Pergamon

Tetrahedron Letters 39 (1998) 1529–1532

TETRAHEDRON
LETTERS

Zirconium and Hafnium Cations Rapidly Cleave Model Phosphodiesters in Acidic Aqueous Solutions

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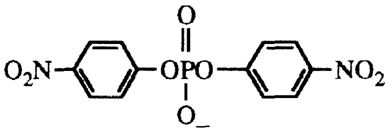
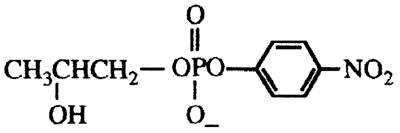
Received 9 December 1997; revised 2 January 1998; accepted 5 January 1998

Abstract. Aqueous solutions of 20-fold excess Zr^{4+} and Hf^{4+} at pH 3.5 (37 °C) hydrolyze phosphodiester substrates **1-3** very rapidly. The reactivity of Zr^{4+} (with rate constants up to 0.015 s^{-1} for **2** at 1 mM Zr^{4+}) exceeds that of most other transition metal cations. © 1998 Elsevier Science Ltd. All rights reserved.

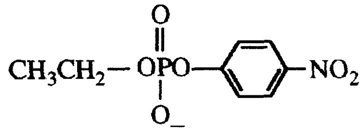
Attempts to develop synthetic nucleases have stimulated intensive study of the metal-catalyzed scission of organic phosphates, particularly phosphodiesters.[1] Their effective mediation of substrate hydrolysis has marked the lanthanides,[1,2] and especially Ce^{4+}, as cations for special attention.[3,4] Recently, we have shown that the actinide cations, UO_2^{2+} and Th^{4+}, are particularly active against phosphodiesters.[5,6] Th^{4+} in aqueous Brij micellar solution educes an acceleration of nearly 3 billion-fold in the hydrolysis of bis(*p*-nitrophenyl)phosphate (**1**, BNPP)[6] similar to that afforded by the Ce^{4+}-palmitate complex.[4]



1 (BNPP)



2 (HNPP)



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Lanthanides and actinides do not occur in metalloenzymes, however. In phosphotriesterase, for example, Zn^{2+} is an essential component of the active site apparatus, and can be replaced by other transition metal cations, including Co^{2+}, Ni^{2+}, Cd^{2+} or Mn^{2+}.[7] Various transition metal cations have also been examined in the mediated cleavages of phosphotriesters (e.g., Zn^{2+} and Cu^{2+})[8,9] and phosphodiesters, especially BNPP. In the latter case, cations of Cu, Ni, Zn, Co, and Cd have been employed,[10,11] and similar studies have been undertaken with the "RNA model" 2-hydroxypropyl *p*-nitrophenyl phosphate (**2**, HNPP).

In phosphodiester cleavage, the cations function simultaneously as acidic and basic catalysts. Acting as Lewis acids, they bind the substrate's $P\text{-}O^-$ and lessen its negative charge; at the same time, they provide a metal-bound OH to attack the phosphoryl group. Because Lewis acidity and the acidity of $M\text{-}OH_2$ both increase with increasing positive charge density on the metal cation, highly charged cations, such as Ce^{4+} and Th^{4+} are particularly reactive toward BNPP.[4,6]

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PII: S0040-4039(98)00074-4

Among the transition metals, zirconium and hafnium nominally afford tetravalent cations, and exhibit chemistry that is mutually similar[12] and similar to that of thorium.[13] Given the exceptional reactivity of Th^{4+},[6] we began a study of the cleavages of BNPP, HNPP, and the less activated ethyl *p*-nitrophenyl phosphate (**3**), by aqueous Zr(IV) and Hf(IV). We are pleased to report that at pH 3.5, where conditions are optimal, the reactivity of Zr^{4+} exceeds that of other transition metal cations (at higher pH's), and rivals that of Th^{4+}.

The pK_a's of metal bound water molecules are ≤ 0.6 for aqueous Zr(IV) and ≤ 1.1 for Hf(IV),[14a] so that M-OH nucleophiles should be available from these cations even in acidic aqueous solutions. However, the precise constitutions of the cations are unclear; it is likely that dimeric $[M_2(OH)_2^{6+}]$ or higher polynuclear Zr and Hf species exist in moderately concentrated aqueous acid.[12b,14b] For simplicity, we will represent the M(IV) species present at pH 3.5, our optimal operating condition, as M^{4+}, while acknowledging that the actual reactant cations are likely to be more complicated.

Kinetic studies of substrates **1-3** were carried out at 37 °C in unbuffered aqueous solutions adjusted at 25 °C to pH 3.5 with KOH or HCl. Post-reaction measurements revealed small downward pH drifts, with final readings of 3.15-3.25 (for Zr^{4+}) or 3.3-3.4 (For Hf^{4+} or Ce^{4+}); these values remain far above the pK_a's of $M^{4+}(H_2O)$; see above and ref. 14c. Under "standard" conditions, Zr^{4+} or Hf^{4+} (supplied as MCl_4, Strem) were used in 20-fold excess (1×10^{-3} M) over substrate (5×10^{-5} M), and the release of *p*-nitrophenol was followed at 317 nm.[15] Reactions were monitored over 8 half-lives; rate constants of duplicate or triplicate runs agreed to within ±5% with $r > 0.997$ for pseudo-first-order kinetic correlations. Kinetic data for Zr^{4+}, Hf^{4+}, and Ce^{4+} appear in Table 1, together with pH 6 data for Th^{4+}.[6,16] Attempts to measure rate constants for Eu^{3+} or Ce^{3+} cleavages at pH 3-5 were frustrated by the very slow kinetics: less than 10% of BNPP hydrolysis was observed after 12 h. The cleavage of BNPP by Zr^{4+} or Hf^{4+} under the conditions of Table 1 proceeded with liberation of 2 equivalents of *p*-nitrophenol. Separate experiments showed that cleavage of the intermediate, *p*-nitrophenyl phosphate, was more rapid that that of BNPP: rate constants were 0.012 s^{-1} (Zr^{4+}) and 0.0038 s^{-1} (Hf^{4+}).

Table 1. Rate Constants ($10^3 k$, s^{-1}) for the Cleavage of Phosphodiesters[a]

Substrate	Zr^{4+}	Hf^{4+}	Ce^{4+}	Th^{4+} [b]
BNPP	8.1	2.9	80	28
HNPP	15	3.9	58	13
3	7.3	2.1	11	0.85

[a]Conditions: pH 3.5, 37 °C, [substrate] = 5×10^{-5} M, $[M^{4+}] = 1 \times 10^{-3}$ M. [b]At pH 6.0, reference 6.

The data of Table 1 reveal that Zr^{4+} and Hf^{4+} are very reactive toward the *p*-nitrophenyl phosphodiesters **1-3** at pH 3.5. The cations' reactivities decrease at pH 4.5, and Zr^{4+} precipitates; Hf^{4+} precipitates at pH 5.5. Solubilization of both cations can be achieved at pH 5.5 using 2 mM Brij-35 micellar solutions, but rate constants are reduced relative to the pH 3.5 aqueous system.

The smaller Zr^{4+} cation (ionic radius 59 pm) is clearly more reactive than Hf^{4+} (71 pm) toward all 3 substrates, although the active species may not be the mononuclear cation. The reactivity of Zr^{4+} is similar to that of Th^{4+} (determined at pH 6[6]), but is inferior to that of Ce^{4+}.[4] Both Zr^{4+} and Hf^{4+} appear to be more reactive than such lanthanide cations as Eu^{3+} and Ce^{3+} at pH 3.5, where the lanthanides ($pK_a > 4$)[14c] bear H_2O, rather than OH ligands.

Comparisons of the Zr^{4+} kinetic data to that for other transition metal cations in the literature must consider changes of conditions. Even so, Zr^{4+} appears to be 1-2 orders of magnitude more reactive than Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, or Cu^{2+} (toward HNPP at pH 6.85, $\mu = 0.1$).[1] To sharpen these comparisons we determined the second order rate constant for the Zr^{4+} hydrolysis of HNPP in 0.1 M $NaNO_3$ solution at pH 3.5, 37 °C. With [HNPP] fixed at 5×10^{-5} M, the concentration of Zr^{4+} was varied between 2.5×10^{-4} M and 1×10^{-3} M. k_2 was obtained as 2.2 $M^{-1}s^{-1}$ from the linear ($r > 0.99$) correlation of pseudo-first-order rate constants vs. $[Zr^{4+}]$. Our value of $k_2 = 2.2\ M^{-1}s^{-1}$ for Zr^{4+} can be compared to k_2 values for other transition metal cations ($M^{-1}s^{-1}$) determined at pH 6.85 in 0.01 M HEPES buffer, $\mu = 0.1$ ($NaNO_3$): 0.04 (Cu^{2+}), 0.018 (Zn^{2+}), 0.026 (Co^{2+}), 0.0024 (Mn^{2+}), or 0.0016 (Ni^{2+}).[1] Lanthanide cations are more reactive under these conditions, but still inferior to Zr^{4+}: 0.13 (La^{3+}), 0.26 (Nd^{3+}), 0.32 (Eu^{3+}), and 0.57 (Tb^{3+}).[1]

Other comparisons of Zr^{4+} with various complexes of Cu^{2+}, Ni^{2+}, Cd^{2+}, or Zn^{2+} generally indicate significant kinetic advantages for Zr^{4+}.[10,17] Indeed, relative to the uncatalyzed hydrolysis of BNPP ($k_o \sim 1.1 \times 10^{-11}$ at 25 °C, pH 7),[18] one can estimate an acceleration of $\sim 7.4 \times 10^8$ for Zr^{4+} and $\sim 2.6 \times 10^8$ for Hf^{4+} under the conditions of Table 1.

Although the results given here are preliminary, and involve only the properties of simple aqueous solutions of Zr^{4+} or Hf^{4+}, they suggest that the kinetic proclivities of appropriately designed complexes of these highly charged transition metal cations could be of considerable interest.

Acknowledgment. We are grateful to the U.S. Army Research Office for financial support.

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(15) In experiments with Ce^{4+} [delivered as $Ce(NH_4)_2(NO_3)_6$] *p*-nitrophenol was monitored at 370 nm because of interference from Ce^{4+} at the lower wavelength.

(16) Kinetic results for Th^{4+} at pH 3.5 were not very reproducible, which may be related to its pK_a falling near this pH (pK_a = 2.4-5.0).[6]

(17) Only the Cu complex of Kövari and Krämer[10b] approaches Zr^{4+} in its reactivity (toward BNPP) among the dipositive transition metal cations. Complexes of Co^{3+}, however, appear to be kinetically more competitive.[11]

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