A novel europium(III) complex with versatility in excitation ranging from infrared to ultraviolet

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One- and two-photon absorption properties of a novel europium(III) complex containing a hemicyanine cation are investigated in this paper; in which the sensitization wavelength of europium(III), induced by beta-diketonate ligands, is in the ultraviolet range, and the hemicyanine(aminostyrylpyridinium) cation extends the sensitization wavelength of Eu(III) to visible region. Furthermore, under 1.06 μm ultrashort pulse laser excitation, the complex exhibits effective europium(III) luminescence induced by the two-photon absorption of the hemicyanine cation. Therefore, this new europium(III) complex with excitation bands in the near infrared, visible and ultraviolet ranges. Since the 1.06-μm ultrafast laser is one of the most frequently used laser sources and 1.06 μm is an ‘optical window’ for cells and tissues, the results suggest that this kind of complex has promising applications in deep-penetrating and high quality bioimaging in vivo.

1. Introduction

Lanthanide ions and their coordination complexes have been attracting considerable attention owing to their unique optical properties, such as high color purity and long lifetimes,1,2 which give rise to many potential applications, including biological imaging3–5 and electroluminescent devices.6 Along with the development of two-photon fluorescent materials7–9 and ultrafast lasers, two-photon scanning microscopy have been used extensively. Two-photon absorption (TPA) extends the excitation wavelength to the long-wavelength region, which is in favor of less-harmful labeling and deep-penetrating bioimaging applications. Recently, with a combination of the advantages of both lanthanide and two-photon scanning microscopy, two-photon sensitized luminescence of lanthanide complexes attracts great attention, in which the luminescence is aroused via TPA of chromophores and subsequent energy transfer to the lanthanide ions.

In this new challenging field, since coordination chemistry of europium(III) shows similarity to that of Ca2+ and Fe3+, europium(III) ion is the most commonly used lanthanide ion as luminescent probe in biomolecular systems. Therefore, developing europium(III) complexes with a large TPA cross-section for two-photon scanning microscopy is expected. Now, many effective europium(III) complexes with TPA wavelength in the range of 700–900 nm have been reported.10–16 However, the europium(III) complex with TPA wavelength at 1.06 μm has not been reported. The 1.06-μm ultrashort pulse laser is one of the most commonly used laser sources,17 and 600–1100 nm is the ‘optical window’ of cells and tissues.18 With a red-shift of the excitation wavelength, the photolytic damage of living cells is reduced.19 Therefore, there is an urgent need to develop the europium(III) complex with efficient luminescence at the excitation wavelength of 1.06 μm.

In this paper, we report a novel europium(III) complex (trans-4-[p-(N,N-diethy lamino)styryl]-N-methylpyridinium tetrakis(η-thenoyl trifluoroacetonato) europium(III)), [Eu(tta)4DEASPI] (as shown in Scheme 1) that meets the urgent need described above. In this ternary complex Eu(tta)4·DEASPI, the important and innovative point is using trans-4-[p-(N,N-diethy lamino)styryl]-N-methylpyridinium (DEASPI) as a one- and two-photon sensitizer for europium ion, which makes the excitation band of this europium(III) complex cover the range from the near infrared and visible to the ultraviolet. i.e. DEASPI cation extends the one-photon sensitization wavelength of europium ion to visible region and TPA to 1.06 μm. The photophysical properties also demonstrate that the energy transfer from DEASPI to europium(III) is through the charge transfer (CT) states of DEASPI.

2. Experimental

2.1 Syntheses and structures of the molecules

The europium(III) complex [Eu(tta)4·DEASPI] was synthesized by using DEASPI as counterion to combine with a europium(III)–thenoyl trifluoroacetonato (ttta) moiety.20 The synthesis route of Eu(tta)4·DEASPI is shown in Scheme 1. Here, DEASPI is a stilbene-type chromophore that shows donor–π-bridge–acceptor (D–π–A) character. The D–π–A structure is a typical CT structure. In DEASPI, the nitrogen of the pyridinium group loses an electron and makes the group prone to attract electrons from the amino group on another end of the DEASPI, i.e. pyridinium group plays as an acceptor
and the amino group plays as a donor. DEASPI is synthesized by the reaction of 4-(N,N-diethylamino)benzaldehyde with 4-methyl-N-methylpyridinium iodide (Scheme 1); and it is first proposed as a TPA sensitizer for emission of Eu\(^{3+}\). The molecular structure of Eu(tta)\(_4\)/C\(_1\)DEASPI is as follows: the Eu\(^{3+}\) ion is coordinated by eight oxygens from four bidentate deprotonated tta (as shown in Scheme 2). The anion Eu(tta)\(_4\)/C\(_0\) is balanced in charge by the pyridine-cation. DEASPI is close to europium ion through Coulombic interaction.

2.2 Methods

Nuclear magnetic resonance spectra were measured with a FX-90Q NMR spectrometer; and element analyses were performed with a Perkin 2400(II) autoanalyser. The melting points and decomposition temperatures were measured with a Perkin Elmer DTA 1700 differential thermal analyzer and a Perkin Elmer TGS-2 thermogravimetric analyzer at a heating rate of 20\(^\circ\)C min\(^{-1}\) under nitrogen atmosphere, respectively.

**Synthesis of 4-methyl-N-methyl-pyridinium iodide (A).** Using a three-neck flask fitted with a stirrer, thermometer, and condenser, 4-picoline (1 equiv.) and methyl iodide (1.1 equiv.) were mixed in toluene. The solution was stirred at room temperature for 4 h and then refluxed for 30 min. After cooling, the solution was filtered and the solid was washed with ethyl ether. The pale yellow solid was dried under vacuum, yield 21.2 g (90%). \(^1\)H NMR (DMSO-d\(_2\)) \(\delta\) 2.56 (s, 3H), 4.20 (s, 3H), 7.0 (d, 2H), 8.90 (d, 2H) ppm.

**Synthesis of trans-4-[p-(N,N-diethylamino)styryl]-N-methyl-pyridinium iodide (B).** A mixture of A (1 equiv.) and 4-(N,N-dimethylamino)-benzaldehyde (1 equiv.) in dried ethanol was treated with piperidine and was refluxed for 4 h. The resulting suspension was cooled. The precipitate was filtered off. Yield (82%) and \(T_d\) 264.7 \(^\circ\)C. Purification by column chromatography on silica gel using ethanol as eluent. \(^1\)H NMR (DMSO-d\(_6\)) \(\delta\): 8.67 (2H, d, \(J\) 6.84 Hz), 8.04 (2H, d, \(J\) 6.84 Hz), 7.91 (1H, d, \(J\) 15.61 Hz), 7.58 (2H, d, \(J\) 8.79 Hz), 7.12 (1H, d, \(J\) 15.63 Hz), 6.75 (2H, d, \(J\) 8.80 Hz), 4.18 (3H, s), 3.43 (4H, q, 6.84), 1.13 (6H, 6.84). Anal. calcd for C\(_{18}\)H\(_{23}\)N\(_2\)I: C 54.83, H 5.88, N 7.10; found: C 54.94, H 5.92, N 7.06.

**Synthesis of trans-4-[p-(N,N-diethylamino)styryl]-N-methyl-pyridinium tetrakis(\(\alpha\)-thenoyltrifluoroacetanato)europium(III).** To a mixture of B (1 equiv.) and of HTTA (4 equiv.) neutralized with aqueous ethanol–NaOH solution (4 equiv.) was added dropwise of aqueous Eu(NO\(_3\))\(_3\) (1 equiv.) under constant stirring. The resulting precipitate was filtered off. A nearly saturated solution of the precipitate in 95% ethanol was allowed to evaporate slowly, and after 24 h red powder was obtained. Anal. calcd for C\(_{50}\)H\(_{43}\)N\(_2\)F\(_{12}\)O\(_8\)S\(_4\)Eu: C 45.91, H 3.31, N 2.14; found: C 45.94, H 3.52, N 2.46.

The UV-vis absorption spectra were recorded by a UV-Vis-NIR scanning spectrophotometer (Shimadzu, model UV-3101PC). The fluorescent spectra were measured by a fluorescence spectrophotometer (Hitachi, model F-4500). The quantum yield was measured by the standard comparison method\(^{21}\) using Rhodamine 6G as the reference standard.

For the measurement of transient behavior, a Nd:YAG picosecond pulse laser with a repetition rate of 10 Hz was used as an excitation source, its pulse duration was 40 ps. The
excitation wavelength was 1.06 μm. An optical multi-channels analyzer was used as the recorder. The ultrafast laser beam passed firstly through a couple of Nicol’s prisms, which were used as an attenuator to obtain a tunable excitation intensity. The laser beam was focused into the sample by a lens of f = 5 cm. The fluorescence was collected by a telescope system, at the perpendicular direction of the pump beam. Before the input slit of optical multi-channels analyzer, a HA30 filter was inserted for cut off the excitation laser. To minimize the re-absorption effect, the excitation beam was adjusted as closely as possible to the front wall of the cell.

The TPA cross section δ was determined by comparing its TPA induced up-conversion fluorescence to that of Rhodamine 6G according to eqn (1):22

\[
\delta = \delta_{\text{cal}} \frac{F_{\text{HEC}}}{F_{\text{cal}}} \frac{\phi_{\text{cal}}}{\phi}
\]

Where, φ is the fluorescence quantum yield, c is the concentration, n is the refractive index, and F is the intensity of up-conversed fluorescence. The cal subscript refers to the standard reference solution.

3. Results and discussion

3.1 Linear spectroscopy

The UV-vis absorption spectra of DEASPI and Eu(tta)\textsubscript{4}\textsuperscript{−} in acetonitrile at the concentration of 1 × 10\textsuperscript{−5} mol L\textsuperscript{−1} are shown in Fig. 1(a). The absorption band of Eu(tta)\textsubscript{4}− is shorter than 410 nm; it demonstrates that, under our experimental conditions and at the wavelength longer than 410 nm, the f–f transitions of Eu\textsuperscript{3+} are negligible compared with the high absorption coefficient of DEASPI. The absorption peak of DEASPI is around 485 nm. While, Fig. 1(b) shows the UV-vis absorption spectrum and fluorescent emission spectra of Eu(tta)\textsubscript{4}−. In the absorption spectrum of Eu(tta)\textsubscript{4}−, DEASPI, two absorption bands around 340 nm and 485 nm are observed, in which, the ultraviolet absorption band is attributed to tta,23 and the blue one results from DEASPI. It is found that the molar absorption coefficient of DEASPI in Eu(tta)\textsubscript{4}− has the same order of magnitude with tta.

In the fluorescent spectra of Eu(tta)\textsubscript{4}−, DEASPI (dash-dot line for λ\textsubscript{ex} = 340 nm, dashed line for λ\textsubscript{ex} = 485 nm in Fig. 1(b)), the distinct emission peak of 611 nm corresponds to the hypersensitive transition \textit{5}D\textsubscript{0} → \textit{7}F\textsubscript{2} of Eu\textsuperscript{3+}.

At the excitation wavelength of 340 nm, there are only emission peaks of Eu\textsuperscript{3+} in the emission spectrum. At the excitation wavelength of 485 nm, the emission spectrum of Eu(tta)\textsubscript{4}− consists of not only the emission band of DEASPI, but also the characteristic emission peak \textit{5}D\textsubscript{0} → \textit{7}F\textsubscript{2} of Eu\textsuperscript{3+}. We measured the excitation wavelength dependent emission spectra of Eu(tta)\textsubscript{4}−. In detail: as the excitation wavelength shorter than 480 nm, the emission intensity of Eu(tta)\textsubscript{4}− DEASPI increases with the redshift of the excitation wavelength. While the excitation wavelength longer than 480 nm, the emission intensity decreases with the redshift of the excitation wavelength. For comparison, we also measures the emission of Eu(tta)\textsubscript{4}−, but it was too weak to be measured in this excitation wavelength range. Therefore, it can be concluded that the fluorescence of Eu\textsuperscript{3+} in Eu(tta)\textsubscript{4}− DEASPI under 485 nm excitation is sensitized by DEASPI rather than f–f transitions of Eu(III). The fluorescence quantum yield of Eu(tta)\textsubscript{4}− DEASPI excited at 352 nm is 0.28. The results above demonstrate that Eu(tta)\textsubscript{4}− DEASPI extends the excitation wavelength of europium(III) from UV of Eu(tta)\textsubscript{4}− to the visible range.

![Fig. 1](image1.png)

**Fig. 1** (a) The UV-vis absorption spectra of Eu(tta)\textsubscript{4}− (solid) and DEASPI (dash) in acetonitrile (1 × 10\textsuperscript{−5} mol L\textsuperscript{−1}). (b) The UV-vis absorption spectrum and fluorescent emission spectra of Eu(tta)\textsubscript{4}− DEASPI in acetonitrile (1 × 10\textsuperscript{−5} mol L\textsuperscript{−1}), in the fluorescent emission spectra of Eu(tta)\textsubscript{4}− DEASPI, solid line: λ\textsubscript{ex} = 485 nm; dash-dot line: λ\textsubscript{ex} = 340 nm.

![Fig. 2](image2.png)

**Fig. 2** The excitation wavelength dependent emission spectra of Eu(tta)\textsubscript{4}− DEASPI.
3.2 Relationship between molecular structure and the efficiency of energy transfer

DEASPI is a D–π–A structure. Changing the terminal group can affect the strength of CT dipole moment and the efficiency of energy transfer. In order to study the effect of molecular architecture on the efficiency of antenna–Eu$^{3+}$ energy transfer, we prepared a family of ternary europium(III) complexes 1–4 (Eu(tta)$_4$DEASPI as complex 1) by changing the length of the alkyl chain in the terminal group of cations and the substituting group in β-diketone ligands (see Scheme 3). For purposes of comparison, complex 1, 2, 3 were synthesized with the same ligand, and the terminal donor groups of cations are ethyl, methyl and propyl chains, respectively; and a new substituting group of ligand was introduced in complex 4. The emission spectral band structures of complexes 1–4 (see Fig. 3) are similar but with different peak intensities. The intensity ratio between Eu$^{3+}$ ion and cations can be considered as a good measure of energy transfer efficiency. The experimental results show that the energy transfer efficiency of complex 1 is the highest while complex 4 is the lowest, and efficiency of complex 2 is approximately equal to complex 3. It can be explained as follows: the ethyl chain of complex 1 compared with the methyl chain of complex 2 extends the arm length of dipole moment, so it has a larger CT dipole moment, which leads to the higher energy transfer efficiency. However, the long alkyl chain of complex 3 is curved due to charge attraction, the curve shortens the arm length of dipole moment, which results the decrease of CT dipole moment. In complex 4, the complicated structure increases the distance between donor and acceptor, which makes the energy transfer efficiency decrease.

3.3 Two-photon absorption spectroscopy

Because DEASPI possesses a large TPA cross-section at 1.06 μm, and europium(III) can be sensitized by the energy transfer from DEASPI, it is an important characteristic of these molecules that luminescence of europium(III) induced by the TPA of DEASPI with 1.06 μm laser. Excitation by 1.06 μm ultrashort pulses laser, a distinct red emission of Eu$^{3+}$ ion in Eu(tta)$_4$DEASPI is observed (Fig. 4). Fig. 5 shows the relationship between fluorescent intensity and excitation density. The excitation–output relationship is an exponential line with exponent of 2.04, which indicates that the up-converted fluorescence is induced by a three-order nonlinear optical process, i.e. a TPA process. In ref. 10, the TPA wavelength of Eu$^{3+}$ is around 800 nm. For comparison, we measured the emission spectra of Eu(tta)$_4$ at the same condition as that for Eu(tta)$_4$DEASPI. The results show that the luminescent intensity of Eu(tta)$_4$ is too weak to be measured in our system; so, the possibility of sensitization via f–f transition can be ruled out. Therefore, the TPA-induced luminescence of Eu(tta)$_4$DEASPI is sensitized by DEASPI. The TPA cross-section of Eu(tta)$_4$DEASPI is 112 GM at 1064 nm (1 GM = 10$^{-50}$ cm$^4$ photon$^{-1}$ s$^{-1}$). Compared Fig. 3 with Fig. 4, it shows that the two-photon sensitized luminescent structures.
states. The luminescence of Eu$^{3+}$ sensitized by TPA of DEASPI to 1.06 μm laser satisfies the need of less-harmful labeling and in vivo processes of Eu(tta)$_4$-DEASPI are the same. Fig. 6 describes the sensitization processes from DEASPI and tta to Eu$^{3+}$.

4. Conclusions

We synthesize a new kind of europium(III) complex, Eu(tta)$_4$-DEASPI. Our experimental results demonstrate that the excitation band of this new europium(III) complex covers the range from the near infrared and visible to the ultraviolet. In this new molecule, the functional counterion DEASPI plays an important role: its one-photon absorption extends the sensitization wavelength of Eu(tta)$_4$-DEASPI to visible region; and its TPA extends the sensitization wavelength of Eu(tta)$_4$-DEASPI to 1.06 μm. The experimental results demonstrate that the antenna-europium ion energy transfer is through CT states. The luminescence of Eu$^{3+}$ sensitized by TPA of 1.06 μm laser satisfies the need of less-harmful labeling and high-quality deep-penetrating bioimaging in vivo.

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