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A comparative-study on the interaction between moxifloxacin and bovine serum albumin / bovine hemoglobin

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Abstract : The interaction between moxifloxacin (MXFX) and Bovine serum albumin (BSA) or Bovine Hemoglobin (BHb) was investigated at different temperatures by fluorescence spectroscopy. Results showed that the quenching mechanism of MXFX on BSA/BHb was a static quenching process with Förester spectroscopy energy transfer. MXFX could bind to protein through electrostatic force especially BSA. 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 primary binding site for MXFX was located at Trp-212 in sub-domain IIA of BSA and β-37 Tryptophan residue in hydrophobic cavity of BHb, respectively. The binding distance (r) was less than 3 nm. In addition, the values of n_H were slightly less than 1 which indicated negative cooperativeness in the interaction of MXFX with protein. Synchronous fluorescence spectra clearly revealed that the microenvironment of amino acid residues and the conformation of protein were changed during the binding reaction.

Keywords : Bovine serum albumin; Bovine hemoglobin; Moxifloxacin; Fluorescence.

INTRODUCTION

Serum albumins are the most extensively studied and applied proteins because of their availability, low cost, stability and unusual ligand binding properties. For this reason, a huge number of papers dealing with albumins have been reviewed so far[1,2]. 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[3]. 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 maintain the total concentration and effective concentrations of blood medicine in plasma. In addition, binding of drugs to plasma proteins con-

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trols 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 proteins might strongly affect its distribution, elimination, as well as its pharmacodynamics and toxic properties[4]. Bovine serum albumin (BSA) is a single-chain 582 amino acid globular nonglycoprotein crosslinked with 17 cystine residues (eight disulfide bonds and one free thiol)[5]. It has three homologous domains (I, II and III), each divided into two subdomains (A and B), and in the pocket IIA and IIIA the majority of small ligands are bound[6-9]. Hemoglobin (Hb), the major protein component in erythrocytes, exists as a tetramer of globins chains that is composed of two α and two β subunits; Hb is well known for its function in the vascular system of animals, being a carrier of oxygen. It also aids, both directly and indirectly, the transport of carbon dioxide and regulates the pH of blood[10]. Bovine hemoglobin (BHb), which shares 90% amino acid sequence homology with human hemoglobin, has a few advantages over its human counterpart. BHb is a better oxygen carrier than human hemoglobin[11]. It is a tetrameric protein with 574 amino acid residues, consisting of two identical α-chains of 141 amino acids each and two identical β-chains of 146 amino acids each. In addition, the α1β2 subunit interface is considered to play a pivotal role in the quaternary structure transition[12]. Studying the binding mechanism is much important for life science, chemical, pharmaceutical and clinical medicine.

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 single protein and many drugs have been investigated successfully in bio-medical domain[13]. However, a comparative-study on the interaction of two proteins has not been investigated. In this report, we provided investigations on the interaction of MXFX with two proteins respectively by fluorescence spectroscopy under physiological pH 7.40. 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.

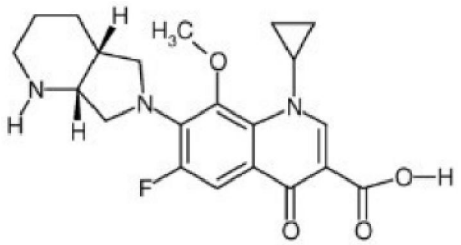

Figure 1 : Chemical structure of moxifloxacin

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). BSA and BHb were purchased from Sigma Company (no less than 99% pure). Stock solutions of BSA (1.0×10^{-4} mol/l), BHb (1.0×10^{-4} mol/l) and MXFX (2.0×10^{-3} mol/l) were prepared. And all the stock solutions were further diluted as working solutions prior to use. Tris-HCl buffer solution containing NaCl (0.15 mol/l) 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[14]:

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

where F_{cor} and F_{obs} are the corrected and observed fluorescence intensities, respectively. A_{ex} and A_{em} are

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the absorbance values of MXFX at excitation and emission wavelengths, respectively. The fluorescence intensity used in this paper was corrected.

Procedures

In a typical fluorescence measurement, 1.0 mL of pH =7.40 Tris-HCl, 1.0 mL of 1.0×10^{-5} mol/l BSA (or 4.0×10^{-5} mol/l BHb) 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 protein 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 for BSA (340 nm for BHb). The fluorescence intensity of protein without MXFX was used as F_0. Meanwhile, the fluorescence intensity of compound without single MXFX ($F_{\text{Protein-MXFX}}$-F_{MXFX}) was used as F because MXFX has slight fluorescence emission at 343 nm and 340 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.

RESULTS AND DISCUSSION

Fluorescence quenching in protein-MXFX system

Based on the fluorescence experiments marked 2.3, we have drawed the fluorescence spectra of protein (BSA or BHb) as well as different MXFX added into protein (BSA or BHb) respectively. When the excitation wavelengths were at 280 nm and 295 nm, the emission peaks for BSA were both located at 343 nm (340 nm for BHb). In this part, we also found that MXFX had fluorescence at 462 nm obviously, but it had weak fluorescence at 343 nm and 340 nm. According to this phenomenon, we scanned the solution on the fluorophotometer with the range of 300-450 nm for BSA (315-390 nm for BHb) and recorded the fluorescence spectra, the fluorescence spectra of BSA-MXFX without MXFX which were shown in Figure 2, it was similar for BHb-MXFX system. 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. Meanwhile, the fluorescence intensity of BHb decreased regularly and there was almost no shift of the emission wavelength with the addition of MXFX. This result implied that MXFX could quench the intrinsic fluorescence of protein strongly, and the interaction between MXFX and protein resulted from the formation of a ground-state complex between this drug and protein.

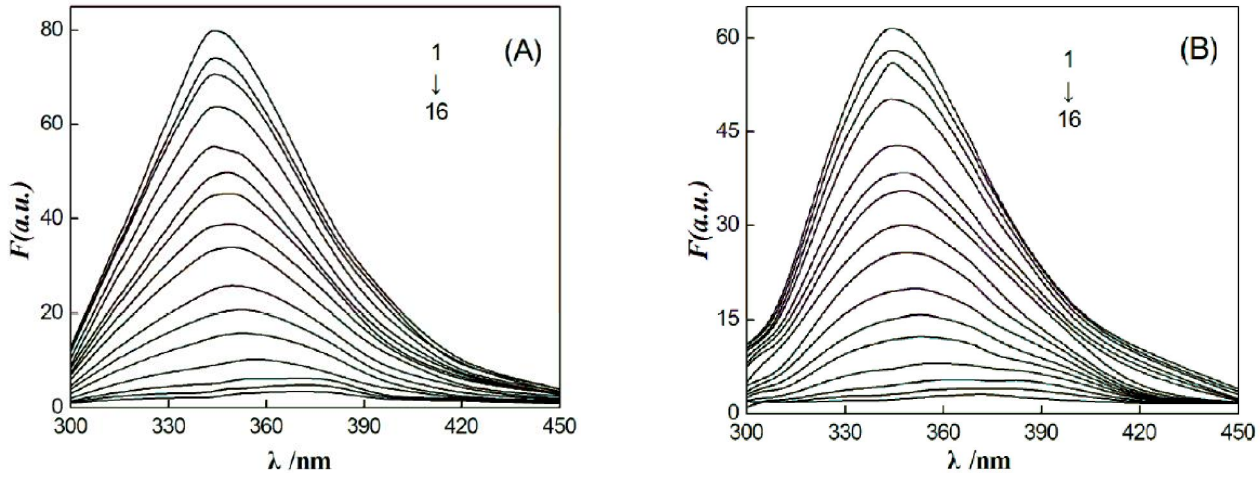

Figure 2: Fluorescence spectra of BSA-MXFX system (T =293 K). C_{BSA} =1.0×10^{-6} mol/l, 1~16 C_{MXFX} = (0.0, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.5, 15.0, 20.0, 25.0, 30.0, 40.0, 50.0, 60.0, 70.0) × 10^{-6} mol/l; (A) λ_{ex} =280 nm; (B) λ_{ex} =295 nm

Fluorescence quenching mechanism of protein-MXFX system

In order to confirm the quenching mechanism, the fluorescence quenching data are analyzed by the Stern-

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Volmer equation[15]:

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

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-Volmer 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[16]. 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 protein and MXFX was initiated by complex formation rather than by dynamic collision[17]. In addition, all the values of K_q were much greater than the maximum scatter collision quenching constant of various quenchers (2×10^{10} l mol^{-1} s^{-1}), this also suggested that the quenching was a static process[18]. For static quenching process, the relationship between the fluorescence intensity and the concentration of quencher can be usually described by Eq.(3) to obtain the binding constant (K_a) and the number of binging sites (n) in most paper[19]:

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

where [D_f] is the free concentration of ligand. In order to calculate conveniently, the free ligand concentration is often replaced by the total concentration in processing the data, because it is difficult to obtain during the experiment. Thus it must affect the accuracy of the results. In view of this situation, we used Eq.(4)[20] to describe, which is more accurate:

$$\log\left(\frac{F_0 - F}{F}\right) = n \log K_a + n \log\left\{\left[D_t\right] - n\frac{F_0 - F}{F_0}\left[B_t\right]\right\} \quad (4)$$

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 $\log(F_0\text{-}F)/F$ versus $\log\{[D_t]\text{-}[B_t](F_0\text{-}F)/F_0\}$ 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 $\log(F_0\text{-}F)/F$ versus $\log\{[D_t]\text{-}n[B_t](F_0\text{-}F)/F_0\}$ 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.

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

λ_{ex}	T / K	K_q / (l mol^{-1} s^{-1})	r_1	K_a / (l mol^{-1})	n	r_2
280	293	2.81×10^{12}	0.9935	2.78×10^{4}	0.95	0.9945
	303	2.46×10^{12}	0.9964	2.38×10^{4}	0.82	0.9932
	313	2.00×10^{12}	0.9919	1.86×10^{4}	0.77	0.9914
295	293	3.41×10^{12}	0.9959	3.43×10^{4}	1.02	0.9970
	303	2.52×10^{12}	0.9977	2.79×10^{4}	0.88	0.9935
	313	2.04×10^{12}	0.9950	2.34×10^{4}	0.81	0.9947

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

As seen in TABLE 1, the values of n were approximately equal to 1 at different temperatures, which indicated the existence of just a main binding site in protein for MXFX. The order of magnitude of binding constants (K_a) was 10^4 indicated the existence of strong interaction between protein and MXFX. Meanwhile, the values of K_a decreased with the increasing temperature, further suggested that the quenching was a static process[21], 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

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MXFX would mainly quench the fluorescence of Trp from protein. In addition, values of K_a were greater for BSA-MXFX system, than that for BHb-MXFX system. It was evident that MXFX could bind to BSA as well as BHb, but the binding ability is stronger for BSA, than that for BHb. It was also proved that BSA is the main transport carrier for drugs in blood plasma protein of organism.

Type of interaction force of protein-MXFX systems

Ross and Subramanian[22] 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[23]. The thermodynamic parameters can be calculated on the basis of the following equation:

$$\mathbf{R \ln K = \Delta S - \Delta H / T} \quad \mathbf{(5)}$$

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

According to the binding constants K_a of MXFX to protein at different temperatures above (TABLE 1), the thermodynamic parameters were obtained conveniently. Therefore, the values of ΔH, ΔS and ΔG for BSA-MXFX system 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[24].

For BHb-MXFX system, the values of ΔH, ΔS and ΔG were -1.67 kJ mol^{-1}, 88.5 J mol^{-1} K^{-1}, -27.6 kJ mol^{-1} (T =293 K), respectively. The negative value of ΔG clarified an automatic reaction between MXFX and BHb. The negative value of ΔH and positive value of ΔS showed that MXFX mainly bound to BHb by the electrostatic attraction.

Identification of the binding sites of MXFX on the protein

Participation of tyrosine (Tyr) and tryptophan (Trp) groups in drug-protein complexes is assessed using different excitation wavelengths. At 280 nm wavelength the Trp and Tyr residues in protein are excited, whereas the 295 nm wavelength excites only Trp residues. 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[25].

As seen in Figure 3, the quenching curves of protein excited at 280 nm and 295 nm in the presence of MXFX overlap below the molar ratio MXFX:BSA at 80:1 and MXFX:BHb 10:1. This phenomenon showed that Trp residue played an important role in the interaction of MXFX with protein. Tyr residue might take part in the interaction on the high concentration of MXFX; however, it is slighter than Try residue.

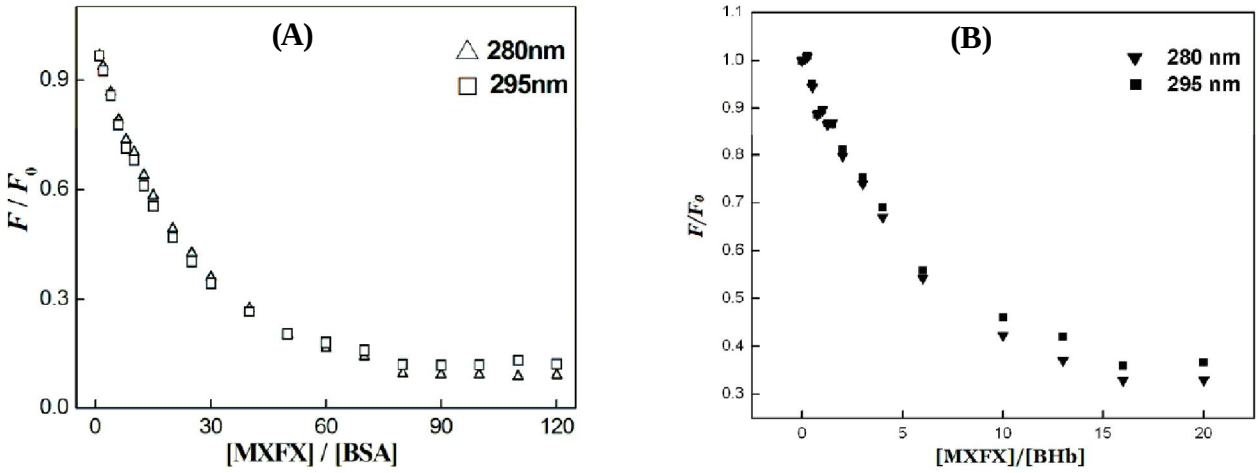

Figure 3 : Quenching curves of Protein-MXFX system at λ_{ex}=280 nm and 295 nm (T =293 K). (A): C_{BSA} = 1.0×10^{-6} mol/l, C_{MXFX} = 1.0×10^{-6} ~ 1.2×10^{-4}mol/l; (B): C_{BHb} = 4.0×10^{-6} mol/l, C_{MXFX} = 1.0×10^{-6} ~ 8.0×10^{-5} mol/l.

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For BSA, there were two Trp moieties (Trp-134 and Trp-212) located in subdomains IB and IIA, respectively, and 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[26]. In this way, it could confirm that the primary binding site for MXFX in BSA was located at Trp-212 in sub-domain IIA of BSA. Meanwhile, Hb is a tetrameric protein composed of two α and two β subunits. There are three Tyr and one Trp residues in α subunit, three Tyr and two Trp residues in β subunit, respectively. α-14Trp and β-15Trp expose to the subunit surface. However, β-37Trp, α-42Tyr, α-140Tyr, and β-145Tyr are located at the α1β2 subunit interface remained invariant throughout the evolution of α and β subunits, they are indispensable for the maintenance of structure and function of Hb, and are also considered as the main binding sites for small drug molecule[27]. Therefore, β-37Trp residue in BHb was confirmed the primary binding site for MXFX in BHb.

Hill's coefficient of protein-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[28]:

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

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:

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

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 protein, 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 protein, that was, the ability of drug bounding to protein has decreased with the previous ligand (MXFX) bounding to protein gradually. Furthermore, this negative cooperativity became more powerful with increasing temperature, reducing the amount of MXFX that could be bound to protein. It was also one of the reasons which led to the reduced K_a with increasing temperature. Comparing the values of n_H of BSA-MXFX system with BHb-MXFX system, it can infered that the negative cooperativity of BSA-MXFX system is more powerful than BHb-MXFX system, which resulted from the more stable binding ability for BSA-MXFX system.

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

T / K	λ_{ex} / 280 nm		λ_{ex} / 295 nm	
	n_H	r_3	n_H	r_3
293	0.987	0.9931	0.958	0.9925
303	0.955	0.9933	0.897	0.9927
313	0.709	0.9910	0.669	0.9908

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

Binding distances between MXFX and protein

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[29]:

$$E = 1 - F/F_0 = r_0^6/(r_0^6 + r^6) \quad (9)$$

$$r_0^6 = 8.78\times10^{-25} K^2 \Phi N^{-4} J \quad (10)$$

$$J = \sum F(\lambda)\varepsilon(\lambda)\lambda^4\Delta\lambda / \sum F(\lambda)\Delta\lambda \quad (11)$$

where K^2 is the orientation factor, Φ is the fluorescence quantum yield of the donor, N is a refractive

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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 (λ_{ex} =280 nm) were shown in Figure 4, it was similar for MXFX and BHb. Under these experimental conditions, it has been reported that K^2 =2/3, N =1.336 and Φ =0.118 for BSA[30], Φ =0.062 for BHb[31], respectively. 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 protein to MXFX occured with high possibility[32]. Further the value of r was greater than r_0 in this study which suggested that MXFX could strongly quench the intrinsic fluorescence of protein by a static quenching mechanism[33]. 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.

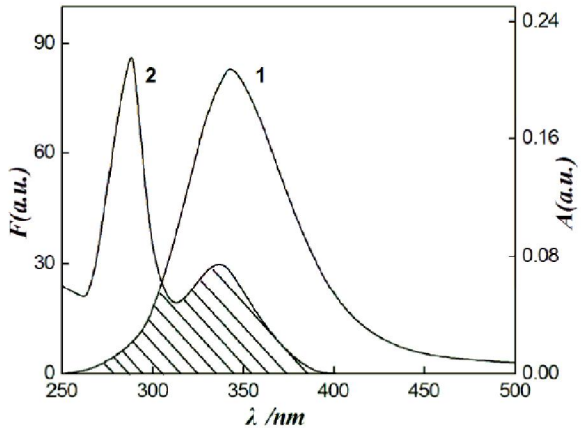

Figure 4 : Fluorescence emission spectra for BSA (1) and UV absorbance spectra for MXFX (2). T = 293 K; $C_{MXFX} = C_{BSA}$ =6.0×10^{-6} mol/l.

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

T / K	E (%)	J / (l mol^{-1} cm^3)	R_0 / nm	r / nm
293	23.13	1.41×10^{-14}	2.33	2.84
303	22.55	1.40×10^{-14}	2.32	2.86
313	21.68	1.36×10^{-14}	2.31	2.87

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.

Conformation investigation of protein

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[34]. 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 hydrophobic environment. These red or blue shifts indicated that the conformation of protein has been changed[35].

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 5. It can be seen from Figure 5 that the fluorescence intensities of Tyr and Trp residues in BSA decreased regularly with increasing concentration of MXFX. At the same 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[36].

It was similar to BSA-MXFX system, the fluorescence intensities of Tyr and Trp residues in BHb decreased regularly with increasing concentration of MXFX. At the same time, the maximum emission wavelength kept the position at the investigated concentrations range when $\Delta\lambda$ =15 nm, while the maximum emission wavelength had an obvious blue shift when $\Delta\lambda$ =60 nm. It revealed that the amino acids microenvironment also changed due to the reaction of MXFX and BHb, that was, MXFX forced into the hydrophobic cavity of BHb through electrostatic attraction and mainly quenched the fluorescence of β-37Trp. All of these led

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to the polarity around Trp residues weakened and the hydrophobicity strengthened, making the quaternary structure of BHb change, thereby affecting the oxygen-carrying function of BHb[37].

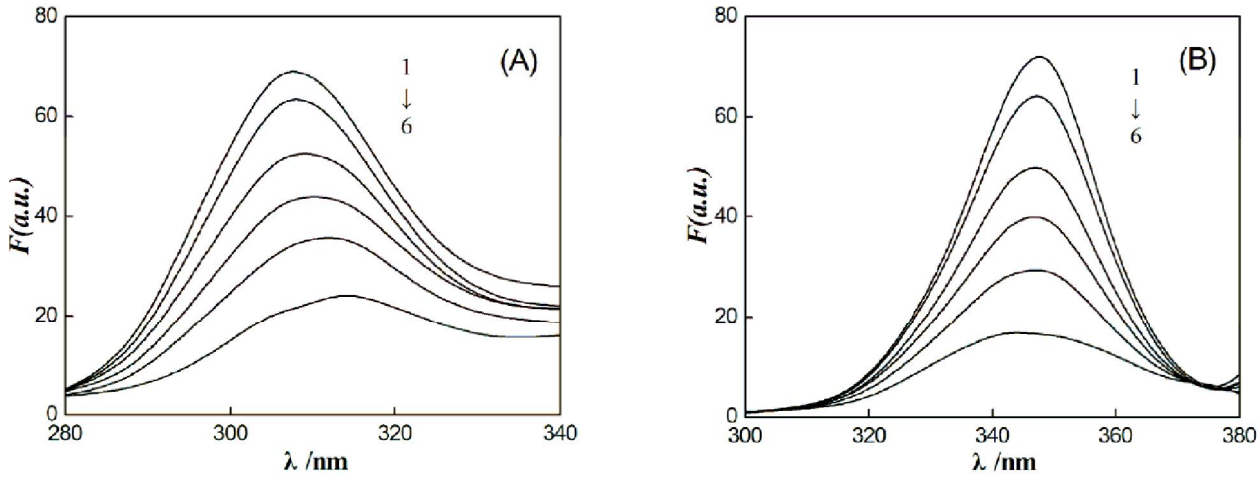

Figure 5 : Synchronous fluorescence spectra of BSA-MXFX system (T = 293 K). C_{BSA} = 1.0×10^{-6} mol/l, 1 ~ 5 C_{MXFX} = (0.0, 2.0, 6.0, 10.0, 15.0, 25.0)×10^{-6} mol/l; (A) $\Delta\lambda$ =15 nm; (B) $\Delta\lambda$ =60 nm

CONCLUSIONS

Using the quenching fluorescence method we studied the interaction between MXFX and BSA as well as BHb. The experimental results indicated that MXFX had embedded into the hydrophobic cavity through electrostatic force and bound to Trp in protein, and the interaction with BSA was stronger than BHb. The donor-to-acceptor distance *r* was less than 7 nm, indicating non-radiation energy transfer. The negative cooperativity existed in protein-MXFX system for subsequent ligand and it was more powerful in BSA. From the synchronous fluorescence spectra, it could be shown that the conformational change of protein was induced by the interaction of MXFX with the amino acid micro-region of protein molecules. The study will extend the use of fluorescence spectroscopy; meanwhile, it provides important information to understand their pharmacokinetic and toxicological profile, and also provides useful insights into the future clinical medicine.

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