Long-Lived Two-Photon Excited Luminescence of Water Soluble Europium Complex: Applications in Biological Imaging using Two-Photon Scanning Microscopy.

Alexandre Picot, Anthony D’Aleo, Patrice L. Baldeck, Alexei Grichine, Alain Duperray, Chantal Andraud, and Olivier Maury

The unique luminescence properties of Ln³⁺ ions (sharp emission, large Stokes shift, insensitivity to oxygen) and particularly their long excited state lifetime ranging from µs (Yb, Nd) to ms (Eu, Tb) triggered the development of time-resolved spectroscopy or microscopy for applications in biological, environmental or clinical analysis. These techniques consist in the introduction of a time delay before the detection of the lanthanide luminescence in order to eliminate parasitic scattering and short-lived luminescence resulting in an enhanced signal/noise ratio. However, UV-light is needed for the metal sensitization, which is a drawback for biological applications. Indeed, these wavelengths are scattered/absorbed by the medium, which limits the investigation depth and presents some phototoxicity for the biological samples. Two (or multi-) photon excitation, that is simultaneous absorption of two photons of half energy, is an elegant way to circumvent the use of UV-light. Following pioneering works of Webb in the 1990’s, confocal bi-photonics microscopy is becoming even more popular with the commercialization of tunable Ti:sapphire source. This technique allows the use of irradiation wavelengths located in the near infra-red (700-900 nm), a spectral range where the biological media are transparent. In addition, its intrinsic confocal character strongly limits the photo-damage and gives rise to 3D resolved spectroscopy and microscopy. Whereas numerous works have been devoted to the optimization of the luminescence properties of lanthanide complexes for imaging or sensing applications, their two-photon sensitization is becoming an emerging field of research. The proof-of-concept of two-photon antenna effect has already been demonstrated in biological media with complexes featuring very low (generally not measured) two-photon absorption (2PA) cross-section, σ2ph. On the other hand, significant σ2ph values are reported in the cases of Eu³⁺ complexes only stable in non aqueous solvents, not suitable for any practical applications as biological probes. In this article, we report the design of new functionalized tris-dipicolinate Eu³⁺ complexes (Chart 1) that are (i) soluble and stable in aqueous media, (ii) luminescent with long excited lifetime and (iii) that present significant two-photon absorption cross-section in the biological window.

Recently, we described the two-photon antenna effect of tricarboxylic [EuL₃]³⁺(OTf)₂ complex (σ₃₉₀ ~ 96 GM at 720 nm), where L₃ is a alkylarylpyrrolepyrrole functionalized pyridine-dicarboxamide ligand (Chart 1). In very low stability prevented any use in aqueous media and prompted us to switch to pyridine-dicarboxylic acid (DPA) analogous known to present a enough stability in water for spectroscopic studies. DPA was functionalized by extended π-system featuring weak electron donor alkyl group (L₁ Chart 1) and the hydrophilic property was ensured by 3,4,5-tris(triethyleneglycyl) phenyl moieties (1k). However, ligand synthesis is reported in supporting information. The corresponding complex [Na₂[Eu(L₁)₃]], was prepared in water by mixing three equiv. of ligand in basic media with EuCl₃·6H₂O and isolated by extraction with dichloromethane. The spectroscopic characterizations are in agreement with the proposed structures (SI) and the complex is stable and soluble in organic solvents and water.

All the photophysical properties of the ligand and complex were recorded in water and the stability of the complex in aqueous media is similar to that of the non functionalized parent analogues (see SI for details). L₁ presents a broad transition by UV-visible spectroscopy centred at 318 nm (25000 L·mol⁻¹·cm⁻¹) in water at pH = 5 assigned to charge transfer (CT) transition from the alkyl donor to the pyridinic acceptor moieties. Complexation to Eu³⁺ results in a small bathochromic shift of the CT transition (λₘₐₓ = 332 nm (78700 L·mol⁻¹·cm⁻¹) Figure 1), the lanthanide Lewis acidity effect being partially compensated by the trianionic nature of the complex, and is accompanied by a threefold excitation of the extinction coefficient in agreement with the 3:1 ligand:metal ratio. Emission spectra reveal the characteristic Eu³⁺ emission profile with the very intense T₁D₂→F₂ transition upon excitation in the ligand CT transition (Figure 2). The quantum yield of the complex is good, about 15.7%, the lifetime is long (1.062 ms in water, figure 52) and remains far higher than that of other organic or endogen chromophores (few ns). The two-photon excitation spectrum was recorded, in the 700-900 nm range using a femtosecond Tri-sapphire laser source. The 2PA process is unambiguously confirmed by the quadratic dependence (experimental coeff. 1.59)
of the 613 nm band with the laser power ($\lambda_{\text{exc}} = 720$ nm, Figure S5). The 2PA spectrum matches almost perfectly the wavelength doubled one absorption spectra (IPA) with a maximum 2PA wavelength around 700 nm (Figure 1), indicating that the excited states involved in the IPA and 2PA processes are the same. In the measured range, the maximal 2PA cross-section is significant, about 92 GM at 700 nm. It is worth underlying that this value is in the same range than that of other lanthanide compounds, only stable in organic solvents and far higher than that of the water stable complexes.\cite{66c}

Finally, two-photon scanning microscopy experiments were carried out using T24 cancer cells fixed with ethanol at $-20^\circ$C and loaded with [Na$_2$][Eu(L$_3$)$_3$] in PBS solution (concentration in the sample about 2.10$^3$ mol.L$^{-1}$cm$^{-2}$, see SI for details). The images shown in figure 3 were taken on a biophotonic laser scanning microscope upon $\lambda$-760 nm irradiation ($\gamma_{2PA}$ (760) = 19 GM). The red Eu luminescence was integrated in 503-695 nm spectral range. Comparison with phase contrast image clearly indicates that the complex is mainly localized in a perinuclear region and its distribution is similar to that of the endoplasmic reticulum. In addition, bright spots are observed in the nucleus indicating that the complex preferentially targeted small organelles called nucleoli, similarly to a recent study of Parker and co-workers.\cite{66c}

In conclusion, we report the design of a new europium complex that fulfills all the requirements for bio-imaging application: (i) good solubility and stability in water, (ii) intense emission in the red (616 nm), (iii) long luminescence lifetime and (iv) significant two-photon absorption cross-section induced by two-photon antenna effect in the biological window. Furthermore, we describe the first two-photon scanning microscopy bio-imaging experiments using a lanthanide complex that can be considered as a new generation of molecular probes. Further studies are currently carried out (i) to study localization of the complex in the cells, (ii) to increase the molecular two-photon cross-section and water stability of the complexes and (iii) to develop biological imaging using biphotonic time-resolved microscopy.

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Supporting Information Available. Experimental data, figures S1-5 are available free of charge via the Internet at http://pub.acs.org.

References


(d) Values from critical stability constant (vol 1)

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Abstract. A new europium complex presenting good solubility and stability in water, intense emission in the red (616 nm), long luminescence lifetime and significant two-photon absorption cross-section in the biological window has been designed and successfully used for two-photon scanning microscopy bio-imaging experiments on fixed cancer cells.