Interaction between carminic acid and bovine serum albumin

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Abstract. The binding reaction between carminic acid (CA) and bovine serum albumin (BSA) was studied by fluorescence and absorption spectrometry. It is shown that there is a powerful ability in CA to quench the fluorescence intensity of BSA, and there is an overlap between the fluorescence spectrum of BSA and the absorption spectrum of CA. The results also indicated that CA could bind with BSA strongly at molar ratio 1: 1 and the equilibrium constant were3.0×10^6. The action distance(r=3.04nm) between donor (BSA) and acceptorcCA was calculated according to Förster′s nonradiative energy transfer mechanism. The thermodynamic parameters indicate that CA molecule combines with BSA through the electrostatic force.

Introduction

It is known that the distribution, free concentration and the metabolism of various drugs are strongly affected by drug–protein interactions in the blood stream. This type of interaction can also influence the drug stability and toxicity during the chemotherapeutic process [1]. Serum albumins are the most abundant protein in the circulatory system of a wide variety of organisms, being the major macromolecule contributing to the osmotic blood pressure [2]. They can play a dominant role in the drug disposition and the efficiency [3]. Many drugs and other bioactive small molecules bind reversibly to albumin and other serum components, which then function as carriers. Serum albumin
often increases the apparent solubility of hydrophobic drug in plasma and modulates their delivery to cell in the in vivo and in vitro. Consequently, it is important to study the interaction of a drug with serumalbumin.Carminicacid(CA),(7-a-D-glucopyranosyl-9,10-dihydro-3,5,6,8-tetrahydroxy-1-methyl-9,10-anthracenecar-boxylic acid) [4], the principal component of the food dye cochineal, is an anionic, anthraquine-based food coloring dye. CA belongs to a kind of antitumor and antibiotic anthracycline derivatives. They are believed to develop their cytotoxic effect by penetrating into the tumor cell nucleus and interacting there with DNA [5–7].

Experimental

Apparatus. Normal fluorescence measurements were recorded with a F-2500 spectrofluorimeter (Hitachi, Japan). All pH measurements were made with a pHS-2F digital acidity meter (Leici, Shanghai). All absorption spectra were recorded with an UV-2401 spectrophotometer (Shimadzu, Japan).

Reagents. Stock solutions of proteins were prepared by dissolving commercial bovine serum albumin (BSA) (1.00×10^{-5} mol·L^{-1}) (Shanghai Boao Biochemical Technology Co., China) in deionized water.

A stock solution of CA (1.00×10^{-5} mol·L^{-1}) was first prepared by dissolving 0.00049g of CA in deionized water., and then diluted to proper concentration with deionized water.

These solutions needed to be stored at 0–4˚C.

The BR-NaOH buffer solutions (pH 5.0, 0.1 mol·L^{-1}) were used for the pH adjustment.

All the chemicals used were of analytical reagents grade and doubly deionized water was used throughout

Procedures. To a 10 mL test-tube, solutions were added as the following order: 1.00 mL BR-NaOH (pH5.0); 1.00 mL of BSA (1.00×10^{-5} mol·L^{-1}), definite standard CA. The mixture was

diluted to 10 mL with water and allowed to stand for 40 min. The fluorescence intensities were measured in a 1 cm quartz cell at kex/kem = 280 nm/340 nm and with slit at 10.0 nm for the excitation and emission. All the UV absorption spectra were measured by a UV-2401 spectrophotometer at the same time.

Results and discussion.

Fluorescence quenching Spectra. The intrinsic fluorescence of BSA was obtained at 340 nm when excited by the ultraviolet wavelength (k) 280 nm, while there is not in RF. The intrinsic fluorescence of BSA is mainly due to the presence of the two tryptophan residues: Trp-134 and Trp-214. Of both tryptophans in BSA, Trp-134 is more exposed to hydrophilic environment, whereas Trp-214 is deeply buried in the hydrophobic loop. Another frequent amino acid in proteins, tyrosine, shows high fluorescence intensity when in pure solution, however, it presents weak emission when part of a protein chains, in special at wavelength actually used (290 nm). Fluorescence spectra of BSA were determined in the presence of increasing amount of CA at room temperature, as shown in Fig. 1. It can be seen that CA was observed to quench the fluorescence of BSA. Furthermore, the little blue shifts were observed when the concentration of CA was increased, which suggesting the fluorescence chromophore was placed in a more hydrophobic environment after the addition of CA. All these indicated that CA could interact with BSA.

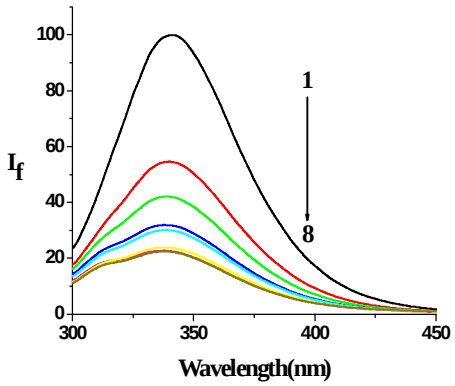

Fig. 1. Fluorescence quenching spectra of BSA as CA were added. From 1to 8: CA ($\times10^{-6}$ mol·L^{-1}) : 0.00, 0.20, 0.40, 0.60, 0.80, 1.00, 1.20, 1.40 ; BSA: 1.00×10^{-6}mol·L^{-1} ; pH=5.00

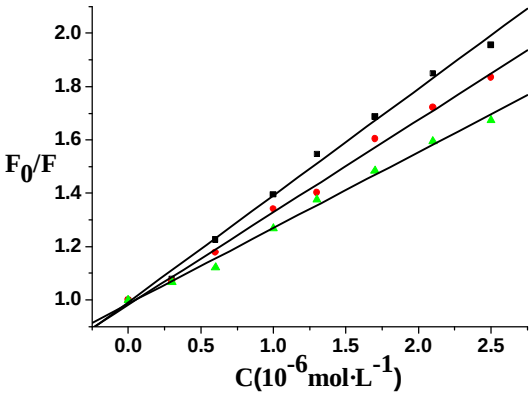

Fig. 2. The Stern–Volmer curves of BSA as CA were added at differen t ■: 283k; ●293k; ▲309k CA ($\times10^{-6}$ mol·L^{-1}) : 0.00, 0.30,0.60 , 1.00,1.30,1.70,2.10,2.50 pH=5.00 , BSA: 1.00×10^{-6}mol·L^{-1}

Fluorescence quenching constant Fluorescence quenching refers to any process that decreases the fluorescence intensity of a sample such as excited state reactions, energy transfers, ground-state complexes formation and collisional process [8]. Collisional quenching, or dynamic quenching, results from collision between fluorophores and a quencher. Static quenching is due to ground-state complex formation between fluorophores and a quencher. Collisional quenching is described by Stern–Volmer equation, which gives the ratio between fluorescence intensities in the absence or presence of a quencher as a function of its concentratio. From this equation, the Stern–Volmer or quenching constant can be calculated, which are use to distinguish dynamic from static quenching [9]. The quenching equation is presented by:

$$F_0/F=1+kq\ \tau_0[Q]=1+Ksv[Q]$$

where F and F_0 are the fluorescence intensity with and without quencher, respectively. [Q] is the concentration of quencher and [Q] is the concentration of the CA in this paper. Ksv is the Stern-Volmer quenching constant and also is static quenching constant when it is static quenching

reaction. The quantitative analysis of the binding BSA-CA was carried out using the Stern–Volmer equation at various temperatures. The plots of F_0/F versus [Q] (intercept = 1, slope = Ksv) are shown in Fig. 2 and the quenching constant Kq and Ksv are listed in Table 1. However, the maximum scatter collision quenching constant Kq of various quenchers with the biopolymer is$2\times10^{10}L\cdot mol^{-1}\cdot s^{-1}$[10] From Table 1, the rate constantsof protein quenching procedures initiated by CAare all greater than the Kq of the scatter procedure. Obviously, the quenching constant of BSA quenching procedure initiated by CA decreased along with the rise of the temperature. This means that the quenching is not initiated by dynamic collision but from the formation of a complex.

Table 1The quenching constants

T (K)	Stern-Volmer quenching	r	$K_q/10^{14}$($L\cdot mol^{-1}$)	$Ksv/10^6$ ($L\cdot mol^{-1}$)
283	Y=0.9916+0.4002x	0.9976	0.4002	0.4002
293	Y=0.9822+0.3468x	0.9976	0.3468	0.3468
309	Y=0.9853+0.2844x	0.9969	0.2844	0.2844

Binding parameters When molecules binding dependently to a set of equivalent sites on a fluorescence molecule, the equilibrium between free and bound molecules is given by the Eq. (2)

$$\lg (F_0-F)/F=\lg K + n\lg C_Q \quad (2)$$

where K and n are the binding constant and the number of binding sites, respectively, which in CA-BSA system are listed in Table 2. It can be seen that 1 BSA molecule combine with 1 CA molecule. In order to elucidate the interaction of BSA with CA, the thermodynamic parameters were calculated from the van't Hoff plots. If the enthalpy change (ΔH°) and the entropy change (ΔS°) do not vary significantly over the temperature range studied, then their value can be determined from the van't Hoff equation:

$$\ln K =-\Delta H°/RT+\Delta S°/R \quad (3)$$

In Eq. (3), K is the binding constant at the corresponding temperature and R is the gas constant. The temperatures used were 283, 293 and 309 K. The enthalpy change (ΔH°) and the entropy change (ΔS°) were calculated from the slope of the van't Hoff relationship, and they are listed in Table 2. The free energy change (ΔG°) was estimated from the following relationship:

$$\Delta G°=\Delta H°-T\Delta G° \quad (4)$$

The experimental results indicate that the probable quenching mechanism of fluorescence of BSA by CA is a static quenching procedure and the bind mode various with the concentration of CA.

Table 2 The binding constants of CA with BSA and the thermodynamic parameters.

T (K)	n	$K_A(10^6)$	$\Delta G(KJ\cdot mol^{-1})$	$\Delta H(KJ\cdot mol^{-1})$	$\Delta S(J\cdot mol^{-1}\cdot K^{-1})$
283	1.17	3.91	-35.71		
293	1.16	2.89	-36.24	-17.46	64.33
309	1.15	2.12	-37.42		

Synchronous fluorescence spectroscopy It is well known that among all the amino acid residues in BSA, only tryptophan and tyrosine residues can contribute to fluorescence spectra. However, tryptophan and tyrosine have similar excitation spectra and their conventional fluorescence spectra overlap strongly. While the synchronous fluorescence technique can be applied to the resolutionbetween tryptophan and tyrosine. It was found that at small wavelength intervals (such as Δλ=15nm), the synchronous fluorescence spectra of a tryptophan–tyrosine mixture solution are characteristic of tyrosine as shown in Fig. 3a, whereas at large wavelength intervals (such as Δλ=60nm), the spectra are attributed to that of tryptophan as shown in Fig. 3b [11,12]. From Fig. 3a and b, it has been seen that when CAis dded into BSA, the intrinsic fluorescence intensity of both tryptophan and tyrosine are all quenched by RF but significant for tryptophan. It indicates that RF is vicinal to tryptophan residues in BSA.

Conclusion

In this paper, the interaction of CA with BSA was studied by spectroscopic methods including

fluorescence spectroscopy and UV–Vis absorption spectroscopy. The experimental results indicate that the probable quenching mechanism of fluorescence of BSA by RF is a static quenching procedure and the bind mode various with the concentration of CA. The main purpose of this research is to study the binding properties between BSA and CA for the great importance in pharmacy, pharmacology and biochemistry.

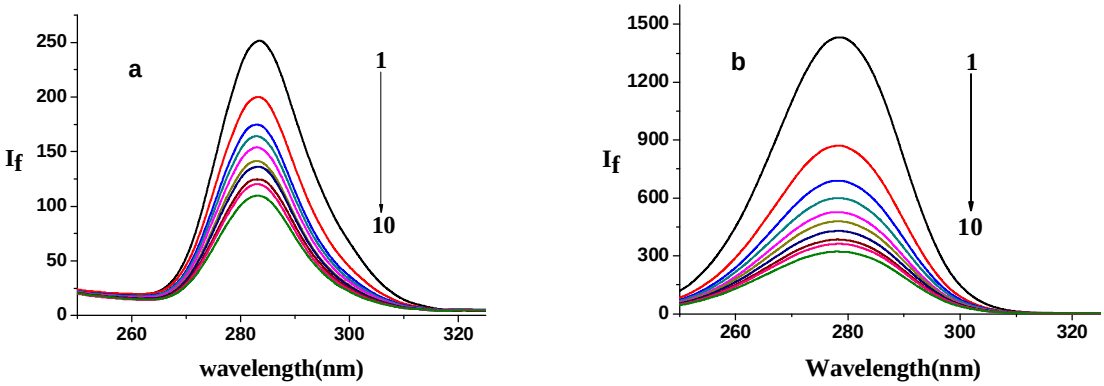

Fig. 3. Synchronous fluorescence spectra (a)Δλ=15nm; (b)Δλ=60nm
pH=5.00; BSA: 1.00×10^{-6} mol·L^{-1}CA:($\times10^{-5}$ mol·L^{-1})1-10:0.00, 0.20,0.40, 0.60,0.80,1.00,1.20,1.40,1.60,1.80;

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