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Spectroscopic studies on the interaction between troxerutin and bovine serum albumin

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ABSTRACT

The interaction between troxerutin and bovine serum albumin (BSA) was investigated by fluorescence and absorption spectroscopy under simulative physiological conditions. Results show that troxerutin causes the fluorescence quenching of BSA through a static quenching procedure. The binding constant K_A and number of binding sites n of troxerutin with BSA were obtained. Positive values of thermodynamic parameters enthalpy change (ΔH) and entropy change (ΔS) indicate that the interaction between troxerutin and BSA is driven mainly by hydrophobic forces. It seems that the binding is spontaneous at standard state for the change in standard Gibbs free energy (ΔG) value is negative. The binding distance between the donor (BSA) and the acceptor (troxerutin) was calculated to be about 4.21 nm based on the Förster theory. The effect of troxerutin on the conformation of BSA was also analyzed by using synchronous fluorescence spectroscopy.

1. Introduction

It is well known that the serum albumin is a major soluble protein constituent in the circulatory system, which plays an important role in the transportation and deposition of many drug molecules in the blood [1–3]. The binding ability of drug–albumin in blood stream may have a significant impact on distribution, free concentration and metabolism of drug. Therefore, many efforts have been paid on the interaction of drugs and serum albumins for knowing the transportation and distribution in the body, as well as for clarifying the action mechanism and pharmaceutical dynamics [4,5]. In this work, bovine serum albumin (BSA) is selected as our protein model because of its medical importance, stability, low cost, unusual ligand-binding properties and structural homology with human serum albumin [6].

Troxerutin, namely {2-[3,4-bis(2-hydroxyethoxy)phenyl]-3[(6-deoxy-α-L-manno-pyranos-yl)-β-(D-glucopyranosyl)]oxy]-5-hydoxy-7-(2-hydroxyethoxy)-4H-1-benzopyran-4-one}, is a flavonoid present in tea, coffee, cereal grains and a variety of fruits and vegetables [7–9]. Troxerutin has been commonly used in the treatment of Chronic Venous Insufficiency (CVI), varicosity and capillary fragility [10]. It exerts a great impact on improving capillary function, reducing capillary fragility and abnormal leakage, and it has anti-erythrocytic, anti-thrombotic, fibrinolytic, oedema-protective and rheological activity [11]. Recently, many studies have been carried out to investigate the characteristics and applications of troxerutin due to its broad pharmacological activities. For example, Fan et al. [12] studied the protective role of troxerutin against D-galactose-induced renal injury in mice. Adam et al. [13] investigated the hepatic protective mechanisms of troxerutin for preventing possible coumarin-induced liver injuries in the rat-model. To the best of our knowledge, few investigations have been conducted to study the interaction between troxerutin and BSA, such as the binding mechanism, binding site, binding distance and so on. However, these parameters are very important in the interaction of troxerutin to BSA, when troxerutin is used as a drug. Hence, investigating the interaction between troxerutin and BSA will help in understanding the troxerutin's toxicity and its distribution in the organism.

Fluorescence spectroscopy is essentially a probe technique sensing changes in the local environment of the fluorophore, which has been widely used for drug–protein studies [14–17]. In this paper, the binding of troxerutin to BSA was studied under physiological buffer solution (pH 7.4) by fluorescence and absorption spectroscopy. The binding constants were calculated and binding mechanism was proposed. In addition, the effect of troxerutin on the conformational change of BSA was also studied. We hope this work cannot only provide useful information for appropriately understanding the troxerutin's toxicity, but also illustrate its binding mechanisms at a molecular level.

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2. Experimental

2.1. Materials

Bovine serum albumin was purchased from Boquan Biochemical Technology Co., Ltd. (Nanjing, China) and used without further purification. Troxerutin was obtained from Shanxi Yabao Pharmaceutical Group Co., Ltd. (Shanxi, China). BSA stock solution (1.0×10^{-6} mol L^{-1}) was prepared in pH 7.4 phosphate buffer solution containing 0.1 mol L^{-1} NaCl. The troxerutin solution (6.0×10^{-5} mol L^{-1}) was prepared in pH 7.4 phosphate buffer solution, respectively. All other chemicals were of analytical reagent grade and double distilled water was used throughout.

2.2. Apparatus

All the fluorescence spectra were performed on a RF-5301PC spectrofluorophotometer (Shimadzu, Japan) equipped with a 150-W Xenon lamp, using a quartz cell of 1.0 cm path length. The absorption spectrum was recorded with a UV-3600PC spectrophotometer (Shimadzu, Japan) equipped with 1.0 cm quartz cell.

2.3. Procedures

A 3-ml solution, containing appropriate concentration of BSA (1.0×10^{-6} mol L^{-1}), was titrated by successive additions of 6.0×10^{-5} mol L^{-1} stock solution of troxerutin (to give a final concentration of 4.0×10^{-6} mol L^{-1}). Titrations were done manually by using micro-injector. The fluorescence emission spectra were recorded at different temperatures (299 and 309 K) in the wavelength range of 300–410 nm with exciting wavelength at 290 nm. The temperature of samples was maintained by recycled water throughout.

3. Results and discussion

3.1. Fluorescence quenching of BSA by troxerutin in physiological condition

Fluorescence quenching is due to the decrease of the quantum yield of fluorescence from a fluorophore induced by a variety of molecular interactions with quencher molecule, including excited-state reactions, molecular rearrangements, energy transfer, ground-state complex formation and collisional quenching processes [18]. It is known that there are two quenching mechanisms involved in quenching process, which are usually classified as dynamic quenching and static quenching [19]. In general, dynamic and static quenching can be distinguished by their different dependence on temperature and viscosity [19]. The quenching rate constants decrease with increasing temperature for static quenching, but the reverse effect is observed for dynamic quenching.

The effect of troxerutin on BSA fluorescence intensity is shown in Fig. 1. It is obvious that BSA has a strong fluorescence emission peak at 339 nm after being excited with a wavelength of 290 nm. The fluorescence intensity of BSA gradually decreases as increasing the concentration of troxerutin, indicating the binding of troxerutin to BSA. Furthermore, there was a slight blue shift at the maximum wavelength of BSA (from 339 to 329 nm) after the addition of troxerutin. It suggests that the microenvironment of tryptophan residue was changed after addition of troxerutin [20].

In order to clarify the quenching mechanism, the fluorescence quenching data are analyzed by the Stern–Volmer equation [19]:

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

$$k_q = K_{SV}/\tau_0 \quad (2)$$

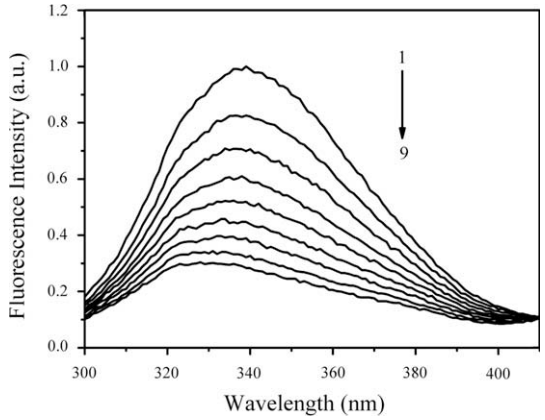

Fig. 1. The fluorescence quenching spectra of BSA at different concentrations of troxerutin (T = 299 K, pH 7.4 and λ_{ex} = 290 nm); from curve 1–9. $C_{BSA} = 1.0 \times 10^{-6}$ mol L^{-1}, troxerutin concentrations: 0, 0.5, 1.0, 1.5, 2.0–2.5, 3.0, 3.5 and 4.0×10^{-6} mol L^{-1}, respectively.

where F_0 and F are the fluorescence intensities before and after the addition of the quencher, respectively. k_q, K_{SV}, τ_0 and [Q] are the quenching rate constant of the biomolecule, the Stern–Volmer dynamic quenching constant, average life-time of the biomolecule without quencher ($\tau_0 = 10^{-8}$ s [21]) and the concentration of the quencher, respectively.

Fig. 2 shows the Stern–Volmer plots of the quenching of BSA fluorescence by troxerutin at different temperatures. The plots show a good linear relationship within the investigated concentrations. The Stern–Volmer quenching constants K_{SV} and the correlation coefficient of each curve were calculated from the slope of the regression curves: 5.81×10^5 L mol^{-1}, R = 0.9982 at 299 K; 5.24×10^5 L mol^{-1}, R = 0.9987 at 309 K. Since the typical fluorescence life-time of biopolymers was about 10^{-8} s, thus k_q were obtained as $k_q = 5.81 \times 10^{13}$ L mol^{-1} s^{-1} at 299 K and 5.24×10^{13} L mol^{-1} s^{-1} at 309 K, based on Eq. (2). The results showed that the values of Stern–Volmer quenching constants K_{SV} and k_q decreased with increasing temperature and the values of k_q were much greater than the limiting diffusion rate constant of the biomolecule (2.0×10^{10} L mol^{-1} s^{-1}) [22], which indicated that the probable quenching mechanism of BSA–troxerutin interactions was initiated by complex formation rather than by dynamic collision [23]. In other words, the fluorescence quenching of BSA results from complex formation predominantly, while that dynamic collision could be negligible.

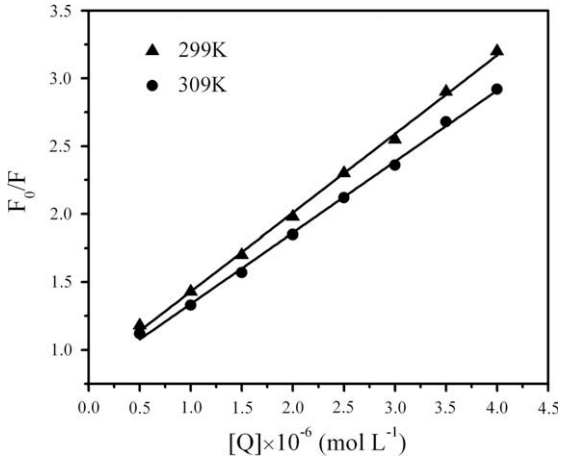

Fig. 2. The Stern–Volmer plots for the quenching of BSA by troxerutin at different temperatures. $C_{BSA} = 1.0 \times 10^{-6}$ mol L^{-1}; λ_{ex} = 290 nm; pH 7.4.

UV absorption spectrum is a very useful method to explore the structural change and the complex formation [24]. In the present study, the UV absorption spectra of BSA in the presence and absence of troxerutin were measured. As shown in Fig. 3, the maximum absorption peak of BSA and troxerutin appeared at 278.5 and 252.0 nm, respectively. With the addition of troxerutin, a new peak appeared at 271.5 nm. Meanwhile, the absorption intensity of BSA was enhanced. The changing of the UV absorption spectrum of BSA was due to forming a ground-state complex (BSA–troxerutin). These results confirmed that the fluorescence quenching type of BSA initiated by troxerutin was static quenching.

3.2. The binding constant and the number of binding site

For static quenching, the relationship between fluorescence quenching intensity and the concentration of quenchers can be described by the binding constant formula [25]:

$$\log(F_0 - F)/F = \log K_A + n \log[Q] \quad (3)$$

where K_A and n are the binding constant and the number of binding sites, respectively. Fig. 4 shows the double-logarithm curve and Table 1 gives the corresponding calculated results. The high linear correlation coefficient R indicates that the interaction between troxerutin and BSA agrees well with the site-binding model underlies in Eq. (3). The results show that troxerutin can bind to BSA and the binding constant increased with the increasing temperature, indicating that the capacity of troxerutin binding to BSA is enhanced. The values of n approximately equal to 1 indicating that there is one binding site in BSA for troxerutin. BSA has two tryptophan residues that possess intrinsic fluorescence: Trp-134 and Trp-212. Trp-134 is embedded in the first sub-domain IB and is more exposed to hydrophilic environment, whereas Trp-212 is embedded in sub-domain IIA and deeply buried in the hydrophobic loop. So, from the value of n, troxerutin most likely binds to the hydrophobic pocket located in sub-domain IIA [26].

3.3. Thermodynamic parameters and nature of the binding forces

The interaction forces between a small molecule and macromolecule commonly include hydrophobic force, electrostatic interactions, van der Waals interactions, hydrogen bonds, etc. [27]. The thermodynamic parameters, enthalpy change (ΔH), free energy (ΔG) and entropy change (ΔS) of reaction, are important for confirming acting force. For this reason, the temperature dependence of the binding constant was studied.

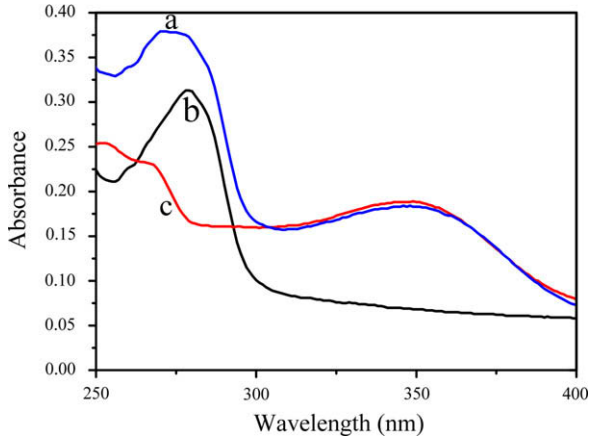

Fig. 3. The UV absorption spectra of troxerutin, BSA and troxerutin–BSA system. (a) The absorption spectra of troxerutin–BSA; (b) the absorption spectrum of BSA only; (c) the absorption spectrum of troxerutin only; $C_{troxerutin} = 6.0 \times 10^{-6}$ mol L^{-1}, $C_{BSA} = 6.0 \times 10^{-6}$ mol L^{-1}; pH 7.4; $T = 299$ K.

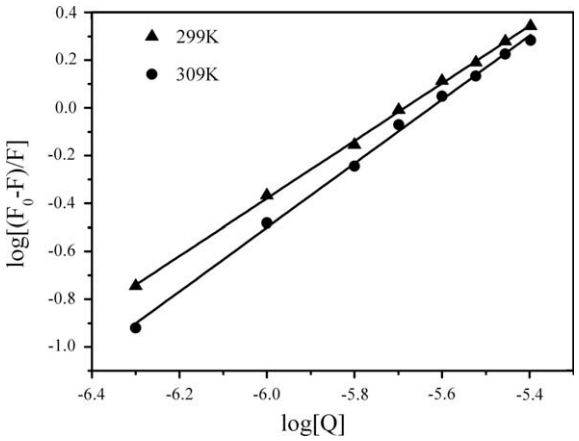

Fig. 4. Double-logarithm plot of troxerutin quenching effect on BSA fluorescence at 299 K and 309 K.

Table 1
The binding parameters, number of binding sites and thermodynamic parameters for BSA–troxerutin complex at different temperatures.

T (K)	K_A (mol^{-1} L)	n	R^a	ΔH (kJ mol^{-1})	ΔG (kJ mol^{-1})	ΔS (J mol^{-1} K^{-1})
299	6.86×10^6	1.20	0.9989	20.08	−39.13	198.03
309	8.91×10^6	1.33	0.9978	20.08	−41.11	198.03

[a] R is the correlation coefficient for the K_A values.

If the temperature does not vary significantly, the enthalpy change can be regarded as a constant. The thermodynamic parameters were calculated from the Van't Hoff equation:

$$\ln \frac{(K_A)_2}{(K_A)_1} = \left(\frac{1}{T_1} - \frac{1}{T_2}\right) \frac{\Delta H}{R} \quad (4)$$

$$\Delta G = -RT \ln K_A \quad (5)$$

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

where R is the gas constant, T is the experimental temperature and K_A is the binding constants at corresponding T. Table 1 gives the thermodynamic parameters for the interaction of troxerutin with BSA. The negative sign for ΔG means the interaction process is spontaneous. The positive ΔH and ΔS values indicate that hydrophobic force plays a major role in the binding between troxerutin and BSA [28].

3.4. Energy transfer from BSA to troxerutin

According to Förster's theory, the energy transfer-efficiency is defined as the following equation:

$$E = 1 - \frac{F}{F_0} = \frac{R_0^6}{R_0^6 + r^6} \quad (7)$$

where F and F_0 are the fluorescence intensities of BSA in the presence and absence of troxerutin, r is the distance between acceptor and donor and R_0 is the critical distance when the transfer efficiency is 50% [29]. R_0 is given by the following equation:

$$R_0^6 = 8.79 \times 10^{-25} k^2 N^{-4} \varphi J \quad (8)$$

where k^2 is the spatial orientation factor of the dipole, N is the refractive index of medium, φ is the quantum yield of the donor

and J is the overlap integral of the fluorescence emission spectrum of the donor with the absorption spectrum of the acceptor (Fig. 5), which can be calculated by the equation:

$$J = \frac{\sum F(\lambda)\varepsilon(\lambda)\lambda^4\Delta\lambda}{\sum F(\lambda)\Delta\lambda} \quad (9)$$

where $F(\lambda)$ is the fluorescence intensity of the donor in the wavelength range λ to $\lambda + \Delta\lambda$, $\varepsilon(\lambda)$ is the molar absorption coefficient of the acceptor at λ. In the presence case, $k^2 = 2/3$, $N = 1.336$ and $\varphi = 0.118$ [30,31]. According to Eqs. (7)–(9), we could obtain that $J = 2.38 \times 10^{-13}$ cm^3 L mol^{-1}, $R_0 = 4.16$ nm, $E = 0.48$ and $r = 4.21$ nm. The donor-to-acceptor distance is much smaller than 8 nm, which implies a high probability of the energy transfer from BSA to troxerutin [32]. It is in accordance with the conditions of Förster's non-radiative energy transfer theory, confirming the static quenching interaction between troxerutin and BSA.

3.5. Conformation investigation

The synchronous fluorescence spectra give information about the molecular environment in a vicinity of the chromosphere molecules and have several advantages, such as sensitivity, spectral simplification, spectral bandwidth reduction and avoiding different perturbing effects [33]. It is applicable 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 [20]. 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 [34].

The effect of troxerutin on BSA synchronous fluorescence spectroscopy is shown in Fig. 6. It is obvious that the maximum emission wavelength of tyrosine residues does not have a significant shift. In contrast, a slight blue shift (from 340 to 338 nm) of tryptophan residues was observed. The blue shift of the maximum emission wavelength indicates that the conformation of BSA was changed and the polarity around the tryptophan residues was decreased and the hydrophobicity was increased [33], but the interaction of BSA with troxerutin does not obviously effect conformation of tyrosine micro-region [35].

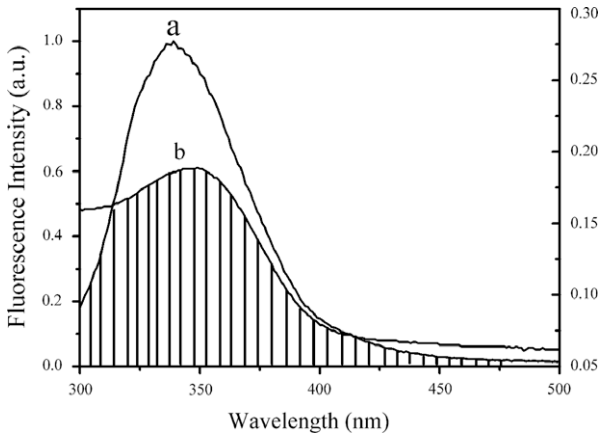

Fig. 5. The overlap of the fluorescence spectrum of BSA (a) and the absorbance spectrum of troxerutin (b): $C_{BSA} = C_{troxerutin} = 1.0 \times 10^{-6}$ mol L^{-1}.

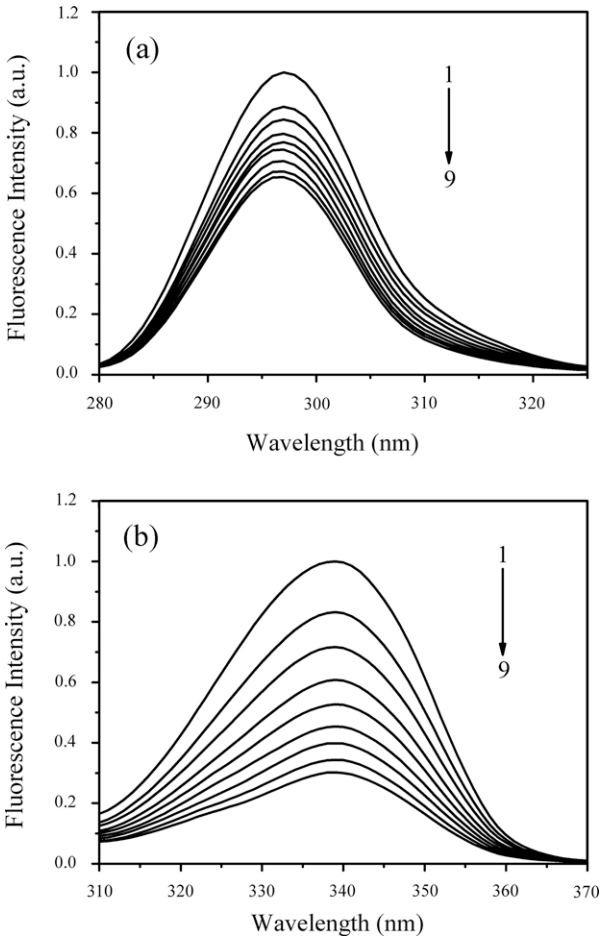

Fig. 6. Synchronous fluorescence spectra of BSA ($T = 299$ K, pH 7.4), from curve 1–9. $C_{BSA} = 1 \times 10^{-6}$ mol L^{-1}, $C_{troxerutin} = 0$, 0.5, 1.0, 1.5, 2.0–2.5, 3.0, 3.5 and 4.0×10^{-6} mol L^{-1}, respectively. (a) $\Delta\lambda = 15$ nm and (b) $\Delta\lambda = 60$ nm.

4. Conclusions

The interaction between troxerutin and BSA in physiological buffer solution was studied by fluorescence and absorption spectroscopy. The results showed that the fluorescence of BSA was quenched by troxerutin through a static quenching process. The binding constants K_A were obtained to be 6.86×10^6 and 8.91×10^6 L mol^{-1} at 299 and 309 K, respectively. The thermodynamic parameters calculated from van't Hoff equation indicate that the hydrophobic force plays an important role in stabilizing the complex and the binding reaction is a spontaneous process. The average binding distance between the donor (BSA) and the acceptor (troxerutin) was about 4.21 nm. The results of synchronous fluorescence spectroscopy indicate that the interaction of BSA with troxerutin affects the conformation of tryptophan residues' microregion.

Acknowledgements

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