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Lanthanide/ATP Interaction in Water Mediated by Luminescent Hemispherical-Shaped Complexes

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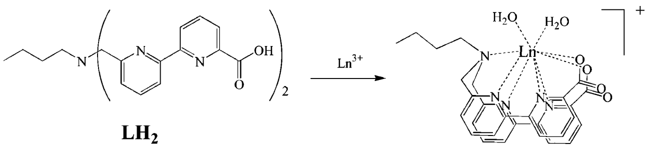
Ligand **L**H_2, composed of two bipyridylcarboxylate fragments linked to an amino butyl chain, reacts with europium and terbium to form luminescent complexes in water at neutral pH. When testing these unsaturated complexes as anion sensors with NO_3^-, HPO_4^{2-}, AMP, ADP, and ATP, a marked selectivity is observed for HPO_4^{2-} and ATP^{4-} at pH = 7.0. The interaction of these anions with the complex was investigated by means of absorption and emission spectroscopies. With ATP^{4-}, ES-MS and ^{31}P NMR experiments revealed the formation of a $[Ln\cdot \mathbf{L}\cdot(ATP)]^{3-}$ ternary species.

Interest in the development of luminescent labels and chemodosimeters has grown continuously over the last few decades and currently engages innumerable research groups around the world. The most flourishing applications involve tags for fluorescence microscopy and chromogenic sensors for cationic, anionic, or neutral chemical species.[1,2] The design of sensitive and selective fluorescent chemodosimeters for biological anion recognition, such as adenosine triphosphate (ATP), presents a particularly daunting challenge.[3] Since the hydrolysis of ATP to ADP and phosphate is one of the most important pathways for energy supply in living organisms,[4] the availability of water-compatible ATP chemodosimeters would permit the analysis of how ATP levels change in response to stress or stimulus to biological cells.

We have now developed luminescent lanthanide complexes whose optical properties can be tuned over a modest range and for which the positively charged metallic core provides electrostatic attraction for incoming anions.[5] This first generation of anion dosimeters takes advantage of the remarkable luminescence properties of Eu and Tb,[6] combined with the antenna effect provided by bipyridyl (bipy) fragments. The presence of different anions could be monitored by absorbance and luminescence changes resulting from solvent-to-anion exchange within the incompletely saturated coordination sphere around the Ln, similarly to the principle of water exchange in Gd-based relaxation agents for NMR imaging.[7] Unfortunately, at that time we were unable to identify complexes having sufficient stability and solubility in water and possessing good recognition for biological anions. Here, we target Eu and Tb complexes coordinated to a novel ligand **L**H_2 composed of two bipy arms for the photon absorption with a carboxylate function on each 6′ position that enables strong coordination to the Ln atom. These two arms are linked to an *n*-butylamine through methylene bridges in the 6 position. These bridges allow formation of three five-membered rings per bipy arm when complexed to Ln. The increased hydrophilicity of **L**H_2, provided by the two carboxylate groups at neutral pH, favors water solubility and high stability of the resultant complexes. Assuming a coordination number of 8−9 for Ln,[6] the seven heteroatoms of **L**H_2 should leave free coordination sites for polyanionic moieties.



LH_2

Ligand **L**H_2 was obtained in seven synthetic steps (see Supporting Information). Complexation with Ln cations was

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Table 1. Conditional Association Constants (log K_{xyz}) for the Formation of the Different Species Depicted in Equations 1−4[a]

	log K_{110} (eq 1)	log K_{120} (eq 2)	log K_{210} (eq 3)	log K_{111} (eq 4)				
				Na_2HPO_4	KNO_3	AMP	ADP	ATP
Eu	5.7 ± 0.4	4.7 ± 0.6	4.1 ± 0.3	4.0 ± 0.1	*b*	*b*	*b*	4.2 ± 0.2
Tb	5.6 ± 0.2	*b*	4.5 ± 0.4	4.5 ± 0.2	*b*	*b*	*b*	4.6 ± 0.4

[a] Average values obtained for 3−5 independent titration experiments in H_2O, 10^{-2} M TRIS/$HClO_4$, at pH = 7.0. [b] Too weak to be measured by absorption spectroscopy.

Table 2. Photophysical Data for [Ln·**L**]Cl Complexes

	λ_{abs} (nm)[a]	ϵ ($M^{-1}\cdot cm^{-1}$)[a]	τ (ms)[a]	Φ_{abs} (%)[a,d]	τ_{H_2O} (τ_{D_2O}) (ms)[e]	n_{H_2O}[f]
Eu	307	18 000	0.28[b]	4.6	0.36 (1.75)	2.3
Tb	308	18 150	0.74[c]	0.4	0.78 (1.15)	1.8

[a] Measured in H_2O, pH = 7.0 (0.01 M TRIS/$HClO_4$). [b] $^5D_0 \rightarrow {}^7F_2$ transition centered on Eu at 620 nm. [c] $^5D_4 \rightarrow {}^7F_5$ transition centered on Tb at 545 nm. [d] Using $[Ru(bipy)_3]Cl_2$ in oxygen equilibrated water, ref 11 (uncertainty ± 15%). [e] Measured in electrolyte free water and D_2O. [f] Number of water molecules calculated according to ref 9a with second sphere interaction corrections 9b (estimated error ± 0.5 water molecules).

monitored by UV−vis spectroscopy, where the evolution of the absorption spectra of the ligand in buffered aqueous media (pH = 7.0, 0.01 M TRIS/$HClO_4$) was followed as a function of increasing amounts of $LnCl_3\cdot 6H_2O$ (Ln = Eu and Tb). The absorption maximum for $\mathbf{L}^{2-}$ at 287 nm (ϵ = 21 040 $M^{-1}\cdot cm^{-1}$) shifts gradually toward lower energy until 1 equiv of cation is added. Factorial analysis of the titration revealed the presence of up to three new absorbing species, and the spectra were fitted[8] to the following model (for full experimental details see Supporting Information):

$$Ln^{3+} + \mathbf{L}^{2-} \leftrightarrow [Ln\cdot\mathbf{L}]^{+} \quad K_{110} = [Ln\cdot\mathbf{L}]/([Ln][\mathbf{L}]) \quad (1)$$

$$[Ln\cdot\mathbf{L}]^{+} + \mathbf{L}^{2-} \leftrightarrow [Ln\cdot\mathbf{L}_2]^{-} \quad K_{120} = [Ln\cdot\mathbf{L}_2]/([Ln\cdot\mathbf{L}][\mathbf{L}]) \quad (2)$$

$$[Ln\cdot\mathbf{L}]^{+} + Ln^{3+} \leftrightarrow [Ln_2\cdot\mathbf{L}]^{4+} \quad K_{210} = [Ln_2\cdot\mathbf{L}]/([Ln\cdot\mathbf{L}][Ln]) \quad (3)$$

Values for the conditional association constants are collected in Table 1. The complexation process can also be monitored by luminescence spectroscopy, where the broad emission band centered at 410 nm (24 400 cm^{-1}) for the free ligand and attributed to a $^3\pi\pi^*$ state vanishes at the expense of the typical emission lines of Ln complexes. Such behavior is clearly indicative of efficient ligand-to-metal energy transfer[6] and was confirmed by excitation spectra.

Mixing equimolar amounts of $LnCl_3\cdot 6H_2O$ salts (Ln = Eu and Tb) with $\mathbf{L}H_2$ in methanol in the presence of a small excess of triethylamine lead after recrystallization to complexes of $[Ln\cdot\mathbf{L}(H_2O)_2]Cl$ formula, characterized by elemental analysis, mass spectrometry, and UV−vis, and IR spectroscopies. The photophysical properties of these complexes are given in Table 2. Using the relationship developed by Horrocks and Südnick[9] on the basis of luminescence lifetime measurements made in water and D_2O, it was calculated that two water molecules complete the first coordination sphere of the cationic complexes, as expected on the basis of a coordination number of 9 with a heptadentate ligand.

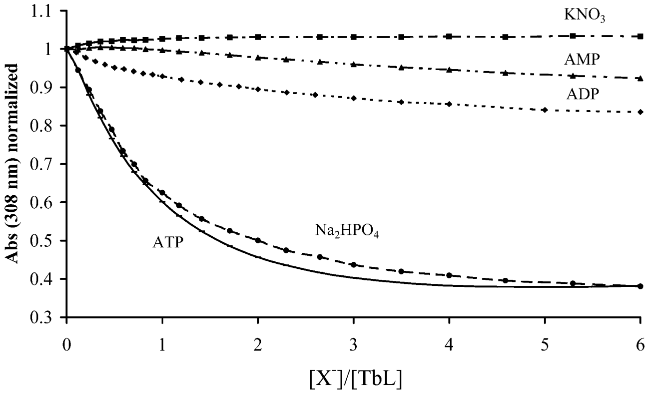

Figure 1. Evolution of the normalized absorbance of [Tb·**L**]Cl upon addition of different anions (0.01 M TRIS/$HClO_4$ in water, pH = 7.0, $[Tb\cdot\mathbf{L}]_{ini}$ = 1.0 to 5.0 × 10^{-5} M).

The most interesting features arose when these complexes were tested toward incoming anions by spectrophotometric titrations in buffered water. While no significant changes could be observed in the UV−vis absorption spectra upon titration with KNO_3, AMP and ADP, titration with Na_2HPO_4 and ATP revealed the formation of a new species (Figure 1). Control luminescence measurements during titration of Eu salts by ATP in the absence of $\mathbf{L}H_2$ show no luminescence. The fact that the species formed upon addition of ATP or Na_2HPO_4 to the $[Eu\cdot\mathbf{L}]^+$ complex is still luminescent rules out competitive decomplexation and suggests the ternary nature of the complex (Ln/X/**L**). As such, the formation of the latter complex was fitted according to eq 4:

$$[Ln\cdot\mathbf{L}]^{+} + X^{n-} \leftrightarrow [Ln\cdot\mathbf{L}\cdot(X)]^{(n-1)-} \quad K_{111} = [Ln\cdot\mathbf{L}\cdot(X)]/([Ln\cdot\mathbf{L}][X]) \quad (4)$$

The calculated UV−vis absorption spectra of the ternary species (Figure 2) show strong absorption bands at 259 and 300 nm, attributed, respectively, to transitions centered on the adenine moiety (λ = 258 nm for free ATP) and on the bipy moieties. The hypsochromic shift observed for this last transition, compared to the $[Eu\cdot\mathbf{L}]^+$ complex, is attributed to a shift of one bipy arm in the second coordination sphere,[5] leading to an absorption maximum for the bipy moieties in the ternary species, which is the average value of the complexed and partially ligated form of the bipy arms. The absence of a significant absorption spectral shift for the adenine moiety upon complexation suggests that this part of the ATP molecule is not implicated in the coordination process.

Upon ATP complexation (5-fold excess), the luminescence intensity (λ_{exc} = 300 nm) of the Ln cations decreases to 20% of its initial value (Figure 3). In contrast, the measured excited-state lifetime of the Eu complex (10^{-4} M in the same

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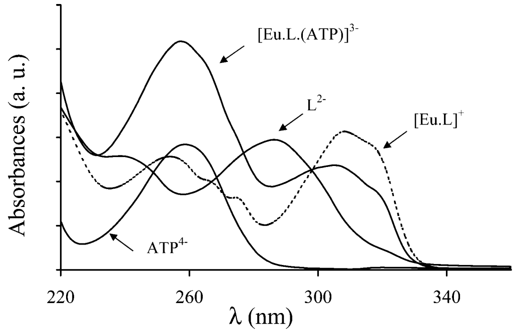

Figure 2. Calculated UV−vis absorption spectra of the species observed upon titration of the Eu complex by ATP (0.01 M TRIS/$HClO_4$ in water, pH = 7.0).

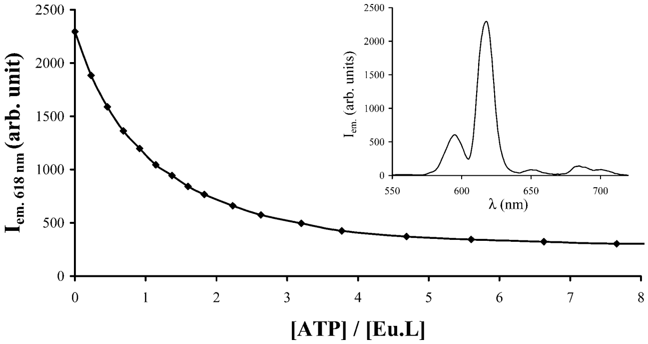

Figure 3. Evolution of the intensity of emission (λ_{exc} = 300 nm, λ_{em} = 618 nm) of a solution of [Eu·**L**]$^+$ upon addition of increasing amounts of ATP ($c = 4.2 \times 10^{-5}$ M, H_2O, pH = 7.0, TRIS/$HClO_4$, 0.01 M). Inset: fluorescence spectrum of the [Eu·**L**]$^+$ complex.

buffer) increases, respectively, from 0.28 to 0.58 and 0.65 ms upon addition of 0, 10, and 20 equiv of ATP. This behavior indicates less efficient energy transfer processes, but substantial decrease of the detrimental nonradiative deactivation pathways in the ternary species. In turn, this result can be interpreted in terms of the displacement of water molecules from the first coordination sphere of the cation caused by complexation of ATP. This situation would lead to a better shielding of Eu from the solvent. This effect is less pronounced with Tb (τ = 0.74 and 0.76 ms, respectively, with 0 and 10 equiv of ATP). Additionally, the ESI-mass spectra, in water at pH = 7 (adjusted with aqueous ammonia), unambiguously confirm the presence of the ternary adduct by mixing a 1:1 solution of the Eu complex and ATP solution. In a negative mode of analysis, intense peaks at 575.9 and 587.2 corresponding, respectively, to [Eu·**L**·(ATP·H)]$^{2-}$ and [Eu·**L**·(ATP·Na)]$^{2-}$, with the expected isotopic profile, were resolved. Additional fragmentation peaks are in keeping with the expected Eu/L/ATP supramolecular species. The strong interaction between ATP and the Eu complex is corroborated by ^{31}P NMR which reveals a pronounced upfield shift and a large broadening of the three peaks due to free ATP seen at δ −9.5, −10.0, and −21.7 ppm,[10] to δ −39.0 and −72.1 (broad, 2P) ppm upon anion binding.

Although the coordination of HPO_4^{2-} and ATP^{4-} resulted in similar photophysical perturbations, it is surmised that the coordination behavior of these two species is not the same. While coordination of ATP can occur through the formation of one or two six-membered chelate rings implicating the oxygen atoms of the phosphorylated chain,[12] coordination of the hydrogenophosphate may happen through a monodentate binding mode or potentially as a bidentate ligand,[13] especially if the remaining hydrogen atom of the hydroxy function is implicated in an intramolecular hydrogen bond with either the nitrogen atom or the carboxylate functions of the ligand.

In summary, complexes of **L**H_2 with Eu^{3+} and Tb^{3+} interact strongly with ATP at physiological pH in water when compared to the other adenosine derivatives with shorter phosphorylated chains. It is surmised that this selectivity is due to the higher negative charge of the substrate (4−) and to the ability to form an increasing number of six-membered chelate rings with the longer phosphorylated chains. The similar behaviors observed for HPO_4^{2-} and ATP^{4-} may be ascribed to two different coordination mechanisms. Our efforts are now being directed toward the provision of additional functions on the ligand that should facilitate increased ATP recognition in water so as to better discriminate ATP from HPO_4^{2-}.

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Supporting Information Available: Experimental details for the new compounds and their characterization. UV−vis titration experiments and ^{31}P NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) Measured in a 50/50 mixture of D_2O and H_2O buffered with 0.1 M TRIS/$HClO_4$ at pH = 7.0.

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