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Fluorimetric study the interaction between asiatic acid and bovine serum albumin

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Abstract : The mutual interaction of asiatic acid(AA) with bovine serum albumin(BSA) was investigated using fluorescence spectroscopy. The results revealed that asiatic acid(AA) caused the fluorescence quenching of bovine serum albumin(BSA) through a static quenching procedure. The Stern-Volmer quenching constant were calculated at different temperature. The binding site, apparent binding constant and corresponding thermodynamic parameters ΔG^o, ΔH^o, ΔS^o were calculated and the Hydrogen bond and Van der Waals force play an important role in stabilizing the complex. Besides, the effect of Zn^{2+}, Cu^{2+}, Ni^{2+}, $Mn^{2+}$and Co^{2+} on the binding constants between asiatic acid(AA) and bovine serum albumin(BSA) were studied.
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Keywords : Asiatic acid(AA); Bovine serum albumin(BSA); Fluorescence quenching; Thermodynamic parameters.

INTRODUCTION

Asiatic acid (AA, Figure 1) is a triterpenoid of *Centella asiatica*[1]. It is reported that it possess a wide range of biological functions, such as antioxidant[2], hepatoprotective[3], anticancer[4], anti-inflammation[5], neurotoxicity activity[6], Hypoglycemic[7-8] and so on. Many reports indicated that AA induces cell cycle arrest and anti-proliferative effects on human breast, gastric, and utrine cancer cells[9]. Recently, people pay much more attention on asiatic acid, because of its wide biological activity[8-12]. However, despite of studies showing anticancer effect of asiatic acid in vitro, the effect of asiatic acid on carcinogenesis remains unknown.

The pharmacological behavior of therapeutic drug molecule plays an important role in deciding its fate in blood stream. The biodis-tribution, availability and metabolism of therapeutic agents strongly depend on their interaction with proteins in blood

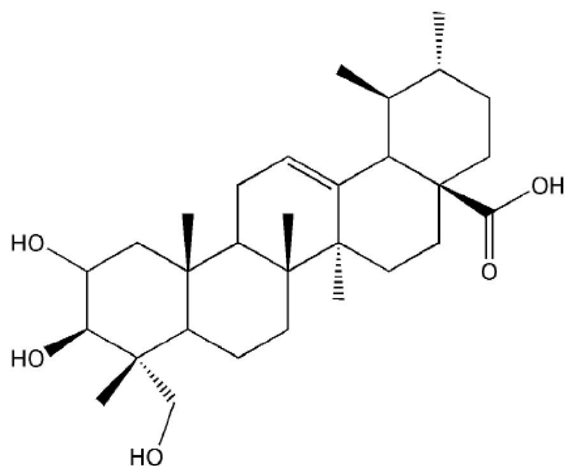

Figure 1 : The structure of asiatic acid

[13]. Bovine serum albumin (BSA), which is one of the most important proteins in the plasma, and has many physiological functions [14-16]. Its main function is to transport various metabolites and drugs such as anesthetics, anticoagulants and sedatives in the circulatory system[13]. So it is necessary to understand the interaction deeply between BSA and drugs. Besides, the molecular structure of BSA is similar to that of human serum albumin (HSA) with 76% identify, so the results of the studies conducted here are applicable to HAS[17-19]. So it was selected as a target protein molecule to research the efficacy of drug. Many of studies on the interactions between BSA and pharmaceutical molecules have reported and enlarged the perspective on the scientific research of drugs in interdisciplinary field[20-23].

Here, we report a spectroscopic investigation on the intermolecular interaction between AA and BSA. The purpose of this effort is to clarify the following aspects: 1) deeply study the intermolecular interaction between BSA and AA, including fluorescence quenching mechanism, interaction nature, binding constants, binding sites, thermodynamic parameters, and so on; 2) research the effect of some metal ions (Zn^{2+}, Cu^{2+}, Ni^{2+}, Mn^{2+} and Co^{2+}) on the interaction of BSA with AA. We believe that this work will assist to enrich the knowledge on these intermolecular interactions, spur further study in this area, and help to provide useful information of the structural features that determine the therapeutic effectiveness of drugs and design of dosage forms.

EXPERIMENTAL

Materials

BSA was purchased from Sino-Biotechnology Company (Shanghai, China). Asiatic acid was purchased from Sigma (USA), the purity of which is not less than 98%. The buffer Tris-HCl, NaCl, HCl, etc were purchased commercially and used without further purification.

Physical measurement

Fluorescence emission spectra were recorded on RF-5301 Spectrophotometer (Shimadzu, Japan) with 1.0cm quartz cells. The emission and excitation slits were 5nm and 3nm, respectively. Fluorescence quenching spectra were measured in the range of 300 - 500 nm with the excitation wavelength of 285nm at four temperatures (293K, 298K, 303K and 310K). The pH value of Tris-HCl was measured using a pH-2500 pH-meter.

Procedures

For titration experiments, aliquots of the AA were added to a solution, containing appropriate concentration of BSA is 1.0×10^{-6} M (2 mL, in 25 mM Tris-HCl, pH 7.40). The mixture was left to equilibrate for 5 min at four temperatures (293 K, 298 K, 303 K and 310 K). Fluorescence spectra were measured in the range of 300 - 500nm at the excitation wavelength of 285nm. In addition, the fluorescence spectra of BSA were also recorded in the presence of 1.0×10^{-6} M metal ion, which contain Zn^{2+}, Cu^{2+}, Ni^{2+}, Mn^{2+} and Co^{2+} at 310K in the range of 300 - 500 nm at excitation wavelength of 285 nm, the overall concentration of BSA was fixed at 1.0×10^{-6} M, and the common metal ion was maintained at 4.0×10^{-6} M.

RESULTS AND DISCUSS

Fluorescence quenching

When 280 nm is used as the excitation wavelength, BSA can emit strong fluorescence at the wavelength of 340 nm due to the tryptophan residues which possess intrinsic fluorescence[14, 24]. So, the intrinsic fluorescence of proteins can provide considerable information about their structure and

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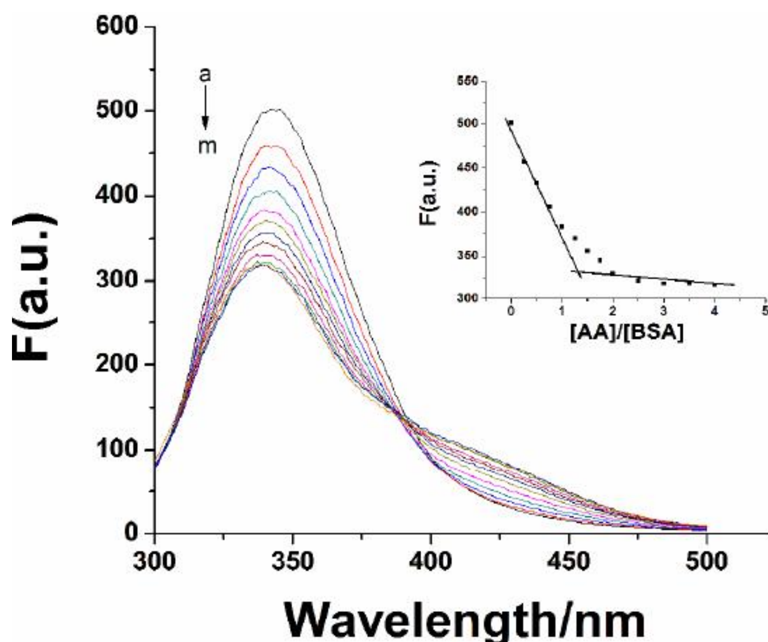

Figure 2 : Effect of AA on fluorescence spectrum of BSA. Conditions: T = 310 K, pH = 7.4, λ_{ex} = 285 nm). [BSA]=1.0×10^{-6}molRþL^{-1}, [AA]: (a)-(m), 0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.5, 3.0, 3.5, 4.0(×10^{-6} mol . L^{-1}), respectively. Inset: Fluorescence intensity at 345 nm is plotted against the ratio of [AA] / [BSA]

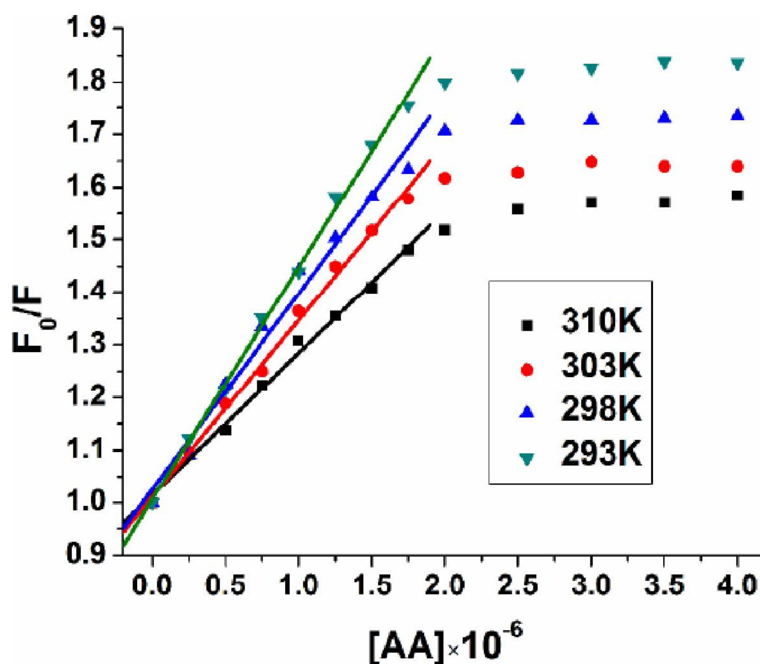

Figure 3 Stern–Volmer plots for the quenching of BSA by AA at different temperatures. [BSA]=1.0×10^{-6}mol . L^{-1}; pH = 7.40; λ_{ex} = 285 nm, λ_{em} = 345 nm; ▼, 293K; ▲, 298K; •, 303K; ■, 310K

dynamics, and is often used to the study of protein folding and association reactions.

The effect of AA on BSA fluorescence intensity is shown in Figure 2. With gradual addition of AA into BSA solution at 310K, the fluorescence intensity of BSA at 345nm remarkably decrease accompanied by an increase in intensity at 425nm. An isosbestic point was located at 387nm, suggesting a specific binding between AA and BSA.

The fluorescence quenching was caused by many reasons, such as excited-state reactions, molecular rearrangements, energy transfer, ground-state complex formation, and collisional quenching[25]. Fluorescence quenching can occur by dynamic quench-

ing, resulting from collisional encounters between the fluorophor and static quenching, resulting from the formation of a ground state complex between the fluorophore and quencher[26]. The fluorescence quenching in the AA and BSA system can be described by Stern-Volmer equation[27]:

$$\frac{F_0}{F} = 1 + k_q \times \tau_0 \times [AA] = 1 + k_{sv} \times [AA] \quad (1)$$

Where F_0 and F are the fluorescence intensities of BSA in the absence and presence of AA, respectively, k_q is the bimolecular quenching rate constant in $M^{-1}\cdot s^{-1}$, τ_0 is the average lifetime of the protein in the absence of a quencher, K_{sv} is the Stern–Volmer quenching constant in M^{-1}, and $[AA]$ is the molar concentration of the respective quencher. Generally, τ_0 is about (10^{-8} s) for a biopolymer[27]. From Eq.(1), K_{sv} can be determine by linear regression of a plot of F_0/F against $[AA]$.

The Stern-Volmer plots of the quenching of BSA fluorescence by AA at different temperatures are displayed in Figure 3. According to Figure 3, it is shown that in the lower AA concentration range of 0 to 2.0×10^{-6}mol·L^{-1}, a good linearity of F_0/F versus $[AA]$ was obviously exhibited and the values of k_q(the values of K_{SV} and k_q were illustrated in TABLE 1) is much greater than 2.0×10^{10} M^{-1} s^{-1}, indicating that the quenching mechanism of BSA by asiatic acid was not initiated by dynamic collision but by static quenching interaction[28].

The binding constant and binding sites

It has been reported that there are independent binding sites in the biomolecule, and the binding constant Ka, as well as number of binding site n could be calculated by using the double logarithm, shown in following equation[29-30]:

$$\log\frac{[C_{BSA}]_b}{[C_{BSA}]_u} = \log\frac{F_0 - F}{F} = \log K_a + n\log[C_1]_u \quad (2)$$

where $[C_{BSA}]_b$ is molar concentration of BSA with a quencher bound and $[C_{BSA}]_u$ is molar concentration

TABLE 1 : Binding and quenching constants according to stern–volmer curves

*p*H	*T*(K)	K_{SV}±SD(10^4L . mol^{-1})	k_q±SD(10^{12}L . mol^{-1} . S^{-1})	*r*
7.40	293	3.417 ± 0.013	3.417 ± 0.013	0.996
	298	2.236 ± 0.020	2.236 ± 0.020	0.997
	303	1.262 ± 0.009	1.262 ± 0.008	0.994
	310	1.091± 0.009	1.091 ± 0.009	0.995

r **is the correlation coefficient; SD is the standard deviation**

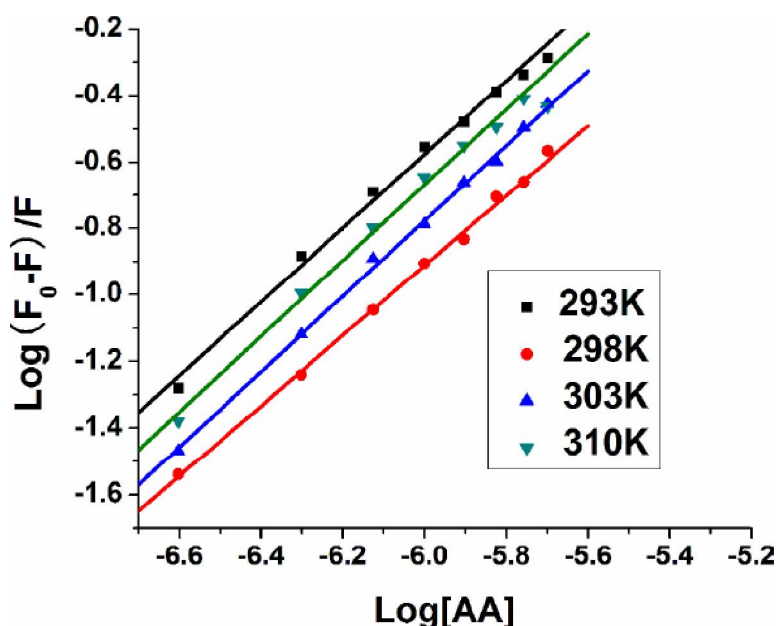

Figure 4 : The plots of lg[(F_0-F)/F] versus lg[AA] at four different temperatures

TABLE 2 : The binding constants and the number of binding sits of AA–BSA interaction at pH 7.4

T	K_a± SD(10^4L . mol^{-1})	n ± SD	r
293	3.06±0.011	0.923±0.012	0.998
298	1.95±0.009	1.164±0.006	0.996
303	1.33±0.007	1.023±0.010	0.999
310	0.93±0.015	0.878±0.014	0.999

r is the correlation coefficient.; SD is the standard deviation.

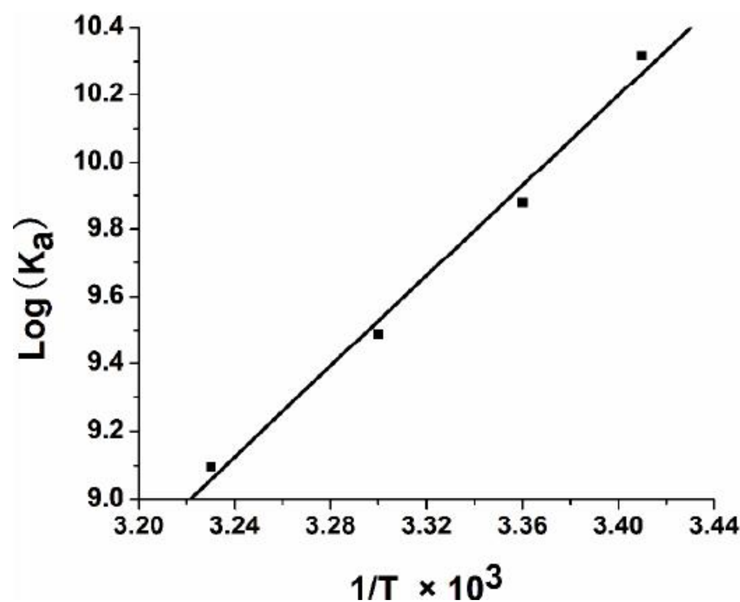

Figure 5 : The plot of 1/T versus log(K_a)

TABLE 3 : Thermodynamic parameters of AA–BSA interaction at pH 7.4

T	ΔH° (KJ . mol^{-1})	ΔG° (KJ . mol^{-1})	ΔS° (KJ . mol^{-1} . K^{-1})
293	-38.64	-25.16	-0.19
298		-24.48	
303		-23.92	
310		-23.55	

of free BSA (with no quencher bound), F_0 and F for fluorescence intensities of BSA in the absence and presence of AA, K_a for the cumulative binding constant, n for the number of binding sites, and $[C_l]_u$ for the molar concentration of free AA. Figure 4 shows the plots of lg[(F_0-F)/F] versus lg[AA] for the AA-BSA system at four different temperatures, the calculated results are presented in TABLE 2. Seen from TABLE 2, there is only a single class of binding sites on BSA for AA, and the binding constant(K_a) decrease with rising temperature because the stability of the complex reduce with a raised in temperature.

Thermodynamic parameters and the nature of the binding forces

The molecular forces contributing to protein interactions with small molecular substrates may be the van der Waals interaction, hydrogen bonds, electrostatic and hydrophobic interactions and so on[30]. The signs and magnitudes of thermodynamic parameters for protein reactions can be accounted for the main forces contributing to protein stability. If the enthalpies 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 equations[31-32]:

$$\ln \frac{K_{a1}}{K_{a2}} = \frac{\Delta H^\circ}{R} \left(\frac{T_2 - T_1}{T_2 T_1} \right) \quad (3)$$

Gibbs free energy change (ΔG) can be obtained

from

$$\Delta G = -RT \ln Ka = \Delta H - T\Delta S \quad (4)$$

where R is the gas constant.

In Eq.(3), K_a is the binding constant at corresponding temperature and R is the gas constant. The fitted curve of lnK_a versus 1/T was shown in Figure 5. As shown in Figure 5, the enthalpy change (ΔH) is calculated from the slope of the van't Hoff relationship and the free energy change (ΔG) is estimated from Eq.(4) and summarized in TABLE 3. The negative values of ³%G indicates that the binding process is spontaneous, while the negative values of ΔH and ΔS mean that the van der Waals interaction and hydrogen bonds are the main impetus in the intermolecular interaction between AA and BSA[33-34].

The effect of metal ions on the binding constant between AA and BSA

There are some metal ions, such as Cu^{2+}, Zn^{2+}, Ni^{2+}, Co^{2+} and etc. in plasma, can form complexes with BSA which could affect the reactions between the drugs and BSA. So, in this paper, the effect of metal ions on binding constants of AA-BSA was studied at 310K. Figure 6 shows the fluorescence spectra in the presence of Cu2+ ions, which could decrease the fluorescence intensity of the BSA-AA. The results of Zn^{2+}, Ni^{2+}, Mn^{2+}, Co^{2+} affected on AA-BSA are listed in TABLE 4. From TABLE 4, the binding constant between BSA and AA increased in presence of Zn^{2+}, Cu^{2+}, Ni^{2+}, Mn^{2+}, Co^{2+}, the binding constans augment 9.18, 9.26, 8.81, 8.63, 7.68, respectively. The results indicated that with the pres-

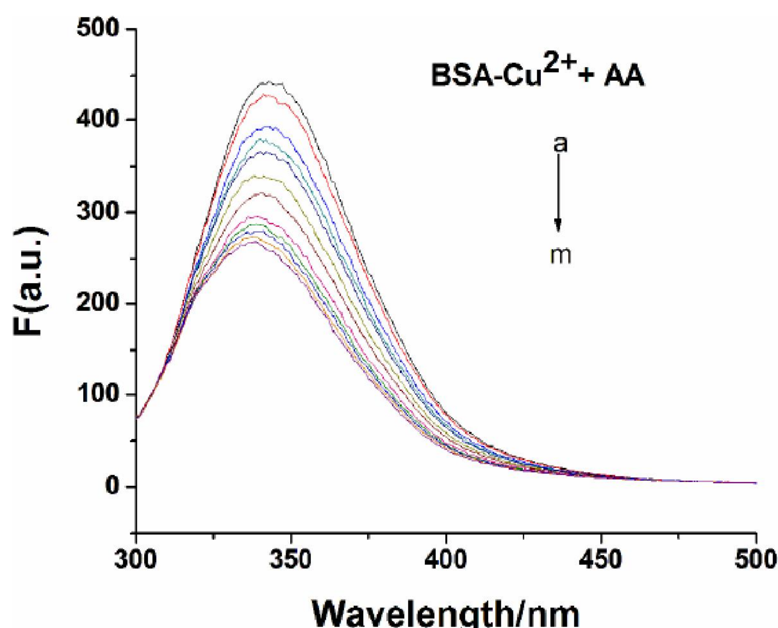

Figure 6 : The fluorescence spectra of the BSA -Cu^{2+} + AA system at 310 K., λex = 285 nm; [BSA] = [Cu^{2+}] = 1.0 × 10^{-6} mol·L^{-1}; [AA]: (a)-(m), 0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.5, 3.0, 3.5, 4.0(×10^{-6} mol . L^{-1}), respectively

TABLE 4 : Effects of some metal ions on the binding constants between AA and BSA at 310K

System	K_a±SD(10^4L . mol^{-1})	n ± SD	r	K_a' / K_a
BSA-AA	0.93±0.015	0.878±0.014	0.999	1
BSA-AA- Zn^{2+}	8.54±0.011	0.932±0.026	0.999	9.18
BSA-AA- Cu^{2+}	8.61±0.023	0.951±0.012	0.998	9.26
BSA-AA- Ni^{2+}	8.19±0.008	1.151±0.018	0.998	8.81
BSA-AA- Mn^{2+}	8.03±0.007	0.920±0.020	0.996	8.63
BSA-AA- Co^{2+}	7.14±0.032	0.899±0.028	0.999	7.68

K_a' and K_a are the values of binding constants in the absence and presence of metal ions, respectively; r is the correlation coefficient. SD is the standard deviation

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ence of M^{2+} (M = metal ions), the stability of BSA could enhanced[35].

CONCLUSIONS

In a word, the intermolecular interaction between BSA and AA has been investigated through fluorescence. The results showed that the static quenching played a main role in the binding process. The thermodynamic parameters demonstrated that the binding was a spontaneous process and the van der Waals interaction and hydrogen bonds played an important role. The association constant, binding potential point and binding site between BSA with AA was discussed. The binding of BSA and AA was strengthened in the presence of Zn^{2+}, Cu^{2+}, Ni^{2+}, Mn^{2+}, Co^{2+} and the binding constans augment 9.18, 9.26, 8.81, 8.63, 7.68, respectively. We believe that this work will help to enrich the knowledge on these intermolecular interactions, spur further study in this area, and assist to provide more useful informations of the structural features on determination the therapeutic effectiveness of drugs and design of dosage forms.

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