A Coordination Chemistry Approach to Fine-Tune the Physicochemical Parameters of Lanthanide Complexes Relevant to Medical Applications


Dedicated to Professors Carlos F. G. C. Geraldes and Lothar Helm on the occasion of their retirement

Abstract: The geometric features of two pyclen-based ligands possessing identical donor atoms but different site organization have a profound impact on their complexation properties toward lanthanide ions. The ligand containing two acetate groups and a picolinate arm arranged in a symmetrical fashion (L1) forms a Gd³⁺ complex being two orders of magnitude less stable than its dissymmetric analogue GdL2. Besides, GdL1 experiences a much faster dissociation following the acid-catalyzed mechanism than GdL2. On the contrary, GdL1 exhibits a lower exchange rate of the coordinated water molecule compared to GdL2. These very different properties are related to different strengths of the Gd-ligand bonds associated to steric effects, which hinder the coordination of a water molecule in GdL2 and the binding of acetate groups in GdL1.

The lanthanides are a group of elements with very important technological applications often associated to their peculiar optical and magnetic properties. Several of these applications require the input of coordination chemistry to obtain lanthanide complexes with the desired properties, particularly regarding the design of optical and magnetic probes for medical diagnosis and bioanalytical assays. The lanthanide(III) (Ln³⁺) ions represent a series of hard Lewis acids that have a strong preference to form stable complexes with polydentate ligands containing hard oxygen donor atoms (i.e. polyaminopolycarboxylate ligands), generally adopting high coordination numbers (typically 8-9). The Ln³⁺-ligand interaction is considered to be largely electrostatic in origin, so that the metal coordination environment is dictated by a subtle interplay between electrostatic interactions and steric constraints.[10] As a result, there has been a limited success in establishing relationships between the structures and properties of Ln³⁺ complexes in solution or designing complexes with predetermined properties, though some major advances were reported for some specific systems[11] or with respect to some specific properties (i.e. relaxivity).[12] For instance, the water exchange kinetics in Ln³⁺ (often Gd³⁺) complexes were accelerated by increasing the steric compression around the water binding site, which can be achieved by replacing carboxylate groups by bulkier phosphonate units,[13] or by increasing the size of one of the chelate rings from 5 to 6-membered.[14] On the contrary, water exchange was decelerated by replacing carboxylate groups by amide donors,[15] as this increases the positive charge of the complex resulting in a stronger Ln³⁺-O_water bond. Increasing the size of the chelate and replacing carboxylate groups by amides is detrimental for complex stability, although DOTA-tetraamide Ln³⁺ complexes were found to possess higher kinetic inertness than the parent DOTA complexes.[10,11] Some structural modification on macrocyclic cyclen-based ligands were also found to accelerate the dissociation of the Gd³⁺ complexes, demonstrating that the kinetic inertness of some macrocyclic complexes cannot be generalized to all macrocyclic chelates.[12]

Herein we show that subtle changes in the arrangement of the ligand donor atoms have a deep impact in important features of the complexes, such as their thermodynamic stabilities and dissociation and solvent exchange kinetics. For this purpose, we have chosen the Gd³⁺ complexes of the recently reported macrocyclic ligands L1 and L2,[13] which differ in the relative positions of the pendant arms attached to the pyclen (12-py-N4)

Scheme 1. Ligands discussed in the present work.
We have selected Gd$^{3+}$ because of the relevance of complexes of this metal ion as contrast agents in MRI. The GdL1 and GdL2 complexes, as well as the corresponding Eu$^{3+}$ and Yb$^{3+}$ analogues, were obtained in excellent yields (89-98%) by reaction of the ligand with the corresponding lanthanide chloride salt in water at pH 5 (see Supporting Information).

Table 1. Relaxivities, stability constants, dissociation rate constants ($k_1$) and water exchange rates ($k_{ex}$) determined for GdL1 and GdL2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>GdL1$^{[a]}$</th>
<th>GdL2$^{[a]}$</th>
<th>GdPCTA$^{[a]}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r_1p / \text{mM}^{-1} \text{s}^{-1}$</td>
<td>4.74</td>
<td>4.95</td>
<td>7.09$^{[a]}$</td>
</tr>
<tr>
<td>$\log K_G$</td>
<td>20.49 ± 0.02</td>
<td>22.37 ± 0.03</td>
<td>18.28±0.03$^{[a]}$</td>
</tr>
<tr>
<td>$pGd$</td>
<td>17.74</td>
<td>20.25</td>
<td>16.62$^{[a]}$</td>
</tr>
<tr>
<td>$k_1 / \text{M}^{-1} \text{s}^{-1}$</td>
<td>$(6.9 \pm 0.1) \times 10^{-4}$</td>
<td>$(2.13 \pm 0.08) \times 10^{-4}$</td>
<td>$5.08 \times 10^{-4}$</td>
</tr>
<tr>
<td>$t_{1/2} / \text{min}$</td>
<td>167</td>
<td>542</td>
<td>231</td>
</tr>
<tr>
<td>$k_{ex} / 10^6 \text{s}^{-1}$</td>
<td>1.08 ± 0.02</td>
<td>22.5 ± 2.3</td>
<td>14.3</td>
</tr>
<tr>
<td>$\Delta H^\ddagger / \text{kJ mol}^{-1}$</td>
<td>28.6 ± 0.6</td>
<td>37.5 ± 0.2</td>
<td>45</td>
</tr>
</tbody>
</table>

[a] This work. [b] At 20 MHz, 25 ºC. [c] Calculated at pH = 7.4 using $c_{\text{ligand}} = 10^{-5} \text{M}$ and $c_{\text{Gd}} = 10^{-6} \text{M}$. [d] Rate constants characterizing the proton-assisted dissociation of the complexes. Data for GdPCTA was taken from reference [17] and correspond to the Eu complex. [e] Half-lives calculated from the rate constants 0.1 M proton concentration.

Given the results obtained from the thermodynamic studies, we next analyzed whether these complexes present significantly different dissociation kinetics. The dissociation of Ln$^{3+}$ complexes with macrocyclic ligands generally takes place following the acid-catalyzed mechanism. Thus, the dissociation of the complexes was followed under pseudo-first order conditions (proton concentration range 0.1 – 1 M) by following the changes in the UV absorption band of the picolinate group at 279 nm. The observed rate constants ($k_{obs}$) show a linear dependence with proton concentration (Figure 2). The rate constants ($k_{obs}$) of GdL2 are clearly lower than those of GdL1 at a given proton concentration, indicating that the complex of L2 presents a higher kinetic inertness. The kinetic data was analyzed by fitting the experimental data to $k_{obs} = k_0 + k_1[H^+]$, where $k_0$ and $k_1$ represent the rate constants characterizing the spontaneous and proton-assisted dissociation, respectively. The linear least-squares fits of the
data provided very small values for $k_s$ with large statistical errors, indicating that the spontaneous dissociation does not contribute to the overall dissociation reaction under the conditions applied. The $k_e$ values obtained (Table 1) reveal a considerably faster proton-assisted dissociation of GdL1 compared to GdL2, the latter showing a half-life ~3 times longer at a proton concentration of 0.1 M. The GdL2 complex is considerably more inert than EuPCTA (Scheme 1), for which an acid-catalyzed rate constant of 5.08 × 10^-4 M^-1 s^-1 was reported. Besides being considerably more inert, GdL2 also presents much faster complexation kinetics than GdL1 (Supporting Information).

The absorption spectra of the Eu^{3+} complexes of L1 and L2 recorded in H_2O solution show an absorption band with a maximum at 274 nm characteristic of the pyridyl chromophores (Supporting Information). The emission spectra were recorded in H_2O and D_2O solutions of the Eu^{3+} complexes (pH 7.45, tris buffer). The corresponding excitation spectra recorded upon analyzing at the maxima of the metal-centered emission are very similar to the corresponding absorption spectra. This indicates sensitization of the metal ion through energy transfer from the ligand to the metal ion. The complexes present the red emission associated to the 5D_0 → 7F_J (J = 0-4) transitions of this metal ion. A detailed comparison of the emission spectra recorded for EuL1 and EuL2 evidences different splitting patterns of the $\Delta J = 1$ and the hypersensitive $\Delta J = 2$ transitions (Supporting Information), reflecting significantly different coordination environments in the two complexes. The emission lifetimes of the Eu^{3+} (5D_0) excited states were measured in H_2O and D_2O solutions to determine the number of water molecules coordinated to the metal ion (Supporting Information). These studies provided hydration numbers close to 1 using the methodology proposed by Beeby, which indicates the presence of a water molecule coordinated to the metal ion.

The water exchange rates of the coordinated water molecules in the GdL1 and GdL2 complexes were determined by measuring the $^{17}$O NMR transverse relaxation rates of aqueous solutions of the complexes at different temperatures (see Supporting Information for details). A qualitative analysis of the reduced transverse relaxation rates ($1/T_2$) can be performed using the following simplified equation (Eq [1]) that neglects the chemical shift difference between bound and bulk water:

$$\frac{1}{T_2}\sim\frac{1}{T_m^2}+\frac{1}{T_e}\tag{1}$$

In this equation, $T_m$ is the mean residence time of a water molecule in the inner coordination sphere of Gd^{3+} and $T_e$ is the transverse relaxation time of the bound water molecule. Generally $T_m$ and $T_e$ present an opposite temperature dependence, as water exchange is accelerated with increasing temperature ($T_m = 1/k_m$ decreases) while $T_e$ increases with increasing temperature. The $^{17}$O NMR data obtained for GdL1 and GdL2 show opposite temperature dependences, which evidences very different water exchange rates for these complexes. The $1/T_2$ values increase with increasing temperature for GdL1, which shows that $T_m$ dominates the denominator of Eq [1], pointing to a rather slow water exchange.

The $^{17}$O NMR transverse relaxation rates were fitted using standard procedures. In this analysis we fixed the $^{17}$O hyperfine coupling constant to the standard value of -3.8 × 10^6 rad s^-1. The longitudinal electron spin relaxation rate $1/T_{1e}$ was assumed to follow an Arrhenius-type temperature dependence with an activation energy of 1 kJ mol^-1. The parameters obtained from the least-squares fit of the data are shown in Table 1, while the fitted curves are presented in Figure 3. The water exchange of the coordinated water molecule was found to be 20 times faster for GdL2 compared to GdL1, highlighting that the different arrangement of the donor atoms of the ligand around the metal ion has a striking impact in the exchange rate of the coordinated water molecule. The water exchange rate obtained for GdL1 is about four times slower than that of GdDOTA ($k_{ex}^{298} = 4.1 \times 10^6$ s^-1) while the exchange rate obtained for GdL2 is faster than the bis-aquated GdPCTA complex ($k_{ex}^{298} = 14.3 \times 10^6$ s^-1). The analysis of the $^{17}$O NMR data also provided the relaxation times of the electron spin, which turned out to be similar for GdL1 and GdL2 ($T_{1e}^{298} = 49.4 \pm 1.4$ and 35.9 ± 2.7 ns, respectively, at 298 K and 9.4 T).

The structure of the YbL2 complex in the solid state was established using single-crystal X-ray diffraction measurements. The crystal structure (Figure 4) shows eight coordination of the metal ion by the ligand, with coordination number nine being completed by the presence of an inner-sphere water molecule. The metal coordination environment can be best described as tricapped trigonal prismatic, where the capping tripod is defined by the coordinated water molecule O1 and the amine nitrogen atoms N31 and N11. Atoms O31, O51 and N41 define the upper tripod while O11, N21 and N51 delineate the lower tripod (see labelling Figure 4).
To rationalize the very different properties of the GdL1 and GdL2 complexes we performed DFT calculations using standard methods (See Supporting Information). The theoretical structure of GdL2 is very similar to that observed in the solid state for the Yb3+ analogue. A comparison of the bond distances of the metal coordination environment calculated for GdL1 and GdL2 reveals significant differences. In particular, the distance involving the coordinated water molecule is clearly longer in GdL2 (Gd-O1 = 2.519 Å) than in GdL1 (2.450 Å). It is also worth noting that the average Gd⋯H distances involving the coordinated water molecules are virtually identical in the two complexes (3.010 Å), as a result of a different orientation of the HOH plane of the coordinated water molecule with respect to the Gd-O1 vector. Another important difference concerns the Gd-O3l distances (2.458 and 2.407 Å for GdL1 and GdL2, respectively), which suggest a weaker coordination of an acetate arm in GdL1. A more detailed analysis of the Gd3+ coordination environment was carried out by calculating the electron density (\( \rho \)) at the bond critical points (BCPs). Both \( \rho_{BCP} \) and ELF\(_{BCP}\) can be correlated to the strength of the Gd-donor bonds. The results (Figure 4, see also Supporting Information) show that the Gd-O bonds are generally stronger than the Gd-N ones, as would be expected. The values of \( \rho_{BCP} \) calculated for the Gd-O bonds involving carboxylate oxygen atoms (O1l, O3l and O5l) evidence stronger bonds in the case of GdL2 compared to GdL1, in line with the higher stability of the former. However, the inner-sphere water molecule is more tightly bound in GdL1, resulting in a lower water exchange rate.

In conclusion, we have shown that subtle changes in the arrangement of the donor atoms around the central metal ion have profound consequences in important properties of lanthanide complexes, including: i) their thermodynamic stability; ii) complexation kinetics; iii) kinetic inertness with respect to complex dissociation, and iv) the lability of coordinated water molecules. All these parameters must be optimized to develop efficient diagnostic and therapeutic agents relying on the use of Ln(III) ions. This effect is related to the high coordination numbers adopted by the Ln\(^{3+}\) ions and the fact that some donor atoms might be hindered by the environment. In GdL2 the coordinated water binding site is sterically compressed, which results in a fast water exchange. However, in GdL1 the oxygen atoms of carboxylate groups provide weaker interactions with the metal ion, which results in a lower thermodynamic stability. The faster proton-assisted dissociation kinetics of GdL1 is likely caused by the protonation of the weakly coordinated carboxylate, which triggers complex dissociation. A similar effect is probably responsible for the different water exchange rates and stabilities observed for the bis-hydrated Gd(DTTA-Me)\(_2\) regioisomers (DTTA-Me = N-methyl diethylenetriamine tetraacetate), as the complex with the methyl group on the terminal nitrogen is two orders of magnitude more stable than the derivative with the methyl group at the central nitrogen, while the terminal N-Me isomer has about 100-fold slower water exchange kinetics compared to the central N-Me isomer. Thus, the results of this study provide the basis for the rational design and prediction of the geometric features of multidentate ligands for efficient lanthanide complexation, an issue with great potential impact in many areas where the coordination chemistry of the rare-earths is present.

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**Keywords:** Lanthanides • MRI • Macrocycles • NMR spectroscopy • Water Exchange


An apparently innocent change in the arrangement of the ligand donor atoms around the central Ln$^{3+}$ ion has profound consequences in the thermodynamic stability and dissociation and water exchange kinetics of the complexes.


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