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Harnessing thorium(IV) as a catalyst: RNA and phosphate diester cleavage by a thorium(IV) macrocyclic complex†

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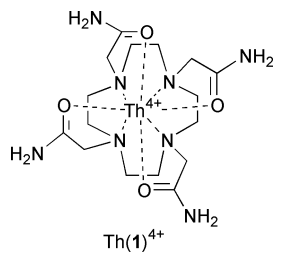
A robust macrocyclic tetraamide complex of Th(IV) binds phosphate diesters and promotes cleavage of RNA and phosphate diesters at 37 °C in the pH range 5.00–7.90.

Tetravalent cations such as Ce(IV), Th(IV) and Zr(IV) are among the most active catalysts for phosphate diester cleavage.[1–7] There are several reports that these metal ions promote the hydrolysis of phosphate diesters with poor leaving groups including dimethylphosphate and DNA.[1–5] Yet, the basis for this exceptional reactivity is not well understood. Studies reported to date on Ce(IV) or Th(IV) employ free metal ion or weak metal ion complexes and most studies are carried out at acidic pH to prevent precipitation of metal hydroxide complexes.[1–7] The complicated solution speciation of these systems makes mechanistic analysis difficult. Few attempts have been made to prepare well defined, catalytically active complexes of these metal ions.[4,6,7] Here, we report an unusual example of a Th(IV) complex of a neutral macrocycle which is highly resistant to dissociation in aqueous solutions. The complex efficiently promotes RNA cleavage and transesterification of a phosphate diester at neutral pH. Rate constants for RNA cleavage compare favorably to other metal ion macrocyclic complex catalysts including those containing lanthanide ions.[8–13]

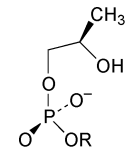


Th(**1**)$^{4+}$

The tetraamide macrocycle **1** binds to 12 members of the lanthanide(III) series with Sm(III) and Eu(III) being the most strongly bound.[12] Given that the ionic radius and coordination geometry of Th(IV) complexes are similar to those of the middle lanthanide(III) ions, **1** was studied as a ligand for Th(IV). Th(**1**)$(NO_3)_4$ was prepared in dry methanol from the free base form of **1** and $Th(NO_3)_4$.‡ NMR studies were carried out to characterize Th(**1**)$^{4+}$ in solution. The six broad ^{1}H NMR resonances observed at room temperature in D_2O sharpened but remained slightly broadened at 4 °C (ESI†).§ The ^{1}H NMR spectrum is analogous to that observed for La(**1**)$^{3+}$ which has **1** coordinated as an octadentate ligand with donor groups coordinated in a square antiprismatic arrangement.¶ The two sets of amide pendent group methylene protons appear as two doublets, the two sets of equatorial ethylene protons give rise to two doublets and the two sets of axial ethylene protons give rise to two triplets. Unfortunately, it was not possible to study the fluxional process further for Th(**1**)$^{4+}$ due to ligand decomposition at high temperatures and the insolubility of the complex in solvents other than water. However, this fluxional process likely involves ring inversion and cooperative pendent group rotation which interconverts the two carbons of the macrocyclic ring, the two sets of lower ring protons, the two sets of upper ring protons and the methylene protons in analogous lanthanide(III) complexes.[13,14] Consistent with such a fluxional process occurring, the ^{13}C NMR spectrum of Th(**1**)$^{4+}$ in D_2O at 22 °C has only three resonances‡ suggesting that, on the ^{13}C NMR timescale, the ethylene carbons are in fast exchange as has been observed for La(**1**)$^{3+}$.[13]

The Th(**1**)$^{4+}$ complex is resistant to dissociation in aqueous solution. Dissociation of Th(**1**)$^{4+}$ was studied in the presence of excess Cu(II) by monitoring formation of the Cu(**1**)$^{2+}$ complex by UV–VIS spectroscopy.[13] No dissociation of the complex (1.00 mM) was observed at pH 6.00 in a buffered solution in the presence of a 10-fold excess of Cu(II) over a period of one week at 37 °C. Remarkably, incubation of the Th(**1**)$^{4+}$ complex in the presence of an equivalent of EDTA at 37 °C and pH 6.00 did not induce dissociation of the complex over a period of a week. This inertness to metal ion release is reminiscent of the Eu(**1**)$^{3+}$ complex which did not release Eu^{3+} over a period of 6 weeks under similar conditions.[13] That this resistance to dissociation is a kinetic effect and not due to an exceptionally high formation constant for Th(**1**)$^{4+}$ relative to Cu(**1**)$^{2+}$ is suggested by competition experiments containing free **1**, $Th(NO_3)_4$ and $Cu(NO_3)_2$. When **1** (1.00 mM) was incubated simultaneously with $Th(NO_3)_4$ (1.00 mM) and $Cu(NO_3)_2$ (10.0 mM) at pH 6.00, 37 °C, all of **1** was complexed as Cu(**1**)$^{2+}$.

The ability to bind phosphate diesters is an integral part of catalysis; lanthanide(III) macrocyclic complexes that do not bind to phosphate diesters are not catalysts for cleavage.[10,13] Binding was studied in solutions containing 5.00 mM diethylphosphate and up to 40 mM Th(**1**)$^{4+}$ at pH 6.90, 21 °C with 0.10 M tetramethylammonium chloride, 5.0 mM triethylphosphate as a reference and 0.100 M Hepes buffer. Two ^{31}P NMR resonances were observed for free (0.81 ppm) and bound phosphate diester (−4.61 ppm), consistent with slow exchange kinetics on the NMR timescale. Fitting of the binding isotherm obtained by plotting the fraction of bound phosphate diester *vs.* concentration of free Th(**1**)$^{4+}$ gave a binding constant of 17 M^{-1}. Th(**1**)$^{4+}$ also bound the RNA analog **2** under similar conditions, but only a few percent was bound at pH 6.90 in the presence of 15 mM Th(**1**)$^{4+}$. In contrast, under similar conditions, diethylphosphate binding to Eu(**1**)$^{3+}$ and La(**1**)$^{3+}$ is too weak to determine binding constants and dissociation of diethylphosphate from La(**1**)$^{3+}$ is rapid on the ^{31}P NMR timescale.[13] The stronger binding and slower exchange rates of Th(**1**)$^{4+}$ are consistent with the higher charge of this actinide complex. These results are consistent with previous studies showing that ligand exchange rates for Th(IV) are slower than those of trivalent cations.[15]



2 R = Ph
3 R= 4-NO_2Ph

Although exchange of bound and free phosphate diester is slow on the NMR timescale, ligand exchange is not likely the

† Electronic supplementary information (ESI) available: ^{1}H NMR spectra for [Th(**1**)]$(NO_3)_4$ in D_2O at 21 and 4 °C. See http://www.rsc.org/suppdata/cc/b0/b005866l

rate determining step in the cleavage studies described below. Binding of Th(**1**)$^{4+}$ (15 mM) to diethyl phosphate (10 mM), pH 6.90, 0.10 M Hepes buffer at 22 °C, is complete within the 90 s it takes to record the ^{31}P NMR spectrum. In comparison, cleavage of RNA analog **3** by 15 mM Th(**1**)$^{4+}$ in 0.10 M Hepes at 22 °C has a half-life of 4.5 min.

Th(**1**)$^{4+}$ promotes transesterification of the phosphate diester **3** to form the cyclic phosphate diester and 4-nitrophenylate as determined by use of ^{31}P NMR and UV–VIS spectroscopy.‖ At pH 7.30, 37 °C with 1.00 mM complex and 0.100 mM **3**, the reaction exhibited good first-order kinetics in **3** for greater than four half-lives. Transesterification of **3** is first-order in Th(**1**)$^{4+}$ complex in the concentration range 0.60–2.00 mM with a second-order rate constant of 0.65 M^{-1} s^{-1}. Addition of 10% EDTA (based on complex concentration) to reaction solutions did not reduce the pseudo-first-order rate constant, suggesting that the reaction is not catalyzed by a small amount of free Th(IV) ion. Transesterification of **3** by Th(**1**)$^{4+}$ is essentially independent of pH in the pH range 5.0–7.9. Phosphate diester cleavage by metal ion complexes is typically pH dependent owing to the formation of metal hydroxide complexes.[6,8–11] That cleavage of **3** by Th(**1**)$^{3+}$ is not pH dependent suggests that the speciation of the Th(IV) complex does not change in this pH range. Pseudo-first-order rate constants for Th(**1**)$^{4+}$ and analogous Ln(III) complexes are listed in Table 1. Th(**1**)$^{4+}$ is 40-times more active than La(**1**)$^{3+}$, the most active lanthanide complex in the series. In addition, Th(**1**)$^{4+}$ promotes cleavage of adenylic acid oligomers more rapidly than does La(**1**)$^{3+}$ under similar conditions.

How does Th(**1**)$^{4+}$ promote RNA cleavage and why is the complex more efficient than analogous Ln(**1**)$^{3+}$ complexes? Given that the ionic radii of Th(IV) and Eu(III) are nearly identical, it is likely that Th(**1**)$^{4+}$ is nine-coordinate with a single site for the binding substrate, analogous to Eu(**1**)$^{3+}$.[13] Thus, Th(**1**)$^{4+}$ activates the phosphate diester to cleavage most probably through interaction at a single coordination site. The greater reactivity of the Th(IV) complex compared to its lanthanide(III) analogs is attributed to the greater Lewis acidity of the Th(IV) center as suggested by its strong interaction with phosphate diesters. In conclusion, we show here that it is feasible to prepare a Th(IV) complex of a neutral macrocycle which is highly resistant to dissociation in neutral aqueous solutions and is active in the cleavage of phosphate diesters and RNA. We demonstrate that the catalytic power of a tetravalent cation can be harnessed in a macrocyclic complex.

Table 1 Pseudo-first-order rate constants for cleavage of RNA and phosphate diester **3** by thorium(IV) and lanthanide(III) complexes of **1** at 37 °C

Complex	Substrate[a,b,c]	$k_{obs}/10^{-4}$ s^{-1}
Th(**1**)$^{4+}$	**3**	7.5[a]
Th(**1**)$^{4+}$	A_{10}	9.2
La(**1**)$^{3+}$	**3**	0.16
La(**1**)$^{3+}$	A_{12}–A_{18}	1.6
Eu(**1**)$^{3+}$	**3**	NR[d]
Eu(**1**)$^{3+}$	A_{12}–A_{18}	NR[d]

[a] For substrate **3** conditions were pH 7.3, 1.00 mM complex, 0.100 mM **3**, 10 mM Hepes buffer, 100 mM $NaNO_3$. [b] For A_{10} conditions were pH 7.4, 0.200 mM complex, 5 mM Hepes buffer, 0.013 mM A_{10} (adenosine concentration). [c] For A_{12}–A_{18} conditions were pH 7.6, 0.200 mM complex, 5 mM Hepes buffer, 0.08 mM A_{12}–A_{18} (adenosine concentration).[13] [d] No cleavage observed under the conditions given above.[13]

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Notes and references

‡ Equimolar amounts of TCMC and $Th(NO_3)_4$ were heated to reflux in dry methanol for 1.5 h. The methanol was removed and the complex was isolated as a white solid in 43% yield following precipitation twice from a methanol–diethyl ether mixture. Anal. Calc. for $C_{20}H_{42}N_{12}O_{17}Th$ [Th(**1**)-(Et_2O)]$(NO_3)_4$: C, 25.16; H, 4.43; N, 17.60. Found: C, 25.02; H, 4.38; N, 17.27. FAB MS: *m*/*z* 818 [Th(**1**)$(NO_3)_3^+$]. The ^{13}C NMR spectrum of Th(**1**)$^{4+}$ in D_2O showed resonances at 52.5, 57.3, and 177.3 ppm assigned to carbons of the ethylene moiety, methylene and carbonyl carbons, respectively.

§ Only one major diastereomeric form of the complex is observed by ^{1}H or ^{13}C NMR spectroscopy. In contrast, Eu(**1**)$^{3+}$ has two diastereomers present in solution and solid state (see refs. 13 and 14).

¶ In the solid state, [La(**1**)(CF_3SO_3)(EtOH)]$(CF_3SO_3)_2$ is a ten-coordinate complex with an unusual 1,5,4 geometry while [Eu(**1**)(H_2O)]$(CF_3SO_3)_3$ is a nine-coordinate complex with a monocapped distorted square antiprism geometry.[13]

‖ The kinetics of transesterification of **3** were monitored by use of UV–VIS spectroscopy. At pH values > 6.0, the production of 4-nitrophenylate was monitored at 412 nm. At pH values < 6.0, the decrease in the absorbance of **3** at 300 nm was monitored.

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