Fluorescence Studies on the Interactions of Barbaloin with Bovine Serum Albumin

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The fluorescence quenching reactions of barbaloin with bovine serum albumin (BSA) in pH 7.20 Tris–HCl buffer solution were studied. The quenching mechanism of BSA by barbaloin was interpreted using the Stern–Volmer (S–V) mechanism. The binding constant *K* values were 2.78×10^5 (293 K), 1.87×10^5 (310 K), 1.25×10^5 (318 K), and the number of binding sites (*n*) were 1.18, 1.14, and 1.09, respectively. In addition, the thermodynamic functions enthalpy ($\Delta H°$) and entropy ($\Delta S°$) for the reaction were also calculated according to Vant's Hoff equation were −23.7 kJ/mol and 23.6 J/mol, respectively. Plausible explanations of the quenching mechanism are discussed on the basis of a hydrophobic interaction between barbaloin and BSA.

Key words fluorescence; barbaloin; interaction; bovine serum albumin

Serum albumins are the most abundant proteins in plasma. As the major soluble protein constituents of the circulatory system, they have many physiological functions. They contribute to colloid osmotic blood pressure and are chiefly responsible for the maintenance of blood pH.[1] The most outstanding property of albumins is their ability to reversibly bind a large variety of endogenous and exogenous ligands. The binding involves hydrophobic, hydrophilic, and cationic substances. The knowledge about proteins has profited from the use of these different ligands. The molecular interactions are often monitored using optical techniques because these methods are sensitive and relatively easy to use. Among these, fluorescence spectroscopy is a valuable technique to study the binding of ligands to proteins. With bovine serum albumin (BSA), different compounds have been used successfully as probes such as dyes[2] and metal complexes.[3,4] The interactions between albumin and drugs are very important in the pharmacokinetics, pharmacodynamics, and toxicology of drugs.[5,6] However, the binding of the components of natural plant medicine to proteins has seldom been investigated.

Barbaloin (Chart 1) (BAR, 19(10*H*)-anthracenore, 10-β-D-glucopyranosyl-1,8-dihydroxy-3-hydroxymethyl) is the main medicinal composition of the Chinese traditional medicine aloe vera that belongs to the liliaceous plant. It has the ability to diminish inflammation, anbiosis, and disinfection.[7] BAR has widely been used in light industry, especially in cosmetics and food products, but its interaction mechanism with protein has not been reported. In this paper, the binding of BAR to BSA was investigated under physiological conditions using a fluorescence method, and the binding constants (K) and number of binding sites (n) were determined. The experimental results indicate the quenching of BAR to BSA is static quenching and that it takes place mainly *via* hydrophobic interaction.

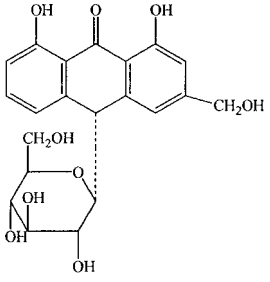

Chart 1

Experimental

Chemicals and Instrument BSA (fatty acid free <0.05%) was purchased from Sino-American Biotechnology Company and used without further purification. BAR (standard) was obtained from the National Institute for Control of Pharmaceutical and Products, China. A 0.5 mol/l NaCl solution was used to keep the ion strength at 0.1. Tris–HCl buffer was selected to maintain the pH of the solution at 7.20. BSA solution (1.00 mg/ml) was prepared by dissolving 1.0000 g of BSA in de-ionized water. Barbaloin solution was obtained by dissolving barbaloin in a small amount of methanol and diluting to volume with de-ionized water. Other chemicals were of analytical reagent grade.

Fluorescence spectra were recorded using a Hitachi-850 spectrofluorophotometer (Japan) with a 150 W Xenon lamp and a 1 cm quartz cell. The excitation and emission band widths were both 5 nm. The absorption spectra were recorded on a Tu-1901 spectrophotometer. Sample temperature was maintained by recycling water throughout the experiment. Circular dichroism (CD) was measured with a Jasco-20c automatic recording spectropolarimeter (Japan), using a 2 mm cell at 293 K. The induced ellipticity was defined as the ellipticity of the drug–BSA mixture minus the ellipicity of drug alone at the same wavelength.

Methods Barbaloin, 0.5 mol/l NaCl, and buffer solution were added to a fixed concentration of BSA solution, and diluted to 10 ml with de-ionized water. The fluorescence intensity was then measured at a wavelength of 340 nm setting the excited wavelength at 285 nm.

Results and Discussion

Interaction of BAR with BSA The aim of the present work was aimed to investigate whether BAR interacts with BSA and changes the conformation of BSA. The conformation changes in BSA were evaluated by measuring the intrinsic fluorescence intensity of protein tryptophan residues before and after addition of BAR. BSA, a 65 kDa molecular weight protein, contains two tryptophan residues (Trp-213 and Trp-314).[8] The fluorescence spectra of BSA–BAR are shown in Fig. 1. The addition of quencher changed neither the shape nor the maximum wavelength of the protein emission spectra, indicating that no conformation changes are induced in BSA by BAR under the conditions studied here. There is a significant decrease in the fluorescence intensity at 340 nm when increased amounts of BAR are added to a fixed

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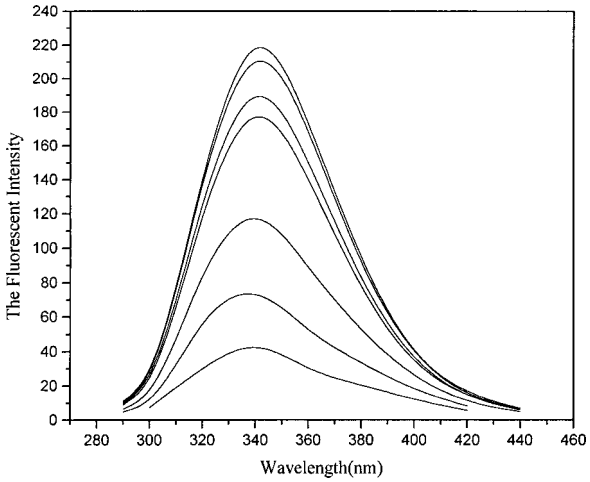

Fig. 1. Fluorescence Emission Spectra of the BSA–BAR System

The concentration of BSA was 7.70×10^{-6} mol/l, the BAR concentration increased from 0 to 5.975×10^{-5} mol/l, from the top to the bottom. $\lambda_{ex}=285$ nm. $T=293$ K. pH=7.20.

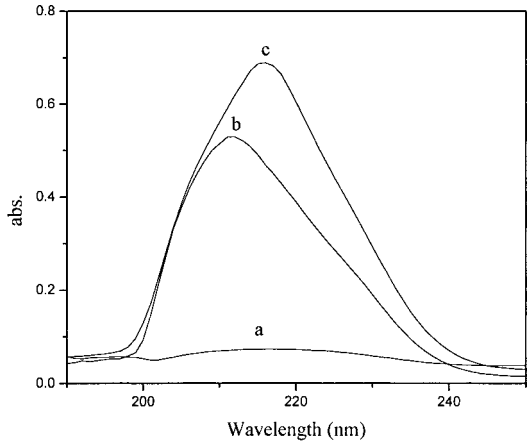

Fig. 2. Absorption Spectra of the BSA–BAR System

The concentration of BSA was 3.0×10^{-7} mol/l, the BAR concentration was 2.30×10^{-6} mol/l, $T=293$ K. pH=7.20. (a) BAR; (b) BSA; (c) BAR+BSA.

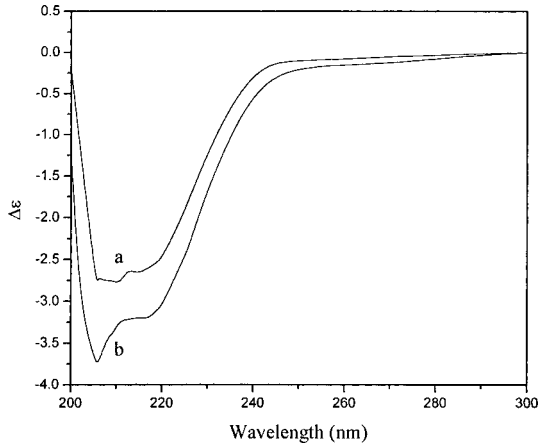

Fig. 3. CD Spectra of the BSA–BAR System

The concentration of BSA was 3.0×10^{-6} mol/l, the BAR concentration was 1.50×10^{-6} mol/l, $T=293$ K. pH=7.20. (a) BSA; (b) BAR+BSA.

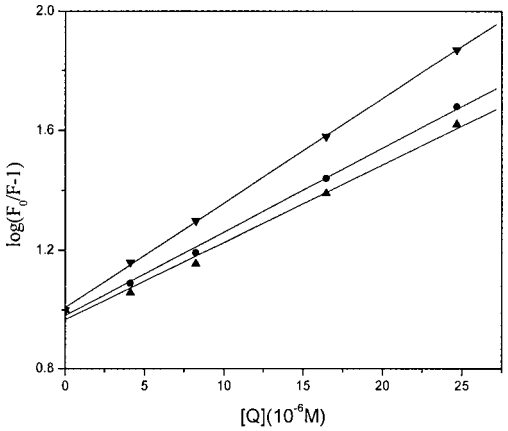

Fig. 4. Stern–Volmer Plots of the Quenching of BSA Tryptophan Residue Fluorescence by BAR

▼ 293 K; ● 310 K; ▲ 318 K. [BSA]=7.70×10^{-6} mol/l, $\lambda_{ex}=285$ nm, $\lambda_{em}=340$ nm, pH=7.20.

concentration of BSA. Figure 2 is the absorption spectra for BAR and BSA. The absorption of BSA (about 210 nm) represents the helix structure of BSA.[9] From Fig. 2, the binding of BAR to BSA resulted in a slight shift of the BSA absorption spectrum toward a longer wavelength, indicating the helix structure of BSA was changed. The signs of the Cotton effects of the CD spectrum of the BAR–BSA complex are shown in Fig. 3. The BSA exhibited a CD spectrum with a negative bands at 207 nm and 218 nm. As BAR was added to the solution of BSA, the intensity of negative Cotton effect of BSA at about 207 nm showed a significant change which indicated the helix structure of BSA was changed and it may be the result of the formation of complex between the BSA and BAR.

The fluorescence quenching data are usually analyzed by the Stern–Volmer equation[10]

$$F_0/F=1+K_{SV}[Q]$$

where F_0 and F are the fluorescence intensity in the absence of a quencher and in the presence at $[Q]$ concentration, respectively, and K_{SV} is the Stern–Volmer dynamic quenching constant. As can be seen from Fig. 4, the slopes decrease with increasing temperature, which is consistent with the static type of quenching mechanism. Static quenching arises from the formation of a dark complex between the fluorophore and quenching agent, and the quenching constant can be interpreted as the association constant or binding constant of the complexation reaction.

Binding Constant *K* and Number of Binding Sites *n*

For static quenching, the equation[11]

$$\log[(F_0-F)/F]=\log K+n\log[Q]$$

where F_0 and F are the fluorescence intensity without and with the quencher [BAR], respectively, and K and n are the binding constant and number of binding sites, respectively, is used. The values of K and n can be obtained by plotting the fluorescence intensity *vs.* the concentration of quencher according to the above equation. The binding of BAR to BSA results in a decrease of the BSA fluorescence emission at 340 nm. Thus, the fluorescence spectroscopy data were used to calculate the binding parameters. The results at different temperatures, shown in Fig. 5 and Table 1, indicate that it has a single class of binding sites on the BSA for BAR, and that

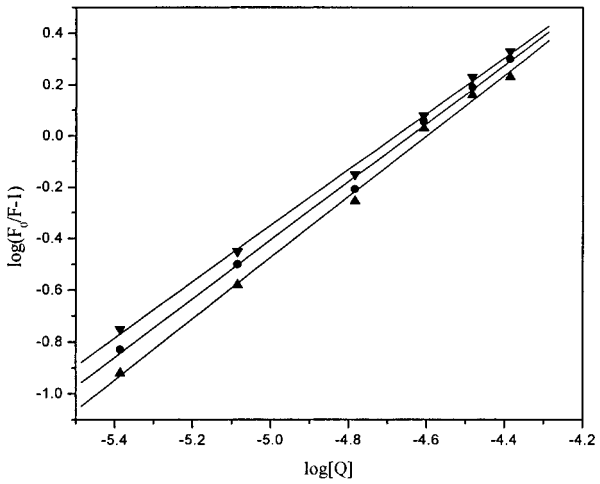

Fig. 5. Binding Constant K and Number of Binding Sites n of the BSA–BAR

▲ 293 K; ● 310 K; ▼ 318 K. [BSA]=7.70×10^{-6} mol/l, λ_{ex}=285 nm, λ_{em}=340 nm, pH=7.20.

Table 1. Binding Constant (K), Numbers of Binding Sites (n), and Thermodynamic Parameters for the Binding of BAR to BSA

Temperature (K)	$K\times10^5$	n	$\Delta G°$ (kJ/mol)	$\Delta S°$ (J/mol·K)	$\Delta H°$ (kJ/mol)
293	2.78	1.18	−30.62	23.63	−23.7
310	1.87	1.14	−31.03	23.63	−23.7
318	1.25	1.09	−31.21	23.63	−23.7

the binding constant decreases with rising temperature because the stability of the complex decreases with an increase in temperature.

Thermodynamic Analysis From the temperature dependence of the binding constant it is possible to estimate the values for the thermodynamic functions involved in the binding process. If the $\Delta H°$ value does not vary significantly over the temperature range studied, then its value and that of $\Delta S°$ can be determined from Vant Hoff's equation:

$$\ln K_T = -\Delta H°/RT + \Delta S°/R$$

K_T is the binding constant at temperature T and R is the gas constant. Fitting the equation to the data shows that the assumption of near-constant $\Delta H°$ is justified. Table 1 shows the value of $\Delta H°$ and $\Delta S°$ obtained from the slopes and ordinates at the origin of the fitted lines and lists the corresponding value of $\Delta G°$ as calculated from the relation

$$\Delta G° = \Delta H° - T\Delta S°$$

Based on the sign and magnitude of the thermodynamic parameters, many authors have discussed the individual kinds of interaction that may take place in the protein association process.[12] As shown in Table 1, the formation of BAR–BSA complexes is an exothermic process, accompanied by negative enthalpy and positive entropy changes. From the point of view of water structure, a positive $\Delta S°$ value is frequently taken as evidence for hydrophobic interactions. Furthermore, specific electrostatic interactions between ionic species in aqueous solution are characterized by a positive value of $\Delta S°$ and a negative $\Delta H°$ value. However, at pH around 7.20, under the conditions used in the present study,

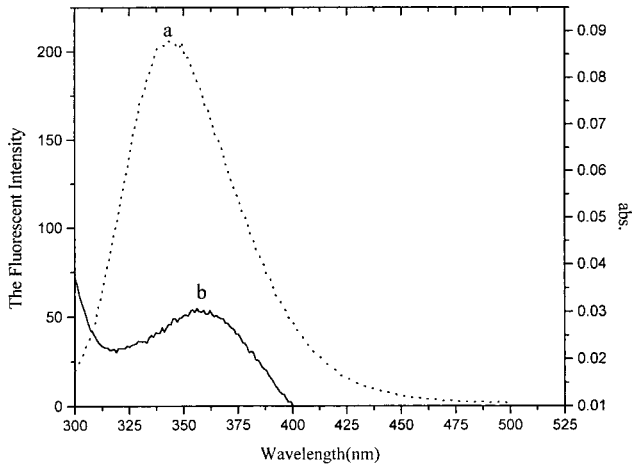

Fig. 6. Fluorescence Spectra and Absorption Spectra of BSA–BAR

(a) Fluorescence spectra of BSA; (b) absorbance spectra of BAR [BSA]/[BAR]=1 : 1, pH=7.20, T=293 K.

BAR is considered to be largely un-ionized according to its structure (Chart 1). Therefore, ionic interaction cannot play a major role in the binding. This view is also supported by the rather large negative value of $\Delta H°$. If the nature of the interaction is mainly electrostatic, the main source of $\Delta G°$ should be the large contribution of $\Delta S°$ with little contribution from $\Delta H°$. It is clear from the values of $\Delta S°$ and $\Delta H°$ presented in Table 1 that the binding of BAR to BSA is mainly stabilized by hydrophobic interaction.

The Donor–Acceptor Distance The distance from the tryptophan residue (donor) to the bound drug (acceptor) in BSA can be calculated according to Förster's theory.[13] The efficiency of energy transfer, E, is given by

$$E = 1 - F/F_0 = R_0^6/(R_0^6 + r^6)$$

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

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

where K^2 is the orientation factor related to the geometry of the donor–acceptor of the dipole and $K^2=2/3$[14] for random orientation as in fluid solution, n is the refractive index of the medium, Φ is the fluorescence quantum yield of the donor, and J is the spectra overlap of the donor emission and the acceptor absorption. J is given by

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

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

Figure 6 is the overlap of the fluorescence spectra of BSA and the absorption spectra of BAR when the molar ratio is 1 : 1. The fluorescence emission spectrum of the tryptophan residue in BSA was quenched by the addition of BAR. Therefore, the fluorescence energy transfer in the spectra may depend on the distance between the tryptophan residue and BAR bound to BSA. From Fig. 6, the overlap integral calculated according to the above relationship is 2.985×10^{-15} cm^3·l/mol. Therefore, the value of R_0 is 2.40 nm and

the r is 2.84 nm.

Conclusion

Studies on the BSA fluorescence quenching reaction have been presented. The results show that BAR is a strong quencher and has strong affinity for BSA. The results indicate that BAR quenches BSA fluorescence mainly through a static quenching mechanism, and that the interaction force is mainly a hydrophobic interaction.

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