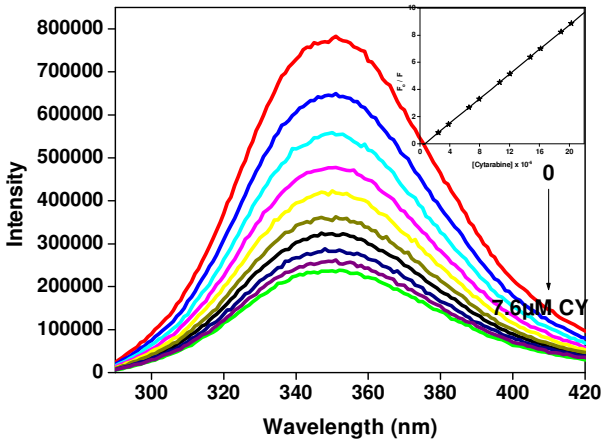


Figure-5
Effect of cytarabine on the fluorescence spectrum of BSA [10^{-5} $molL^{-1}$] (298K) Inset: Stern-Volmer plot for quenching of BSA fluorescence by cytarabine

3 UV-vis. absorption spectra: UV-vis absorption spectra can be used to understand the structural changes and the formation of complexes. In order to confirm the quenching nature, the UV-vis. absorption spectra of the BSA-drug system were taken and the steady state absorption spectra of BSA in the presence and absence of drugs are presented in figure 6 - 8.

The figure 6 shows the absorption of BSA in the absence and presence of 5-FU. BSA has strong absorbance with a peak at 279 nm and the absorbance of BSA increased with the addition of 5-FU. The absorbance peak at 279 nm is raised and blue shifted (from 279 nm to 266 nm) on adding 5-FU. These observations indicate that BSA molecules formed BSA-5-FU complex with 5-Fluoro uracil and also the hydrophobicity increased [15, 16].

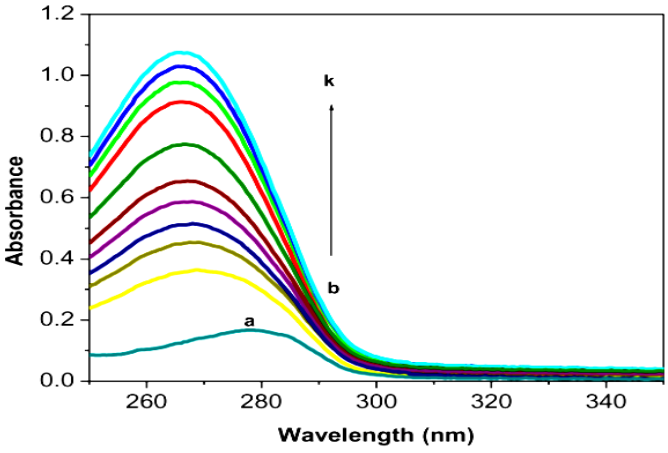


Figure-6
The absorption spectra of BSA (a) and BSA-5-FU (b - k)

The figure 7 and 8 show the absorption of BSA in the absence and presence of azacitidine and cytarabine. In the case of AZ and CY also the absorbance peak at 279 nm is raised and blue shifted (from 279 to 254 nm in the case of AZ and from 279 to 272 nm in the case of CY indicating complex formation with these drugs [17].

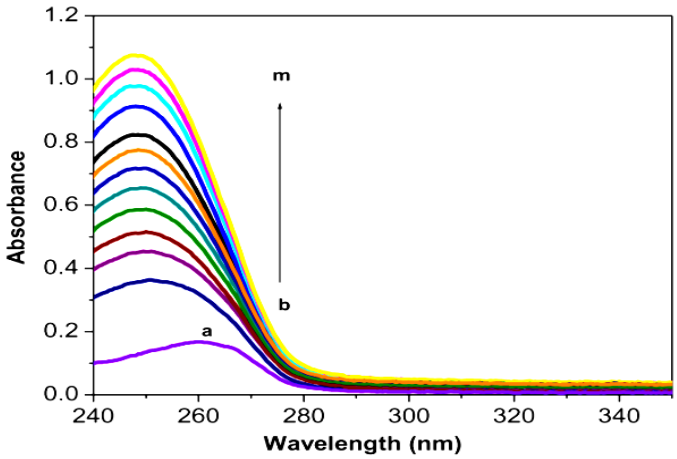


Figure-7
The absorption spectra of BSA (a) and BSA- AZ (b - m)

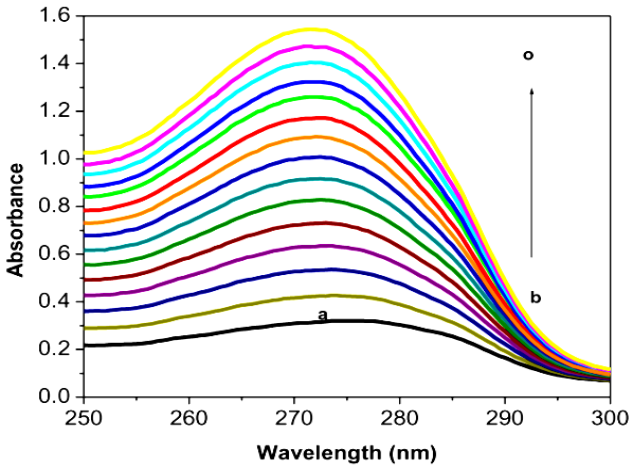


Figure-8
The absorption spectra of BSA (a) and BSA- CY (b - o)

Identification of binding sites of drugs on BSA: The binding constant calculated by using the following equation in static quenching mechanism[18].

$$\log (F_o\text{-}F)/F = \log k_b + n \log [Q] \quad (3)$$

where k_b and n are the apparent binding constant and the number of binding sites respectively for the BSA-drug system. Thus, the intercept and the slope value of the plot, log $(F_o\text{-}F)/F$ versus log [Q] give the k_b and n values. Here the result suggests that there is one class of binding site between drug and BSA and summarized in table 2.

Thermodynamic parameters and nature of the binding forces: In the protein drug- drug interaction the forces between them is calculated which include hydrogen bond, vander Waals force, electrostatic force, and hydrophobic forces[19]. It is obtained from thermodynamic parameters can be calculated from van't Hoff equation:

$$\ln \frac{k_{b_2}}{k_{b_1}} = [\frac{1}{T_1} - \frac{1}{T_2}] \Delta H / R \quad (4)$$

$$\Delta G = -RT \ln kb \quad (5)$$

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

where k_b is the binding constant at the corresponding temperature and R is the gas constant. ΔH, ΔG and ΔS are enthalpy change, free energy change and entropy change, respectively. The experiments were carried out at 298, and 310 K, and the thermodynamic parameters were calculated and summarized in table 3, where the negative value of ΔH indicated that the binding interaction of drugs and BSA is exothermic (table 2). The formation of the negative values of ΔH indicated that both hydrogen bond and vander Waals forces played an important role in the binding of drugs and BSA. The negative value of ΔG revealed that the interaction process was spontaneous. The negative value ΔS indicated that the reaction is disordered.

Fluorescence Resonance Energy Transfer: The distance between the protein residue (donor) and the bound drug (acceptor) can be determined using Fluorescence Resonance Energy Transfer (FRET) [20,21]. The distance between the donor and acceptor and extent of spectral overlap determines the extent of energy transfer. Generally FRET occurs whenever the emission spectrum of a fluorophore (donor i.e.; BSA) overlaps with the absorption spectrum of acceptor (ie; here any of the drugs: 5-FU, AZ, and CY). The spectral overlap between the UV absorption spectrum of the 5-FU and the fluorescence emission spectrum of BSA is displayed in the figure 9. The distance between the donor and acceptor can be calculated according to Foster theory of dipole-dipole energy transfer. The efficiency of energy transfer E is calculated using equation

$$E = 1 - (F/F_o) = R_o^6 / (R_o^6 + r^6) \quad (7)$$

Where F and F_o are the fluorescence intensities of BSA in the presence and absence of drugs, r is the distance between acceptor and donor and R_o is the critical distance when the transfer efficiency is 50%. The values are listed in the table 4. The results suggest that the maximum distance between the donor and acceptor occurs three drugs. Here distance which is in the range 2-6 nm. The donor to acceptor distance, $r < 8$ nm indicated that the energy transfer from BSA and drugs occurs with high possibility.

Table-1
Stern-Volmer quenching constants (K_{sv}) of BSA - drug complexes at two levels of temperatures

Drugs	T(K)		k_{sv} (M^{-1})	k_q ($M^{-1}S^{-1}$)
5-Fluorouracil	298	310	0.1222 x 10^6 0.1456 x 10^6	1.2 x 10^{13} 1.4 x 10^{13}
Azacitidine	298	310	0.1534 x 10^6 0.2234 x 10^6	1.53 x10^{13} 2.2 x 10^{13}
Cytarabine	298	310	0.2495 x 10^6 0.3214 x 10^6	2.495 x10^{13} 3.2 x 10^{13}

Table-2
Binding constants (k_b) and binding sites (n) of BSA with 5-FU, AZ and CY at two levels of temperature

Drugs	T (K)	k_b $Lmol^{-1}$	No: of binding sites (n)	R^2
5-Fluoro uracil	298 310	3.99 x10^4 2.41 x 10^4	1.14 1.00	0.998 0.999
Azacitidine	298 310	5.76 x 10^4 4.32 x 10^4	1.03 1.02	0.987 0.992
Cytarabine	298 310	2.74 x10^4 1.75 x10^4	1.45 1.32	0.999 0.985

Table-3
Thermodynamic parameters of pyramidine derivatives - BSA complexes

Drugs	T(K)	**ΔH** (kJ mol^{-1})	**ΔG** (kJ mol^{-1})	**ΔS** (kJ mol^{-1})
5-Fluoro uracil	298 310	-21.99	-34.42 -22.67	155.03
Azacitidine	298 310	-25.34	-43.35 -37.77	378.43
Cytarabine	298 310	-20.37	-24.94 -14.42	161.35

Table-4
The values of overlap integral and Forster distance for BSA-drugs energy transfer

Drugs	J (cm^6)	Ro (nm)	r (nm)	E
5-Fluoro uracil	1.22 x 10^{-15}	1.66	2.17	0.12
Azacitidine	1.31 x 10^{-15}	1.74	2.30	0.15
Cytarabine	1.25 x 10^{-15}	1.62	2.20	0.14

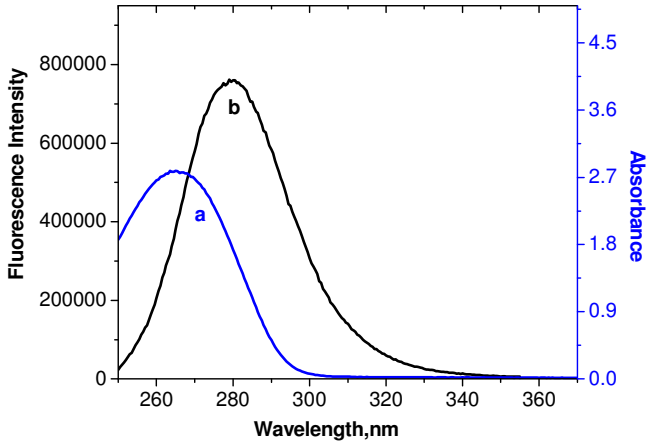


Figure-9
Overlap of the absorption spectrum of 5-FU (a) and fluorescence spectrum of BSA (b) (298 K)

Conclusion

In the three pyramidine analogue such as 5-fluoro uracil, azacitidine and cytarabine has been studied under drug to protein binding under physiological conditions. Fluorescent studies reveal that there is strong binding between drugs to protein take place. UV absorption spectra also confirmed that the ground state complex formation. The binding study of drugs by BSA is of great importance in understanding bio-chemical interactions for biochemistry and pharmacology. The distance between donor and acceptor is obtained according to Forster's theory of non radiation resonance energy transfer. For 5-fluoro uracil, azacitidine and cytarabine the quenching is via non radiative energy transfer mechanism. Furthermore, these studies are expected to provide important insight into the interactions of the physiologically important protein BSA with important drugs used in various therapeutic regims.

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