Research Article

LUMINESCENCE
The Journal of Biological and Chemical Luminescence

Received: 28 August 2008, Revised: 20 December 2008, Accepted: 25 December 2008, Published online in Wiley Interscience: 9 July 2009

(www.interscience.wiley.com) DOI 10.1002/bio.1122

Sensitive determination of protein based on the fluorescence enhancement effect of terbium (III)–epinephrine–protein–sodium dodecylsulfate system

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ABSTRACT: It was found that the fluorescence of Tb^{3+}–epinephrine (E) complex can be enhanced by both bovine serum albumin (BSA) and sodium dodecylsulfate (SDS), and stabilized by ascorbic acid (AA). It is considered that the fluorescence enhancement of the Tb^{3+}–E–BSA–AA–SDS system originates not only from the hydrophobic microenvironment provided by BSA–SDS, but also from the energy transfer from BSA to Tb^{3+} in this system. Therefore, a new fluorescence method for the determination of protein concentrations as low as 1.3×10^{-9} g mL^{-1} BSA is established using Tb^{3+}–epinephrine complex as probe. The method has been applied for the determination of BSA and human serum albumin in actual samples, and the results obtained are satisfactory. Compared with other fluorescence methods, this method is simpler and more sensitive for the determination of protein. The mechanism of the fluorescence enhancement of the system is studied in detail. Copyright © 2009 John Wiley & Sons, Ltd.

Keywords: terbium (III); epinephrine (E); bovine serum albumin (BSA); fluorescence probe

Introduction

The detection and quantification of proteins at or below the ng mL^{-1} concentration range are of critical importance for biochemical studies, biological techniques, clinical diagnosis and analysis of food nutrition. This has been achieved by the development of high-sensitivity detection methods based on the fluorescence probes of proteins.[1]

The luminescence of the lanthanides Eu^{3+} and Tb^{3+} arises from the transition between their f orbital, which is shielded by the $5s^2$ and $5p^6$ sub-shell, so that they can emit narrow fluorescence spectra with long luminescence lifetime, and their intensities are not easily quenched by oxygen. Because of their low extinction coefficients, the lanthanides are not usually excited directly. It is well known that Eu^{3+} and Tb^{3+} can bind to biological molecules that contain the fluorophore. The energy from the fluorophore can be transferred to Eu^{3+} and Tb^{3+}, resulting in the enhancement of their characteristic fluorescence.[2] Based on the above properties, Eu^{3+} and Tb^{3+} are usually used as fluorescence probes to study nucleic acids,[3–5] proteins,[6,7] and drugs.[8–10] However, based on the only rare earth ions as probes, the sensitivity is not adequate. Recently, the rare earth ion complexes as fluorescence probes have been used for the determination of proteins.[11–23] However, the improvement of the sensitivities is limited because the fluorescence enhancement of rare earth ion complexes in these systems originates only from the hydrophobic microenvironment provided by protein. Therefore, it is imperative to find new rare earth ion complex as sensitive fluorescence probe for the determination of proteins.

Our previous research[24] indicated that Tb^{3+} could bind with epinephrine (E) and form Tb^{3+}–E complex, which emitted the characteristic fluorescence of Tb^{3+}. In the present report it is found that the fluorescence of Tb^{3+}–E complex can be enhanced by both bovine serum albumin (BSA) and sodium dodecyl sulfate (SDS), and stabilized by ascorbic acid (AA). It is considered that the fluorescence enhancement of the Tb^{3+}–E–BSA–AA–SDS system originates from both the hydrophobic microenvironment provided by BSA–SDS and the energy transfer of this system. Therefore, a new fluorescence method for the determination of proteins as low as 1.3×10^{-9} g mL^{-1} BSA is established using Tb^{3+}–epinephrine complex as probe. The method was applied for the determination of BSA and HSA in actual samples, and the results obtained were satisfactory. Compared with other fluorescence methods, this method is simpler and more sensitive for the determination of protein. The mechanism of the fluorescence enhancement of the system is studied in detail.

Experimental

Apparatus

Normal fluorescence, synchronous fluorescence and fluorescence polarization measurements were made on an FL-4500 spectrofluorimeter (Hitachi, Japan) and a PE-LS55 spectrofluorimeter (Perkin-Elmer). Luminescence lifetimes were measured on an Edinburgh F900 spectrofluorimeter (UK). CD spectra were recorded using a Jasco J-810S circular dichroism spectropolarimeter

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(Japan). All pH measurements were made on a Delta 320-S pH meter (Mettler Toledo).

Regents and solutions

Stock solution of BSA (1.30×10^{-3} g mL^{-1}) was prepared by dissolving 0.130 g of commercial BSA (Shanghai Boao Biochemical Technology Co., China) in a 100 mL volumetric flask with deionized water. This stock solution was stored at 0–4°C.

An epinephrine standard stock solution (1.36×10^{-3} mol L^{-1}) was prepared by dissolving 0.025 g epinephrine (Fluka, HPLC-grade) in 100 mL of 0.01 mol L^{-1} hydrochloric acid and stored in a refrigerator at 4°C. Working solutions were prepared by diluting with doubly deionized water. Stock standard solutions (1.00×10^{-2} mol L^{-1}) of rare-earth ions were prepared by dissolving the corresponding oxides (Yuelong Chemical Co., Shanghai, 99.9% pure) in hydrochloric acid and diluting with deionized water. A stock solution of SDS (1.0×10^{-2} mol L^{-1}) was prepared by dissolving 0.721 g SDS in a 250 mL volumetric flask with deionized water.

A solution of AA (0.15%, w/v) was prepared by dissolving 0.150 g AA in 100 mL with distilled water. A 0.20 mol L^{-1} Tris–HCl buffer solution was prepared by dissolving 12.5 g of Tris in 500 mL deionized water, and adjusting the pH = 9.0 with HCl (6.0 mol L^{-1}). All the chemicals used are of analytical grade and doubly deionized water was used throughout.

Procedure

To a 25 mL test tube, solutions were added in the following order: E, AA, Tb^{3+}, BSA, SDS and Tris–HCl buffer. The mixture was diluted to 10 mL with doubly deionized water, and then the solution was shaken and allowed to stand for 20 min at room temperature. The fluorescence intensity was measured in a 1 cm quartz cell with excitation and emission wavelengths of 300 and 548 nm, respectively. The excitation and emission slits were both 10 nm.

Results and discussion

Fluorescence spectra

Excitation and emission spectra of Tb^{3+} (1), Tb^{3+}–AA (2), Tb^{3+}–BSA (3), Tb^{3+}–SDS (4), Tb^{3+}–E (5), Tb^{3+}–E–BSA–AA (6), Tb^{3+}–E–AA–SDS (7), Tb^{3+}–E–HSA–AA–SDS (8) and Tb^{3+}–E–BSA–AA–SDS (9) systems are shown in Fig. 1. It can be seen that all these systems have the same emission peaks at 493 and 548 nm, corresponding to the transitions of $^5D_4$–$^7F_6$ and $^5D_4$–$^7F_5$ of Tb^{3+}, respectively, and the intensity at 548 nm is the strongest. From Fig. 1, it can be seen that protein (BSA or HSA) and SDS can greatly enhance the fluorescence intensity of the Tb^{3+}–E system in the presence of AA and the excitation peak moves from 320 to 300 nm. Here we choose 300 and 548 nm as the excitation and emission wavelengths for further research.

Optimization of the general procedure

Effects of pH and buffers solution. The effect of pH on the fluorescence intensity of the system is shown in Fig. 2. The results indicate that the fluorescence intensity remains the maximum when the pH is in range 8.8–9.2. Therefore, pH = 9.0 is fixed for further research. The effect of following buffers on the fluorescence intensity of this system is tested: Tris–HCl, $Na_2B_4O_7$–NaOH, NH_4Cl–NH_3, $NaHCO_3$–Na_2CO_3 and NH_2CH_2COOH–NaOH. The results indicate that the buffers also have a large effect on the fluorescence intensity of the system, and 0.020 mol L^{-1} Tris–HCl (pH = 9.0) is the best buffer.

Effect of Tb^{3+} concentration. The effect of Tb^{3+} concentration on the fluorescence intensity of this system was studied. The results indicate that the fluorescence intensity of the system reached the maximum and remained constant when the concentration of Tb^{3+} was in range $3.0–7.0 \times 10^{-5}$ mol L^{-1}. Therefore, 4.0×10^{-5} mol L^{-1} Tb^{3+} was chosen.

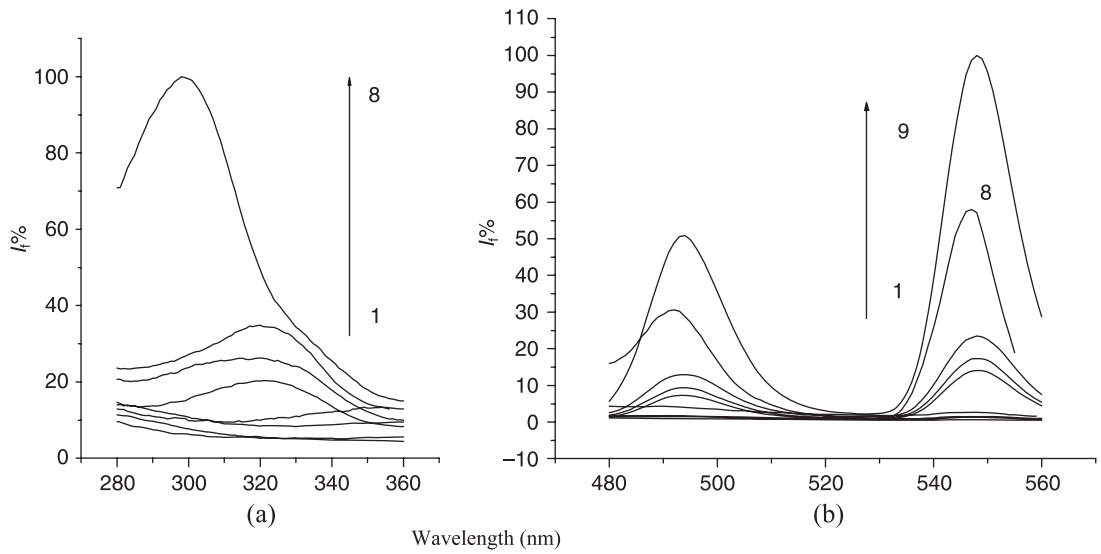

Figure 1. Fluorescence spectra. (a) Excitation spectra (λ_{em} = 497 nm). (b) Emission spectra (λ_{ex} = 300 nm). (1) Tb^{3+}; (2) Tb^{3+}–AA; (3) Tb^{3+}–BSA; (40 Tb^{3+}–SDS; (5) Tb^{3+}–E; (6) Tb^{3+}–E–BSA–AA; (7) Tb^{3+}–E–AA–SDS; (8) Tb^{3+}–E–HSA–AA–SDS; (9) Tb^{3+}–E–BSA–AA–SDS. Conditions: E, 1.0×10^{-4} mol L^{-1}; AA, 4.5×10^{-6} g mL^{-1}; Tb^{3+}, 4.0×10^{-5} mol L^{-1}; BSA, 1.3×10^{-5} g mL^{-1}; SDS, 1.0×10^{-3} mol L^{-1}; Tris–HCl, 0.020 mol L^{-1}, pH = 9.0.

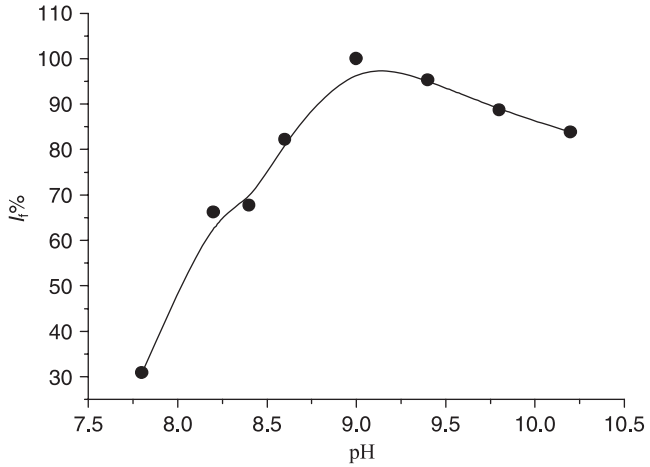

Figure 2. Effect of pH. Conditions: E, 1.0×10^{-4} mol L^{-1}; AA, 4.5×10^{-6} g mL^{-1}; Tb^{3+}, 4.0×10^{-5} mol L^{-1}; BSA, 1.3×10^{-5} g mL^{-1}; SDS, 1.0×10^{-3} mol L^{-1}; Tris–HCl, 0.020 mol L^{-1}.

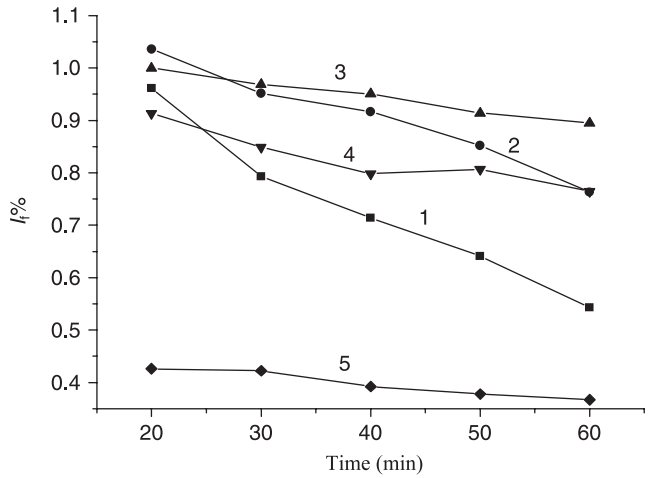

Figure 3. Effect of AA concentration. AA ($\times 10^{-6}$ g mL^{-1}): (1) 0; (2) 1.5; (3) 4.5; (4) 7.5; (5) 13.5. Conditions: E, 1.0×10^{-4} mol L^{-1}; Tb^{3+}, 4.0×10^{-5} mol L^{-1}; BSA, 1.3×10^{-5} g mL^{-1}; SDS, 1.0×10^{-3} mol L^{-1}; Tris–HCl, 0.020 mol L^{-1}, pH = 9.00.

Effect of E concentration. The effect of E concentration on the fluorescence intensity of this system was studied. The results showed that the fluorescence intensity of the system reached the maximum and remained constant when the concentration of E was in range 0.8×10^{-4} to 1.2×10^{-4} mol L^{-1}. Thus, 1.0×10^{-4} mol L^{-1} E was chosen for this research.

Effect of AA concentration. From Fig. 3, it can be seen that the fluorescence intensity of the system without AA is not stable, which is attributed to the oxidation of E by the oxygen dissolved in an alkaline solution,[25] while the addition of AA can stabilize the fluorescence intensity of this system. The experiment shows that the fluorescence intensity of the system was the most stable when the concentration of AA was 4.5×10^{-4} g mL^{-1}.

Effect of SDS concentration. Different surfactants can influence the fluorescence intensity of the system, and the results are shown in Table 1. The fluorescence intensity of this system was the strongest when SDS was used as the surfactant. The effect of SDS concentration on the fluorescence intensity of this system was studied. The result indicated that the fluorescence intensity

Table 1. Effect of the different surfactants

	SDS[a]	SDBS[b]	CTAB[c]	CPB[d]
$\Delta I_f\%$	100	67.4	1.74	0.64

[a] Sodium dodecylsulfate; [b] sodium dodecyl benzene sulfonate; [c] cetyltrimethylammonium bromide; [d] cetylpyridine bromide.

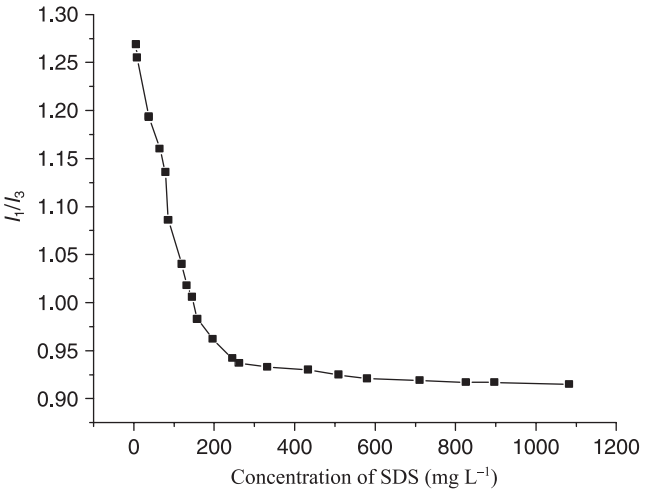

Figure 4. The determination of SDS CMC. Conditions: E, 1.0×10^{-4} mol L^{-1}; AA, 4.5×10^{-6} g mL^{-1}; Tb^{3+}, 4.0×10^{-5} mol L^{-1}; BSA, 1.3×10^{-5} g mL^{-1}; Tris–HCl, 0.020 mol L^{-1}, pH = 9.0; pyrene, 2.0×10^{-7} mol L^{-1}.

was the strongest when the concentration of SDS was in range of 0.5×10^{-3} to 1.2×10^{-3} mol L^{-1}. Therefore, 1.0×10^{-3} mol L^{-1} SDS was chosen for this research.

Based on the change in the ratio of the first to the third fluorescence bands of pyrene monomer (I_1/I_3) with the increase in SDS concentration, the critical micelle concentration (CMC) of SDS in this system was determined.[26] From Fig. 4, the CMC of SDS in this system was considered to be 217.2 mg L^{-1}, i.e. 7.5×10^{-4} mol L^{-1}, which was lower than the optimum concentration of SDS (1.0×10^{-3} mol L^{-1}) in this experiment. Therefore, it was considered that SDS exists as the micellar form in this system.

Addition order and stability of this system. The effect of the adding order on the fluorescence intensity of this system was tested. The result indicated that the addition order of E, AA, Tb^{3+}, BSA, SDS and Tris–HCl buffer was the best. It was considered that early addition of AA prevented oxidation of E by the oxygen dissolved in alkaline solution in favor of the formation of Tb–E complex; then both BSA and SDS were added for the formation of BSA–SDS composite. Finally, the addition of Tris–HCl provided a suitable buffer for both Tb–E complex and BSA–SDS composite to combine into Tb–E–BSA–SDS ionic association.

Under the optimum conditions, the effect of time on the fluorescence intensity was studied. The result shows that the fluorescence intensity reached a maximum within 20 min after all the reagents were added and remained stable for 40 min.

Effect of foreign substances. The interference of foreign substances was tested for 1.3×10^{-5} g mL^{-1} BSA, and the results are

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Table 2. Interference from foreign substances

Foreign substances	Coexisting concentration ($\times 10^{-4}$ mol L^{-1})	Change of ΔI_f (%)
$AlCl_3$	4.0	−5.6
$BaCl_2$	2.0	−4.7
$CaCl_2$	3.5	−3.3
KCl	3.0	−4.2
$MgSO_4$	3.0	−4.7
Na Cl	3.0	−5.7
NH_4Cl	2.0	−4.5
$ZnCl_2$	3.5	+5.0
Dopamine	0.045	+4.8
NE	0.10	+4.6
Trp	0.10	+4.6
Typ	1.5	−4.9
Glucose	0.01%	−4.8
yRNA	1.5×10^{-6} g mL^{-1}	−5.4
ctDNA	2.0×10^{-6} g mL^{-1}	−4.7
fsDNA	2.0×10^{-6} g mL^{-1}	−3.6

Table 3. Analytical parameters of this method

Protein	Linear range (g mL^{-1})	Correlation coefficient	Limit of detection (ng mL^{-1})
BSA	5.2×10^{-9} to 1.3×10^{-5}	0.9997	1.3
HSA	1.7×10^{-8} to 5.3×10^{-5}	0.9986	8.5

shown in Table 2. It can be seen that most of the materials tested except norepinephrine (NE), dopamine, Trp and glucose had little effect on the determination of protein under the limit of ±5% relative error.

Analytical applications

Calibration curve and detection limit

Under the optimum conditions defined here, the calibration graphs for BSA and HSA were obtained and are shown in Table 3. It can be seen that there is a linear relationship between the fluorescence intensity of this system and the concentration of proteins in the range of 5.2×10^{-9} to 1.3×10^{-5} g mL^{-1} for BSA and 1.7×10^{-8} to 5.3×10^{-5} g mL^{-1} for HSA, and their detection limits (S/N = 3) are 1.3×10^{-9} g mL^{-1} and 8.5×10^{-9} g mL^{-1}, respectively. A comparison of this method with other fluorescence methods is summarized in Table 4. It shows that the sensitivity of the proposed method is higher than those of most other methods.

Sample determination

Considering the effects of foreign substances on the fluorescence intensity of the system, an actual sample of human blood serum was tested by the standard addition method under the optimum conditions defined here. This sample of human serum was obtained from the Hospital of Shandong University. The results are shown in Table 5. Compared with ultraviolet spectrophotometry, the accuracy and precision of this method are satisfactory.

Interaction mechanism of the system

Formation of the Tb–E–BSA–SDS complex

Resonance light scattering spectra. Resonance light scattering (RLS) spectra of Tb^{3+}–AA (1), Tb^{3+}–E–AA (2), Tb^{3+}–E–BSA–AA (3) and Tb^{3+}–E–BSA–AA–SDS (4) systems are shown in Fig. 5. It can be seen that when E, E–BSA and E–BSA–SDS were added to the Tb^{3+}–AA system, the RLS intensities of the systems were

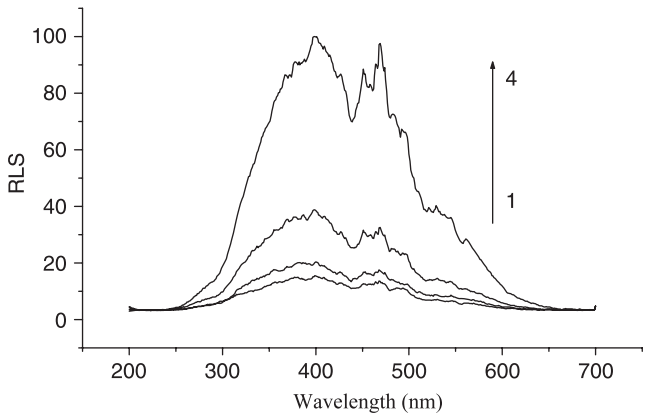

Figure 5. Resonance light scattering (RLS) spectra of the systems. (1) Tb^{3+}–AA–Tris; (2) Tb^{3+}–E–AA–Tris; (3) Tb^{3+}–E–BSA–AA–Tris; (4) Tb^{3+}–E–BSA–AA–SDS–Tris. Conditions: E, 1.0×10^{-4} mol L^{-1}; AA, 4.5×10^{-6} g mL^{-1}; Tb^{3+}, 4.0×10^{-5} mol L^{-1}. BSA, 1.3×10^{-5} g mL^{-1}; SDS, 1.0×10^{-3} mol L^{-1}; Tris–HCl, 0.020 mol L^{-1}, pH = 9.0

Table 4. Comparison with other methods

Method	Linear range of BSA (μg mL^{-1})	Detection limit of BSA (ng mL^{-1})	References
This method	0.0052–0.013	1.3	
Eu(III)–chlorotetracycline	0.20–10	8.9	(20)
Yttrium(III)–sodium lauryl sulfate–rutin	0.0050–10.0	1.6	(23)
Curcumin and SDS	0.0050–20.0	1.4	(27)
Morin–Al^{3+}–cetyltrimerhylammomium bromide	0.0050–20.0	2.6	(28)
Magdala Red	0.1–4.0	100	(29)
Functionalized CdS nanoparticles	0.1–3.0	10	(30)
Terbium–gadolinium–protein –cetylpyridine bromide	0.060–70	23	(6)

Table 5. The analytical results of HSA from actual human blood serum

Method	Determination value (1.3×10^{-5} g mL^{-1})	Average (1.3×10^{-5} g mL^{-1})	RSD (%)
Proposed method	0.323, 0.309, 0.327, 0.330, 0.319	0.322	1.2
UV–spectrophotometric method	0.310, 0.328, 0.336, 0.306, 0.303	0.317	2.3

enhanced. However the RLS intensity of Tb^{3+}–E–BSA–AA–SDS system was the largest. It is considered that a large ionic association was formed in this system. It is well known that E can combine with Tb^{3+} to form Tb^{3+}–E complex with positive charge,[24] whereas SDS is able to combine with BSA to form SDS–BSA composite with negative charge.[6,31] We consider that there is an interaction between SDS–BSA and Tb^{3+}–E through electrostatic attraction to form a large ionic association in this system, which causes the RLS intensity of the system to be greatly enhanced.

CD spectra of BSA. Far-UV CD spectroscopy can give the quantitative information in details on the secondary structure change of protein. Figure 6 shows the far-UV CD spectra of BSA in different systems. The spectra (normalized to residue molar ellipticity) were analyzed with the software DICROPROT by a self-consistent method, and the results are shown in Table 6. It can be seen that, when Tb^{3+}–E and Tb^{3+}–E–SDS were respectively added to BSA–AA system, the two troughs (208.6 and 220.8 nm) in the CD spectrum of BSA decreased and were moved towards long wavelengths. This shows a change in the secondary structure of BSA, i.e. α-helix and β-turn contents increased, whereas β-sheet and random contents decreased.

Luminescence enhancement mechanism

Hydrophobility of the system. Crystal structure analysis has revealed that BSA is made up of three homologous domains: I (residues 1–183), II (184–376) and III (377–583), each containing two subdomains (A and B), which are divided into nine loops by 17 disulfide bonds, each one formed by six helices. Almost all

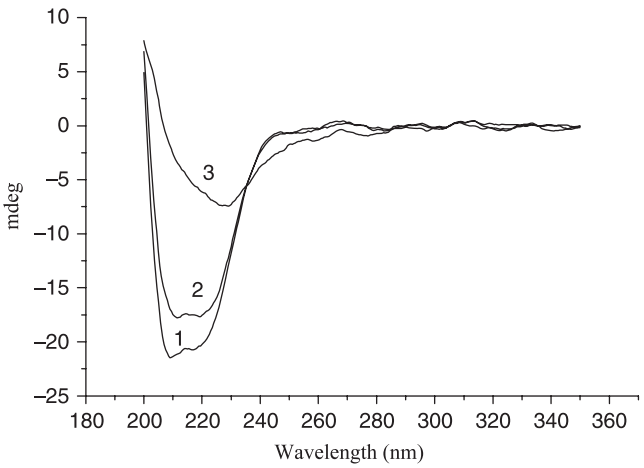

Figure 6. CD spectra of BSA in the systems. (1) BSA–AA; (2) Tb^{3+}–E–BSA–AA; (3) Tb^{3+}–E–BSA–SDS–AA. Conditions: E, 1.0×10^{-4} mol L^{-1}; AA, 4.5×10^{-6} g mL^{-1}; BSA, 1.3×10^{-5} g mL^{-1}; SDS, 1.0×10^{-3} mol L^{-1}; Tris–HCl, 0.020 mol L^{-1}, pH = 9.0.

Table 6. The analytical results for CD spectra of BSA in different systems

	BSA–AA	Tb^{3+}–E–BSA–AA	Tb^{3+}–E–BSA–SDS–AA
α-Helix (%)	26.1	37.2	75.2
β-Sheet (%)	29.0	16.6	0.0
β-Turn (%)	15.4	17.8	24.8
Random (%)	29.5	28.5	0.0
Total (%)	100.0	100	100

hydrophobic amide acids are embedded in the cylinders forming hydrophobic cavities, which play an important role on absorption, metabolism and transportation of biomolecules. The principal regions of ligand binding to BSA are located in hydrophobic cavities in subdomains IIA and IIIA, and one tryptophan residue (Trp 212) of BSA is in subdomain IIA.[33] The interaction between SDS and BSA could induce the unfolding of BSA.[31] As a result, this makes the hydrophobic part of the BSA molecule exposed outside,[6,32] which is favorable for the Tb^{3+}–E complex to bind with the Trp residues (Trp 212) of BSA in the hydrophobic subdomain IIA. The hydrophobic microenvironment provided by both BSA and SDS can be proved by both the polarity and fluorescence polarization of the system in Table 7.

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 change of a system experienced by the pyrene probe.[34] A low value reflects a lower polar environment.

According to the Perrin equation, the microviscosity (η) of the microenvironment can be estimated using the fluorescence polarization of fluorescence probe.[35] A large value reflects a larger microviscosity.

Table 7. The polarity and luminescence polarization of different systems

System	Polarization (P)	I_1/I_3
Tb^{3+}–E–Tris	0.0672	1.557
Tb^{3+}–E–AA–Tris	0.0496	1.631
Tb^{3+}–E–BSA–AA–Tris	0.0913	1.325
Tb^{3+}–E–SDS–AA–Tris	0.194	1.202
Tb^{3+}–E–BSA–SDS–AA–Tris	0.271	1.074

Conditions: E, 1.0×10^{-4} mol L^{-1}; AA, 4.5×10^{-6} g mL^{-1}; Tb^{3+}, 4.0×10^{-5} mol L^{-1}; BSA, 1.3×10^{-5} g mL^{-1}; SDS, 1.0×10^{-3} mol L^{-1}; Tris–HCl, 0.020 mol L^{-1}, pH = 9.0; pyrene, 2.0×10^{-7} mol L^{-1}.

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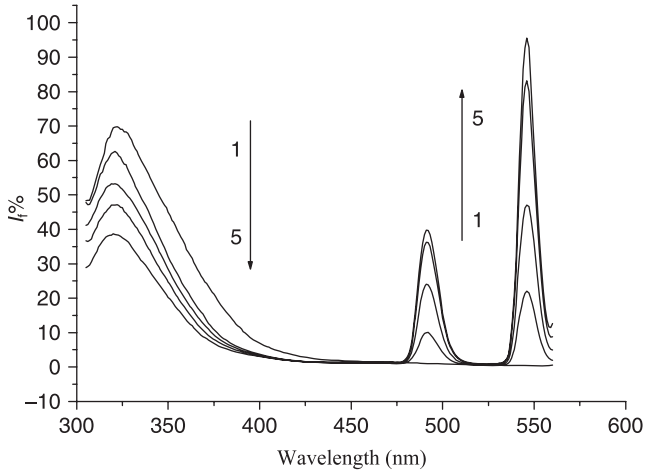

Figure 7. Energy transfer of between BSA and Tb^{3+} (λ_{ex} = 290 nm). (1) BSA–E–AA–SDS; (2–5) Tb^{3+}–BSA–E–AA–SDS. Tb^{3+} (× 10^{-5} mol L^{-1}): (1) 0; (2–5) 0.4, 0.8, 1.2 and 1.6. Conditions: E, 1.0 × 10^{-4} mol L^{-1}; AA, 4.5 × 10^{-6} g mL^{-1}; BSA, 6.5 × 10^{-5} g mL^{-1}; SDS, 1.0 × 10^{-3} mol L^{-1}; Tris–HCl, 0.020 mol L^{-1}, pH = 9.0.

$$\eta = 2P/(0.46 - P)$$

where P refers to the observed fluorescence polarization of the probe.

From Table 7, we can see that the value of I_1/I_3 decreased and the P value increased when BSA, SDS and BSA–SDS were added to the Tb^{3+}–E–AA–Tris system. Based on the above facts, it was considered that BSA–SDS composite not only replaced the water molecules coordinated around Tb^{3+} but also provided an optimum hydrophobic environment with low polarity and large viscosity for Tb–E complex. It was considered that the hydrophobic microenvironment provided by BSA–SDS could prevent the collision between Tb–E complex and water molecules, and decrease the energy loss of the system. Thus, the fluorescence quantum yield was improved and the luminescence intensity of Tb^{3+}–E complex was significantly enhanced.

Energy transfer of the system. The fluorescence peak of 326 nm in this system was attributed to the emission of both E and BSA. Fig. 7 indicates that, after continuously adding Tb^{3+} to the BSA–E–AA–SDS system, the fluorescence intensity of 326 nm was gradually weakened and the characteristic fluorescence of Tb^{3+} was gradually enhanced. This indicates that there is intramolecular energy transfer from both E and BSA to Tb^{3+} in the system. Therefore, the energy transfer between BSA and Tb^{3+} is another reason for the fluorescence enhancement in this system.

In order to testify to above conclusion, the synchronous fluorescence spectra of the systems were tested. The synchronous fluorescence of $\Delta\lambda$ = 15 or 60 nm gave the characteristic information on tyrosine or tryptophan residues of protein. From Fig. 8, it can be seen that, when Tb^{3+} was added into the BSA–E–AA–SDS system, the fluorescence intensities of tyrosine and tryptophan residues of BSA (both $\Delta\lambda$ = 15 and 60 nm) decreased, whereas the characteristic fluorescence of Tb^{3+} was enhanced (as shown in Fig. 8). This indicates that there is intramolecular energy transfer from BSA to Tb^{3+} in the system, resulting in fluorescence enhancement.

The above mechanism of fluorescence enhancement can be proved by the fluorescence lifetime of Tb^{3+} in different systems. The results show that, when SDS and BSA are respectively added to the Tb–E–AA system, the fluorescence lifetime of Tb^{3+} is delayed from 19.06 to 78.77 and 628.79 μs. BSA not only can provide the hydrophobic environment for Tb–E complex, but also acts as the donor of the energy. Thus, the fluorescence lifetime of Tb^{3+} in the Tb–E–AA–BSA system is much longer than that in the Tb–E–AA–SDS system. When BSA and SDS are together added to the Tb–E–AA system, the fluorescence lifetime of Tb^{3+} is greatly prolonged to 860.68 μs. The prolongation of the fluorescence lifetime is attributed to both the protection of the BSA–SDS composite for Tb–E complex and the energy transfer from BSA to Tb^{3+}.

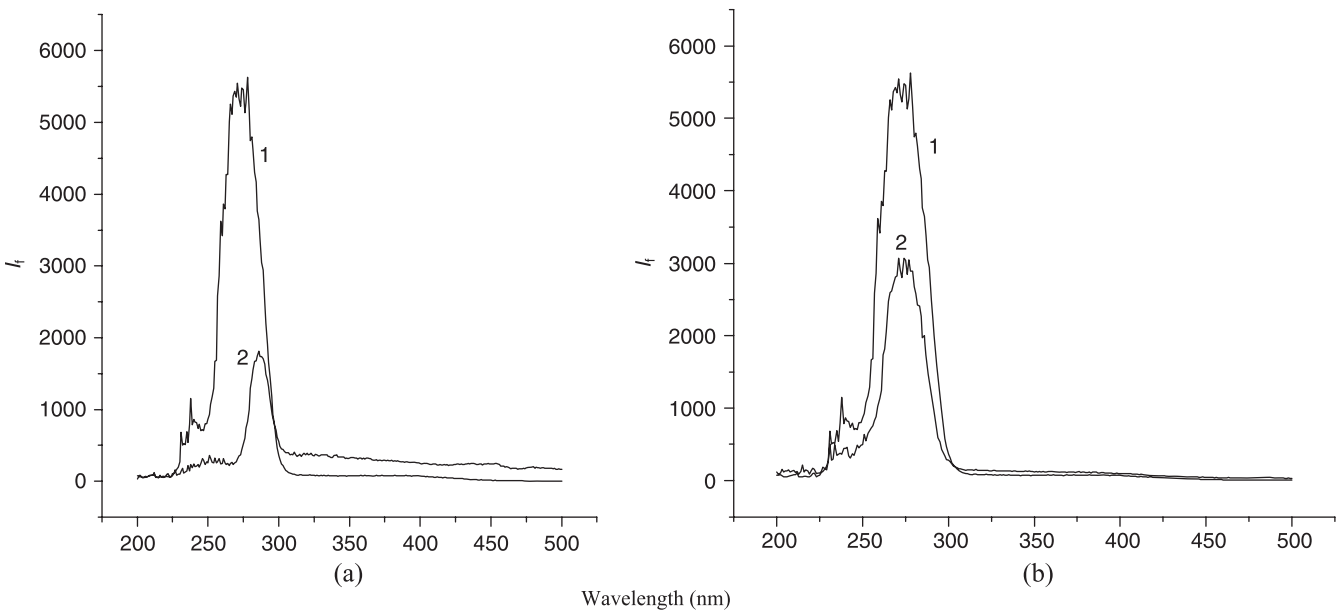

Figure 8. Synchronous spectra of the systems. (a) $\Delta\lambda$ = 15 nm; (b) $\Delta\lambda$ = 60 nm. (1) E–BSA–AA–SDS; (2) Tb^{3+}–E–BSA–AA–SDS. Conditions: E, 1.0 × 10^{-4} mol L^{-1}; AA, 4.5 × 10^{-6} g mL^{-1}; Tb^{3+}, 4.0 × 10^{-5} mol L^{-1}; BSA, 1.3 × 10^{-5} g mL^{-1}; SDS, 1.0 × 10^{-3} mol L^{-1}; Tris–HCl, 0.020 mol L^{-1}, pH = 9.0.

Conclusion

Based on the fluorescence enhancement, a new fluorescence method for the determination of proteins has been reported in this paper. Compared with other luminescence methods, the advantages of the method include low detection limits, simple assay process and wide linear range in response. Therefore, it is considered that Tb^{3+}–E complex can be used as fluorescence probe of proteins and shows potential for application in biochemical investigation, clinical diagnosis and food nutrition.

The mechanism of both the interaction and fluorescence enhancement in Tb^{3+}–E–BSA–AA–SDS system has been studied in detail. There is interaction between SDS–BSA and Tb^{3+}–E through electrostatic attraction to form a large ionic association in this system; the fluorescence enhancement of this system is attributed to both the hydrophobic environment provided by the BSA–SDS composite for Tb–E complex and the energy transfer from BSA to Tb^{3+}. The study is helpful for us to understand the interaction and energy transfer between protein and small molecules. This work will be valuable for exploring new fluorescence probes for proteins.

Acknowledgements

This work was supported by Natural Science Foundations of China (20 575 035) and Shandong Province (Y2003B02).

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