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Study on the Interaction of Ketoconazole with Human and Bovine Serum Albumins by Fluorescence Spectroscopy

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The binding of ketoconazole to human serum albumin and bovine serum albumin was studied by using fluorescence and ultraviolet spectroscopy. The measurements were performed in 0.1 mol•L^{-1} phosphate buffer solution at pH＝7.40±0.1. Decreasing of quenching constant was observed in association with temperature increase. Our findings show that the quenching mechanism of fluorescence of serum albumins by ketoconazole was static quenching because of compound formation. The thermodynamic parameters ΔG, ΔH, and ΔS at different temperatures were calculated, showing that the electrostatic interactions and hydrophobic interaction are the main forces for the binding of ketoconazole to serum albumins. The distance r between the donor (Trp-214) and acceptor (ketoconazole) was obtained according to fluorescence resonance energy transfer theory.

Keywords ketoconazole, bovine serum albumin (BSA), human serum albumin (HSA), fluorescence quenching, UV-Vis spectroscopy, thermodynamic parameter

Introduction

Triazole antifungals that act by inhibiting lanosterol cytochrome P450 14α-demethylase (CYP51) have now become the most rapidly expanding group of antifungal compounds.[1] Antifungal drugs are widely prescribed for the treatment of systemic fungal infections, particularly in immunocompromised and cancer patients.[2]

Ketoconazole (Figure 1) is one of the most famous antifungal medications and a potent inhibitor against the enzyme cytochrome P450 (CYP3A4); several statins, including simvastatin and lovastatin, interact with this hepatic microsomal enzyme, which is responsible in significant part for statin clearance.[3] It is often used to treat fungal infections that can spread to different parts of the body through the bloodstream such as yeast infections of the mouth, skin, urinary tract, and blood, and certain fungal infections that begin on the skin or in the lungs and can spread through the body. Ketoconazole works by slowing the growth of fungi which may cause infection. It is used to treat a variety of fungal infections such as candida infections of the skin or mouth (thrush), blastomycosis, histoplasmosis, coccidiomycosis, and others.

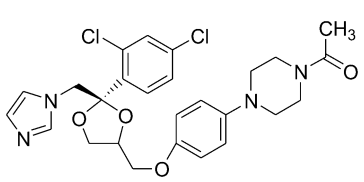

Figure 1 Structure of ketoconazole.

Protein is an important component of cell and the executor of life activities. It is a frontier topic to study the function of protein in life science. Studying the thermodynamics characteristics and mechanism of the interaction of a small organic molecule such as medicament with biological macromolecules is an important component of life sciences.

Serum albumins are the most abundant proteins in plasma.[4] As the major soluble protein constituents of the circulatory system, they have many physiological functions. They contribute to colloid osmotic blood pressure and are primarily responsible for the maintenance of blood pH;[5-7] they can play a dominant role in drug metabolism, efficacy and disposition.[8] Many drugs and other bioactivity small molecules bind reversibly to albumin,[9-11] which implicates a serum albumin role as carriers. Consequently, it is important to study the interactions of drugs with this protein. These studies may provide information about the structural features that determine the therapeutic effectiveness of drugs, and have become an important research field in the life sciences, chemistry and clinical medicine. The crystal structure analyses of human serum albumin (HSA) and bovine serum albumin (BSA) have revealed that the

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drug binding sites are located in subdomains IIA and IIIA.[12] A large hydrophobic cavity is present in subdomain IIA. The geometry of the pocket in subdomain IIA is quite different from that found for subdomain IIIA. HSA has one tryptophan (Trp-214) in subdomain IIA, whereas BSA has two tryptophan moieties (Trp-135 and Trp-214) located in subdomains IA and IIA, respectively.[13] Trp-212 is located within a hydrophobic binding pocket of the protein.

However, detailed investigations of the interaction of HSA and BSA with ketoconazole are yet to be conducted. In this work, these two serum albumins are selected as our protein models because of their medical importance, low cost, ready availability, unusual ligand-binding properties, and the results of all the studies are consistent with the fact that bovine and human serum albumins are homologous proteins.[7,14]

Fluorescence spectroscopy is a powerful tool for the study of the reaction of chemical and biological systems since it allows non-intrusive measurements of substances at a low concentration under physiological conditions.[15] Quenching measurement of albumin fluorescence is an important method to study the interactions of drugs with protein.[16,17] The effectiveness of drugs depends on their binding ability with albumins, so it is significant to study the interactions of drugs with these proteins.[18] These studies can reveal the accessibility of quenchers to albumin fluorophore groups, help understand albumin binding mechanisms to drugs, and provide clues to the nature of the binding phenomenon. In the present work, we demonstrated the interactions between ketoconazole and serum albumins by fluorescence spectroscopy. The effect of the energy transfer was studied according to fluorescence resonance energy transfer (FRET) theory. The aim of our work was to determine the affinity of ketoconazole for serum albumins, and to investigate the thermodynamics of their interaction. In the previous drug-protein investigations, this drug was a little researched and the interpretation of its fluorescence information was incomprehensive. So we made a detailed and insightful analysis of the fluorescence information exhibited by these spectra in the present work. It is very useful to research the pharmacological properties and the mechanism of interaction between drug and protein.

Experimental

Apparatus

All fluorescence measurements were carried out on an F-2500 FL spectrophotometer (Hitachi, Tokyo, Japan) equipped with a Xenon lamp source, a thermostat system and 1.0 cm quartz cells, using 5.0 nm slit width. A TU-1901 UV-Vis recording spectrophotometer (Puxi Analytic Instrument Ltd. of Beijing, China) equipped with 1.0 cm quartz cells was used for scanning the UV spectrum.

Chemicals

Bovine serum albumin and human serum albumin (molecular weight of 67000) were purchased from the company of Bo'ao Biotechnology Company (Shanghai, China) and used without further purification. All other materials were of analytical reagent grade. Water was distilled and deionized.

Procedures

Sample preparation All standard serum albumin (BSA and HSA) solutions of 2×10^{-6} mol•L^{-1} were prepared by dissolving pure samples in 0.1 mol•L^{-1} phosphate buffer solution and making up the volume to 100 mL in a calibration flask and kept in the cool and dark condition.

Ketoconazole was accurately weighed and placed in two 10 mL cuvettes respectively and dissolved with 5 mL of DMF for fluorescence detection. The standard solutions of 2×10^{-4} mol•L^{-1} were kept in the cool and dark condition.

In the same way, ketoconazole was accurately weighed and placed in two 10 mL cuvettes respectively and dissolved with 5 mL of THF for UV absorption detection. The standard solutions of 2×10^{-3} mol•L^{-1} were kept in the cool and dark.

0.1 mol/L phosphate buffer solution of pH = 7.40 ± 0.1 was prepared by dissolving appropriate amounts of NaH_2PO_4-NaOH.

Fluorescence and UV spectra Titrations were done manually by using trace syringes, and the fluorescence spectra (excitation at 285 nm and emission wavelengths of 300—450 nm) and absorption spectra were registered. The temperatures chosen were 298, 304 and 310 K, so that all the serum albumin did not undergo any structural degradation. The UV spectra of the serum albumin were recorded in the range of 200—450 nm.

Quenching measurement of albumin fluorescence is an important method to study interactions of several substances with protein.[14-16] Fluorescence quenching refers to any process that decreases the fluorescence intensity of a sample. A variety of molecular interactions can result in quenching, including excited-state reactions, molecular rearrangements, energy transfer, ground-state complex formation, and collisional quenching. The different mechanisms of quenching are usually classified as either dynamic quenching or static quenching.

The quenching constants were calculated according to Stern-Volmer equation and Lehrer equation,[19,20] which is a modified Stern-Volmer equation:

$$\frac{F_0}{F}=1+k_q\tau_0[Q]=1+K_{SV}[Q] \quad (1)$$

$$\frac{F_0}{\Delta F}=\frac{1}{f_a K_a}\frac{1}{[Q]}+\frac{1}{f_a} \quad (2)$$

where F and F_0 are current and initial fluorescence intensity, respectively, [Q] is ligand concentration, k_q is

the quenching rate constant of the biomolecule, K_{SV} is the Stern-Volmer quenching constant, τ_0 (10^{-8} s^{-1}) is the average lifetime of the fluorescent substance without quencher,[21] ΔF is the relative margin of fluorescence intensities of protein in the absence and presence of quencher, respectively, f is the fractional maximum fluorescence intensity of protein summed up and K_a is the static quenching constant.

If the binding reaction in the serum albumin molecule happens for the static quenching interaction, there are similar and independent binding sites in the serum albumin. The apparent binding constant K and binding sites z can be found from equation:[22]

$$\lg\left(\frac{F_0-F}{F}\right)=\lg K+z\lg[Q] \quad (3)$$

where K is the apparent binding constant for the protein-ligand complex, and z is number of the binding sites.

Method 2 mL of serum albumin solutions at 2×10^{-6} mol•L^{-1} were titrated by ketoconazole standard solutions. And the concentration of ketoconazole varied from 0 to 1.0×10^{-6} mol•L^{-1} at increments of 2.0×10^{-7} mol•L^{-1}, and from 1.0×10^{-6} to 2.6×10^{-6} mol•L^{-1} at increments of 4.0×10^{-7} mol•L^{-1}.

All experiments were repeated several times, and the present method has good reproducibility.

Results and discussion

Fluorescence characteristics of serum albumins

The fluorescence quencher interacts in the protein subdomain which contains tryptophan residues. From the spectroscopic point of view, one of the main differences between the two proteins is that a BSA molecule is formed by 582 amino acid residues, and contains a first tryptophan residue at position 135, in subdomain IB of the albumin molecule, and a second tryptophan residue at position 214, in sub domain IIA,[23] while HSA has only one tryptophan residue in position 214. A large hydrophobic cavity is present in the subdomain IIA, and a wide variety of arrangements can take place in this subdomain. So subdomain IIA is the target of ketoconazole binding. At the same time, the tryptophan residues in the vicinity of W135 interact directly with the polar head group of ketoconazole possibly, which causes effect difference between the two proteins.

The fluorescence spectra of the serum albumin were recorded before and after titration with a series of concentrations of ketoconazole in a physiological condition (pH=7.40±0.1) at λ_{ex}=285 nm (Figure 2). It could be seen from Figure 2 that there was little fluorescence emission for ketoconazole in the range measured.

The effect of ketoconazole on serum albumin fluorescence intensity was shown in Figure 3. It could be known that the fluorescence intensity of albumins decreased gradually with the increase of ketoconazole concentration, and the emission maximum and shape of the peaks also changed gradually. A slight blue shift of nearly 2.0 nm was observed in the maximum emission wavelength of emission spectra of BSA with increasing transition ketoconazole concentration. And there was a biggish blue shift in the maximum emission wavelength of HSA, which was from 337 to 327 nm. It was probably owing to the aggrandizement of the compact structure of hydrophobic subdomain. This suggested an increased hydrophobicity of the region surrounding the tryptophan site.[24,25]

These results indicated that there were interactions between ketoconazole and the serum albumins. The fluorescence quenching effect was due to the formation of fluorescent complexes.

Figure 4 displays the Stern-Volmer plots (F_0/F against [Q]) of the quenching of serum albumin fluorescence by ketoconazole at 298 K. As can be seen from Figure 4, the linear relationships are very awful in both figures of BSA and HSA. There is an inflexion which makes two distinct slopes before and after it. Therefore, we cannot deal with the data and get the quenching constant by Stern-Volmer equation.

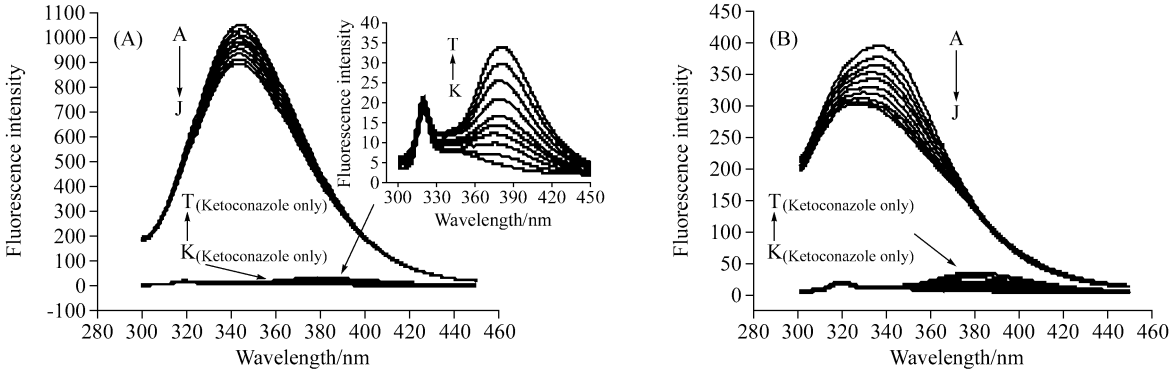

Figure 2 Emission spectra of BSA (A) and HSA (B) in the presence of various concentration ketoconazole (T=298 K, λ_{ex}=285 nm). c(serum albumin)=2.0×10^{-6} mol•L^{-1}; c(ketoconazole), A—J: 0, 0.2×10^{-6}, 0.4×10^{-6}, 0.6×10^{-6}, 0.8×10^{-6}, 1.0×10^{-6}, 1.4×10^{-6}, 1.8×10^{-6}, 2.2×10^{-6}, 2.6×10^{-6} mol•L^{-1}; curves of K—T show the emission spectra of ketoconazole only, and concentrations of ketoconazole of K—T are the same as those of A—J.

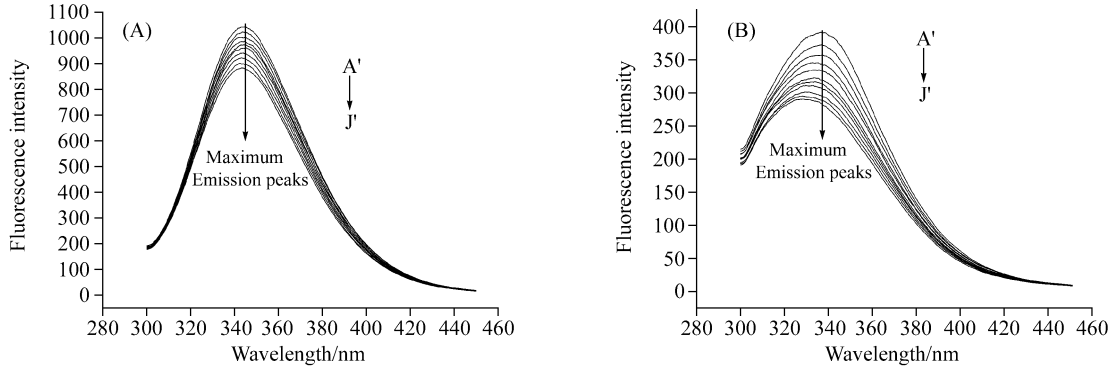

Figure 3 Emission spectra of BSA (A) and HSA (B) in the presence of various concentration ketoconazole from which the spectra of ketoconazole only have been deducted (T=298 K, λ_{ex}=285 nm). c(serum albumin)=2.0×10^{-6} mol•L^{-1}; c(ketoconazole), A—J: 0, 0.2×10^{-6}, 0.4×10^{-6}, 0.6×10^{-6}, 0.8×10^{-6}, 1.0×10^{-6}, 1.4×10^{-6}, 1.8×10^{-6}, 2.2×10^{-6}, 2.6×10^{-6} mol•L^{-1}.

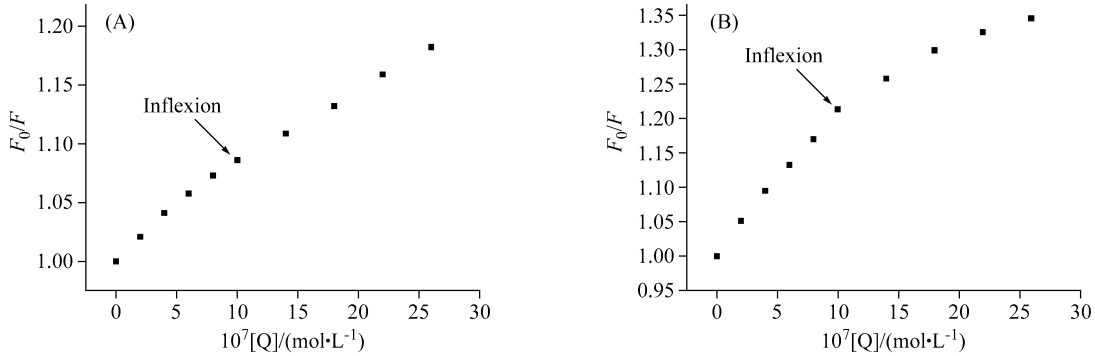

Figure 4 A group of Stern-Volmer dots for ketoconazole-albumins, BSA (A) and HSA (B). c(serum albumin)=2.0×10^{-6} mol•L^{-1}, pH=7.40±0.1, λ_{ex}=285 nm and T=298 K.

But it does not mean the result of Stern-Volmer plots is neither rhyme nor reason. We can calculate the cursory values of k_q that are larger than 10^{13} by Eq. (1).[26] It is much greater than the value of the maximum scatter collision quenching constant (2.0×10^{10} mol^{-1}•s^{-1}).[27,28] So the static quenching may be present here. And the concentration of ketoconazole which the inflexion corresponds to is 1.0×10^{-6} mol•L^{-1}, namely, ketoconazole/albumin concentration ratio is 1∶2. Thus, we can deduce the number of binding sites is during 0.5 to 1.0.

Figure 5 displays the modified Stern-Volmer plots [Eq. (2)] ($F_0/\Delta F$ against [Q]) of the quenching of serum albumin fluorescence by ketoconazole at 298 K. Compared with the Stern-Volmer equation, the linear relationships processed by the modified Stern-Volmer equation are quite good.

It can be found from Figure 6 and Table 1 that the modified Stern-Volmer plots were linear and the slopes decreased with temperature increasing. The interaction was weakened and the quenching rate constants decreased with increasing temperature. So the results showed the modified Stern-Volmer quenching constant K_a was inversely correlated with temperature, which indicated that the probable quenching mechanism of fluorescence of the serum albumins by ketoconazole was not initiated by dynamic collision but from complex formation.[15] Also according to the $F_0/(F-F_0)$ values showed in Figure 6, we can learn about that ketoconazole has the similar effects on the two serum albumins at the same temperature. For both of them, temperature had a significant effect on the fluorescence quenching process.

The great difference in fluorescence intensities between BSA and HSA is due to the additional tryptophan residue found in BSA. The results showed that the quenching of aqueous solution of tryptophan was the same as the quenching of BSA and HSA. That is to say, the binding site between drugs and the two serum albumins is tryptophan. HSA has only one tryptophan residue (Trp-214) in its molecule and this would facilitate the interpretation of the results. BSA contains two tryptophan residues with intrinsic fluorescence; Trp-212 is located with a hydrophobic pocket of the protein and Trp-135 is located on the surface of the molecule. The similarities of the quenching process between BSA and HSA led us to the conclusion that the primary binding site for ketoconazole on the BSA and HSA chains should be located in similar regions. Therefore, the BSA tryptophan residue quenched by ketoconazole should be located at position 212.

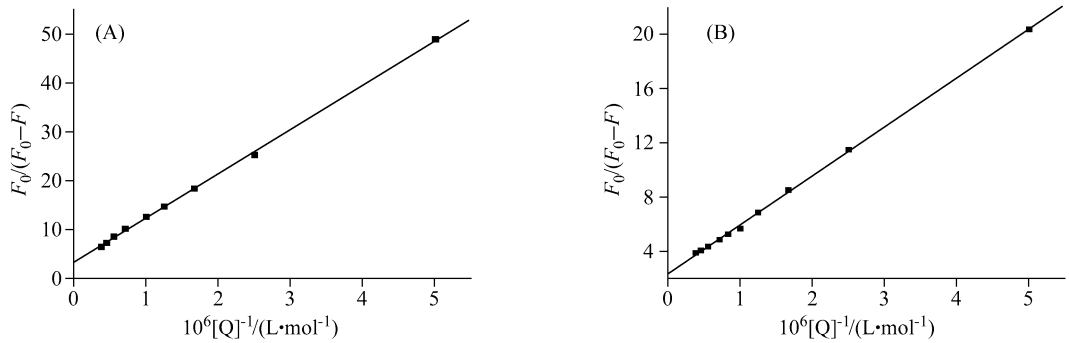

Figure 5 Modified Stern-Volmer plots for ketoconazole-albumins, BSA (A) and HSA (B). c(serum albumin)$=2.0\times10^{-6}$ mol•L^{-1}, pH$=7.40\pm0.1$, $\lambda_{ex}=285$ nm and $T=298$ K.

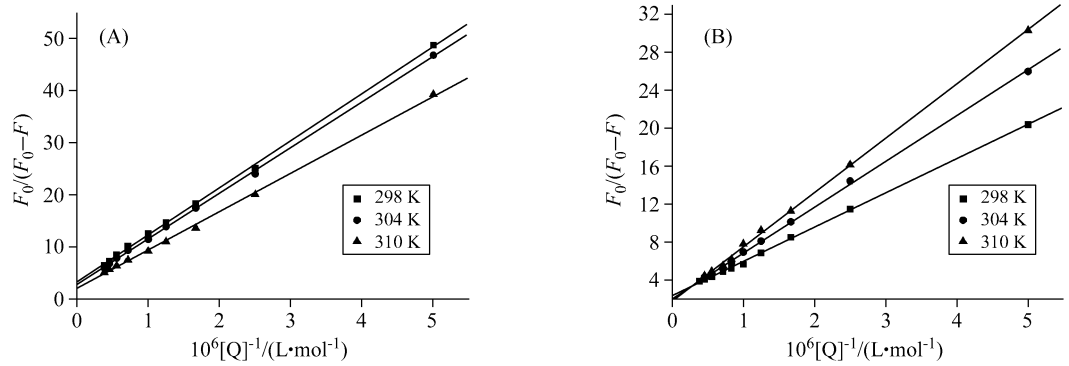

Figure 6 Modified Stern-Volmer plots for ketoconazole-albumins, BSA (A) and HSA (B) at different temperatures. c(serum albumin)$=2.0\times10^{-6}$ mol•L^{-1}, pH$=7.40\pm0.1$, $\lambda_{ex}=285$ nm, temperature (K) as mentioned in the legend.

Table 1 Binding and quenching constants according to modified Stern-Volmer curves

T/K	K_a(BSA)/ (10^5 L•mol^{-1})	R	K_a(HSA)/ (10^4 L•mol^{-1})	R
298	3.68	0.9997	6.48	0.9997
304	3.24	0.9998	5.13	0.9998
310	2.78	0.9994	4.04	0.9999

It is noted that a complex was possibly formed between ketoconazole and the serum albumin, which is responsible to the quenching of the fluorescence of albumin.

Binding constants and binding modes

Analysis of modified Stern-Volmer plots in this regime yields equilibrium expressions for static quenching, K_a, which are analogous to associative binding constants for the quencher-acceptor system.[29]

The interaction forces between drugs and biomolecules may include electrostatic interactions, multiple hydrogen bonds, van der Waals interactions and hydrophobic interactions, and so on.[30] Considering the dependence of binding constant on temperature, a thermodynamic process was considered to be responsible for the formation of the complex. Therefore, the dependence of the thermodynamic parameters on temperature was analyzed in order to further characterize the acting forces between ketoconazole and the serum albumins.

Hydrophobic force may increase ΔH and ΔS of a system, while hydrogen bond and van der Waals force may decrease them, and electrostatic force usually makes $\Delta H\approx0$ or <0 and $\Delta S>0$.

If the enthalpy change (ΔH) does not vary significantly over the temperature range studied, then its value and that of entropy change (ΔS) can be determined from the van't Hoff equation:[31]

$$\ln K=-\frac{\Delta H}{RT}+\frac{\Delta S}{R} \tag{4}$$

where associative binding constants K are analogous to the effective quenching constants K_a at the corresponding temperature and R is the gas constant. The temperatures used were 298, 304 and 310 K. The entropy change (ΔS) and enthalpy change (ΔH) were calculated from the slope of the van't Hoff relationship. The free energy change (ΔG) was then estimated from the following relationship:

$$\Delta G=-RT\ln K \tag{5}$$

Figure 7, by fitting the data of Tables 2 and 3, shows that assumption of nearly constant ΔH is justified. Tables 2 and 3 show the values of ΔH and ΔS obtained for the binding site from the slopes and ordinates at the origin of the fitted lines.

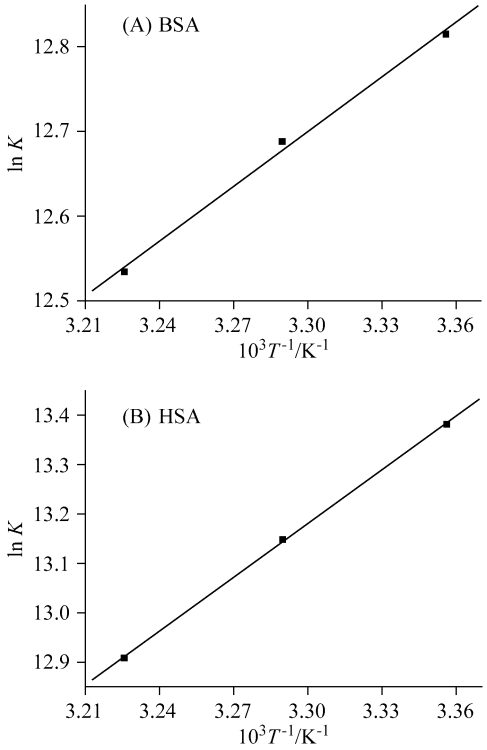

Figure 7 Van't Hoff plots, pH=7.40±0.1, *c*(serum albumin)=2.0×10^{-6} mol•L^{-1}.

Table 2 Modified Stern-Volmer association constant K_a and relative thermodynamic parameters of the system of ketoconazole-BSA

T/K	Δ*H*/ (kJ•mol^{-1})	Δ*S*/ (J•mol^{-1}•K^{-1})	Δ*G*/ (kJ•mol^{-1})
298			−31.75
304	−17.96	46.31	−32.07
310			−32.30

Table 3 Modified Stern-Volmer association constant K_a and relative thermodynamic parameters of the system of ketoconazole-HSA

T/K	Δ*H*/ (kJ•mol^{-1})	Δ*S*/ (J•mol^{-1}•K^{-1})	Δ*G*/ (kJ•mol^{-1})
298			−33.15
304	−30.25	9.76	−33.23
310			−33.27

It can be found from Tables 2 and 3 that the negative values of free energy (Δ*G*), supports the assertion that the binding process is spontaneous. The negative enthalpy (Δ*H*) and bigger positive entropy (Δ*S*) values of the interaction of ketoconazole with BSA indicate that the binding is mainly enthalpy driven, the electrostatic interactions played a major role in the reaction, in addition to the hydrophobic association. The negative enthalpy (Δ*H*) and very small positive entropy (Δ*S*) values of the interaction of ketoconazole and HSA indicate that the binding is synergy of the hydrophobic forces and electrostatic interactions. It can be presumed that this interaction is due to the entropy-enthalpy compensation happened between the two reactions and the ketoconazole molecule entered the hydrophobic cavities partly which led to extrude out the water molecule from BSA and HSA.[32]

Binding sites

So binding reaction in serum albumin molecules happened for the static quenching interaction, and there were similar and independent binding sites in the BSA and HSA.

According to the relationship of $\lg\left(\frac{F_0-F}{F}\right)$ vs. lg[Q], the fit to the fluorescence data using equation (3) for the system of ketoconazole and the serum albumins was found by setting $z=0.848\pm0.037$ for BSA, and $z=0.866\pm0.024$ for HSA, respectively.

The energy transfer between ketoconazole and serum albumins

If we assumed the binding reaction in the serum albumin molecules happened in a sequential manner, the distance between the binding site and the fluorophore in the protein could be evaluated according to the Förster mechanism of non-radiation energy transfer.[33]

The efficiency of energy transfer *E* is given by

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

where *r* is the distance between a donor and an acceptor and R_0 is the distance at 50% transfer efficiency.

$$R_0^6=8.8\times10^{-25}K^2n^{-4}\Phi J \tag{7}$$

where K^2 is the orientation factor related to the geometry of the donor-acceptor of dipole and $K^2=2/3$ for random orientation as that in fluid solution, n (=1.4) is the refractive index of medium, Φ (=0.10) is the fluorescence quantum yield of the donor,[34] and *J* is the spectral overlap of the donor emission and the acceptor absorption. *J* is given by

$$J=\Sigma F(\lambda)\varepsilon(\lambda)\lambda^4\Delta\lambda/\Sigma F(\lambda)\Delta\lambda \tag{8}$$

where $F(\lambda)$ is the fluorescence intensity of fluorescence reagent when the wavelength is λ, and $\varepsilon(\lambda)$ is the molar absorbance coefficient at the wavelength of λ. From these relationships, *J*, *E* and R_0 can be calculated; so the value of *r* can also be calculated.

From Figure 8, the overlap integral calculated according to above relationship is 5.06×10^{-15} cm^3•L•mol^{-1} for BSA, and 5.49×10^{-15} cm^3•L•mol^{-1} for HSA. So the value of *E* is 0.09, the value of R_0 is 2.29 nm, and the value of *r* is 3.4 nm (<7 nm) for BSA; the value of *E* is 0.19, the value of R_0 is 2.32 nm, and the value of *r* is 2.9 nm (<7 nm) for HSA. So the real distance between ke-

toconazole and the chromophore in BSA is 3.4 nm, and the real distance is 2.9 nm for HSA. Obviously, this result accorded with conditions of non-radiative energy transfer theory, indicating again the static quenching interaction between ketoconazole and the serum albumins.

Conformation investigation

To explore the structural change of BSA and HSA by addition of ketoconazole, we measured UV-Vis spectra (Figure 9) and synchronous fluorescence spectra (Figures 10, 11) of serum albumins with various amounts of ketoconazole.

UV-Vis absorption measurement is a simple method, which is appropriate to explore the structural change and to know the complex formation.[35,36] Figure 9 displays the UV-Vis absorbance spectra of BSA (A) and HSA (B) at different contents of ketoconazole. It is clear that intensity of the UV-Vis absorbance spectra are raised at 340—240 nm and the maximum absorption wavelength has a little red shift, indicating that interactions occur between ketoconazole and serum albumins. The albumins molecules bind with ketoconazole to form ketoconazole-HSA complex, while the peptide strands of BSA and HSA molecules shrinked more and the hydrophobicity was increased.

The synchronous fluorescence spectra give information about the molecular environment in a vicinity of the chromosphere molecules and have several advantages, such as spectral simplification, spectral bandwidth reduction, sensitivity and avoiding different perturbing effects.[36] Yuan *et al*. suggested a useful method to study the environment of amino acid residues by measuring the possible shift in wavelength emission maximum λ_{max}, the shift in position of emission maximum corresponding to the changes of the polarity around the chromophore molecule.[37] When the D-value ($\Delta\lambda$) between excitation wavelength and emission wavelength were stabilized at 15 or 60 nm, the synchronous fluorescence gives the characteristic information of tyrosine residues or tryptophan residues.[38] The effect of synchronous fluorescence spectroscopies of ketoconazole on serum albumins are shown in Figures 10 and 11. It is apparent from these figures that the maximum emission wavelength moderate shifts towards short wave when $\Delta\lambda=60$ nm. The shift effect expresses that the conformations of BSA and HSA were changed. It is also indicated that the polarity around the tryptophan residues was decreased and the hydrophobicity was increased.[39]

For reconfirming the structural change of serum albumins by addition of ketoconazole, we measured the synchronous fluorescence spectroscopies of BSA and HSA with various amounts of ketoconazole. The conclusion agrees with the result of conformational changes by UV-Vis spectra.

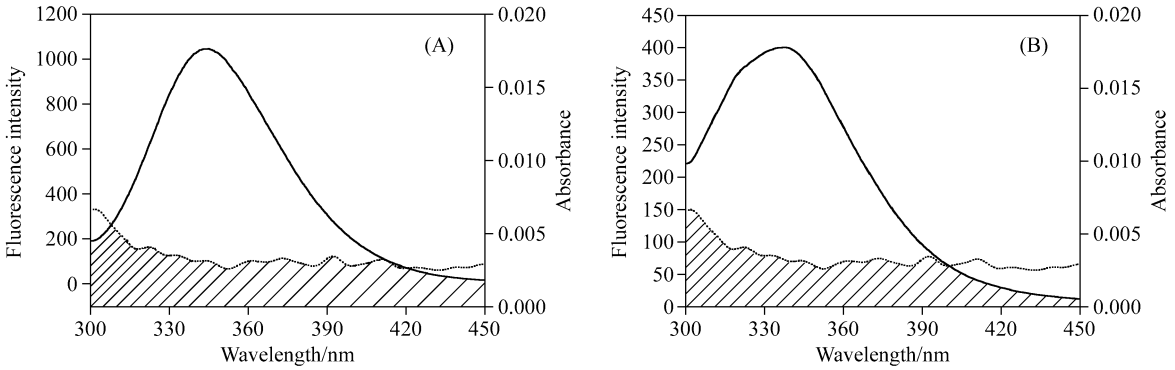

Figure 8 The overlap of the fluorescence spectra of BSA (A, real line) and HSA (B, real line) and the absorption spectra of ketoconazole (dashed line). $c_{albumin}=c_{drug}=2.0\times10^{-6}$ mol•L^{-1}, pH=7.40±0.1, T=298 K.

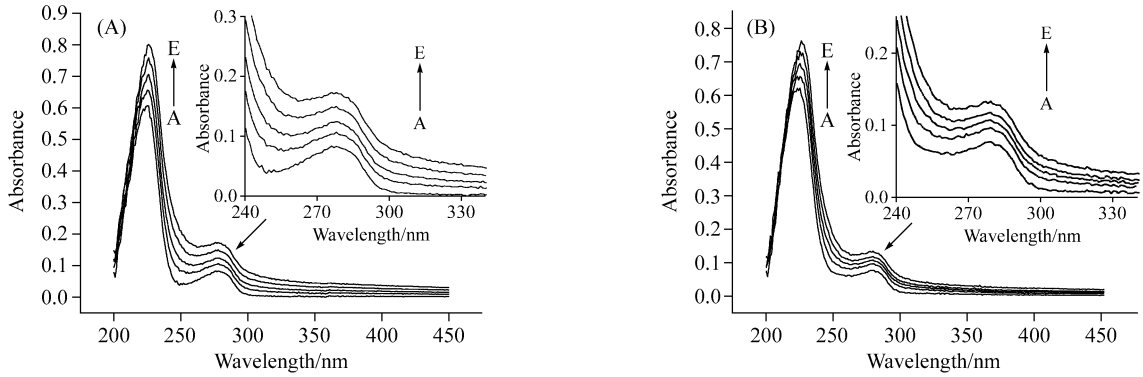

Figure 9 UV-Vis spectras of BSA (A) and HSA (B) in the presence of various concentrations of ketoconazole (T=298 K, ketoconazole was dissolved in THF). c(BSA)=2.0×10^{-6} mol•L^{-1}; c(Ketoconazole), A—E: 0, 2.0×10^{-6}, 4.0×10^{-6}, 6.0×10^{-6}, 8.0×10^{-6} mol•L^{-1}.

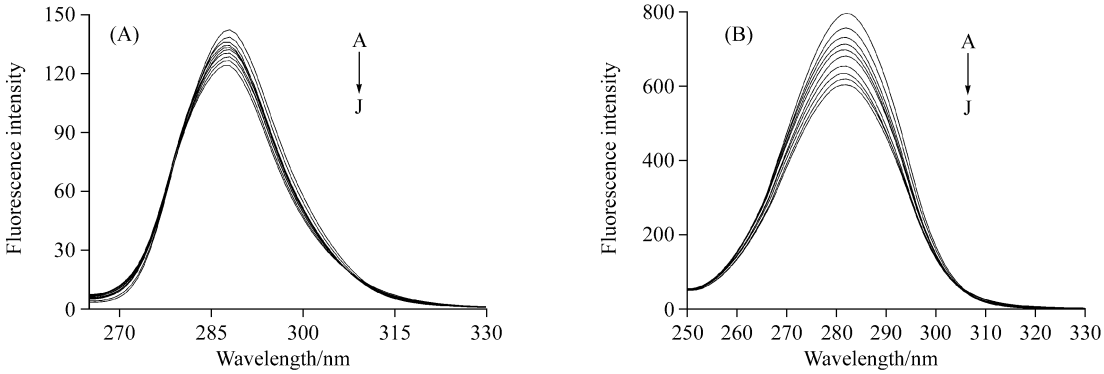

Figure 10 Synchronous fluorescence spectrum of BSA: (A) $\Delta\lambda = 15$ nm; (B) $\Delta\lambda = 60$ nm. c(BSA) $= 2.0 \times 10^{-6}$ mol•L^{-1}; c(Ketoconazole), A—J: 0, 0.2×10^{-6}, 0.4×10^{-6}, 0.6×10^{-6}, 0.8×10^{-6}, 1.0×10^{-6}, 1.4×10^{-6}, 1.8×10^{-6}, 2.2×10^{-6}, 2.6×10^{-6} mol•L^{-1}.

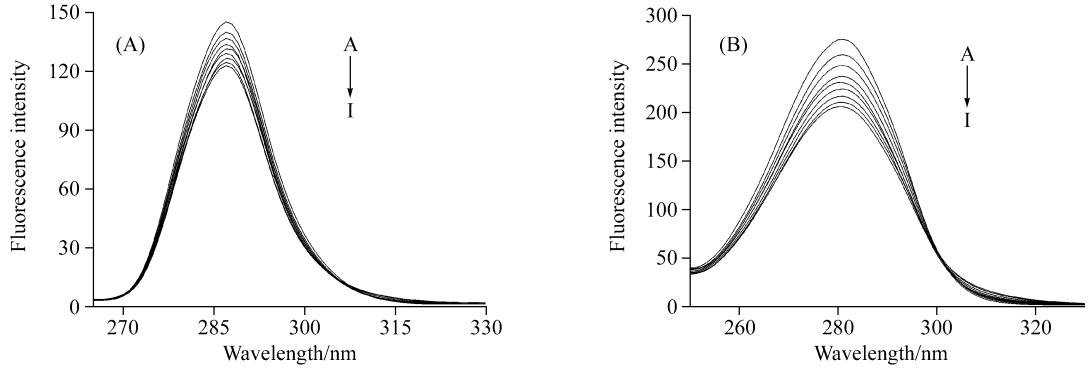

Figure 11 Synchronous fluorescence spectrum of HSA: (A) $\Delta\lambda$=15 nm; (B) $\Delta\lambda$=60 nm. c(HSA)=2.0×10^{-6} mol•L^{-1}; c(Ketoconazole), A—I: 0, 0.2×10^{-6}, 0.4×10^{-6}, 0.6×10^{-6}, 0.8×10^{-6}, 1.0×10^{-6}, 1.4×10^{-6}, 1.8×10^{-6}, 2.2×10^{-6} mol•L^{-1}.

Conclusion

In this paper, the interactions of ketoconazole with the two kinds of serum albumins were studied by spectroscopic methods including fluorescence spectroscopy and UV-Vis absorption spectroscopy. The results show that the modified Stern-Volmer quenching constant K_a is inversely correlated with temperature, which indicates that the probable quenching mechanism of the ketoconazole-serum albumin binding reactions is initiated by complex formation. The results of synchronous fluorescence spectroscopies and ultraviolet spectra indicate that the secondary structures of albumin molecules are changed dramatically in the presence of ketoconazole. The experimental results also indicate that the probable quenching mechanisms of fluorescence of the albumins by ketoconazole are static quenching procedure. The binding reaction of ketoconazole with BSA is mainly enthalpy driven, the electrostatic interactions play a major role in the reaction, in addition to the hydrophobic association. The interaction of ketoconazole and HSA indicates that the binding is synergy of the hydrophobic forces and electrostatic interactions.

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