Remarkably Rapid Cleavage of a Model Phosphodiester by Complexed Ceric Ions in Aqueous Micellar Solutions

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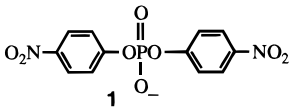
The 1990's have witnessed intense interest in the use of lanthanide (Ln) cations to facilitate the hydrolysis of phosphodiesters. "Early" explorations of model DNA and RNA hydrolyses, accelerated by various lanthanides,[1] were followed by comparative kinetic studies focused on bis(*p*-nitrophenyl)-phosphate (**1**, BNPP), which has become an informal "standard" phosphodiester substrate.[2,3] Using BNPP, one can readily



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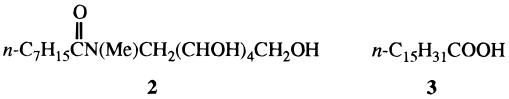
compare the accelerations provided by simple complexes of Ln cations,[2] La^{3+}/H_2O_2,[3,4] Ln/non-Ln clusters,[5] hydroxyl-functionalized azamacrocyclic Ln complexes,[6] and paired Ln cations in macrocylic complexes.[7] Other recent applications of Ln ion mediation have been reported for hydrolyses of DNA,[8] RNA,[9] hydroxyquinoline phosphodiesters or phosphonates,[10] and liposomal phosphodiesters.[11]

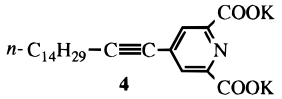
Mechanistically, the Ln cations serve a dual purpose: as Lewis acids to bind and charge-neutralize the phosphodiester's P−O^-, while simultaneously furnishing a metal-bound OH nucleophile to attack the substrate's phosphonyl group. Increasing the metal cation's charge density should enhance both its Lewis acid proclivity and the acidity of its waters of hydration, so that particular attention centers on Ce(IV), the only lanthanide with a readily available +4 oxidation state. Applications of Ce(IV) to DNA cleavage have been emphasized.[12,13]

Unfortunately, above pH 4, the formation and precipitation of Ce(IV)−hydroxide gels hinders kinetic studies of Ce(IV)-mediated phosphodiester hydrolyses. Complexation of the Ce(IV) could solve this problem, but strongly bound ligands would mitigate the metal's activity. Komiyama has used a weak complex of Ce(IV) and γ-cyclodextrin to obtain homogeneous, neutral, aqueous solutions for the cleavage of nucleoside 3′,5′-cyclic monophosphates[13a] and peptides.[13d] Now, we describe three new ligands which provide stabilized, highly reactive Ce(IV) in nonionic Brij-35 micelles.[14] These systems mediate the cleavage of BNPP over a broad pH range (4.0−12.0) at rates clearly surpassing those previously reported for other Ln cations.

The ligands we employed included *N*-octanoyl-*N*-methyl-*D*-glucamine (**2**), palmitic acid/palmitate (**3**), and 4-(1-hexadecynyl)-2,6-pyridinedicarboxylate (**4**). Ligands **2** and **3** were



2 **3**



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commercially available, whereas **4** was readily prepared by Cu/$Pd(PPh_3)_2$ catalyzed coupling of hexadecyne with diethyl 4-bromo-2,6-pyridinedicarboxylate[15] (75 °C, 3 h, 72%), followed by saponification (aq KOH, MeOH, 40 °C, 36 h, 84%). Ligand **4** was characterized by NMR and elemental analysis.

Hydrolytic media were prepared in 2×10^{-3} M (for **2**) or 5×10^{-3} M sonicated (for **3** or **4**) aqueous Brij-35 solutions that also contained 1.0 mM ligand and 0.01 M KCl. The solutions were buffered with 0.01 M MES, HEPES, CHES, or CAPS according to pH requirements, but solutions at pH 4, 5, or 12 were unbuffered. To initiate hydrolyses, 1.0 or 2.0 mM Ce$(NH_4)_2(NO_3)_6$ was added, the pH was adjusted with KOH, and then 5×10^{-5} M BNPP was added. Importantly, stock solutions of Ce(IV) were prepared at pH ≤1.5 to avoid hydroxide gel formation and an accompanying reactivity decrease. Delivery of the Ce(IV) into the ligand/Brij medium appears to stabilize the Ce(IV) against hydroxide at higher pH.

Control experiments demonstrate that *both* ligand and Brij are required to provide homogeneous Ce(IV) solutions above pH 5.[16] The critical micelle concentration of Brij-35 is 0.06−0.09 mM,[14] so that our reaction solutions are micellar. For example, dynamic light scattering analysis of **2** + Brij or **2** + Brij + Ce(IV) solutions reveal aggregates of ∼15-nm diameter, consistent with micellar aggregation.

Rate constants for the hydrolyses of BNPP mediated by Ce(IV) and ligands **2**−**4** at various pH's appear in Table 1. At pH >6, kinetics were monitored at 400 nm, following released *p*-nitrophenylate ions. (HPLC demonstrated that *p*-nitrophenol was the sole, final organic product.) Below pH 6, *p*-nitrophenol formation (317 nm) and BNPP disappearance (290 nm) were

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(16) Brij oxygens should assist the ligands in binding Ce(IV); crown and cryptand ether oxygens bind Ln cations weakly in water; for example, $K = 4$ for the binding of Eu^{3+} by sorbitol.[2b] Carboxylate groups (as in **3** or **4**) are more effective: Arnaud-Neu, F. *Chem. Soc. Rev.* **1994**, 235. In addition, Ce−ligand coordination energies might be altered in the Brij micelles, and the reactivity of water could be enhanced. The micellar pseudophase will also concentrate the substrate and Ce complex into a small reaction volume, thus further enhancing the rate.

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Table 1. Rate Constants for Ce(IV)-Mediated Hydrolyses of BNPP[a,b]

		2		**3**		**4**[d]	
pH	no ligand[c]	1:1[e]	2:1[f]	1:1[e]	2:1[f]	1:1[e]	2:1[f]
4.0[g]	173	162	79.8	2.5	44		
5.0[g]	107	102	39.7				
6.0	*h*	43.6	21.9		180		
7.0	*h*	18.1	11.2	6.0	260	7.5	
8.0	*h*	11.0	7.8	7.0	190	9.0	
9.0	*h*	10.0	3.9	9.5	97	13.1	51
10.0	*h*		2.4	15.5	74	22.0	160
11.0			1.9	22.0		64.1	200
12.0[g]						98.0	

[a] Rate constants are multiplied by 10^4, units are s^{-1}. [b] Conditions: [Brij] = 2×10^{-3} M (for **2**) or 5×10^{-3} M (for **3**, **4**); [Ce(IV)] = 1 or 2×10^{-3} M; [ligand] = 1×10^{-3} M; [BNPP] = 5×10^{-5} M; [buffer] = 0.01 M (see text for buffers); 0.01 M KCl, 37 °C. [c] Brij and other components are present. [d] Rate constant for BNPP to nitrophenylphosphate. [e] Ce(IV):ligand = 1:1, [Ce(IV)] = 1 mM. [f] Ce(IV):ligand = 2:1, [Ce(IV)] = 2 mM. [g] No buffer. [h] Ce(IV) precipitates above pH 5 in the absence of ligand.

followed. Pseudo-first-order rate constants (>8 half-lives[17]) were obtained as means of triplicate runs with $r > 0.997$ and reproducibilities within ±10%.

Table 1 indicates that, at selected pH's, 1−2 mM Ce(IV) provides rate constants exceeding 0.01 s^{-1} for the cleavage of BNPP in Brij micelles. At pH 4, the cation itself affords $k = 1.7 \times 10^{-2}$ s^{-1}, and the Ce(IV)−glucamine (**2**) complex is comparable. The palmitate (**3**) preparation provides $k = 2.6 \times 10^{-2}$ s^{-1} for 2:1 Ce(IV)/ligand at pH 7, whereas $k = 2.0 \times 10^{-2}$ s^{-1} for 2:1 Ce(IV)/**4** at pH 11.[18]

A rate constant of 2.6×10^{-2} s^{-1} represents an enhancement of about *2.4 billion* in BNPP cleavage, relative to $k_0 \sim 1.1 \times 10^{-11}$ at 25 °C.[3b] This appears to be the largest metal ion induced acceleration reported for BNPP. Ce(IV), either alone in Brij at pH 4, or at optimal pH in the presence of ligands **2**−**4**, is ≥50 times more reactive toward BNPP than any of the other lanthanides, including Eu^{3+},[2b,3b,6] La^{3+},[3b,5] or Pr^{3+}.[7] It is also somewhat more reactive than the La^{3+}/H_2O_2 systems studied by Chin[3b] and Breslow.[4]

The enormous accelerations of BNPP hydrolyses fostered by Ce(IV) are surely related to the cation's high charge/size ratio. The +4 oxidation state makes Ce(IV) a "hard" Lewis acid that efficiently interacts with the substrate's P−O^- and acidifies coordinated water molecules so that they are available as OH nucleophiles even under acidic conditions.[19] As anticipated,[12b] Ce(IV) is more reactive toward BNPP than Ce(III); for example, 1:1 Ce(IV)/**4** is 32 times more reactive than Ce(III) under comparable conditions at pH 11.

The pH dependences of the several Ce(IV)−ligand combinations vary with both ligand identity and the Ce/ligand ratio. With glucamine **2**, ceric reactivity decreases with increasing pH, presumably due to (microscopic) hydroxide gel formation. However, 2:1 Ce(IV)/**3** exhibits a reactivity maximum at pH 7, whereas the reactivities of 1:1 Ce(IV)/**3** and Ce(IV)/**4** continue to increase up to pH 11−12. The pH titration of Ce(IV) in the presence of 1 equiv of **3** requires almost 3 equiv of base between pH 8 and 11, suggesting the deprotonation of several water molecules coordinated to the same metal ion. Similar phenomena presumably attend Ce(IV)/**4**.

The high reactivity of 2:1 Ce(IV) with ligands **3** or **4** (but not **2**) may reflect electrophilic/nucleophilic cooperativity between the 2 Ce(IV) centers. Related synergism is known with other lanthanides,[5,7] as well as Ce(IV),[13c] and, of course, with phosphatase enzymes.[3,10,20] In the present case, the 2:1 systems might involve hyroxide bridged Ce(IV) centers,[21] additionally stabilized by Brij and carboxylate oxygens.[16]

Both 1:1 Ce(IV)/**2** and Ce(IV)/**3** mediate the hydrolysis of BNPP with the release of 2 equiv of *p*-nitrophenol; the first step is slower than the cleavage of mononitrophenylphosphate (MNPP), and is rate-determining. In the former case, 2-fold *excess* BNPP can be completely cleaved at pH 7.5, 45 °C, with $k = 9.6 \times 10^{-4}$ s^{-1} and turnover. In the latter case (pH 11, 37 °C), 2 *p*-nitrophenols are liberated from a single substrate molecule with $k = 2.2 \times 10^{-3}$ s^{-1},[22] but binding of inorganic phosphate[16] inhibits subsequent reactions (as demonstrated by controls). Surprisingly, 1:1 Ce(IV)/**4** releases only 1 equiv of *p*-nitrophenol from BNPP at pH 11 ($k = 6.4 \times 10^{-3}$ s^{-1}); cleavage of the MNPP is 105 times slower. These differences in selectivity and reactivity between Ce(IV) complexed with ligands **3** or **4** illustrate the sensitivity of the hydrolytic processes to the identity and disposition of ligand donor atoms around the Ce(IV) catalytic center(s) within the Brij micelles.

In conclusion, dilute aqueous solutions of Ce(IV), solubilized with simple ligands in Brij micelles, provide extraordinary accelerations in the hyrolysis of BNPP, the most commonly employed model phosphodiester for DNA. The observed kinetic behavior is very sensitive to ligand structure and pH, so that further development and optimization of these systems should be possible.

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(17) Infinity titers reflected stoichiometric cleavage of both *p*-nitrophenol moieties with Ce(IV) and ligands **2** and **3**, but of only a single *p*-nitrophenol with ligand **4**; see below.

(18) Cleavage is extraordinarily slow at pH 11 in the absence of Ce(IV).

(19) The pK_a of $Ce^{IV}-OH_2$ is 0.7, whereas that of Ce(III) is 9.0: Burgess, J. *Metal Ions in Solution*; Halstead Press: New York, 1978; p 267.

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(22) Controls show that 1:1 Ce^{IV}/**3** cleaves mononitrophenylphosphate 1.4 times faster than BNPP at pH 11 in Brij.