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Binding studies of a new copper (II) complex containing mixed aliphatic and aromatic dinitrogen ligands with bovine serum albumin using different instrumental methods

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ABSTRACT

The new copper (II) complex, $[Cu(N–N)(L)(EtOH)](NO_3)_2{\cdot}2H_2O$; in which N–N = 2,9-dimethyl-1,10-phenanthroline and L = N,N-dimethyltrimethylenediamine; has been synthesized and characterized by ^1H and ^{13}C NMR, absorption spectroscopy and elemental analysis (CHN). The interaction of this complex with bovine serum albumin (BSA) was investigated under physiological condition in 0.01 M phosphate buffer using spectroscopic methods including fluorescence, UV–vis absorption and circular dichroism (CD). The results of fluorescence titration revealed that the complex strongly quench the intrinsic fluorescence of BSA through a static quenching procedure. Binding constants (K_b), association constants (K_a) and the number of binding sites ($n \approx 1$) were calculated using modified Stern–Volmer equations. The thermodynamic parameters indicate that the hydrophobic and hydrogen bonding interactions play a major role in BSA–Cu complex association. The process of binding was spontaneous, in which Gibbs free energy change (ΔG) was negative. The distance r_0 between donor (BSA) and acceptor (copper complex) was obtained to be 1.9 nm according to Foster's non-radiative energy transfer theory and showed that that the energy transfer from BSA to the Cu(II) complex occurs with high probability. Other results also revealed that binding of the complex could induce the conformational changes in BSA.

1. Introduction

Copper is a physiologically important metal element that plays an important role in the endogenous oxidative DNA damage associated with aging and cancer [1]. Some of copper (II) complexes are found to exhibit a variety of pharmacological activity and superoxide dismutase activity [2]. Among the various copper complexes so far investigated, those containing phenanthroline and its derivatives have attracted much attention for their various biological activities such as anti Candida [3], antimycobacterial [4] and antimicrobial [5] activities. Furthermore, it is well known that 1,10-phenanthroline and $[Cu(phen)_2(mal)]{\cdot}2H_2O$ are capable of decreasing cancer cell viability through an inhibition of DNA synthesis [6].

Serum albumins are the most extensively studied and applied proteins because of their availability, low cost, stability and unusual ligand binding properties. For this reason, a huge number of papers dealing with albumins have been reviewed so far [7,8]. In addition, albumin is the most multifunctional transport protein and play an important role in the transport and deposition of a variety of endogenous and exogenous substances in blood [9]. The interaction of drugs with protein result in the formation of a stable drug–protein complex, which can exert important effect on the distribution, free concentration and metabolism of the drug in the blood stream. Thus the drug–albumin complex, may be considered as a model for gaining fundamental insights into the drug–protein interactions. Therefore, studies on the binding of drug with protein will facilitate interpretation of the metabolism and transporting process of a drug and will help to explain the relationship between structures and functions of a protein. In this regard, bovine serum albumin (BSA) was studied extensively in the past years, partly because of its structural homology with human serum albumin (HAS) [10,11].

Despite the numerous studies on copper complexes and their interactions with bio-macromolecules, in this study, we have reported the synthesis of a new water-soluble copper (II) complex containing mixed aliphatic and aromatic ligands. The binding properties of this complex with bovine serum albumin have been carried out using different instrumental methods and the binding mode is discussed. Some techniques commonly used to detect interaction between drugs and serum albumin include fluorescence spectroscopy [12,13], UV-spectrophotometry [14,15], and circular dichroism spectroscopy [16].

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2. Experimental

2.1. Materials

Bovine serum albumin (BSA) was purchased from Sigma–Aldrich, N,N-dimethyltrimethylenediamine (L) was purchased from Merck, 2,9-dimethyl-1,10-phenanthroline (NN) was purchased from Riedel-deHaën.

2.1.1. Synthesis of copper (II) complex

The Cu(II) complex was synthesized by the modification of the method which was used by Rupesh et al. [17].

2.1.1.1. Preparation of $[Cu(L)(NO_3)_2]\cdot H_2O$. To 10 mmol (2.416 g) of copper (II) nitrate taken in 25 mL of distilled water, 10 mmol (1.022 g) of N,N-dimethyltrimethylenediamine (L_1) in 50 mL ethanol was added and stirred for about 2 h. The resulting blue solution was reduced to 20 mL to precipitate; the desired complex was filtered, recrystallized from acetonitrile/ethanol and dried under vacuum. Yield (2.35 g, 81%).

2.1.1.2. Preparation of [Cu(L)(2,9-dimethyl-1,10-phenanthroline)(EtOH)] $(NO_3)_2\cdot 2H_2O$. To 10 mmol (2.34 g) of $[Cu(L_1)(NO_3)_2]\cdot H_2O$ complex in 50 mL water, 10 mmol (2.09 g) of *2,9-dimethyl-1,10-phenanthroline* in 100 mL ethanol was added dropwise and stirred for 4 h. The resulting brown colored solution was reduced to 20 mL. The precipitate was filtered and dried in the desiccator. Yield (4.28 g, 74%).

Analytical data (%) for $CuC_{21}H_{35}N_6O_9$, found (calculated); C, 44.1 (43.56); H, 6.5 (6.11); N, 14.9 (14.51).

^{1}H NMR (200 MHz, $CDCl_3$); aromatic dinitrogen ligand; 8.48 (d, 2H), 8.01 (s, 2H), 7.75 (d, 2H), 2.39 (s, 6H); aliphatic diamine ligand; 2.46 (s, 6H), 0.87 (t, 2H), 1.21 (t, 2H), 1.28 (q, 2H), 4.17 (br NH_2).

^{13}C NMR (200 MHz, $CDCl_3$); aromatic dinitrogen ligand; 125.61, 127.64, 128.79, 130.95, 143.03, 157.62 (6 carbon atoms of phen ligand), 25.88 (Me groups of phen ligand); aliphatic dinitrogen ligand; 22.96, 29.68, 25.88, 14.06 (3 carbon atoms and Me groups, respectively).

2.2. Reagents

A new copper (II) complex and BSA solutions were prepared in the buffer solution adjusted to pH 7.00 with 0.01 M Na_2HPO_4 and NaH_2PO_4 in pure aqueous medium. BSA stock solution (1.00×10^{-5} M, based on its molecular weight of 66,000) was prepared in 0.01 M phosphate buffer of pH 7.00 and was kept in the dark at 277 K. Triply distilled water was used throughout the experiment.

2.3. Methods

2.3.1. UV-spectrophotometry

Absorbance spectra were recorded using an hp spectrophotometer (agilent 8453), equipped with a thermostated bath (Huber polysat cc1). Absorption titration experiments were carried out by keeping the concentration of BSA constant (1×10^{-5} M) while varying the Cu(II) complex concentration from 0 to 1.2×10^{-5} M (ri = [complex]/[BSA] = 0.0, 0.35, 0.5, 0.7, 1, 1.2).

Absorbance values were recorded after each successive addition of BSA solution and equilibration (ca. 10 min).

2.3.2. CD studies

Circular dichroism (CD) measurements were recorded on a JASCO (J-810) spectropolarimeter by keeping the concentration of BSA (1×10^{-5} M) while varying the Cu(II) complex concentration from 0 to 3×10^{5} M (ri = [Cu(II)]/[BSA]) = 0.0, 0.5, 1, 1.5, 2).

2.3.3. Fluorescence spectra

Fluorescence measurements were carried out with a JASCO spectrofluorimeter (FP 6200) by keeping the concentration of BSA constant (1×10^{-5} M) while varying the Cu(II) complex concentration from 0 to 2×10^{-5} M (ri = [Cu(II)]/[BSA]) = 0.0, 0.2, 0.5, 0.7, 1.0, 1.35, 1.5, 1.7, 2) at three different temperatures (288, 298, 308 K).

3. Results and discussion

The synthesis of water-soluble complexes is an important first step for possible applications in biological systems; to this aim, we synthesized a new copper (II) complex, which has a good solubility in water (Fig. 1).

Serum albumin often increases the apparent solubility of hydrophobic drugs in plasma and modulates their delivery to cell in vivo and in vitro. Consequently, it is important, to understand the mechanism of interaction of a bioactive compound with protein. The nature and magnitude of drug–protein interaction influence the biological activity (efficacy and rate of delivery) of the drug [18]. It is then important to study the binding parameters in order to know and try to control the pharmacological response of drugs and design of dosage forms. This kind of studies may provide salient information on the structural features that determine the therapeutic effectiveness of drugs and hence become an important research field in chemistry life science and clinical medicine [19]. Serum albumin is considered as a model for studying drug–protein interaction in vitro since it is the major binding protein for drugs and other physiological substances.

Bovine serum albumin (BSA) is constituted by 582 amino acid residues and based on the distribution of the disulfide bridges and of the amino acid sequence, it seems possible to regard BSA as composed of three homologous domains linked together. The domains can all be subdivided into two sub-domains. As proposed by Kragh-Hansen [20], there are at least six binding regions and another characteristic feature of albumin–ligand interactions seems to be the presence of one or two high affinity binding sites (primary sites) and a number of sites with lower affinity.

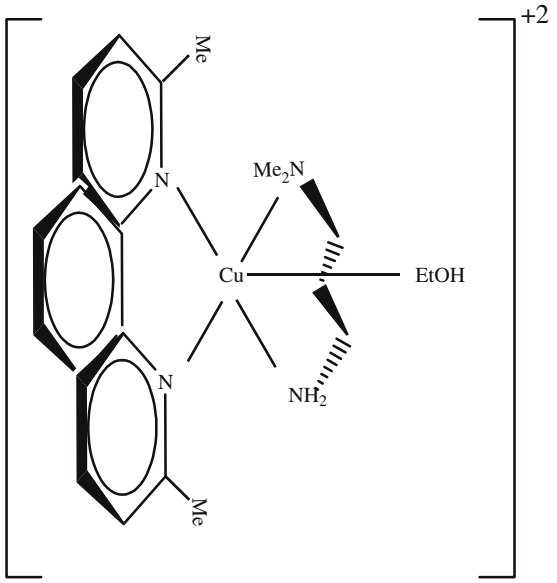

Fig. 1. Structure of the [Cu(2,9-dimethyl-phenanthroline)(N,N-dimethyltrimethylene diamine)(EtOH)] $(NO_3)_2\cdot 2H_2O$ complex.

3.1. Fluorescence spectroscopy

Qualitative analysis of binding of chemical compounds to BSA can be detected by examining fluorescence spectra. Generally, the fluorescence of protein is caused by three intrinsic flours present in the protein, i.e. tryptophan, tyrosine, and phenyl alanine residues. Actually, the intrinsic fluorescence of many proteins is mainly contributed by tryptophan alone. Due to a very low quantum yield of phenylalanine, fluorescence of a tyrosine is almost completely quenched if that tyrosine is ionized, near an amino group, a carboxyl group, or a tryptophan. Fluorescence quenching refers to any process, which is a decrease of the fluorescence intensity from a fluorophore due to a variety of molecular interaction. These include excited-state reactions, molecular rearrangements, energy transfer ground-state complex formation, and collisional quenching. Quenching can occur by different mechanisms, which usually classified as dynamic quenching and static quenching; dynamic quenching refers to a process that the fluorophore and the quencher come into contact during the transient existence of the exited state. Static quenching refers to fluorophore–quencher complex formation. In general, dynamic and static quenching can be distinguished by their differing dependence on temperature and excited state lifetime [21]. As, in both cases the fluorescence intensity is related to the concentration of the quencher. Therefore, the quenched fluorophore can serve as an indicator for quenching agent [22].

The effect of Cu(II) complex on BSA fluorescence intensity is shown in Fig. 2. The concentration of BSA was stabilized at 1×10^{-5} mol L^{-1} and the concentration of Cu(II) complex varied from 0 to 2×10^{-5} mol L^{-1}. It is obvious that BSA has a strong fluorescence emission peaked at 346 nm. When BSA was titrated with different amount of Cu(II) complex, a remarkable intrinsic fluorescence decrease of BSA was observed. Fluorescence quenching is described by the Stern–Volmer equation [23]:

$$F_0/F = 1 + k_q\tau_0[Q] = 1 + K_{sv}[Q] \quad (1)$$

where F_0 and F represent the fluorescence intensities in the absence and in the presence of quencher, respectively. k_q is the quenching rate constant of biomolecule, K_{sv} *is* the dynamic quenching constant, τ_0 is the average lifetime of the biomolecule without quencher (τ_0 = 6.2 ns [21]), and [Q] is the concentration of quencher.

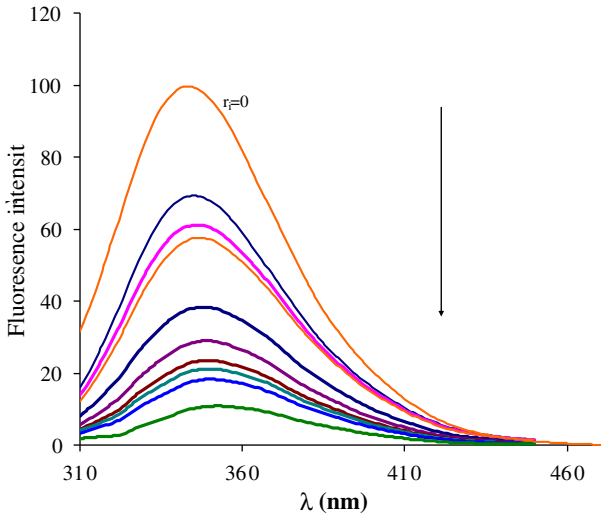

Fig. 2. Fluorescence spectra of BSA in the various of concentrations of Cu(II) complex, c(BSA) = 1.0×10^{-5}: c(Cu(II) complex)/c(BSA); 0, 0.2, 0.5, 0.7, 1, 1.2, 1.35, 1.5, 1.7, 2, respectively.

Fig. 3 displays the Stern–Volmer plots of the quenching of BSA fluorescence by Cu(II) complex at different temperatures. As we see, the plot of F_0/F for BSA versus [complex] ranging from 0 to 1.5×10^{-5} L mol^{-1} is linear. This observation may suggest that a single quenching mechanism, either static or dynamic is occurred at these concentrations [24,25].

Dynamic and static quenching can be distinguished by their different dependence on temperature. Dynamic quenching depends upon diffusion. Since higher temperatures result in higher diffusion coefficients, the bimolecular quenching constants are expected to increase with increasing temperature. In contrast, increased temperature is likely to result in decreased stability of complexes, and thus lower values of the static quenching constants [26]. The results in Table 1 indicate that the probable quenching mechanism of fluorescence of BSA by Cu(II) complex is a static quenching procedure, because K_{sv} is decreased with increasing temperature [13].

In addition, according to $k_q = K_{sv}/\tau_0$ the quenching rate constant k_q can be calculated. The values of k_q are of the order of 10^{13} L mol^{-1} s^{-1} (Table 1). The maximum scatter collision-quenching constant, k_q of various quenching with the biopolymer is 2×10^{10} L mol^{-1} s^{-1} [27]. Obviously, the constants of the protein quenching procedure initiated by Cu(II) complex are greater than the maximum scatter collision-quenching constant. This indicates that the quenching is not initiated by dynamic collision but from the formation of the complex. Therefore, in the present case, the static quenching equation (Linweaver–Burk equation; Eq. (2)) can be used [28]:

$$(F_0 - F)^{-1} = F_0^{-1} + K_a^{-1}F_0^{-1}[Q]^{-1} \quad (2)$$

where K_a is the binding constant of Cu(II) complex with BSA, which can be determined by the slope of the Linweaver–Burk $(1/F_0 - F)$ versus $1/[Q]$ curves (Fig. 4, Table 2). As shown in Fig. 4 the curves of $(F_0 - F)^{-1}$ versus $[Q]^{-1}$ are linear which demonstrate the static quenching [29]. Furthermore, the binding constants in Table 2 indicate strong binding between BSA and Cu(II) complex. As a result, the quenching of BSA by the titled complex is reduced significantly as temperature increases. Thus, the Cu(II) complex can be stored and moved by the protein in the body [30,31].

3.2. Binding constants and the number of binding sites

For the static quenching interaction, if it is assumed that there are similar and independent binding sites in the biomolecule, the binding constant (K_b) and the number of binding sites (n) can be determined according to the method described by Chipman et al. [32], using the following equation:

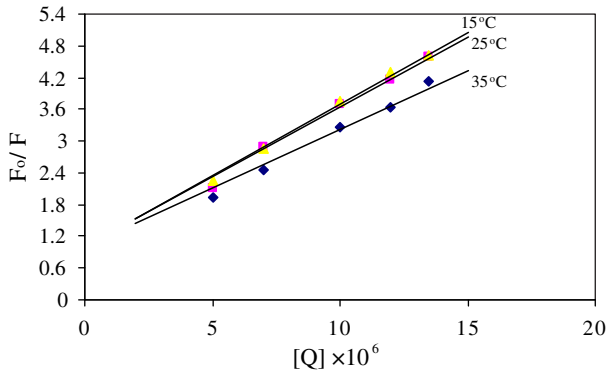

Fig. 3. Stern–Volmer plots for the interaction of Cu(II) complex with BSA at three different temperatures (T = 288, 298, and 318 K).

Table 1
The quenching constants of BSA by Cu(II) complex at different temperatures ranging from 5 to 13.5 μM of Cu(II) complex.

T (K)	R^2	K_{sv} (L mol^{-1}) × 10^5	k_q (L mol^{-1}) × 10^{13}
288	0.9990	2.71	4.5
298	0.9969	2.66	4.3
308	0.9988	2.22	3.6

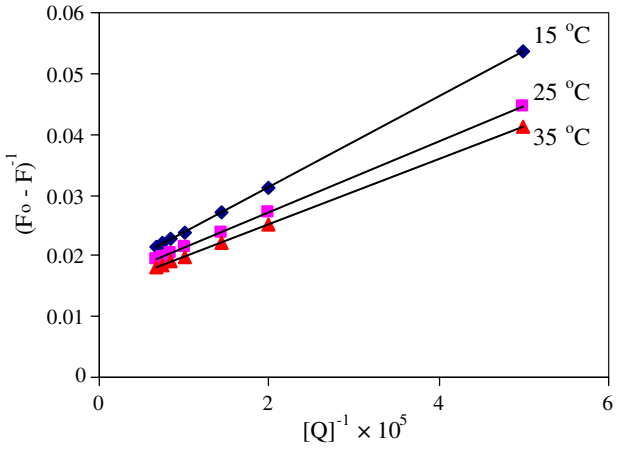

Fig. 4. The Linweaver–Burk plot for the binding of BSA with Cu(II) complex at different temperatures.

$$\log(F_0 - F)/(F - F_\infty) = \log K_b + n\log[Q] \quad (3)$$

where, in the present case, K_b is the binding constant for the complex–protein interaction and n is the number of binding sites per albumin molecule, which can be determined by the slope and the intercept of double logarithm regression curve of $\log(F_0 - F)/(F - F_\infty)$ versus log[complex] based on the Eq. (3) (Fig. 5 and Table 2). The relative fluorescence intensity of BSA saturated with Cu(II) complex, F_∞, was extrapolated from the experimental data by plotting $1/F_0 - F_\infty$ against 1/[complex] where F is measured fluorescence of a solution containing the BSA with a given Cu(II) complex concentration and F_0 is the fluorescence of a solution of BSA alone.

The correlation coefficients are larger than 0.99, indicating that the assumption underlying the deviation of Eq. (3) is satisfactory. The values of n at the experimental temperatures approximately equal to 1, which indicates that there is just single binding site in BSA for Cu(II) complex. Hence the Cu(II) complex most likely binds to hydrophobic pocket located in sub-domain IIA, that is to say, Trp-212 is near or within the binding site [33].

Table 2
Modified Stern–Volmer association constants (K_a), binding constants (K_b), number of binding sites (n) and relative thermodynamic parameters of the BSA–Cu(II) complex system.

T (K)	$K_a \times 10^{5a}$ (L mol^{-1})	$K_b \times 10^{5b}$ (L mol^{-1})	n	ΔG (kJ mol^{-1})	ΔH (kJ mol^{-1})	ΔS (J mol^{-1} K^{-1})
288	2.89	2.70	0.988	−22.64	−7.32	78.59
298	2.49	2.59	0.990	−23.43		
308	2.11	2.21	1.004	−24.21		

[a] Linweaver–Burk plot.
[b] $\log(F_0 - F)/(F - F_\infty)$ versus log[Cu(II)] plot.

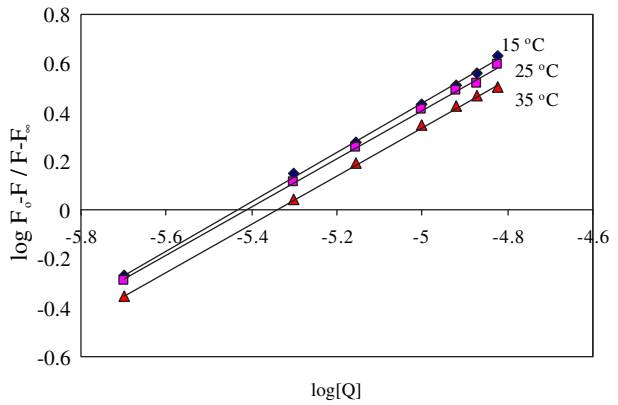

Fig. 5. Plot of $\log(F_0 - F)/(F - F_\infty)$ versus log[Q]. c(BSA) = 1 × 10^{-5}; mol c(Cu(II))/(1 × 10^{-5} mol L^{-1}): 0.5, 0.7, 1, 1.2, 1.35 and 1.5, respectively.

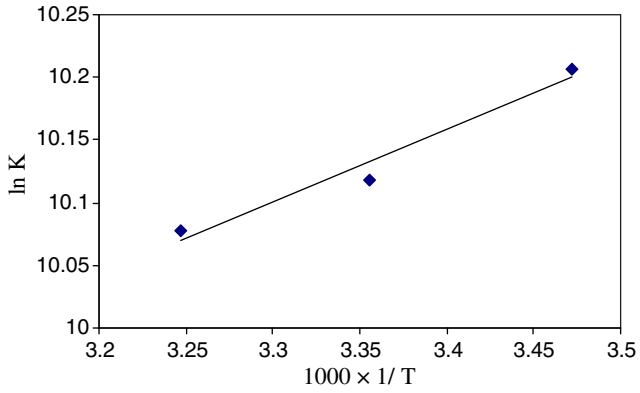

Fig. 6. Van't Hoff plot for the interaction of BSA and Cu(II) complex at pH 7.0.

3.3. Binding mode between Cu(II) complex and BSA

The interaction forces between drug and biomolecules may involve hydrophobic forces, electrostatic interactions, van der Waals interactions, hydrogen bonds, etc. [34,35]. In order to elucidate the interaction of Cu(II) complex with BSA, the thermodynamic parameters were calculated. The plot of log K_b versus $1/T$ (Fig. 6) allows the determination of enthalpy change (ΔH) and entropy change (ΔS). If the temperature does not vary significantly, the enthalpy change (ΔH) can be regarded as a constant. Based on the binding constants at different temperatures, the free energy change (ΔG) can be estimate by following equation:

$$\ln K = -\Delta H/RT + \Delta S/R \quad (4)$$

where K is the binding constant (K_b) at the corresponding temperatures and R is the gas constant. The temperatures used were 288, 298, 308 K. Table 2 shows the values of ΔH, and ΔS. According to the data of enthalpy change (ΔH) and entropy change (ΔS), the model of interaction between drug and biomolecule can be concluded [36]: (1) The positive entropy change is frequently regarded as an evidence for a hydrophobic interaction [19] because the water molecules that are arranged in an orderly fashion around the drug and protein acquire a more random configuration. (2) The negative ΔH value observed cannot be mainly attributed to electrostatic interactions since for electrostatic interactions ΔH is very small, almost zero [37,38]. A negative ΔH value will be obtained whenever there is a hydrogen bonding in the binding [38]. Therefore, hydrophobic forces and hydrogen bonds may play main role in binding of Cu(II) complex to BSA [29,38,39]. The negative value of ΔG reveals that the interaction process is spontaneous (Fig. 6 and Table 2).

3.4. Energy transfer between Cu(II) complex and BSA

Energy transfer phenomena have wide applications in energy conversion process [40]. According to Foster non-radiative energy transfer theory (FRET) [41] the rate of energy transfer depends on (1) the relative orientation of the donor and acceptor dipoles, (2) the extent of overlap of fluorescence emission spectrum of the donor with the absorption spectrum of the acceptor and (3) the distance between the donor and the acceptor. In addition, the energy transfer will take place under these conditions: (1) the donor can produce fluorescent light that has sufficiently long lifetime; (2) the fluorescence emission spectrum of the donor and the UV absorbance spectrum of the acceptor have sufficient overlap; (3) the distance between the donor and the acceptor is less than 8 nm [42].

The energy transfer effect is related not only to the distance between the donor and acceptor, but also to the critical energy transfer distance, that is, calculated by the following equation [43]:

$$E = 1 - F/F_0 = R_0^6/R_0^6 + r_0^6 \quad (5)$$

where r_0 represents the distance between the donor and the acceptor and R_0 is the critical distance at which transfer efficiency equals to 50%. The value of R_0 is calculated using the following equation [43]:

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

where K^2 is an orientation factor dependent on the alignment of the donor and acceptor dipoles, n is the refractive index of the medium, Φ is the luminescence quantum yield in the absence of energy transfer and J is the overlap between the luminescence spectrum of the donor and the absorption spectrum of the acceptor, J is given by [3]

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

where $F(\lambda)$ is the corrected fluorescence intensity of the donor in the wavelength λ to $\lambda + \Delta\lambda$, ε is the extinction coefficient of the acceptor at λ.

The overlap of the UV absorption spectrum of Cu(II) complex with the fluorescence emission spectrum of the BSA is shown in Fig. 7. From the overlapping spectrum, J can be evaluated by integrating the spectra for $\lambda = 300$–450 nm and $J = 4.56 \times 10^{-15}$ cm^3 L mol^{-1}. Under these experimental calculations, we have found $E = 0.85$, $R_0 = 2.53$ nm from Eq. (6) using $K = 2/3$, $n = 1.336$ and $\Phi = 0.15$ for BSA [44] and $r_0 = 1.9$ nm from Eq. (5). Obviously, the donor–acceptor distance, $r < 8$ [45] indicates that the energy transfer from BSA to Cu(II) complex occurs with high possibility.

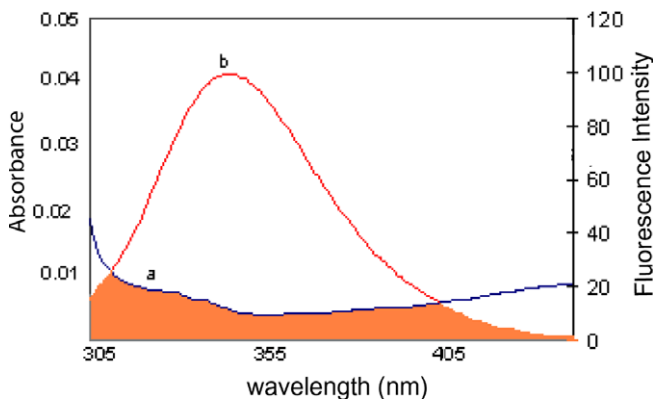

Fig. 7. Spectral overlap of Cu(II) complex absorption (a) with BSA fluorescence (b); c(BSA) = c(Cu(II) complex) = 1.0×10^5 mol L^{-1} (T = 298 K).

These accord with the conditions of FRET, indicating again the static quenching interaction between Cu(II) complex and BSA [46].

3.5. Conformation investigation

3.5.1. UV–vis spectroscopy

UV–vis absorption measurement is a simple but effective method in detecting complex formation [33,47]. In the present study, to explore the structural changes of BSA by addition of Cu(II) complex, we measured UV–vis spectra of BSA with various amounts of the titled complex (Fig. 8). This figure shows that the UV absorbance intensity of BSA was increased with the addition of Cu(II) complex, which indicates formation of a complex between BSA and Cu(II) complex and change in protein conformation [48]. Furthermore, the changes in absorption spectroscopy confirmed that the quenching of BSA by the Cu(II) complex is static. In other words, the fluorescence quenching of BSA resulting from complex formation is predominant, while from dynamic collision could be negligible [49]. It is also apparent that the maximum absorption wavelength moderate shifts towards shorter wave, when $\Delta\lambda = 4$ nm. This shift effect expresses that the conformation of

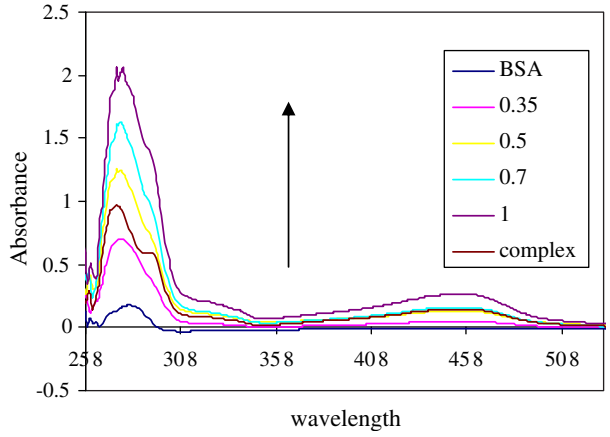

Fig. 8. The UV–vis absorption spectra of BSA in the various concentrations of Cu(II) complex, c(BSA) = 1.0×10^{-5}: c(Cu(II) complex)/c(BSA): 0, 0.35, 0.5, 0.7, 1, 1.2, respectively.

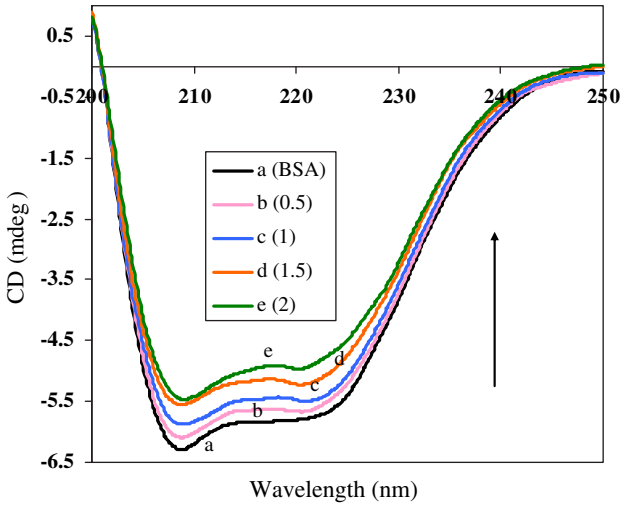

Fig. 9. CD spectra of BSA in the presence of various concentrations of Cu(II) complex (T = 298 K). c(BSA) = 3×10^{-6} M. The concentration ratios of BSA to complex (a–e) were 1:0, 1:0.5, 1:1, 1:1.5 and 1:2, respectively.

Table 3
Fractional contents of the secondary structure of BSA (3×10^{-6} M) with and without Cu(II) complex.

Concentration ratio (BSA/complex)	α-Helix (f_α)	β-Sheet (f_β)	Turn (f_{turn})	Random (f_{random})
1/0	15.7	15.5	35.5	33.3
1/0.5	15.4	15.6	35.7	33.3
1/1	15	16	35.7	33.3
1/1.5	14.5	16.7	35.9	32.9

BSA was changed and the polarity around the tryptophan residues was increased and the hydrophobicity was decreased [50]. This conclusion agrees with the other results of conformational changes by UV–vis spectra [47,51].

3.5.2. Circular dichroism

CD is a sensitive technique to monitor conformational changes of protein upon interaction with small molecules. Bovine serum albumin has a high percentage of α-helical structure which shows a characteristic strong double minimum signals at 222 and 208 nm [52,53] (Fig. 9, curve a). The intensities of two double minimum reflect the amount of helicity of BSA and further these indicate that BSA contains more than 50% of α-helical structure. Upon addition of the Cu(II) complex to BSA the extent of α-helicity of the protein decreased and hence, the intensity of double minimum was reduced. This is indicative of change in helicity when the Cu(II) is completely bound to BSA. According to previous studies, the titled Cu(II) complex does not any CD signals in this region [54].

Fractional contents of the secondary structure of BSA (f_α, f_β, f_{turn} and f_{random}) with and without Cu(II) complex are calculated utilizing circular dichroism spectroscopy and are shown in Table 3.

The CD results show that the addition of the Cu(II) complex to BSA caused the reduction of the α-helix fraction (f_α) from 15.7 to 14.5. Also changes in the fraction of β-sheet (f_β), turn (f_{turn}) and random coil (f_{random}) was observed on the addition of the titled complex to BSA (Table 3). These results are in agreement with the previous studies on the interaction of albumin with small molecules [55], which suggests the interaction of Cu(II) complex with BSA.

The CD spectra of BSA in the presence and absence of the Cu(II) complex were observed to be similar in shape, which meant that the structure of BSA was also predominantly of α-helix [56]. Therefore, we can conclude that the binding of complex–BSA induced some secondary-structure changes in BSA.

4. Conclusions

The main purpose of this research was to synthesis of a new water-soluble copper (II) complex containing different dinitrogen ligands (aliphatic and aromatic). Different instrumental methods were used to finding the interaction mechanism of this complex with bovine serum albumin (BSA). The following results support the fact that the Cu(II) complex can bind to BSA and transport in the body:

1. Fluorescence results show that the Cu(II) complex is a strong quencher and interacts with BSA through a static quenching procedure.
2. The results in Table 1 indicate that the probable quenching mechanism of fluorescence of BSA by Cu(II) complex is a static quenching procedure, because K_{sv} is decreased with increasing temperature [13].
3. The negative value of ΔH, positive value of ΔS and the negative value of ΔG indicate that hydrophobic and hydrogen bonding interactions playing a major role in the binding.
4. According to the FRET theory, the critical distance between BSA and the Cu(II) complex (r_0) is 1.9 nm, in the 2–8 nm scale, and shows that the energy transfer from BSA to the Cu(II) complex occurs with high probability.
5. Increase of the absorption intensity of BSA spectrum in the presence of various amounts of the complex indicates that the polarity around the tryptophan residues is increased and hydrophobicity is decreased.
6. The results of CD spectra indicate that the conformation of BSA molecule is changed significantly in the presence of the Cu(II) complex.

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