BioCHEMISTRY

An Indian Journal

Trade Science Inc.

Regular Paper

Interaction of moxifloxacin with bovine serum albumin and effect of the coexistent drugs on the reaction

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ABSTRACT

The interaction between moxifloxacin (MXFX) and bovine serum albumin (BSA) was investigated at different temperatures by fluorescence spectroscopy. Results showed that MXFX could quench the intrinsic fluorescence of BSA strongly, and the quenching mechanism was a static quenching process with Förester spectroscopy energy transfer. The electrostatic force played an important role on the conjugation reaction between BSA and MXFX. The order of magnitude of binding constants (K_a) was 10^4, and the number of binding site (n) in the binary system was approximately equal to 1. The binding distance (r) was less than 3 nm and the primary binding site for MXFX was located in sub-domain IIA of BSA. Synchronous fluorescence spectra clearly revealed that the microenvironment of amino acid residues and the conformation of BSA were changed during the binding reaction. In addition, the effect of some antibiotics on the binding constant of MXFX with BSA was also studied.   © 2011 Trade Science Inc. - INDIA

KEYWORDS

INTRODUCTION

Most drugs are able to bind to plasma protein when they entrance in blood plasma system of organism, and serum albumin is the most abundant protein in blood plasma and serves as a depot protein and transport protein for numerous endogenous and exogenous compounds[1]. Generally speaking, drugs could bind with serum albumin mostly through the formation of noncovalent complexes reversibly. The drug-protein complex can be regarded as a form of drug in the biology temporary storage, it can effectively avoid drug was eliminate from metabolism so quickly that it can main-

tain the total concentration and effective concentrations of blood medicine in plasma. In addition, binding of drugs to plasma proteins controls their free, active concentrations and provides a reservoir for a longer action, the binding of drugs is responsible for the protective role of albumin. Therefore, interaction of a drug with, and competition for, the binding sites on plasma proteins might strongly affect its distribution, elimination, as well as its pharmacodynamics and toxic properties[2]. Competition between two drugs for their binding to plasma protein can strongly affect the drug disposition of both drugs, with possible serious physiological consequences. Binding parameters are indeed funda-

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mental factors in determining the overall pharmacological activity of a drug and in this context the determination of the binding parameters of drugs to albumin has become essential to understand their pharmacokinetic, pharmacodynamic and toxicological profile.

Moxifloxacin (MXFX) is a new generation fluoroquinolone antibacterial agent, which is used to treat a wide variety of bacterial infections including pneumonia, sinusitis, and worsening of chronic bronchitis (the structure shown in Figure 1). This medicine gradually played a significant role in clinical cure. At present, the molecular interactions between BSA and many drugs have been investigated successfully in bio-medical domain[3]. However, the interaction between MXFX and BSA has not been investigated, especially the effect of the common antibiotics on the binding of drugs to BSA. In this report, we provided investigations on the interaction of MXFX with BSA by fluorescence spectroscopy under physiological pH 7.40 as well as the effect of the coexistent drugs. This study is expected to provide important insight into the essence, potential toxicity between drugs and protein in real terms, and can also provide a useful clinical reference for future combination therapy.
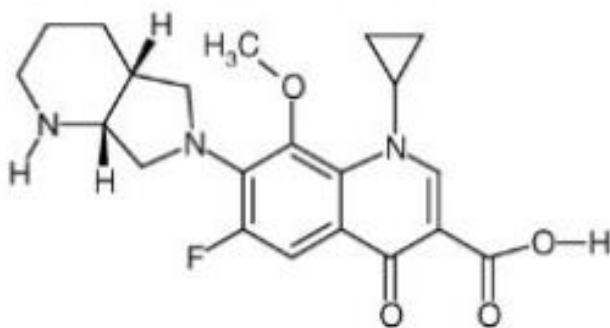

Figure 1: Chemical structure of moxifloxacin

EXPERIMENTAL

Apparatus

All fluorescence spectra were recorded with a Shimadzu RF-540 spectrofluorophotometer and a Hitachi F-4500 spectrofluorophotometer. Absorption was measured with an UV-vis recording spectrophotometer (UV-265 Shimadzu, Japan). All pH measurements were made with a pHS-3C precision acidity meter (Leici, Shanghai). All temperatures were controlled by a CS501 super-heated water bath (Nantong Science Instrument Factory).

Materials

Moxifloxacin (CAS#, 186826-86-8) was obtained from Monitor of Chinese Veterinary Medicine (no less than 99.9% pure). Bovine serum albumin (BSA, no less than 99% pure) was purchased from Sigma Company. Warfarin (WF), ibuprofen (IB) and digitoxin (DG) were all obtained from Chinese Institute of Drug and Biological Products. Stock solutions of BSA (1.0×10^{-4} M) and MXFX (2.0×10^{-3} M) were prepared. And all the stock solutions were further diluted as working solutions prior to use. All the common antibiotics were diluted to 1.0×10^{-3} M. Tris-HCl buffer solution containing NaCl (0.15 M) was used to keep the pH of the solution at 7.40. NaCl solution was used to maintain the ionic strength of the solution. All other reagents were of analytical grade and all aqueous solutions were prepared with newly double-distilled water and stored at 277K.

The fluorescence intensities were corrected for the absorption of excitation light and re-absorption of emitted light to decrease the inner filter using the following relationship[4]:

$$F_{cor} = F_{obs} \times e^{(A_{ex}+A_{em})/2} \qquad (1)$$

where F_{cor} and F_{obs} are the corrected and observed fluorescence intensities, respectively. A_{ex} and A_{em} are the absorbance values of MXFX at excitation and emission wavelengths, respectively. The fluorescence intensity used in this paper was corrected.

Procedures

Fluorescence experiments

In a typical fluorescence measurement, 1.0 mL of pH =7.40 Tris-HCl, 1.0 mL of 1.0×10^{-5} M BSA solution and different concentrations of MXFX were added into a 10 mL colorimetric tube successively. The samples were diluted to scaled volume with water, mixed thoroughly by shaking, and kept static for 15 min at different temperatures (293, 303 and 313 K). The excitation wavelength for BSA was 280 nm (or 295 nm), with the excitation and emission slit widths set at 5 nm. The solution was subsequently scanned on the fluorophotometer and determined the fluorescent intensity at 343 nm. The fluorescence intensity of BSA without MXFX was used as F_0. Meanwhile, the fluores-

cence intensity of compound without single MXFX ($F_{BSA-MXFX}$-F_{MXFX}) was used as F because MXFX has slight fluorescence emission at 343 nm. The fluorescence intensities in this paper were corrected by Eq.(1). Otherwise, we recorded the fluorescence spectra when the $\Delta\lambda$ value between the excitation and emission wavelengths was stabilized at 15 and 60 nm, respectively.

Probe displace experiments

At 293 K, different concentrations of probe drug WF (or IB, DG) were added to the mixture of MXFX-BSA systems. The molar ratio of MXFX and BSA was kept at 1, both of concentrations are 1.0×10^{-6} M in MXFX-BSA systems. The mixtures were diluted to scaled volume with water, mixed thoroughly by shaking, and kept static for 30 min. The excitation wavelength for BSA was 280 nm, with the excitation and emission slit widths set at 5 nm. The fluorescence intensity of BSA only with MXFX was used as F_0 to study the quenching effect of probe drugs to MXFX-BSA system.

RESULTS AND DISCUSSION

Fluorescence quenching spectra of BSA-MXFX system

Based on the fluorescence experiments marked 2.3.1, we have drawn the fluorescence spectra of BSA as well as different MXFX added into BSA respectively. When the excitation wavelengths were at 280 nm and 295 nm, the emission peaks for BSA were both located at 343 nm. In this part, we also found that MXFX had fluorescence at 462 nm obviously, but it had weak fluorescence at 343 nm. According to this phenomenon, we scanned the solution on the fluorophotometer with the range of 300-450 nm and recorded the fluorescence spectra of BSA-MXFX without MXFX which were shown in Figure 2. As can be seen from Figure 2, the fluorescence intensity of BSA decreased regularly with the addition of MXFX, and the maximum emission wavelength had 9 nm red shifts from 343 nm to 352 nm. This result implied that MXFX could quench the intrinsic fluorescence of BSA strongly, and the interaction between MXFX and BSA resulted from the formation of a ground-state complex between this drug and protein.

Fluorescence quenching mechanism of BSA-MXFX system
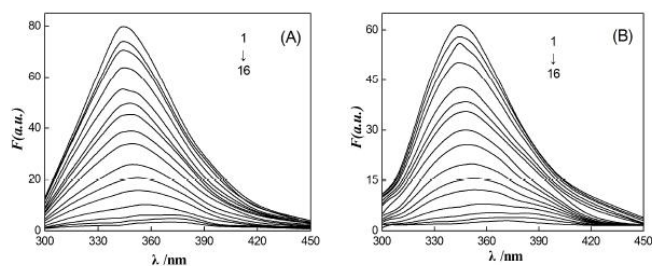
Fluorescence quenching can occur by different

Figure 2: Fluorescence spectra of BSA-MXFX system (T =293 K) C_{BSA} =1.0×10^{-6} M, 1~16 C_{MXFX} =(0.0, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.5, 15.0, 20.0, 25.0, 30.0, 40.0, 50.0, 60.0, 70.0)$\times 10^{-6}$ M; (A) λ_{ex} =280 nm; (B) λ_{ex} =295 nm

mechanisms, it may be dynamic quenching, resulting from the collisional encounter between the drug and protein, or static quenching, resulting from the formation of a ground-state complex between the drug and protein. In order to confirm the quenching mechanism, the fluorescence quenching data are analyzed by the Stern-Volmer equation[5]:

$$F_0 / F = 1 + K_q\tau_0[L] = 1 + K_{sv}[L] \qquad (2)$$

where F_0 and F are the fluorescence intensities in the absence and presence of ligand, respectively. τ_0 is the average lifetime of fluorescence without ligand, which is about 10^{-8} s. K_{sv} is the Stern- quenching constant. K_q is the quenching rate constant of biomolecule, and [L] is the concentration of the ligand. Based on the linear fit plot of F_0/F versus [L], the K_q values can be obtained. The calculated results were shown in TABLE 1. Higher temperature would result in faster diffusion and typically the dissociation of weekly bound complexes, leading to larger amount of dynamic quenching and smaller amounts of static quenching, respectively[6]. The Stern-Volmer plots of BSA-MXFX system at different temperatures were shown in Figure 3. It appeared that the values of K_{sv} decreased with the increase in temperature for all systems, which indicated that the probable quenching mechanism of the interaction between BSA and MXFX was initiated by complex formation rather than by dynamic collision[7]. In addition, all the values of K_q were much greater than the maximum scatter collision quenching constant of various quenchers (2×10^{10} M^{-1} s^{-1})[8], This also suggested that

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the quenching was a static process[9].

For static quenching process, the relationship between the fluorescence intensity and the concentration of quencher can be usually described by derived Eq.
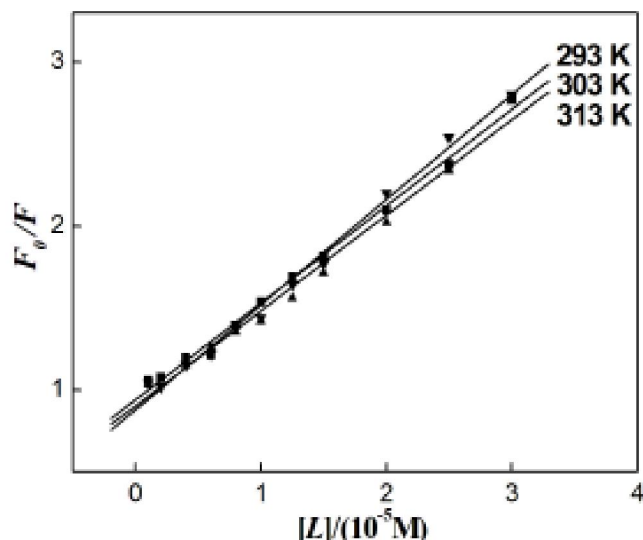

Figure 3: Stern-Volmer plots of BSA-MXFX system at different temperatures
$C_{BSA} = 1.0 \times 10^{-6}$ M, $C_{MXFX} = 1.0 \times 10^{-6} \sim 3.0 \times 10^{-5}$ M, $\lambda_{ex} = 280$ nm, $T = 293$ K

TABLE 1: Quenching reactive parameters of BSA and MXFX at different temperatures

λ_{ex}	T / K	K_q / ($M^{-1}s^{-1}$)	r_1	K_a / (M^{-1})	n	r_2
	293	5.81×10^{12}	0.9943	5.89×10^4	1.26	0.9971
280	303	5.49×10^{12}	0.9938	5.75×10^4	1.23	0.9903
	313	4.86×10^{12}	0.9951	5.62×10^4	1.24	0.9913
	293	6.35×10^{12}	0.9956	6.02×10^4	1.18	0.9992
295	303	6.12×10^{12}	0.9941	5.89×10^4	1.18	0.9937
	313	5.54×10^{12}	0.9960	5.75×10^4	1.43	0.9954

r_1 **is the linear relative coefficient of** F_0/F Å ‘[L]; r_2 **is the linear relative coefficient of** $\log(F_0-F)/F$ Å ‘$\log\{[D_t]-n[B_t](F_0-F)/F_0\}$. K_q **is the quenching rate constant;** K_a **is the binding constant;** n **is the number of binding site.**

(3)[10] to obtain the binding constant (K_a) and the number of binging sites (n) in most paper:

$$\log\left(\frac{F_0 - F}{F}\right) = n \log K_a + n \log\left\{[D_t] - n\frac{F_0 - F}{F_0}[B_t]\right\} \quad (3)$$

where $[D_t]$ and $[B_t]$ are the total concentrations of MXFX and protein, respectively. On the assumption that n in the bracket is equal to 1, the curve of $\log(F_0-F)/F$ versus $\log\{[D_t]-[B_t](F_0-F)/F_0\}$ is drawn and fitted linearly, then the value of n can be obtained from the slope of the plot. If the n value obtained is not equal

to 1, then it is substituted into the bracket and the curve of $\log(F_0-F)/F$ versus $\log\{[D_t]-n[B_t](F_0-F)/F_0\}$ is drawn again. The above process is repeated again and again till n obtained is only a single value or a circulating value. Based on the n obtained the binding constant K_a can be also obtained. In the work, a calculation program was developed. The calculation process can be finished with calculator based on the simple program and the calculating results can be obtained by inputting F, $[D_t]$ and $[B_t]$. The calculated results were shown in TABLE 1.

As seen in TABLE 1, the values of n were approximately equal to 1 at different temperatures, which indicated the existence of just one main binding site in BSA for MXFX. The order of magnitude of binding constants (K_a) was 10^4 indicated the existence of strong interaction between BSA and MXFX. Meanwhile, the values of K_a decreased with the increasing temperature, further suggested that the quenching was a static process[11], hence it led to the reduced of the stability of binary systems. In the other hand, comparing the data of 280 nm with 295 nm, values of K_a and n had less difference at different temperatures, it explained that MXFX would mainly quench the fluorescence of tryptophanyl residues from BSA.

Type of interaction force of the binary systems

Generally, the interaction forces between the small drug molecule and biological macromolecule include hydrogen bond, Van der Waals force, electrostatic interactions and hydrophobic force, etc[12]. Ross and Subramanian[13] have characterized the sign and magnitude of the thermodynamic parameter, enthalpy change (ΔH), free energy (ΔG) and entropy change (ΔS) of reaction, associated with various individual kinds of interaction. The reaction ΔH can be regarded as constant if the temperature changes little. Negative ΔH and positive ΔS indicate electrostatic interaction plays a major role in the binding reaction. Positive ΔH and ΔS are generally considered as the evidence for typical hydrophobic interactions. In addition, Van der Waals force and hydrogen bonding formation in low dielectric media are characterized by negative ΔH and ΔS[14]. The thermodynamic parameters can be calculated on the basis of the following equation:

$$R \ln K = \Delta S - \Delta H / T \quad (4)$$

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

According to the binding constants K_a of MXFX to BSA at different temperatures above (TABLE 1), the thermodynamic parameters were obtained conveniently. Therefore, the values of ΔH, ΔS and ΔG were -1.79 kJ mol^{-1}, 85.2 J mol^{-1} K^{-1}, -26.8 kJ mol^{-1} (T =293 K), respectively. The negative value of ΔG clarified an automatic reaction between MXFX and BSA. The negative value of ΔH and positive value of ΔS showed that MXFX mainly bound to BSA by the electrostatic attraction.

Identification of the binding sites of MXFX on the BSA

Participation of tyrosine (Tyr) and tryptophan (Trp) groups in drug-serum albumin complexes is assessed using different excitation wavelengths. At 280 nm wavelength the Trp and Tyr residues in serum albumin are excited, whereas the 295 nm wavelength excites only Trp residues. In the sub-domains of BSA, IIA subunit (Trp and Tyr) and IIIA (Tyr) are also considered as the main binding sites for small drug molecule[15]. Based on the Stern-Volmer equation, comparing the fluorescence quenching of protein excited at 280 nm and 295 nm allows to estimate the participation of Trp and Tyr groups in the complex[16]. As seen in Figure 4, the quenching curves of BSA excited at 280 nm and 295 nm in the presence of MXFX overlap below the molar ratio MXFX:BSA 80:1. This means that if there are fewer than 80 MXFX molecules for 1 BSA molecules, only Trp residues take part in the interaction of MXFX with BSA, and protein fluorescence was quenched by almost 80% at the molar ratio 80:1. The quenching of BSA fluorescence exited at 280 nm above this molar ratio was slightly higher than that excited at 295 nm which means that Tyr residues began to take part in the interaction. According to this conclusion, it could infer that MXFX molecules only take interaction with Trp residues of BSA at low concentration whereas both Trp and Tyr residues at high concentration, this conclusion can also expound why the value of n is slightly more than 1 which has been shown in TABLE 1.

BSA has two Trp moieties (Trp-134 and Trp-212) located in subdomains IB and IIA, respectively Å Cand only Trp-212 is located within a hydrophobic binding pocket of the protein which usually bind many small

ligands, especially heterocyclic ligands of average size and small aromatic carboxylic acids[17]. In this way, it could confirm that the primary binding site for MXFX was located in sub-domain IIA of BSA.
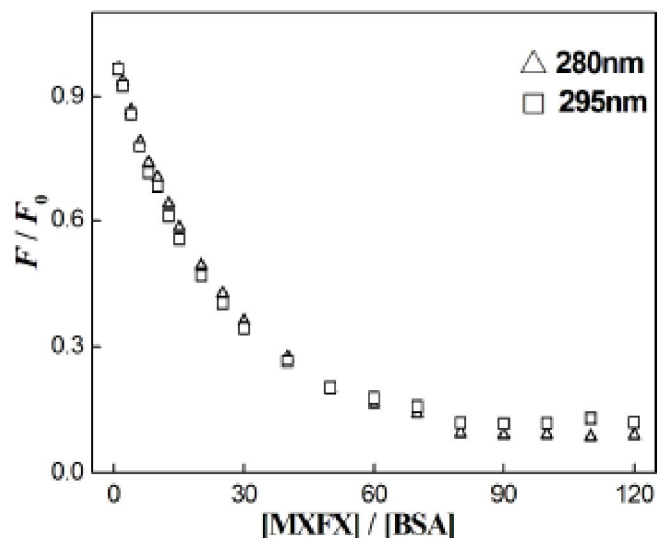

Figure 4: Quenching curves of BSA-MXFX system at λ_{ex} **=280 nm and 295 nm (** T **=293 K)**
$C_{BSA} = 1.0 \times 10^{-6}$ M, $C_{MXFX} = 1.0 \times 10^{-6} \sim 1.2 \times 10^{-4}$ M

Site marker fluorescent probes are useful for rapid identification of drug binding sites[18, 19]. In order to estimate the drug binding sites on BSA, Sudlow et al. has been proposed the percentage of probe replacement method using the following equation[20]:

$$\text{probe displacement}(\%) = F_2 / F_1 \times 100 \qquad (6)$$

The concentrations of BSA and MXFX were kept constant (1×10^{-6} M), while that different concentrations of various probe drugs each were added into it. In the study of the substitution reaction, F_1 and F_2 are the fluorescence intensities in the absence and presence probe drug. In this paper, we have considered WF, CP and DG as probe durgs which bind specifically to sites I, II and III, respectively. The processed results were shown in Figure 5 by Eq.(6).

λ_{ex} =280 nm; T =293 K

As seen in Figure 5, relative fluorescence intensities decreased obviously with increasing concentration of WF whereas it almost kept constant with the adding of CP and DG. It indicated that MXFX was pronouncedly displaced by WF rather than CP or DG, hence it could be inferred that the binding site for MXFX was site I which located in sub-domain IIA of BSA.

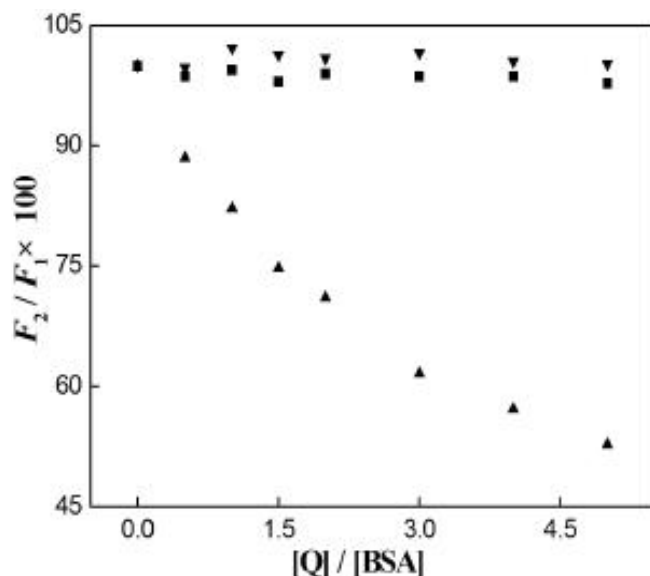At room temperature (293 K), control the con-

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Figure 5: Effect of site maker probe on the fluorescence intensities of BSA-MXFX system.(T =293 K)
$C_{MXFX} = C_{BSA}$ = 1.0×10^{-6} M; C_Q = (0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0)×10^{-6}M. [Q]:?WF;¦CP;?DG;

centrations of BSA and various probe drugs each at 1×10^{-6} M. According to the method has described in 2.3.1, successively adding different concentrations of MXFX. The experimental data was calculated by Eq.(3). The values of binding constants K_a were 4.68×10^4, 5.50×10^4 and 5.37×10^4 M^{-1} in presence of WF, CP and DG, respectively. Compared with the binding constant of MXFX-BSA without any probe drug (5.89×10^4 M^{-1}), it can be seen that the binding constant between MXFX and BSA decreased most obviously in the presence of WF, this result indicated that binding of MXFX with BSA was affected by adding WF, which compete the same binding site of BSA. Hence, it also can be proposed that the binding of MXFX primarily occurred in sub-domain IIA (site I).

Hill's coefficient of BSA-MXFX system

In biochemistry, the binding of a ligand molecule at one site of a macromolecule often influences the affinity for other ligand molecules at additional sites. This is known as cooperative binding. It is classified into positive cooperativity, negative cooperativity and non-cooperativity according to the promotion or inhibition to the affinity for other ligand molecules. Hill's coefficient provides a way to quantify this effect and is calculated graphically on the basis of the following equation[21]:

$$\lg \frac{Y}{1-Y} = \lg K + n_H \lg[L] \qquad (7)$$

where Y is the fractional binding saturation; K is the binding constant and n_H is the Hill's coefficient. Hill's coefficient is greater than one, which exhibits positive cooperativity. Conversely, Hill's coefficient is less than one, which exhibits negative cooperativity. A coefficient of 1 indicates non-cooperative reaction.

For fluorescence measurement:

$$\frac{Y}{1-Y} = \frac{Q}{Q_m - Q} \qquad (8)$$

where $Q = (F_0 - F)/F_0$; $1/Q_m$ = intercept of the plot $1/Q$ versus $1/[L]$. Hill's coefficients were presented in TABLE 2. The values of n_H were slightly less than 1 in the systems both at excitation wavelengths 280 nm and 295 nm at different temperatures, which indicated negative cooperativeness in the interaction of MXFX with BSA, but they were weak. In addition, the values of n_H were inversely correlated with increasing temperature illustrated the existence of negative cooperativity between MXFX and BSA, that was, the ability of drug bounding to BSA has decreased with the previous ligand (MXFX) bounding to BSA gradually. Furthermore, this negative cooperativity became more powerful with increasing temperature, reducing the amount of MXFX that could be bound to BSA. It was also one of the reasons which led to the reduced K_a with increasing temperature.

Binding distances between MXFX and BSA

TABLE 2 : Hill's coefficients n_H of BSA-MXFX at different temperatures

T / K	λ_{ex} / 280 nm		λ_{ex} / 295 nm	
	n_H	r_3	n_H	r_3
293	0.89	0.9938	0.86	0.9932
303	0.74	0.9959	0.72	0.9953
313	0.70	0.9913	0.69	0.9906

r_3 **is the linear relative coefficient of lg[Y/(1–Y)]~lg[L].**

Fluorescence energy transfer is categorized as radiation energy transfer and non-radiation energy transfer. The fluorescence spectrum will deform if radiation energy transfer occurred. The fluorescence spectrum in Figure 1 has no deformation, thus the energy transfer of BSA and MXFX should be non-radiation energy

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transfer.

According to Förster's non-radiative energy transfer theory, energy efficiency E, critical energy-transfer distance R_0 ($E = 50\%$), the energy donor and the energy acceptor distance r and the overlap integral between the fluorescence emission spectrum of the donor and the absorption spectrum of the acceptor J can be calculated by the formulas[22]:

$$E = 1 - F / F_0 = R_0^6 / (R_0^6 + r^6) \tag{9}$$

$$R_0^6 = 8.78 \times 10^{-25} K^2 \Phi N^{-4} J \tag{10}$$

$$J = \sum F(\lambda)\varepsilon(\lambda)\lambda^4 \Delta\lambda / \sum F(\lambda)\Delta\lambda \tag{11}$$

where K^2 is the orientation factor, Φ is the fluorescence quantum yield of the donor, N is a refractive index of the medium, $F(\lambda)$ is the fluorescence intensity of the fluorescence donor at wavelength λ and $\varepsilon(\lambda)$ is the molar absorption coefficient of the acceptor at this wavelength. The overlap of UV-vis absorption spectra of MXFX and the fluorescence emission spectra of BSA ($\lambda_{ex} = 280$ nm) were shown in Figure 6. Under these experimental conditions, it has been reported that $K^2 = 2/3$, $N = 1.336$ and $\Phi = 0.118$[23]. Thus J, E, R_0 and r were calculated and shown in TABLE 3. The donor-to-acceptor distance $r < 7$ nm indicated that the energy transfer from BSA to MXFX occured with high possibility[24]. Further the value of r was greater than R_0 in this study which suggested that MXFX could strongly quench the intrinsic fluorescence of BSA by a static quenching mechanism[25]. Moreover, the distance r increased and the energy efficiency E decreased with increasing temperature (TABLE 3), which resulted in the reduced stability of the binary systems and the values of K_a.

Conformation Investigation of BSA

Synchronous fluorescence spectra are used to investigate the protein conformational change, as it has been shown to give narrow and simple spectra. For the synchronous fluorescence spectra of protein, when the $\Delta\lambda$ value between the excitation and emission wavelengths is stabilized at either 15 or 60 nm, the synchronous fluorescence gives characteristic information for Tyr residues or Trp residues[26]. Because of the red shifts of maximum emission wavelengths of both Tyr and Trp with the less hydrophobic environment, blue shifts of maximun emission wavelengths with the more hydro-
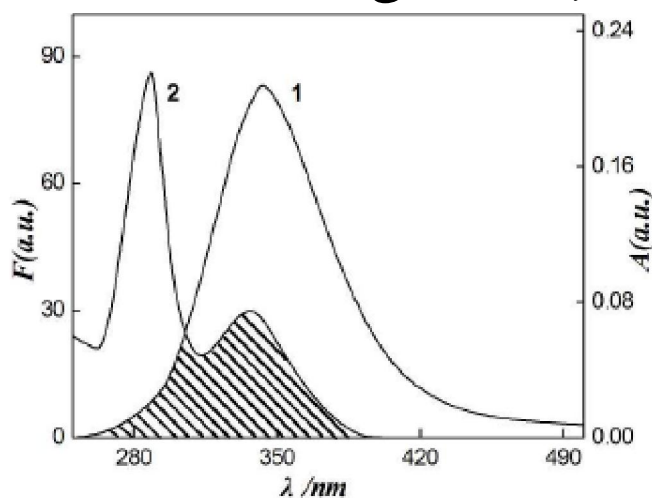

Figure 6: Fluorescence emission spectra for BSA (1) and UV absorbance spectra for MXFX (2) $T = 293$ K; $C_{MXFX} = C_{BSA} = 6.0 \times 10^{-6}$ M

TABLE 3 : Parameters of E, J, r, R_0 between MXFX and BSA at different temperatures

T / K	E (%)	J / (M^{-1} cm^3)	R_0 / nm	r / nm
293	28.04	1.07×10^{-14}	2.48	2.90
303	26.66	1.04×10^{-14}	2.47	2.92
313	25.60	1.02×10^{-14}	2.46	2.95

R_0 is the critical distance when E is 50%; r is the distance between acceptor and donor; J is the overlap integral between the fluorescence emission spectrum of donor and the absorption spectrum of the acceptor.

phobic environment. These red or blue shifts indicated that the conformation of BSA has been changed[27]. In order to further study the effect on the conformation of BSA, the synchronous fluorescence spectra were measured when the $\Delta\lambda = 15$ nm and $\Delta\lambda = 60$ nm as shown in Figure 7.
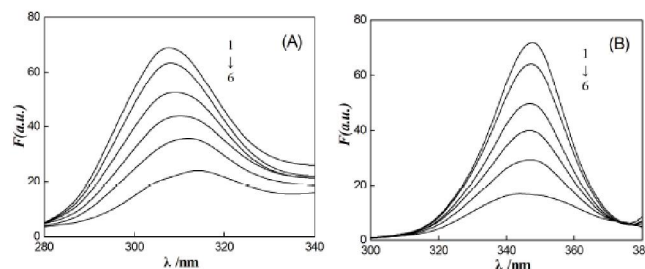
It can be seen from Figure 7 that the fluorescence

Figure 7: Synchronous fluorescence spectra of BSA-MXFX system ($T = 293$ K)
$C_{BSA} = 1.0 \times 10^{-6}$ M, 1 ~ 5 $C_{MXFX} = (0.0, 2.0, 6.0, 10.0, 15.0, 25.0) \times 10^{-6}$ M; (A) $\Delta\lambda = 15$ nm; (B) $\Delta\lambda = 60$ nm

intensities of Tyr and Trp residues decreased regularly with increasing concentration of MXFX. At the same

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time, the maximum emission wavelength had 4 nm blue shifts at the investigated concentrations range when $\Delta\lambda$ =60 nm, while the maximum emission wavelength had 7 nm red shifts when $\Delta\lambda$ =15 nm. This revealed that the amino acids microenvironment changed due to the reaction of MXFX and BSA, making the hydrophobicity of Trp residues strengthened whereas the hydrophobic environment of Tyr residues more polar. High concentration of drugs makes protein molecules extend, thus reducing energy transfer between amino acids, reducing the fluorescence intensity[28].

Effect of the coexistent drugs on the binding of MXFX and BSA

According to this method about the percentage of probe replacement method described in 3.4, we have taken assays to study the effects of some common antibiotics on the binding of BSA-MXFX system. The concentrations of BSA and MXFX were kept constant (1×10^{-6} M), while that different concentrations of various antibiotics each were added into it. In the study of the substitution reaction, F_1 and F_2 are the fluorescence intensities in the absence and presence antibiotice. It supposed that there was no effect on the binding of BSA-MXFX system when the value of $(F_2-F_1)/F_1$ was less than ±5% by Eq.(6), that was, this antibiotice could coexist with MXFX. The results showed that it allowed 500 times of gentamicin, thiamphenicol, 300 times of sulfamethoxazole, erythromycin and streptomycin, 100 times of ampicillin, neomycin and kanamycin, 20 times of penicillin G sodium salt and acetyl spiramycin, and 10 times of cefotaxime sodium, cefoperazone sodium, ceftriaxone and chloramphenicol to coexist with MXFX, but a bit of ciprofloxacin, norfloxacin could disturb the stability of BSA-MXFX system, making the fluorescence intensity changed a lot. It might result that MXFX and ciprofloxacin, norfloxacin all belong to a class of drugs called quinolone antibiotics, having much similar structures, hence, there were certain competition among them for the protein. The competition would lead to binding constant K_a changed spontaneously. It could infer that there were possible effects on the stayed-time from the blood, as well as plasma concentration and effective concentration, having effects on the efficacy of drugs when the antibiotics such as ciprofloxacin and norfloxacin coexist with MXFX on the binding reaction

of BSA. However, aminoglycosides, macrolides, sulfonamides and penicillins antibiotics have no effects on the binary system, that is, those antibiotics will not affect the transportation of MXFX though BSA when they coexist with MXFX, and also have no effects on the efficacy of MXFX.

CONCLUSIONS

In this paper, the interaction of MXFX with BSA was studied at different temperatures by fluorescence spectroscopic methods. The experimental results indicated that MXFX molecular had embedded into the hydrophobic cavity through electrostatic force and was mainly bound to Trp-212 in BSA. The donor-to-acceptor distance r was less than 7 nm, indicating non-radiation energy transfer. The negative cooperativity existed in BSA-MXFX system for subsequent ligand. From the synchronous fluorescence spectra, it could be shown that the quaternary conformational change of BSA was induced by the interaction of MXFX with the Trp micro-region of BSA molecules. The method liked percentage of probe replacement clearly revealed the existence of effects of common antibiotics on the binding of BSA-MXFX system. The study will extend the use of fluorescence spectroscopy; meanwhile, it provides important insights into the future clinical medicine.

ACKNOWLEDGEMENTS

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