Zn Sensitive Luminescent Sensors

Selective Detection of Zinc Ions with Novel Luminescent Lanthanide Probes**

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The zinc ion (Zn^{II}) is the second most abundant heavy metal ion, after iron, in the human body and plays important roles in various biological systems. For example, it is an essential component of many enzymes, transcription factors, and synaptic vesicles in excitatory nerve terminals.[1,2] Zinc is also an important factor in regulating synaptic transmission and cell death.[3]

So far, several Zn^{II}-selective fluorescent sensor molecules have been reported.[4] However, there are still few reports about long-lifetime fluorescent sensors for Zn^{II} ions. The fluorescence lifetimes of typical organic compounds, including common zinc-selective fluorescent sensor molecules, are in the nanosecond region. On the other hand, luminescent lanthanide complexes, in particular Tb^{III} and Eu^{III} complexes, have large Stokes shifts and long luminescence lifetimes in the

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order of milliseconds.[5] These long-lived luminescent compounds have the advantage that short-lived background fluorescence and scattered light decay to negligible levels when a pulse of excitation light is applied and the emitted light is collected after an appropriate delay time.

Herein we report novel sensitive luminescent sensors for Zn^{II} ions, that is, Tb^{III} and Eu^{III} diethylenetriaminepentaacetic acid (DTPA)-bisamide complexes, [Tb**L**$_1$] and [Eu**L**$_1$] (Figure 1). It is known that *N,N,N′,N′*-tetrakis(2-pyridylmethyl)ethylenediamine (TPEN) complexes readily with Zn^{II} ions, but hardly at all with Ca^{II} and Mg^{II} ions.[6] In the characterization of the new compound [Tb**L**$_1$], we observed that the time-resolved emission intensity of Tb^{III} ions increased upon addition of Zn^{II} ions between 0 and 1 equiv in 100 mM HEPES buffer at pH 7.4 (Figure 2). The luminescence emission showed three bands at 490 nm, 546 nm, and 587 nm arising from transitions from the $^5D_4$ excited state to the $^7F_6$, $^7F_5$ and $^7F_4$ ground manifold, respectively.[7] The emission intensity of [Eu**L**$_1$] upon adding Zn^{II} ions changed in a similar way to that of [Tb**L**$_1$], but with only a sixth of the intensity. The luminescence emission data also revealed very high selectivity for Zn^{II} ions compared with Ca^{II} and Mg^{II} ions. The luminescence emission enhancement in 100 mM HEPES buffer (pH 7.4) containing 5 mM Ca^{II} or Mg^{II} ions showed that these ions had no effect in the presence or absence of Zn^{II} ions (Figure 3). The complex [Tb**L**$_1$] also showed high selectivity for Zn^{II} compared with heavy metal ions (Fe^{II}, Fe^{III}, Cu^{II}, Ni^{II}, Co^{II}, Mn^{II}) and some anions (Cl^-, carbonate, and phosphate). The luminescent intensities of [Tb**L**$_1$] in 100 mM HEPES buffer (pH 7.4) were not increased by the addition of any of the above heavy metal ions (1 equiv to [Tb**L**$_1$]) or other anions (100 mM Cl^-, 10 mM carbonate, and 10 mM phosphate; see Supporting Information). We also added one equivalent of Cu^{II} ions to the solution of [Tb**L**$_1$] (100 mM HEPES buffer; pH 7.4) containing one equivalent of Zn^{II} ions. In this experiment, the luminescence emission intensity decreased to a fifth of the emission enhancement upon adding one equivalent of Zn^{II} ions only. The emission lifetime of [Tb**L**$_1$] after the addition of 1.0 equivalent of Zn^{II} ions was measured in 100 mM HEPES buffer (pH 7.4). The emission signal showed a single exponential decay with a lifetime of 1.45 ms. The compound [Tb**L**$_1$] showed an apparent dissociation constant for Zn^{II} of 2.6 nM (pH 7.4, 295 K). Furthermore, at the excitation wavelength of 260 nm with the emission wavelength of 545 nm, the correlation between the Zn^{II} ion concentration and the emission intensity was determined and was found to be linear in the range from 0 to 1.0×10^{-4} M. The detection limit (3σ) in 100 mM HEPES buffer (pH 7.4) by using 100 μM of [Tb**L**$_1$] was calculated (see Supporting Information), and found to be 4.8×10^{-6} M.

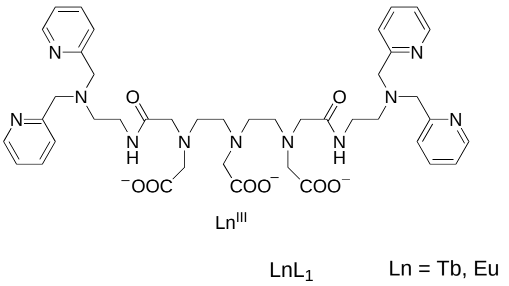

Figure 1. Structures of Tb^{III} and Eu^{III} DTPA-bisamide complexes, [Tb**L**$_1$] and [Eu**L**$_1$].

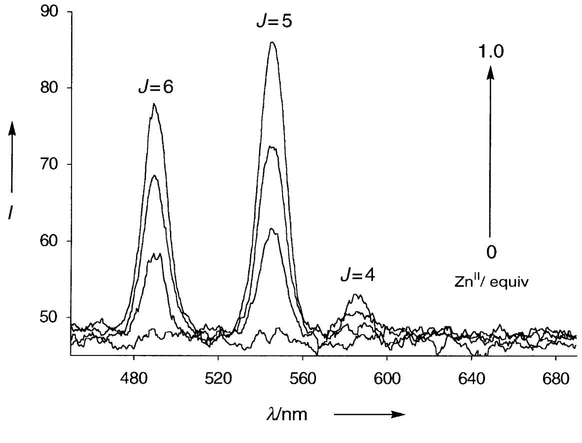

Figure 2. Emission spectra (excitation at 260 nm) of [Tb**L**$_1$] (100 μM) in the presence of various concentrations of Zn^{II} ions: 0–1.0 equivalents Zn^{II} ions with respect to [Tb**L**$_1$]. These spectra were measured at pH 7.4 (100 mM HEPES buffer) and 22 °C by using a delay time of 0.05 ms and a gate time of 1.00 ms. The bands arise from $^5D_4 \rightarrow ^7F_J$ transitions; the *J* values of the bands are labeled. *I* = Intensity (arbitrary units).

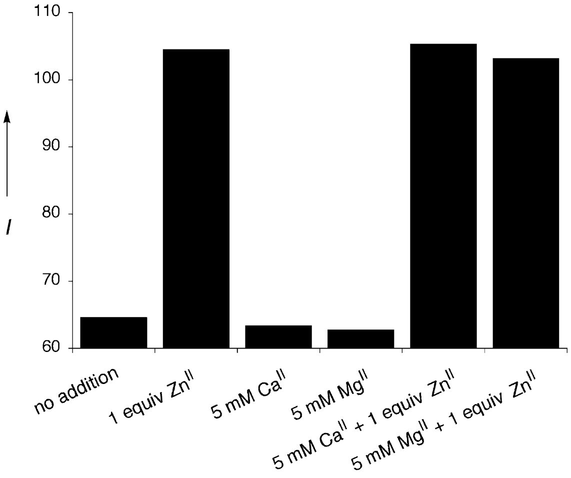

Figure 3. The luminescence intensity of [Tb**L**$_1$] (100 μM) at 545 nm upon the addition of Zn^{II}, Ca^{II} or Mg^{II} ions (excitation at 260 nm) in 100 mM HEPES buffer (pH 7.4) at 22 °C. Cations were added as $ZnCl_2$ (1 equiv to [Tb**L**$_1$]), $CaCl_2$ or $MgCl_2$ (5 mM). *I* = Intensity (arbitrary units).

We examined the absorption spectra of a solution of [Tb**L**$_1$] in the presence of various concentrations of Zn^{II} ions (Figure 4). The absorbance between 230 and 300 nm changed linearly with an increase in the concentration of Zn^{II} ions up to a molar ratio of 1:1 Zn^{II}/[Tb**L**$_1$], and remained at a plateau upon adding more Zn^{II} ions. The Job plot of the luminescence

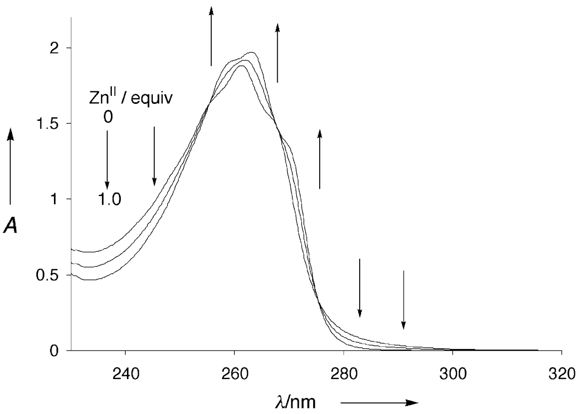

Figure 4. UV/Vis absorption spectra of [TbL$_1$] (150 μM) in HEPES buffer (100 mM; pH 7.4) in the presence of various concentrations of Zn^{II} ions: 0, 0.5, 1.0 equiv On the addition of between 1.0 and 3.0 equivalents of Zn^{II} ions, the absorption spectra hardly changed. *A* = Absorbance.

emission intensity of Tb^{III} for complexation between [Tb**L$_1$**] and Zn^{II} has a maximum at a mole fraction of 0.5, thus indicative of the formation of a 1:1 complex.[8] Figure 5 shows the proposed conformational change of the structure in the presence of Zn^{II}. The light-conversion process of absorption to emission by energy transfer in [Tb**L$_1$**] is also shown (Figure 5).[9,10] The previously reported Zn^{II}-sensitive luminescent lanthanide probe operates through a PET (photo-induced electron transfer) process.[10a] We investigated the mechanism of luminescence increase of our probes by measuring the effects of D_2O and pH. We measured the luminescence emission spectra of [Tb**L$_1$**] in D_2O solution at pD 7.40 (100 mM HEPES buffer) in the presence of various concentrations of Zn^{II} ions. The emission spectra in D_2O solution changed in the same manner as those in H_2O solution. It can be considered from this result that the increase of the emission intensity upon addition of Zn^{II} does not mainly because of a change in the direct interaction of water molecules with the Tb^{III} ion.[10b,11] Further, there was no effect of H^+ ions on the emission spectra of [Tb**L$_1$**] between pH 3.0 and 10.0 in the absence of Zn^{II} ions. The $\log K_A$ values of TPEN are 7.12, 4.81, 3.30 and 2.88.[12] Therefore, it is unlikely that the increase of the emission intensity of [Tb**L$_1$**] generated by the addition of Zn^{II} ions can be ascribed to the cessation of the PET process, which occurs from the tertiary amine of the TPEN moiety to a pyridyl group. From these results, the enhancement of the emission intensity of our probes upon Zn^{II}-binding appears to be the result of efficient intramolecular energy transfer from the pyridyl group to the Tb^{III} ion. Further investigations are underway to establish the mechanism of the luminescence enhancement.

In summary, the novel Zn^{II}-ion-sensitive luminescent probe [Tb**L$_1$**] showed a remarkable increase in the luminescence emission of Tb^{III} ion upon the addition of Zn^{II} ions, with a long emission lifetime in the order of milliseconds. The novel probes described herein should be excellent lead complexes for the further development of highly selective, Zn^{II}-ion-sensitive luminescent chemosensors with long luminescence lifetimes and a remarkable "on-off" switching of emission.

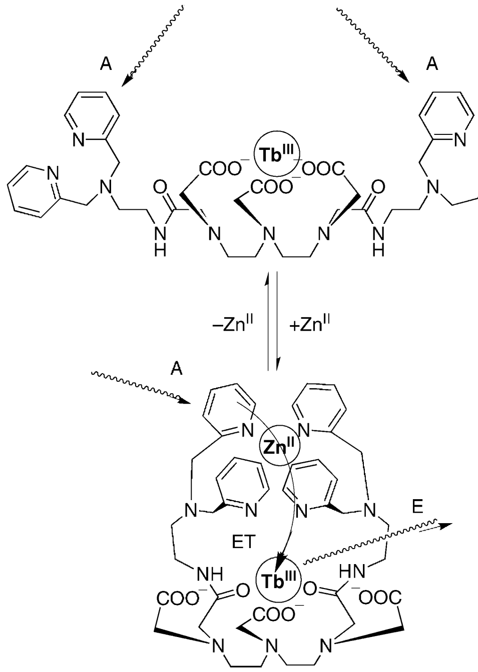

Figure 5. Schematic of [TbL$_1$] representing the proposed conformational alteration of the structure in the presence and absence of Zn^{II} ions. The light-conversion process of absorption-energy transfer-emission, A-ET-E, performed by [TbL$_1$], is also represented.

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