Fluorescence spectral studies on interaction of SEGS with BSA

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Abstract. The seeds of Gleditsia sinensis is one of the most effective drugs for the treatment of esophageal carcinoma. To definite the active ingredients of the seeds of gleditsia sinensis, SEGS was extracted from the seeds of Gleditsia sinensis. Investigations the interaction between the active ingredient of traditional Chinese medicine and BSA is helpful for explaining the medicine mechanism. Under the simulative human physiological conditions, the interaction between SEGS and bovine serum albumin (BSA) was investigated by fluorescence spectroscopy. The quenching mechanism of SEGS with BSA is dynamic quenching, SEGS bound with BSA by van der Waals forces or hydrogen bond. The binding constants and the number of binding sites between SEGS and BSA at different temperatures (298 and 308 K) were obtained.

1 Introduction

Bovine Serum Albumin (BSA) is the most abundant soluble protein in the blood plasma, and can help to keep colloidal osmotic blood pressure and act as the transportation and disposition of many drugs, and is capable of binding, delivering an extraordinarily diverse range of endogenous and exogenous compounds like fatty acids, nutrients, steroids, certain metal ions, hormones, enzymes, surfactants and a variety of therapeutic drugs, through the bloodstream to their target organs[1].Therefore, BSA plays an important role in the transportation, distribution, free concentration, excretion, metabolism and interaction with the target tissues of these ligands[2]. Investigations the interaction between the active ingredient of traditional Chinese medicine and BSA is helpful for explaining the medicine mechanism. It is especially useful for select anti-medicine vitro, and can giving theory guide for anti-medicine design[3].

Gleditsia sinensis is a widely used traditional Chinese herb that belongs to the Caesalpinioideae family[4]. For the past 10 years, our group have carried out purposeful studies of anti-esophageal

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carcinoma medicine from over 2000 kinds Chinese traditional herbs. The seeds of Gleditsia sinensis (Chinese honey locust) is one of the most effective drugs for the treatment of esophageal carcinoma[5]. The seeds of Gleditsia sinensis were performed extraction by 95% ethanol. The crude extract was dried and suspended in water and successively treated with chloroform, ethyl acetate and n-butanol. The n-butanol fraction showed good cytotoxic activity to the esophageal carcinoma cells, Eca109, Ec9706 and TE1, by MTT assay. And then the n-butanol fraction subjected to Dianion HP-20 column and successively eluted with H_2O, 30%, 50%, 70%, 90%(v/v)EtOH-H_2O, respectively. The 70% EtOH fraction was chromatographed on silica gel. Sub-fraction was subjected to repeating column chromatography on silica geland further purified with recrystallization, obtained colourless power(SEGS).

2 Experimental

2.1 Apparatus and Reagents

F-7000 fluorescence spectrophotometer (Hitachi, Japan), pHS-3C digital pH-meter (Shanghai REX Instrument Corp., China) Bovine serum albumin, BSA (Beijing Olympic nemesis Biological Technology Co. Ltd.China, relative molecular mass 65000), was dissolved daily in 0.1 mol/L Tris–HCl buffer solution (pH =7.3) to prepare a solution (1×10^{-5}mol/L), SEGS to prepare a solution (80mg/L), All chemicals were analytical-reagent grade. All solutions were prepared with double-distilled water.

2.2 Methods

Fluorescence spectra were carried out on a Hitachi F-7000 fluorescence spectrophotometer using 1.0 cm quartz cells. The excitation wavelength of BSA studied in this work was 280 nm.

3 Results and discussion

3.1 Fluorescence quenching of BSA

Fluorescence technique is wide spread application in investigating of interactions between the active ingredient of traditional Chinese medicine and protein molecules[6]. It is well known that BSA has intrinsic fluorescence due to the presence of amino acids, mainly tryptophan (Trp) and tyrosine (Tyr) residues. The interaction between SEGS and BSA has been mornitored upon exciting BSA at 280 nm where both Trp and Tyr residues get excited. The variations of emission spectra was shown in Figure 1. The fluorescence quenching spectra of solutions containing a BSA fixed concentration and different concentrations of SEGS at 298 K and 308 K, It can be observed that the fluorescence intensity of BSA decreases regularly with the increase addition of SEGS, under the experimental condition, the SEGS does not produce any emission in the specified range of study, and there is no significant emission wavelength shift. These results suggest that SEGS interact with BSA can quench BSA intrinsic fluorescence.

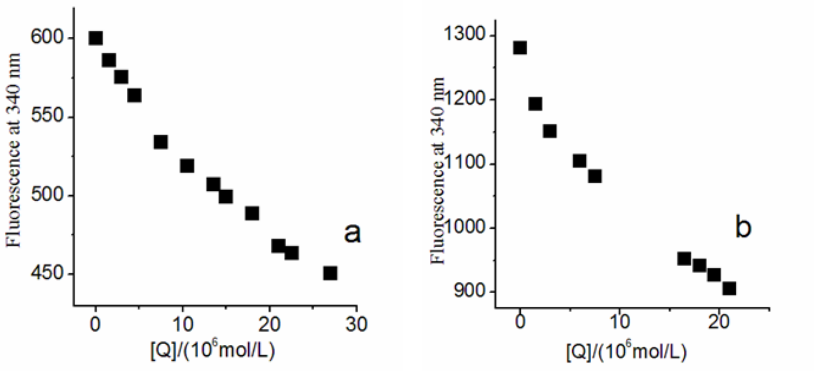

Figure 1. Changes in the emission spectra (λex = 280 nm) of the BSA (1.0×10^{-5} mol/L) upon increasing concentrations of SEGS (a) 298 K and (b) 308K

3.2 Determination of quenching mechanism

There are two quenching types in characterizing the mechanism of the interaction between quenchers and macromolecules: static and dynamic quenching. Static quenching refers to the formation of a non-fluorescence fluorophore-quencher complex. With temperature increasing, the stability of fluorophore-quencher complex reduced, the quenching constant decreased. However, opposite feature exists in dynamic quenching, Dynamic quenching refers to the quencher diffusing towards the fluorophore during the lifetime of the excited state and, upon contact, the fluorophore returns to the ground state without emission of a photon. Dynamic quenching mechanism was probed using the Stern-Volmer equation[7]:

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

Where F_0 and F represent the fluorescence intensities of BSA in the absence and in the presence of the external quencher, Kq is the bimolecular quenching rate constant, K_{SV} is the Stern–Volmer quenching constant, τ_0 is the average lifetime of biopolymers without quencher, the fluorescence lifetime of the biopolymers is 10^{-8} s [8], [Q] is the concentration of the quencher.

This work studied the fluorescence spectrum of the action between BSA and SEGS, obtained the plot of F_0/F against [Q] at different temperature shown in Figure 2.

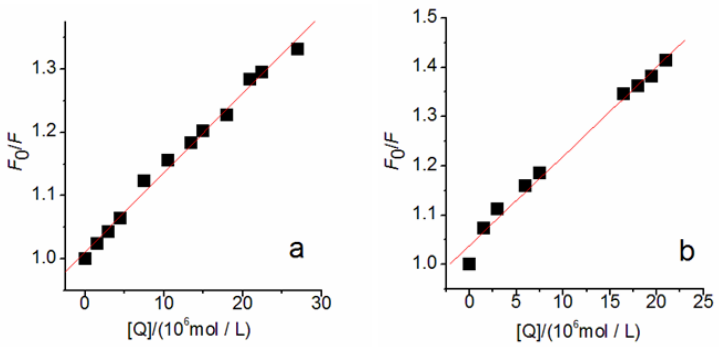

Figure 2. Stern-Volmer plot for the quenching of BSA by SEGS at different temperatures (a) 298 K and (b) 308K

Equation (1) was applied to determine K_{SV} by linear regression from a plot of F_0/F against [Q], hence, Kq can be caculate by Kq=K_{SV}/τ_0. As shown in Table 1.

Table 1. Constant of the systems of interaction between SEGS and BSA at different temperatures

T/K	Ksv/(L/mol)	Kq/(L/mol•s)
298	1.25×10^{-2}	1.25×10^{6}
308	1.81×10^{-2}	1.81×10^{6}

For the SEGS–BSA systems, the values of K_{SV} and Kq =K_{SV}/τ_0 obtained from the plots at 298 K and 308 K are shown in Table 1. According to the literatures[9-10], which suggest that the fluorescence quenching mechanism is mainly dynamic quenching.

3.3 The binding constant Kb and the number of binding sites n

The relationship between fluorescence quenching intensity and the concentration of quenchers can be used to obtain the binding constant and the number of binding sites[11].

$$\mathrm{Lg}(F_0\text{-}F)/F=\mathrm{Lg}K_b+n\mathrm{Lg}[Q] \quad (2)$$

The values of K_b and n at 298K and 308K were obtained from the double logarithm regression curve (Figure 3).
The values of K_b and n at 298K and 308K are listed in Table 2.

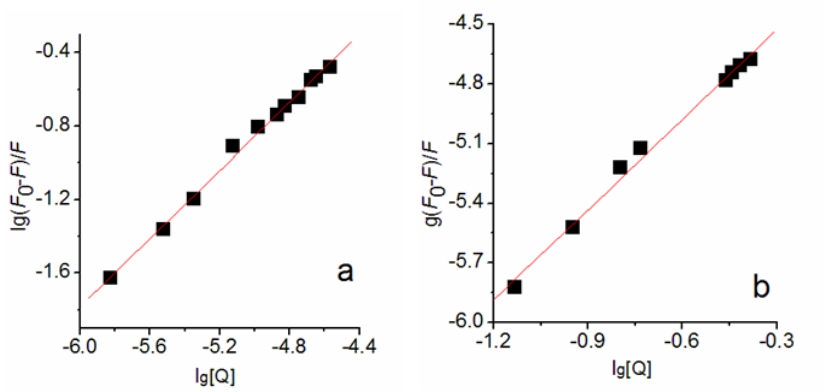

Figure 3. The plots of lg(F_0-F)/F vs. lg[Q] at different temperatures (a) 298 K and (b) 308K
The values of K_b and n at 298K and 308K are listed in Table 2.

Table 2. The binding constant K_b, and the number of binding sites n

T/K	double logarithm regression curve	r	k_b	n
298	lg(F_0-F)/F=3.7811+0.9273lg[Q]	0.99746	6.041×10^{2}	0.9273
308	lg(F_0-F)/F=2.6680+0.6565lg[Q]	0.99648	4.656×10^{2}	0.6565

The n values are nearly 1, and thus indicate the existence of a single binding site in BSA for SEGS. The interactions of SEGS with BSA decreased when rising the temperature, which suggest that interactions of SEGS with BSA were exothermic[12].

3.4 Binding modes

The acting forces between drug and biomolecule are composed of weak interactions of molecules such as hydrogen bond formation, van der Waals forces, electrostatic forces, and hydrophobic interaction. The thermodynamic parameters could be calculated by equation (3), (4) and (5).

$$\mathrm{Ln}(K_2/K_1) = \Delta\mathrm{rHm}(1/\mathrm{T1}-1/\mathrm{T2})/\mathrm{R} \quad (3)$$

$$\Delta\mathrm{rGm} = -\mathrm{RTLnK} \quad (4)$$

$$\Delta\mathrm{rSm} = (\Delta\mathrm{rHm}-\Delta\mathrm{rGm})/\mathrm{T} \quad (5)$$

The thermodynamic parameters are presented in Table 3.

Table 3. Thermodynamic parameters for the association of SEGS with BSA

T/K	ΔrHm/(KJ/mol)	ΔrSm/(J/K)	ΔrGm/(KJ/mol)
298	-19.78	-13.42	-15.87
308		-13.44	-15.73

The negative signs for ΔrHm, ΔrSm and ΔrGm reveal that the binding processes are influenced by temperature, low temperature are spontaneous, high temperature are non-spontaneous. According to the literature[13,14,15], The negative ΔH and ΔS values suggest the van der Waals forces or hydrogen bond formation exist in SEGS and BSA.

4 Conclusion

The interaction between SEGS and BSA has been investigated under simulated physiological conditions (pH 7.3, ionic strength 0.1 mol/L) using fluorescence methodology. The constant *K*q of the SEGS and BSA is less than 2×10^{10} $\mathrm{L\cdot mol^{-1}\cdot s^{-1}}$, which suggest that the fluorescence quenching mechanism of BSA by SEGS was consistent with dynamic quenching. The binding reaction was influenced by temperature for Δ rHm, Δ rSm and Δ rGm is negative. ΔH and ΔS is negative suggest that Van der Waals forces or hydrogen bond play a major role in the SEGS–BSA interaction.

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