Remarkable acceleration of dimethyl phosphate hydrolysis by ceric cations

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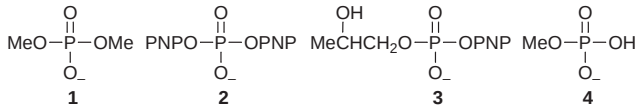
Ce^{4+} cations in aqueous solution at pH 1.8 and 60 °C reduce the half-life of dimethyl phosphate hydrolysis from 8454 years (pH 7, 60 °C) to 22 min.

Dimethyl phosphate **1** (DMP) strongly resists hydrolysis. A recent meticulous study reports $k = 1.6 \times 10^{-13}$ s^{-1} at 25 °C, equivalent to a half-life of ~137 000 years for the pH 7 uncatalyzed cleavage (by Me–O scission) of DMP by water.[1] This extraordinary stability makes the phosphodiester linkage ideal for its role in the backbone of DNA and RNA.[1,2]

The hydrolysis of DMP can be accelerated; below pH 5, acid catalysis is apparent.[1] Bunton *et al.* reported that at pH 1.24 and 100 °C, the P–O$^-$ of DMP was protonated, and hydrolysis of neutral **1** proceeded with $k = 3.13 \times 10^{-6}$ s^{-1} ($t_{1/2}$ = 2.5 days, 78% Me–O scission).[3] More recently, Kim and Chin found that $[(cyclen)Co(OH_2)_2]^{3+}$ at pD 6.3 and 60 °C catalyzed the hydrolysis of DMP (presumably by P–O scission) with $k = 2 \times 10^{-7}$ s^{-1} ($t_{1/2}$ ~40 days).[2]



1 **2** **3** **4**

Various transition metal, lanthanide and actinide cations are known to accelerate the hydrolytic cleavage of phosphodiesters.[4] Our own studies of the metal-ion mediated hydrolysis of phosphodiesters have focused on Th^{4+}, Ce^{4+} and Zr^{4+} cleavage of activated substrates such as bis(*p*-nitrophenyl) phosphate **2** (BNPP) and 2-hydroxypropyl (*p*-nitrophenyl) phosphate **3** (HNPP).[5–7] Rate enhancements $>10^9$, relative to the uncatalyzed hydrolyses, were observed in the Th^{4+} or Ce^{4+} cleavage of BNPP.[5,6] A high value of the charge/cation diameter ratio appears necessary for optimal cation reactivity in phosphodiester hydrolysis.[5–8]

In view of our results with substrate **2**,[6] and the known ability of Ce^{4+} to accelerate the cleavage of DNA (at P–O),[9] we have now examined the Ce^{4+} acidic hydrolysis of DMP. The remarkable kinetic results indicate an acceleration of 2×10^8, relative to the uncatalyzed hydrolysis at 60 °C, and a reduction in $t_{1/2}$ from ~8450 years to 22 min.

Hydrolyses of DMP **1** and of methyl phosphate **4** (MP) mediated by ceric ammonium nitrate (CAN) were followed in D_2O at 60 °C by 400 MHz proton NMR spectroscopy, monitoring the disappearance of the Me signals at δ 3.9, relative to an internal pyrazine standard. Solutions of CAN were not buffered, but were adjusted to pH 1.6 or 1.8 (pD 2.0 or 2.2) by addition of pD 13.4 NaOD solution prior to reaction. The pH typically declined by 0.25 during the reaction. Reactions were followed for two half-lives and the infinity titer was obtained after 24 h at 60 °C. Rate constants were duplicated and agreed to ±7%.

With 1 mM DMP at pH 1.6,‡ the measured hydrolytic rate constants varied from 9.3×10^{-5} s^{-1} at [Ce^{4+}] = 2.5 mM to 4.25×10^{-4} s^{-1} at [Ce^{4+}] = 20 mM. A graphical representation of the dependence of k on [Ce^{4+}] appears in Fig. 1, where 'saturation' behavior is apparent. Michaelis–Menten analysis provides K_m ~0.0105 M and a binding constant of ~95 M^{-1}, with k_{cat} ~6.5×10^{-4} s^{-1}.§ Unfortunately, the fit of the data in Fig. 1 to the Michaelis–Menten equation is not very precise. Although saturation (binding of DMP to Ce^{4+}) is observed, the K_m and k_{cat} are approximate.

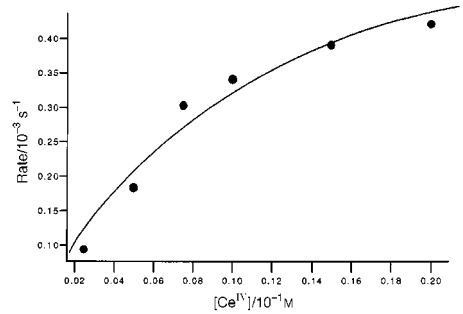


Fig. 1. Pseudo-first order rate constants (s^{-1}) for the hydrolysis of 1 mM DMP by CAN as a function of [Ce^{4+}] at pH 1.6. The points are the experimental values; the solid line is generated from the Michaelis–Menten equation with $K_M = 0.0105$ M and $k_{cat} = 6.48 \times 10^{-4}$ s^{-1}.

Our highest observed rate constant for the cleavage of 1 mM DMP by 10 mM Ce^{4+} was at pH 1.8, 60 °C, where k = $5.28(\pm0.38) \times 10^{-4}$ s^{-1}. At higher pH, precipitation of Ce was apparent.

Comparisons of the hydrolytic kinetics of DMP under neutral,[1] acidic,[3] Co^{3+}-assisted,[2] or Ce^{4+}-mediated conditions appear in Table 1, where the extraordinary acceleration due to Ce^{4+} is manifest. At 200 million, the rate enhancement by acidic Ce^{4+} exceeds those provided by H^+ alone or Co^{3+}-cyclen by factors of 10^3–10^4. The 8454 year 'benchmark' half-life of DMP under neutral conditions at 60 °C is reduced to 22 min by Ce^{4+} at pH 1.8.

The Ce^{4+} cleavage of DMP produces 2 equiv. of MeOH (NMR), so that the reaction is hydrolytic, not oxidative; hydrolysis also occurs in the Ce^{4+} cleavage of deoxyribodinucleotides.[10] Hydrolysis of 1 mM MP **4** by 10 mM CAN at pH 1.8 and 60 °C liberates 1 equiv. of MeOH and proceeds with $k = 6.3 \times 10^{-4}$ s^{-1}, about 1.2 times faster than the corresponding cleavage of DMP. Accordingly, MP does not accumulate during the hydrolysis of DMP. A similar rate ordering prevails during the acid catalyzed hydrolyses of DMP and MP.[3,11]

We briefly examined several other sets of conditions and catalysts for the hydrolysis of DMP. A ten-fold excess of Ce^{3+} at pH 8 (gel) also cleaved DMP at 60 °C, but much more slowly than Ce^{4+} at pH 1.8; the Ce^{3+} hydrolysis was only ~50% complete after 48 h. Surprisingly, Th^{4+} (10 mM) was unreactive toward DMP at pH 3.5 and 60 °C, while Zr^{4+} (10 mM, pH 2.0,

Table 1 Comparison of DMP hydrolyses at 60 °C[a]

Conditions	k_{hydrol}/s^{-1}	k_{rel}	$t_{1/2}$
Neutral[b]	2.6×10^{-12}	1.0	8454 years
H^+, pH 1.24[c]	5.2×10^{-8}	2.0×10^4	154 days
Co^{3+}-cyclen[d]	2.0×10^{-7}	7.7×10^4	40 days
Ce^{4+}, pH 1.8[e]	5.3×10^{-4}	2.0×10^8	22 min

[a] The first two entries are extrapolated to 60 °C from data in the original references. [b] Ref. 1. [c] Ref. 3. [d] Ref. 2; pH 5.9. [e] This work.

60 °C) cleaved DMP slowly ($k \sim 7.6 \times 10^{-6}$ s^{-1}). Addition of 5 mM $PrCl_3$ to 10 mM CAN did not significantly accelerate the cleavage of DMP at pH 2.5, 60 °C; $k = 5.7 \times 10^{-4}$ s^{-1}, comparable to the rate constant with Ce^{4+} alone (Table 1). Rate enhancement due to Pr^{3+}–Ce^{4+} clusters[12] did not occur. Finally, neither added chloride or nitrate ions (120 mM) further enhanced the Ce^{4+} hydrolysis of DMP.

The mechanism of the Ce^{4+}-mediated hydrolysis of DMP is not yet fully defined. The saturation behavior (Fig. 1) implicates binding of substrate P–O$^-$ by Ce^{4+}. The pK_a of the waters of hydration bound to Ce^{4+} is ~0.7,[13] so that the Ce^{4+}-DMP complex will have Ce-bound OH nucleophiles available for attack at the substrate P. One can imagine a P–O(Me) scission mechanistically analogous to that suggested for the Co^{3+}-cyclen/DMP reaction.[2] However, the neutral (> 99.5%)[1] and the acid catalyzed (78%)[3] hydrolyses of DMP occur mainly by O–Me cleavage. Although it seems unlikely that Ce^{4+} complexation of DMP would enhance subsequent H_2O attack at the MeO (rather than the P–O) linkage by the enormous factors observed here, a definitive mechanism requires $H_2{}^{18}O$ studies of the Ce^{4+} hydrolyses of both DMP and MP. These studies, together with comparable examinations of phosphonate monoester hydrolyses, are in progress.

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Notes and References

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‡ The pK_a of DMP is 1.06 at 60 °C (extrapolated from the data in ref. 3), so that > 77% of the DMP will be in the monoanionic form at pH 1.6, available for binding to Ce^{4+}.

§ From the initial four points of Fig. 1, [Ce^{4+}] = 2.5–10 mM, we can estimate a second order rate constant for the Ce^{4+}/DMP cleavage: $k_2 = 3.5 \times 10^{-2}$ M^{-1} s^{-1} at pH 1.6 and 60 °C. This may be compared with $k_2 = 6.2 \times 10^{-7}$ M^{-1} s^{-1} at pD 6.3 and 60 °C for the Co^{3+}-cyclen/DMP reaction (ref. 2).

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