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Binding of the scutellarin to albumin using tryptophan fluorescence quenching, CD and FT-IR spectra

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Abstract: The binding of scutellarin with bovine serum albumin (BSA) was investigated at three temperatures, 296k, 310K, and 318K, by the fluorescence, circular dichroism (CD) and fourier transform infrared spectroscopy (FT-IR) at pH 7.40. The binding parameters were determined by Stern-Volmer equation. The thermodynamic parameters were calculated according to the dependence of enthalpy change on the temperature as follows: ΔH^0 and ΔS^0 is negative value (-13.34 KJ/mol) and positive value (47.88J/mol·K), respectively. Quenching of the fluorescence BSA in the presence of scutellarin was observed. Data obtained by fluorescence spectroscopy and CD experiment, FT-IR experiment suggested that scutellarin can strongly bind to the BSA and it is considered that scutellarin binds to BSA mainly by hydrophobic interaction. The distance between the tryptophan residues in BSA and scutellarin was estimated to be 2.41 nm using Förster's equation on the basis of fluorescence energy transfer.

Key words: Bovine serum albumin, circular dichroism (CD), fluorescence, fourier transform infrared spectroscopy (FT-IR), interaction, scutellarin

INTRODUCTION

Serum albumin are the most abundant proteins I plasma. As the major soluble protein constituents of circulatory system, they have many physiological functions and play a key role in the transport of many endogenous and exogenous ligands. For many drugs binding to serum albumin is a critical determination of their distribution and pharmacokinetics [1]. BSA possesses a high helical content (about 67%) and molecular weight of 66.4 KDa (calculated form the amino acid composition). BSA consists of 583 amino acids in a single polypeptide chain. It is built from three structurally homologous domains (I, II and III). Each domain is the product of two subdomains (IA, IB etc.)[2]. Serum albumin is postulated to have a heart-shaped structure with dimensions of 80×80×30 Å [3] and many binding sites are available for the binding of ligands. Hence, the nature of binding of a ligand with BSA is different for different ligands [4-5]. Furthermore, the binding of active component of natural herb medicine with serum albumin has seldom been studied [6-7].

The flavonoids comprise an important group of naturally occurring bioactive polyphenolics, ubiquitous in plants of higher genera [8-9]. However, till today, very little is known about the mode of interactions of these compounds with their respective target proteins at the molecular level. Furthermore, the interaction of scutellarin with bovine serum albumin (BSA) has not been reported.

In this paper, the interaction of scutellarin (Huangqin Dai, scheme I, β-D-Glucopyranosiduronic acid), which is the main active component of Traditional Chinese medicine *Scutellaria altissima L.* and *Baicalensis Georgi*, with BSA was studied at pH 7.40 by spectroscopic methods including circular dichroism (CD), fluorescence spectroscopy, and fourier transform infrared spectroscopy (FT-IR) spectra.

MATERIALS AND METHODS

Bovine serum albumin (BSA, Sino-American Biotechnology Company) solution of 1.5×10^{-5} mol/L was prepared in pH 7.40 Tris-HCl buffer solution. Scutellarin (National Institute for Control of Pharmaceutical and Products, China, 1.0×10^{-3} mol/L) solution was obtained by dissolving it in 50mL ethanol. All other chemicals were of analytical reagent grade.

Fluorescence spectra were recorded using a RF-5301 PC spectrofluorophotometer (Shimadzu) with a 150 W Xenon lamp and a 1 cm quartz cell. The excitation and emission bandwidths were both 5 nm. Circular diachronic (CD) measurements were made on a Jasco-20c automatic recording spectropolarimeter (Japan), using a 2 mm cell at 296K. The induced ellipticity was defined as the ellipticity of the drug-BSA mixture minus the ellipicity of drug alone at the same wavelength and the content of α-helix was calculated [10].FI-IR measurements were carried out at room temperature on a Nicolet Nexus 670 FT-IR spectrometer (America) equipped with a Germanium attenuated total reflection (ATR) accessory, a DTGS

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KBr detector and a KBr beam splitter. All spectra were taken via the Attenuated Total Reflection (ATR) method with resolution of 4 cm^{-1} and 60 scans.

RESULTS

Spectroscopic study

The conformational changes of BSA were evaluated by the measurement of intrinsic fluorescence intensity of protein before and after addition of drug. The effect of drug on BSA fluorescence intensity is shown in Fig. 1. The decrease in the fluorescence intensity was the most marked change in the fluorescence spectrum observed upon addition of scutellarin (Fig. 1). From Fig. 1, when the solution of scutellarin was titrated into a fixed concentration of BSA, a remarkable fluorescence decreasing of BSA was observed. Furthermore, a slight red shift of the maximum emission wavelength was observed that it could be deduced that conformational changes induced by the interaction lead to a further exposure of tryptophan residues to the polar solvent, that is, the interaction of scutellarin with BSA induced the alteration of the tertiary structure of BSA. [11]

Fig. 2 shows the CD spectra of the BSA and BSA-scutellarin complex obtained at pH 7.40. The CD spectra of BSA exhibited two negative minima at 208 and 217 nm, which is typical of the α/β helix structure of class proteins. The spectra of scutellarin-BSA are similar in shape with BSA, but the interaction between scutellarin and BSA caused a decrease in band intensity at all wavelengths of the far-UV CD without any significant shift of the peaks, indicating that this drug induces a slight decrease in the helix structure content of the protein. The α-helix content of BSA was decreased from 43.4% to 40.7%. From CD and fluorescence spectra results, we can conclude that the interaction of scutellarin with BSA induced the slight unfolding of the constitutive polypeptides of protein, which resulted in a conformational change of the protein that increased the exposure of some hydrophobic regions which were previously buried. Conformation of this explanation is also given by the red shift observe in the intrinsic fluorescence of the protein.

Fig. 3 showed the FT-IR spectrum of free BSA in Tris-HCl buffer and the difference spectra after binding with scutellarin. The peak position of amide I moved from 1645 to 1659 $cm^{-1}$and amide II moved from 1558 to 1541 cm^{-1} in BSA infrared spectrum after interaction with scutellarin, which indicate that the secondary structure of BSA has been changed because of the interaction of scutellarin with BSA. This result was also in agreement with the result of CD experiment.

The binding constants obtained from Stern-Volmer method are $7.13\times10^{4}M^{-1}$,$5.13\times10^{4}M^{-1}$ and $4.87\times10^{4}M^{-1}$ for 296,310,318 K, respectively. It can be seen that scutellarin can strongly bind to serum albumin and temperature has effect on it. If the enthalpy changes (ΔH^{0}) does not vary significantly over the temperature range studied, then its value and that of ΔS^{0} can be determined from the Van't Hoff equation. The enthalpy change (ΔH^{0}) is calculated from the slope of the van't Hoff relationship. The ΔH^{0} and ΔS^{0} is small negative value (-13.34 KJ/mol) and positive value (47.88 J/mol·K), respectively, which indicates that the binding processes are entropically driven. Furthermore, the main source of ΔG^{0} value is derived from a large contribution of ΔS^{0} term with little contribution from the ΔH^{0} factor, so the main interaction is hydrophobic contact, but the electrostatic interaction cannot be excluded.

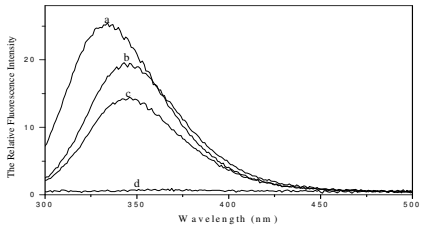

Fig. 1 The fluorescence spectra of scutellarin-BSA system. [BSA]=1.5×10^{-6} mol/L, [scutellarin]=0, 1.8, 2.4×10^{-5} mol/L from the a to the c, d [scutellarin] = 1.8×10^{-5} mol/L. pH 7.40; λex = 280 nm, λem = 342 nm

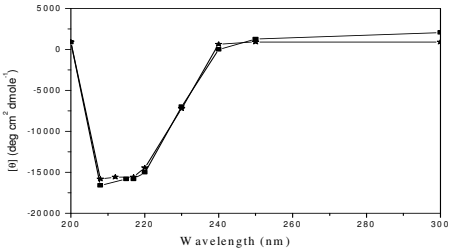

Fig. 2 CD spectra of the scutellarin-BSA complex. Drug to BSA (1.5×10^{-6}mol/L) ratios, ■: 0:1, ★: 2:1

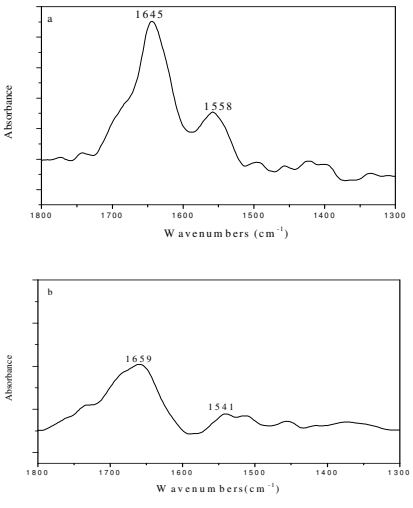

Fig. 3 FT-IR spectra and difference spectra [(BSA solution + scutellarin solution)- (scutellarin solution)] of free BSA (a) and its scutellarin complexes (b) [scutellarin] = 4.0×10^{-5}mol/L, [BSA] = 3.0×10^{-5}mol/L

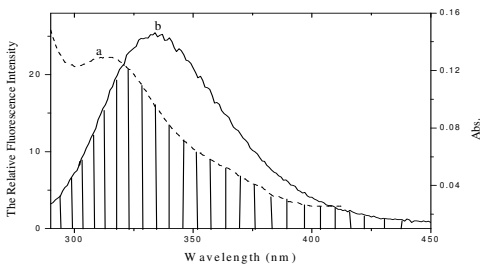

Fig. 4 The fluorescence spectra of BSA(straight line) and the absorption spectra of of scutellarin(dash line) [BSA] = 1.5×10^{-6}mol/L; [scutellarin] = 1.2×10^{-5}mol/L

Fig. 4 is the overlap of the fluorescence spectra of BSA and the absorption spectra of scutellarin. Under these experimental conditions, we found R_0 is 1.69 nm, using K^2=2/3, N=1.336, Φ=0.15, the distance between scutellarin and BSA is 2.41 nm.

CONCLUSION

The results of fluorescence quenching measurements and CD, FR-IR experiments suggested that scutellarin can binds to BSA mainly by hydrophobic interaction and the interaction of scutellarin with BSA can induce the change of secondary structure of BSA. The distance between scutellarin and BSA is calculated using the Förster's energy transfer theory.

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