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Research Article

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Investigation of the interaction between baicalin and human serum albumin by a spectroscopic method and molecular modeling

Kun Qin[1] and Caihong Liu[2*]

[1]College of Chemical Engineering, Taishan Medical University, Tai'an, P.R. China
[2]College of Pharmaceutical, Taishan Medical University, Tai'an, P.R. China

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ABSTRACT

Baicalin is a widely used compound in Chinese traditional medicine and exhibits many pharmacological activities. Under simulated physiological conditions, the interaction between baicalin and human serum albumin(HSA), had been investigated by fluorescence spectroscopic, ultraviolet spectrum(UV), Fourier transform infrared(FT-IR), circular dichroism(CD) and molecular modeling. The results indicated that baicalin caused a static quenching of intrinsic fluorescence of HSA. The binding constants were 1.28×10^5 and 0.91×10^5 $L\cdot mol^{-1}$ at 299 and 309 K respectively. The thermodynamic analysis found the enthalpy change (ΔH) and the entropy change (ΔS) were -26.2 $kJ\cdot mol^{-1}$and 10.1 $J\cdot mol^{-1}\cdot K^{-1}$ respectively, which suggested that hydrophobic was the predominant forces in the baicalin-HSA complex. The alterations of protein secondary structure in the presence of baicalin in aqueous solution were estimated by the evidences from CD and FT-IR. The results from synchronous fluorescence indicated microenvironment around tryptophan (Trp) had a slight trend of polarity increasing. Molecular modeling suggested baicalin was located in subdomain IIA by hydrophobic forces，which was agreed well with the corresponding experimental results.

Keywords: Baicalin; Human serum albumin; Spectroscopy; Molecular modeling

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INTRODUCTION

Human serum albumin (HSA), the most abundant protein in blood serum, acts as a disposer and transporter of many endogenous and exogenous compounds [1–5]. HSA binds a number of endogenous compounds such as unesterified bile acids, fatty acids, and bilirubin. HSA also binds many xenobiotics such as drugs. The studies of drug-protein interacting have a important effect on the distribution, free concentration and the metabolism of a drug in the blood stream. Therefore, studying the interaction of active components in Chinese herbs with HSA has major biochemical importance and can be used as a model for gaining fundamental insights into drug-protein interactions.

Baicalin (Scheme 1), one of flavonoid compound, is a traditional Chinese medicine. Like other flavonoid compounds, this drug has a broad spectrum of biological activities such as antioxidant activity [6], preventive effect against ischemic stroke [7], antipyretic effects [8]. It also has important therapeutic applications such as a prophylactic agent for heatstroke and protect against cerebrovascular dysfunction and brain inflammation in heatstroke [9].

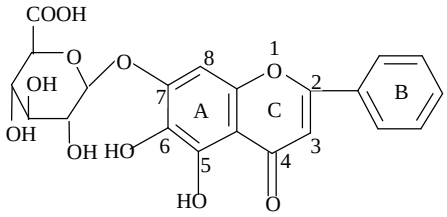

Fig1. Chemical structure of baicalin

To better understand the pharmacological activities of baicalin at molecular level, we investigated the interaction between baicalin and HSA by different spectroscopic methods such as fluorescence spectroscopic (FS), ultraviolet spectroscopy (UV), Fourier transform infrared (FT-IR), circular dichroism (CD). Binding parameters were calculated and binding mode was discussed. The location of baicalin on HSA was identified by molecular docking.

EXPERIMENTAL SECTION

2.1 Materials and chemicals

Human serum albumin (HSA, fatty acid free <0.05%) was purchased from Sigma Chemical Co. Baicalin (>99.0%) was purchased from the National Institute for Control of Pharmaceutical and Bioproducts, China, and the stock solution(1×10^{-3}mol·L^{-1}) was prepared in ethanol. All HSA solution was prepared in the pH 7.40 buffer solution and stored in refrigerator prior to use. Tris-HCl buffer (0.20mol·L^{-1}, pH 7.40) containing 0.10mol·L^{-1}NaCl was prepared. All other reagents and solvents were of analytical reagent grade. All aqueous solutions were prepared using newly double-distilled water.

2.2 Apparatus and methods

The UV absorption spectra of the baicalin-HSA with concentrations of baicalin from 0 to 2.4 $\times10^{-5}$ mol·L^{-1} were recorded on a UV-1700 spectrophotometer (Shimadzu Corporation, Japan) from 200-500nm.

A Nicolet Nexus 670 FT-IR spectrometer (America) FT-IR was used to measure the spectra of baicalin-HSA. Spectra were taken with resolution of 4 cm^{-1} and 60 scans. Spectra processing procedures: spectra of buffer solution were collected at the same condition. Then, subtract the absorbance of buffer solution from the spectra of sample solution to get the FT-IR spectra of proteins. The subtraction criterion was that the original spectrum of protein solution between 2200 and1800 cm^{-1} was featureless [10].

Circular dichroism (CD) measurements were carried out on a JASCO J-820 automatic recording spectropolarimeter (Japan) using a 300 μl cell. Data were recorded from 200-250 nm with a scan speed of 100 nm/min. HSA was mixed with different concentration of baicalin before the measurement and the concentration of HSA was maintained at 3×10^{-6} mol·L^{-1}.

Fluorescence spectra of HSA were recorded on a RF-5301PC fluorescence spectrometer (Shimadzu Corporation, Japan) from 300-500 nm at an excitation wavelength of 280 nm with different concentration of drug as reference. Synchronous fluorescence spectra of HSA in the absence and presence of increasing amount of baicalin (0-7.0×10^{-5} mol·L^{-1}) were recorded.

The program AutoDock 3.05 was used in this docking study, in which Lamarckian genetic algorithm was used to search for the optimum binding site [11]. The crystal structure of HSA in complex with Dig (digitoxin) was taken from the Brookhaven Protein Data Bank (entry codes 1h9z)

[12].The potential of the 3-D structure of HSA was assigned according the Amber 4.0 force field with Kollman-all-atom charges. The initial structures of all the molecules were generated by molecular modeling software Sybyl 6.9. During docking process, a maximum of 10 conformers was considered for the drug. The conformer with the lowest binding free energy was used for further analysis.

RESULTS AND DISCUSSION

3.1 Change of HSA secondary structure induced by baicalin

Fig. 2 showed the UV absorption of HSA in the absence and presence of baicalin. As shown in Fig. 2, HSA had two absorption peaks at 211 and 278nm. The absorption of HSA at 211 nm represented the content of α-helix in the protein [13]. With adding baicalin to HSA, the absorbance intensity at 211 nm was decreased, which indicated a

perturbation of α-helix induced by a specific interaction between baicalin and HSA.

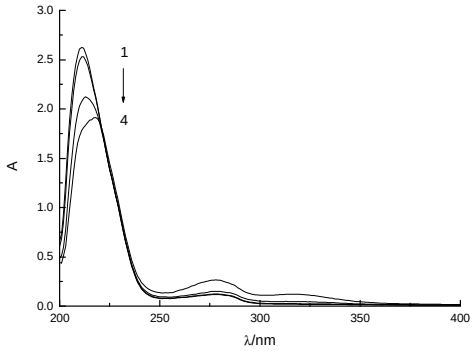

Fig2. UV absorbance of HSA with different concentrations of baicalin
$C_{HSA}=3\times10^{-6}$mol.L^{-1}
1-4：$C_{baicalin}/C_{HSA}$=0,1,5,8

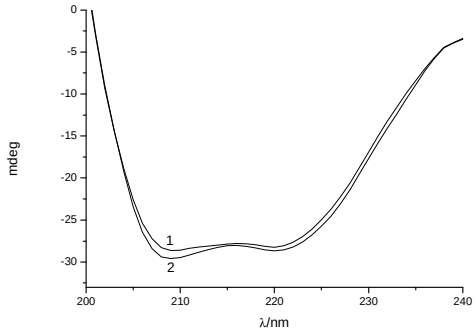

Fig3. CD spectra of HSA-baicalin
$C_{HSA}=3\times10^{-6}$ mol·L^{-1}
1-2：$C_{baicalin}$/ C_{HSA}= 0,1

To ascertain the possible influence of drug binding on the secondary structure of HSA, CD studies were performed in the presence of different concentrations of baicalin. The result (in Fig. 3) of CD showed that HSA had two negative binds in the far UV region at 208 and 220 nm, which were typical characterization of α-helix structure in protein [14]. The negative peaks between 208 -209 nm and 222-223 nm are contributed to n-π* transfer for the peptide bond of α-helical. In the presence of baicalin, the intensity of negative binds at 208 nm was increased, without any significant shift of peaks. The results suggested structure of HSA was also predominantly α-helix. The α-helix contents of free and combined HSA were calculated using the flowing equation [15]:

$$[\theta]=\frac{\theta_\lambda}{C_p\times n\times l\times 10} \quad (1)$$

$$\alpha-helix(\%)=\frac{-[\theta]_{208}-4000}{33000-4000}\times 100 \quad (2)$$

where C_P is the concentration of protein, n is the number of residues in protein (HAS, 585), l is the path length (0.1 cm). According to the calculated results, the α-helix contents of HSA were 41.77% and 43.94% with the molar ratio baicalin–HSA of 0:1 and 1:1 respectively. It suggested that binding of baicalin to HSA had slightly altered the secondary structure of the protein.

Additional evidence regarding the baicalin-HSA came from FT-IR spectroscopy results obtained for drug-protein complexes. Fig.4 showed the FT-IR spectroscopy of HSA in the absence and presence of baicalin. As shown in Fig.4, the peak position of amide I band (1670 cm^{-1}) and amide II band (1529 cm^{-1}) in the HSA infrared spectrum had shift to 1666 cm^{-1} and 1525 cm^{-1} respectively. The peak shapes of them were also changed. These indicated that the secondary structure of HSA is changed.

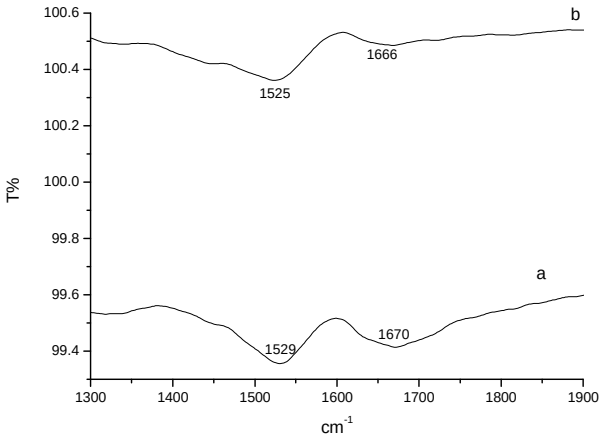

Fig4. FT-IR spectra of HSA and different spectra of HSA after binding with baicalin
(a)HSA （b）HSA-baicalin

3.2 Conformational changes investigated by synchronous fluorescence

Synchronous fluorescence is a useful tool to investigate the microenvironments around the fluorophore functional groups. The fluorescence of HSA with Δλ ($\Delta\lambda=\lambda_{em}-\lambda_{ex}$) of 60 and 15 nm are characteristic of tryptophan(Try) and tyrosine(Tyr) respectively[16]. As shown in Fig.5, with the addition of baicalin, the fluorescence intensity of Tyr (Fig. 5b) was weak and no shift of maximum emission wavelength (λ_{max}) was observed. The fluorescence intensity of Try (Fig. 5a) was weak and the λ_{max} shifted from 338 to 341 nm. According to reporte, λ_{max} at 330-332 nm indicated the Try residues were located in an apolar region, namely they were buried in a hydrophobic cavity. The λ_{max} at 350-352 nm showed Try residues were exposed to water, namely the hydrophobic cavity in HSA was disagglomerated and the structure of HAS was looser. As shown in Fig.5, the result of red shift of λ_{max} suggested baicalin bound to a hydrophobic cavity in HAS and the polarity around Try increased while the hydrophobicity decreased.

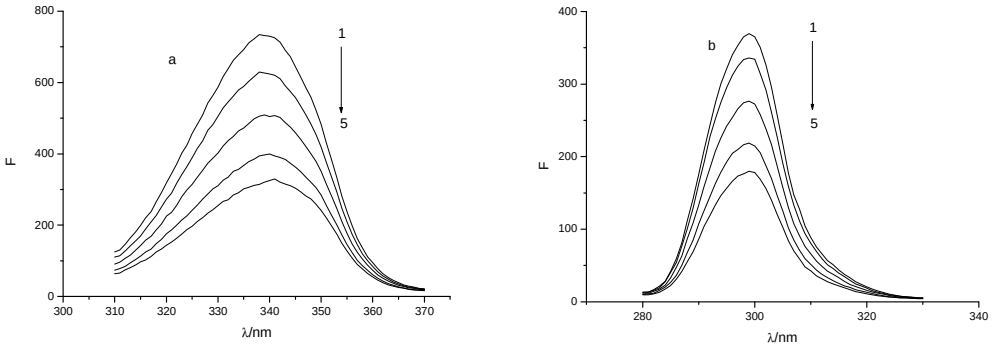

Fig5. Synchronous fluorescence spectra of HSA (a)△λ=60 nm; (b) △λ=15 nm
$C_{HSA}=1\times10^{-6}$ mol·L^{-1}; 1-5:$C_{baicalin}$/ C_{HSA} = 0,1,3,5,7

3.3 Analysis of fluorescence quenching of HSA by baicalin

As shown in Fig.6, when the excitation was 280 nm, the fluorescence intensity decreased gradually and the maximum emission wavelength showed a red shift from 337 to 344 nm, with the addition of baicalin. The quenching indicated the binding of baicalin to HSA. The red shift of the maximum emission wavelength suggested the hydrophobicity in HSA decreased.

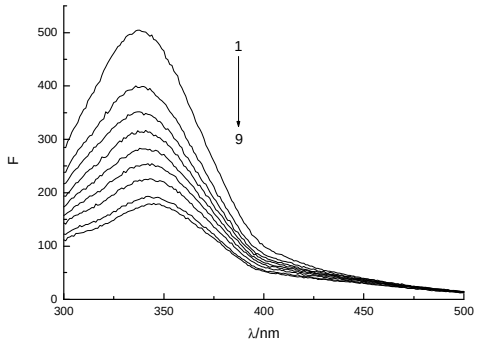

Fig6. Fluorescence quenching spectra of baicalin-HSA
1-9: $C_{baicalin}$/ C_{HSA} = 0, 0.5, 1.0, , 2.0, 3.0, 4.0, 5.0，6.0，7.0
C_{HSA} =1.0×10^{-6}mol·L^{-1}

Stern-Volmer equation was used to analyze the mechanism of fluorescence quenching by baicalin[17]:

$$\frac{F_0}{F} = 1 + K_{SV} C_q \qquad (3)$$

where F_0 and F are the fluorescence intensities in the absence and presence of baicalin, C_q is the drug concentration, K_{SV} is the quenching constant. According to Eq. (3), we calculated the value of K_{SV} (Table1). As shown in table 1, the value of K_{SV} was decreased with increasing of temperature, indicating a static quenching mechanism by the specific interaction of baicalin with HSA.

Table 1 The Stern-Volmer K_{SV} of the baicalin-HSA system

T/K	R^2	K_{SV}（10^3 L·mol^{-1}）
299	0.9916	2.48
309	0.9949	1.95

3.4 Binding constant

To get the binding constant, quenching data from the fluorescence titration were analyzed according to the equation (4) [18]:

$$\lg [(F_0-F)/F] = \lg K + n \lg [Q] \qquad (4)$$

where F_0 and F are the fluorescence intensities in the absence and presence of baicalin, [Q] is the drug concentration, K and n are the binding constant and number of binding sites, respectively. The results in Table2 indicated that the binding between baicalin and HSA was very strong.

Table2 Binding parameters and thermodynamic parameters of baicalin-HSA

T/K	K(10^5)	n	R^2	ΔH/(kJ·mol^{-1})	ΔS/(J·mol^{-1}·K^{-1})	ΔG/(kJ·mol^{-1})
299	1.28	0.94	0.9913	-26.2	10.1	-23.2
309	0.91	0.92	0.9979			-23.1

3.5 Thermodynamic analysis and the binding force

The molecular forces contributing to drug-protein binding process included van der Waals forces, hydrogen bonds, electrostatic and hydrophobic interactions. According to the data of enthalpy change (ΔH) and entropy change (ΔS), the molecular forces of interaction between a drug and protein could be concluded. If the enthalpies change (ΔH) did not vary significantly over the temperature range studied, then thermodynamic parameters could be determined from the van′t Hoff equation.

$$\ln K = -\Delta H/RT + \Delta S/R \qquad (5)$$

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

where K is the binding constant at corresponding temperature and R is the gas constant. The thermodynamic

parameters for the interaction of baicalin with HSA were shown in Table 2. The negative sign for ΔG meant that the interaction process was spontaneous. A positive ΔS value indicated that hydrophobic forces might play a major role in the binding of baicalin to HSA [19]. A negative ΔH value indicated electrostatic forces might exist in the binding of baicalin to HSA. Meanwhile, it was found that the major contribution to ΔG arised from the ΔH rather than from ΔS. Thus the hydrophobic interaction might play a major role in the binding baicalin to HSA.

3.6 Molecule modeling

Autodock3.05 was applied to deduce the right site and interaction forces of baicalin binding to HSA. As shown in Fig.7, baicalin bound to the drug site I located in subdomain IIA. The baicalin moiety was located within the binding pocket and the A- and C-rings were practically coplanar. It was worth noting that the Trp214 residue of HSA was in close proximity to the A-ring of baicalin suggesting the existence of hydrophobic interaction between them. The finding also explained the reason why the efficient fluorescence quenching of HSA emission in the presence of baicalin. The calculated binding Gibbs free energy (ΔG) was -20.9 kJ·mol^{-1}, which was agreed well with the experimental data (-23.2 kJ·mol^{-1}).

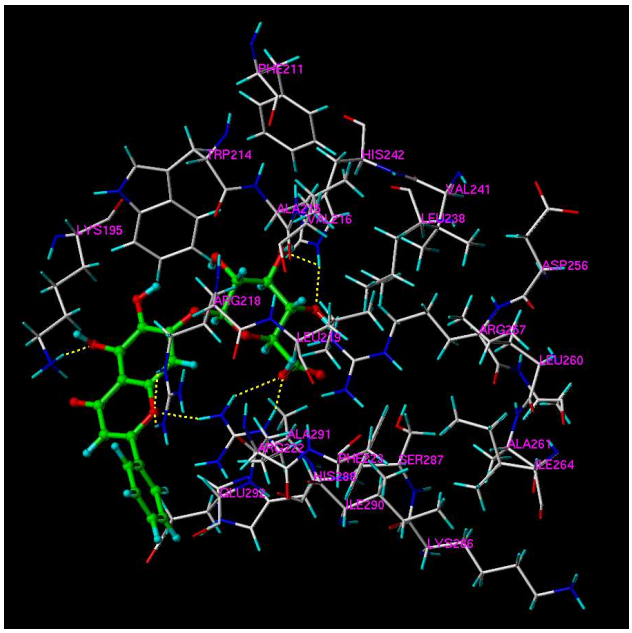

Fig.7 Molecular docking of the baicalin-HSA complex, only residues around 8 Å the ligand are displayed
The hydrogen bonds are shown by broken lined

CONCLUSION

By spectroscopic and modeling methods, the interaction between baicalin and HSA had been investigated under simulative physiological conditions. Experimental results showed that the intrinsic fluorescence of HSA was quenched through static quenching mechanism. The results also suggested that binding of baicalin to HSA induced a conformational change of HSA and a slight increase in α-helical contents. The microenvironment around HSA also became less hydrophobic according to data from synchronous fluorescence. Molecule modeling results indicated the binding site was located in a hydrophobic cavity near the drug binding site I. The work provides some valuable information for the transportation and distribution of baicalin in vivo, which is helpful for clarifying toxicity and dynamics of baicalin.

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REFERENCES

[1] Z Lu; Y Zhang; H Liu; J Yuan; Z Zheng; G Zou, *J Fluoresc*, **2007**, 17(5), 580-587.
[2] YJ Hu; Y Liu; LX Zhang, *J Mol Struct*, **2005**, 750(1-3), 174-178.
[3] C Dufour; O Dangles, *Biochim Biophys Acta*, **2005**, 1721(1-3), 164-173.
[4] MX Xie; M Long; Y Liu; C Qin; YD Wang, *Biochim Biophys Acta*, **2006**, 1760(8), 1184-1191.
[5] HG Mahesha; SA Sighn; N Srinivasan; RG Appu, *Febs J.*, **2006**, 273(3), 451-467.

[6] T Akao; M Hanada; Y Sakashita; K Sato; M Morita; T Imanaka, *J Pharm. Pharmacol*., **2007**, 59(1), 87-93.
[7] ZJ Zhang; P Li; Z Wang; PT Li; WS Zhang; ZH Sun; XJ Zhang; YY Wang, *Brain Res*., **2006**, 1123(1), 188-195.
[8] CC Tsai; MT Lin; JJ Wang; JF Liao; WT Huang, *Neuropharmacology*, **2006**, 51(4), 709-917.
[9] CP Chang; WT Huang; BC Cheng; CC Hsu; MT Lin, *Neuropharmacology*, **2007**, 52(3), 1024-1033.
[10] AC Dong; P Huang; WS Caughey, *Biochemistry*, **1990**, 29(13), 3303-3308.
[11] I Petitpas; AA Bhattacharya; S Twine; M East; S Curry, *J Biol. Chem*., **2001**, 276, 22804-22809.
[12] HM Berman; J Westbrook; Z Feng; G Gilliland; TN Bhat; H Weissig; IN Shindyalov; PE Bourne, *Nucleic Acids Res.*, **2000**, 28, 235-242.
[13] YL Wang; HF Wang, Universitatis Pekinensia Scientiarum Naturalium, **2002**, 38, 159-164.
[14] JY Lee; M Hirose, *J Biol. Chem*., **1999**, 274, 29303-29310.
[15] YH Chen; JT Yang; HM Martinez, *Biochemistry*, **1972**, 11, 4120-4131.
[16] JN Miller, *Proc Anal Div Chem. Soc*., **1979**, 16, 203-208.
[17] A Papadopoulou; RJ Green; RA Frazier; J *Agric*, *Food Chem*., **2005**, 53, 158-163.
[18] C Wang; QH Wu; Z Wang; J Zhao, *Anal Sci.*, **2006**, 22, 435-438.
[19] PD Ross; S Sburamannian, *Biochemistry*, **1981**, 20(11), 3096-3102.