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Original article

Study on the binding interaction between carnitine optical isomer and bovine serum albumin

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Abstract

The reaction between carnitine and bovine serum albumin (BSA) in aqueous solution has been studied by fluorescence spectroscopy and absorbance spectra. The binding interaction between optical isomer, D-carnitine and L-carnitine, with BSA has been compared. Based on the site-binding model and florescence quenching, practical formulas for small molecular ligand binding to bio-macromolecule have been used, and the binding parameters were measured. The binding distance, the energy transfer efficiency between carnitine and BSA was also obtained by virtue of the Förster theory of non-radiative energy transfer. The effect of carnitine on the BSA conformation has been analyzed by using synchronous fluorescence spectroscopy. The influence of Fe^{3+} on the interactions between carnitine optical isomer and bovine serum albumin were also explored in this work. As a conclusion, molecular identification of BSA to carnitine isomer has been suggested preliminary.

Keywords: Carnitine; Bovine serum albumin; Fluorescence spectroscopy; Ultraviolet spectroscopy

1. Introduction

Carnitine (Fig. 1), which was first isolated from meat as a compound, is a vitamin-like substance, and also called vitamin B_T. Carnitine mediates the transport of medium/long-chain fatty acids across mitochondrial membranes, and facilitates their oxidation with subsequent energy production; in addition, it also facilitates the transport of intermediate toxic compounds out of the mitochondria to prevent their accumulation. Carnitine is studied extensively in part because of the important role it plays in fatty acid oxidation and energy production, and it's a well-tolerated and generally safe therapeutic agent [1–4]. Carnitine occurs in two forms, known as D and L; they are mirror images (isomers) of each other with L-carnitine as the biological active form [5–7]. The D-isomer and DL raceme carnitine, which is not biologically active, can compete with the L-isomer so as to potentially increase the risk of L-carnitine deficiency. Therefore, the range of applications of L-carnitine in pharmaceuticals, food and feed industry are widening and the demand for L-carnitine is increasing. Hence carnitine is becoming a hot issue and more researches are undertaken on it than before [8–10].

Both food-derived and liver-synthesized carnitine are transported around the body by the blood stream. Most organic compounds (including carnitine) in the blood stream are mainly carried by transported proteins while only a minor fraction is dissolved in the serum. Serum albumin is the most abundant and most versatile of those transported proteins [11,12]. The exogenous substances and endogenous compounds (ligands) are bound to serum albumin with a high affinity in the processing of transportation, and this interaction results in a stable protein–ligand complex formed. However, the micrometal ions, which are stored in blood plasma, may affect the binding reaction of protein–ligand molecule complex. So it is necessary to investigate the interaction of protein–ligand both in the presence and absence of metal ions. The binding data of serum albumin differ from species to species. Animal experiments are indispensable in providing basic information on the pharmacological actions, bio-transformation, bio-distribution, etc. of a ligand. The BSA is well suited

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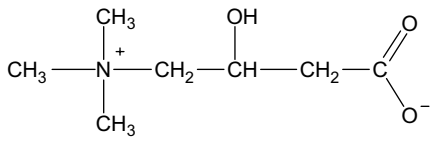


Fig. 1. Chemical structure of carnitine.

to these initial studies since it has been extensively characterized. The study on binding phenomena will also help to explain the relationship between structures and functions of proteins [13–15]. To our knowledge, no attempts have been made on the binding interaction between isomer carnitine and transportation protein.

Fluorescence spectroscopy is an appropriate method to investigate the interaction between small molecular ligands and bio-macromolecules. According to the measurement and analysis of the emission peak, the transfer efficiency of energy, the lifetime, fluorescence polarization etc., a vast amount of information about the structural fluctuations and the microenvironment surrounding the fluorophore in the macromolecules can be given [16,17]. In this paper, the reaction with isomer carnitine binding to BSA was investigated both in the presence and absence of metal ions Fe^{3+}, and the binding parameters and transfer efficiency of energy were measured. Another main purpose of this work was to check the isomer carnitine effect on the conformational changes of BSA. After the characteristics of binding of BSA with different isomer of carnitine were checked, the basic interpretation of biological activity was provided for different isomers of carnitine in the process of transportation.

2. Principle of binding equations for ligand-binding studies

2.1. Binding equation and binding parameters

For experiments carried out at large molar protein/ligand ratios, it was assumed that only strong sites were active in the binding ligand. For simplicity, these strong binding sites were also assumed to be identical and act independently. If these assumptions are valid, the site-binding model could be constructed as follows [18]:

$$\nu = nK[D]/(1 + K[D]), \tag{1}$$

where ν is the average number of ligand molecules bound per protein molecule, n is the number of (strong) binding sites, K is the intrinsic (microscopic) binding (association) constant, and $[D]$ is the concentration of free (unbound) ligand. Eq. (1) can be written in the following form which has been mentioned in some literatures [2,16,19,20] as Scatchard equation:

$$\nu/[D] = K(n - \nu). \tag{2}$$

As to the Scatchard equation, the assay of $[D]$ is a problem in many cases, so a practical formula for small molecular ligand interaction with bio-macromolecule has been derived based on the Scatchard's site-binding model and florescence quenching.

In the case of the fluorescence caused only by protein at the selected wavelength, the relationship between the concentration of protein and the fluorescence intensity can be described as

$$F_0/F = [P_t]/[P]. \tag{3}$$

According to the definition of ν, another equation is also known:

$$\nu = ([D_t] - [D])/[P_t] = n([P_t] - [P])/[P_t] = n(F_0 - F)/F_0 = n\Delta F/F_0, \tag{4}$$

where $[P_t]$ is the total protein concentration, $[D_t]$ is the final ligand concentration, F_0 and F are, respectively, the fluorescence intensity in the absence of a quencher and in its presence at $[D]$ concentration, $\Delta F = F_0 - F$.

The following equation is obtained by combining Eqs. (2) and (4) [14,15,21]:

$$F_0/F = K[D_t]F_0/(F_0 - F) - Kn[P_t]. \tag{5}$$

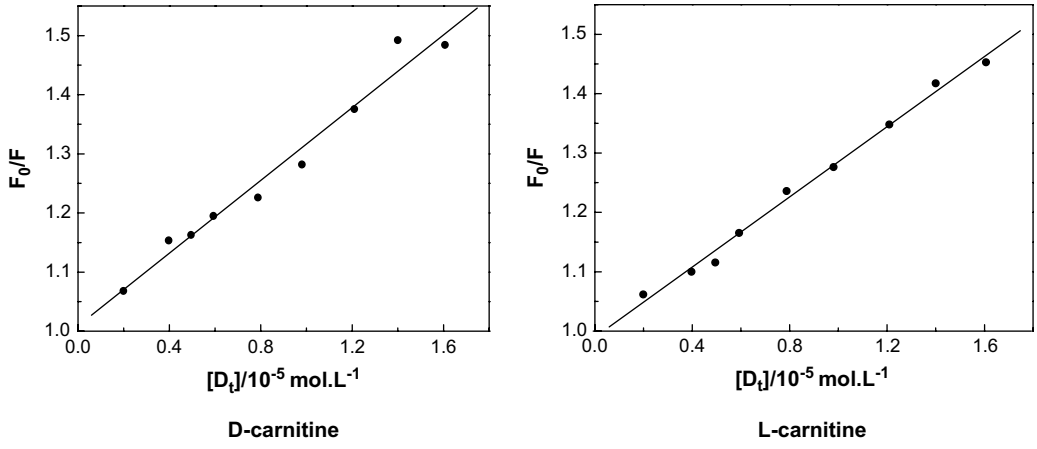


Fig. 2. The Stern–Volmer curve of carnitine binding with BSA. $c_{(BSA)} = 1.0 \times 10^{-5}$ mol L^{-1}, $\lambda_{ex} = 282$ nm.

Table 1
The Stern–Volmer parameters of carnitine binding with BSA

Solution system	Algorithm	K_{sv}	K_q	Correlation coefficient (r^2)
(D) Carnitine–BSA	Least-squares	3.08×10^4 L mol^{-1}	3.08×10^{12} L mol^{-1}	0.9928
(L) Carnitine–BSA	Least-squares	2.96×10^4 L mol^{-1}	2.96×10^{12} L mol^{-1}	0.9982

If the interaction between small molecular ligand and biomacromolecule acts in accordance with the theory of site-binding, and if the concentration of protein is fixed and a range of the dose for ligand is prepared, the binding constant K and the binding site n can be estimated at the same time by using a Least-squares algorithm for data fitting according to Eq. (5). The fitting model (5) does not consider the term [D].

2.2. Energy transfer between ligand and BSA

According to Förster non-radiative energy transfer theory [22,23], the energy transfer efficiency is not only related to the distance between the acceptor and donor (r), but also to the critical energy transfer distance (R_0), i.e.,

$$E = R_0^6/\left(R_0^6 + r^6\right), \tag{6}$$

where R_0 is the critical distance in the case of the transfer efficiency, 50%.

$$R_0^6 = 8.8 \times 10^{-25} K^2 N^{-4} \phi J, \tag{7}$$

where K^2 is the spatial orientation factor of the dipole, N is the refractive index of the medium, Φ is the fluorescence quantum yield of the donor, J is the overlap integral of the fluorescence emission spectrum of the donor and the absorption spectrum of the acceptor. Therefore,

$$J = \int_0^\infty F(\lambda)\varepsilon(\lambda)\lambda^4 \mathrm{d}\lambda / \int_0^\infty F(\lambda)\mathrm{d}\lambda, \tag{8}$$

where $F(\lambda)$ is the fluorescence intensity of the donor and $\varepsilon(\lambda)$ is the molar absorptivity of the acceptor when the wavelength is λ. Thus, the energy transfer efficiency is

$$E = 1 - F/F_0. \tag{9}$$

3. Results and discussion

3.1. The quenching mechanism and quenching constants

The fluorescence quenching mechanism is usually divided into static quenching and dynamic quenching. The dynamic quenching mechanism can be described by Stern–Volmer equation. The present work is aimed at investigating whether carnitine interacts with BSA (a protein containing two tryptophan residues, Trp-213 and Trp-134) and identifying the quenching mechanism. Increasing carnitine concentrations caused a reducing fluorescence of the tryptophan residues, which is interpreted as the compounds come into being from carnitine and BSA. In order to confirm the quenching mechanism, the procedure that the fluorescence quenching data was disposed by us was assumed to be a dynamic quenching. The Stern–Volmer equation [24] is

$$F_0/F = 1 + K_q \tau_0 [D] = 1 + K_{sv}[D], \tag{10}$$

where K_q, K_{sv}, τ_0 and [D] are the quenching rate constant of the bimolecule, the dynamic quenching constant, the average lifetime of molecule without quencher and the concentration of quencher, respectively. The dynamic quenching parameters of carnitine and BSA, K_{sv} could be obtained from the experimental data by using Stern–Volmer equation. For the fluorescence lifetime of the biopolymer is 10^{-8} s [25,26], the quenching constant (K_q: L mol^{-1} s^{-1}) can be obtained from $K_q = K_{sv}/\tau_0$. Fig. 2 shows the fitting curve and Table 1 shows the results.

A favorable relationship is shown by Stern–Volmer curve on certain concentration condition. Because the maximum

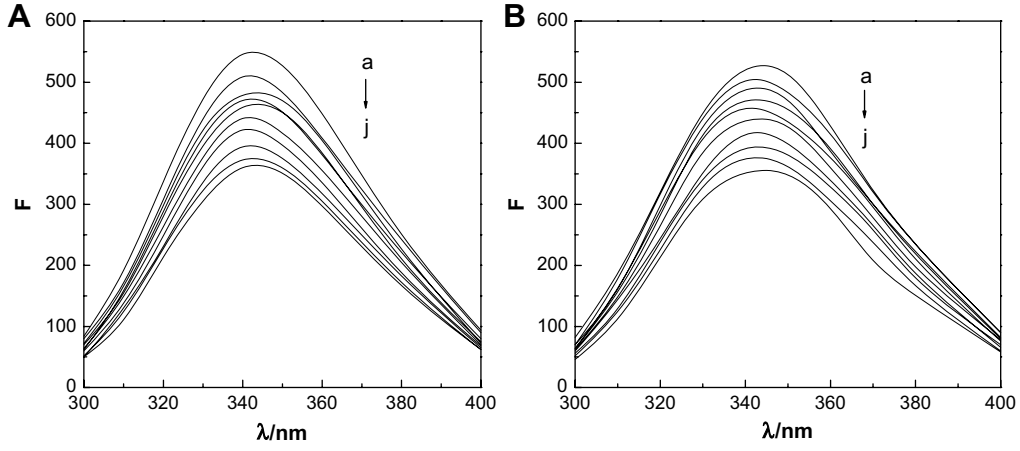


Fig. 3. Effect of carnitine on quenching of BSA fluorescence. (A) D-carnitine. (B) L-carnitine. $c_{(BSA)} = 1.0 \times 10^{-5}$ mol L^{-1}, $c_{[D,\ L\text{-carnitine}]} = 5 \times 10^{-6}$ mol L^{-1}, a to j: 0, 0.4, 0.8, 1.0, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2.

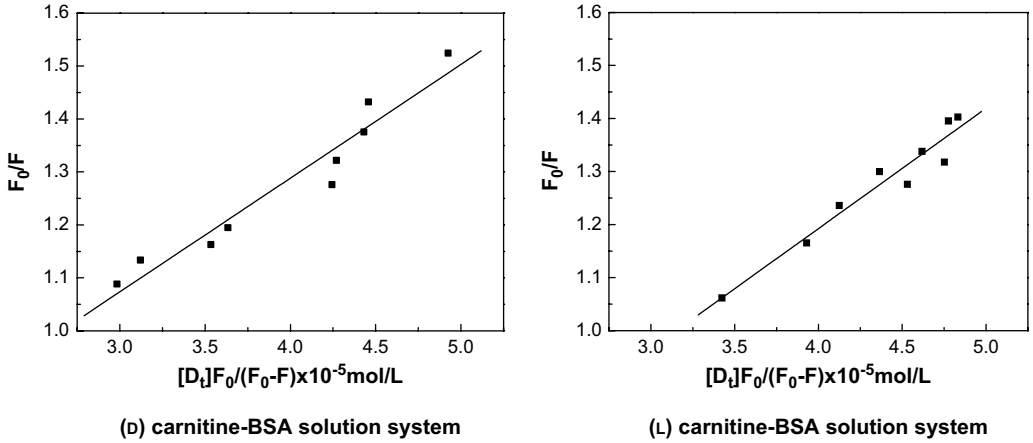


Fig. 4. The fitting curve F_0/F *vs.* $[D_t]F_0/(F_0 - F)$ of carnitine–protein solution system.

scatter collision quenching constant of various quenchers with the biopolymer is 2.0×10^{10} L mol^{-1} s^{-1}[26,27] and the rate constant of the protein quenching procedure initiated by carnitine is much greater than the K_q value of the scatter procedure, quenching is not initiated by dynamic quenching, but by compound formation.

It is also observed from Fig. 2 and Table 1 that the quenching mechanism and quenching constants of optical isomers, D-carnitine, L-carnitine to BSA do not have significant difference due to experimental conditions.

3.2. The fluorescence spectra, binding constant and binding sites

The fluorescence quenching spectra of BSA in Tris–HCl buffer with increasing D-carnitine, L-carnitine concentration and fixed BSA concentration (1.00×10^{-5} mol L^{-1}) are shown in Fig. 3. It is obvious that the concentration of D-carnitine and L-carnitine gradually increases with the titration, and the fluorescence intensity of BSA gradually decreases, which shows that there is a binding interaction of carnitine to BSA and the energy transfer occurs.

From Fig. 3, we can also find that the maximum emission peak of fluorescence almost occurs in the same position under a series of titration in the range of 300–500 nm when the excitation wavelength is 282 nm. At the same time, none of enhanced fluorescence emission peak of carnitine emerges, which means it is a non-radiative energy transfer.

After the measurements of fluorescence quenching on BSA at λ_{max} were fetched, the Least-squares algorithm for data fitting was introduced by Eq. (5). Fig. 4 shows the fitting curve and Table 2 shows the fitting results.

A favorable relationship between the experimental points and the calculated values shows that the interaction of carnitine bound to BSA agrees with the model of site-binding. It also indicates that there is a stronger binding force between carnitine and BSA. From Table 2, we can find that the binding parameters of isomer, D-carnitine, L-carnitine to BSA are almost of the same value. This may account for the existing no binding difference in the process of transport.

3.3. Binding distance between the carnitine and the amino acid residues of BSA

The overlap of the carnitine absorption spectrum and the BSA fluorescence emission spectrum is shown in Fig. 5.

The overlap integral, J can be evaluated by integrating the spectra in Fig. 5. In this paper, J is given by the following Eq. (11) [26,28] when $\lambda = 250$–500 nm.

$$J = \sum F(\lambda)\varepsilon(\lambda)\lambda^4 \Delta\lambda / \sum F(\lambda)\Delta\lambda . \quad (11)$$

In the experimental conditions, the distance $R_{0,}$ corresponding to 50% energy transfer from BSA to (D, L) carnitine, can be calculated by Eq. (7) using $K^2 = 2/3$, $N = 1.336$, $\Phi = 0.118$ [28–31]. Moreover, the energy transfer efficiency E, the binding distance between (D, L) carnitine and amino acid residues of BSA, can be gained by Eqs. (9) and (6) separately. Table 3 shows the results.

3.4. The effect of carnitine on BSA conformation

The conformational changes of BSA were evaluated by the measurement of synchronous fluorescence intensity of protein

Table 2
The binding parameters for the system of carnitine–BSA

Solution system	Algorithm	Binding constant K	Binding site n	Correlation coefficient (r^2)
(D) Carnitine–BSA	Least-squares	2.15×10^4 L mol^{-1}	2.00	0.9845
(L) Carnitine–BSA	Least-squares	2.27×10^4 L mol^{-1}	1.62	0.9851

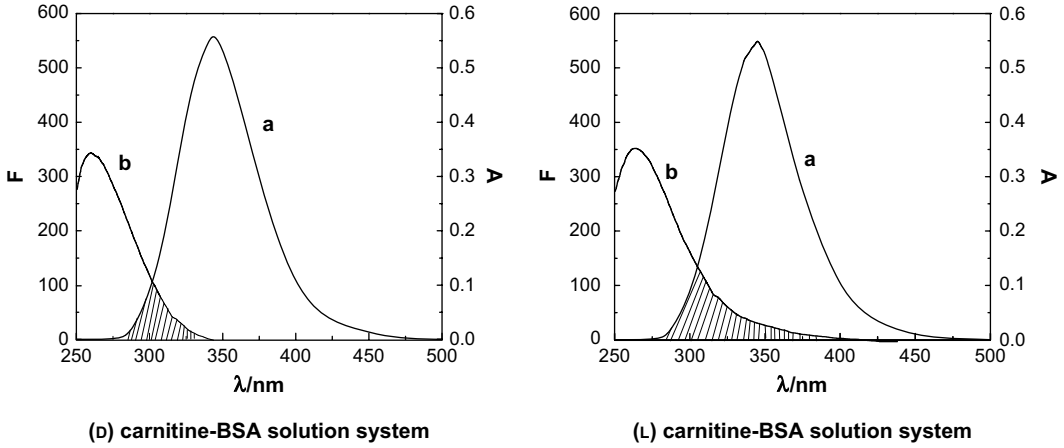


Fig. 5. Overlap of the fluorescence emission spectrum of BSA (a) with the absorption spectrum of carnitine (b).

amino acid residues before and after the addition of carnitine. In this work, synchronous fluorescence spectroscopy was used to study the synchronous fluorescence characteristics of BSA at different scanning intervals ($\Delta\lambda = \lambda_{emission} - \lambda_{excitation}$). When $\Delta\lambda = 15$ nm, the spectrum characteristic of protein tyrosine residues is observed, and when $\Delta\lambda = 60$ nm, the spectrum characteristic of protein tryptophan residues is observed. A useful method suggested by the authors [32,33] to study the environment of amino acid residues is to measure the possible shift in the wavelength emission maximum (λ_{max}). The shift in the position of the emission maximum corresponds to the changes in the polarity around the chromophore molecule. Thus, the conformation changes of BSA can be evaluated by the measurement.

With the unchanged concentration of the protein and the increasing concentration of carnitine, the synchronous spectroscopy was scanned at $\Delta\lambda = 15$ nm, $\Delta\lambda = 60$ nm. Fig. 6 shows L-carnitine synchronous spectroscopy (D-carnitine shows a similar figure). The obtained synchronous spectroscopy shows that the main contribution to the fluorescence intensity of BSA is tryptophan residues.

Fig. 6 also shows the effect of carnitine on the emission maximum with the process of titration. A slightly stronger blue-shift of tryptophan fluorescence upon the addition of the carnitine was observed, and the emission maximum of tyrosine was kept in the position. This shift indicated that tryptophan residues were placed in a more hydrophobic environment, and less exposed to the solvent. This may be due to the carnitine inserting to BSA molecules, and consequently rearrange the tryptophan microenvironment. It also suggests that the environments of tryptophan residues in pure albumin aqueous solution are relatively polar. Binding of the carnitine changes the environments to be apolar ones. The shift in polarity brought about the conformational changes in the interaction between albumin and the ligand molecule.

3.5. The influence of Fe^{3+} ions on the binding interaction between carnitine and the protein

The structure of carnitine suggested that a stable carnitine–Fe^{3+} ion complex might have been formed in Tris–HCl buffer. However, researches have previously indicated that there is a binding reaction between metal ions and protein, and the presence of metal ions directly affects the binding between the ligand and the protein [34,35]. Further, in order to study the effect of metal ions on the binding between carnitine and BSA, the carnitine–metal ion solution (1:1) were prepared by mixing the solution of Fe^{3+} ions and carnitine in the buffer. The fluorescence quenching spectra of carnitine–ion–BSA solution system was carried out in the same apparatus condition as mentioned above, and Fig. 7 shows the fluorescence quenching spectra of (L) carnitine–Fe^{3+}–BSA solution system ((D) carnitine–Fe^{3+}– BSA solution system shows a similar figure). The phenomena of BSA fluorescence declining regularly were also observed.

In order to investigate the influence of Fe^{3+} ions on the fluorescence intensity of BSA, the fluorescence spectra of protein and Fe^{3+} ions were also made and shown in the upper right of Fig. 7. This experiment illustrates that there was a tiny quenching between BSA and the ions though Fe^{3+} was not an effective quencher for BSA. With the titration of Fe^{3+} ions, and the concentration of ion is in sufficient excess the protein molecules, the quenching effect is gradually noticeable, which implies that a match between carnitine and ions on binding reaction may be true in carnitine–ion–BSA system.

Table 3
The non-radiative energy transferred parameters for the system of carnitine–BSA

Solution system	J ($cm^3\,dm^3\,mol^{-1}$)	R_0 (nm)	E	r (nm)
(D) Carnitine–BSA	1.35×10^{-15}	1.75	$E_1 = 0.2198$, $E_2 = 0.3262$	$r_1 = 2.16$, $r_2 = 1.97$
(L) Carnitine–BSA	1.29×10^{-15}	1.74	$E_1 = 0.2028$, $E_2 = 0.3149$	$r_1 = 2.19$, $r_2 = 1.98$

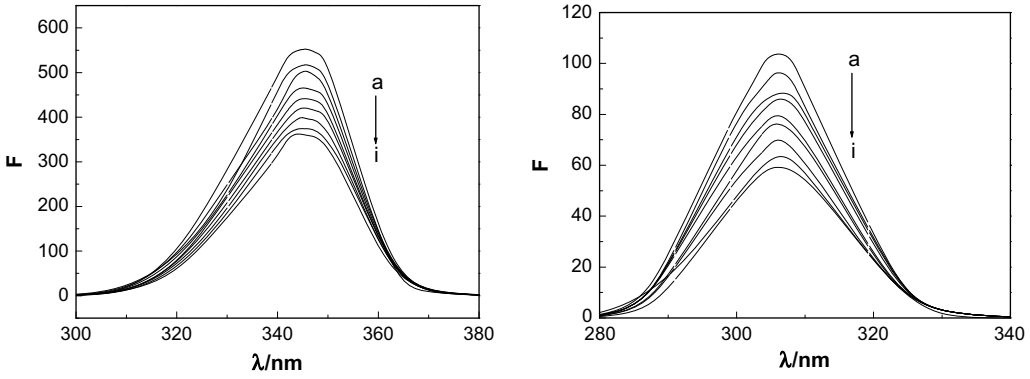


Fig. 6. Effect of the L-carnitine on the synchronous fluorescence spectra of BSA. $c_{(BSA)} = 1.0 \times 10^{-5}$ mol L^{-1}, $c_{[L\text{-carnitine}]} = 10^{-5}$ mol L^{-1}.

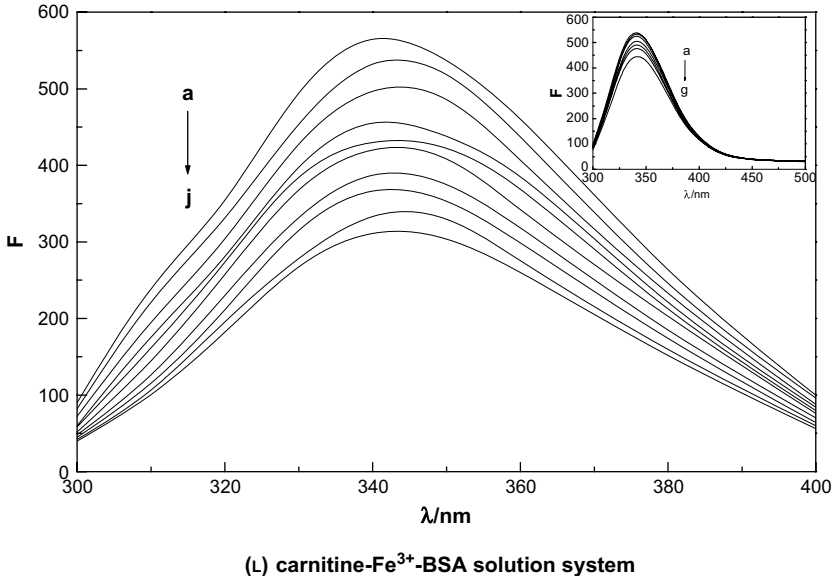


Fig. 7. Effect of carnitine–Fe^{3+} complex on the quenching of BSA fluorescence. $c_{(BSA)} = 1.0 \times 10^{-5}$ mol L^{-1}, $c_{[carnitine-Fe^{3+}]} = 5 \times 10^{-6}$ mol L^{-1}, a to j: 0, 0.8, 1.6, 2.0, 2.4, 3.2, 4.0, 4.8, 5.6, 6.4. Upper right: $c_{(Fe^{3+})}/10^5$ mol L^{-1}, a to g: 0, 0.8, 1.6, 3.2, 4.0, 4.8, 6.4.

In order to confirm the quenching mechanism, the same procedure was taken as carnitine–BSA system, and the fluorescence quenching data disposed by us was assumed to be a dynamic quenching in the three-component system of carnitine–Fe^{3+}–BSA. Fig. 8 shows Stern–Volmer curve. The Stern–Volmer parameters were deduced from Fig. 8 according to Eq. (10), and Table 4 shows the results.

A favorable relationship is shown by Stern–Volmer curve in certain concentration condition. Because the maximum scatter collision quenching constant of various quenchers with the biopolymer is 2.0×10^{10} L mol^{-1} s^{-1}[26,27] and the rate constant of the protein quenching procedure initiated by carnitine–Fe^{3+} is much greater than the K_q value of the scatter procedure, quenching is initiated by compound formation.

The fluorescence quenching data of (D) carnitine–Fe^{3+}–BSA, (L)carnitine–Fe^{3+}–BSA at λ_{max} was measured, respectively, and the Least-squares algorithm for data fitting was assessed by Eq. (5). Fig. 9 shows the fitting curve and Table 5 shows the fitting results.

Table 5 shows that the combination of metal ions and the ligand increased the binding constants of the ligand–protein, which suggested that the ternary complex was formed between

Table 4
The Stern–Volmer parameters of carnitine–Fe^{3+} binding with BSA

Solution system	Algorithm	K_{sv}	K_q	Correlation coefficient (r^2)
(D) Carnitine–Fe^{3+}–BSA	Least-squares	2.41×10^4 L mol^{-1}	2.41×10^{12} L mol^{-1}	0.9995
(L) Carnitine–Fe^{3+}–BSA	Least-squares	2.53×10^4 L mol^{-1}	2.53×10^{12} L mol^{-1}	0.9976

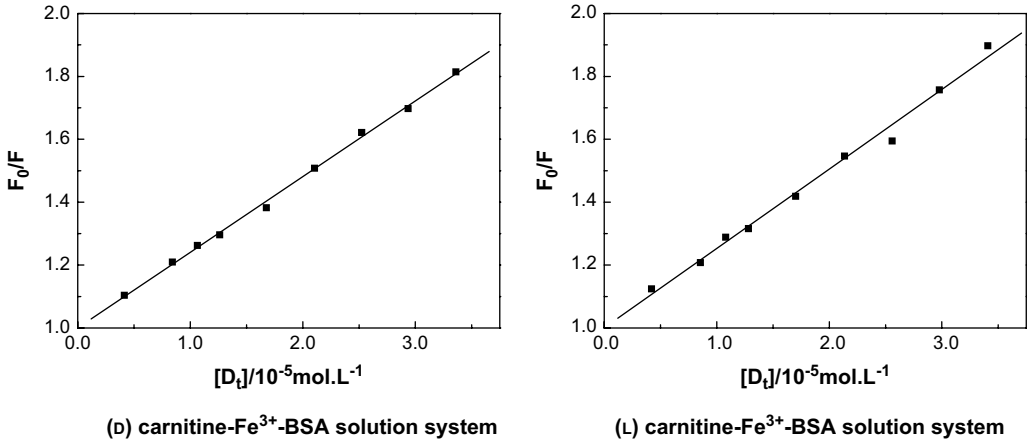


Fig. 8. The Stern–Volmer curve of carnitine–Fe^{3+} binding with BSA.

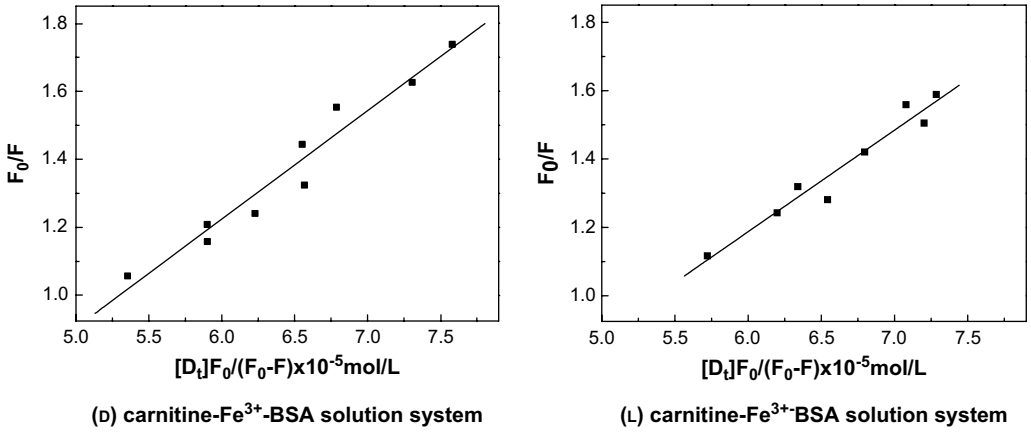


Fig. 9. The fitting curve F_0/F *vs.* $[D_t]F_0/(F_0 - F)$ of carnitine–Fe^{3+}–protein solution system.

BSA and carnitine–protein complex. Though the evidence of ternary complex in this study is not sufficient and the crystal structure has not been obtained till now, the existing of metal ions effect on the binding between carnitine and BSA can be verified through this type of experiment.

The overlap of fluorescence emission spectrum of BSA and the absorption spectrum of carnitine–Fe^{3+} ions (1:1) is similar to BSA–carnitine solution system. Some non-radiative energy transfer parameters were determined by Eqs. (7), (9) and (6) in the presence of Fe^{3+} at 25 °C. The results are shown in Table 6.

3.6. Effect of the carnitine–Fe^{3+} on the BSA conformation

Fig. 10 shows the synchronous spectra of (L) carnitine–Fe^{3+}–BSA system ((D) carnitine–Fe^{3+}–BSA solution shows a similar figure). The same results of conformational changes of BSA can be reached by comparing Fig. 6.

The synchronous spectra of carnitine–ion–BSA system shows that the combination between Fe^{3+} and carnitine has caused a non-remarkable change in the synchronous fluorescence of carnitine–protein. In the process of binding reaction, though metal ions can bind to the amino acid residues of protein, the carnitine plays the primary role in binding interaction to fluorophore functional groups in carnitine–Fe^{3+}–protein solution system.

4. Conclusions

Results of the present experiment demonstrated that the (D, L) carnitines caused the fluorescence quenching of BSA in the presence or absence of metal ion, Fe^{3+}. The mechanism of fluorescence quenching was static quenching, which was induced by the formation of the compound. BSA contains two tryptophan residues per molecule (in contrast to human serum albumin which contains only one such residue). The binding

Table 5
The binding parameters for the system of carnitine–Fe^{3+}–BSA

Solution system	Algorithm	Binding constant K	Binding site n	Correlation coefficient (r^2)
(D) Carnitine–Fe^{3+}–BSA	Least-squares	3.18×10^4 L mol^{-1}	2.15	0.9875
(L) Carnitine–Fe^{3+}–BSA	Least-squares	2.97×10^4 L mol^{-1}	2.00	0.9865

Table 6
The non-radiative energy transferred parameters for the system of carnitine–Fe^{3+}–BSA

Solution system	J ($cm^3\,dm^3\,mol^{-1}$)	R_0 (nm)	E	r (nm)
(D) Carnitine–Fe^{3+}–BSA	1.52×10^{-15}	1.79	$E_1 = 0.2161$, $E_2 = 0.3342$	$r_1 = 2.22$, $r_2 = 2.01$
(L) Carnitine–Fe^{3+}–BSA	0.97×10^{-15}	1.66	$E_1 = 0.1783$, $E_2 = 0.3151$	$r_1 = 2.14$, $r_2 = 1.89$

constant (K) and the number of binding sites (n) were measured by the site-binding model, which indicated that there is a stronger fluorescence quenching to BSA. Additionally, the binding distance and transfer rate of energy of donator–acceptor was determined according to the Förster non-radiative energy transfer theory. The binding distance of (D, L) carnitines to BSA is less than 7 nm ($r < 7$ nm), and the condition of protein fluorescence quenching is reached. The binding distance, r approaches to R_0 (corresponding to 50% energy transfer from protein to ligand), which can be interpreted as the higher relative probability of non-radioactive energy transfer induced to protein fluorescence quenching. Data obtained by the spectrum technology also shows that BSA has no significant function of molecular identification to differentiate D-isomers from L-isomers of carnitine, however, it is in the presence or absence of metal ion, Fe^{3+}. No significant difference in binding reaction between BSA and different isomers could be used to deduce that the difference of physiological activity between D-isomer and L-isomer of carnitine was probably not caused by the difference in binding reaction between transporting protein (BSA) and isomer carnitine during the process of transporting. However, the micrometal ions Fe^{3+}, which is stored in blood plasma, may have no significant effect on the binding between carnitine isomers and BSA. The present study may help to better understand the chemical mechanisms involved in the binding reaction between BSA and different isomers, and it is also looking forward for a further supplement and perfection.

5. Experimental

5.1. Apparatus and reagents

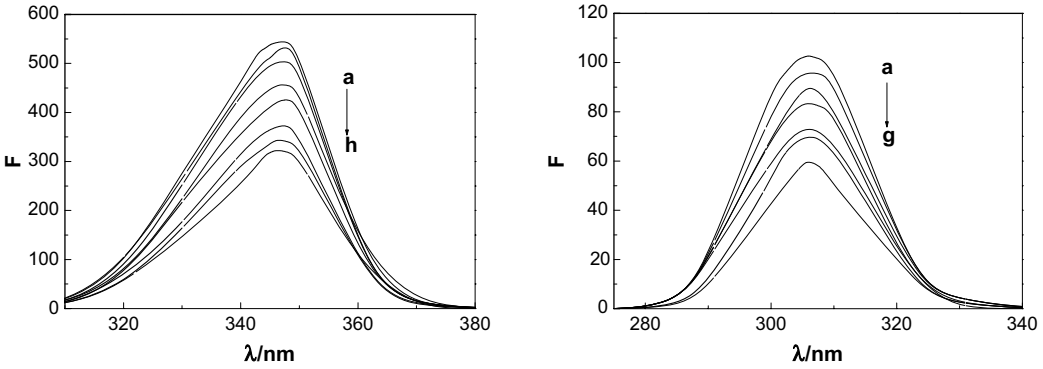
All fluorescence measurements were carried out on a RF-5000 recording spectrofluorimeter (Shimadzu) equipped with a xenon lamp source and 1.0 cm cells. A UV-240 recording spectrophotometer (Shimadzu) equipped with 1.0 cm cuvette was used for scanning the UV spectrum. All pH measurements were made with a pHS-2 digital pH-meter (Shanghai Lei Ci Device works, Shanghai China) with a combinal glass calomel electrode.

The BSA was purchased from Huamei Biotechnology Co. (Essentially fatty acid free, ≥96%, lyophilized powder, Shanghai China). L-Carnitine (≥98.0) and D-carnitine (≥98.0, Sigma) were used. The solutions of metal ions (1.00×10^{-3} mol L^{-1}) and 0.05 mol L^{-1} Tris–HCl buffer of pH = 7.4 (0.1 mol L^{-1} NaCl was used to keep the ionic strength constant) were prepared. All regents were of analytical grade and double distilled water was used throughout.

5.2. Procedures

The BSA and (D, L) carnitine were dissolved in Tris–HCl buffer. The concentrations of BSA and carnitine were 1.00×10^{-5} mol L^{-1} and 0.50×10^{-3} mol L^{-1}, respectively. The carnitine–metal ion solution (in the ratio = 1:1) was prepared by mixing the metal ion solution and the carnitine in the buffer. The mixing samples contained 0.50×10^{-3} mol L^{-1} solutes.

To a 1.0 cm quartz cell, 2.5 mL of BSA solution was added , and a range of carnitine solutions were gradually titrated into the cell using micro-injector. The accumulated volume in μL were 10, 20, 30, 40, 50, 62, 72, 83 (the total accumulated volume is less than 200 μL). In order to study the effect of metal ions, the carnitine–metal ion concentration was varied according to the same scheme. The fluorescence quenching spectra were obtained at excitation and emission wavelengths of 282 and 250–500 nm, respectively. The range of synchronous scanning was λ_{ex}: 250–275 nm, λ_{em}: 310–280 nm, where the difference of wavelengths $\Delta\lambda$ were 60 and 15 nm, respectively.



Δλ=60nm, a to h : 0, 0.8, 1.6, 2.4, 3.2, 4.8, 5.6, 6.4.

Δλ=15nm, a to g : 0, 0.8, 1.6, 2.4, 3.2, 4.0, 6.4.

Fig. 10. Effect of the (L) carnitine–Fe^{3+} on the synchronous fluorescence spectra of BSA $c_{(BSA)} = 1.0 \times 10^{-5}$ mol L^{-1}, $c_{[L\text{-carnitine}]} = 5 \times 10^{-6}$ mol L^{-1}.

For absorption spectra (UV) experiments, the samples of carnitine, carnitine–Fe^{3+}(1:1) were brought to 1.0 cm cuvette *vs*. a blank buffer. Absorbances were read and spectral scanning curve was made.

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