Three-Photon Induced Luminescence of Europium Acetylacetonate Type Complexes

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We measured three-photon absorption spectra of two europium acetylacetonate type complexes. Three-photon absorption peak wavelengths of tris(hexafluoroacetylacetonate)europium(III) (Eu(hfa)) and tris(naphthoyltrifluoroacetonate)europium(III) (Eu(ntfa)) were 925 and 1025 nm, respectively. The peak values of 3PA cross-sections of Eu(hfa) and Eu(ntfa) were 1.9 and 13.9 GM (1 GM = 10^{-82} cm^6 s^2), respectively. Using Eu(ntfa) as a probe, a three-photon microscope image of a living cell was obtained with a 1030 nm operating femto-second fiber laser as the light-source. Thus, Eu(ntfa) is a fiber laser excitable luminescent probe useful for three-photon microscopy.

Multi-photon absorption is a nonlinear optical phenomenon in which a material absorbs multiple photons simultaneously inducing an electronic excitation. For example, in a two-photon process, two photons with an energy of half the energy gap between ground and excited states of a material can be used for excitation. In a three-photon process, the photon energy is one third of the energy gap. The absorption rates have a quadratic or cubic dependence on the intensity of incident irradiation in two- or three-photon processes, respectively. Therefore, multi-photon absorption can only be observed under an intense field. Thus in these processes, absorption can be limited to a focal point chosen by irradiation with a focused laser beam. The size of the excited area of three-photon processes is reduced compared with two-photon processes[1]. The three-photon absorption cross-section ($\sigma^{(3)}$) of materials are considerably lower than the two-photon absorption cross-section. This characteristic is an advantage for three-dimensional bio-imaging,[2] which is one of the important applications of nonlinear absorption processes. Several studies on bio-imaging utilizing three-photon absorption of organic dyes[3,4,5] or quantum dots[6] have been reported.

The sharp spectral peak of rare earth metal complexes enables three-dimensional multi-color imaging[7]. In the case of rare earth metal complexes, luminescence is known to occur after energy transfer from excited state of a ligand[8]. To obtain a high quantum yield rare earth metal complex, the excitation energy level of central metal should be located as far as the excited state of ligand[9]. As represented in figure 1, such a situation can more easily be achieved by through three-photon excitation than two-photon excitation. Three-photon luminescence imaging using [Tb(N-[2-(bis{2-[(3-methoxybenzoyl)amino]-ethyl}amino)ethyl]-3-methoxybenzamide)$(NO_3)_3$][10] and methoxy functionalized polyoxyethylene beard tris(dipicolinate) chelate Eu complex[11] have been demonstrated.

Considering the small cross-section of three-photon absorption, design of a complex exhibiting a relatively large

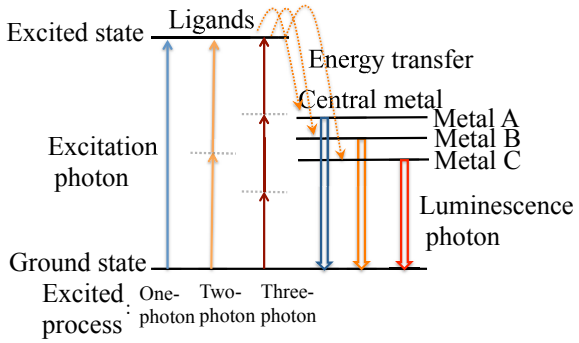

Figure 1. Schematic representation of one-, two- and three-photon induced luminescence from rare earth metal complex.

$\sigma^{(3)}$ at a desired wavelength is an important issue. As a light source for the three-photon and multi-color imaging, a recently developed Yb-doped femto-second fiber laser which oscillates at ca. 1050 nm has favorable characteristics. Therefore, in this study, we explore a rare earth metal complex three-photon absorption with a Yb-doped femto-second fiber laser.

According to the selection rules, the lowest three-photon allowed excited state should be the same as the lowest one-photon allowed excited state. Therefore, the wavelength at which three-photon excitation is effective should be at three times the one-photon absorption maximum wavelength. We measured the three-photon absorption spectrum of tris(naphthoyltrifluoroacetonate)europium(III) Eu(ntfa) (figure 2 (a)),[12] the one-photon absorption maximum of which is 333 nm. For the comparison, the three-photon characteristics of tris(hexafluoroacetylacetonate)europium(III) (Eu(hfa)) (figure 2 (b))[13] was also investigated.

Absorption and luminescence spectra of Eu complexes are shown in figure 3. Absorption peak wavelength of Eu(ntfa) and Eu(hfa) were 333 and 303 nm, respectively. The wavelength of the absorption peak of Eu(ntfa) was 10 nm shorter than that of the free ligand. This blue-shift has been attributed to a change of the intermolecular charge transfer of the ligand due to coordination[14]. Except for this small shift,

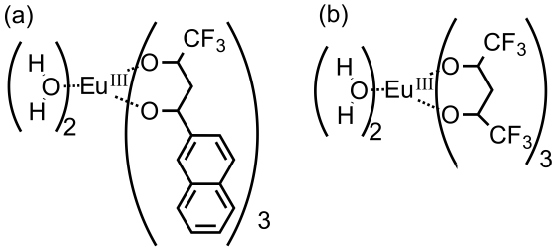

Figure 2 Chemical structure of (a)Eu(ntfa) and (b)Eu(hfa)

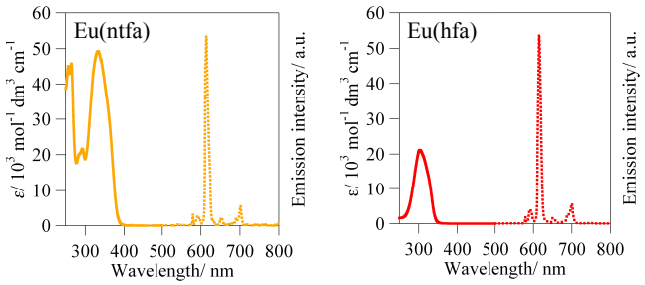

Figure 3. Absorption (solid line) and luminescence (doted line) spectra of Eu(ntfa) and Eu(hfa).

the absorption bands of the complexes were almost three times as intense as that of the ligands. Molar absorption coefficients at the absorption peak of Eu(ntfa) and Eu(hfa) were 54000 and 23000, respectively. This shift, and the difference between the molar absorption coefficients, were attributed to difference of the size of the π-electron systems of the respective ligands. Generally, a large three-photon absorption cross-section is observed from a molecule with a large molar absorption coefficient.[15] Therefore, Eu(ntfa) should exhibit a larger three-photon absorption cross-section than Eu(hfa). Similar luminescence spectra were observed for Eu(hfa) and Eu(ntfa) as was expected.[7]

Three-photon absorption spectra of Eu(ntfa) and Eu(hfa) measured by three-photon induced luminescence technique[16] are shown in figure 4. Luminescence spectra of complexes excited through a three-photon process were essentially the same as those through one-photon process. We confirmed a cubic dependence of the luminescence intensity on excitation intensity for all plots. Wavelengths of three-photon absorption peaks of Eu(ntfa) and Eu(hfa) were 1025 and 925 nm, respectively. As expected, these wavelengths were almost three times as long as the observed one-photon absorption peaks. This indicated that the rare earth metal complexes obey the general selection rule for state transitions.

Peak values of $\sigma^{(3)}$ of Eu(ntfa) and Eu(hfa) were 13.9 and 1.9 GM (1 GM = 10^{-82} cm^6 s^2) (2-(4-amidinophenyl)-1*H*-indole-6-carboxamidine used as standard)[16]. $\sigma^{(3)}$ of Eu (ntfa) was observed to be 7.3 times larger than that of Eu(hfa), demonstrating a larger molar absorption coefficient for Eu(ntfa). For Eu(ntfa), an additional three-photon absorption peak corresponding to the one photon absorption band at around 280 nm was observed below 925 nm. This band may provide a three-photon absorption peak at ca. 840 nm. In

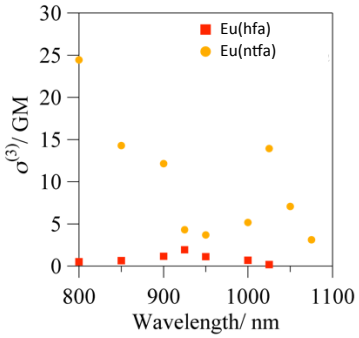

Figure 4. Three-photon absorption cross-section of Eu(ntfa) and Eu(hfa) at respective wavelength of excitation light source.

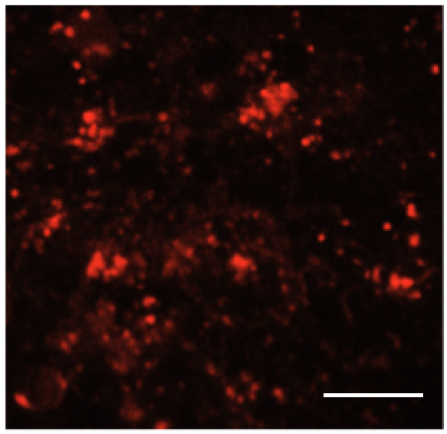

Figure 5. Three-photon microscope image of HEK293 cell stained with Eu(ntfa). Scale bar represents 10 μm.

addition, edge of two-photon absorption band corresponded to the one-photon absorption band at around 333 nm should exist at wavelength region below 800 nm. Therefore, the increase of $\sigma^{(3)}$ at 800 nm is though to be due to sum of additional three-photon absorption band and resonance enhancement of three-photon absorption to the edge of two-photon absorption band. Actually cubic dependence of luminescence intensity of Eu(ntfa) was observed at the excitation wavelength of 800 nm. However, the luminescence lower than the excitation wavelength of 800 nm showed a quadratic dependence, rather than a cubic dependence, on the intensity of the excitation beam. Therefore, we could not evaluate $\sigma^{(3)}$ of Eu(ntfa) below 800 nm.

A three-photon microscope image of Human Embryonic Kidney 293 (HEK293) cell stained with Eu(ntfa) is shown in figure 5. As an excitation light source, a Yb-doped femto-second fiber laser oscillating at 1030 nm was employed. Luminescence from Eu(ntfa) seems to be observed at a endosome (see figure S3). Reflecting the efficient three-photon absorption characteristic of Eu(ntfa), a three-photon induced luminescence signal from a living cell was observed even by using fiber laser. Thus, Eu(ntfa) should be considered to be a promising three-photon absorption probe.

In summary, we have measured the three-photon absorption spectra of two rare earth complexes, Eu(hfa) and Eu(ntfa). We have confirmed that three-photon absorption bands were observed at nearly three times the wavelength of the one-photon absorption peaks of the metal complexes. One of the complexes, Eu(ntfa) was effectively excited using a femto-second pulsed fiber laser operating at 1030 nm. Designing a rare earth metal complex with a ligand, which exhibits absorption band at a one third of the desired three-photon excitation wavelength, should therefore be an effective means for obtaining useful three-photon excitation probes.

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