Extraordinary acceleration of phosphodiester hydrolyses by thorium cations

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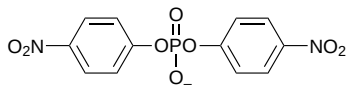
Th^{IV} cations in aqueous Brij micelles strongly promote the hydrolyses of several phosphodiester substrates; in the case of bis(*p*-nitrophenyl)phosphate 1, the observed rate constant (0.028 s^{-1}) represents an acceleration of *ca* 2.8 billion.

Much attention is deservedly focused on the remarkable acceleration of phosphodiester hydrolyses educed by lanthanide (Ln) cations.[1] Acting as Lewis acids, they bind the substrate's P–O^- and mitigate its negative charge, while simultaneously furnishing a metal-bound hydroxide nucleophile to attack the phosphodiester's phosphoryl group. Both Lewis acid strength and the acidity of the cation's water of hydration increase with increasing positive charge density, so that Ce^{IV}, the only lanthanide with a readily accessible +4 oxidation state, is of particular interest for the mediated hydrolysis of *e.g.* DNA.[2] Indeed, an excess of a 2 : 1 palmitate–Ce^{IV} complex in aqueous Brij micelles at pH 7 and 37 °C cleaves the model phosphodiester substrate, bis(*p*-nitrophenyl)phosphate **1**, (BNPP), with



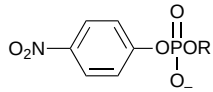
1 (BNPP)

$k = 2.6 \times 10^{-2}$ s^{-1},[3] representing a record enhancement of *ca.* 2×10^9 relative to the uncatalysed reaction at 25 °C.[1h]

Similarly, we examined hydrolyses mediated by the actinide uranyl cation.[4] Uranium offers a +6 oxidation state, but exists in water as UO_2^{2+}, which is less reactive than Ce^{IV}; *e.g.* $k = 9.5 \times 10^{-6}$ s^{-1} for hydrolysis of BNPP by excess UO_2^{2+}–*N*-hexadecyl-*N*,*N*′,*N*′,- trimethylethylenediamine (HTMED, pH 4.9, 37 °C).[4] Although this is equivalent to a rate enhancement of at least 8.6×10^5 relative to the uncatalysed reaction, UO_2^{2+} is inferior to Ce^{IV} by a kinetic factor of *ca.* 1100 in the cleavage of BNPP.

The actinide thorium, however, affords a stable +4 oxidation state in aqueous solution with multiple coordinated water molecules.[5] The 'hard acid' Th^{4+} readily coordinates with oxygen ligands (including phosphates),[5] so that we anticipate nonoxidative,[6] hydrolytic potency toward phosphodiesters. Indeed, Th^{4+} accelerates the hydrolysis of plasmid DNA and nucleotides at pH 4 or 5, with rate constants approaching those of Ce^{IV}.[6]

In order to quantitatively evaluate Th^{4+} relative to UO_2^{2+} and various Ln promoters of phosphodiester cleavage, we have now determined the reactivity of Th^{4+} toward BNPP and additional substrates **2**–**4**. We are pleased to report conditions under which



2 R = $CH_2CH(OH)Me$
3 R = $C_{16}H_{33}$
4 R = Et

the reactivity of Th^{4+} far exceeds that of UO_2^{2+}, is comparable to that of Ce^{IV}, and surpasses that of other metal cations.

Substrates **1**–**4** were hydrolysed readily in the presence of aqueous $Th(NO_3)_4$. Optimal conditions (see below) comprised 2 mM Brij-35, 10 mM HEPES buffer, pH 6.0 (10 mM KCl, 37 °C). We employed a 20-fold excess of Th^{4+} (1 mM) over substrate (0.05 mM). Kinetics were followed spectrophotometrically, monitoring the disappearance of the substrate's *p*-nitrophenyl moiety (290 nm) and the concomitant appearance of *p*-nitrophenol (317 nm). Reactions were observed for more than eight half-lives and pseudo-first order rate constants were obtained as the means of triplicate runs with $r > 0.997$ and reproducibilities within ±5%.

Rate constants appear in Table 1, where k for BNPP hydrolysis, 0.028 s^{-1}, represents an acceleration of *ca.* 2.8 *billion* relative to the uncatalysed reaction at pH 7,[1h] whilst the entry for the intramolecularly assisted cleavage of RNA model **2**, $k = 0.013$ s^{-1}, is *ca.* 3.9×10^5 greater than in the absence of metal cations at pH 7.[1a] These enhancements appear to be the largest reported to date for Ln or actinide cations with these commonly employed model substrates. We note, however, that the 20-fold excess of Th^{4+} is necessary; k_{obs} slows markedly as $[Th^{4+}]$: [BNPP] is reduced below 15 : 1 at pH 6. Control experiments also reveal inhibition of the hydrolysis by (added) inorganic phosphate.

The hydrolysis of BNPP was examined in detail: pH was varied from 3.5–7.0 in water, aqueous HEPES buffer, or HEPES–micellar Brij; added ligands included *N*-octyl-D-glucamine.[3] In HEPES– Brij, k_{obs} increased steadily from 9.3×10^{-4} s^{-1} at pH 3.5 to a maximum of 2.8×10^{-2} s^{-1} at pH 6.0, before declining slightly at pH 6.5 or 7.0. Above pH 7, precipitation of OH-bridged Th oligomers[5] was observed. A similar pH dependence was seen in water, with $k_{obs} = 5.2 \times 10^{-3}$ s^{-1} at pH 3.5 and 2.2×10^{-2} s^{-1} at pH 6.0 (maximum). The pK_a for water bound to Th^{4+} varies from 2.4 to 5.0, depending on the exact conditions;[7] a recent report[6] gives $pK_{a1} = 3.89$ and $pK_{a2} = 4.20$. These data are consistent with our observation of a reactivity maximum around pH 6.

The Th^{4+}-mediated hydrolysis of BNPP occurs with release of both *p*-nitrophenyl groups. Kinetic experiments under the conditions of Table 1 reveal that Th^{4+} hydrolysis of the presumptive intermediate, *p*-nitrophenylphosphate, is twice as fast ($k = 5.5 \times 10^{-2}$ s^{-1}) as that of BNPP. The reported rate constants therefore describe the hydrolysis of the first *p*-nitrophenyl group of BNPP, which is the rate-limiting step.

Brij micelles enhance the Th^{4+} reactivity: k_{obs} increases steadily at pH 6 in HEPES buffer from 5.5×10^{-3} s^{-1} in the absence of Brij, to a maximum of 2.8×10^{-2} s^{-1} at 2.0 mM Brij. Not only does micellar Brij potentiate the reactivity of Th^{4+} (presumably by binding substrate molecules and cations), but its oxygen atoms appear to solubilize the Th^{4+} cations or oligomers

Table 1 Kinetics of Th^{4+} mediated hydrolyses[a]

Substrate	$k_{obs}/10^{-4}$ s^{-1}
1	282[b]
2	132
3	11.9
4	8.54

[a] At pH 6.0, 37 °C; other conditions are detailed in the text. Rate constants are reported as means of three runs with $r > 0.997$ and reproducibilities within ±5%. [b] Both *p*-nitrophenyl groups were cleaved.

above pH 5. Brij-35 has been previously used to stabilize metal cation catalysts for phosphodiester hydrolysis.[4,8]

In contrast to Ce^{IV}, which requires both a complexing ligand and micellar Brij to remain in solution above pH 4–5, Th^{IV} is stabilized by Brij alone; added ligands merely sap its reactivity. For example, $k_{obs} = 2.8 \times 10^{-2}$ s^{-1} (Th^{4+}–BNPP in aqueous Brij at pH 6) is reduced to 1.4×10^{-3} s^{-1} upon the addition of 1 equiv. of *N*-octyl-D-glucamine, a ligand useful in the stabilization of Ce^{IV}.[3]

Th^{4+} is much more reactive than UO_2^{2+} toward substrates **1** or **2**. The rate constants of Table 1 can be compared with *k*(pH 4.9) = 9.5×10^{-6} s^{-1} for **1** (UO_2^{2+}–HTMED) or 2.2×10^{-4} s^{-1} (UO_2^{2+}) for **2**.† Using BNPP hydrolysis as a reactivity measure, Th^{4+} is comparable to Ce^{IV}–palmitate in Brij ($k = 2.6 \times 10^{-2}$, pH 7),[3] but more reactive than Eu^{3+} ($k = 1.7 \times 10^{-4}$ s^{-1}, pH 7, 50 °C),[1e,f] or various Eu^{3+} complexes (2.0–6.7 $\times$ 10^{-4} s^{-1}, pH 7–7.4, 25–50 °C),[1f,9] La^{3+} (1.4×10^{-7} s^{-1})[1g] or La^{3+}–H_2O_2 (4.8×10^{-3} s^{-1}, both at pH 7, 25 °C),[1g] 1 : 1 La^{3+}–Fe^{3+} (2.8×10^{-4} s^{-1}, pH 7, 50 °C),[10] or binuclear azamacrocyclic complexes of Eu^{3+} (1.4×10^{-3} s^{-1}, pH 7, 50 °C) or Pr^{3+} (8.4×10^{-4} s^{-1}, pH 7, 50 °C).[11]

It is clear from these comparisons that Th^{IV} and Ce^{IV} are the most reactive actinide or lanthanide cations yet reported for the acceleration of BNPP hydrolysis. A similar conclusion follows from the reactivity of Th^{IV} toward substrate **2**. Indeed, Th^{IV} and Ce^{IV} appear to be more reactive toward BNPP or **2**[1c] than any other metal cations thus far described, including (complexes of) cobalt[12] and copper.[13]

Toward long-chain phosphodiester substrate **3**, comicellized with 2 mM Brij-35 (critical micelle concentration, 0.06–0.09 mM[8a]), excess Th^{4+} affords $k_{obs} = 1.2 \times 10^{-3}$ s^{-1}. Comparisons are lacking except for UO_2^{2+}–HTMED where $k_{obs} = 1.1 \times 10^{-4}$ s^{-1}.[4] Perhaps a fairer comparison of Th^{4+}–Brij *vs.* UO_2^{2+}–HTMED is with the short-chain substrate **4**, where Th^{4+} (Table 1) manifests a 500-fold rate advantage over UO_2^{2+} ($k = 1.7 \times 10^{-6}$ s^{-1}).‡

In conclusion, Th^{IV} cations are an extraordinarily reactive, readily employed promoter of the hydrolysis of a variety of model phosphodiester substrates. We are continuing to probe their application to challenging problems in this area.

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Footnotes and References

† No attempt is made in the kinetic comparisons to correct for differing conditions or cation–substrate ratios. Rather, the maximum rate constants available in the literature are compared to those of Th^{4+}.

‡ The minimal rate advantage for Th^{4+} cleavage of micellized substrate **3**, relative to nonmicellar analogue **4**, contrasts with rate enhancements of 50–70 previously observed with Eu^{3+} or UO_2^{2+} toward aggregated substrates (refs. 4, 14). The differing behaviour may be due to the Brij comicelles employed with Th^{4+}.

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