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Study on the interaction between antibacterial drug and bovine serum albumin: A spectroscopic approach

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ABSTRACT

The binding of sulfamethoxazole (SMZ) to bovine serum albumin (BSA) was investigated by spectroscopic methods *viz.*, fluorescence, FT-IR and UV–vis absorption techniques. The binding parameters have been evaluated by fluorescence quenching method. The thermodynamic parameters, ΔH°, $\Delta S^{\circ}$and ΔG° were observed to be $-58.0\,kJ\,mol^{-1}$, $-111\,J\,K^{-1}\,mol^{-1}$ and $-24\,kJ\,mol^{-1}$, respectively. These indicated that the hydrogen bonding and weak van der Waals forces played a major role in the interaction. Based on the Forster's theory of non-radiation energy transfer, the binding average distance, *r*, between the donor (BSA) and acceptor (SMZ) was evaluated and found to be 4.12 nm. Spectral results showed the binding of SMZ to BSA induced conformational changes in BSA. The effect of common ions and some of the polymers used in drug delivery for control release was also tested on the binding of SMZ to BSA. The effect of common ions revealed that there is adverse effect on the binding of SMZ to BSA.

1. Introduction

It is known that the distribution, free concentration and the metabolism of various drugs are strongly affected by drug–protein interactions in the blood stream [1–4]. This type of interaction can also influence the drug stability and toxicity during the chemotherapeutic process [1]. Serum albumin is the most abundant protein in the circulatory system of a wide variety of organisms, being the major macromolecule contributing to the osmotic blood pressure [5]. It can play a dominant role in the drug disposition and the efficiency [6]. Many drugs and other bioactive small molecules bind reversibly to albumin and other serum components, which then function as carriers. Serum albumin often increases the apparent solubility of hydrophobic drugs in plasma and modulates their delivery to cell *in vivo* and *in vitro*. Animal experiments are indispensable in providing basic information on the pharmacological actions, biotransformation and biodistribution of drugs [7,8]. Bovine serum albumin (BSA) is well suited to these initial studies, since it has been extensively characterized [8]. In addition, drug–albumin complex may be considered as a model for gaining general fundamental insights into drug–protein binding.

Sulfamethoxazole (SMZ) [N-(5-methyl-3-iso-xazolyl) sulfanilamide] (Fig. 1) is a well known antibacterial drug. It was mainly used in treating urinary tract and lower respiratory tract infections and gets readily absorbed in the body [9]. We find it worthwhile to study the binding of SMZ with BSA using various techniques *viz.*, UV–vis, fluorescence and FT-IR techniques, as it is not reported so far.

2. Experimental

2.1. Reagents

Bovine serum albumin (BSA, Fraction V, approximately 99%; protease free and essentially γ-globulin free) was obtained from Sigma Chemical Company, St. Louis, USA. Sulfamethoxazole was obtained from Indian Bulk Drugs unit of CSIR, India. The solutions of SMZ and BSA were prepared in 0.1 M sodium phosphate buffer, pH 7.4 containing 0.15 M NaCl. BSA solution was prepared based on its molecular weight of 65,000 [2,3]. All other materials were of analytical reagent grade and double distilled water was used throughout.

2.2. Apparatus

Fluorescence spectra were recorded using a RF-5301 PC spectrofluorophotometer Model F-2000 (Hitachi, Japan) with a 150-W xenon lamp, a 1-cm quartz cell and thermostatic cuvette holder. The excitation and emission bandwidths were both 5 nm. The temperature of the sample was maintained by recycling water throughout the experiment. The absorption spectra were recorded on a double beam CARY 50-BIO UV–vis spectrophotometer. FT-IR Nicolet-5700, USA was used for the additional evidence regarding SMZ–BSA complexations.

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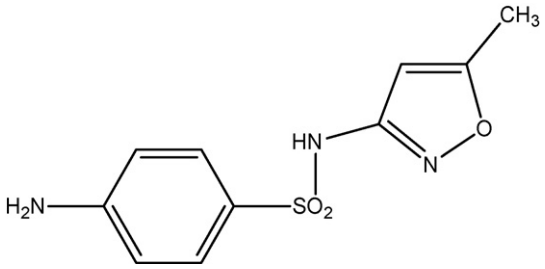

Fig. 1. Structure of sulfamethoxazole.

2.3. Procedures

2.3.1. SMZ–BSA interaction study

An appropriate volume of the BSA solution (40 μM from 250 μM stock), SMZ solution (40 μM from 250 μM stock) were transferred into a 5-mL conical flask. The mixtures were diluted with phosphate buffer solution (pH 7.4) to make the total volume (2 mL) and then were shaken. On the basis of preliminary experiments, BSA concentration was fixed at 5 μM and drug concentration was varied from 5 to 40 μM. Fluorescence spectra were recorded at room temperature (25 °C) in the range 290–450 nm upon excitation at 280 nm in each case.

2.3.2. Effects of some common ions

The fluorescence spectra of SMZ–BSA were recorded in the presence of various common ions *viz.*, SO_4^{2-}, NO_3^-, CH_3COO^-, Mg^{2+}, Co^{2+}, K^+, Ni^{2+} and V^{5+} at 340 nm upon excitation at 280 nm. The concentration of BSA was fixed at 5 μM and that of common ion was maintained at 5 μM.

2.3.3. Effects of some polymers

The fluorescence spectra of SMZ–BSA were recorded in the presence of various polymers *viz.*, poly vinyl acrylate (PVA), methyl cellulose (MC), sodium alginate (Na-ALG), guargum and hydroxy ethyl cellulose (HAC) at 340 nm upon excitation at 280 nm. The concentration of BSA was fixed at 5 μM and that of polymer was maintained at 20 μM from 2% stock solution.

2.3.4. Fourier transform infrared (FT-IR) spectroscopy

FT-IR spectra of buffer and protein (BSA) solution were collected under similar condition. Then, the absorbance values of buffer solution were subtracted from the values of protein solution to get the FT-IR spectra of protein. FT-IR spectra of protein–SMZ system were obtained in a similar way by subtracting the absorbance values of the SMZ + buffer from that of the SMZ-bound form.

3. Results and discussion

3.1. Fluorescence studies

The conformational changes of BSA were evaluated by the measurement of intrinsic fluorescence intensity of protein before and after addition of drug. Fluorescence measurements give information about the molecular environment in a vicinity of the fluorophore molecules. The effect of drug (SMZ) on BSA fluorescence intensity was shown in Fig. 2. When different concentration of SMZ solution was titrated into a fixed concentration of BSA, a remarkable decrease of fluorescence intensity of BSA was observed, which indicated that SMZ could interact with BSA. Furthermore, from Fig. 2, the maximum wavelength of BSA shifted from 344 to 340 nm after the addition of SMZ, so a slight blue shift of the maximum emission wavelength was observed and it could be deduced that the fluorophore of protein was placed in a more hydrophobic

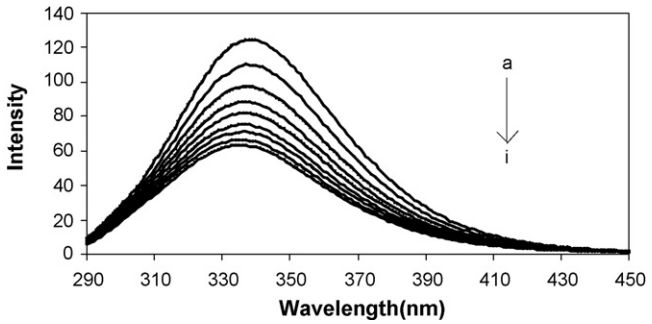

Fig. 2. Fluorescence spectra of BSA in the presence of SMZ. BSA concentration was 5 μM (a), SMZ concentration was at 5 μM (b), 10 μM (c), 15 μM (d), 20 μM (e), 25 μM (f), 30 μM (g), 35 μM (h) and 40 μM (i).

environment after the addition of SMZ. It was probably owing to the loss of the compact structure of hydrophobic subdomain IIA where tryptophan is placed [10]. The quantitative analysis of the binding of SMZ to BSA was carried out using the fluorescence quenching at 344 nm at various temperatures. With the increase of the concentration of SMZ, the fluorescence intensity of system gradually decreased, and with the further addition of SMZ, the fluorescence intensity of system decreased tardily in each titration curve which indicates the beginning of saturation of the BSA binding site.

Dynamic and static quenching are the different mechanisms of quenching and they can be distinguished by their differing dependence on temperature [11]. The quenching rate constants decrease with increasing temperature for static quenching, but the reverse effect is observed in the case of dynamic quenching [12]. A possible quenching mechanism is evident from the Stern–Volmer plots (Fig. 3) of BSA with SMZ at different temperatures (288, 298 and 308 K) [2–4]. The Stern–Volmer plots were linear with decreasing slopes with increase in temperature. The values of K_{SV} at different temperatures were found to be 3.312×10^4, 2.80×10^4 and 2.42×10^4 L mol^{-1}, respectively. This indicates the static quenching interaction between SMZ and BSA. In order to invoke this possibility, the mechanism is assumed to involve dynamic quenching. The equation for dynamic quenching [11] is presented by

$$\frac{F_0}{F} = 1 + K_{SV}[Q] = 1 + K_q \tau_o [Q] \quad (1)$$

where F and F_0 are the fluorescence intensity of BSA with and without quencher (drug), respectively. K_q, K_{SV}, τ_o and [Q] are the quenching rate constant of the biomolecule, the dynamic quenching constant, the average lifetime of the biomolecule without quencher and the concentration of quencher, respectively. Obviously,

$$K_q = \frac{K_{SV}}{\Gamma_o} \quad (2)$$

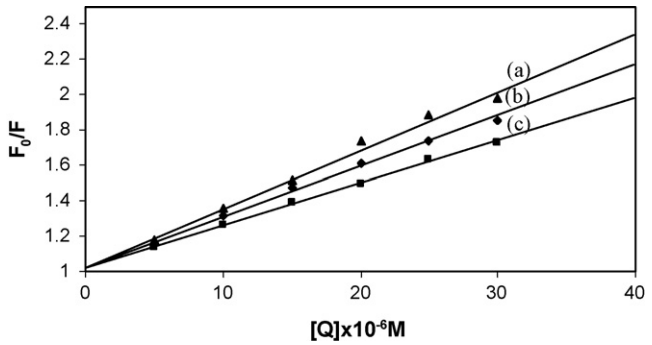

Fig. 3. The Stern–Volmer curves for the binding of SMZ with BSA at 288 K (a), 298 K (b) and 308 K (c). λ_{ex} = 280 nm; λ_{em} = 344 nm and c(BSA) = 5 μM.

Table 1
Thermodynamic parameters of SMZ–BSA system.

Temperature (K)	Binding constant ($K \times 10^{-4}$ L mol^{-1})	Number of binding sites (n)	ΔH° (kJ mol^{-1})	ΔS° (J K^{-1} mol^{-1})	ΔG° (kJ mol^{-1})
288	2.30 ± 0.001	0.962	−57.6 ± 3	−111 ± 1	−24 ± 3
298	1.52 ± 0.003	0.937			
308	1.07 ± 0.002	0.956			

Since the fluorescence lifetime of biopolymer is 10^{-8} s [12], the value of K_q was observed to be 2.80×10^{12} L mol^{-1} s^{-1} at 298 K. However, the maximum scatter collision quenching constant, K_q of various quenchers with the biopolymer is 2×10^{10} L mol^{-1} s^{-1} [13]. Thus, the rate constant calculated by protein quenching procedure is greater than K_q of scatter procedure. This indicates that a static quenching mechanism is operative [11,14].

3.2. Analysis of binding equilibria

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

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

where K and n are the binding constant and the number of binding sites, respectively. Thus, the slope and intercept value of the plot, $\log(F_0 - F)/F$ versus $\log[Q]$ give the K and n values (Table 1). The value of K indicates that there is interaction and the formation of complex between SMZ and BSA. From the value of n, it was found that there is one independent class of binding sites on BSA for SMZ. SMZ most likely binds to the hydrophobic pocket located in subdomain IIA; that is to say tryptophan-214 is near or within the binding site [16].

3.3. Determination of acting force between SMZ and BSA

Considering the dependence of binding constant on temperature, a thermodynamic process was considered to be responsible for the formation of a complex. The acting forces between a small molecule and macromolecule include hydrogen bond, van der Waals force, electrostatic force, hydrophobic force, and so on [15]. The thermodynamic parameters were determined using van't Hoff equation:

$$\log K = -\frac{\Delta H^\circ}{2.303\,RT} + \frac{\Delta S^\circ}{2.303\,R} \quad (4)$$

The $\log K$ versus $1/T$ plot enabled the determination of ΔH° and ΔS° for the binding process. The value of ΔG° was calculated from the relation:

$$\Delta G^\circ = \Delta H^\circ - T\,\Delta S^\circ \quad (5)$$

where ΔH°, ΔG° and ΔS° are, respectively, standard enthalpy change, standard free energy change and standard entropy change. The binding studies were carried out at 288, 298 and 308 K and the values are given in Table 1. At these temperatures the BSA does not undergo structural degradation. Ross and Subramanian [17] have characterized the sign and magnitude of the thermodynamic parameter associated with various individual kinds of interaction. From Table 2 it can be seen that ΔH° and ΔS° has a negative value (−57.6 kJ mol^{-1}) and a negative value (−111.0 J mol^{-1} K^{-1}), respectively. The negative value of ΔG° reveals that the interaction process is spontaneous. An important source of negative contribution to $\Delta H^\circ$and ΔS° will arise if a hydrogen bond is formed. The negative $\Delta H^\circ$and ΔS° values for the interaction of SMZ and BSA indicate that the binding is mainly enthalpy driven and entropy is unfavorable for it, and that the hydrogen bonding and weak van der Waals forces played major role in the interaction [15,17,18].

Table 2
Effect of anions and cations on binding constant of BSA–SMZ system.

Systems	Binding constant (L mol^{-1})
BSA + SMZ	$1.52 \pm 0.003 \times 10^4$
BSA + SMZ + Mg^{2+}	$1.16 \pm 0.008 \times 10^2$
BSA + SMZ + Co^{2+}	$1.45 \pm 0.006 \times 10^3$
BSA + SMZ + Ni^{2+}	$1.25 \pm 0.003 \times 10^3$
BSA + SMZ + V^{5+}	$3.44 \pm 0.003 \times 10^3$
BSA + SMZ + K^+	$2.07 \pm 0.004 \times 10^3$
BSA + SMZ + NO_3^-	$3.94 \pm 0.001 \times 10^3$
BSA + SMZ + SO_4^{2-}	$1.85 \pm 0.006 \times 10^3$
BSA + SMZ + CH_3COO^-	$1.29 \pm 0.005 \times 10^3$

3.4. Energy transfer between SMZ and BSA

The overlap of the UV absorption spectra of SMZ with the fluorescence emission spectra of BSA is shown in Fig. 4. The importance of the energy transfer in biochemistry is that, the efficiency of transfer can be used to evaluate the distance, r, between the ligand and tryptophan residues in the protein. According to Förster's non-radiative energy transfer theory [19], the rate of energy transfer depends on (i) the relative orientation of the donor and acceptor dipoles, (ii) the extent of overlap of fluorescence emission spectrum of the donor with the absorption spectrum of the acceptor and (iii) the distance between the donor and the acceptor. The energy transfer effect is related not only to the distance between the acceptor and donor, but also to the critical energy transfer distance, R_0, and the efficiency of energy transfer, E, was studied according to Förster's energy transfer theory. The value of E is calculated using the equation:

$$E = 1 - \frac{F}{F_0} = \frac{R_0^6}{R_0^6 + r^6} \quad (6)$$

where F and F_0 are the fluorescence intensities of BSA in the presence and absence of SMZ, r is the distance between acceptor and donor and R_0 is the critical distance when the transfer efficiency is

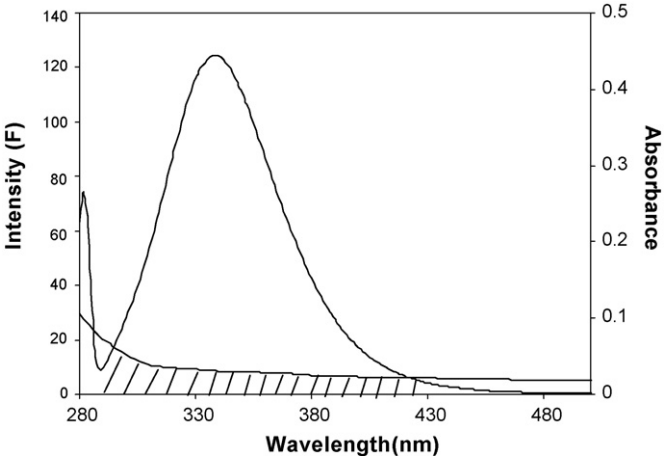

Fig. 4. The overlap of the fluorescence spectrum of BSA and the absorbance spectrum of SMZ (λ_{ex} = 280 nm, λ_{em} = 344 nm and c(BSA)/c(SMZ) = 1:1. The fluorescence spectrum of BSA (5 μM) (a) and the absorption spectrum of SMZ (5 μM) (b).

Table 3
Effect of polymers on binding constant of BSA–SMZ system.

Systems	Binding constant ($L\,mol^{-1}$)
BSA + SMZ	$1.529 \pm 0.003 \times 10^4$
BSA + SMZ + HAC	$6.343 \pm 0.002 \times 10^2$
BSA + SMZ + MC	$2.267 \pm 0.004 \times 10^3$
BSA + SMZ + PVA	$6.661 \pm 0.002 \times 10^2$
BSA + SMZ + GUARGUM	$4.428 \pm 0.006 \times 10^2$
BSA + SMZ + SODIUM ALGINATE	$1.701 \pm 0.002 \times 10^3$

50%. R_0^6 can be calculated by the equation:

$$R_0^6 = 8.8 \times 10^{-25} k^2 N^{-4} \Phi J \quad (7)$$

where k^2 is the spatial orientation factor of the dipole, N is the refractive index of the medium, Φ is the fluorescence quantum yield of the donor and J is the overlap integral of the fluorescence emission spectrum of the donor and the absorption spectrum of the acceptor. J is given by:

$$J = \frac{\sum F(\lambda)\varepsilon(\lambda)\lambda^4\ \Delta\lambda}{\sum F(\lambda)\,\Delta\lambda} \quad (8)$$

where $F(\lambda)$ is the fluorescence intensity of the fluorescent donor of wavelength, λ, $\varepsilon(\lambda)$ is the molar absorption coefficient of the acceptor at wavelength, λ. In the present case, $k^2 = 2/3$, $N = 1.336$ and $\Phi = 0.15$ [20]. From Eqs. (6)–(8) we could able to calculate that $J = 4.3 \times 10^{-15}\ cm^3\,L\,mol^{-1}$, $R_0 = 3.041$ nm, $E = 0.139$ and $r = 4.12$ nm. The donor-to-acceptor distance, $r < 7$ nm [8,20,21] indicated that the energy transfer from BSA to SMZ occurs with high possibility. Larger BSA–SMZ distance, r compared to that of R_0 value observed in the present study also reveals the presence of static type quenching mechanism to a larger extent [22].

3.5. The effects of common ions and polymers on the binding constant

The effect of common ions *viz.*, SO_4^{2-}, NO_3^-, CH_3COO^-, Mg^{2+}, Co^{2+}, K^+, Ni^{2+} and V^{5+} on the binding constant of SMZ–BSA system was investigated by recording the fluorescence intensity in the range of 300–500 nm upon excitation at 280 nm [20,23]. The fluorescence emission spectrum of SMZ in the presence of cation/anion shows that there is no interaction between the cation/anion and SMZ, but, there is a binding reaction between the cation/anion and BSA and thus the presence of cation/anion directly affects the binding between SMZ and BSA. As evident from Table 2, the presence of common ions reduced the SMZ–BSA binding constant, indicating SMZ to be quickly released from the blood, which may lead to the need for more doses of SMZ to achieve the desired therapeutic effect [24] in the presence of these common ions. Also the interaction between different polymers which are used in controlled drug delivery was studied to know their effect on BSA–SMZ system. It was observed that in the presence of polymer *viz.*, poly vinyl acrylate (PVA), methyl cellulose (MC), sodium alginate (Na-ALG), guargum and hydroxy ethyl cellulose (HAC), the binding constant of BSA–SMZ system was decreased (Table 3). This indicates that these polymers may compete for the same binding sites on BSA.

3.6. UV–vis absorption spectroscopy

UV–vis absorption measurement is a very simple method and applicable to know the change in hydrophobicity [15] and to know the complex formation [25]. In the present study, we have observed the change in UV absorption spectra of SMZ, BSA and SMZ–BSA system (Fig. 5). It is suggested possibly that the complex was formed between SMZ and BSA [18]. It is evident that the UV absorption intensity of BSA increased regularly with the variation of SMZ concentration. The maximum peak position of SMZ–BSA was shifted slightly towards lower wavelength region. The change in λ_{max} indicates the change in polarity around the tryptophan residue and the change in peptide strand of BSA molecules and hence the change in hydrophobicity [20]. These above observations signify that with the addition of SMZ, the peptide strands of BSA molecules were extended more and hydrophobicity was decreased [15].

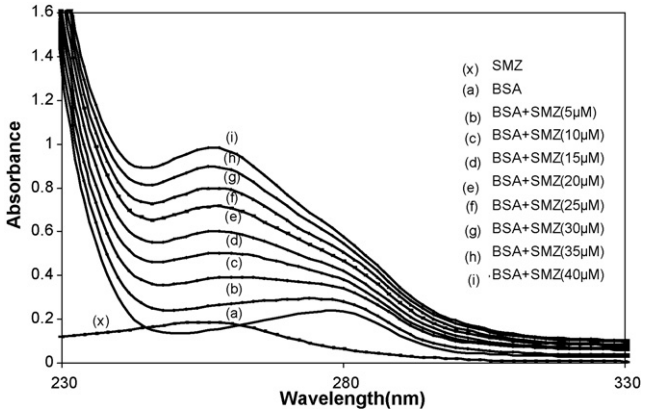

Fig. 5. Absorbance spectra of BSA, SMZ and BSA–SMZ system. BSA concentration was at 5 μM (a). SMZ concentration for SMZ–BSA system was at 5 μM (b), 10 μM (c), 15 μM (d), 20 μM (e), 25 μM (f), 30 μM (g), 35 μM (h) and 40 μM (i). A concentration of 10 μM SMZ (x) was used for SMZ only.

3.7. FT-IR measurements

Infrared spectra of proteins exhibit a number of amide bands due to different vibrations of the peptide moiety. The protein

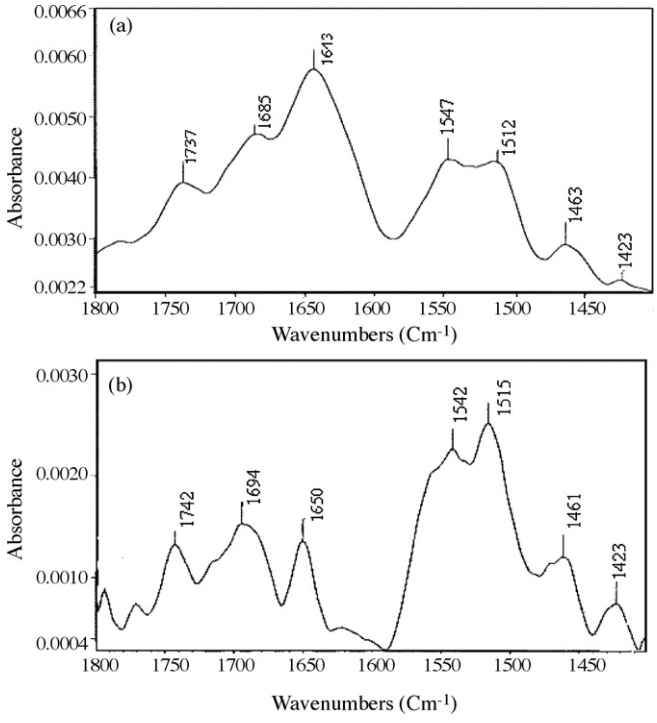

Fig. 6. FT-IR spectrum of BSA obtained by subtracting the spectrum of buffer (a) and FT-IR difference spectrum of BSA obtained by subtracting the spectrum of the SMZ + buffer from that of the SMZ-bound form in the region of 1800–1450 cm^{-1} at physiological pH (BSA: 5 μM; SMZ: 5.0 μM) (b).

amide I band ≈1653 cm^{-1} (mainly C=O stretch) and amide II band ≈1548 cm^{-1} (C–N stretch coupled with N–H bending mode) both have a relationship with the secondary structure of the protein. Of all the amide modes of the peptide group, the single most widely used one in studies of protein secondary structure is amide I, which is more sensitive to the change of protein secondary structure than amide II [25,26]. Fig. 6a and b shows the FT-IR spectra of the SMZ free and SMZ-bound form of BSA with its difference absorption spectrum. The spectrum in Fig. 6a was obtained by subtracting the absorption of phosphate buffer from the absorption of protein solution. Difference spectrum in present paper (Fig. 6b) was obtained by subtracting the absorption of the SMZ free + buffer from that of the SMZ-bound form. The peak position of amide I has remarkably changed from 1643 to 1650 cm^{-1} in the BSA infrared spectrum after interaction with SMZ and the appearance of new peaks in Fig. 6b indicates that SMZ can interact with BSA and the secondary structure of BSA has been changed because of the interaction of SMZ with BSA.

4. Conclusions

The present work provides an approach for studying the interactions of BSA with SMZ using absorption, fluorescence and FT-IR techniques under physiological conditions. The results show that BSA fluorescence was quenched by SMZ through static quenching mechanism. SMZ interacted with BSA through hydrogen bond and weak van der Waals forces. The decreased K values of SMZ–BSA complexes in the presence of common ions and polymers indicated that these common ions and polymers might compete for the same binding sites on BSA. This work also reports the distance between BSA and bound SMZ based on Forster's energy transfer theory. The remarkable change of amide I peak position in the BSA infrared spectrum after interaction with SMZ indicated that secondary structure of BSA has been changed. Since, the pharmaceutical firms need standardized screens for protein binding in the first step of new drug design, this kind of study of interaction between BSA with SMZ would be useful in pharmaceutical industry, life sciences and clinical medicine.

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References

[1] Y.J. Hu, Y. Liu, X.S. Shen, X.Y. Fang, S.S. Qu, J. Mol. Struct. 738 (2005) 143–147.
[2] B.P. Kamat, J. Pharm. Biomed. Anal. 39 (2005) 1046–1050.
[3] B.P. Kamat, J. Photosci. 12 (1) (2005) 11–15.
[4] J. Seetharamappa, B.P. Kamat, Chem. Pharm. Bull. 52 (9) (2004) 1053–1057.
[5] D.C. Cater, J.X. Ho, Adv. Protein Chem. 45 (1994) 153–203.
[6] R.E. Olson, D.D. Christ, Ann. Rep. Med. Chem. 31 (1996) 327–337.
[7] X.M. He, D.C. Carter, Nature 358 (1992) 209–215.
[8] M. Guo, J.W. Zou, P.G. Yi, Z.C. Shang, G.X. Hu, Q.S. Yu, Anal. Sci. 20 (2004) 465–470.
[9] G.R. Chatwal, Medicinal Chemistry, 1st ed., Himalaya Publishing House, New Delhi, 1998, p. 409.
[10] A. Sulkowska, J. Mol. Struct. 614 (2002) 227–232.
[11] J.R. Lakowica, Principles of Fluorescence Spectroscopy, 2nd ed., Plenum Press, New York, 1999, pp. 237–265.
[12] G.Z. Chen, X.Z. Huang, J.G. Xu, Z.Z. Zheng, Z.B. Wang, The Methods of Fluorescence Analysis, 2nd ed, Beijing Science Press, 1990, pp. 2–39.
[13] J.R. Lakowicz, G. Weber, Biochemistry 12 (1973) 4161–4170.
[14] S. Deepa, A.K. Mishra, J. Pharm. Biomed. Anal. 38 (2005) 556–563.
[15] H. Yan-Jun, Y. Liu, W.J. Bo, X.X. He, Q.S. Sheng, J. Pharm. Biomed. Anal. 36 (2004) 915–919.
[16] Y.C. Ying, A.X. Hou, L. Yi, T. Hui, S.S. Qu, J. Pharm. Biomed. Anal. 39 (2005) 263–267.
[17] D.P. Ross, S. Subramanian, Biochemistry 20 (1981) 3096–3102.
[18] S.M.T. Shaikh, J. Seetharamappa, P.B. Kandagal, D.H. Manjunath, S. Ashoka, Dyes Pigments 74 (2007) 665–671.
[19] T. Förster, in: O. Sinanoglu (Ed.), Modern Quantum Chemistry, vol. 3, Academic Press, New York, 1996, p. 93.
[20] B. Valeur, J.C. Brochon, New Trends in Fluorescence Spectroscopy, 6th ed., Springer Press, Berlin, 1999, p. 25.
[21] F.L. Cui, J. Fan, J.P. Li, Z. Hu, Bioorg. Med. Chem. 12 (2004) 151–157.
[22] F.L. Cui, J. Fan, D.L. Ma, M.C. Liu, X.G. Chen, Z. Hu, Anal. Lett. 36 (2003) 2151–2166.
[23] L. Ying, H. Wenying, L. Jiaqin, S. Fenling, Z. Hu, C. Xingguo, Biochim. Biophys. Acta 1722 (2005) 15–21.
[24] B. Shuyun, S. Daqian, T. Yuan, Z. Xin, L. Zhongying, Z. Hanqi, Spectrochim. Acta, Part A 61 (2005) 629–636.
[25] K.S. Witold, H.M. Henry, C. Dennis, Biochemistry 32 (1993) 389–394.
[26] W. He, Y. Li, C. Xuen, Z. Hu, X. Chen, F. Sheng, Bioorg. Med. Chem. 13 (2005) 1837–1845.