Lanthanide Luminescence

Efficient Two-Photon-Sensitized Luminescence of a Europium(III) Complex**

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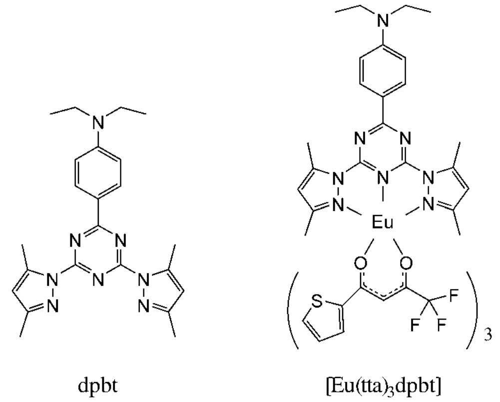
The luminescence of lanthanide(III) coordination complexes is unique in their high color purity, long lifetimes, and insensitivity to environmental quenching.[1] These advantages have attracted intensive research efforts in the development of photosensitized and electrochemically driven luminescent materials[2] and luminescent biolabels.[3] Whereas the poor absorptivity of lanthanide ions owing to the parity-forbidden nature of the inner-shell f–f transition is readily overcome by the introduction of chelating chromophores with large extinction coefficients, it has long been a challenge to extend the wavelength range of photosensitization which is inherently hampered by the restriction of ligand-to-metal excitation energy transfer (EET) from the triplet excited state of the ligand.[4] Very recently, we successfully extended the sensitization wavelength up to 460 nm by incorporating a novel ligand, 2-(*N*,*N*-diethylanilin-4-yl)-4,6-bis(3,5-dimethylpyrazol-1-yl)-1,3,5-triazine (dpbt), into a europium(III)–thenoyltrifluoroacetonato (tta) complex, [Eu(tta)$_3$dpbt] (Scheme 1). The luminescence of the Eu^{III} ion in this complex, with a quantum yield of 0.52 ($\lambda_{em} = 614$ nm), was sensitized by efficient EET from the lowest singlet excited state (S$_1$) of dpbt to the Eu^{III} center.[5] The *N*,*N*-diethylaniline group in dpbt functions as an electron donor (D) and the dipyrazolyltriazine moiety acts as an electron acceptor (A). Therefore, dpbt is a polar chromophore that shows D–A character, which is known to be the structural basis for an efficient two-photon absorber.[6]

As a newly emerging frontier with important application prospects, two-photon excitation (TPE) of a light-harvesting chromophore and subsequent EET to another functional chromophore in close proximity has been brought into practice by the use of femtosecond-pulsed laser sources.[7] Recently, the TPE–EET strategy has found an increasing number of applications. For instance, in photosynthetic pigment–protein complexes, two-photon-excited carotenoids transfer excitation energy to fluorescent (bacterio)chlorophylls—this was used to detect the optically forbidden excited states of carotenoids which play key roles in the photochemistry of photosynthetic carotenoids.[8] In a newly reported porphyrin-centered dendritic complex, porphyrin was activated by fluorescence resonance energy transfer (FRET) from the covalently bonded TPE peripheral chromophores and sensitized the production of singlet oxygen, which is crucial in photodynamic therapy.[7a] Lanthanide(III) ions directly coordinated to proteins, nucleic acids, and biologically relevant chromophores have been shown to act as luminescent biolabels upon TPE sensitization.[9] Here, TPE–EET provides a new way for extending the wavelengths needed for sensitization which may lead to less-harmful and deep-penetrating bioimaging applications.



Scheme 1. Structures of the ligand dpbt and the [Eu(tta)$_3$dpbt] complex.

Intrinsic biological fluorophores directly chelated to lanthanide ions generally have low efficiencies of two-photon sensitization owing to their low two-photon-absorption cross sections: for example, the two-photon-absorption cross sections of amino acids such as tyrosine residues in a Tb^{III}–protein complex lie between 0.4–7.4 GM in the spectral region of 502–566 nm.[9c,10] This low efficiency may be an inherent restriction for the intrinsic TPE sensitizers. Herein, we report the efficient TPE–EET sensitization of Eu^{III} luminescence in the [Eu(tta)$_3$dpbt] complex. The two-photon-absorption cross sections of dpbt in both the free form and when chelated to Eu^{III} were measured over the spectral region of 730–830 nm by the use of femtosecond laser pulses: the maximal values are 185 and 157 GM for dpbt and [Eu(tta)$_3$dpbt], respectively. This Eu complex displays both efficient two-photon sensitization and high-purity red emission.

In Figure 1 we see that the fluorescence excitation spectrum of dpbt in toluene is identical to the one-photon-absorption spectrum. This suggests that the absorption band between 300 nm and 400 nm results mainly from the absorptive transition from the ground state (S$_0$) to the S$_1$ state, which undergoes efficient vibrational relaxation upon one-photon excitation. Importantly, the absorption/excitation and the

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[**] We are grateful for the Grants-in-Aid from the NSFC (grants 20273077, 20433010, 90206011, and 29925308) and the Major State Basic Research Development Program (G2000077503).

DOI: 10.1002/ange.200462382

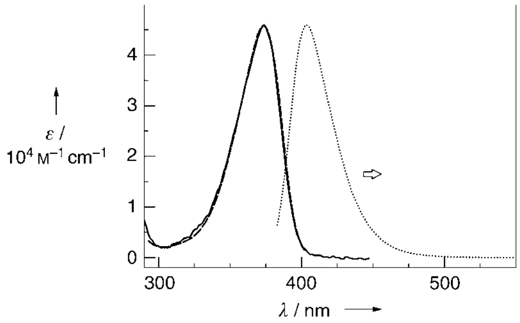

Figure 1. Room temperature UV/Vis absorption (——), fluorescence excitation (- - -; $\lambda_{em}=405$ nm), and fluorescence emission (•••••; $\lambda_{ex}=375$ nm) spectra for dpbt in toluene (7.2×10^{-6} M). Spectra are normalized to the absorption maximum.

fluorescence spectra display a perfect mirror-image relationship, which indicates little change in the molecular configurations of a dpbt molecule in either the S_0 or S_1 states. This structural rigidity meets the requirement for an efficient two-photon absorber.

Spectroscopic data (linear absorption and fluorescence) for dpbt in six organic solvents of different polarities are provided in Table 1. Upon increasing the solvent polarity, the absorption and the fluorescence maximal wavelengths show systematic bathochromic shifts and the Stokes shift increases in parallel, whereas the fluorescence quantum yield drops significantly. This spectroscopic behavior reflects the intramolecular charge transfer (CT) character of the S_1 state[11,12] and is strongly supported by a theoretical calculation on the molecular orbitals. The promotion of an electron in dpbt from the HOMO into the LUMO (see Figure 2) results in a significant change of the electron-density distribution over the diethylaniline and the dipyrazoletriazine moieties. Therefore, the corresponding optical excitation clearly has a photon-induced CT character, and a significant change in the static dipole moment may be expected. Furthermore, the dipole moment obtained from the absorption spectrum of dpbt (Figure 1) corresponding to the $S_1\leftarrow S_0$ transition is about 6.9 D. Thus, the strong CT character of the S_1 state and the considerably large transition dipole moment suggest a significant contribution of this state to the process of two-photon absorption.[6c,8c]

Table 1: Absorption and fluorescence maximal wavelengths (λ_A and λ_F), Stokes shift $\Delta\lambda$, and fluorescence quantum yields (Φ_F) of dpbt dissolved in organic solvents with a polarity factor $P(\varepsilon)$.

Solvent	$P(\varepsilon)$[a]	λ_A [nm]	λ_F [nm]	$\Delta\lambda$ [nm][b]	Φ_F
n-hexane	0.229	368	375	7	0.81[c]
toluene	0.343	374	405	31	0.75
diethyl ether	0.526	370	404	34	0.67
tetrahydrofuran	0.687	378	422	44	0.27
ethanol	0.886	384	440	56	0.02
N,*N*-dimethylformamide	0.922	381	444	63	0.03

[a] Polarity factor is defined as $P(\varepsilon)=(\varepsilon-1)/(\varepsilon+2)$, where ε is the dielectric constant. [b] Stokes shift is defined as $\Delta\lambda=\lambda_F-\lambda_A$. [c] Poor solubility in this solvent.

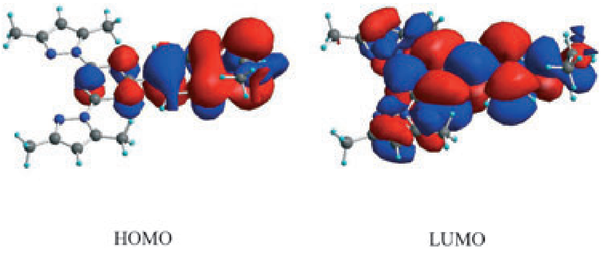

Figure 2. Frontier orbitals of HOMO and LUMO for dpbt. The geometry optimization and the frontier orbital population analysis were performed at the B3LYP/6-31G level. All calculations were carried out by using the GAUSSIAN03 program package.[13]

In Figure 3, the band at $\lambda=402$ nm is assigned to the absorptive transition of the ligand-centered S_1 state of dpbt in the [Eu(tta)$_3$dpbt] complex. This transition is red-shifted by approximately 30 nm with respect to free dpbt (Figure 1) and is accounted for by the strong polarization effect of the Eu^{III} ion field on the S_1 state. Furthermore, the transition dipole moment estimated from the dpbt-centered absorption band in Figure 3 is 8.2 D, which is slightly larger than that of free dpbt. The agreement between the fluorescence excitation spectrum and the linear absorption spectrum in the dpbt-centered $S_1\leftarrow S_0$ spectral region suggests efficient dpbt-to-Eu^{III} excitation energy transfer. However, the tta-centered absorption at 350 nm and the UV absorption at 280 nm show relatively low one-photon-sensitization efficiencies.

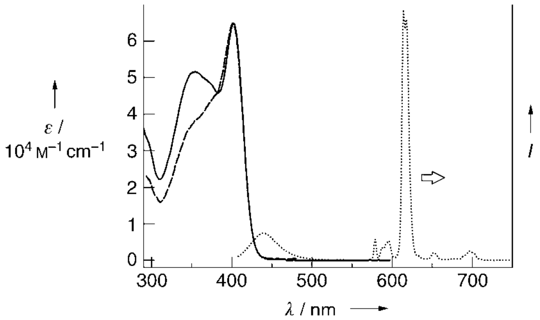

Figure 3. Room temperature UV/Vis absorption (——), fluorescence excitation (- - -; $\lambda_{em}=614$ nm), and fluorescence emission (•••••; $\lambda_{ex}=402$ nm) spectra of [Eu(tta)$_3$dpbt] in toluene (4.3×10^{-6} M). Spectra are normalized to the absorption maximum.

Figure 4 shows the excitation power dependence of TPE-sensitized luminescence intensity for both dpbt and [Eu(tta)$_3$dpbt] in toluene. In both cases, the experimental points fit nicely to a linear relationship with a similar slope of 1.98 ± 0.04. This value indicates that the excitation power dependence is quadratic and, therefore, confirms the TPE origin of the luminescence. The excitation power dependence was examined for all the other near-infrared excitation wavelengths used in the subsequent determination of the two-photon-absorption cross section and it was found that

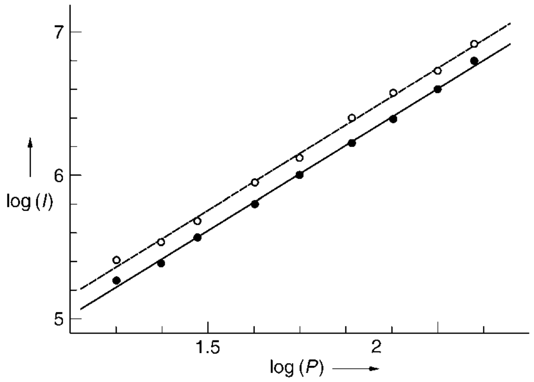

Figure 4. TPE-sensitized luminescence intensity (*I*) versus the incident laser power (*P*) for dpbt (○; $\lambda_{ex} = 745$ nm, $\lambda_{em} = 405$ nm) and [Eu(tta)$_3$dpbt] (●; $\lambda_{ex} = 799$ nm, $\lambda_{em} = 614$ nm) in toluene. The slopes found from the linear fitting (—— and – – –) are both 1.98 ± 0.04.

they all maintain the quadratic dependence with slopes of 1.95–2.05.

The maximal two-photon-absorption cross sections of dpbt and [Eu(tta)$_3$dpbt] are 185 GM at 735 nm and 157 GM at 808 nm, respectively (see Figure 5). The value of the Eu complex is comparable to those of Rhodamine B in the same spectral range[15] and is one or two orders of magnitude larger than native biological fluorophores such as green fluorescent protein[10] and tyrosine residues.[9c] Besides the high luminescence quantum yield following one-photon-sensitization for [Eu(tta)$_3$dpbt] ($\Phi = 0.52$ at $\lambda_{em} = 614$ nm),[5] this complex is unique also with regards to the high efficiency of two-photon sensitization and in the high purity of the red emission.

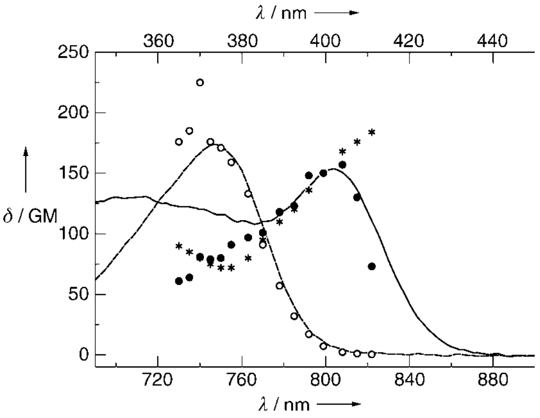

Figure 5. Two-photon-absorption cross sections (δ) for [Eu(tta)$_3$dpbt] (●; $\lambda_{em} = 614$ nm) and dpbt (○; $\lambda_{em} = 405$ nm) in toluene (1×10^{-4} M). Solid and dash lines are the corresponding one-photon-absorption spectra, whose wavelengths are shown as the upper abscissa, and the peak intensities are arbitrarily scaled. δ values for Rhodamine B in methanol (asterisk) taken from ref. [15] are shown for comparison. 1 GM = 10^{-50} cm^4 s photon^{-1} molecule^{-1}.[14]

The TPE-sensitized luminescence from [Eu(tta)$_3$dpbt] at 614 nm was mainly attributed to the ligand dpbt rather than tta because in the spectral region below 380 nm (Figure 5), the two-photon-excitation spectrum deviates significantly from the wavelength-doubled one-photon absorption of tta. Most importantly, for both dpbt and [Eu(tta)$_3$dpbt], the experimental points of two-photon-excitation spectra agree with the wavelength-doubled linear absorption spectra of dpbt. Moreover, for both dpbt and [Eu(tta)$_3$dpbt] in toluene, one- and two-photon-induced luminescence spectra show no appreciable differences in the positions and shapes of the bands (data not shown). These results suggest that the S_1 state is both one- and two-photon allowed, which is normally seen for polar chromophores that undergo a significant change of static dipole moments and have large transition dipole moments upon optical excitation.[6c,16] We have previously shown that the one-photon sensitization of [Eu(tta)$_3$dpbt] by visible light is dominated by efficient dpbt(S_1)-to-Eu^{III} energy transfer.[5] It is then most likely that the same mechanism of energy transfer is in operation for the present two-photon-sensitized Eu^{III} luminescence in the [Eu(tta)$_3$dpbt] complex.

To summarize, efficient two-photon sensitization of Eu^{III} luminescence in [Eu(tta)$_3$dpbt] has been demonstrated in the present work. The two-photon-absorption cross sections for dpbt and [Eu(tta)$_3$dpbt] in toluene were determined over the spectral range of 730–830 nm by using femtosecond laser pulses and the maximal values obtained are 185 and 157 GM, respectively. Complex [Eu(tta)$_3$dpbt] combines the advantages of two-photon sensitization and high-purity red emission and, therefore, points to a new direction for the synthesis of luminescent biolabels for less harmful and better quality bioimaging and for other biochemical applications.

Experimental Section

The ligand dpbt and the complex [Eu(tta)$_3$dpbt] were synthesized essentially according to the methods that we reported recently.[5] All the spectroscopic measurements described below were performed at room temperature.

UV/Vis absorption and photoluminescence measurements were carried out on an absorption spectrometer (U-3310, Hitachi) and a fluorescence spectrophotometer (F-2500, Hitachi), respectively. Luminescence quantum yields (Φ_F) of dpbt and [Eu(tta)$_3$dpbt] were determined by following the method described by Demas and Grosby,[17] for which anthracene ($\Phi_F \approx 0.36$)[18] and 4-dicyanomethylene-2-methyl-6-*p*-dimethylaminostyryl-4*H*-pyran (DCM; $\Phi_F \approx 0.71$),[19] respectively, were used as the references. Ligand dpbt and complex [Eu(tta)$_3$dpbt] were dissolved in toluene, anthracene was dissolved in cyclohexane, and DCM was dissolved in absolute ethanol, all at a concentration of approximately 5×10^{-6} M. The two-photon-absorption cross sections of dpbt and [Eu(tta)$_3$dpbt] were determined by the method of two-photon-induced fluorescence by using Rhodamine B as a standard with known two-photon-absorption cross sections.[15] The output from a mode-locked Ti:Sapphire laser (730–830 nm, ≈80 fs, 82 MHz; Tsunami, Spectra Physics) was focused into a quartz cuvette with an optical path length of 10 mm. The intensity of the incident laser beam was adjusted by using a variable neutral density filter. The two-photon-induced fluorescence was collected and sent to a polychromator (Spectropro-550i, Acton) equipped with a liquid-nitrogen-cooled CCD detector (SPEC-10-400B/LbN, Roper Scientific). To reject the interference of stray laser light, a 10-mm-long sample of a saturated aqueous solution of $CuSO_4$ was placed in front of the entrance slit. Ligand dpbt and complex [Eu(tta)$_3$dpbt] were dissolved in toluene at a concentration of 10^{-4} M, and Rhodamine B was dissolved in ethanol at the same concentration. Spectral response of the spectrograph including filters was calibrated with a standard blackbody lamp. In our experiments, the sample and the reference pigments were dissolved in different solvents. The two-photon-absorption cross sections (δ) were obtained according to [Eq. (1)],[15,20] in which subscripts S and R denote the sample and the reference, respectively, F represents the intensity of two-photon-induced fluorescence, Φ is the fluorescence quantum yield, c denotes the concentration, and n represents the refractive index of the solvents:

$$\delta_{\mathrm{S}} = \frac{F_{\mathrm{S}}}{F_{\mathrm{R}}}\left[\frac{\Phi_{\mathrm{R}}\, c_{\mathrm{R}}\, n_{\mathrm{S}}}{\Phi_{\mathrm{S}}\, c_{\mathrm{S}}\, n_{\mathrm{R}}}\right]\delta_{\mathrm{R}} \qquad (1)$$

Received: October 21, 2004

Keywords: energy transfer · europium · luminescence · sensitizers · two-photon excitation

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