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ORIGINAL PAPER

Determination of Protein by Fluorescence Enhancement of Curcumin in Lanthanum-Curcumin-Sodium Dodecyl Benzene Sulfonate-Protein System

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Abstract We found that the fluorescence intensity of the lanthanum (La^{3+})-curcumin (CU) complex can be highly enhanced by proteins in the presence of sodium dodecyl benzene sulphonate (SDBS). Based on this finding, a new fluorimetric method for the determination of protein was developed. Under optimized conditions, the enhanced intensities of fluorescence are quantitatively in proportion to the concentrations of proteins in the range 0.0080–20.0 $\mu g \cdot mL^{-1}$ for bovine serum albumin (BSA) and 0.00080–20.0 $\mu g \cdot mL^{-1}$ for human serum albumin (HSA) with excitation of 425 nm, and 0.00020–20.0 $\mu g \cdot mL^{-1}$ for bovine serum albumin (BSA) and 0.00080–20.0 $\mu g \cdot mL^{-1}$for human serum albumin (HSA) with excitation of 280 nm, while corresponding qualitative detection limits (S/N≥3) are as low as 5.368, 0.573, 0.049, 0.562 $\mu g \cdot mL^{-1}$, respectively. Study on reaction mechanism reveals that proteins can bind with La^{3+}, CU and SDBS through self-assembling function with electrostatic attraction, hydrogen bonding, hydrophobic interaction and van der Waals forces, etc. The proteins form a supermolecular association with multilayer structure, in which La^{3+}-CU is clamped between BSA and SDBS. The unique high fluorescence enhancement of CU is resulted through synergic effects of favorable hydrophobic microenvironment provided by BSA and SDBS, and efficient intermolecular energy transfer among BSA, SDBS and CU. In energy transfer process, La^{3+} plays a crucial role because it not only shortens the distance between SDBS and CU, but also acts as a "bridge" for transferring the energy from BSA to CU.

Keywords Fluorescence · Spectral analysis · Protein · La^{3+} · Curcumin · Surfactant

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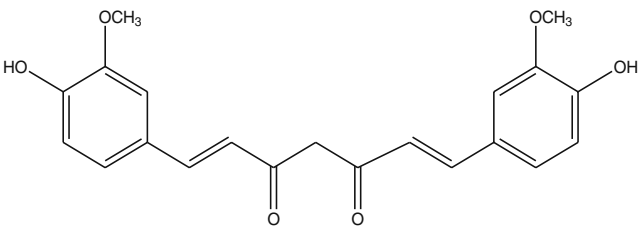
Introduction

The qualitative and quantitative analysis of proteins is becoming more and more important for the investigation of proteins and their interactions with drugs. Many chemical analysis methods have been developed for the concentration determination of proteins. However, the traditional methods for the determination of proteins have some limitations. For example, the Lowry [1] assay, which is based on a colored complex formed with tyrosine or tryptophan, can only be used for protein concentrations greater than 20 $\mu g \cdot mL^{-1}$. The drawback of the Bradford [2] assay, which is based on the binding of coomassie blue to protein, is that it contaminates vessels. The silver staining method [3] is complicated as multiple steps are involved. Furthermore, current methods have a limited sensitivity because they are based on absorption

measurements [4, 5]. Some other advanced methods, such as fluorescence, resonance light scattering (RLS) [6–8] and chemiluminescence [9] have improved sensitivity and selectivity, and thus have been applied for protein determinations. Among these methods, the fluorimetric analyses based on covalent or noncovalent probe have been widely used since they not only possess high sensitivity and selectivity, but also provide valuable information on molecular interactions, as reviewed in literatures [10–12]. The covalent fluorescent probe is highly sensitive, but only suitable for labeling the N-terminus or other functioned groups of proteins, and thus problems arise from inefficient chemistry practices and multiple derivatives. In comparison with covalent fluorescent labeling, noncovalent fluorescent labeling is very simple, fast and inexpensive. Most of the compounds serving as noncovalent fluorescent probes of protein are anionic dyes and their detection limits are in the range of 0.1–0.01 $\mu g \cdot mL^{-1}$, only very limited complexes (like morin–Al^{3+} and Eu^{3+}-chlorotetracycline et al.) have been studied as noncovalent fluorescent probes of protein [13, 14]. In this paper, we present the lanthanum-curcumin- sodium dodecyl benzene sulfonate (La^{3+}-CU-SSBS) complex as a new noncovalent fluorescent probe for protein analysis.

Curcumin [1.7-bis (4-hydroxy-3methoxyphenyl)-1, 6-heptadiene-3, 5-Dione], the main constituent of rhizomes of plant curcumin longa, is a common ingredient used in spices, cosmetics and traditional Chinese medicine. It is a natural antioxidant, which is considered to be a very useful compound in health matters, and has been applied in treatment of cardiovascular and arthritic illnesses. It is also used as anti-inflammatory, antioxidant and anticarcinogenic agents [15–21]. The structure of curcumin is as follows:



In previous studies, we have found that the fluorescence intensity of CU could be enhanced through solubilization in the SDBS-BSA complex by forming a ternary complex of CU-SDBS-BSA [22]. In the present research, our experiments demonstrate that the fluorescence intensity of La^{3+}-CU-SDBS system can be further greatly enhanced by proteins, and the enhanced fluorescence intensity is proportional to the concentration of proteins. Evaluation with real protein samples indicates that this method is able to determine proteins at low concentrations of $ng \cdot mL^{-1}$ level.

Experimental

Chemicals

Protein bovine serum albumin (BSA) was purchased from Shanghai Boao Biochemical Technology Co., and human serum albumin (HSA) was obtained from Sigma. La_2O_3 (99.99%) came from Shanghai Yuelong Chemical Co. SDBS and sodium citrate were Aldrich product. All the chemicals used were of analytical reagent grade and deionized water was used for all experiments.

Stock solutions

Stock solutions of BSA and HSA at 100.0 $\mu g \cdot mL^{-1}$ were prepared by dissolving them in water. Stock solution of La^{3+} at 1.00×10^{-2} $mol \cdot L^{-1}$ was prepared by first dissolving 0.3258 g La_2O_3 in 6 M HCl solution, then evaporating the solution until nearly dried, and finally diluting it to 100 mL with water. CU solution at 1.00×10^{-4} $mol \cdot L^{-1}$ was prepared by dissolving CU in ethanol, and SDBS solution at 1.00×10^{-4} $mol \cdot L^{-1}$ was prepared by dissolving SDBS in deionized water. A series of citric acid-sodium citrate buffer solutions (0.5 $mol \cdot L^{-1}$) was used for the pH adjustment. The above solutions were stored at 4 °C for further experiments.

Measurement

Fluorescence spectra were recorded with an F-2500 spectrofluorometer (Hitachi, Japan) and absorption spectra were taken with an UV-2401 spectrophotometer (Shimadzu, Japan). For spectral measurement, all samples were prepared according to the following procedure: 1.00 mL citric acid-sodium citrate (pH=4.00), 1.40 mL of La (1.00×10^{-4} $mol \cdot L^{-1}$), 0.30 mL of CU (1.00×10^{-4} $mol \cdot L^{-1}$), 0.60 mL of SDBS (1.00×10^{-3} $mol \cdot L^{-1}$) and certain standard BSA (or sample solution) in turn, and then the mixture was diluted to 10 mL with water and allowed to stand for 15 min. The fluorescence intensities were measured with quartz cell (1-cm pass length) at $\lambda_{ex}/\lambda_{em}$= 425 nm/506 nm and $\lambda_{ex}/\lambda_{em}$=280 nm/506 nm and with a slit at 10.0 nm. The enhanced fluorescence intensity of La^{3+}-CU-SDBS-BSA system is represented as $\Delta I_f = I_f - I_f^o$. Here, I_f and I_f^o were the intensities of the systems with and without proteins, respectively. The resonance light scattering (RLS) spectra were obtained by simultaneously scanning the excitation and emission monochromators (namely, $\Delta\lambda$=0 nm) of spectrofluo-

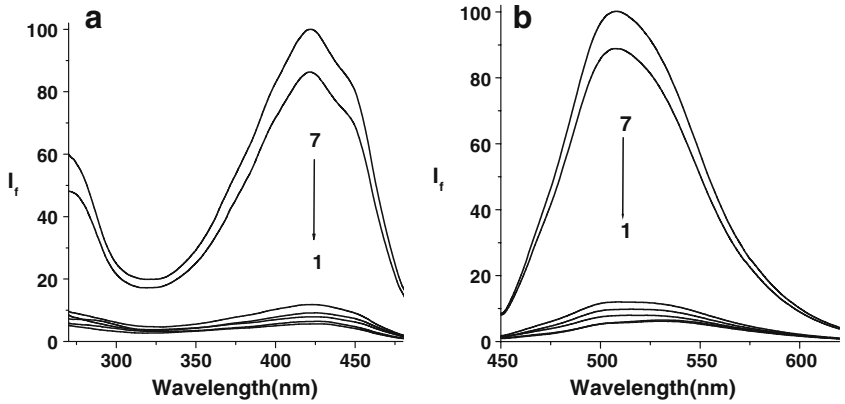


Fig. 1 (**a**) Fluorescence excitation spectra (λ_{em}=506 nm) and (**b**) emission spectra (λ_{ex}=425 nm) of the systems: (1) CU, (2) La^{3+}-CU, (3) La^{3+}-CU-BSA, (4) CU-SDBS, (5) La^{3+}-CU-SDBS, (6) CU-SDBS-BSA, and (7) La^{3+}-CU- SDBS-BSA. Conditions: CU at 3.00×10^{-6} mol L^{-1}, SDBS at 6.00×10^{-5} mol L^{-1}, La^{3+} at 1.40×10^{-5} mol L^{-1}, pH at 4.00, and BSA at 10 μg mL^{-1}

rometer from 220 to 600 nm in 1-cm quartz cell with a slit at 2.5 nm.

Results and discussion

Fluorescence behavior and optimization of reaction condition

Fluorescence spectra of the systems are shown in Fig. 1. It can be seen that in a citric-sodium citrate buffer at pH=4.00, CU only emits very weak fluorescence with an excitation peak of 425 nm and an emission peak of 530 nm. After the addition of La^{3+}, SDBS or BSA, the fluorescence of CU has little enhancement. However, when La^{3+}, SDBS and BSA are all added together into the system, fluorescence intensity of the CU-La^{3+}-BSA-SDBS system is much higher than those of the La^{3+}-CU, La^{3+}-CU-SDBS and La^{3+}-CU- BSA systems. Also, the fluorescence peak has a blue-shift to 506 nm; in addition, there is a new excitation peak at 280 nm in the La^{3+}-CU-BSA and La^{3+}-CU-SDBS-BSA systems. These phenomena clearly reveal an interaction among BSA, CU, La^{3+} and SDBS. With the CU-La^{3+}-BSA-SDBS system, experimental conditions were optimized for the fluorescence enhancement by finely tuning the pH value, type of buffers, type of rare earth ions, concentrations of CU and surfactant, and reaction time.

Effect of pH and buffers

Effect of pH on fluorescence intensity of the system is shown in Fig. 2. It can be seen that the pH change significantly influences I_f and ΔI_f, but not I_f^0. The maximum ΔI_f is obtained in the pH range of 2.0–4.0. Experiments indicate that different buffers also have a large effect on fluorescence intensity of the system. The ΔI_f (%) for BR, HMTA, citric acid-sodium citrate, HOAc- NaOAc, NaH_2PO_4-Na_2HPO_4, and Tris-HCl at pH of 4.00 is 48.6, 45.5, 100.0, 54.3, 72.8 and 45.4, respectively. The results demonstrate that the citric acid-sodium citrate is the best of the buffers tested. Thus, the citric acid-sodium citrate buffer (pH=4.00) was selected for the assay and the optimum volume of citric acid-sodium citrate buffer is 1.00 mL.

Effect of ions

Experiments also indicate that different rare earth (RE) ions have different effects on fluorescence intensity (I_f) of the system. The ΔI_f values for La^{3+}, Gd^{3+}, Sm^{3+}, $Dy^{3+}$and Y^{3+}at 1.40×10^{-5} mol·L^{-1} are shown in Table 1. The results suggest that La^{3+} was the best in the ions tested, so La^{3+} was selected for further assay use. The effect of La^{3+} concentration is shown in Fig. 3. It is found that the fluorescence intensity reached its maximum when the

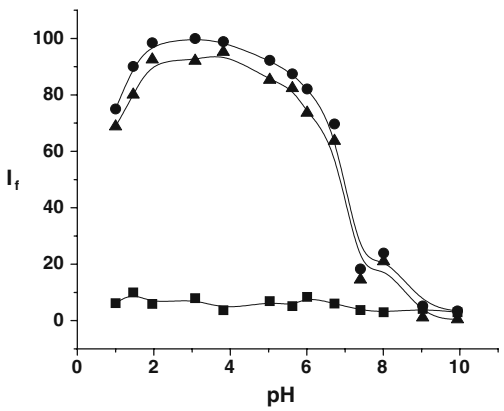


Fig. 2 Effect of pH on intensity of fluorescence in the La^{3+}-CU-SDBS-BSA system: ■: I_f^o; ●: I_f; ▲: ΔI_f. Conditions: CU at 3.00×10^{-6} mol L^{-1}, SDBS at 6.00×10^{-5} mol L^{-1}, La^{3+} at 1.40×10^{-5} mol L^{-1}, and BSA at 10 μg mL^{-1}

Table 1 Effects of different rare earth (RE) ions on fluorescence of the CU-BSA-SDBS system

Metal	Gd^{3+}	Y^{3+}	Sm^{3+}	La^{3+}	No metal
ΔI_f	73.4	95.6	91.6	100	58.0

concentration of La^{3+} was 1.40×10^{-5} mol·L^{-1}. Thus 1.40×10^{-5} mol·L^{-1} of La^{3+} was fixed for further experiment.

Effect of CU concentration

From Fig. 4 it can be seen that the fluorescence intensity of the system reached a maximum when the concentration of CU was in the range of 2.00×10^{-6}–6.00×10^{-6} mol·L^{-1}. So 3.00×10^{-6} mol·L^{-1} of the CU was used for the further experiment.

Effect of surfactants

The effect of surfactants on the fluorescence intensity was tested. The results are shown in Table 2. It can be seen that the surfactants obviously enhances the fluorescence, whereas the enhancement of SDBS is the most remarkable. Figure 5 shows that the concentration of SDBS above 6.00×10^{-5} mol·L^{-1} gives the maximum ΔI_f. So, 6.00×10^{-5} mol·L^{-1} SDBS was chosen for further use.

Effect of reaction time

Tests show that the ΔI_f reached a maximum within 15 minutes after reagents had been added, and the ΔI_f remained stable for at least 2 h. Therefore the system exhibited good stability. In this research, 15 min of sample incubate time was set for all the fluorescence measurements.

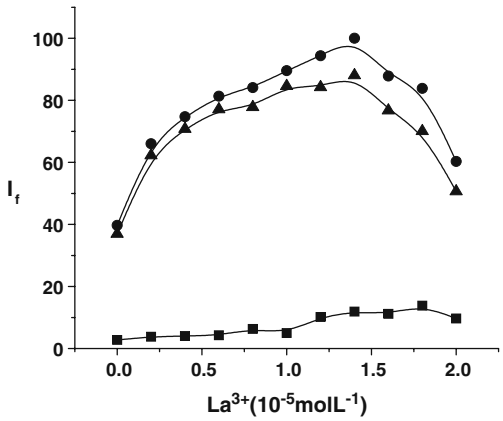


Fig. 3 Effect of the concentration of La^{3+} on intensity of fluorescence in the La^{3+}-CU- SDBS-BSA system: ■: I_f^o; ●: I_f; ▲: ΔI_f. Conditions: CU at 3.00×10^{-6} mol L^{-1}, SDBS at 6.00×10^{-5} mol L^{-1}, pH at 4.00, and BSA at 10 μg mL^{-1}

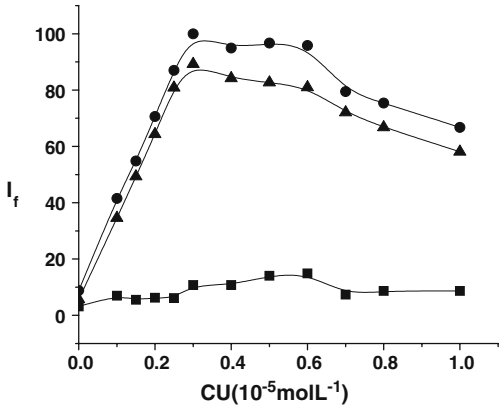


Fig. 4 Effect of concentration of CU on intensity of fluorescence in the La^{3+}-CU- SDBS-BSA system: ■: I_f^o; ●: I_f; ▲: ΔI_f. Conditions: SDBS at 6.00×10^{-5} mol L^{-1}; La^{3+} at 1.40×10^{-5} mol L^{-1}, pH at 4.00, BSA at 10 μg mL^{-1}

Analytical performance

With the standard analytical procedure established at above optimized condition, interference of foreign substances including various ions and biomolecules on fluorescence of the CU-La^{3+}-BSA-SDBS system was evaluated. The results in Table 3 indicate these foreign substances had little or no effect on the determination of proteins under the permission of ±5% relative errors, and thus the proposed method exhibits good selectivity in protein measurement.

Under the optimum condition defined, the calibration graphs for BSA and HSA were obtained, and a linear relationship between the ΔI_f and the concentration of proteins was quantified. It shows that this method has high sensitivity and wide linear range (Table 4).

A comparison between this method with other luminescent methods for proteins in sensitivity and linear range is summarized in Table 5. This method exhibits higher sensitivity and wider linear range than most other methods reported in literatures for protein measurement

The proposed method was used for the determination of HSA in a real sample of human serum. The measured results coincide very well with those obtained from UV spectrophotometric method, as shown in Table 6, while this method could achieve much lower detection limit.

Table 2 Effects of different surfactants on fluorescence of the CU-La^{3+}-BSA system

Surfactants	CTAB	SDBS	OP	SDS	CPB	Without surfactants
ΔI_f	6.2	100	7.6	79.6	3.2	26.5

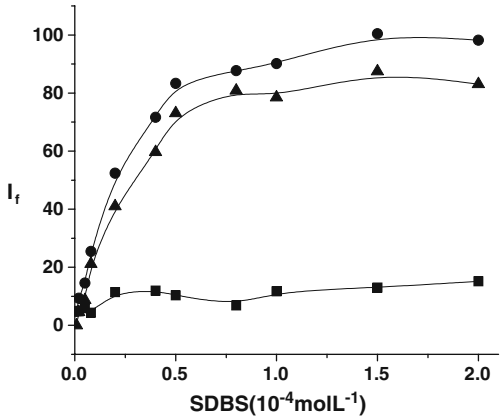


Fig. 5 Effects of the concentration of SDBS on intensity of fluorescence in the La^{3+}-CU- SDBS-BSA system: ■: I_f^o; ●: I_f; ▲: ΔI_f. Conditions: CU at 3.00×10^{-6} mol L^{-1}, La^{3+} at 1.40×10^{-5} mol L^{-1}, pH at 4.00, and BSA at 10 μg mL^{-1}

Table 3 Effects of foreign interference substances on fluorescence of the CU-La^{3+}-BSA-SDBS system

Foreign substances	Concentration ($\times10^{-5}$mol·L^{-1})	ΔI_f (%)
KCl	20	5.0
KNO_3	20	4.2
$MgSO_4$	20	5.0
$MnSO_4$	70	4.9
$ZnCl_2$	70	4.9
KNO_3	20	4.7
NaCl	50	5.0
$Fe_2(SO_4)_3$	50	4.8
$CuCl_2$	20	4.6
NH_4Cl	50	4.4
Na_2SO_4	100	4.8
$CaCl_2$	100	5.0
$FeSO_4$	50	4.8
sucrose	20	4.7
fructose	20	4.2
glucose	10	4.7
L-α-Ala	9	5.0
L-Leu	10	3.3
L-Arg	30	4.1
L-Pro	20	4.6
L-Asp	5	5.0
L-Glu	5	4.5
DNA [a]	10	4.5
RNA [a]	10	5.0

Conditions: CU at 3.00×10^{-6} mol L^{-1}, SDBS at 6.00×10^{-5} mol L^{-1}, La^{3+} at 1.40×10^{-5} mol L^{-1}, pH=4.00, and BSA at 8.0×10^{-8} g mL^{-1}

[a] Concentration at $\times10^{-7}$ g.mL^{-1}

Table 4 Work range and detection limit of the proposed method

Excitation wavelength (nm)	Proteins	Linear range (μg·mL^{-1})	Correlation coefficient (r)	Limit of detection (ng·mL^{-1})
425	BSA	0.0080–20.0	0.9994	5.368
	HSA	0.00080–20.0	0.9993	0.573
280	BSA	0.0002–20.0	0.9929	0.049
	HSA	0.0008–20.0	0.9931	1.262

Mechanism of fluorescence enhancement

Form our previous research, it is found that fluorescence intensities of the La^{3+}-SDBS-CU and CU-SDBS-BSA systems [22, 32] are stronger than that of the single CU, which also can be seen in Fig. 1. Furthermore, it is shown that when La^{3+}-SDBS-BSA is added to CU, the fluorescence intensity of La^{3+}-CU-SDBS-BSA system can be further enhanced, which is much stronger than those of La^{3+}-CU-SDBS, La^{3+}-CU-BSA and CU-SDBS-BSA systems. Obviously, the fluorescence enhancement is due to synergic effects of BSA, SDBS, La^{3+} and CU.

Formation of La^{3+}-CU-SDBS-BSA complex

Resonance light scattering (RLS) spectra of the systems are shown in Fig. 6. According to the theory of RLS [33, 34], the peaks of light scattering at 280 nm, 380 nm and 450 nm are ascribed to the absorption bands of CU at 200–250 nm, 360–370 nm and about 430 nm, respectively. It can be seen that the RLS intensities of the CU are enhanced when La^{3+}, BSA and SDBS are added into the CU system and especially that the RLS intensity of the La^{3+}-CU–SDBS–BSA system is the highest among all the studied systems. The RLS spectra are closely correlated to some intrinsic behaviors of complex, and reflect that large La^{3+}-CU–SDBS–BSA quaternary complex may form in the system.

The earlier publication [35] shows that rare earth ions can bind with complexes that contain the structure of β-diketone, and such as CU. In this system, La^{3+} may form complex with CU through carbonyl groups, which increases rigidity of plate structure of the CU and thus enhances the fluorescence emission through L*-L type of energy transfer. La^{3+} and CU form the complex at 1:1 molar ratio of La^{3+}/CU, as determined by continuous variation method of equivalent mole (Fig. 7). Because binding number of La^{3+} is still unsaturated in the La^{3+}- CU system, the La^{3+} is able to simultaneously bind with BSA [36] to form a BSA- La^{3+}- CU complex. As known the isoelectric point of BSA at pH 4.7, BSA is positively charged at pH<4.7. Therefore, the entire surface of ternary complex BSA- La^{3+}- CU is positively charged in this

Table 5 Comparison on sensitivity with other luminescent methods reported in literature

Probe	$\lambda_{ex}/\lambda_{em}$ (nm)	Protein	LOD ($\mu g \cdot mL^{-1}$)	Linear range ($\mu g \cdot mL^{-1}$)	Reference
Erythrosin	–/550	BSA	–	1.36–20.4	[23]
Magdala Red	–/556	BSA	0.1	0.1–4.0	[24]
		EGG	–	0.4–4.0	
Functionalized CdS nanoparticles	–/–	BSA	0.01	0.1–3.0	[25]
		HSA	0.019	0.1–1.4	
5-(4-Carboxyphenylazo)-8-(4-methoxy)-Benzyl ideneaminoquinoline	240/358	HSA	0.028	0.1–4.5	[26]
Nile Blue-SDBS	592/664	HSA	0.020	0–16	[27]
Calix [8] arenas and Ce(III)	254/361	BSA	2.83×10^{-3}	1.1–11.4	[28]
Acrdine Orange-Rhodamine 6G	450–556	BSA	0.32	0–31.25	[29]
			0.33	0–30	
Acrdine Orange- SDBS	280/490	BSA	0.0026	0.0050–20.0	[30]
		HSA	0.014	0.020–15.0	
Curcumin- SDBS	528/530	BSA	1.4	0.0050–20.0	[22]
		HSA	20	0.080–20.0	
Y^{3+}-rutin-SDBS	425/520	BSA	1.6×10^{-3}	0.005–10	[31]
		HSA	9.8×10^{-3}	0.030–10	
This method	425/506	BSA	5.368	0.008–20.0	
		HSA	0.573	0.0008–20.0	
	280/506	BSA	0.049	0.0002–20.0	
		HSA	0.562	0.0008–20.0	

system of pH 4.0. It is natural that anionic surfactant SDBS can aggregate in the positively BSA- La^{3+}-CU complex through electrostatic attraction to form a large La^{3+}-CU-SDBS-BSA complex, which could be a supermolecular association with multilayer structure.

The influence of ionic strength on the fluorescence intensity of this system was investigated. When NaCl is introduced into the system, the fluorescence intensity of the system has obviously decreased (Fig. 8). Thus, it is assumed that the interactions among La^{3+}, CU, SDBS and BSA in this system forming the quaternary complex are mostly through electrostatic attractions. At high salt concentration, the fluorescence intensity of this system decreased because charges could be screened and the interaction via electric attraction weakened.

It is well know that among all the amino acid residues in BSA, only tryptophan and tyrosine residues can contribute to fluorescence spectra. However, tryptophan and tyrosine have similar excitation spectra and their conventional fluorescence spectra overlap heavily. Here we take advantage of the synchronous fluorescence technique identify the tryptophan and tyrosine. At large wavelength intervals (such as λ=60 nm), the synchronous fluorescence spectra of a tryptophan–tyrosine mixture solution are characteristic of tryptophan, whereas at small wavelength intervals (such as λ=15 nm), the spectra are attributed to that of tyrosine [37, 38]. From Fig. 9(a) and (b), the fluorescence intensity of BSA is decreased for both $\Delta\lambda$=60 nm and $\Delta\lambda$=15 nm when CU, La^{3+} and SDBS are added, but the decrease of the intensity for $\Delta\lambda$=60 nm is much larger than that for $\Delta\lambda$= 15 nm. This suggests that La^{3+}, CU and SDBS is closer to tryptophan residues compared to tyrosine residues and the main interaction occurred between the tryptophan residue and La^{3+}-CU -SDBS. When adding SDBS to the system, the intrinsic fluorescence intensity of both tryptophan and tyrosine have a little blue shift, which may attribute to a SDBS induced change of hydrophobic micro-environment for both tryptophan and tyrosine residues in BSA. It is known that the peak at ~190 nm can reflect the framework conformation of protein [39]. In UV-visible absorption spectra in Fig. 10, the peak of BSA at 190 nm increases its intensity when CU, La^{3+} and SDBS are added into protein

Table 6 Results on HSA determination in a real sample of human serum

Samples	Methods	Concentration ($mg \cdot mL^{-1}$)	Average($mg \cdot mL^{-1}$)	RSD(%)
HSA	This method	78.6, 77.6, 79.0 , 78.2, 77.9	78.3	0.55
	UV method	75.2, 76.7, 75.9, 76.0., 75.8	75.9	0.54

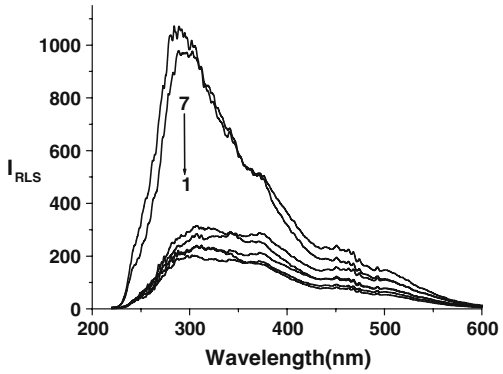


Fig. 6 Resonance light scattering spectra of the systems: (1) CU, (2) CU-SDBS, (3) La^{3+}-CU, (4) La^{3+}-CU-BSA, (5) La^{3+}-CU- SDBS, (6) CU-SDBS-BSA, and (7) La^{3+}-CU-SDBS-BSA. Condition: CU at 3.00×10^{-6} mol L^{-1}, SDBS at 6.00×10^{-5} mol L^{-1}, La^{3+} at 1.40×10^{-5} mol L^{-1}, pH at 4.00, and BSA at 10 μg mL^{-1}

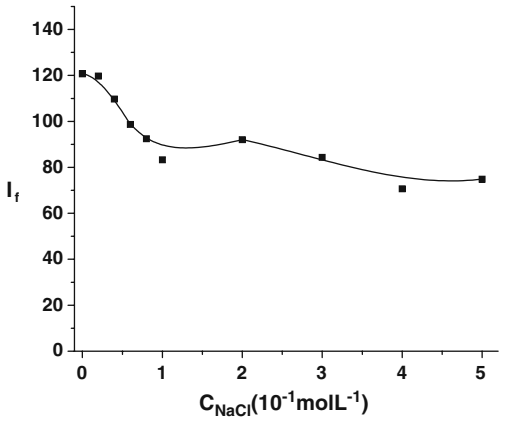


Fig. 8 Effect of ionic strengths on intensity of fluorescence in the La^{3+}-CU- SDBS-BSA system. Conditions: pH at 4.00, CU at 3.00×10^{-6} mol L^{-1}, SDBS at 6.00×10^{-5} mol L^{-1}, La^{3+} at 1.40×10^{-5} mol L^{-1}, and BSA at 10 μg mL^{-1}

solution. Meantime, the peak shifts to longer wavelengths when SDBS presents, revealing strong interactions between the CU, La^{3+}, BSA and the SDBS. The above phenomena suggest that the interaction may lead to substantial changes of protein conformation and the major conformation may have reconstructed into a β-folding. In addition, the absorption peak red-shift indicates that the polarity of the microenvironment around BSA is decreased and the addition of SDBS can stabilize the folded structure of BSA. These changes in absorption spectra are coincident with the band blue-shifts in fluorescence spectra.

Based on these spectral analyses, it reasonably hypothesize that in the La^{3+}-CU-SDBS-BSA system, a quaternary complex La^{3+}-CU-SDBS-BSA formed. In the complex formation, it is considered that the self-assembled protein serves as a template and the La^{3+}, CU and SDBS associate with the BSA scaffolding through electrostatic attraction. During formation of the large complex of La^{3+}-CU-SDBS-BSA, the secondary structure of BSA changes into β-folding, and meantime an aromatic ring stacking effect exhibits, caused by with the tryptophan residue of BSA, the aromatic ring of SDBS and CU come along with the [40]. This is the reason for the enhanced absorption at 220 nm in absorption spectra.

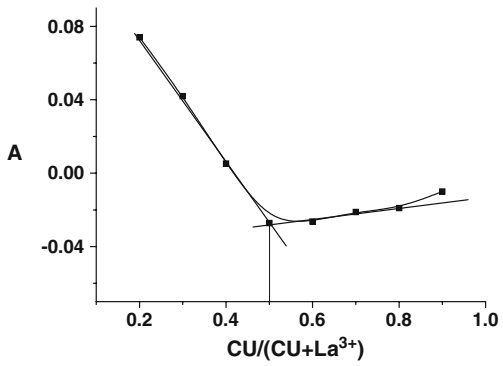


Fig. 7 Ratio of La^{3+} and CU in the CU-La^{3+}-SDBS-BSA system by continuous variation method of equivalent mole. Conditions: pH at 4.00, SDBS at 6.00×10^{-5} mol L^{-1}, and BSA at 10 μg mL^{-1}

Energy transfer in La^{3+}-CU-SDBS-BSA

Emission peaks are at 332 nm for BSA and 335 nm for SDBS with excitation at 280 nm, as shown in Fig. 11. When CU is added into BSA or SDBS, the fluorescence of BSA or SDBS decreases and the characteristic fluorescence peak of CU increases. An overlap between the absorption spectrum of CU and the emission spectra of BSA and SDBS exists (Fig. 12), and thus an intermolecular energy transfer from BSA or SDBS to CU occurs under the excitation of 280 nm. However, the weak fluorescence intensity implies that energy transfer is not efficient. When La^{3+}, SDBS and BSA is added into the CU system, the fluorescence of both BSA and SDBS are quenched and the characteristic fluorescence of CU is greatly enhanced, which indicates that energies of both SDBS and BSA are transferred to CU in the complex of La^{3+}-CU-SDBS -BSA. To understand this process, the energy transfer efficiency (E_a) and the interaction distance (r) between donor and acceptor can be evaluated using Főrster model [41, 42]:

$$E_a = \frac{A_a}{A_d}\left(\frac{I_{ad}}{I_a} - 1\right) \quad (1)$$

$$E_a = 1 - \frac{I_{da}}{I_d} \quad (2)$$

$$R_0^6 = 8.8 \times 10^{-25} \times k^2 \times n^{-4} \times \phi_d \times J \quad (3)$$

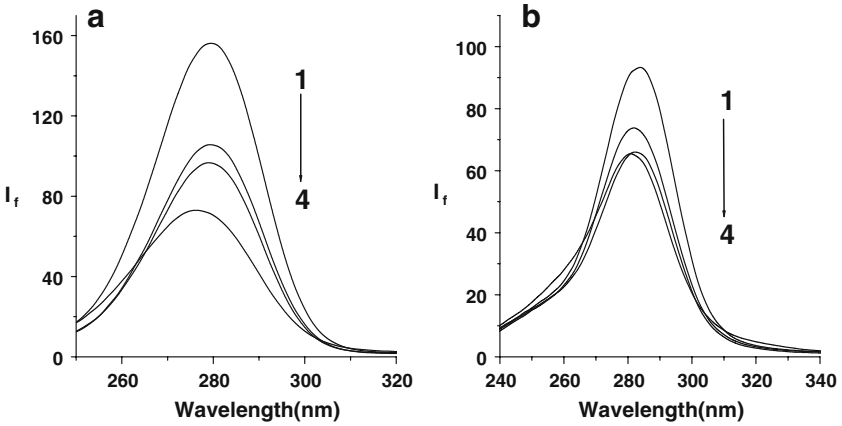


Fig. 9 Synchronous fluorescence spectra at (a) Δλ=60 nm and (b) Δλ=15 nm for the systems: (1) BSA, (2) CU-BSA, (3) La^{3+}-CU-BSA, and (4) La^{3+}-CU -SDBS-BSA. Conditions: pH at 4.00, CU at 3.00×10^{-6}mol L^{-1}, SDBS at 6.00×10^{-5}mol L^{-1}, La^{3+} at 1.40×10^{-5}mol L^{-1}, and BSA at 10 μg mL^{-1}

$$E_a = \frac{R_0^6}{R_0^6 + r^6} \tag{4}$$

where the energy transfer efficiency E_a and the critical transfer radius R_0 are calculated, A_a and A_d are the absorbance of energy donor and acceptor, respectively; I_{ad}, I_a, I_{da} and I_d are the fluorescence intensities of the acceptor in the presence of donor, acceptor in absence of donor, donor in the presence of acceptor and donor in absence of acceptor, respectively. In Eq. (3) k^2 is a factor describing the relative orientation in space of the transition dipoles of donor and acceptor; Φ_d is the fluorescence quantum yield of the donor in the absence of acceptor; n is the refractive index of the solvent; J is the spectral overlap integral between the emission spectrum of donor and the absorption spectrum of acceptor. The choice of Eq. (1) or Eq. (2) is based on the degree of the energy transfer efficiency in order to decrease calculated error. The values of E_a, R_0 and r are listed in Table 7.

From Table 7, it can be seen that the energy transfer efficiency between BSA and CU or SDBS and CU is remarkably enhanced when SDBS-BSA adding into CU or La^{3+}-CU. In case of the quaternary complex La^{3+}-CU-SDBS-BSA, energy transfer between any two species is more efficient than that in ternary complex CU-SDBS-BSA. It is considered that CU can be solubilized in SDBS-BSA premicelle-like cluster complexes, which shortens the distance between BSA and CU or SDBS and CU in CU-SDBS-BSA system [22]. Synergistic interactions among the four species result in the most efficient energy transfer between any two substances in quaternary system. Although the Főrster model is an approximate evaluation [43], the calculation indicates that

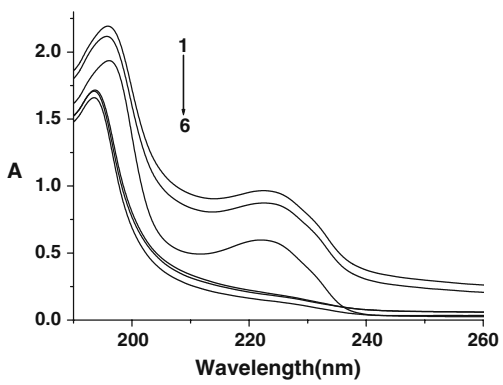


Fig. 10 UV absorption spectra of BSA in the systems: (1) La^{3+}-CU-SDBS-BSA, (2) La^{3+}-BSA-SDBS, (3) SDBS-BSA, (4) La^{3+} -CU-BSA, (5) CU-BSA, and (6) BSA. Conditions: pH at 4.00, CU at 3.00×10^{-6} mol L^{-1}, SDBS at 6.00×10^{-5} mol L^{-1}, La^{3+} at 1.40×10^{-5} mol L^{-1}, and BSA at 10 μg mL^{-1}

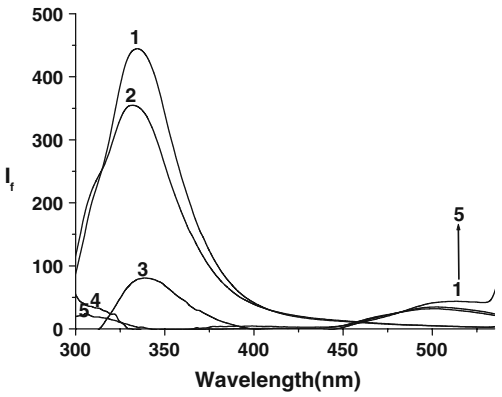


Fig. 11 Fluorescence spectra of the systems: (1) SDBS, (2) BSA, (3) BSA-SDBS-CU (*vs.* SDBS-CU), (4) BSA-SDBS-CU (*vs.* BSA –CU), and (5) La^{3+}-CU-SDBS-BSA (*vs.* La^{3+}-CU-SDBS). Conditions: pH at 4.00, CU at 3.0×10^{-6} mol L^{-1}, SDBS at 6.0×10^{-5} mol L^{-1}, La^{3+} at 1.40×10^{-5} mol L^{-1}, and BSA at 10 μg mL^{-1}

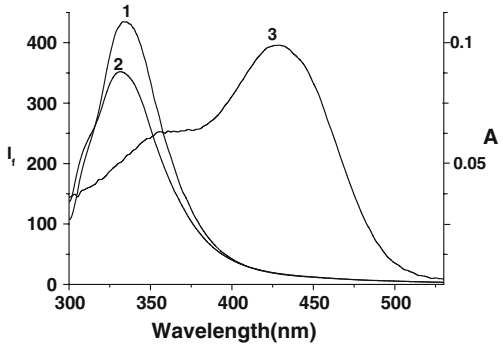


Fig. 12 Absorption and fluorescence spectra of (1) SDBS emission, (2) BSA emission, and (3) CU absorption. Note the overlap between the emissions of SDBS and BSA (donors) and the absorption of CU (acceptor). Conditions: pH at 4.00, CU at 3.0×10^{-6} mol L^{-1}, SDBS at 6.0×10^{-5} mol L^{-1}, La^{3+} at 1.40×10^{-5} mol L^{-1}, and BSA at 10 μg mL^{-1}

the presence of La^{3+} could shorten distance between SDBS and CU from 3.20 nm to 2.62 nm, which may promote efficiency in energy transfer. Unusual increase of the distance between BSA and CU form 1.18 nm to 1.71 nm in existence of La^{3+} resulted in an increase in the energy transfer efficiency of them. Most likely, the La^{3+} ion inserts into the midst of BSA and CU in quaternary complex, i.e., BSA could be bound to CU through La^{3+} resulting in the increase of distance between BSA and CU. The important result of the addition of positive metal ion La^{3+} reduces the electron density of the aromatic rings and enhances the electron transfer between aromatic rings, which make the "aromatic ring stacking" effect stronger. In this model, metal ion La^{3+} became a more expedient energy transfer mediator from BSA to CU. At the same time, the interaction decreases the distance between molecules and increases the intermolecular forces including hydrogen bond, hydrophobic interaction and van der Waals force, which gets the association become compact and the interaction become strong. Therefore, it has higher sensitivity of the La^{3+}-CU-SDBS system than the CU-SDBS system in terms of forming complex with protein for fluorescent determination.

Table 8 Polarity (I_1/I_3) and fluorescence quantum yield (y) of CU in different systems

System	CU	CU - BSA	La^{3+}-CU-BSA	CU-SDBS-BSA	La^{3+}-CU - SDBS-BSA
I_1/I_3	1.61	1.60	1.59	1.23	1.12
y	0.0187	0.0393	0.0650	0.159	0.207

Hydrophobic microenvironment

As shown in Fig. 1, with excitation of 425 nm, the La^{3+}-CU system emits the intrinsic fluorescence of CU with the emission peak of 530 nm. The intensity is greatly enhanced by BSA in the presence of SDBS and the emission peak has a blue shift to 506 nm. These changes may be related to the change in microenvironment for the different systems. As shown in Table 8, polarity is different for the system containing different components.

The ratio of the first to the third fluorescence bands of pyrene monomer (I_1/I_3) is a well established parameter which reflects the polarity changes of a system experienced by the pyrene probe [44, 45]. A low value reflects a lower polar environment than a high value. In Table 8, when SDBS and BSA are added to the CU or La^{3+}-CU system, the value of I_1/I_3 decreased. Therefore, it is considered that BSA–SDBS system provides an optimum hydrophobic environment with low polarity for CU or La^{3+}-CU complex, which makes the fluorescence intensity of them enhanced, especially the latter. In addition, the hydrophobic environment of BSA-SDBS system can also prevent the collision between complex and water and decrease the energy loss of the La^{3+}-CU-SDBS- BSA system. Thus, the fluorescence quantum yield of CU in the system is determined based on the reference method [22, 46]. The results in Table 8 show that the fluorescence quantum yield of CU increases to 0.207 in the La^{3+}-CU-SDBS-BSA system. It is clear that the hydrophobic microenvironment can prevent the collision between CU and water, and decrease the energy loss of the La^{3+}-CU-SDBS-BSA system. The improved fluorescence quantum yield makes the fluorescence intensity highly enhanced for the CU-La^{3+}-BSA-SDBS system.

Table 7 Efficiency of energy transfer ($_{Ea}$) and the critical transfer radius (R_o)

System	Donor	Acceptor	E_a	J ($10^{-14}cm^6M^{-1}$)	R_0 (nm)	r (nm)
CU-BSA	BSA	CU	0.23	2.898	4.31	5.28
CU-SDBS	SDBS	CU	0.09	2.904	3.02	4.49
CU-BSA-SDBS	BSA	CU	0.96	1.323	2.00	1.18
	SDBS	CU	0.57	1.604	2.74	3.20
La^{3+}-CU-SDBS-BSA	BSA	CU	0.99	2.219	4.12	1.71
	SDBS	CU	0.61	1.904	2.82	2.62

Conclusion

In this work, fluorescence property for La^{3+}-CU-SDBS-BSA system has been systematically investigated by using multi-techniques including UV absorbance spectroscopy, fluorescence spectroscopy, and resonance light scattering technique. Results shown that the fluorescence of CU can be highly enhanced by La^{3+}, SDBS and BSA and the enhancement in fluorescence intensity is quantitatively proportional to concentration of proteins in a wide range. Based on this observation, a sensitive fluorescence method for determination of protein at $ng \cdot mL^{-1}$ level is established, and evaluated by real samples, the HSA in human blood. Mechanism studies shown that in La^{3+}-CU-SDBS-BSA system, protein can bind with La^{3+}, CU and SDBS through self-assembling function with electrostatic attraction, hydrogen bond, hydrophobic interaction and van der Waals force etc, and forms a supermolecular association with multilayer structure, in which La^{3+}-CU is clamped between BSA and SDBS. The fluorescence enhancement of CU is considered to originate from (a) the hydrophobic microenvironment provided by BSA and SDBS, and (b) the intermolecular energy transfer between BSA, SDBS and CU. In the energy transfer process, La^{3+} ions play crucial roles. The La^{3+} and CU could form a L*-L type of complex, and the La^{3+} also shortens the distance between SDBS and CU and acts as a "bridge" to transfer the energy from BSA to CU.

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