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Spectroscopic study on the inherent binding information of cationic perfluorinated surfactant with bovine serum albumin

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ABSTRACT

UV–vis, FT-IR, fluorescence and synchronous fluorescence spectra are applied to discuss the inherent binding information of model protein bovine serum albumin (BSA) with perfluorinated surfactant trimethyl-1-propanaminium iodide (FC-134). According to the results analyzed from Stern–Volmer equation, FC-134 can quench the fluorescence intensity of BSA via a dynamic quenching mechanism with complex formation. The thermodynamic parameters are calculated, revealing that hydrophobic force is the main interaction driven force. The binding constants and number of binding sites are also obtained. With the aid of site markers—warfarin and ibuprofen, we first report that FC-134 primarily binds to tryptophan residue Trp-214 of BSA within site I (sub-domain IIA).

1. Introduction

Protein is the main target of all medicines in organism and it is an important chemical substance in our life [1]. Serum albumin, the most abundant protein in blood plasma and the circulatory system, has been one of the most extensively studied proteins and has many physiological functions [1–3]. From a biopharmacological point of view, serum albumin is extremely important because it is the major transporter of non-esterified fatty acids and different drugs and metabolites to different tissues [4]. It is synthesized in the liver, exported as a non-glycosylated protein [1]. In this work, bovine serum albumin (BSA) is selected as our model protein because of its abundance, low cost, ease of purification, unusual ligand-binding properties [5,6] and especially of its structural homology with human serum albumin [6,7]. BSA is a heart-shaped helical molecule and is made up of three homologous domains (I, II and III) [3] and each domain includes two sub-domains called A and B. The accurate binding site of other compounds in BSA can be detected with the aid of site markers warfarin and ibuprofen.

Surfactant–protein interaction is of immense importance since it is to some extent comparable to lipid–protein interaction in the membrane of living cells [8] and can account for the transport of metabolites in body fluids [9]. Previous works mainly focused on traditional surfactant–protein interactions while perfluorinated surfactant–protein interactions remained rarely studied. Due to the highest electronegativity of fluorine (4.0) and its strong carbon–fluorine (C–F) covalent bond, perfluorinated surfactant has some unique properties such as water and oil repellency, high surface activity and excellent spreading characteristics [10,11]. It will exhibit more excellent functions than traditional amphiphilic surfactant. Thus, study on the perfluorinated surfactant–protein interaction is critical and undoubtedly needed.

Fluorescence quenching is a rapid and sensitive technique [12] and has been successfully and widely employed in the studies of protein–drug binding interactions due to aromatic acid residues such as tryptophan [13–15]. Analysis of quenching of albumin's natural fluorescence intensity can help understand albumin's binding mechanism to drugs, and provide clues to the essence of the binding phenomenon [16]. However, the application of fluorescence quenching technique and other spectroscopic methods to discuss the interaction of BSA with perfluorinated surfactant has rarely been reported.

Therefore, in present study, we chose a typical perfluorinated surfactant trimethyl-1-propanaminium iodide and bovine serum albumin to study the inherent binding information. Trimethyl-1-propanaminium iodide ($C_{14}H_{16}F_{17}IN_2O_2S$, molecular structure shown in Fig. 1), also known as perfluoroalkylsulfonyl quaternary ammonium iodide, is a kind of perfluoro cation surfactant. It is short for FC-134 according to the trademark against 3M US Company. We utilized UV–vis, FT-IR, synchronous fluorescence and fluorescence emission spectra to investigate the interaction of FC-134 with BSA. The fluorescence quenching mechanism,

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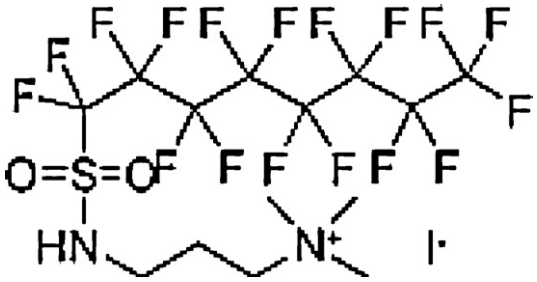

Fig. 1. Molecular structure of trimethyl-1-propanaminium iodide (FC-134).

thermodynamic parameters, driven binding forces, binding constants and number of binding sites were all obtained by fluorescence quenching technique. With the aid of warfarin and ibuprofen, the binding site of FC-134 in BSA was confirmed.

2. Results and discussion

2.1. UV–vis absorption spectra

In order to confirm the occurrence of binding interaction, we have recorded the influence of FC-134 on the absorption spectra of BSA (Fig. 2) and that of BSA on FC-134 (Fig. 3). Fig. 2 displays the absorbance of BSA (277 nm) at fixed concentration titrated against increasing concentrations of FC-134, indicating a slight blue shift (4–7 nm) with increase in absorbance. Fig. 3 illustrates the absorbance of FC-134 (225 nm) at fixed concentration titrated against rising amount of BSA, indicating a gradual disappearance of maximum absorption band with increase in absorbance. The blue shift in Fig. 2 is owing to the change of environment around BSA upon binding while the disappearance of band in Fig. 3 is a result of the influence of interaction with BSA. Increases in absorbance are due to the gradual addition of FC-134 and BSA, respectively. Figs. 2 and 3 clearly prove that an interaction between BSA and FC-134 has occurred.

2.2. FT-IR spectra

FT-IR technique is applicable in exploring the structural changes and confirming the complex formation and thus will provide more proof on the interaction. The FTIR-spectra of BSA and

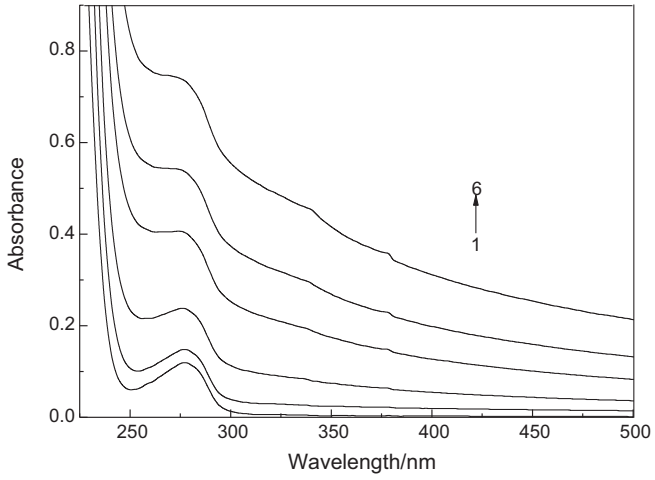

Fig. 2. Effect of FC-134 on UV–vis absorption spectra of BSA. 1 → 6: C_{BSA} = 3.33 × 10^{-6} mol L^{-1}; $C_{FC\text{-}134}$ (×10^{-5} mol L^{-1}): 0, 1.43, 4.18, 5.50, 6.79 and 9.87.

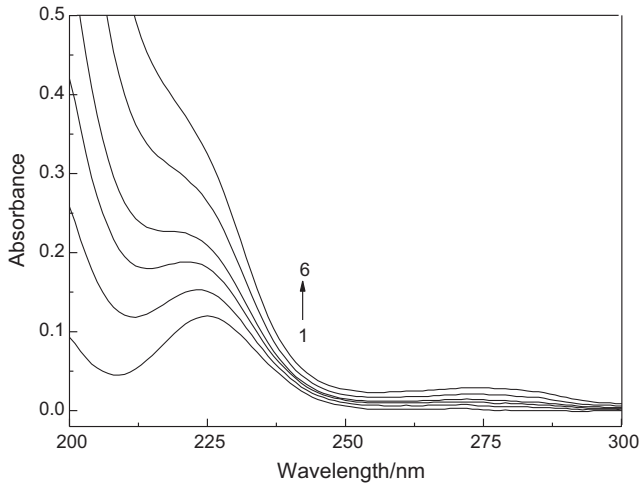

Fig. 3. Effect of BSA on UV–vis absorption spectra of FC-134. 1 → 6: $C_{FC\text{-}134}$ = 1.086 × 10^{-5} mol L^{-1}; C_{BSA} (×10^{-7} mol L^{-1}): 0, 0.662, 1.32, 1.96, 3.23 and 4.46.

BSA-FC-134 complex are shown in Fig. 4. There are two regions, 1700–1600 (mainly C=O stretch) and 1550–1500 cm^{-1} (C–N stretch coupled with N–H bending mode), in the FT-IR spectrum of native BSA unique to BSA secondary structure, called amides I and II [17]. The main absorption bands of BSA are listed as follows: 3409 cm^{-1}, 2960 cm^{-1}, 1653 cm^{-1} and 1540 cm^{-1}, corresponding to the stretching vibration of –OH, the amide A′ band, the amide I band and the amide II band, respectively [18]. However, in the presence of FC-134, these bands shift to 3431 cm^{-1}, 3066 cm^{-1}, 1644 cm^{-1} and 1486 cm^{-1}, respectively. As the amide I band is sensitive to change of protein conformation, from the 9 nm shift (from 1653 cm^{-1} to 1644 cm^{-1}) in peak position, we confirm that the conformation of BSA has been affected by the addition of FC-134 [19].

2.3. Synchronous fluorescence spectra

Synchronous fluorescence (SF) technique is another way to analyze the conformational change of BSA. When Δλ between excitation and emission wavelength are set at 15 or 60 nm, the SF spectra exhibit spectral character only of tyrosine or tryptophan residues in BSA, respectively. SF spectra of BSA upon addition of FC-134 gained at Δλ = 15 and 60 nm are shown in Fig. 5. The emission intensity of tyrosine residues increased slightly with a 8 nm red

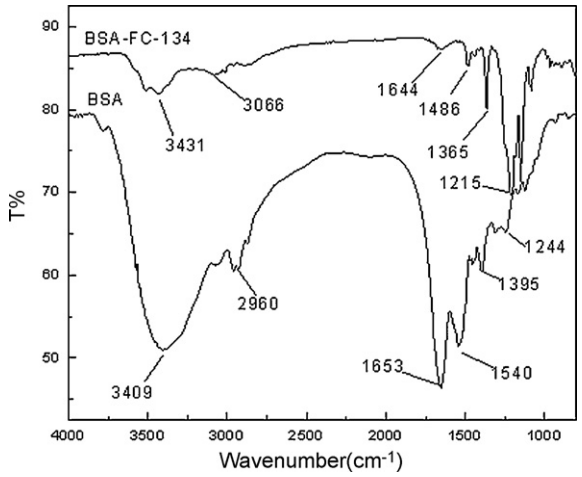

Fig. 4. FT-IR spectra of BSA and FC-134–BSA systems.

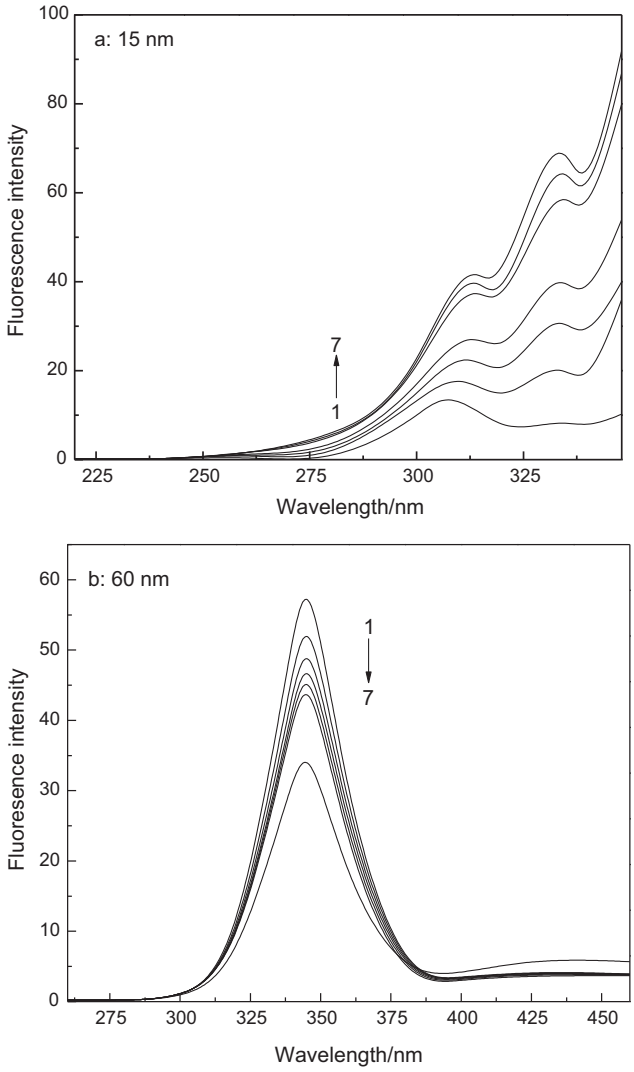

Fig. 5. Synchronous fluorescence spectra of BSA in the presence of FC-134. (a) $\Delta\lambda$ = 15 nm, 1 → 7, $C_{BSA} = 6 \times 10^{-7}$ mol L^{-1}; $C_{FC\text{-}134}$ ($\times 10^{-4}$ mol L^{-1}): 0, 0.451, 0.773, 1.08, 1.36, 1.63 and 2.13; (b) $\Delta\lambda$ = 60 nm, 1 → 7, $C_{BSA} = 6 \times 10^{-7}$ mol L^{-1}; $C_{FC\text{-}134}$ ($\times 10^{-5}$ mol L^{-1}): 0, 0.361, 0.719, 1.07, 1.43, 1.78 and 27.1.

shift (Fig. 5a, from 306 nm to 314 nm) while that of tryptophan residues decreased significantly (fluorescence intensity from 63.7 to 37.2) with no notable shift (Fig. 5b), indicating tyrosine residues are placed in a less hydrophobic environment with increase polarity while the environment around tryptophan residues has not been affected [20]. Thus, the binding of FC-134 with BSA has changed the conformation of BSA.

2.4. Fluorescence quenching spectra

As is known to all, the intrinsic fluorescence of BSA is mainly contributed by tryptophan residues. When excited at 280 nm, the maximum emission band of BSA locates at 350 nm. Fig. 6 (1 → 8) reveals that successive addition of FC-134 results in a concentration dependent quenching of fluorescence intensity of BSA with a significant 8 nm blue shift of the emission maximum (350.2 nm → 342 nm). As fluorescence quenching, the decrease of fluorescence quantum, is induced by a variety of molecular interactions with quencher molecule [20], the quenching of BSA fluorescence indicates that the formation of FC-134–BSA complex occurs, which in turn affects the microenvironment around

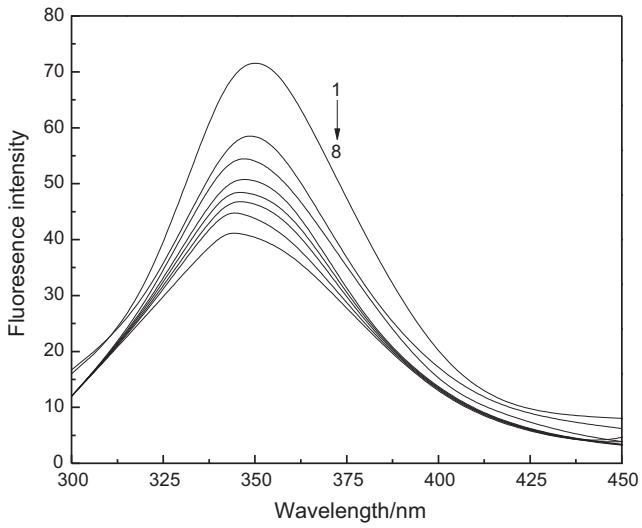

Fig. 6. Effect of FC-134 on fluorescence emission spectra of BSA. 1 → 8: $C_{BSA} = 6 \times 10^{-7}$ mol L^{-1}; $C_{FC\text{-}134}$ ($\times 10^{-5}$ mol L^{-1}): 0, 0.361, 0.719, 1.07, 1.43, 1.78, 6.79 and 27.1.

tryptophan and changes the conformation of BSA. From FT-IR, SF, UV–vis and fluorescence spectra, we may conclude now that the interaction of FC-134 with BSA occurs with FC-134–BSA complex formation and the binding has affected the conformation of BSA. Apart from that, we may apply the fluorescence quenching technique to discuss the inherent binding information in following study.

2.5. Fluorescence quenching mechanism

Generally, fluorescence quenching mechanism is classified as dynamic quenching or static quenching, which can be distinguished by their different dependence on temperature: the quenching constants decrease with increasing temperature for the static quenching, but the reversed effect is observed for the dynamic quenching [1]. In order to confirm the quenching mechanism, the procedure is assumed to be dynamic quenching and the quenching equation is presented by the well-known Stern–Volmer equation [1,3]:

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

where F and F_0 are the fluorescence intensity of BSA at 350 nm with and without quencher (FC-134), respectively. K_q, K_{sv}, τ_0 and [Q] are the quenching rate constant of the bimolecular, the Stern–Volmer dynamic quenching constant, the average lifetime of molecule without quencher and the concentration of the quencher, respectively. Eq. (1) was applied to determine K_{sv} by linear regression of a plot of ($F_0/F - 1$) against [Q]. Obviously

$$K_q = \frac{K_{sv}}{\tau_0} \quad (2)$$

Taking average lifetime of molecule fluorescence (τ_0) as around 10^{-8} s [1,3], the quenching rate constant (K_q, L mol^{-1} s^{-1}) could be obtained according to Eq. (2).

Fig. 7 displayed the Stern–Volmer plots for the quenching of BSA by FC-134 at three different temperatures (293 K, 303 K and 313 K). The K_{sv}, K_q and R^2 at different temperatures were listed in Table 1. Results showed that the Stern–Volmer plots were linear and the slopes (K_{sv}) enhanced with increasing temperature, which indicated that the predominant quenching mechanism was dynamic quenching.

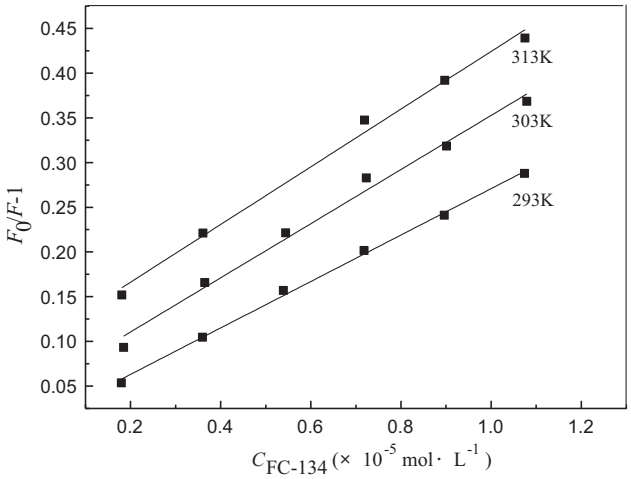

Fig. 7. Stern–Volmer plots for the quenching of BSA by FC-134 at three different temperatures. $C_{BSA} = 6.0 \times 10^{-7}$ mol L^{-1}.

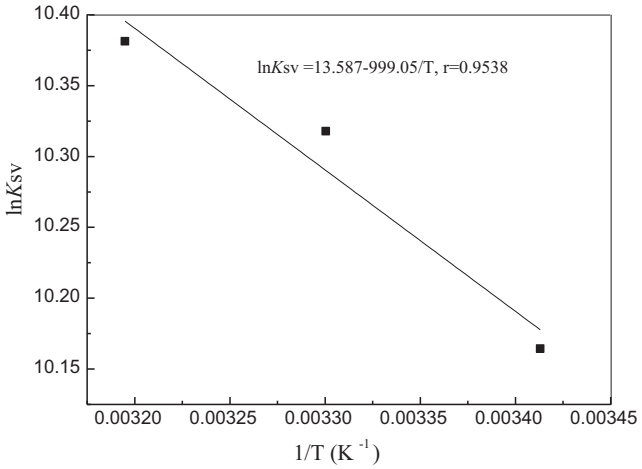

Fig. 8. The Van't Hoff plot of ln K_{sv} vs. $1/T$.

However, the static quenching effect of complex formation could not be completely precluded in the present study. On the one hand, spectroscopic studies above have confirmed the formation of FC-134–BSA complex. On the other hand, the maximum scatter collision quenching constant (K_q) of various quenchers with the biopolymer is 2×10^{10} L mol^{-1} s^{-1} [1] while that of BSA quenching procedure initiated by FC-134 is greater than this value. Therefore, in conclusion, the fluorescence quenching mechanism of BSA by FC-134 is a combination of dynamic quenching with ground complex formation.

2.6. Thermodynamic parameters and driven binding forces

The acting forces between quencher and biomolecules are mainly composed of hydrogen bond, Van der Waals forces, electrostatic forces, and the hydrophobic interaction [1]. The thermodynamic parameters, enthalpy change (ΔH) and entropy change (ΔS) were calculated to confirm binding modes. If ΔH is regarded as a constant over the temperature range investigated, the value of ΔH and that of ΔS can be determined from the following equation [21]:

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

where K is analogous to the Stern–Volmer quenching constants K_{sv} at corresponding temperature (293 K, 303 K and 313 K); R is the gas constant. ΔH and ΔS were obtained from the slope ($-\Delta H/R$) and intercept ($\Delta S/R$) of the fitted curve of ln K_{sv} against $1/T$ (shown in Fig. 8), respectively. The free energy change (ΔG) at three temperatures are estimated from the following relationship:

$$\Delta G = \Delta H - T\Delta S \tag{4}$$

The values of ΔH, ΔS and ΔG at 293 K, 303 K and 313 K are listed in Table 1. According to the theory proposed by Ross and Subramanian [22], from the positive values of ΔH and ΔS observed in the present study, it can be concluded that the acting driven force is mainly hydrophobic force. Furthermore, the negative ΔG values and the positive ΔS values reveal that the binding interaction of BSA with FC-134 is spontaneous and entropy-driven.

FC-134 is a cationic surfactant, the pH value is less than 4.7. The isoelectric point of BSA is 4.7, so in the experimental conditions, BSA is with positive charge. Generally, cationic surfactants cannot interact with positively charged BSA because of electrostatic repulsion [23]. However, fluorine atoms enhance the hydrophobicity of surfactant proved by our previous research [24], so that there is strong hydrophobic interaction between FC-134 and BSA.

2.7. Binding constants and number of binding sites

The quenching interaction can be presented by Eq. (5) [25]:

$$\log\left[\frac{(F_0 - F)}{F}\right] = \log K_A + n\log[Q] \tag{5}$$

where K_A and n are the apparent binding constant to a site and the number of binding sites, respectively. K_A and n at three different temperatures (293 K, 303 K and 313 K) can be calculated from the intercept ($\log K_A$) and the slope of double logarithm regression curves (Fig. 9) of $\log[(F_0 - F)/F]$ versus log[Q] based on Eq. (5). The results are summarized in Table 2. We can find K_A decreases with rising temperatures, but n is approximately equal to 1, which maybe indicates that FC-134 and BSA form the mol ratio 1:1 complex and this complex will be partly decomposed with rising temperature.

2.8. Binding site with the aid of site markers—warfarin and ibuprofen

The main binding sites in BSA are referred to as site I and site II, locating in the hydrophobic cavities of sub-domains IIA and IIIA, which are marked by typical ligands warfarin and ibuprofen, respectively [25]. In order to compare the effect of warfarin and ibuprofen on the binding of FC-134 to BSA, the fluorescence quenching data of BSA, BSA-warfarin and BSA-ibuprofen systems by FC-134 are all analyzed by the Stern–Volmer equation. The K_{sv}

Table 1
The quenching constants and thermodynamic parameters of BSA–FC-134 system at different temperatures.

T (K)	K_{sv} (L mol^{-1})	K_q (L mol^{-1} s^{-1})	R^2	ΔG (kJ mol^{-1})	ΔH (kJ mol^{-1})	ΔS (J mol^{-1} K^{-1})
293	2.596×10^4	2.596×10^{12}	0.9978	−24.791	8.306	112.96
303	3.027×10^4	3.027×10^{12}	0.9901	−25.921		
313	3.225×10^4	3.225×10^{12}	0.9939	−27.051		

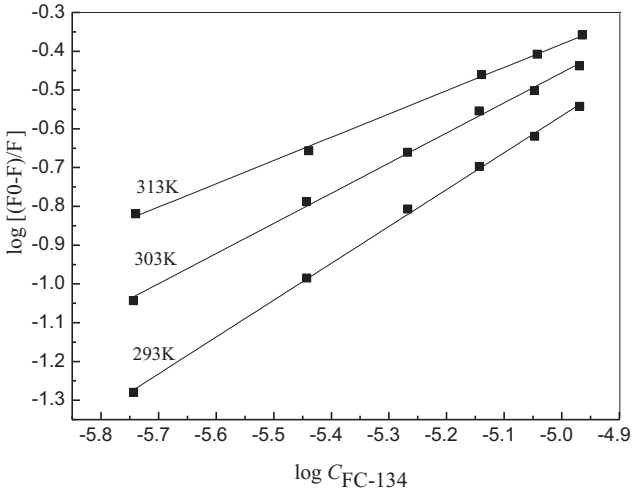

Fig. 9. Plots of $\log[(F_0 - F)/F]$ vs. $\log[Q]$ for BSA–FC-134 system at 3 different temperatures. $C_{BSA} = 6.0 \times 10^{-7}$ mol L^{-1}.

values of BSA, BSA-ibuprofen and BSA-warfarin systems are calculated from the slopes of three plots (F_0/F against [Q], shown in Fig. 10) to be 2.15×10^5 L mol^{-1}, 0.718×10^5 L mol^{-1} and 0.004×10^5 L mol^{-1}.

Obviously, K_{sv} value of BSA-ibuprofen system is almost 33.4% of pure BSA while that of BSA-warfarin system is just 0.186% of free BSA. There is a significant competition between FC-134 and warfarin, while its competition with ibuprofen is relatively low. When we add FC-134 to BSA-warfarin system, the fluorescence intensity has nearly no change and the Stern–Volmer plot is a line. If we assume that site I possess a hydrophobic cavity large enough to accommodate FC-134 or warfarin and the added FC-134 will bind to the same place of BSA as warfarin [25]. So, if warfarin takes that place at first, the fluorescence quenching rate of BSA by FC-134 will decrease significantly (very low) because there is no place to accommodate FC-134. This assumption clearly explains the experimental results in Fig. 10. So the above analysis demonstrates that the binding of FC-134 to BSA mainly locates within site I (sub-domain IIA).

As we all know, BSA has two tryptophans, Trp-135 and Trp-214, located in sub-domains IB and IIA, respectively [26]. Thus, this

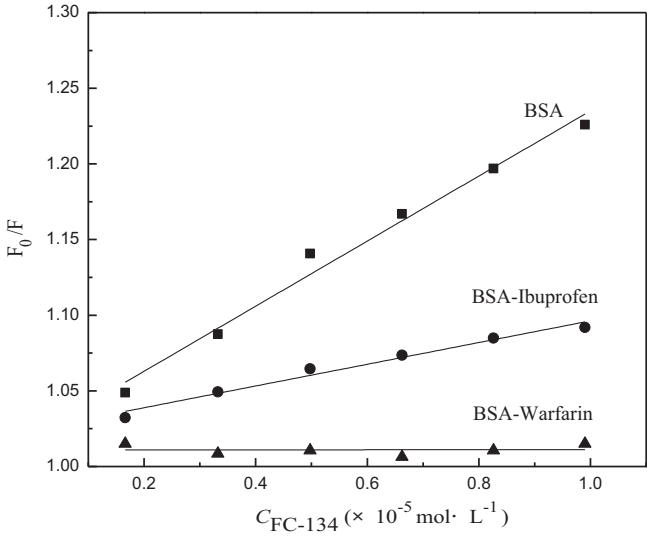

Fig. 10. Competition of FC-134 with warfarin and ibuprofen in quenching of BSA fluorescence intensity. $C_{BSA\text{-}warfarin} = C_{BSA\text{-}ibuprofen} = C_{BSA} = 1 \times 10^{-6}$ mol L^{-1}.

Table 2
The binding constants K_A and binding sites n for BSA–FC-134 system.

T (K)	$\log K_A$	K_A (L mol^{-1})	n	R^2
293	4.1861	1.5349×10^4	0.9505	0.9990
303	3.4332	2.711×10^3	0.7774	0.9978
313	2.6228	4.19×10^2	0.6002	0.9987

work suggests that at low concentration and excited at 280 nm, FC-134 primarily binds to Trp-214 of BSA in site I (sub-domain IIA), which quenches the fluorescence intensity of BSA at 350 nm.

3. Conclusions

Spectroscopic studies, including UV–vis, FT-IR, fluorescence and synchronous fluorescence spectra, indicate that the interaction of FC-134 with BSA occurs with FC-134–BSA complex formation and the binding has affected the conformation of BSA. The inherent binding information is presented by applying the fluorescence quenching technique and site competition study. According to the results analyzed from Stern–Volmer equation, perfluorinated surfactant trimethyl-1-propanaminium iodide (FC-134) can quench the fluorescence intensity of BSA at 350 nm via a dynamic quenching mechanism with complex formation. By calculating the thermodynamic parameters, we may conclude that hydrophobic force is the main interaction driven force and the interaction is spontaneous and entropy-driven. There is one binding site in BSA for FC-134. With the aid of site markers—warfarin and ibuprofen, we first report that FC-134 primarily binds to tryptophan residue Trp-214 of BSA within site I (sub-domain IIA).

4. Experimental details

4.1. Reagents and chemicals

FC-134 was commercially available from Hubei Hengxin Chemical Co., Ltd. (Xiaogan, China) and the stock solution was 1.0×10^{-3} mol L^{-1}. BSA (Fraction V, purity > 99%) was purchased from Wuhan Tianyuan Biotechnology LTD. Co. (Wuhan, China) and used without further purification. 1.0×10^{-5} mol L^{-1} stock solution of BSA was kept at 0–4 °C and the working solution was 6×10^{-7} mol L^{-1}. Warfarin and ibuprofen were bought from Wuhan Kaibo Laboratory Instruments Co. Ltd. (Wuhan, China) and the stock solution were both 2.0×10^{-4} mol L^{-1}. All reagents were of analytical grade and double distilled water was used throughout the experiment.

4.2. Apparatus

Absorption spectra were measured on a UV2300 UV–vis spectrophotometer (Techcomp Bio-Equipment, Ltd., Shanghai, China). FT-IR spectra were taken on a Spectrum One Fourier Transform Infra-Red Spectrometer (P-E Co., America). Fluorescence and synchronous fluorescence spectra were performed on a Shimadzu RF-540 spectrofluorophotometer (Shimadzu, Kyoto, Japan) with a 1 cm × 1 cm cross-section quartz cell. A WH-3 vortex mixer (Huxi Instrumental Co., Shanghai, China) was used to blend the solution. A 5 μL micro-injector was used.

4.3. Standard procedure

FC-134 was successively added to 3 mL BSA working solution, manually by using micro-injector. UV–vis absorption spectra and fluorescence quenching spectra (Ex = 280 nm) of BSA with increasing amounts of FC-134 were then measured. Synchronous fluorescence spectra were also recorded when $\Delta\lambda$ was set at 15 or

60 nm, respectively. The samples of BSA and BSA–FC-134 complex for FT-IR analysis were prepared as thin films by volatilizing the appropriate concentrated solutions on glass slides.

Competitive experiments between FC-134 and two site markers (warfarin and ibuprofen) were performed by fluorescence quenching method. The concentrations of BSA, BSA-warfarin and BSA-ibuprofen solutions were all stabilized at 1.0×10^{-6} mol L^{-1}. FC-134 was then gradually added to the three solutions. Fluorescence quenching data for three systems were recorded (*Ex* = 280 nm).

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