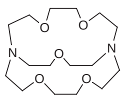
Structure and phosphodiesterase activity of Bis-Tris coordinated lanthanide(III) complexes

Soon Jin Oh, Young-Seo Choi, Seok Hwangbo, Sung Chul Bae, Ja Kang Ku, and Joon Won Park*

Department of Chemistry, Center for Biofunctional Molecules, Pohang University of Science and Technology, San 31 Hyoja-dong, Pohang, 790-784, Korea. E-mail: jwpark@vision.postech.ac.kr

A commonly used buffer, 2,2-bis(hydroxymethyl)-2,2′,2″-nitrilotriethanol (Bis-Tris) coordinates lanthanide(III) ion strongly in water to form molecular species that are highly active for the hydrolysis of a phosphate diester, bis(4-nitrophenyl) phosphate.

Ligand free lanthanide ions have been shown to be highly reactive for hydrolyzing phosphate esters including RNA and DNA.[1] There has been considerable interest in developing organic ligands that bind tightly around lanthanide ions in order to increase the solubility of the metal ions, and to develop artificial restriction enzymes by covalently anchoring the metal to molecules that bind nucleic acids sequence specifically.[2] Over the last several years, many elegant ligands (**1–7**) that bind lanthanide ions have been developed for the purpose of hydrolyzing phosphates.[2–5] Negatively charged ligands tend to bind tightly to lanthanide ions but greatly lower the reactivity of the metal ions for hydrolyzing phosphates.† On the other hand, neutral ligands tend to release the lanthanide ions by deligation (**1**, **2**).[3,4] Here we report a remarkably simple neutral ligand (**8**)[6]‡ that binds tightly around lanthanide ions without lowering their reactivity for hydrolyzing phosphates.



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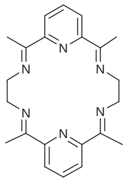

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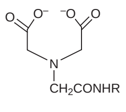

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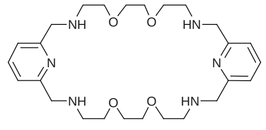

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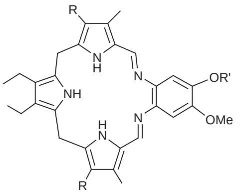

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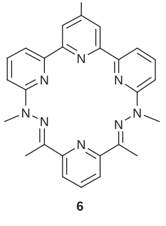

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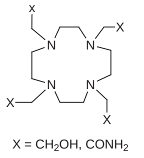

X = CH_2OH, $CONH_2$

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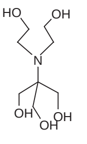

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Compound **8** is a commonly used buffer 2,2-bis(hydroxymethyl)-2,2′,2″-nitrilotriethanol (Bis-Tris). An equimolar mixture of $LaCl_3$ and Bis-Tris in methanol yielded white crystals. The structure of $[La(\mathbf{8})_2]Cl_3$ (Fig. 1) reveals that the metal has ten coordination sites occupied by two molecules of Bis-Tris.§ Each molecule of Bis-Tris acts as a pentacoordinate ligand with four of its oxygen atoms and its nitrogen atom. The remaining oxygen of Bis-Tris cannot coordinate due to its structural position. Although the crystal structure is that of a 2 : 1 ligand to metal complex, other data shown below indicate that the major species in aqueous solution of an equimolar mixture of $LaCl_3$ and Bis-Tris is a 1 : 1 ligand to metal complex.

Potentiometric titration of an equimolar mixture of $LaCl_3$ and Bis-Tris in water showed that there is no ligand-free La(III) ion or metal-free Bis-Tris. The pK_a of Bis-Tris shifts from 6.5 to 4.0 in the presence of an equivalent of $LaCl_3$ (24 mM), indicating that the formation of the complex is quite favorable in the aqueous solution ($K_f = 5 \times 10^4$ M^{-1}).¶ It is rather surprising to observe such a large binding constant for the neutral ligand, given that the formation constant of the europium(III) cryptand 221 complex is smaller than 0.5 M^{-1}.[3a]‖ Other buffers of the similar structure such as Tris, Bis-Tris Propane, Taps, Tapso, and Tes do not bind to lanthanide ions as well as Bis-Tris. In particular, a binding constant of 2.75×10^2 M^{-1} for Tris has been reported.[7]

In the titration of the equimolar mixture, consumption of 2 equiv. OH^- is observed at around pH 9.8. The low steepness of the titration curve suggest that a dimer **9** is formed at the pH. In the absence of any organic ligands, $LaCl_3$ itself also gives a curve of the similar shape. Based on the slope of the titration curve, it has been suggested that ligand-free lanthanide ions also form dimers or higher order aggregates under alkaline conditions. Thus lanthanide ions tend to form gels under alkaline conditions (pH > 9) even at low concentrations (0.5 mM). Interestingly, equimolar mixtures of $LaCl_3$ and Bis-Tris remain in solution even at 40 mM $LaCl_3$ and pH 11.5. It may be that Bis-Tris prevents aggregation of La(III) ions and enhances solubility by capping the two ends of the dimer **9** (Scheme 1).

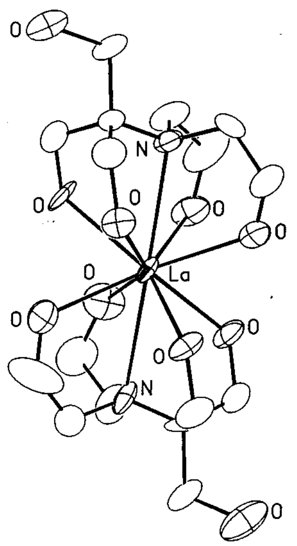
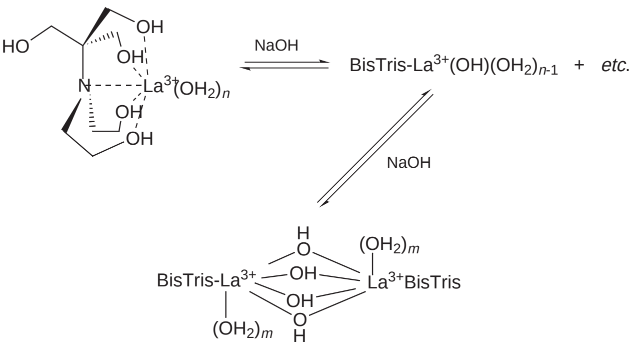


Fig. 1 ORTEP diagram of the lanthanum(III) complex coordinating two molecules of Bis-Tris. Distances between the coordinated oxygen atoms and the metal center are between 2.515(14) and 2.622(13) Å. Distances between the nitrogen atoms and the metal center are between 2.863(14) and 2.948(13) Å. Hydrogen atoms and non-coordinating chloride anions are removed for clarity.



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Scheme 1

Time-resolved luminescence spectroscopy is a valuable method for investigating Eu(III) complexes in aqueous solution.[8] The number of coordinating waters (*q*) per Eu(III) center in solution can be obtained by measuring the luminescence lifetimes of each species in water and deuterated water. Fig. 2 shows the excitation spectra of an equimolar mixture of $EuCl_3$ and Bis-Tris in water with 0–2.5 equiv. NaOH added. In the absence of any added NaOH, a single sharp peak at 579.7 nm with the *q* value of 6 suggests an 1 : 1 Eu to Bis-Tris complex with less than six coordinating water molecules. When two equiv. OH^- is added, a major peak at 580.4 nm with a significantly reduced *q* (2.5) value appears, which is consistent with structure **9**.

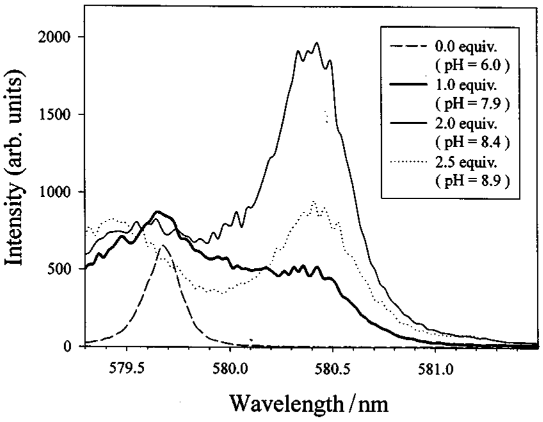
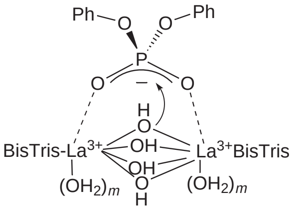


Fig. 2 Excitation spectra of the europium(III) complex in the presence of 1 equiv. of Bis-Tris. Spectra were recorded in water upon addition of (a) 0, (b) 1.0, (c) 2.0 and (d) 2.5 equiv. NaOH.

The reactivity of a 1 : 1 $LaCl_3$ to Bis-Tris solution for hydrolyzing bis(4-nitrophenyl)phosphate (BNPP) initially increases with added NaOH but reaches a maximum at 2 equiv. NaOH. Therefore, complex **9** or its kinetic equivalent is the active species for hydrolyzing BNPP. The phenomenon is observed at a wide range of concentration (0.5–20 mM). Although Bis-Tris increases the solubility of lanthanide ions by tightly complexing them, it does not reduce the reactivity of the metal center for hydrolyzing BNPP. For comparison, the activities were measured at low concentration, where precipitation of free lanthanide ions is not problematic. The hydrolytic activity of a 1 : 1 $LaCl_3$ to Bis-Tris solution (0.5 mM) with 2 equiv. NaOH ($k = 7.3 \times 10^{-3}$ s^{-1}) is comparable to a solution of $LaCl_3$ (0.5 mM) with 2 equiv. NaOH ($k = 8.2 \times 10^{-3}$ s^{-1}). We propose that the hydrolytic mechanism involves nucleophilic attack of the phosphate bridging the two metal centers by one of the bridging hydroxides (**10**).[9]



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Notes and References

† Anionic chelates such as EDTA and maleic acid significantly reduce the hydrolytic activity of lanthanide ions.

‡ Polyalcohols such as glycerol and cyclodextrin have been used for effective chelating ligands for lanthanide ions.

§ *Crystal data* for [La(**8**)$_2$]Cl$_3$: Colorless crystals of $C_{16}H_{38}N_2O_{10}LaCl_3$; triclinic, space group $P\bar{1}$, $a = 13.9502(2)$, $b = 14.4812(2)$, $c = 21.5698(2)$, $\alpha = 84.5980(10)$, $\beta = 71.707(10)$, $\gamma = 71.6110(10)°$, $V = 3925.91(9)$ Å^3, $Z = 6$, $\mu = 19.9$ cm^{-1}, $D_c = 1.684$ g cm^{-3}, $M = 663.74$. There are two asymmetric units in the unit cell and three independent molecules per unit. The final $R_w(F^2) = 0.2326$ with a goodness of fit = 1.152, while the conventional $R(F) = 0.1034$ for 11 239 reflections with $F_o > 4[\sigma(F_o)]$. The high *R* value is mostly due to the large formula weight, unavoidable disorder of the unbound hydroxyl groups, and abnormally large thermal vibrations of C13, C21, C56, C58, N11 and O23 atoms. See Fig. S4 in electronic supplementary information section for a numbering scheme (http://www.rsc.org./suppdata/cc/1998/2189).

¶ A commercially available program, BEST, was employed for the data fitting. See A. E. Martell and R. J. Motekraitis, *Determination and Use of Stability Constants*, 2nd edn., VCH, New York, 1992.

∥ A larger formation constant has been reported by Burns *et al.* (*Inorg. Chem.*, 1981, **20**, 616). Uncertainty in interpreting the *q* values arises from the ill defined influence of the bound OH^- groups.

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