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Characterization of the interaction between Fe(III)-2,9,16, 23-tetracarboxyphthalocyanine and blood proteins

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Abstract

The features of FeTCPc (Fe(III)-2,9,16,23-tetracarboxyphthalocyanine) binding to bovine serum albumin and bovine hemoglobin were investigated by fluorescence and UV/vis absorption spectroscopy. FeTCPc has the ability to quench the intrinsic fluorescence of both bovine serum albumin and bovine hemoglobin through mainly static quenching. The binding site number n, apparent binding constant K_A and the corresponding thermodynamic parameters ΔG^0, ΔH^0, ΔS^0 at different temperatures were calculated; both electrostatic and hydrophobic interactions play a major role in stabilizing the complex. The distance r between the donor (bovine serum albumin or bovine hemoglobin) and acceptor (FeTCPc) was obtained according to fluorescence resonance energy transfer. The effect of FeTCPc on the conformation of the two donors was analyzed using synchronous fluorescence spectroscopy.

Keywords: Fe(III)-2,9,16,23-tetracarboxyphthalocyanine; Bovine serum albumin; Bovine hemoglobin; Fluorescence quenching; Thermodynamic parameter; Spectroscopy

1. Introduction

Phthalocyanines have been one of the most extensively studied classes of organic functional materials because of their aromatic 18-π electron system [1]. They are important blue and green dyes, recently they have been used as photoconducting agents in photocopying machines, as sensing elements in chemical sensors [2,3], electrochromic display devices, and photodynamic reagents for cancer therapy and for other medical applications. Microbicides, which serve to prevent the initial entry of the virus into the cell, offer the possibility of protection against transmission of the virus [4,5]. There have been a number of previous studies on phthalocyanines and metallophthalocyanines as potential microbicides to kill the human immunodeficiency virus [6], the vesicular stomatitis virus [7], bovine viral diarrhea virus [8], pseudorabies virus [8], and herpes simplex virus [9]. All of these studies involve photoactivation of the phthalocyanine to produce species (singlet oxygen or free radical) that kill the virus. Conjugation of sensitizer with biological targeting agents, e.g., monoclonal antibodies or lipoproteins, increases the concentration of sensitizer on targeted tissue over normal tissue, providing additional level of selectivity and possibility to reduce effective sensitizer concentration, and thus phototoxicity to normal tissue [10,11]. To have a full understanding of the modes of drug actions, their interaction with all possible biological targets, including nucleic acids, enzymes, and other proteins, is required.

Serum albumins are the major soluble protein constituents of the circulatory system and have many physiological functions [12]. The most important property of this group of

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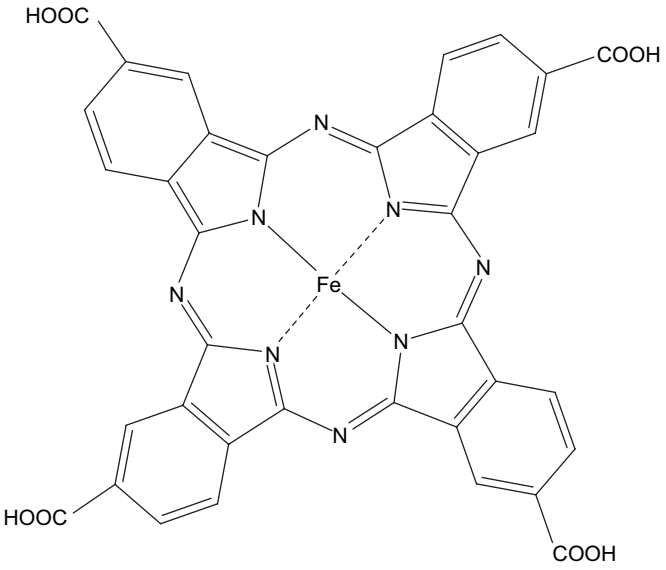
proteins is that they serve as a depot protein and as a transport protein for many drugs and other small bioactive molecules. The molecular interactions of flavonoids [13], isoquinoline alkaloids [14], insecticide [15] and dye molecules [16,17] with albumin have been investigated successfully. Another abundant blood protein, hemoglobin, accounts for 97% of dry erythrocyte weight. Hemoglobin is well known for its function in the vascular system of animals, transporting oxygen from the lungs or gills to peripheral tissues. It also aids, both directly and indirectly, the transport of carbon dioxide and regulates the pH of blood [18]. Several reports were published on the interactions of Pt drugs [19], artemisinins [20], and heteropolyacid compounds [21] with hemoglobin. In the present work, we demonstrated the affinity of Fe(III)-2,9,16,23-tetracarboxyphthalocyanine (FeTCPc) (Scheme 1) to bovine serum albumin (BSA) and bovine hemoglobin (BHb). In order to attain these objectives, we planned to carry out detailed investigation of FeTCPc–BSA and FeTCPc–BHb associations using fluorescence spectroscopy and UV/vis absorption spectroscopy. Through fluorescence resonance energy transfer and synchronous fluorescence spectroscopy, we planned to further investigate the effect of the energy transfer and the effect of FeTCPc on the conformation of BSA and BHb.

2. Materials and methods

2.1. Materials

BSA and BHb were purchased from Sigma (St. Louis, MO, USA) and used without further purification. Dr. L. Wu donated FeTCPc which was synthesized and purified according to the procedures described previously [22]. The buffer Tris was purchased from Acros (Geel, Belgium), and NaCl, HCl, etc. were all of analytical purity. BSA and BHb solutions (3.0×10^{-6} mol L^{-1}) were prepared in pH 7.40 Tris–HCl buffer solution (0.05 mol L^{-1} Tris, 0.1 mol L^{-1} NaCl). The FeTCPc solution (3.75×10^{-4} mol L^{-1}) was prepared in pH 7.40 Tris–HCl buffer containing 50% DMSO (v/v) because of its low solubility.



Scheme 1. Molecular structure of Fe(III)-2,9,16,23-tetracarboxyphthalocyanine (FeTCPc).

2.2. Equipments and spectral measurements

The UV/vis spectrum was recorded at 291 K on a GBC UV/Vis916 spectrophotometer (Australia) equipped with 1.0 cm quartz cells. All fluorescence spectra were recorded on LS-50B Spectrofluorimeter (Perkin–Elmer, USA) equipped with 1.0 cm quartz cells and a thermostat bath.

The widths of both the excitation slit and the emission slit were set to 10.0 nm/2.5 nm for BSA and 10.0 nm/5.0 nm for BHb.

2.3. Procedures

A 2.5 mL solution, containing appropriate concentration of BSA or BHb, was titrated by successive additions of a 3.75×10^{-4} mol L^{-1} stock solution of FeTCPc (to give a final concentration of 1.20×10^{-5} mol L^{-1}). Titrations were done manually by using trace syringes. The fluorescence spectra were then measured (excitation at 280 nm and emission wavelengths of 290–500 nm) at two temperatures (291 K, 305 K). The UV/vis absorbance spectra of FeTCPc with concentration of 3.0×10^{-6} mol L^{-1} were recorded at 291 K.

3. Results and discussion

3.1. UV/vis absorption studies

UV/vis absorption measurement is a very simple method and applicable to explore the structural change and to know the complex formation [23]. As shown in Fig. 1, FeTCPc gives a characteristic Q band absorption of non-aggregated

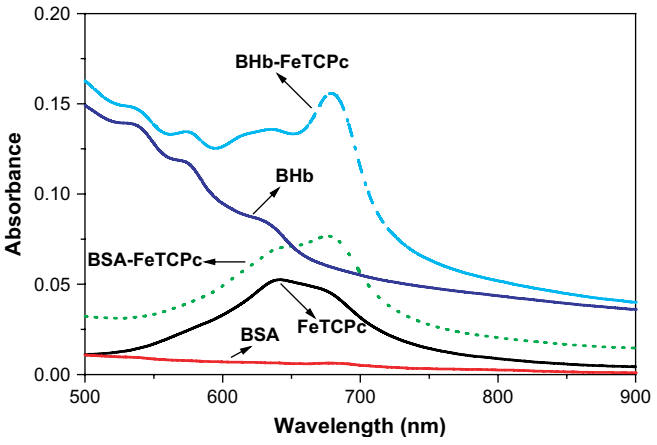


Fig. 1. Absorption spectra of BSA, BHb, FeTCPc, BSA–FeTCPc, and BHb–FeTCPc systems ($T = 291$ K, pH = 7.4). BSA or BHb concentration was 3.0×10^{-6} mol L^{-1}. FeTCPc concentration for BSA–FeTCPc or BHb–FeTCPc system was 3.0×10^{-6} mol L^{-1}. A concentration of 3.0×10^{-6} mol L^{-1} was used for FeTCPc only.

carboxyphthalocyanine at 642 nm. Upon complexation with BSA or BHb, the Q band of FeTCPc experiences an obvious change giving a new absorption at 679 nm. These observations strongly suggest that the complex formation between FeTCPc and blood proteins, BSA and BHb induces aggregation of FeTCPc. Since aggregation can shorten the excited-state lifetime mainly through internal conversion, this is an undesirable process for photosensitization [24].

3.2. Fluorescence quenching of blood proteins by FeTCPc

BSA and BHb have similar molecular weight, and both contain 30 helices [25,26]. However, albumin is a single chain protein, whereas BHb is a tetramer. BSA molecule is formed by 582 amino acid residues, and has two tryptophan residues that possess intrinsic fluorescence: Trp-134 in the first subdomain IB of the albumin molecule and Trp-212 in subdomain IIA. Trp-212 is located within a hydrophobic binding pocket of the protein and Trp-134 is located on the surface of the albumin molecule [27,28]. BHb contains three Trp residues in each αβ dimer, for a total of six in the tetramer: two α-14 Trp, two β-15 Trp, and β-37 Trp [29]. Of the three Trp residues, only β-37 Trp is located at the dimer–dimer interface, wherein the structural difference between quaternary states is the largest [30]. Moreover, β-37 Trp is known to donate protons in hydrogen bonds to Asp α94 residues in the T crystal structure [31]. The intrinsic fluorescence of BHb primarily originates from β-37 Trp that plays a key role in the quaternary state change upon ligand binding [32]. This view implies that the rigidity of the Trp regions in the hemoglobin molecule, compared with other proteins, may be a requirement for the functionality of the heme-iron site. A valuable feature of intrinsic fluorescence of proteins is the high sensitivity of tryptophan to its local environment. Changes in emission spectra of tryptophan are common in response to protein conformational transitions, subunit association, substrate binding, or denaturation [33]. So, the intrinsic fluorescence of proteins can provide considerable information about their structure and dynamics, and is often considered for the study of protein folding and association reactions.

The effect of FeTCPc on tryptophan residues' fluorescence intensity is shown in Fig. 2. As the data show, the fluorescence intensity of BSA and BHb decreased regularly with the increasing concentration of FeTCPc without changing the emission maximum and shape of the peaks. These results indicated that there were interactions between FeTCPc and BSA (or BHb).

Generally speaking, fluorescence quenching is the decrease of the quantum yield of fluorescence from a fluorophore induced by a variety of molecular interactions with quencher molecule, such as excited-state reaction, molecules' rearrangement, energy transfer, ground state complex formation and collision quenching. It is necessary to know quenching procedure and type for researching the mechanism of quenching. Quenching types often include static and dynamic quenching. In this paper, we have used the binding constants' dependence on temperature to elucidate the quenching mechanism.

Fluorescence quenching spectra and quenching type could be described by the well-known Stern–Volmer Eq. (1) [34] and modified Stern–Volmer Eq. (2) [35] to confirm the mechanism.

$$\frac{F_0}{F} = 1 + K_q \tau_0 [Q] = 1 + K_{SV}[Q] \tag{1}$$

$$\frac{F_0}{\Delta F} = \frac{F_0}{F_0 - F} = \frac{1}{f_a} + \frac{1}{f_a K_a [Q]} \tag{2}$$

where F_0 and F are the fluorescence intensities before and after the addition of the quencher, respectively. K_q, K_{SV}, τ_0, K_a, f_a, and [Q] are the quenching rate constants of the biomolecule, the Stern–Volmer dynamic quenching constant, the average lifetime of the biomolecule without quencher ($\tau_0 = 10^{-8}$ s [36]), the fraction of accessible fluorescence, the effected quenching constant for the accessible fluorophores, and the concentration of the quencher, respectively.

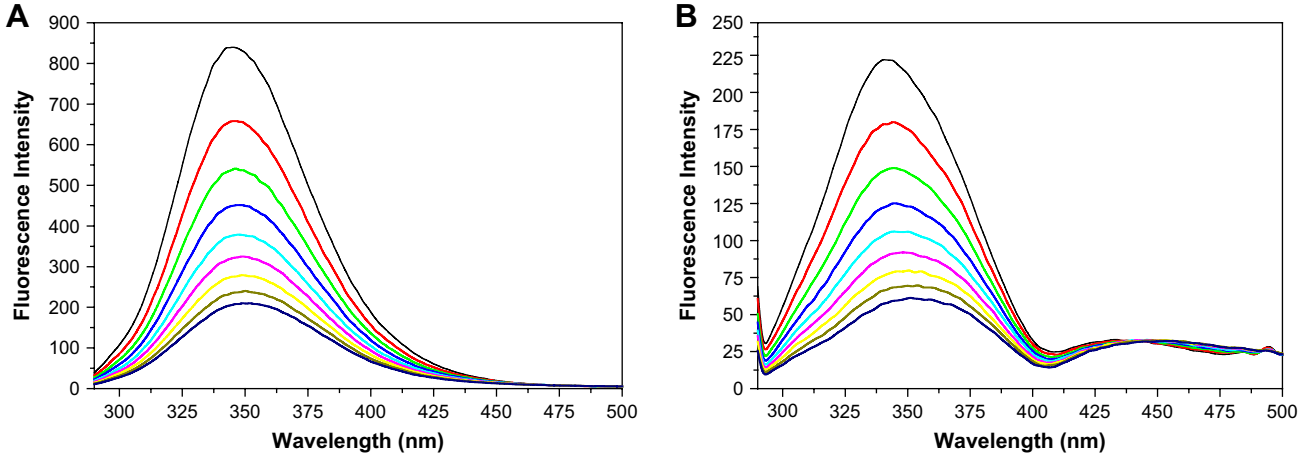


Fig. 2. Effect of FeTCPc on fluorescence spectrum of blood proteins ($T = 291$ K, pH = 7.4 and $\lambda_{ex} = 280$ nm). From up to down, c(BSA) = c(BHb) = 3.0×10^{-6} mol L^{-1}, c(FeTCPc)/(10^{-6} mol L^{-1}): 0, 1.5, 3.0, 4.5, 6.0, 7.5, 9.0, 10.5 and 12.0. BSA (A) and BHb (B).

Within certain concentration, the curve of F_0/F versus [Q] (Stern–Volmer curve) would be linear if the quenching type is single static or dynamic quenching [37]; similarly, the curve of $F_0/(F_0 - F)$ versus 1/[Q] (modified Stern–Volmer curve) would be linear for static quenching [38]. If the quenching type is combined quenching (both static and dynamic), the Stern–Volmer plot is an upward curvature [39].

The Stern–Volmer curves of FeTCPc–BSA and FeTCPc–BHb are shown in Fig. 3(A), it could be shown that when the concentration of FeTCPc was lower, the Stern–Volmer curves were linear, while the concentration of FeTCPc was higher, the Stern–Volmer curves were upward bent. It indicated that the quenching type was probably single quenching (static or dynamic quenching) at lower FeTCPc concentration. But at higher FeTCPc concentration a combined quenching (both static and dynamic) would be obtained [40]. Fig. 3(B) lists the modified Stern–Volmer curves. From Fig. 3(B), it is known that under certain FeTCPc concentration, the curves of $F_0/(F_0 - F)$ versus 1/[Q] were linear. All these indicated that there were obviously characters of static quenching.

As a rule, the K_{SV} values decrease with an increase in temperature for static quenching, but the reverse effect would be observed for dynamic quenching [41]; the maximum scatter collision quenching constant $K_{q,r}$ of various quenchers with the biopolymer was 2×10^{10} L mol^{-1} s^{-1} [38,39]. If $K_q > K_{q,r}$, the fluorescence quenching of biopolymer is surely not due to dynamic quenching. In this paper, K_{SV}, and K_q at two different temperatures are listed in Table 1. It indicated that the K_{SV} values decreased with an increase in temperature and K_q was of the order of 10^{13} L mol^{-1} s^{-1}. Obviously, this indicated that the quenching was not initiated from dynamic collision but from the formation of a compound. In this paper, the association constants obtained from the modified Stern–Volmer curves were applied to analyze the thermodynamic parameter and the nature of the binding forces.

3.3. Binding model

There are essentially four types of noncovalent interactions that could play a role in ligand binding to proteins. These are hydrogen bonds, van der Waals forces, electrostatic and hydrophobic interactions. To obtain such information, the implications of the present results have been discussed in conjunction with thermodynamic characteristics obtained for FeTCPc binding, and the thermodynamic parameters were calculated from Eqs. (3)–(5).

$$\ln \frac{(K_a)_2}{(K_a)_1} = \frac{\Delta H^0}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right) \tag{3}$$

$$\Delta G^0 = -RT \ln K_a \tag{4}$$

$$\Delta S^0 = \frac{\Delta H^0 - \Delta G^0}{T} \tag{5}$$

As can be seen from Table 2, the negative sign for ΔG^0 indicates the spontaneity of the binding of FeTCPc with BSA and BHb. ΔH^0 was a small negative value and ΔS^0 was a positive value. The main source of ΔG^0 value was derived from a large contribution of ΔS^0 term with little contribution from the ΔH^0 factor. Ross and Subramanian [41] have characterized the sign and magnitude of the thermodynamic parameter associated with various individual kinds of interactions that may take place in protein association processes. From the point of view of water structure, a positive ΔS^0 value is frequently taken as evidence for hydrophobic interaction. Tryptophan, tyrosine and phenylalanine in the binding cavity of protein have conjugated π-electrons and easily form charge transfer compounds with other electron-absent or π-electron system [42]. The interaction of FeTCPc with BSA (or BHb) included the hydrophobic forces between the aromatic 18-π electron system and the hydrophobic amino acid residues. Furthermore, specific electrostatic interactions of FeTCPc with BSA (or BHb) in aqueous solution were characterized by a positive value of ΔS^0 and a negative ΔH^0 (or $\Delta H^0 \approx 0$). Accordingly, it was not possible to account for the thermodynamic parameters of FeTCPc–BSA or FeTCPc–BHb coordination compound on the basis of a single intermolecular force model [43]. It was more likely that hydrophobic, electrostatic interactions were involved in its binding process.

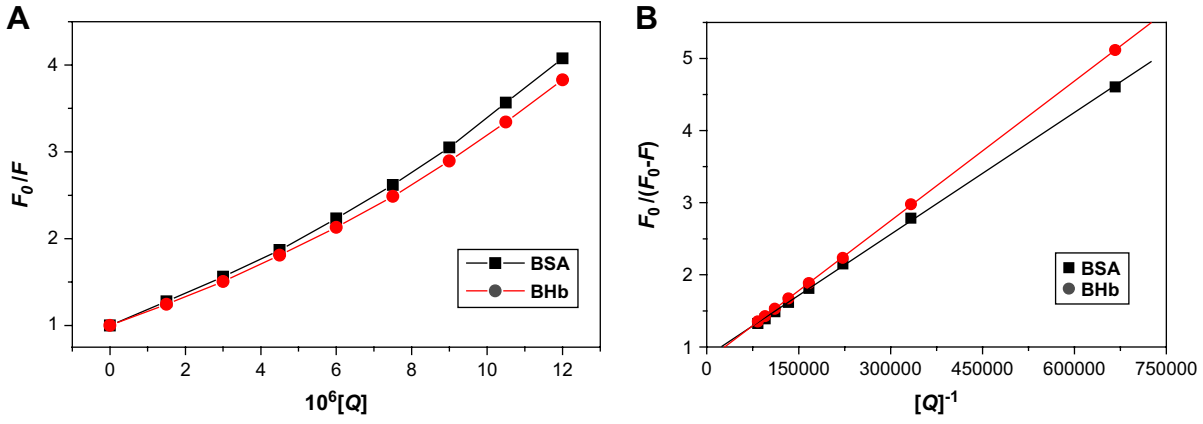


Fig. 3. The Stern–Volmer (A) and modified Stern–Volmer (B) curves of the interaction of FeTCPc with proteins (pH 7.40, $T = 291$ K, c(BSA) = c(BHb) = 3.0×10^{-6} mol L^{-1}, $\lambda_{ex} = 280$ nm).

Table 1
Stern–Volmer quenching constant (K_{SV}) and modified Stern–Volmer association constant (K_a) of the interaction of FeTCPc with proteins at two different temperatures

Protein	T (K)	Eq. (1)				Eq. (2)		
		10^{-5} K_{SV} (L mol^{-1})	10^{-13} K_q (L mol^{-1})	R^a	SD^b	10^{-5} K_a (L mol^{-1})	R^a	SD^b
BSA	291	3.05	3.05	0.9931	0.1321	1.54	0.9998	0.0233
	305	2.97	2.97	0.9928	0.1329	1.53	0.9999	0.0172
BHb	291	2.77	2.77	0.9934	0.1186	1.26	0.9999	0.0070
	305	2.70	2.70	0.9935	0.1109	1.25	0.9996	0.0109

[a] The correlation coefficient.
[b] The standard deviation.

3.4. The association constant and the number of binding sites

In order to calculate the binding constant and number of binding sites, several methods were employed to dispose the fluorescence data. Assuming that small molecules bind independently to a set of equivalent sites on a macromolecule, the equilibrium between free and bound molecules is given by Eq. (6) [39,40], Eq. (7) [44,45], and Eq. (8) [46,47]:

$$\log\left[\frac{F_0 - F}{F}\right] = \log K_A + n\log[Q] \tag{6}$$

$$\frac{F_0}{F} = \frac{K_A[Q_t]F_0}{F_0 - F} - nK[P_t] \tag{7}$$

$$\log\frac{F_0 - F}{F} = n\log K_A - n\log\left(\frac{1}{[Q_t] - (F_0 - F)[P_t]/F_0}\right) \tag{8}$$

where F_0 and F are the fluorescence intensities before and after the addition of the quencher, K_A is the apparent binding constant to a set of sites, and n is the average number of binding sites per BSA. $[Q_t]$ and $[P_t]$ are the total quencher concentration and the total protein concentration, respectively. The results for two different temperatures with these diverse methods are listed in Table 3. As can be seen from Table 3, results from Eq. (7) are closer to Eq. (8) than Eq. (6). Results from Eq. (6) have a bigger deviation, because in Eq. (6), [Q] should have been the free concentration of drug, but for simplification it was replaced by the total concentration of quencher. The data clearly showed that there was the binding site on BSA and BHb for FeTCPc independent of temperature from 291 K to 305 K. The result illustrates that there is a strong binding force between FeTCPc and blood proteins, and a binding site would be formed.

Table 2
Thermodynamic parameters of FeTCPc–BSA and FeTCPc–BHb interactions

Protein	T (K)	ΔH^0 (kJ mol^{-1})	ΔG^0 (kJ mol^{-1})	ΔS^0 (J mol^{-1} K^{-1})
BSA	291	−0.03	−28.90	99.20
	305		−30.27	99.14
BHb	291	−0.04	−28.41	97.49
	305		−29.76	97.44

3.5. Energy transfer from blood proteins to FeTCPc

Fluorescence resonance energy transfer (FRET) is a distance dependent interaction between the different electronic excited states of dye molecules in which excitation energy is transferred from one molecule (donor) to another molecule (acceptor) without emission of a photon from the former molecular system. According to Förster's theory [48], the efficiency of FRET depends mainly on the following factors: (i) the extent of overlap between the donor emission and the acceptor absorption, (ii) the orientation of the transition dipole of donor and acceptor, and (iii) the distance between the donor and the acceptor. FRET is an important technique for investigating a variety of biological phenomena including energy transfer processes [49]. One important consequence of energy transfer is photosensitization, a classic example of which is photosynthesis. Moreover, FRET plays a key role in photodynamic therapy (PDT) of cancer and is extensively used to study the structure, conformation, spatial distribution and assembly of complex protein [50].

There is a spectral overlap between the fluorescence emission spectrum of free BSA or BHb and absorption UV/vis spectra of FeTCPc (Fig. 4). According to Förster's theory the energy transfer efficiency E is defined as the following equation, Eq. (9), where r is the distance from the ligand to the tryptophan residue of the protein, and R_0 is the Förster critical distance, at which 50% of the excitation energy is transferred to the acceptor [48]. It can be calculated from donor emission and acceptor absorption spectra using the Förster formula Eq. (10).

$$E = 1 - \frac{F}{F_0} = \frac{R_0^6}{R_0^6 + r^6} \tag{9}$$

$$R_0^6 = 8.79 \times 10^{-25} K^2 N^{-4} \Phi J \tag{10}$$

$$J = \frac{\int_0^\infty F(\lambda)\varepsilon(\lambda)\lambda^4\,\mathrm{d}\lambda}{\int_0^\infty F(\lambda)\mathrm{d}\lambda} \tag{11}$$

In Eq. (10), K^2 is the orientation factor of dipoles related to the geometry of the donor and acceptor and $K^2 = 2/3$ for random

Table 3
Binding parameters from different methods at different tempreratures

Protein	T (K)	10^{-5} K_A (L mol^{-1})			n		
		Eq. (6) (R^a)	Eq. (7) (R^a)	Eq. (8) (R^a)	Eq. (6)	Eq. (7)	Eq. (8)
BSA	291	14.55 (0.9979)	3.14 (0.9924)	2.98 (0.9978)	1.16	1.14	0.99
	305	13.18 (0.9980)	3.06 (0.9913)	2.93 (0.9979)	1.15	1.12	0.98
BHb	291	16.92 (0.9988)	3.01 (0.9953)	2.65 (0.9990)	1.18	1.32	1.03
	305	16.18 (0.9987)	2.95 (0.9960)	2.59 (0.9980)	1.18	1.32	1.03

[a] The correlation coefficient.

orientation as in fluid solution; N is the average refractive index of medium in the wavelength range where spectral overlap is significant; Φ is the fluorescence quantum yield of the donor; J is the effect of the spectral overlap between the emission spectrum of the donor and the absorption spectrum of the acceptor (Fig. 4), which could be calculated by Eq. (11), where $F(\lambda)$ is the corrected fluorescence intensity of the donor in the wavelength range λ to $\lambda+\Delta\lambda$; $\varepsilon(\lambda)$ is the extinction coefficient of the acceptor at λ. In the present case, $N=1.36$, $K^2=2/3$, and $\Phi=0.15$ [50] for BSA, and $N=1.36$, $K^2=2/3$, and $\Phi=0.06$ [51] for BHb. According to Eqs. (9)–(11), we could calculate that $J=3.64\times10^{-14}$ cm^3 L mol^{-1}, $E=0.36$, $R_0=3.89$ nm, $r=4.28$ nm for BSA and $J=3.60\times10^{-14}$ cm^3 L mol^{-1}, $E=0.34$, $R_0=3.35$ nm, $r=3.75$ nm for BHb. The average distances between a donor fluorophore and acceptor fluorophore was on the 2–8 nm scale, which indicate that the energy transfer from BSA and BHb to FeTCPc occurs with high possibility [52].

3.6. Conformation investigation

Synchronous fluorescence spectroscopy technique was introduced by Lloyd in 1971 [53]. It involves simultaneous scanning of the excitation and emission monochromators while maintaining a constant wavelength interval between them. Synchronous fluorescence spectroscopy gives information about the molecular environment in the vicinity of the chromosphere molecules and has several advantages, such as sensitivity, spectral simplification, spectral bandwidth reduction and avoiding different perturbing effects [23]. Yuan et al. [54] suggested a useful method to study the environment of amino acid residues by measuring the possible shift in wavelength emission maximum λ_{max}, the shift in position of emission maximum corresponding to the changes of the polarity around the chromosphere molecule. When the D-value ($\Delta\lambda$) between excitation wavelength and emission wavelength was stabilized at 15 nm or 60 nm, the synchronous fluorescence gives the characteristic information of tyrosine residues or tryptophan residues [55]. The effect of FeTCPc on the synchronous fluorescence spectroscopy of BSA and BHb is shown in Fig. 5.

The tyrosine residues and the tryptophan residues of the fluorescence spectra of BSA and BHb at various concentrations of FeTCPc are shown in Fig. 5. It can be seen that no significant shift change of the wavelength was observed, which indicated that the interaction of FeTCPc with BSA or BHb does not obviously affect the conformation of tryptophan and tyrosine micro-region.

It has also been shown in Fig. 6(A) that addition of the drug results in strong fluorescence quenching of tyrosine residues of BSA which decrease about 74.60% in fluorescence intensity, while fluorescence strength of tryptophan residues decreases about 79.50%. Thus, there are similar decreasing percentages of fluorescence intensity for tyrosine and tryptophan. In BSA, tryptophans are located in the hydrophobic environment, IB and IIA (Trp-135, Trp-212); tyrosine residues are located in subdomain IA (Tyr-30, Tyr-84), IB (Tyr-140, Tyr-148,

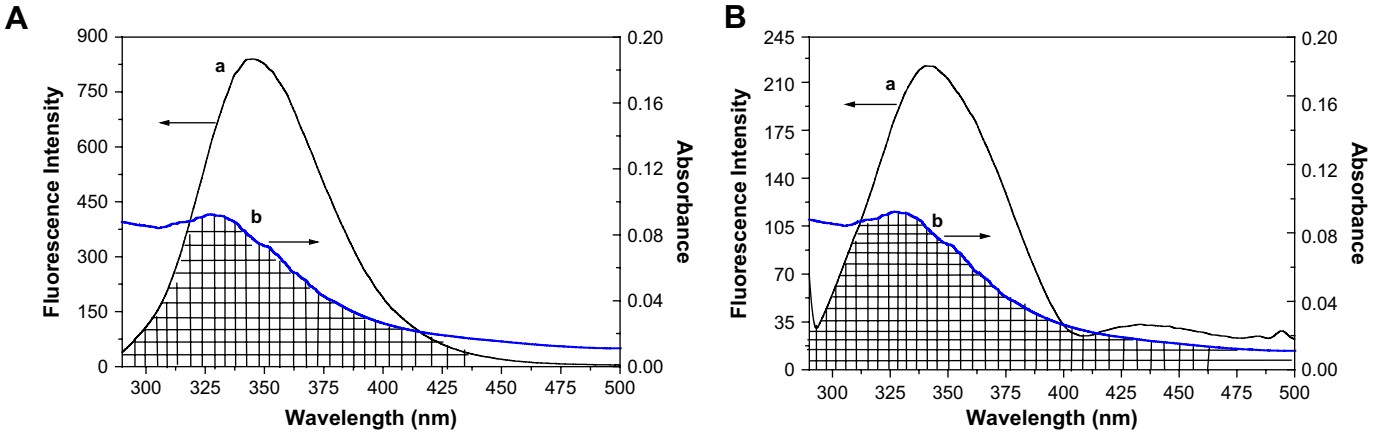


Fig. 4. The overlap of the fluorescence spectrum of protein (a) and the absorbance spectrum of FeTCPc (b), c(BSA) = c(BHb) = c(FeTCPc) = 3.0×10^{-6} mol L^{-1}. BSA (A) and BHb (B).

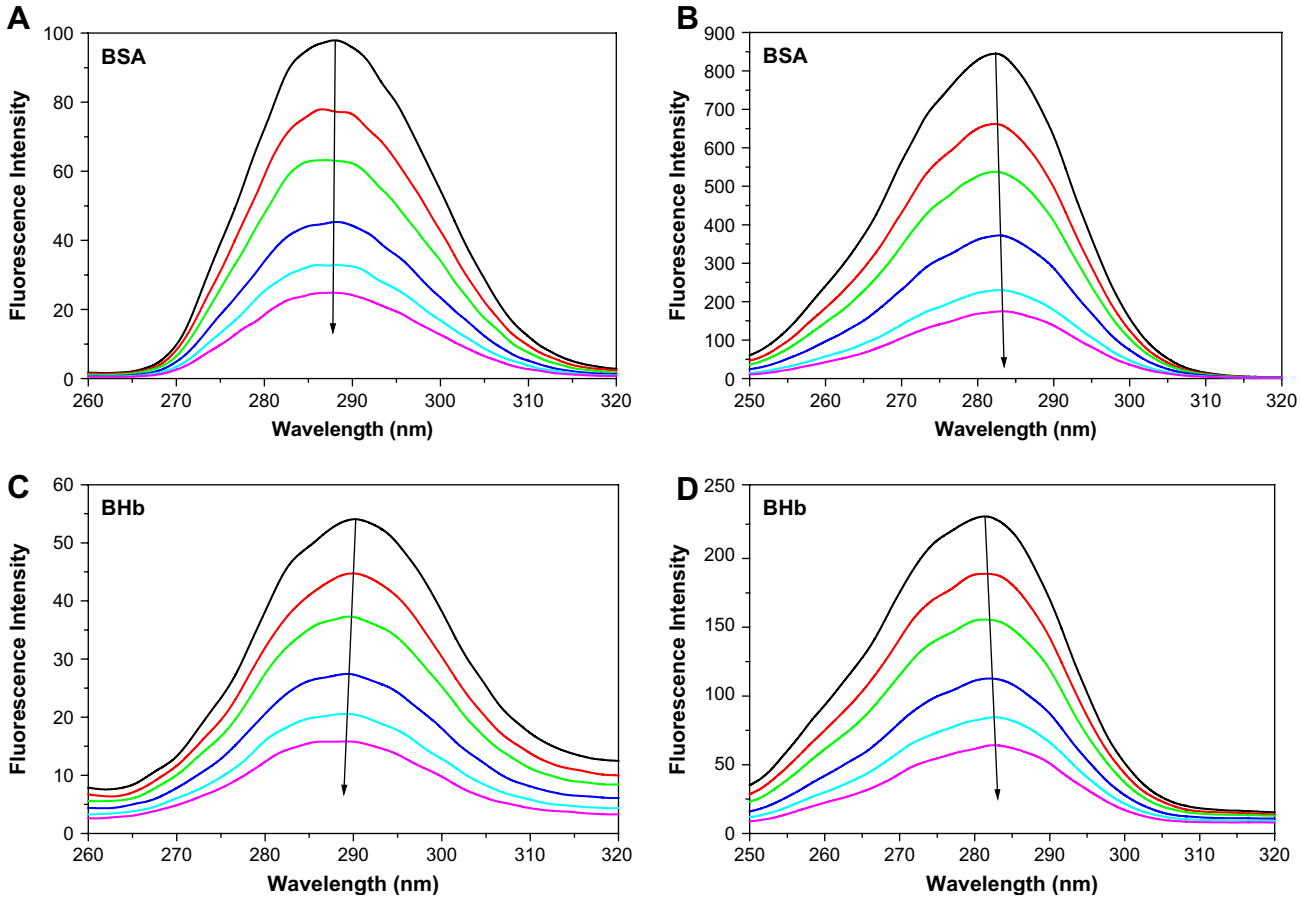


Fig. 5. Synchronous fluorescence spectrum of BSA and BHb ($T = 291$ K, pH = 7.40), c(BSA) = c(BHb) = 3.0×10^{-6} mol L^{-1}; c(FeTCPc)/(10^{-6} mol L^{-1}), from up to down: 0, 1.5, 3.0, 6.0, 9.0, 12.0. $\Delta\lambda = 15$ nm (A); $\Delta\lambda = 60$ nm (B).

Tyr-150, Tyr-156 and Tyr-157) and IIA (Tyr-263) [56]. The above results hint that FeTCPc can bind BSA in these domains and certain kinds of fluorescence groups may have equal accessibility to quencher [57].

In Fig. 6(B), there are also similar decreasing percentages of fluorescence intensity as to tyrosine and tryptophan of BHb. BHb contains three Trp residues in each αβ dimer, for a total of six in the tetramer: two α-14 Trp, two β-15 Trp, and β-37 Trp. The α-14 Trp and β-15 Trp residues are outside the subunit interface [58]. The β-37 Trp residue is located at the $\alpha_1\beta_2$ interface, which has been assigned as the primary source of fluorescence emission [29]. The aromatic residues of α-42 Tyr, α-140 Tyr, and β-145 Tyr are also located at the $\alpha_1\beta_2$ interface [59]. This result implies that the binding site of FeTCPc is located at the $\alpha_1\beta_2$ interface of BHb.

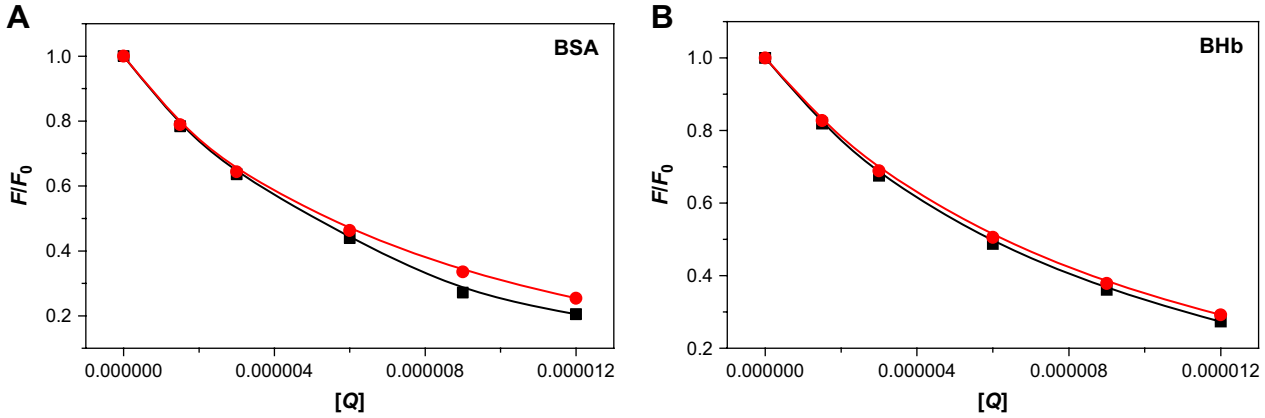


Fig. 6. The quenching of synchronous fluorescence of BSA (A) and BHb (B) by FeTCPc. The concentration of BSA and BHb was 3.0 μM. $\Delta\lambda = 15$ nm (●) and $\Delta\lambda = 60$ nm (■).

4. Conclusions

This paper presents spectroscopic studies on the interaction of FeTCPc with BSA and BHb using fluorescence emission spectrum, synchronous fluorescence spectrum and UV/vis spectrum. It was shown that the fluorescence of BSA (or BHb) has been quenched for reacting with FeTCPc and forming a certain kind of new compound. The quenching belonged to static fluorescence quenching type, with non-radiation energy transfer happening within single molecule. Meanwhile, from the thermodynamic parameter calculation, it was shown that the acting forces were mainly hydrophobic and electrostatic interactions. The results of synchronous fluorescence spectroscopy revealed that tyrosine and tryptophan residues of BSA may have equal accessibility to FeTCPc; the presence of a single class of binding site in the surrounding of β-37 Trp and some Tyr residues at the $\alpha_1\beta_2$ interface of BHb. The interaction of FeTCPc with BSA or BHb does not obviously affect the conformation of tryptophan and tyrosine micro-region. The binding study of photosensitizer with blood 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 blood proteins BSA and BHb with microbicides.

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