The interaction between protocatechuic aldehyde and human serum albumin using three-dimensional fluorescence techniques

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Abstract. The binding mechanism between protocatechuic aldehyde (PA) and human serum albumin (HSA) was investigated by fluorescence spectroscopy at different temperatures. It is proved that the fluorescence quenching of HSA by PA is not results of the formation of PA–HSA complex. The equilibrium constant K and the number of binding sites n were measured at different temperature by fluorescence quenching method. The standard enthalpy change (ΔH) and the standard entropy change (ΔS) of this interaction process were calculated to be $114.02 \text{ kJ} \cdot \text{mol}^{-1}$, $541.92 \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$, respectively. According to the interaction between PA and HSA of thermodynamic parameters ΔH and ΔS , the major acting force can be measured. The synchronous fluorescence spectra shows the microenvironment of tryptophan residue is changed and the hydrophobicity is increased. Through three-dimensional (3D) fluorescence spectra can get some valuable structure changing information.

Keywords: Binding mechanism, protocatechuic aldehyde, human serum albumin, three-dimensional (3D) fluorescence spectra

1. Introduction

Protocatechuic aldehyde (3,4-dihydroxybenzaldehyde, molecular structure shown in Fig. 1) is one of the most effective component in *Salvia miltorrhrza*, a Chinese traditional herbal drug, used for treatment of some diseases, such as cardiodynia, myocardial infarction, phylogenic infection, hypochondriac pain, stomachache dysmenorrhea and trauma with blood stasis. This molecule can treat anti-cardium colic, improve flow in coronary arteries and inhibit the aggregation of platelets caused by ADP (adenosine diphosphate) [6]. It also can be used as a precursor in the vanillin synthesis by biotransformation by *Capsicum frutescens* cell cultures [7].

Human serum albumin (HSA), as the most abundant protein constituent of blood plasma, has a high affinity to an extraordinarily diverse range of materials, such as drugs, metabolites, fatty acids and metal ions [1]. As a kind of storage form of chemicals, it plays an important role on the disposition and transportation of drugs and other exogenous and endogenous compounds to their targets. It serves as a solubilizer and transporter for the materials and it is also responsible for the maintenance of blood pH, the drug disposition and efficacy, and the contribution of colloid osmotic blood pressure [4,5,8].

In this paper, the interaction between protocatechuic aldehyde (PA) and HSA has been investigated with fluorescent spectrum under physiological conditions.

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Fig. 1. Molecular structure of protocatechuic aldehyde.

2. Materials and methods

2.1. Instruments and materials

The fluorescence spectra were recorded on a Perkin Elmer LS-50B fluorophotometer (USA).

Human serum albumin (HSA) was purchased from Sigma Chemical Company. It was used without further purification and its molecular weight was assumed to be 66,500 HSA (0.5 μ M) solutions were prepared in (pH 7.4). Protocatechuic aldehyde was provided by China Pharmaceutical University, with a concentration of 54.8 μ g/ml. All other reagents and solvents were of analytical reagent grade.

2.2. Methods

Fluorescence spectra of HSA (3 ml 0.5 μ M in a 1.0 cm quartz cell) were recorded in the absence and presence of different amount of trace drug solutions.

The pH measurements were carried out on a pH-25 Exact Digital pH meter.

3. Results and discussion

3.1. Fluorescence spectra

3.1.1. Fluorescence emission spectra

Figure 2 shows the emission spectra of HSA in the absence and presence of PA. As can be seen from Fig. 2, HSA has a strong fluorescence emission with a peak at 350 nm upon excitation at 280 nm, and its fluorescence intensity decreases in the presence of PA. It implies that the binding of PA to HSA occurs and the microenvironment around the chromophore of HSA is changed. There is about 25 nm wavelength redshift on line h. Shift of maximum emission ($\lambda_{\max}$) is an indication that the conformation of protein can be affected by the addition of the drug. The results indicate that there are strong interactions between PA and HSA.

3.1.2. The relations between temperatures and fluorescence quenching

In order to confirm the quenching mechanism, the fluorescence quenching was analyzed according to the well-known Stern–Volmer equation:

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

where F_0 and F are the fluorescence intensities in the absence and presence of PA, respectively. $[Q]$ is the concentration of PA and K_{SV} is the Stern–Volmer quenching constant. K_q is the quenching rate constant of the biomolecule, and τ_0 is the average lifetime of the biomolecule without quencher.

Fig. 2. Fluorescence emission spectra (a) 5.0×10^{-7} mol/l HSA, (b)–(h) 5.0×10^{-7} mol/l HAS in the presence of 3.97×10^{-7} mol/l, 7.94×10^{-7} mol/l, 11.91×10^{-7} mol/l, 15.88×10^{-7} mol/l, 19.85×10^{-7} mol/l, 23.82×10^{-7} mol/l, 27.79×10^{-7} mol/l PA, respectively.

Fig. 3. The Stern–Volmer curve of HSA (5.0×10^{-7} mol/l $^{-1}$) quenched by PA (a) 288 K; (b) 298 K; (c) 308 K.

In order to illustrate the fluorescence quenching mechanism of HSA by PA, the fluorescence intensity data was analyzed according to the Stern–Volmer equation by plotting F_0/F versus the PA concentration $[Q]$ at 288, 298 and 308 K, respectively (see Fig. 3). It can be obviously observed that K_{SV} increases while the temperature increasing, therefore, the fluorescence quenching mechanism is dynamic quenching, which resulting from collisional encounters between the fluorophore and quencher. It is also shown that the binding between PA and HSA is remarkable and the effect of temperature is obvious.

3.2. Synchronous fluorescence

The method of synchronous fluorescence is a kind of simple and effective way to measure the fluorescence quenching and the possible shift of the maximum emission wavelength. The fluorescence of HSA comes from tyrosine, tryptophan and phenylalanine residues. According to Miller [3], with large $\Delta\lambda$ values such as 60 nm, the synchronous fluorescence of HSA is characteristic of tryptophan residue. Figure 4 shows the effect of addition of PA on the synchronous fluorescence spectra of HSA when $\Delta\lambda = 60$ nm. It is indicated that addition of PA results in the strong fluorescence quenching of tryptophan residues and significant blue shift is observed when the density of PA increased from 0 to 27.97×10^{-7} mol/l. This shows the microenvironment of tryptophan residue is changed and the hydrophobicity is increased.

Fig. 4. Synchronous fluorescence spectra of HSA (5.0×10^{-7} mol/l) with $\Delta\lambda = 60$ nm in the absence and presence of increasing amount of PA from 1 to 8: 0, 0.397, 0.794, 1.191, 1.588, 1.985, 2.382, 2.779 $\mu\text{mol/l}$.

Table 1

The binding sites number n and binding constant K of PA–HSA at different temperatures

T (K)	n	$K (\times 10^7 \text{ M}^{-1})$
288	1.10	4.43
298	1.17	13.3
308	1.22	97.9

3.3. Determinations of binding sites number and binding constant

From the equation: $\lg[(F_0 - F)/F] = \lg K + n \lg[Q]$ [2], binding sites number n can be obtained and listed in Table 1 together with binding constant K . It can be observed that the average binding sites number n is one, and binding constant K increased while the temperature increased.

3.4. Determination of the acting force

Essentially, there are four types of non-covalent interaction existing in the binding of the ligands to proteins. These are hydrogen bonds, van der Waals forces, hydrophobic and electrostatic interactions [3]. Thermodynamic parameters, free energy (ΔG), standard enthalpy (ΔH) and standard entropy (ΔS) can provide an insight into the binding mode. Among these parameters, ΔG reflects the possibility of reaction, ΔH and ΔS are the main evidence to determine acting forces. When temperature varies in a small range, the ΔH can be considered as a constant.

Therefore, from the following equations:

$$\ln(K_2/K_1) = \Delta H(1/T_1 - 1/T_2)/R, \quad (2)$$

$$\Delta G = \Delta H - T\Delta S = -T \ln K. \quad (3)$$

These thermodynamics parameters are calculated from the linear relationship between $\ln K$ and the $1/T$ (Table 2). ΔH and ΔS of the binding interaction between PA and HSA are calculated to be $114.02 \text{ kJ} \cdot \text{mol}^{-1}$ and $541.92 \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$. Because enthalpy change $\Delta H > 0$ and entropy change $\Delta S > 0$, the acting force, which result in the form of the non-fluorescence complex, are mainly caused by electrostatic and hydrophobic interaction [3,5].

Table 2
Thermodynamic parameters of the system of PA–HAS

Temperature (K)	K ($\times 10^7 \text{ l} \cdot \text{mol}^{-1}$)	$\Delta G^\ominus$ ($\text{kJ} \cdot \text{mol}^{-1}$)	R	$\Delta S^\ominus$ ($\text{J} \cdot \text{mol}^{-1} \text{K}^{-1}$)	$\Delta H^\ominus$ ($\text{kJ} \cdot \text{mol}^{-1}$)
288	4.43	−42.16	0.9974	541.92	114.02
298	13.3	−47.25			
308	97.9	−53.01

Fig. 5. The three-dimensional projections of 2 ml HSA ($C_{\text{HSA}}: 5.0 \times 10^{-7} \text{ mol/l}$, $C_{\text{PA}}: 3.97 \times 10^{-4} \text{ mol/l}$).

3.5. The three-dimensional fluorescence spectra

The three-dimensional fluorescence spectra are a promising fluorescence analysis technique in recent years. Figures 5–7 presented the three-dimensional fluorescence spectrum and their characteristics of PA–HSA, respectively. It was apparent that the shift in position of D -value ($\Delta\lambda$) (between excitation wavelength and emission wavelength) were ranged from about 150–175 nm. The result showed that the emission maximum of tryptophan residues did significant blue shift while tyrosine residues did red shift. The polarity around some of the tyrosine residues decreased while tryptophan residues increased. This may be due to the changes of residue microenvironment with the insertion of PA.

4. Conclusions

This paper indicated that PA can quench the intrinsic fluorescence of HSA, and the quenching mechanisms is dynamic quenching. In addition, the standard enthalpy change (ΔH) and the standard entropy change (ΔS) of this interaction process were calculated to be $114.02 \text{ kJ} \cdot \text{mol}^{-1}$ and $541.92 \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$, which indicated that hydrophobic and electrostatic forces played major roles in the interaction between

Fig. 6. The three-dimensional projections of 2 ml HSA–0.5 ml PA ($C_{\text{HSA}}: 5.0 \times 10^{-7}$ mol/l, $C_{\text{PA}}: 3.97 \times 10^{-4}$ mol/l).

Fig. 7. The three-dimensional projections of 2 ml HSA–1 ml PA ($C_{\text{HSA}}: 5.0 \times 10^{-7}$ mol/l, $C_{\text{PA}}: 3.97 \times 10^{-4}$ mol/l).

PA and HSA. And the result of 3D fluorescence spectra indicated that the polarity around the tyrosine residues decreased while tryptophan residues increased, resulted in a disordered structure of HSA.

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