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Luminescence behavior of a water soluble calix[4]arene derivative complex with terbium ion(III) in gelation solution

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Abstract

The complexation luminescence behavior of a water soluble calix[4]arene derivative, 5,11,17,23-tetra-sulfonate-25,26,27,28-tetra-carboxymethoxycalix[4]arene (L) with lanthanoid ion (Tb^{3+}) has been investigated in gelation solution at 25 °C by using UV–vis and fluorescence spectra. The results obtained indicated that the water soluble calix[4]arene derivative can form an efficient energy transfer complex with terbium ion(III). The fluorescence of L·Tb^{3+} complex is partially quenched by gelatin in gelation solution. The quenching intensity is related to the concentration and the hydrolysis degree of gelatin. Absorption and fluorescence spectra analysis show that the $-COO^-$ groups on gelatin have a definite binding ability to Tb^{3+}, and then, gelatin could compete binding with calix[4]arene derivative upon complexation with Tb^{3+}, leading to the relative fluorescence quenching of the formation complex of terbium(III) ion with calix[4]arene derivative.

Keywords: Calix[4]arene derivative; Terbium(III) complex; Gelatin; Fluorescence behavior

1. Introduction

Calixarenes and their derivatives can be viewed as a molecular receptor selective binding a wide variety of guest molecules/ions, forming the host-guest complexes or suparmolecular systems. One of the current interests with this is the luminescence behavior of the complexes formed by calix[4]arene with lanthanide ions because of their potential uses as probes and labels for protein and DNA analysis [1–4]. However, calix[4]arene and its derivatives possess very limited solubility in aqueous solution. Fortunately, they could be modified, e.g. via sulfonation in the upper rim, and be transformed into water-soluble molecular receptor, i.e. calixarenesulfonates [5–7]. Reinhoudt and Shinkai reported some water soluble calix[4]arenes, which have the lowest excited state sufficiently high for the energy transfer to lanthanide ions, but the stability of the complexes of calix[4]arene with lanthanide ions was seldom discussed in protein solution [8,9]. Recently, we have shown that the water soluble 5,11,17,23-tetra-

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sulfonate-25,26,27,28-tetracarboxymethoxycalix [4]arene (L) can form a stoichiometric 1:1 complex with Tb^{3+} [10]. The fluorescence quantum yield of $L{\cdot}Tb^{3+}$ complex is about 0.31, which indicated that $L{\cdot}Tb^{3+}$ complex is one of Tb^{3+} complexes possessing a high quantum yield ($\varphi > 0.2$) in aqueous solution [10,11]. The complexation stability constant ($\lg K_S = 6.3$) of L with Tb^{3+} is higher than that of lanthanum with some amino acids ($\lg K_S = 3 \sim 5$) [12]. On the other hand, Zhao and his coauthor reported that protein could combine with lanthanoid ions, and this combination is not a superfine coordination but a stochastic process [13]. These results prompted us to investigate the luminescence behavior of $L{\cdot}Tb^{3+}$ complex. In present paper, we wish to report our research results on the luminescence properties of $L{\cdot}Tb^{3+}$ complex in gelation solution. A simple reason for choosing gelation solution is that gelatin molecular consists of 18 kinds amino acids linked by peptide linkage. The chain length of gelatin molecular is related to the degree of hydrolysis, which can be identified by measuring the viscosity of the gelatin solution [14]. The results obtained show the luminescence of $L{\cdot}Tb^{3+}$ complex is partially quenched by gelatin, and the gelatin possessing $-COO^-$ binding sites could compete binding with calix[4]arene derivative for complexation with Tb^{3+} ion, leading to the luminescence quenching of $L{\cdot}Tb^{3+}$ complex. The luminescence data analysis also reveals a number of unexpected results that might help to identify the hydrolysis degree of gelatin by the luminescence quenching intensity of $L{\cdot}Tb^{3+}$ complex.

2. Experimental

Water soluble calix[4]arene derivative was synthesized according to our previous report [15] and identified by IR and element analysis ^1H NMR. Terbium nitrate was prepared by dissolving the corresponding oxides of 99.99% purity (Baotou Rare Earth Chem. Co.) in 50% aqueous nitric acid. After evaporation, the solid residue was dried in vacuo for several days and then dissolved in water. The stock solution of Tb^{3+} was standardized by EDTA titration with xylenol orange as an indicator. $1^{\#}$ gelatin was purchased from Beijing Chem. Factory, its viscosity (5% soln.) is 5.088°E and its average molecular weight is about 10^5. $2^{\#}$ gelatin and $3^{\#}$ gelatin were obtained by enzymolysis of $1^{\#}$ gelatin with papain, their viscosity (5% soln.) are 3.175 and 1.446°E, respectively. Generally, the gelatin concentration is expressed as S (g per 100 cm^3). In other words, the 100 cm^3 solution contains the quantity of gelatin.

UV–vis spectra have been performed on a WFZ800-D3B spectrometer (Beijing Ruili Apparatus Co.). Fluorescence spectra were recorded on a Shimadzu RF-5310 PC spectrofluorimeter, the band passes for the excitation and emission monochromators were set at 3 nm. Solution pH value was adjusted to 7.5 with NaOH and HCl on a pHS-P1 acid meter. All the measurement temperature was at 15 °C.

3. Results and discussion

3.1. The interaction of Tb^{3+} with gelatin

Fig. 1 gives the UV–vis absorption spectra of gelatin and gelatin mixed with Tb^{3+}. As can be seen from Fig. 1, the absorption spectrum of $1^{\#}$ gelatin is composed of one band at 292 nm. Comparison of curve 1 and curve 2 found that the band intensity only slightly increase after adding Tb^{3+} to $1^{\#}$ gelatin. The absorption spec-

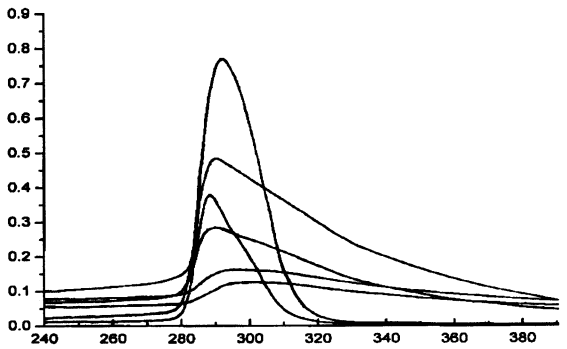

Fig. 1. UV–vis absorption spectra. (1) $1^{\#}$ gel. (0.40 g per 100 cm^3), (2) $2^{\#}$ gel. (0.40 g per 100 cm^3)/Tb^{3+} (1.0×10^{-4} mol l^{-1}), (3) $3^{\#}$ gel. (0.40 g per 100 cm^3), (4) $3^{\#}$ gel. (0.40 g per 100 cm^3)/Tb^{3+} (1.0×10^{-4} mol l^{-1}), (5) L (1.0×10^{-4} mol l^{-1}), (6) L (1.0×10^{-4} mol l^{-1})/Tb^{3+} (1.0×10^{-4} mol l^{-1}).

trum of 3# gelatin has a peak at 292 nm, the maximum absorption wavelength is not shifted, but the intensity has a significant increase after adding Tb^{3+} to 1# gelatin. Comparison of curve 5 and curve 6, the band at 288 nm shifts to 291 nm with concurrent of dramatic increase in intensity after mixing Tb^{3+} with water soluble calix[4]arene (L).

It is well known that the all of phenylalanine, tyrosine, histidine, and tryptophane have UV–vis absorption in main 20 kinds amino acids that form protein. Gelatin is composed of 18 kinds amino acids in which tryptophane is not included. The absorption of gelatin is mainly due to the absorption of tyrosine groups. In aqueous solution, gelatin is charged at different pH and can be expressed as:

$$\underset{pH<5.5}{NH_3{}^+\text{–Gel–COOH}} \overset{H^+}{\leftarrow} \underset{pH\ 5.5}{NH_3{}^+\text{–Gel–COO}^-} \overset{OH^-}{\rightarrow} \times \underset{pH>5.5}{NH_2\text{–Gel–COO}^-}$$

On the other hand, some research indicated that Tb^{3+} ion has a priority to incorporate with oxygen atoms over nitrogen atoms. Therefore, it is suggested the $-COO^-$ groups of gelatin is the main binding sites to Tb^{3+} at pH 7.5. One gelatin molecular has many –COOH groups not only at the side chains (–COOH groups of aspartate and glutamate) but also at C-terminal, and has only some –COOH easy dissociation to be changed to $-COO^-$ at pH 7.5. The $-COO^-$ groups at different position on gelatin are not enough for the coordination number of Tb^{3+}, and then, the binding ability of $-COO^-$ groups with Tb^{3+} is weak, indicating the weak electrostatic interaction between the positively charged Tb^{3+} and the negatively changed $-COO^-$ groups. As compared with 1# gelatin, the molecular chain of 3# gelatin at the same concentration (g per 100 cm^3) is short, and the $-COO^-$ groups at C-terminal have a significant multiplication as the peptide linkage breaking, in other words, the C-terminal binding sites to Tb^{3+} are multiple. Meanwhile, the distance of Tb^{3+} to tyrosine groups is also short, so the absorption intensity of 3# gelatin has a significant increase after adding Tb^{3+}.

Gelatin possessing the tyrosine can produce the relative fluorescence ($\lambda_{em} = 306$ nm) as excited at 278 nm. The emission intensity of gelatin in the presence and absence of Tb^{3+} is listed in Table 1.

As can be seen from Table 1, the emission intensity of gelatin decreases slightly after adding Tb^{3+}. These results are entirely different from the gelatin–Cu^{2+} or gelatin–Fe^{3+} system [16]. Since the strong coordination ability of Cu^{2+} or Fe^{3+} to N and O on peptide linkage, the energy transfer from tyrosine groups to Cu^{2+} or Fe^{3+} should occur as the binding sites approach to tyrosine groups, which results in a significant fluorescence quenching of tyrosine groups [17]. The interaction between Tb^{3+} and $-COO^-$ groups in gelatin is not identified by the characteristic emission of Tb^{3+} at 547 nm as excited at 278 nm, which is a dynamic stochastic process, and then could not efficient affect on the fluorescence behavior of tyrosine groups.

3.2. The fluorescence spectra of $L \cdot Tb^{3+}$ complex in gelation solution

The fluorescence spectra of the complexation of water soluble calix[4]arene (2.0×10^{-6} mol l^{-1}) and Tb^{3+} (2.0×10^{-6} mol l^{-1}) are shown in Fig. 2. The excitation spectra for $L \cdot Tb^{3+}$ complex feature one band at 271 nm as the 547 nm characteristic emission of Tb^{3+} is monitored. The maximum wavelength at 271 nm is not shifted after adding 1# gelatin. The 547 nm emission spectra of $L \cdot Tb^{3+}$ complex are overlapped with the octave band of 271 nm caused by gelatin as excited at 271 nm. So all the emission spectra were measured at 265 nm. The 547 nm emission intensity of $L \cdot Tb^{3+}$ complex in 2# gelation solution and 3# gelation solution are also given in Table 2.

It is clearly seen from Fig. 2 and Table 2 that the emission intensity of $L \cdot Tb^{3+}$ complex is decreased with adding gelatin concentration. But the fluorescence quenching intensity has no linear relationship with gelatin concentration. As shown in Table 2, the emission intensity drop is also markedly dependent upon the hydrolysis degree of gelatin. The decreases in emission intensity of $L \cdot Tb^{3+}$ complex upon the addition of gelatin are mainly

Table 1
Emission intensity ($\lambda_{em} = 306$ nm) of gelatin/Tb^{3+} system ([gel.] = 0.040 g per 100 cm^3)

Tb^{3+} (mol l^{-1})	0.0	1.0×10^{-6}	5.0×10^{-6}	1.0×10^{-5}	5.0×10^{-5}	1.0×10^{-4}
$1^{\#}$ gelatin	29.8	29.6	29.3	29.0	28.8	28.7
$3^{\#}$ gelatin	27.2	26.8	26.4	26.0	25.7	25.3

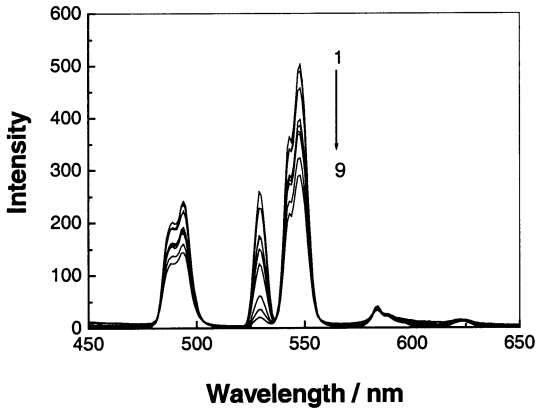

Fig. 2. The emission spectra ($\lambda_{ex} = 265$ nm) of $L \cdot Tb^{3+}$ in the presence and absence of $1^{\#}$ gelatin, from 1 to 9, the amounts of $1^{\#}$ gelatin are 0.005, 0.010, 0.020, 0.060, 0.080, 0.100, 0.160 and 0.200 (g per 100 cm^3), respectively.

attributed to the interaction between gelatin and Tb^{3+}. The combination of Tb^{3+} with gelatin would result in the decrease of the binding ability of calix[4]arene derivative for Tb^{3+}. Furthermore, the peptide chain of $2^{\#}$ or $3^{\#}$ gelatin is shorter than that of $1^{\#}$ gelatin, making the combination ability of $2^{\#}$ or $3^{\#}$ gelatin to free Tb^{3+} enhance, which results in the concentration decrease of $L \cdot Tb^{3+}$ complex.

When [gel.] > [Tb^{3+}], we can suppose that gelatin can form a stoichiometric 1:1 complex with Tb^{3+}. The two dynamic equilibrium equations are established in the mixing system as expressed by Eqs. (1) and (2).

Combining Eqs. (1) and (2) gives Eq. (3).

$$\mathrm{Gel} + \mathrm{Tb}^{3+} \overset{K_1}{\rightleftharpoons} \mathrm{Gel} \cdot \mathrm{Tb}^{3+} \quad (1)$$

$$\mathrm{L} + \mathrm{Tb}^{3+} \overset{K_2}{\rightleftharpoons} \mathrm{L} \cdot \mathrm{Tb}^{3+} \quad (2)$$

$$\mathrm{Gel} + \mathrm{L} \cdot \mathrm{Tb}^{3+} \overset{K_3}{\rightleftharpoons} \mathrm{L} + \mathrm{Gel} \cdot \mathrm{Tb}^{3+} \quad (3)$$

The initial molar concentration of gelatin and $L \cdot Tb^{3+}$ complex are expressed as $[Gel]_0$ and $[L \cdot Tb^{3+}]_0$, respectively, and the equilibrium molar concentration of $Gel \cdot Tb^{3+}$ is expressed as x, K_S for Eq. (3) can be expressed as:

$$K_S = \frac{x^2}{([\mathrm{Gel}]_0 - x)([\mathrm{L} \cdot \mathrm{Tb}^{3+}]_0 - x)} \quad (4)$$

The fluorescence of the mixing system is raised from $L \cdot Tb^{3+}$. It is well known fluorescence intensity (F) is proportional to the concentration of the fluorophore, F can be represented by the general equation.

Table 2
Effects of gelatin ($1^{\#}$, $2^{\#}$ and $3^{\#}$) concentration on the emission intensity of $L \cdot Tb^{3+}$ ([Tb^{3+}] = [L] = 2.0×10^{-6} mol l^{-1})

Gel. concentration (S_0, g per 100 cm^3)	Intensity ($L \cdot Tb^{3+} + 1^{\#}$ gel.)	Intensity ($L \cdot Tb^{3+} + 2^{\#}$ gel.)	Intensity ($L \cdot Tb^{3+} + 3^{\#}$ gel.)
0.000	502.8	502.8	502.8
0.005	490.0	411.5	391.7
0.010	458.2	357.4	276.4
0.020	398.3	334.1	247.1
0.060	386.1	253.5	223.7
0.080	372.8	233.7	186.3
0.100	362.9	212.7	164.1
0.160	323.4	170.8	131.7
0.200	296.5	157.8	110.4

$$F = k([L \cdot Tb^{3+}]_0 - x) \quad (5)$$

$$F_0 = k[L \cdot Tb^{3+}]_0 \quad (6)$$

where F and F_0 are the emission intensity of $L \cdot Tb^{3+}$ complex in the presence and absence of gelatin, K is constant.

When $[Gel]_0 \gg [L \cdot Tb^{3+}]_0$, combining Eqs. (4)–(6) gives Eq. (7)

$$\frac{F}{(F_0 - F)^2} = \frac{1}{(K_S \cdot k[Gel]_0)} \quad (7)$$

Generally, gelatin concentration is expressed as S (g per 100 cm^3), so we can obtain Eq. (8)

$$\frac{F}{(F_0 - F)^2} = \frac{M}{(10K_S \cdot k \cdot S_0)} \quad (8)$$

where K_S is constant, M is the average molar weight of gelatin, and S_0 is the initial concentration of gelatin.

Eq. (8) indicates that $F/(F_0-F)^2$ has a linear relationship with $1/S_0$. The average molar weight of 1# gelatin is about 10^5, 0.020 g per 100 cm^3 is equal to 2.0×10^{-6} mol l^{-1}. For 2# gelatin (or 3# gelatin), 0.020 g per 100 cm^3 means the molar concentration is more than 2.0×10^{-6} mol l^{-1}. In order to keep $[Gel]_0 \gg [L \cdot Tb^{3+}]_0$, only these points ($S_0 \geq 0.060$ g per 100 cm^3) were selected. The linear regression curves are shown in Fig. 3. The linear regression constants for 1# gelatin, 2# gelatin and 3# gelatin are 0.9956, 0.9982 and 0.9916, respectively. The good linearity shows the above supposition is rational.

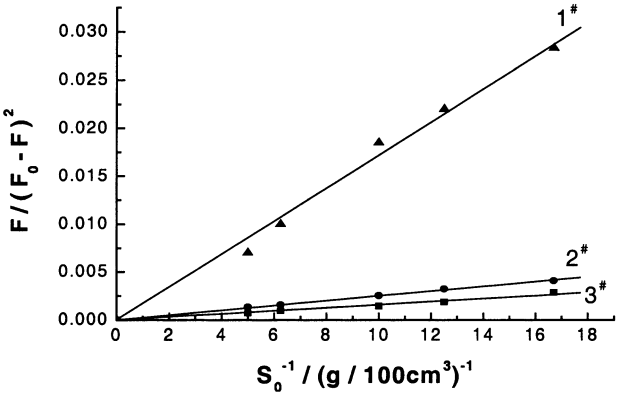

Fig. 3. Linear regression curves of at $F/(F_0-F)^2$ vis $1/S_0$.

4. Conclusion

The investigation on the luminescence behavior of the complex formed by the soluble calix[4]arene derivative with terbium ion(III) in gelation solution indicates that, although gelatin has a definite binding ability to Tb^{3+} and the combination of gelatin with Tb^{3+} is not superfine coordination, gelatin can combine with free Tb^{3+}, which will lead to the fluorescence quenching of $L \cdot Tb^{3+}$ complex. Interestingly, the quenching intensity of $L \cdot Tb^{3+}$ complex is related to the hydrolysis degree of gelatin. Therefore, the calix[4]arene derivative complex with Tb^{3+} can be used as fluorescence probes for the amplifying the interaction beacon of Tb^{3+} with $-COO^-$ groups of gelatin. These results are also suggested that $L \cdot Tb^{3+}$ complex could be used as fluorescence probes for monitoring the enzymolysis process of protein.

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