

## Study on the Interaction Between $\text{Cu}(\text{phen})_3^{2+}$ and Bovine Serum Albumin by Spectroscopic Methods

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**Abstract** In this work, the interaction between  $\text{Cu}(\text{phen})_3^{2+}$  and bovine serum albumin (BSA) was investigated by fluorescence spectroscopy combined with UV–vis absorption and circular dichroism (CD) spectroscopic techniques under physiological conditions. The fluorescence data proved that the fluorescence quenching of BSA by  $\text{Cu}(\text{phen})_3^{2+}$  was the result of the  $\text{Cu}(\text{phen})_3^{2+}$  – BSA complex formation. The binding constants ( $K_a$ ) between  $\text{Cu}(\text{phen})_3^{2+}$  and BSA at four different temperatures were calculated according to the modified Stern–Volmer equation. The enthalpy change ( $\Delta H$ ) and entropy change ( $\Delta S$ ) were calculated to be  $10.74 \text{ kJ mol}^{-1}$  and  $54.35 \text{ J mol}^{-1} \text{ K}^{-1}$ , respectively, which indicated that electrostatic interactions played a major role in the formation of  $\text{Cu}(\text{phen})_3^{2+}$  – BSA complex. The distance  $r$  between the donor (BSA) and acceptor [ $\text{Cu}(\text{phen})_3^{2+}$ ] was obtained to be 3.55 nm based on Förster’s energy transfer theory. The synchronous fluorescence and CD spectroscopy results showed that the polarity of the residues increased and the lost of the  $\alpha$ -helix content of BSA (from 59.84 to 53.70%). These indicated that the microenvironment and conformation of BSA were changed in the presence of  $\text{Cu}(\text{phen})_3^{2+}$ .

**Keywords**  $\text{Cu}(\text{phen})_3^{2+}$  · Bovine serum albumin · Fluorescence quenching · Circular dichroism spectroscopy · Thermodynamic parameters

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## Introduction

Serum albumins, as one of the most abundant carrier proteins, play an important role in the transport and disposition of endogenous and exogenous ligands presented in blood. It is not only the major transport protein for unesterified fatty acids but also capable of binding an extraordinarily diverse range of metabolites, drugs, and organic compounds. And the binding of ligands to serum albumin *in vitro*, considered as a model in protein chemistry to study the binding behavior of proteins, has been an interesting research field studied for many years [1–4]. In this work, bovine serum albumin (BSA) was selected as our protein model because of its low cost, ready availability, unusual ligand-binding properties, and the results of all the studies are consistent with the results that bovine and human serum albumin are homologous proteins [5–7].

Copper has been recognized as an essential trace metal for living organisms since the late 1930s [8]. Among various copper complexes investigated so far, those containing phenanthroline and its derivatives have attracted much attention for their various biological activities, such as anticandida [9], antimycobacterial [10], and antimicrobial [11] activities. As 1,10-phenanthroline is the parent of an important class of chelating agents that form multitude compounds with various metal ions and its potential application as nonradioactive nucleic acid probes, the complexes of 1,10-phenanthroline and other polypyridyls with transition metals have stimulated various researches [12]. Moreover, 1,10-phenanthroline copper complexes are found to have the ability to distinguish and split DNA and could be used as chemical nucleases [13–16], although the synthesis of tris-chelated  $\text{Cu}(\text{II})$  complexes  $[\text{Cu}(\text{phen})_3^{2+}$ , phen = 1, 10 – phenanthroline] is uncommon and the detailed investigations of the interaction of BSA with  $\text{Cu}(\text{phen})_3^{2+}$  are scanty. Thus, the investigation of the interaction of such molecule with BSA is imperative and of fundamental importance.

In this paper, we synthesized  $\text{Cu}(\text{phen})_3^{2+}$  and studied the interaction between  $\text{Cu}(\text{phen})_3^{2+}$  and BSA by multispectroscopic methods including fluorescence spectroscopy, UV–vis absorption, and circular dichroism (CD) spectroscopy. The experimental results indicated that the fluorescence quench of BSA by  $\text{Cu}(\text{phen})_3^{2+}$  was due to the formation of  $\text{Cu}(\text{phen})_3^{2+}$  – BSA complex. In addition, the binding constants were calculated, and the binding mechanism was proposed. Furthermore, synchronous fluorescence and CD spectroscopy results demonstrated that the microenvironment and conformation of BSA were changed in the presence of  $\text{Cu}(\text{phen})_3^{2+}$ .

## Materials and Methods

### Materials and Reagents

BSA (electrophoresis grade reagents) purchased from Sigma was used without further purification. All BSA solution was prepared in the pH 7.40 Tris–HCl buffer solution [0.05 mol  $\text{l}^{-1}$  Tris base (2-amino-2-(hydroxymethyl)-1,3-propanediol), 0.15 mol  $\text{l}^{-1}$  NaCl, pH 7.4] and was kept in the dark at 277 K. The Tris base had a purity of no less than 99.5%, and NaCl, HCl, etc. were all of analytical purity. The synthesis of  $\text{Cu}(\text{phen})_3^{2+}$  was according to the literature [17], and the purity of the compounds was checked by elemental analysis. The stock solution was dissolved in water. Doubly distilled water was used throughout the experiment. The weight measurements were performed with an AY-120 electronic analytic weighting scale (Shimadzu, Japan) with a resolution of 0.1 mg.

## Fluorescence Measurements

Fluorescence spectra were measured with a LS-55 Spectrofluorimeter (Perkin–Elmer, USA) equipped with 1.0-cm quartz cells and a thermostat bath. Fluorescence spectra were recorded at 292, 298, 304, and 310 K in the range of 300–480 nm. Furthermore, the temperature of sample was kept by recycle water in the experiment. The width of the excitation and emission slit was set to 15.0 and 2.5 nm, respectively.

## UV–vis Absorbance Spectroscopy and CD Measurements

The UV–vis absorption spectra were recorded at room temperature on a TU-1901 spectrophotometer (Puxi Analytic Instrument, Beijing, China) equipped with 1.0-cm quartz cells.

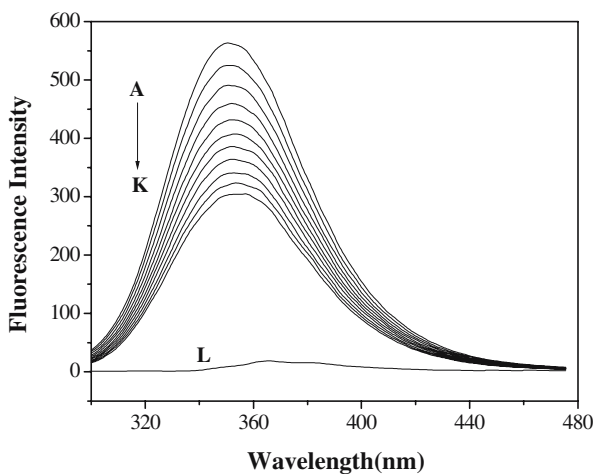
CD measurements were performed on a J-810 Spectropolarimeter (Jasco, Tokyo, Japan) at room temperature. The CD measurements of BSA in the absence and presence of  $\text{Cu}(\text{phen})_3^{2+}$  (1:1, 1:3) were recorded in the range of 260–200 nm. The instrument was controlled by Jasco's Spectra Manage™ software. Quartz cells having path lengths of 0.1 cm were used at a scanning speed of 200 nm/min. The data were expressed in terms of mean residue ellipticity (MRE). Appropriate buffer solution running under the same conditions was taken as blank and subtracted from the sample spectra.

## Result and Discussion

### Fluorescence-Quenching Measurements

Fluorescence is the process of photon emission as a result of the return of an electron in a higher energy orbital back to a lower energy orbital. Furthermore, the fluorescence intensity of a fluorophore can be decreased by a variety of molecular interactions, such as excited-state reactions, energy transfer, ground-state complex formation, and collisional quenching. The quenching mechanisms are usually classified as either dynamic or static quenching. Dynamic quenching mainly results from collision between fluorophore and quencher, and

**Fig. 1** Emission spectra of BSA in the presence of various concentrations of  $\text{Cu}(\text{phen})_3^{2+}$  ( $T = 298 \text{ K}$ ,  $\lambda_{\text{ex}} = 285 \text{ nm}$ ).  $c(\text{BSA}) = 1.0 \times 10^{-5} \text{ mol L}^{-1}$ ;  $c(\text{Cu}(\text{phen})_3^{2+})/(10^{-6} \text{ mol L}^{-1})$ , A–K: 0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, respectively. Curve L shows the emission spectrum of  $\text{Cu}(\text{phen})_3^{2+}$  only,  $c(\text{Cu}(\text{phen})_3^{2+}) = 1.0 \times 10^{-5} \text{ mol L}^{-1}$



higher temperatures can lead to larger diffusion coefficients; the value of the bimolecular quenching constants is expected to be higher with increasing temperature. The static quenching is mainly due to the formation of ground-state complex between the fluorophore and quencher, and the values of static quenching constants are likely to be lower because the stability of complexes decreases with increasing temperature [18].

Figure 1 shows the emission spectra of BSA in the presence of various concentrations of  $\text{Cu}(\text{phen})_3^{2+}$  at pH 7.4. As shown in Fig. 1, the increasing concentration of  $\text{Cu}(\text{phen})_3^{2+}$  caused a progressive reduction in fluorescence intensity, accompanied with an increasing of the maximum emission wavelength ( $\lambda_{\text{max}}$ ). Curve L shows the emission spectrum of  $\text{Cu}(\text{phen})_3^{2+}$  only, which indicated that the effect of  $\text{Cu}(\text{phen})_3^{2+}$  at the investigated concentration range was very small and could be negligible. The red shift of  $\lambda_{\text{max}}$  was ascribed to an increasing in the polarity of the protein environment compared to that of the pure protein solution [19]. Therefore,  $\text{Cu}(\text{phen})_3^{2+}$  could interact with BSA and quench its intrinsic fluorescence.

For dynamic quenching, the mechanism can be analyzed by the well-known Stern–Volmer equation [20]:

$$\frac{F_0}{F} = 1 + K_{\text{SV}}[Q] \quad (1)$$

where  $F_0$  and  $F$  represent the fluorescence intensities in the absence and presence of the quencher, respectively;  $K_{\text{SV}}$  is the Stern–Volmer quenching constant,  $[Q]$  is the concentration of the quencher. Hence, Eq. 1 is applied to determine  $K_{\text{SV}}$  by linear regression of a plot of  $F_0/F$  against  $[Q]$ .

The fluorescence quenching of BSA by  $\text{Cu}(\text{phen})_3^{2+}$  at four different temperatures (292, 298, 304, and 310 K) was then measured. Figure 2a shows the Stern–Volmer plots,  $F_0/F$  versus  $[Q]$ , according to Eq. 1. Furthermore, it can be seen that the linear relationship of the plots is not very well. The calculated quenching constants  $K_{\text{SV}}$  at each temperature were summarized in Table 1. From the analysis of the first segment, the values of  $K_{\text{SV}}$  inversely correlated with temperature indicated that the binding reaction was mainly initiated by compound formation rather than by dynamic collision [20].

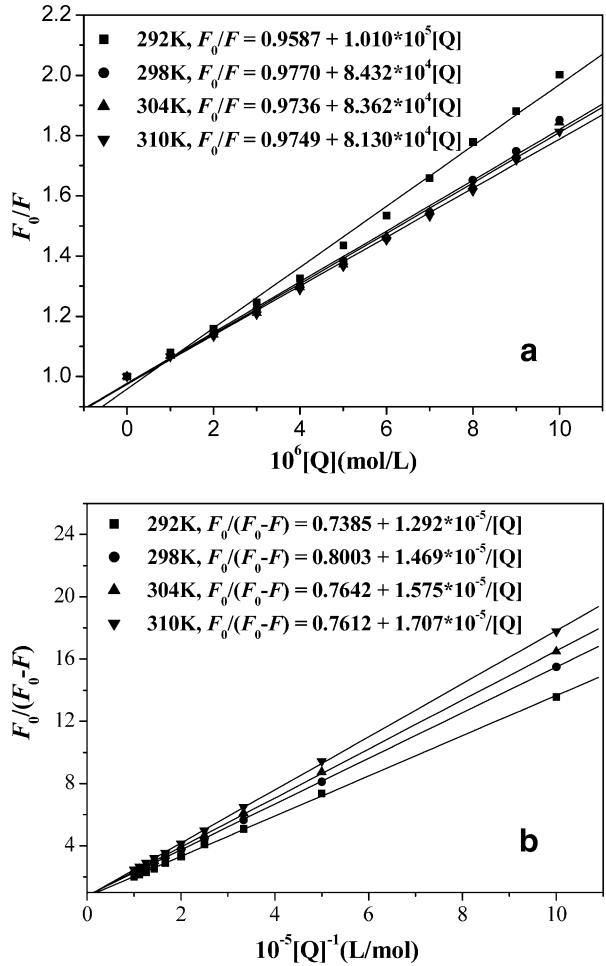
The UV–vis absorption spectra of  $\text{Cu}(\text{phen})_3^{2+}$ , BSA, and the  $\text{Cu}(\text{phen})_3^{2+}$ –BSA system were also investigated to confirm the probable quenching mechanism (Fig. 3). It can be seen from Fig. 3 that the UV–vis absorbance intensity decreased regularly with the increasing concentration of  $\text{Cu}(\text{phen})_3^{2+}$ , indicating that the BSA molecules associated with  $\text{Cu}(\text{phen})_3^{2+}$  and formed a  $\text{Cu}(\text{phen})_3^{2+}$ –BSA complex. This confirmed again a static quenching mechanism.

Therefore, the quenching data were analyzed according to the modified Stern–Volmer equation [21]:

$$\frac{F_0}{\Delta F} = \frac{1}{f_a K_a} \frac{1}{[Q]} + \frac{1}{f_a} \quad (2)$$

In the present case,  $\Delta F$  is the difference in fluorescence intensity in the absence and presence of the quencher at concentration  $[Q]$ ,  $f_a$  is the fraction of accessible fluorescence, and  $K_a$  is the effective quenching constant for the accessible fluorophores. The modified Stern–Volmer plots are shown in Fig. 2b. As can be seen from Fig. 2, the linear relationship of the modified Stern–Volmer plots is much better than that of the Stern–Volmer plots, which further confirmed a static quenching mechanism. The dependence of  $F_0/\Delta F$  on the reciprocal value of the quencher concentration  $[Q]^{-1}$  is linear with slope equal to the value

**Fig. 2** Stern–Volmer plots (a) and modified Stern–Volmer plots (b) for the quenching of BSA by  $\text{Cu}(\text{phen})_3^{2+}$  at four different temperatures



of  $(f_a K_a)^{-1}$ . The quotient of the ordinate  $f_a^{-1}$  and the slope  $(f_a K_a)^{-1}$  is equal to the value of  $K_a$ . The corresponding results at different temperatures were shown in Table 2. The decreasing trend of  $K_a$  with increasing temperature was in accordance with  $K_{\text{SV}}$ 's dependence on temperature as mentioned above. It shows that the binding constant between  $\text{Cu}(\text{phen})_3^{2+}$

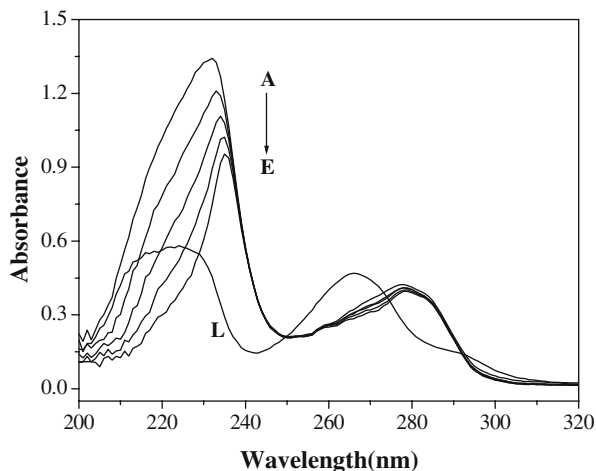
**Table 1** Stern–Volmer Quenching Constants for the Interaction of  $\text{Cu}(\text{phen})_3^{2+}$  with BSA at Four Different Temperatures

pH	$T$ (K)	$K_{\text{SV}} (\times 10^4 \text{ l mol}^{-1})$	$R^a$	SD <sup>b</sup>
7.4	292	10.10	0.9969	0.028
	298	8.432	0.9980	0.019
	304	8.362	0.9980	0.019
	310	8.130	0.9984	0.016

<sup>a</sup>  $R$  is the correlation coefficient.

<sup>b</sup> SD is the standard deviation for the  $K_{\text{SV}}$  values.

**Fig. 3** The UV-vis absorption spectra of BSA and  $\text{Cu}(\text{phen})_3^{2+}$ .  $c(\text{BSA}) = 1.0 \times 10^{-5} \text{ mol l}^{-1}$ ;  $c(\text{Cu}(\text{phen})_3^{2+}) / (10^{-5} \text{ mol l}^{-1})$ ; A-E: 0, 0.5, 1.0, 1.5, 2.0, respectively. Curve L shows the UV-vis spectra of  $\text{Cu}(\text{phen})_3^{2+}$  only;  $c(\text{Cu}(\text{phen})_3^{2+}) = 1.0 \times 10^{-5} \text{ mol l}^{-1}$



and BSA is great and the effect of temperature is small. Thus,  $\text{Cu}(\text{phen})_3^{2+}$  can be stored and carried by protein.

#### Type of Interaction Force Between $\text{Cu}(\text{phen})_3^{2+}$ and BSA

The molecular forces contributing to protein interactions with small molecules may be hydrophobic interactions, hydrogen bonds, van der Waals force, or electrostatic force, etc. The thermodynamic parameters for protein reactions can be accounted for the main forces contributing to protein stability. If the enthalpy changes ( $\Delta H$ ) does not change significantly over the studied temperature range, then it can be regarded as a constant, and its value and that of entropy change ( $\Delta S$ ) can be determined from the van't Hoff equation:

$$\ln K = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \quad (3)$$

where  $K$  is analogous to the effective quenching constants  $K_a$  at the corresponding temperature and  $R$  is the gas constant; the enthalpy change ( $\Delta H$ ) can be calculated from the slope. The free energy change ( $\Delta G$ ) is estimated from the following relationship:

$$\Delta G = \Delta H - T\Delta S = -RT \ln K \quad (4)$$

**Table 2** Modified Stern-Volmer Constants  $K_a$  and Relative Thermodynamic Parameters

$T$ (K)	$K_a (\times 10^4 \text{ l mol}^{-1})$	$R^a$	$\Delta H$ (kJ mol $^{-1}$ )	$\Delta G$ (kJ mol $^{-1}$ )	$\Delta S$ (J mol $^{-1}$ K $^{-1}$ )	$R^b$
292	5.724	0.9999		-26.60		
298	5.439	0.9997	-10.74	-27.02	54.35	0.9897
304	4.848	0.9999		-27.29		
310	4.476	0.9998		-27.60		

<sup>a</sup> $R$  is the correlation coefficient for the  $K_a$  values.

<sup>b</sup> $R$  is the correlation coefficient for the van't Hoff plot.

**Fig. 4** van't Hoff plot for the interaction of BSA and  $\text{Cu}(\text{phen})_3^{2+}$  in Tris buffer at pH 7.4

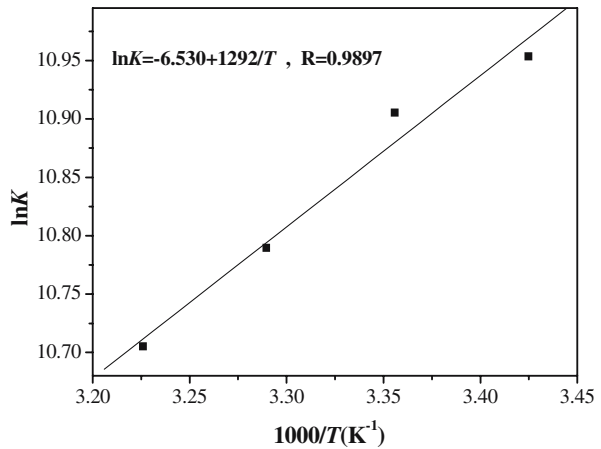


Figure 4, by fitting the data of Table 2, shows that the assumption of near constant  $\Delta H$  is justified. According to the binding constants  $K_a$  at the four temperatures (292, 298, 304, and 310 K), the thermodynamic parameters can be obtained from van't Hoff plot, and the corresponding results were presented in Table 2. As shown in Table 2,  $\Delta H$  and  $\Delta S$  for the binding reaction between  $\text{Cu}(\text{phen})_3^{2+}$  and BSA were calculated to be  $-10.74 \text{ kJ mol}^{-1}$  and  $54.35 \text{ J mol}^{-1} \text{ K}^{-1}$ . Thus, the formation of  $\text{Cu}(\text{phen})_3^{2+}$ –BSA compound was an exothermic reaction accompanied by positive  $\Delta S$  value. The negative sign for free energy ( $\Delta G$ ) means that the binding process was spontaneous.

Ross and Subramanian [22] have characterized the sign and magnitude of the thermodynamic parameters associated with various individual kinds of interaction that may take place in protein association process. The small negative enthalpy change value ( $-10.74 \text{ kJ mol}^{-1}$ ) and the positive entropy change value ( $54.35 \text{ J mol}^{-1} \text{ K}^{-1}$ ) indicated that electrostatic interactions might play a major role in the reaction. Moreover, according to the corresponding literature [23], acidity has some influence on the combination of ligands to BSA, and the isoelectric point of BSA is around pH 4.7. When the value of pH is larger than 4.7, protein has negative net charge because of the ionization of amino acid residues, whereas the electric charge of  $\text{Cu}(\text{phen})_3^{2+}$  is positive, which makes the static attraction between BSA and  $\text{Cu}(\text{phen})_3^{2+}$  be likely to take place. This confirmed that electrostatic interactions played a major role in the binding of  $\text{Cu}(\text{phen})_3^{2+}$  to BSA.

#### Energy Transfer Between $\text{Cu}(\text{phen})_3^{2+}$ and BSA

According to Förster's nonradioactive energy transfer theory [24], a transfer of energy could take place through a direct electrodynamic interaction between the primarily excited molecule and its neighbors [25]. The efficient ligand–protein interaction gives rise to energy data, from which the distance between two interacting molecules can be easily evaluated. Furthermore, the efficiency of energy transfer  $E$  is related to the distance  $R_0$  between donor and acceptor by the following equation:

$$E = 1 - \frac{F}{F_0} = \frac{R_0^6}{R_0^6 + r^6} \quad (5)$$

where  $E$  denotes the efficiency of energy transfer between the donor and acceptor;  $r$  is the distance between the donor and acceptor.  $R_0$ , the critical distance at which the transfer efficiency equals to 50%, is given by the following equation:

$$R_0^6 = 8.79 \times 10^{-25} K^2 n^{-4} \phi J \quad (6)$$

In Eq. 6,  $K^2$  is the orientation factor to the donor and acceptor of dipoles and  $K^2=2/3$  [26] for random orientation as in fluid solution;  $n$  is the refracted index of medium;  $\phi$  is the fluorescence quantum yield of the donor;  $J$  expresses the degree of spectral overlap between the donor emission and the acceptor absorption (Fig. 5) spectrum, which could be calculated by the equation:

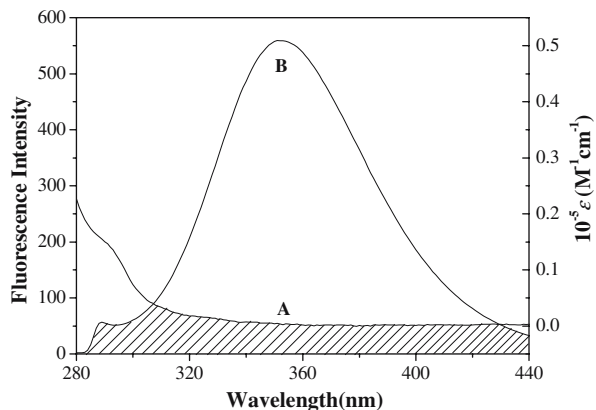
$$J = \frac{\int_0^\infty F(\lambda)\varepsilon(\lambda)\lambda^4 d\lambda}{\int_0^\infty F(\lambda)d\lambda} \quad (7)$$

where  $F(\lambda)$  is the fluorescence intensity of the donor at wavelength range  $\lambda$ ;  $\varepsilon(\lambda)$  is the molar absorption coefficient of the acceptor at wavelength  $\lambda$ . In the present case,  $n=1.36$ ,  $\Phi=0.15$  [27], according to Eqs. 5–7, we calculated out that  $J=5.90 \times 10^{-15} \text{ cm}^3 \text{ l mol}^{-1}$ ,  $R_0=3.44 \text{ nm}$ ,  $E=0.46$ , and  $r=3.55 \text{ nm}$ . The average distance between a donor and acceptor fluorophore was on the 2–8 nm scale and  $0.5R_0 < r < 1.5R_0$ , indicating that the energy transfer from BSA to  $\text{Cu}(\text{phen})_3^{2+}$  occurs with high probability [28].

### Conformational Investigations

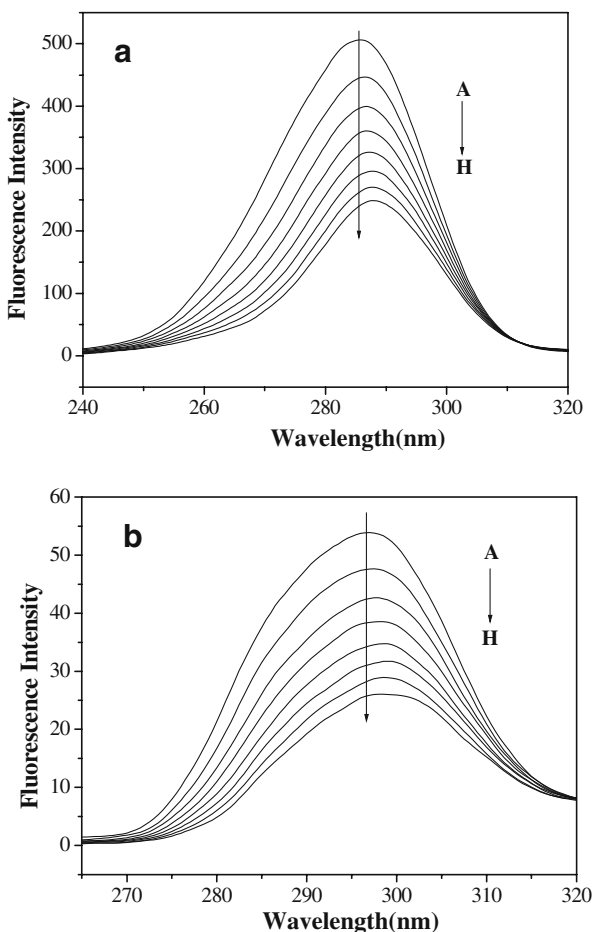
Synchronous fluorescence spectroscopy can give information about the molecular environment in the vicinity of the chromosphere molecules in low concentration under physiological condition. It involves the simultaneous scanning of the excitation and the fluorescence monochromators of a fluorimeter, while maintaining a fixed wavelength difference ( $\Delta\lambda$ ) between them [29]. When the  $D$  value ( $\Delta\lambda$ ) between excitation and emission wavelength is stabilized at 15 or 60 nm, the synchronous fluorescence gives the characteristic information of tyrosine or tryptophan residues [30]. It can be seen from Fig. 6 that the maximum emission wavelength of both tyrosine and tryptophan residues had a slight red-shift in the studied concentration range, which suggested that the conformation of BSA was changed and a more polar (or less hydrophobic) environment of both residues [31].

**Fig. 5** Spectral overlap of  $\text{Cu}(\text{phen})_3^{2+}$  absorption (A) with BSA fluorescence (B) spectrum.  $c(\text{BSA}) = c(\text{Cu}(\text{phen})_3^{2+}) = 1.0 \times 10^{-5} \text{ mol l}^{-1}$  ( $T=298 \text{ K}$ )





**Fig. 6** Synchronous fluorescence spectra of BSA: **a**  $\Delta\lambda = 15$  nm, **b**  $\Delta\lambda = 60$  nm.  $c(\text{BSA}) = 1.0 \times 10^{-5} \text{ mol L}^{-1}$ ,  $c(\text{Cu}(\text{phen})_3^{2+}) / (10^{-5} \text{ mol L}^{-1})$  A–H: 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, respectively



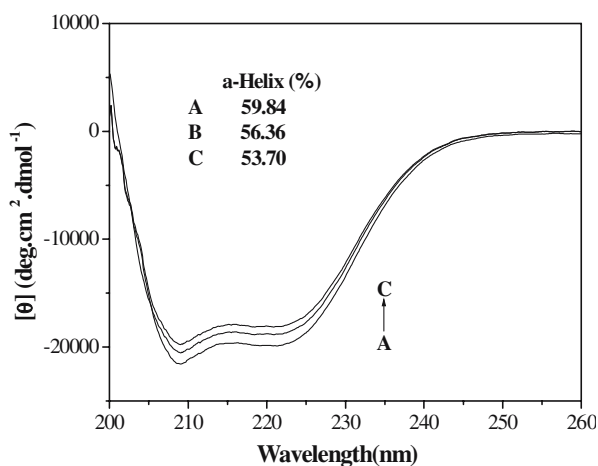
For secondary-structure analysis of protein, CD spectroscopy is a sensitive technique which has been widely used. The CD spectra of BSA exhibits two negative bands in the far-UV region at 208 and 222 nm, which is the typical characterization of the  $\alpha$ -helix structure of protein (Fig. 7, curve A). The reasonable explanation may be that the negative peaks between 208 and 222 nm are both contributed to  $n \rightarrow \pi^*$  transfer for the peptide bond of  $\alpha$ -helix [32]. As can be seen from Fig. 7, with the addition of  $\text{Cu}(\text{phen})_3^{2+}$ , there was a decrease in both bands' intensity. The CD results are usually expressed in terms of MRE in  $\text{deg cm}^2 \text{ dmol}^{-1}$  according to the following equation [33]:

$$\text{MRE} = \frac{\text{ObservedCD}(\text{medg})}{C_p n l \times 10} \quad (8)$$

where  $C_p$  is the molar concentration of the protein,  $n$  is the number of amino acid residues, and  $l$  is the path length. The  $\alpha$ -helical contents of BSA were calculated from the MRE value at 208 nm using the equation:

$$\alpha - \text{Helical}(\%) = \frac{-\text{MRE}_{208} - 4,000}{33,000 - 4,000} \times 100 \quad (9)$$

**Fig. 7** The CD spectra of the  $\text{Cu}(\text{phen})_3^{2+}$  – BSA system obtained in 0.1 mol  $\text{l}^{-1}$  Tris buffer at pH 7.4 and room temperature.  $c(\text{BSA})=1.0 \times 10^{-5}$  mol  $\text{l}^{-1}$ ;  $c(\text{Cu}(\text{phen})_3^{2+})/(10^{-5} \text{ mol l}^{-1})$ ; A–C: 0, 1.0, 3.0, respectively



where  $\text{MRE}_{208}$  is the observed MRE value at 208 nm, 4,000 is the MRE of the  $\beta$ -form and random coil conformation cross at 208 nm, and 33,000 is the MRE value of a pure  $\alpha$ -helix at 208 nm. From the above equation, the quantitative analysis results of the  $\alpha$ -helix content of BSA were obtained and shown in Fig. 7. The  $\alpha$ -helix content differed from that of 59.84% in free BSA to 53.70% when the molar ratio of  $\text{Cu}(\text{phen})_3^{2+}$  to BSA reached 3:1, which was indicative of the loss of  $\alpha$ -helical. It indicated that  $\text{Cu}(\text{phen})_3^{2+}$  bound with the amino acid residues of the main polypeptide chain of protein and destroyed their hydrogen-bonding networks [34]. The CD spectra of BSA in presence and absence of  $\text{Cu}(\text{phen})_3^{2+}$  were similar in shape, which indicated that the structure of BSA was also predominantly of  $\alpha$ -helix. The difference between curve A and curves B and C means the peptide strand extended even more, while the hydrophobicity was decreased. This conclusion agrees with the results of synchronous fluorescence spectra experiment.

## Conclusions

In this paper, the interaction between  $\text{Cu}(\text{phen})_3^{2+}$  and BSA was investigated by spectroscopic methods including fluorescence spectroscopy, UV–vis absorption, and CD spectroscopy. The results showed that the Stern–Volmer quenching constant  $K_{\text{SV}}$  was inversely correlated with temperature, which indicated a static quenching mechanism in the  $\text{Cu}(\text{phen})_3^{2+}$ –BSA reaction. The thermodynamic parameters  $\Delta G < 0$ ,  $\Delta H < 0$ , and  $\Delta S > 0$  at different temperatures indicated that the binding process was spontaneous and electrostatic interactions played a major role in stabilizing the complex. The binding distance was calculated to be 3.55 nm. The synchronous fluorescence and CD spectroscopy also revealed that the microenvironment and conformation of BSA were changed in the presence of  $\text{Cu}(\text{phen})_3^{2+}$ . The binding study of drugs with proteins is of great importance in pharmacy, pharmacology, and biochemistry. This study is expected to provide important insight into the interactions of the physiologically important protein BSA with drugs. Information is also obtained about the effect of environment on BSA structure, which may be correlated to its physiological activity.

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