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PAPER

Fluorescence enhancement by microphase separation-induced chain extension of Eu³⁺ coordination polymers: phenomenon and analysis

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In this paper, we report on the fluorescence enhancement of Eu coordination complexes in dilute solutions through electrostatic complex micelle formation with an oppositely charged block polyelectrolyte. The coordination complexes alone are oligomeric structures which have many ends where partially coordinated europium is exposed to water. In the presence of oppositely charged polyelectrolytes, the local concentration of the coordination complexes is greatly enhanced, so that they transform into polymeric structures and form electrostatically induced micelles with the block polyelectrolytes. This effectively decreases the number of europium–water coordination bonds, which leads to the enhancement of fluorescence emission. This is the first report that utilizes the concentration responsiveness of the smart coordination polymers to promote the function of the colloids made from them.

Introduction

Following recent interests on fluorescent biomedical materials,^{1–3} developing new types of fluorescent assays whose sensitivity is high enough has been the focus of many scientific investigations. Among these, employing the coordination complexes of lanthanide metal ions,^{2–4} especially europium, is one of the main choices, since they possess uniquely narrow and well-defined emission bands and extremely long excited state lifetimes which allow time-resolved detection of luminescence.⁵ Europium ions themselves have very weak fluorescence emissions in aqueous media due to deactivation by water molecules, but the emission can be enhanced by hundreds of times by coordination with proper organic ligands, since the ligands transfer the absorbed UV energy to the center europium ion and replace water in the coordination sphere.⁶ Although many coordination complexes of europium have been proved to have enough luminescent intensity, efforts in enhancing the fluorescence intensity for application in bioassays have never stopped.

In the past decade, methods such as varying ligand structure, using lanthanide mixtures instead of europium alone, as well as utilizing the surface plasmon resonance of noble metals, have

been found efficient in enhancement of fluorescence intensity.^{5,7–9} However, both methods still require the concentration of the fluorescent species to be high enough. In practical biomedical applications, the concentration of fluorescent coordination complexes should be strictly controlled to avoid cytotoxicity. Therefore, it is important to enhance the fluorescence intensity at very low concentrations where equilibrium tends to favor partial coordination. So far, hardly any reported work related to the fluorescence intensity enhancement of europium coordination complexes considered this issue. Here, we report on a new strategy to enhance the fluorescence emission of coordination complexes of europium at very low concentrations.

It is known that many organic ligands containing carboxylic groups are capable of reversibly coordinating with lanthanides. If multitopic ligands are employed, the coordination complexes may form branched polymeric structures with a degree of polymerization which is concentration dependent.^{10,11} At concentrations below a threshold value, only oligomer-like coordination clusters exist, whereas above this critical concentration, branched polymeric structures become dominant which are kept together by reversible lanthanide coordination bonds.¹¹ Like their conventional covalent counterparts, these non-traditional polyelectrolytes are capable of interacting with oppositely charged species. We have reported two typical examples in previous work, where we infer that the coordination oligomers may grow into polymers owing to the enhancement of their local concentration in the presence of the oppositely charged polyelectrolyte block.^{12,13} However, till then we did not have strong proof of such chain extension.

In this paper, we utilize the concentration responsiveness of the coordination polymers to enhance the fluorescence emissions of europium coordination complexes, which can be formed simply by

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mixing $\text{Eu}(\text{NO}_3)_3$ and a bisligand 1,11-bis(2,6-dicarboxypyridin-4-yloxy)-3,6,9-trioxaundecane (L_2EO_4) in aqueous solution. The key point is that we increase their local concentration by introduction of an oppositely charged diblock polyelectrolyte poly(*N*-methyl-2-vinylpyridinium iodide)-*b*-poly(ethylene oxide) (P2MVP₄₁-*b*-PEO₂₀₅, $M_w = 18.5$ K, PDI = 1.05, about 90% quaternized). Therefore, the oligomeric coordination complexes are enriched around the oppositely charged polyelectrolytes and simultaneously transform into polymeric structures. This induces a decrease of the number of coordination ends so that the fraction of fully coordinated europium increases, which consequently enhances the fluorescence intensity of the system. In this process, core-shell like complex coacervate core micelles (C3Ms),¹⁴ which are also known as polyion complexes (PICs)¹⁵ or block ionomer complexes (BICs),¹⁶ are formed. In these structures, the micro-phase separated electrostatic complexes form the micellar core, which is stabilized by a water soluble shell consisting of the charge-neutral block of the block copolymer. Typical C3Ms are easily formed from covalent homo- and block polyelectrolytes.¹⁴ To distinguish our C3Ms formed from coordination polymers, we name them “metallic C3Ms” (MC3Ms). We found that at very low concentrations, formation of MC3Ms from europium coordination complexes indeed enhances the fluorescence emission significantly. What is more striking is that, the lower the concentration of the coordination complexes, the higher the extent of the fluorescence enhancement. This is the first report that explores the concentration responsiveness of the smart coordination polymers to promote the function of the colloids made from them. Meanwhile, it is also strong proof of the microphase separation induced chain extension of reversible coordination polymers. Such an effect had been predicted some time ago,¹⁷ but no direct evidence has so far been obtained.

Experimental section

Materials

The bisligand 1,11-bis(2,6-dicarboxypyridin-4-yloxy)-3,6,9-trioxaundecane (L_2EO_4) and diblock polyelectrolyte poly(*N*-methyl-2-vinylpyridinium iodide)-*b*-poly(ethylene oxide) (P2MVP₄₁-*b*-PEO₂₀₅, $M_w = 18.5$ K, PDI = 1.05, about 90% quaternized) used in this work were prepared according to previously reported procedures.^{10,12} $\text{Eu}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$ (99.99%) was obtained from Sigma.

Stock solutions of P2MVP₄₁-*b*-PEO₂₀₅, L_2EO_4 , and $\text{Eu}(\text{NO}_3)_3$ were prepared at appropriate concentrations. To prepare the Eu coordination complexes, 5 mM L_2EO_4 solution and 10 mM $\text{Eu}(\text{NO}_3)_3$ solution were mixed at desired molar ratios. The coordination complexes were added in stoichiometric amounts to a P2MVP₄₁-*b*-PEO₂₀₅ aqueous solution ($[+] = 1$ mM) to reach charge neutral mixing. Here, $[+]$ stands for the molar concentration of positive charges carried by the polyelectrolyte P2MVP₄₁-*b*-PEO₂₀₅. The positive charge fraction or mixing ratio is defined as $f^+ = [+]/([+] + [-])$, with $[-]$ being the molar concentration of the negative charges of the Eu coordination polymer. Ultra-pure water was used, and no extra salt was added.

Fluorescence spectrometer measurements. A Hitachi F-4500 Fluorescence Spectrometer was used to measure the fluorescence emission of europium(III)-containing solutions. The excitation wavelength was set at 394 nm because this wavelength does no harm to the biological cells. Emission spectra were recorded in the range of 500–700 nm. The emission intensity was normalized with respect to the concentration of europium.

Light scattering measurements. Dynamic light scattering measurements were carried out using a spectrometer of standard design (ALV-5000/E/WIN Multiple Tau Digital Correlator) with a Spectra-Physics 2017 200 mW Ar laser (wavelength: 514.5 nm). The scattering angle was 90° and CONTIN method was used to analyze the distribution of the radii of micelles. Unweighted data were recorded for all experiments. The scattering intensities were recorded and normalized with respect to the total concentration.

Transmission electron microscopy (TEM). A Tecnai F30 TEM was used to observe the morphology of samples. Drops of sample suspensions were deposited onto 230 mesh copper grids that had been coated with formvar, and excess water was removed with filter paper. No staining agents were needed because the systems contain electron-rich metal ions, which give sufficient contrast under TEM.

Results and discussion

Fluorescence enhancement of 2 : 3 mixed $\text{Eu-L}_2\text{EO}_4$ coordination complexes in MC3Ms

Fluorescence emission was negligible for aqueous solutions of $\text{Eu}(\text{NO}_3)_3$ at the concentrations used in our experiments. After mixing aqueous solutions of $\text{Eu}(\text{NO}_3)_3$ and bisligand L_2EO_4 at 2 : 3 molar ratio, a strong fluorescence was observed when the solution was excited by UV light, suggesting the formation of coordination complexes between europium ions and L_2EO_4 . The emission spectra of the complexes display three narrow peaks centered at 595, 617, and 694 nm. According to literature,^{18,19} these peaks can be attributed to $^5\text{D}_0\text{-}^7\text{F}_1$, $^5\text{D}_0\text{-}^7\text{F}_2$, and $^5\text{D}_0\text{-}^7\text{F}_4$ transitions, respectively. If a stoichiometric amount of P2MVP₄₁-*b*-PEO₂₀₅ block copolymer is added to the Eu coordination complexes, the fluorescence emission intensity is

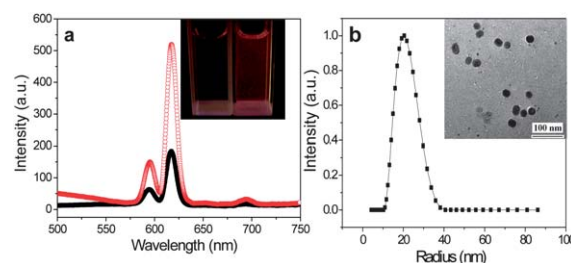


Fig. 1 (a) Comparison of the fluorescence emission of $\text{Eu-L}_2\text{EO}_4$ complexes at a Eu : bisligand ratio of 2 : 3 without (solid symbols) and with P2MVP₄₁-*b*-PEO₂₀₅ (open symbols). The inset shows the photos of before (left) and after (right) addition of P2MVP₄₁-*b*-PEO₂₀₅ in the 0.1 mM $\text{Eu} : \text{L}_2\text{EO}_4$ (2 : 3) complexes. (b) DLS size distribution and TEM image (inset) of the P2MVP₄₁-*b*-PEO₂₀₅/ $\text{Eu-L}_2\text{EO}_4$ (2 : 3) micelles ($[\text{Eu}^{3+}] = 0.295$ mM, $f^+ = [+]/([+] + [-]) = 0.50$).

significantly enhanced (Fig. 1a). It can be clearly seen from the photos of the samples that before addition of P2MVP₄₁-*b*-PEO₂₀₅, the oligomeric Eu-L₂EO₄ complexes exhibit very weak red fluorescence at the measured concentration; whereas strong red fluorescence is observed after addition of P2MVP₄₁-*b*-PEO₂₀₅. It should be pointed out that P2MVP₄₁-*b*-PEO₂₀₅ doesn't bind with Eu³⁺ since the nitrogen in it was quaternized so that it doesn't possess lone electron pairs for coordination any more.

The emission spectra measurements suggest that the fluorescence intensity was enhanced more than two times by mixing the 2 : 3 Eu : bisligand coordination complexes with P2MVP₄₁-*b*-PEO₂₀₅. The unaffected emission peak positions and the considerably enhanced emission intensity suggest that the presence of P2MVP₄₁-*b*-PEO₂₀₅ helps to promote the coordination between europium and the L₂EO₄ bisligand. To further understand the interaction between P2MVP₄₁-*b*-PEO₂₀₅ and the 2 : 3 Eu : bisligand coordination complexes, TEM and DLS measurements were performed on the mixed system. Similar to our previous observations in a zinc system, both results revealed the formation of MC3Ms (Fig. 1b).¹² This is because the microphase separated electrostatic complexes of Eu-L₂EO₄/P2MVP₄₁ are confined and stabilized by the PEO₂₀₅ block, which prevents precipitation. The good contrast of micelles in the TEM image suggests that europium is well confined in the center of the micelles. The size difference for the micelles obtained by DLS and TEM suggests that the thickness of the PEO corona is about 7 nm, whereas the radius of the core which contains the europium coordination complexes is about 10 nm.

Mechanism of the fluorescence enhancement

To verify the origin of the fluorescence enhancement, fluorescence intensities of Eu-L₂EO₄ complexes at various Eu : L₂EO₄ ratios were measured. A significant fluorescence enhancement was observed upon increasing the fraction of L₂EO₄ (Fig. 2a). However, upon addition of P2MVP₄₁-*b*-PEO₂₀₅ to these mixtures, a maximum of the fluorescence enhancement ratio (FER, defined as the ratio of intensities in the presence and in the absence of diblock copolymer) occurs at Eu : L₂EO₄ = 2 : 3; any ratio larger than this leads to a decrease in the extent of the fluorescence enhancement (Fig. 2b). In all these systems with different Eu : L₂EO₄ ratios, addition of P2MVP₄₁-*b*-PEO₂₀₅ leads to MC3Ms, as evidenced by DLS (Fig. 2c) and TEM results (Fig. 2d–e). This suggests that the enhancement of the fluorescence intensity is not caused by the change in environment experienced by the coordination complexes, but by an increase in the number of occupied sites in the coordination shell of the europium ion, since one europium ion can accommodate at most nine coordinating atoms in its shell, three L₂EO₄ ligands fit around one europium ion (Scheme 1c). These ligands then replace the water molecules in the coordination sphere which quenches the fluorescence of europium ions due to the vibration of the OH bond, therefore increasing the fluorescence of Eu. At Eu : L₂EO₄ larger than 1 : 3, all the coordinating sites of all europium ions are supposed to be fully coordinated with L₂EO₄ regardless of the concentration. In this case, chain extension is impossible since the excess L₂EO₄ act as chain stoppers.

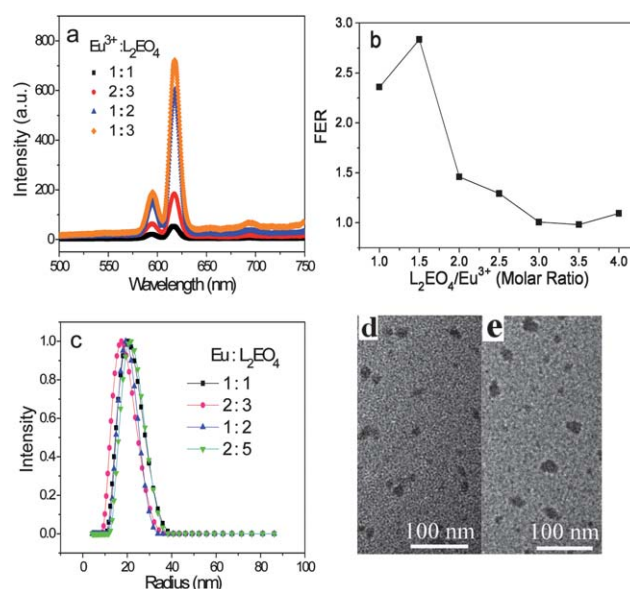
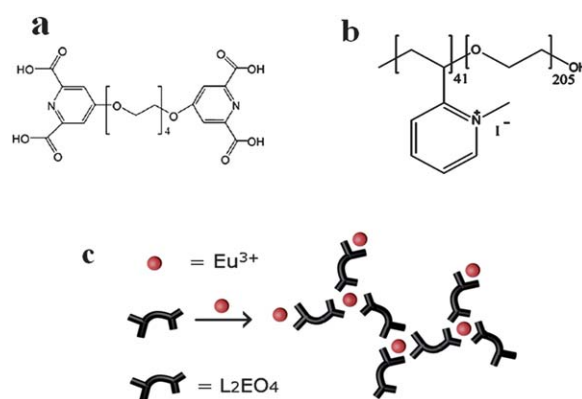


Fig. 2 (a) The emission fluorescence spectra of Eu-L₂EO₄ complexes formed at various molar ratios (without block copolymer). Excitation wavelength = 394 nm, [Eu³⁺] = 0.295 mM. (b) The fluorescence enhancement ratios (FER, defined as the ratio of intensities in the presence and in the absence of diblock copolymer) at various Eu³⁺ : L₂EO₄ ratios. (c) DLS size distribution of the P2MVP₄₁-*b*-PEO₂₀₅/Eu-L₂EO₄ micelles at different Eu : bisligand molar ratios. (d) and (e) TEM images of micelles formed in the P2MVP₄₁-*b*-PEO₂₀₅/Eu-L₂EO₄ mixed systems at different L₂EO₄ : Eu ratios: (d) 2 : 1; (e) 3 : 1.

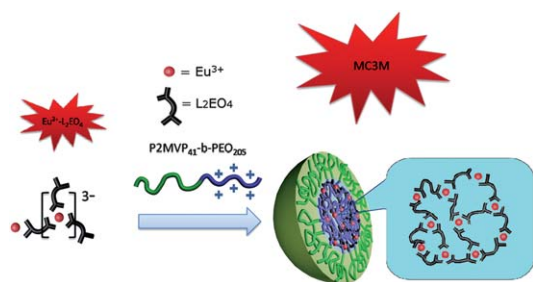
Therefore, no fluorescence enhancement can be achieved upon formation of MC3Ms with P2MVP₄₁-*b*-PEO₂₀₅.

However, for the 2 : 3 mixed Eu : L₂EO₄ system, chain extension may occur inside the micellar core due to the considerably enhanced local concentration. As a result, the initial 'oligomers' transform into 'polymers' in the micellar core, as illustrated in Scheme 2. This induces a decrease in the number of unoccupied sites in the coordination shell of europium ions so that the fraction of fully coordinated europium increases, leading to enhancement of the fluorescence intensity.

This microphase-separation-induced fluorescence enhancement upon formation of MC3Ms was confirmed by experiments in which micelles were destabilized by adding salt (Fig. 3a) or



Scheme 1 Structure of L₂EO₄ (a), P2MVP₄₁-*b*-PEO₂₀₅ (b), and illustration of formation of coordination polymers between Eu³⁺ and L₂EO₄ (c).



Scheme 2 Illustration of the mechanism of the enhancement of fluorescence emission of Eu^{3+} - L_2EO_4 coordination complexes in the presence of $\text{P2MVP}_{41}\text{-}b\text{-PEO}_{205}$. The size of the stars demonstrates the strength of the fluorescence emissions.

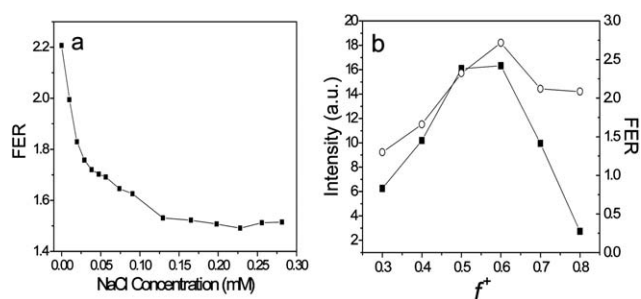


Fig. 3 (a) Effect of NaCl concentration on the fluorescence enhancement of $\text{P2MVP}_{41}\text{-}b\text{-PEO}_{205}/\text{Eu-L}_2\text{EO}_4$ micelles ($\text{Eu} : \text{L}_2\text{EO}_4 = 2 : 3$, $[\text{Eu}^{3+}] = 0.295\text{mM}$, $f^* = 0.50$). (b) Variation of the extent of the fluorescence enhancement (■) and light scattering intensities (○) in $\text{P2MVP}_{41}\text{-}b\text{-PEO}_{205}/\text{Eu-L}_2\text{EO}_4$ micellar systems with mixing ratio f^* . $[\text{Eu}^{3+}] = 1.5\text{mM}$.

shifting the charge ratios in the micellar systems (Fig. 3b). When micellization did not occur, the fluorescence enhancement was significantly reduced. This is as expected: with addition of NaCl, the charges on $\text{P2MVP}_{41}\text{-}b\text{-PEO}_{205}$ and $\text{Eu-L}_2\text{EO}_4$ coordination complexes are screened, so that micelles gradually disintegrate. As a result, the $\text{Eu-L}_2\text{EO}_4$ coordination complexes change from ‘polymers’ to ‘oligomers’. Correspondingly, the fluorescence emission decreases. Similarly, when shifting the charge ratios, the micelles were destroyed as well due to repulsion in the electrostatic complexes. Therefore, a fluorescence maximum can be expected at a proper charge ratio. This normally occurs at charge neutral mixing ratio, namely, $f^* = 0.5$ in the present case. However, as is shown in Fig. 3b, the fluorescence maximum was observed to occur at $f^* = 0.6$, which was probably induced by experimental error. Anyway, this fluorescence maximum agrees very well with that of the strongest light scattering intensity, suggesting that the formation of well-defined micelles is synchronized with the occurrence of the largest FER.

Effect of concentration and pH on the fluorescence intensity

After understanding the mechanism of the fluorescence enhancement in the present MC3M system, it can be expected that the fluorescence enhancement should be more significant at lower concentrations, since there are more partly coordinated europium ions than at higher concentrations. Indeed, as revealed in

Fig. 4a, although the fluorescence intensity decreases with decreasing concentrations, the fluorescence enhancement ratio increases with decreasing concentration. This means that without the formation of MC3Ms, the coordination system itself exhibits much weaker fluorescence at lower concentrations, since the degree of polymerization of coordination polymer decreases with concentration. Therefore, such a fluorescence enhancement is more significant at lower concentrations, which suggests that upon formation of MC3Ms, much lower concentrations of coordination complexes might be used with respect to that of the pure solutions of coordination complexes in order to achieve the same fluorescence intensity.

It is also very relevant to probe the influence of pH on the fluorescence enhancement regarding its potential application in biosystems. Fig. 4b demonstrates that at acidic pH, the fluorescence enhancement can be as large as more than 6 times! What is more significant is, although the fluorescence intensities of the $\text{Eu} : \text{L}_2\text{EO}_4 = 2 : 3$ coordination complexes vary with pH, once they are cooperated into the MC3Ms, the fluorescence intensity turns out to be of a similar level. This means that upon phase separation induced formation of MC3Ms, these fluorescent colloidal objects can be applied in a wide pH range from 4 to 10. The larger FER at lower pH is ascribed to the pH dependent nature of the bisligands: at acidic pH, due to the protonation of COO^- groups which decreases their coordination ability, less coordinating sites of the europium ions are occupied by the ligands, so that these sites are replaced by water which quenches the fluorescence of europium. In contrast, at basic pH, the COO^- groups of the ligand are deprotonated and bind with the europium ions more easily. As a result, the fluorescence of the coordination complexes is relatively higher than at lower pH. However, upon formation of MC3Ms with oppositely charged cblock polyelectrolytes, the transformation of coordination complexes into ‘polymers’ smears the influence of pH, because as revealed in Fig. 4a, this pH influence on the coordination complexes can be completely overcome simply by formation of MC3Ms.

Conclusions

In conclusion, we find that significant fluorescence enhancement of europium coordination complexes in dilute aqueous systems occurs when they are taken up in MC3Ms. The more dilute the

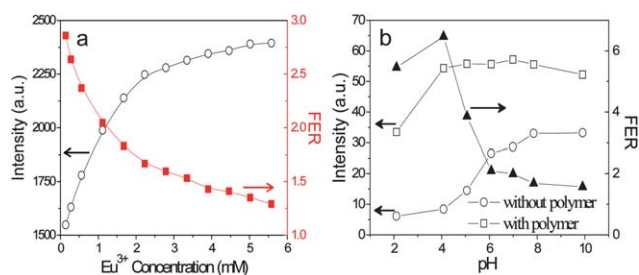


Fig. 4 (a) Effect of the concentration of $\text{Eu-L}_2\text{EO}_4$ ($\text{Eu} : \text{L}_2\text{EO}_4 = 2 : 3$) on the fluorescence enhancement ratio (FER). Spheres (○) and squares (■) are the fluorescence intensities of the $\text{Eu} : \text{L}_2\text{EO}_4 = 2 : 3$ coordination systems with and without $\text{P2MVP}_{41}\text{-}b\text{-PEO}_{205}$ ($f^* = 0.50$). (b) Effect of pH on fluorescence enhancement, in which the left axis refers to fluorescence intensity.

solution is, the stronger the fluorescence enhancement appears. The origin of this effect is the increase in degree of polymerization of the (branched) coordination polymers (driven by the formation of a dense complex coacervate that forms the micellar core), and the concomitant reduction in the number of water ligands in the coordination shell of the lanthanide ions. This is the first example that uses the concentration responsiveness of coordination polymers to promote the function of micelle systems composed of them.

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