Kinetics of phosphodiester cleavage by differently generated cerium(IV) hydroxo species in neutral solutions†

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Neutral aqueous solutions of cerium ammonium nitrate obtained by dilution of their acetonitrile stock solution with imidazole buffer show high catalytic activity in the hydrolysis of bis(*p*-nitrophenyl) phosphate (BNPP) and better reproducibility than other similar systems, but suffer from low stability. The kinetics of catalytic hydrolysis is second-order in Ce(IV), independent of pH in the range 5–8 and tentatively involves the $Ce_2(OH)_7^+$ species as the active form. Attempts to stabilize the active species by different types of added ligands failed, but the use of Ce(IV) complexes pre-synthesized in an organic solvent with potentially stabilizing ligands as precursors of active hydroxo species appeared to be more successful. Three new Ce(IV) complexes, $[Ce(Phen)_2O(NO_3)_2]$, $[Ce(tris)O(NO_3)(OH)]$ and $[Ce(BTP)_2(NO_3)_4]\cdot2H_2O$ (BTP = bis-tris propane, 1,3-bis[tris(hydroxymethyl)methylamino]propane), were prepared by reacting cerium ammonium nitrate with the respective ligands in acetonitrile and were characterized by analytical and spectroscopic techniques. Aqueous solutions of these complexes undergo rapid hydrolysis producing nearly neutral polynuclear Ce(IV) oxo/hydroxo species with high catalytic activity in BNPP hydrolysis. Potentiometric titrations of the solutions obtained from the complex with BTP revealed the formation of $Ce_4(OH)_{15}^+$ species at pH > 7, which are protonated affording $Ce_4(OH)_{14}^{2+}$ and then $Ce_4(OH)_{13}^{3+}$ on a decrease in pH from 7 to 5. The catalytic activity increases strongly on going to species with a higher positive charge. The reaction mechanism involves first- and second-order in catalyst paths as well as intermediate complex formation with the substrate for higher charged species.

Introduction

The cerium(IV) cation is attracting increasing interest as a possible catalyst for various hydrolytic reactions due to its extremely strong Lewis acidity. The form of Ce(IV) used most often is cerium ammonium nitrate which is commercially available as a pure, readily water soluble compound. The use of cerium ammonium nitrate has been reported in the hydrolysis of phosphate diesters,[1] DNA,[2] peptides,[3] cyclic acetals and ketals.[4–6] However, the behavior of this and other Ce(IV) salts in water is very complex due to their hydrolytic and redox instability, posing serious restrictions on the use and further development of new Ce(IV)-based, potentially very active, catalysts. Even in an acid medium Ce(IV) slowly forms hydroxo polymers, which eventually precipitate as CeO_2. In neutral buffered solutions cerium ammonium nitrate rapidly forms a hydroxo gel, surprisingly still active in peptide[3] and DNA[2] hydrolysis, but the use of a heterogeneous system of an uncontrolled composition is rather inconvenient both for practical applications and for research.

Several compounds allowing one to maintain homogeneous solutions of Ce(IV) salts in neutral media were proposed,[1,2] however, the composition of active species in such systems remains unidentified. Complex formation with strongly coordinating EDTA-type ligands solves the problem of stability,[7–9] but although the Ce(IV)–EDTA system is active in the single-stranded DNA hydrolysis,[7] reported catalytic activities of such complexes in the hydrolysis of synthetic activated phosphodiesters like bis(*p*-nitrophenyl) phosphate[8,9] do not surpass those observed with trivalent lanthanides and so the anticipated advantage of Ce(IV) actually disappears (*e.g.*, Ce(IV) and Eu(III)

peptide complexes show similar catalytic activities in DNA hydrolysis[10]).

Speciation of Ce(IV) in solution has been established only in highly acid media.[11] In dilute 0.1 mM Ce(IV) perchlorate solutions the principal species in the pH range 0–1 are mononuclear hydroxocomplexes $Ce(OH)^{3+}$ and $Ce(OH)_2^{2+}$, but in 10 mM solutions binuclear complexes $Ce_2(OH)_3^{5+}$, $Ce_2(OH)_4^{4+}$ and hexanuclear $Ce_6(OH)_{12}^{12+}$ become predominant.[11] In less acid solutions the average number of metal bound OH anions increased to 2.35 at the highest pH of 2, but the type of hydroxo species was not identified.[11] At a pH > 4, Ce(IV) hydroxide starts to precipitate.

The hydrolysis of cyclic nucleotides by cerium ammonium nitrate proceeds with an inversion of configuration consistent with in-line nucleophilic attack on the metal-bound phosphate ester group.[12] On the basis of kinetic results for cAMP hydrolysis in the presence of cerium ammonium nitrate at a pH of around 2, the dinuclear $Ce_2(OH)_4^{4+}$ cation was claimed to be the active species.[2c] It seems, however, highly improbable that such species may still exist and be responsible for the catalytic activity in neutral solutions.
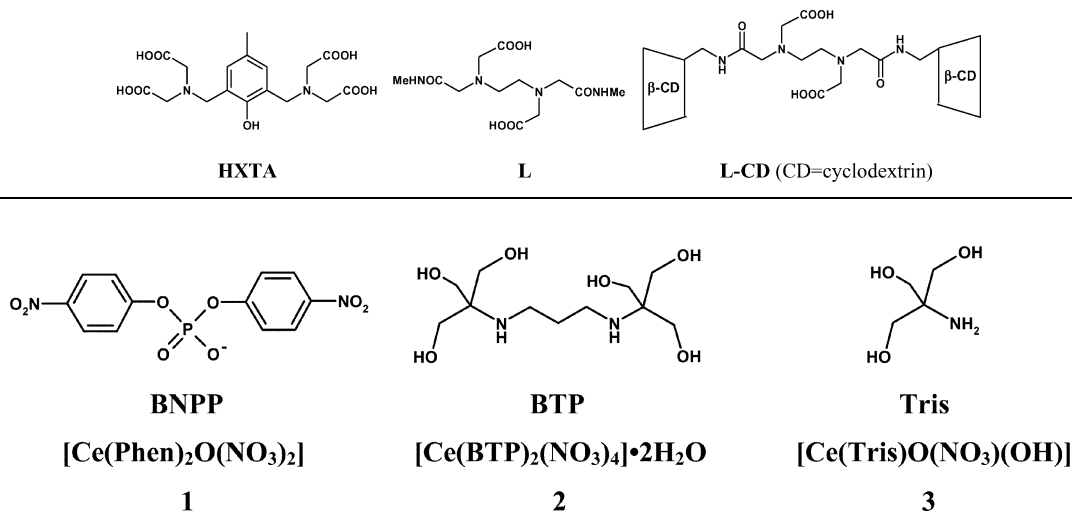
The first homogeneous system studied kinetically with bis(*p*-nitrophenyl) phosphate as a substrate involved cerium ammonium nitrate in the presence of a hydrophobic ligand, *e.g.* palmitate in a neutral micellar solution of nonionic surfactant Brij-35.[1a–c] Very large observed rate constants were reported, see Table 1, and the binuclear $Ce_2(OH)_4^{4+}$ complex was considered to be the active species. The kinetic results for BNPP hydrolysis in this and other Ce(IV) based systems reported in the literature are summarized in Table 1.

All kinetic studies with Ce(IV) reported so far are semi-quantitative in nature. In preliminary experiments attempting to reproduce published results with cerium ammonium nitrate as a source of Ce(IV) we observed extremely poor reproducibility of rate parameters and often it was virtually impossible to handle the reaction system, *e.g.* to vary pH, temperature or concentrations of reactants without causing precipitation or

Table 1 Observed first-order rate constants for BNPP hydrolysis in the presence of Ce(IV) compounds

	Reaction system	$T/^\circ C$	pH	k_{obs}/s^{-1}	Ref.
1	1 mM $(NH_4)_2Ce(NO_3)_6$ + 1 mM palmitate in 5 mM Brij	37	7.0	6.0×10^{-4}	1a
2	2 mM $(NH_4)_2Ce(NO_3)_6$ + 1 mM palmitate in 5 mM Brij	37	7.0	2.6×10^{-2}	1a
3	0.1 mM $Ce_2(HXTA)$	37	8.0	1.0×10^{-5}	8
4	0.275 mM Ce(L)	25	7.0	1.18×10^{-6}	9
5	0.275 mM Ce(L-CD)	25	7.0	6.17×10^{-4}	9
6	0.5 mM $(NH_4)_2Ce(NO_3)_6$ in 10 mM imidazole	25	7.0	0.020	this work
7	1 mM complex **2**	25	7.5	6.9×10^{-4}	this work
8	1 mM complex **2**	25	5.45	0.055	this work
9	1 mM complex **3**	25	5.5	0.023	this work

HXTA   L   L-CD (CD=cyclodextrin)

BNPP   BTP   Tris

$[Ce(Phen)_2O(NO_3)_2]$ $[Ce(BTP)_2(NO_3)_4]\cdot 2H_2O$ $[Ce(Tris)O(NO_3)(OH)]$

1   **2**   **3**

Chart 1

an unexpected drop in activity. The purpose of this paper is to test different ways of preparing neutral homogeneous Ce(IV) solutions, such as the dilution of cerium ammonium nitrate with aqueous buffers containing potentially stabilizing ligands and the use of Ce(IV) complexes pre-synthesized in non-aqueous media as precursors (see Chart 1 for the structures of the substrate and ligands employed in this study), in order to find suitable conditions for a detailed kinetic study aimed at identifying the type of active Ce(IV) species in neutral solutions and to provide a plausible reaction mechanism.

Results and discussion

Reactivity of cerium ammonium nitrate

In preliminary experiments we employed stock solutions of $(NH_4)_2Ce(NO_3)_6$ in 3 M HNO_3 and observed, in line with reported results (see the Introduction), that 0.1–5 mM Ce(IV) solutions obtained by dilution are homogeneous up to pH 4 and show high catalytic activity in the phosphodiester cleavage. At a pH of around 3.5, the first-order rate constants (k_{obs}) observed for the cleavage of BNPP with 0.2 mM cerium ammonium nitrate in unbuffered solutions[13] at 25 °C were about 0.001 s^{-1}, but in less acid solutions the activity rapidly decreased, Fig. 1. No activity at all was observed in heterogeneous conditions with Ce(IV) hydroxide in form of a gel or precipitate. Surprisingly in spite of high acidity and high nitrate concentration, the nitric acid stock solutions underwent rather fast "aging" and after one week lost about 90% of the initial activity. In addition, using these solutions was rather inconvenient because of necessity of neutralizing with large amount of acid. Therefore, we tested other ways of preparing stock solutions and observed the best results using 0.05–0.1 M cerium ammonium nitrate in dry acetonitrile, which conserved full activity over several weeks and did not contain added acid.

Diluted 0.1–1.0 mM cerium ammonium nitrate obtained from these stock solutions by adding water containing variable
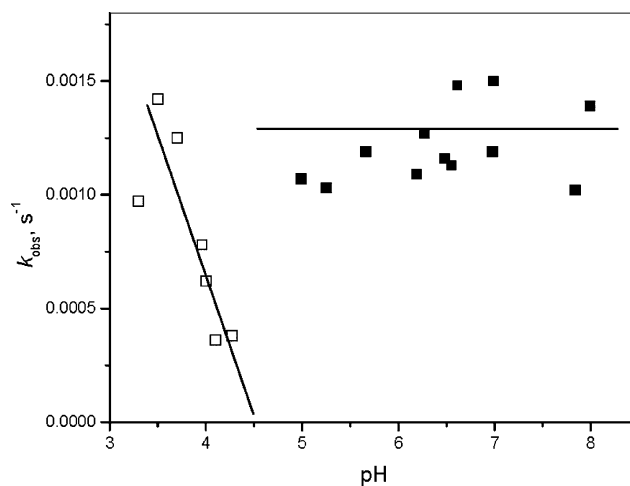

Fig. 1 Observed first-order rate constants for BNPP hydrolysis at 25 °C in the presence of 0.2 mM $(NH_4)_2Ce(NO_3)_6$ from 3 M HNO_3 stock solution diluted with water (open squares) and from the acetonitrile stock solution diluted with 10 mM imidazole buffer (solid squares) as a function of pH.

amounts of alkali remained homogeneous for at least 1 h up to pH 9 and showed a significant, but poorly reproducible catalytic activity in the hydrolysis of BNPP in unbuffered solutions. About 20 different compounds (amino acids, amino alcohols, nitrogen heterocycles, oximes, hydroxamic acids, and Good's buffers) were tested as potentially stabilizing ligands in order to improve the stability and reproducibility of the catalytic system. Some of these compounds (iminodiacetic acid and its derivatives, hydroxamic acids) completely prevented the precipitation of Ce(IV) in neutral and even basic solutions, but also completely inhibited the catalytic activity. The majority of compounds tested did not show any significant effect, but two of them, bis-tris propane (BTP) and imidazole allowed us

to obtain more reproducible kinetic results. Observed activity was higher in imidazole, which also served as a buffer in the pH range 6–8 and for these reasons further experiments were performed in imidazole solutions. Kinetic measurements under these conditions allowed us to obtain reproducible trends in k_{obs} such as the effect of pH or concentration, but the absolute values of the rate constants were still rather inaccurate (see the Experimental section).

Fig. 1 compares k_{obs} for BNPP hydrolysis in the presence of 0.2 mM cerium ammonium nitrate from the acetonitrile stock solution as a function of pH in 10 mM imidazole (solid squares) and in the absence of imidazole from a nitric acid stock solution (open squares). Apparently the use of imidazole allows one to keep the observed activity below pH 3.5, up to pH 8. The results in Fig. 1 show that the reaction rate is independent of pH in the range 5–8. Increased amounts of imidazole produce an inhibitory effect (Fig. 2) consistent with the formation of a weak complex ($K = 100$ M^{-1}) between the active catalyst and the imidazole molecule. Imidazole does not form detectable complexes with trivalent lanthanide cations, but its complexation with the much more electrophilic Ce(IV) cation seems quite probable. In these experiments the variation in pH was achieved by changing the initial pH of the imidazole buffer before the addition of an aliquot of cerium ammonium nitrate stock solution. Attempts to vary pH by adding base or acid to solutions already containing Ce(IV) always resulted in a loss of reproducibility of the kinetic measurements and often in a loss of stability and led to precipitation. Apparently the system is not at true equilibrium, the formation of active Ce(IV) species is controlled by the kinetics rather than by the thermodynamics of the cation hydrolysis, but fortunately it is quite reproducible.
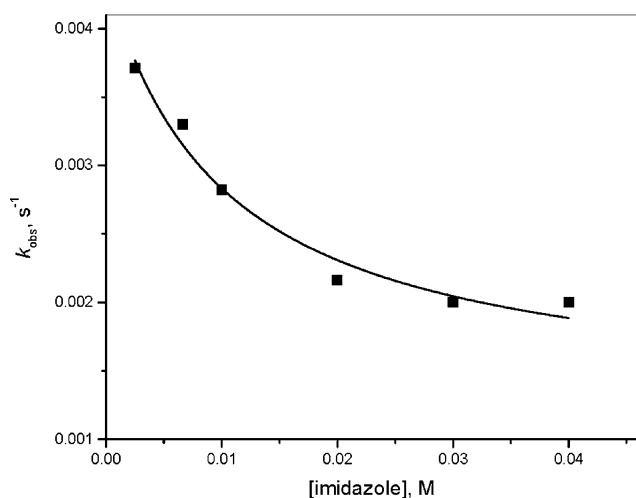
Fig. 2 The effect of imidazole buffer concentration on the reactivity of 0.2 mM $(NH_4)_2Ce(NO_3)_6$ at pH 7.0 and at 25 °C. The value of k_{obs} in 10 mM imidazole is two times higher than the respective point in Fig. 1 due to variations in absolute rates in different series described in the Experimental section.

The instability of the system towards additions of acids and bases makes it impossible to determine the species composition by potentiometric titrations. The following procedure was used instead. To 3–10 mM imidazole buffer solutions adjusted to different pHs from 6–8, portions of the cerium ammonium nitrate stock solution were added to get final Ce(IV) concentrations of 0.1–0.6 mM . These additions induced decreases in pH and by using the known value of pK_a = 7.1 for imidazolium cation, concentrations of protonated imidazole were calculated from pH readings before and after Ce(IV) additions. The difference between these concentrations is equal to the number of protons liberated during the hydrolysis of Ce(IV) and, consequently to the number of OH anions bound to the resulting Ce(IV) hydroxo species. From these results the average number of 3.5 ± 0.2

protons liberated per one Ce(IV) cation in the range of pH 6–8 was estimated (Fig. 3 shows the results of one of the series of such experiments obtained with 10 mM imidazole at initial pH 7.5). This indicates the formation of nearly neutral hydroxo species of an average composition $Ce_2(OH)_7^+$. When the same amounts of cerium ammonium nitrate were added to pure water, the pH dropped to approximately three and calculated numbers of liberated protons varied from 3.0 to 2.3 per one Ce(IV) cation at the highest and lowest pH values (3.22 and 2.86) respectively (Fig. 3).
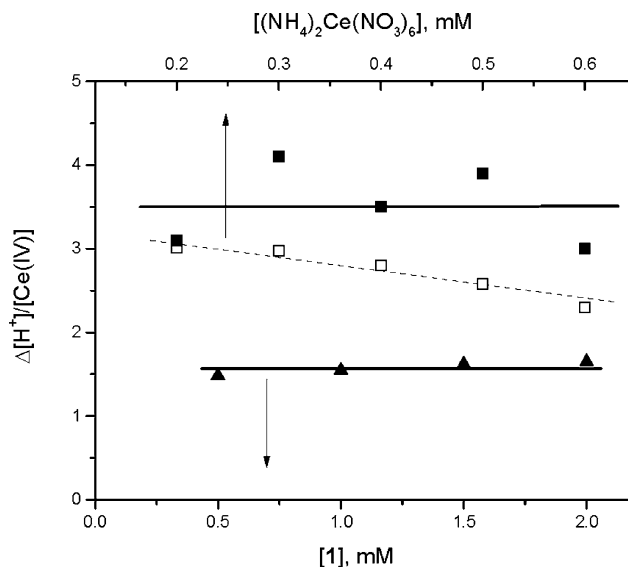

Fig. 3 Number of protons released per one mol of Ce(IV) on addition of $(NH_4)_2Ce(NO_3)_6$ to water (open squares) and to 10 mM imidazole buffer (solid squares) and on addition of complex **1** to 10% v/v aqueous DMSO (solid triangles).

It seems, therefore, that the observed independence of k_{obs} of pH (Fig. 1) results from an approximately constant degree of Ce(IV) hydrolysis above pH 3 and the role of imidazole is to prevent fast precipitation of Ce(IV) hydroxide owing to a weak complexation of active hydroxo species.

In reality hydroxo complexes of Ce(IV) may be of mixed oxo/hydroxo type (it was proposed that the real structure of the $Ce_6(OH)_{12}^{12+}$ species is $Ce_6(O)_4(OH)_4^{12+}$)[11] and may have a higher nuclearity because of the known tendency of oxo and hydroxo ligands to serve as bridges between metal ions. Thus possible structures of $Ce_2(OH)_7^+$ may be deduced by analogy *e.g.* $Ce_2(O)_3(OH)^+$ or $Ce_6(O)_9(OH)_3^{3+}$. Interestingly, in several instances monocationic binuclear hydroxo or alcoxo complexes of trivalent lanthanides, *i.e.* $La_2(OH)_5^+$[14] and $La_2(OMe)_5^+$[15] were identified as the reactive forms for the phosphodiester cleavage in water and methanol respectively. Also the fragment $Ln_2(OH)_5^+$ stabilized by the neutral bis-tris propane ligand was found to be the reactive form in BNPP hydrolysis by several lanthanide ions.[16]

Fig. 4 shows the effect of Ce(IV) concentration on the reaction rate: initially the observed rate constant increases quadratically with increasing in metal concentration (the inset shows the results below 0.4 mM Ce(IV) in logarithmic coordinates, which follow a linear dependence with the slope 1.9 ± 0.2), but then passes through a maximum at 0.5 mM and decreases to an approximately constant level between 1 and 1.5 mM. Apparently this behavior can be attributed to the formation of less reactive aggregates of Ce(IV) hydroxo species at higher concentrations. It is noteworthy that k_{obs} at the optimum Ce(IV) concentration reaches the highest reported value so far of about 0.02 s^{-1}, observed previously at a higher temperature and Ce(IV) concentration in a micellar medium (see Table 1).

Higher than first-order kinetics are not uncommon for lanthanide-catalyzed BNPP hydrolysis: second-order kinetics
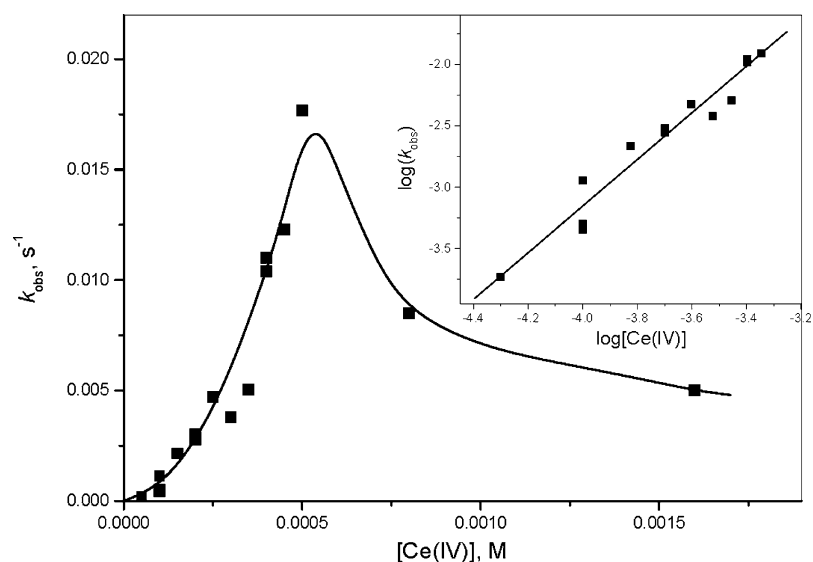
Fig. 4 Observed first-order rate constants for BNPP hydrolysis at 25 °C as a function of $(NH_4)_2Ce(NO_3)_6$ concentration in 10 mM imidazole buffer at pH 7. The inset shows the results at concentrations below 0.4 mM in logarithmic coordinates.

were observed for Y(III) dinuclear hydroxo complexes with tris-type ligands,[17] third-order kinetics were reported for dinuclear complexes of La(III) with a macrocyclic ligand,[18] and second-,[19] third-,[20] and even fourth-order[21] kinetics were observed for lanthanide peroxide complexes of different nuclearities.

The major drawback of this system is its very low stability. Although precipitation of the metal hydroxide from neutral imidazole solutions was only observed after several hours, these solutions had already lost *ca.* 20% of their initial activity (estimated by comparison of initial rates) after 20 min of incubation, *ca.* 50% after 40 min, and after 1 h solutions which were still transparent were practically inactive. This inactivation is not associated with a deeper hydrolysis of Ce(IV) because the pH of cerium ammonium nitrate solutions stabilizes in just few minutes. The reason for inactivation is probably aggregation of hydroxo species to still soluble, but inactive, hydroxo polymers. This instability was manifested perhaps most clearly in the temperature dependence of the observed rate constant: increasing the temperature above 25 °C leads to a decrease rather than an increase in the rate constant, Fig. 5, apparently because of faster inactivation of the reactive species.
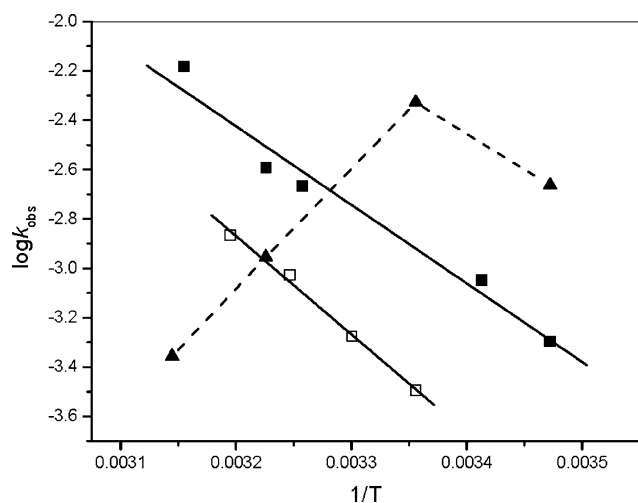
Fig. 5 Arrhenius plots for the rate constants for BNPP hydrolysis in the presence of 0.4 mM $(NH_4)_2Ce(NO_3)_6$ at pH 7.4 (solid triangles), 0.7 mM **2** at pH 7.5 (open squares) and 1.0 mM **3** at pH 7.6 (solid squares).

## Pre-synthesized cerium(IV) complexes with neutral ligands

Testing potentially stabilizing ligands in water showed us that a suitable ligand should be a neutral amine or amino alcohol molecule because more potent anionic ligands decreased the catalytic activity of the Ce(IV) cation too strongly. Even if such a molecule cannot prevent deep hydrolysis of Ce(IV), it may work as a mediator making the cation hydrolysis a slower and more selective process leading to the formation of hydroxo complexes of a more definite composition, as has been demonstrated in the successful realization of the so-called ligand-controlled self-assembly of polynuclear oxo/hydroxo complexes of trivalent lanthanides.[22] In this procedure a weakly coordinating ligand, *e.g.* an amino acid, is present in a solution of lanthanide salt adjusted to the pH of hydroxide precipitation and then di-, tetra- and even pentadecanucelar oxo/hydroxo complexes can be isolated in good yields depending on type of the cation and the ligand. The hydrolysis of Ce(IV) proceeds much more vigorously than that of trivalent lanthanides and when the "controlling" ligand is added to water it probably does not manage to participate in the process. One may expect, however, that if such a ligand was bound beforehand to Ce(IV) in an organic solvent, then when the complex underwent the reaction with water it might act more successfully. Proceeding from these considerations we have prepared three new complexes by reacting cerium ammonium nitrate in acetonitrile with neutral ligands of increasing basicity, namely phenanthroline, tris and bis-tris propane. In complexes with first and second ligands, Ce(IV) had already undergone a partial hydrolysis to oxo/hydroxo forms during preparation, but with all three complexes we indeed obtained catalytic systems which were more stable and reproducible, allowing easier manipulation of the catalyst solutions, in particular, in the performance of potentiometric titrations, and still possessing the high catalytic activity expected for Ce(IV) cations.

Cerium(IV)-phenanthroline complex. The complex of composition $[Ce(Phen)_2O(NO_3)_2]$ (**1**) was prepared by reacting $(NH_4)_2Ce(NO_3)_6$ with two mol equivalents of phenanthroline in acetonitrile. Evidently, Ce(IV) had already undergone a partial hydrolysis during synthesis. Complex **1** is soluble in DMSO, but insoluble in other common organic solvents and water. The proton NMR spectrum of **1** in DMSO shows small up-field shifts for the signals of all ligand protons by *ca.* 0.1 ppm as compared to the free ligand and loss of the multiplicity of the most up-field signal, which appears as a quartet in the spectrum of free phenanthroline (see the electronic supplementary information

(ESI)†). These observations indicate a rather weak interaction of the metal ion with the ligand. In D_2O/DMSO-d_6 solution the spectrum of the ligand practically coincides with that of free phenanthroline indicating a complete loss of the ligand in water.

The kinetics of BNPP cleavage was studied in aqueous DMSO. The catalytic activity increased with decreasing DMSO content and therefore all measurements were performed at a minimum DMSO content of 10% v/v which was sufficient to dissolve **1** at millimolar concentrations. Addition of **1** to water produced a significant acidification of the solution indicative of further hydrolysis of Ce(IV). Changes in pH were smaller than in the case of cerium ammonium nitrate and estimation of the number of protons liberated per one mol of **1** performed in the same way as for $(NH_4)_2Ce(NO_3)_6$ (Fig. 3, solid triangles) gives an average number of 1.57 ± 0.08 protons liberated per one Ce(IV) cation. This means that further hydrolysis of **1** in water is accompanied by the generation of approximately three hydroxo ions per two Ce(IV) cations. Taking into account that **1** already contains one oxo ligand per Ce(IV) cation, the final composition of Ce(IV) species in solution should be close to $Ce_2(O)_2(OH)_3^+$, which is a doubly dehydrated form of $Ce_2(OH)_7^+$. Thus using **1** as a precursor of the active catalyst leads to similar species in neutral solution, but as one can see from Fig. 3, the results with **1** are much less scattered than with cerium ammonium nitrate. Also the kinetic measurements were more reproducible in this case and the catalytic activity was much more stable during the time of incubation.

The kinetics of BNPP cleavage was studied in the pH range 7–9 in 10% DMSO. The reaction rate decreased slightly on increase in pH, changing by a factor of two at extreme pH values. The concentration dependence of k_{obs} is shown in Fig. 6. Evidently, **1** is significantly less active than cerium ammonium nitrate (*cf.* Fig. 4 and Fig. 6), but does not suffer from a decrease in the reaction rate at higher concentrations. Another difference is that the kinetic order with respect to total Ce(IV) is even higher in this case. The solid line in Fig. 6 is a fit to the third-order concentration dependence, and on plotting in logarithmic coordinates gives a reaction order of 3.26 ± 0.09. Thus, although the Ce(IV) hydroxo species generated in solution from **1** have a similar total charge to those generated from cerium ammonium nitrate, they behave differently. This may be attributed to the different structures of these species, probably different nuclearity and/or a different balance of oxo and hydroxo ligands, which are intertransformable by the addition/elimination of water molecules.
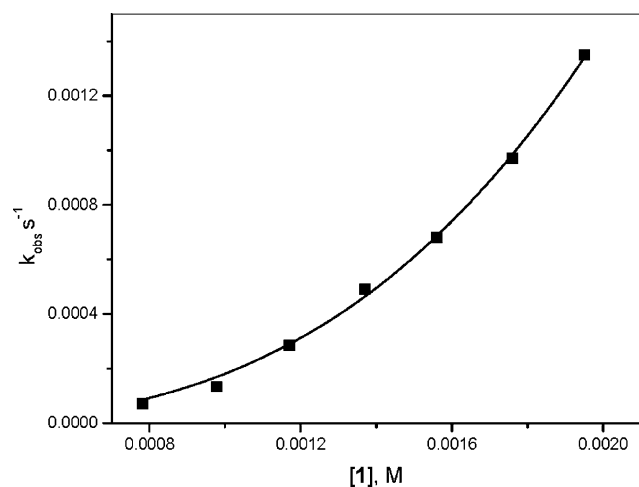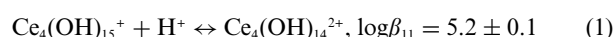

Fig. 6 Observed first-order rate constants for BNPP hydrolysis at 25 °C as a function of concentration of complex **1** in 4 mM BTP buffer containing 10% v/v DMSO at pH 7.5.

Cerium(IV)-tris and bis-tris propane complexes. Two other complexes with tris type ligands were prepared *via* the same
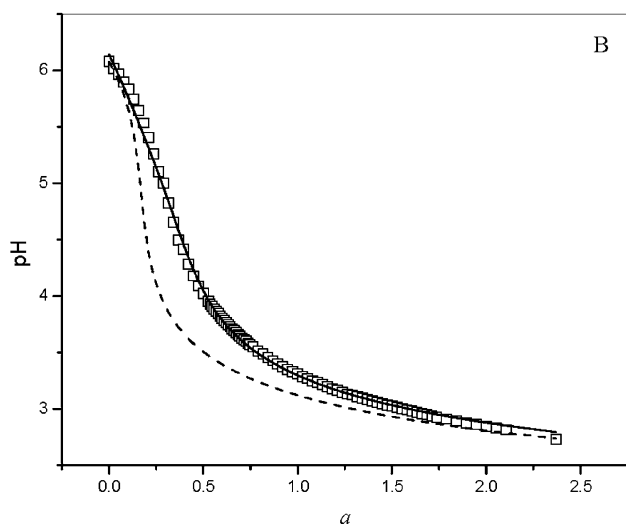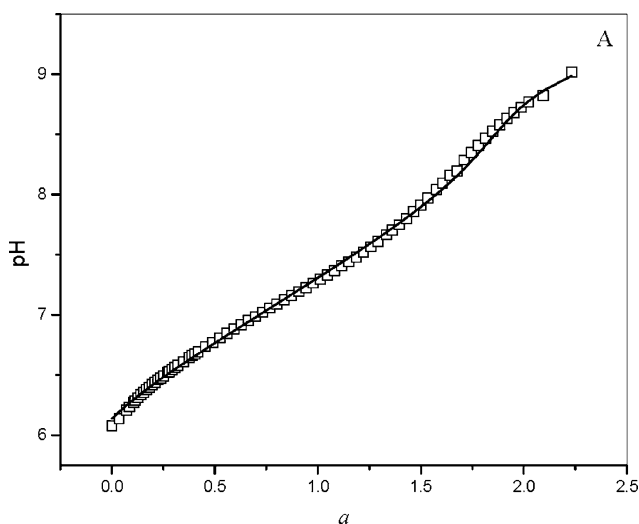
general procedure. The catalytic activity and kinetic behavior was very similar for both complexes. The complex with the bis-tris propane (BTP) ligand, which possessed a more definite and simple composition, was studied in more detail.

The complex of composition $[Ce(BTP)_2(NO_3)_4]\cdot 2H_2O$ (**2**) was prepared in a similar way to **1**. Also, like **1** it was soluble only in DMSO, but was still sufficiently soluble in 5% v/v aqueous DMSO to allow us to make measurements at a lower organic co-solvent content.[23] Comparison of the proton NMR spectra of free BTP and **2** (see the ESI †) indicates a tighter binding of this ligand to the metal ion than observed for phenanthroline. Signals of central propane fragments of BTP and of those adjacent to N methylene groups are shifted downfield by 0.54 and 0.47 ppm, respectively, and nearly lose their multiplicity. The signal of NH protons undergoes a very strong downfield shift by 4.87 ppm due to complexation with the Ce(IV) ion. Much smaller downfield shifts, of 0.24 and 1.06 ppm, are also observed for the signals of methylene and OH protons of CH_2OH groups, respectively, indicating weaker coordination of hydroxyl groups. The reported X-ray structure of the bis-tris complex of La(III)[24] shows that the tris fragment is tridentate (N and two of three OH groups). Therefore, in principle two BTP ligands may occupy 12 coordination sites of Ce(IV); this is the maximum possible coordination number for this cation. However, after dilution of the DMSO solution of **2** with D_2O, the spectrum of BTP becomes identical to that of the free ligand at the same pH indicating complete dissociation of the complex in water.

In contrast to all previously studied systems, the addition of **2** to water does not change the pH. This is explicable by complete neutralization of the acid liberated during the hydrolysis of Ce(IV) by the BTP ligand, which is much more basic then phenathroline. Neutral solutions of complex **2** are stable over *ca.* 2 h and then precipitation of Ce(IV) hydroxide begins. Remarkably, the stability of the Ce(IV) species in this case was so high that it was possible to perform the potentiometric titration of the solution over a wide pH range. Fig. 7 shows the results of typical titration experiments. Titration with NaOH was terminated at pH 9 because of a rapid precipitation of Ce(IV) hydroxide in the more basic media. The titration curve for NaOH (Fig. 7A) coincides exactly with that expected for the titration of protonated BTP ($pK_{a1} = 6.81$, $pK_{a2} = 9.06$)[16] with no additional titratable groups attributable to the Ce(IV) species. From the results of titrations with NaOH we determined that $94.0 \pm 0.8\%$ of the total amount of BTP is protonated in the solution of **2** in water. This means that 3.76 ± 0.03 protons are liberated per one Ce(IV) cation during the hydrolysis of **2**, which corresponds to the formation of a nearly neutral hydroxo complex of the composition $Ce_4(OH)_{15}^+$, probably in a partially dehydrated form. The absence of titratable protons indicates that this species remains unchanged until pH 9 and then deprotonates affording a Ce(IV) hydroxide precipitate. Titration with HCl, however, consumes more acid than is required for the protonation of the remaining 6% of BTP base. The dashed line in Fig. 7B is the expected titration profile for BTP alone. Evidently, the experimental curve occurs at higher pH values indicating additional consumption of added acid attributable to protonation of the Ce(IV) hydroxo species. The results fit satisfactorily to a model involving two consecutive protonation steps represented by eqns. (1) and (2). The protonation constants given below are the mean values from four titrations.
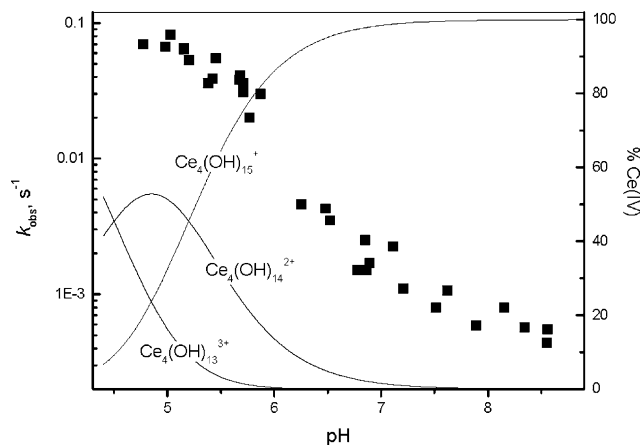
$$Ce_4(OH)_{15}^+ + H^+ \leftrightarrow Ce_4(OH)_{14}^{2+}, \log\beta_{11} = 5.2 \pm 0.1 \qquad (1)$$

$$Ce_4(OH)_{15}^+ + 2\,H^+ \leftrightarrow Ce_4(OH)_{13}^{3+}, \log\beta_{12} = 9.7 \pm 0.3 \qquad (2)$$

In other words, the complex $Ce_4(OH)_{14}^{2+}$ behaves as an acid with a $pK_a = 5.2$, and the complex $Ce_4(OH)_{13}^{3+}$ as an acid with a $pK_a = 4.5$. It is worth noting that stoichiometrically the complex $Ce_4(OH)_{14}^{2+}$ is a dimeric form of $Ce_2(OH)_7^+$ which is the major hydrolysis product of cerium ammonium nitrate (see above).

Fig. 8 Observed first-order rate constants for the BNPP hydrolysis at 25 °C *vs.* pH and the species distribution diagram for 1.0 mM **2**.

Rates *vs.* complex concentration profiles at two pH values, 7.9 when the reactivity is due to $Ce_4(OH)_{15}^+$ species and 5.8 when the major contribution is from $Ce_4(OH)_{14}^{2+}$, are shown in logarithmic coordinates in Fig. 9. The formal linear fit of these profiles gives a fractional reaction order of 1.7 ± 0.1 in **2** at both pH values. This is a lower value than for all previously described systems, but it still indicates a significant second-order contribution.
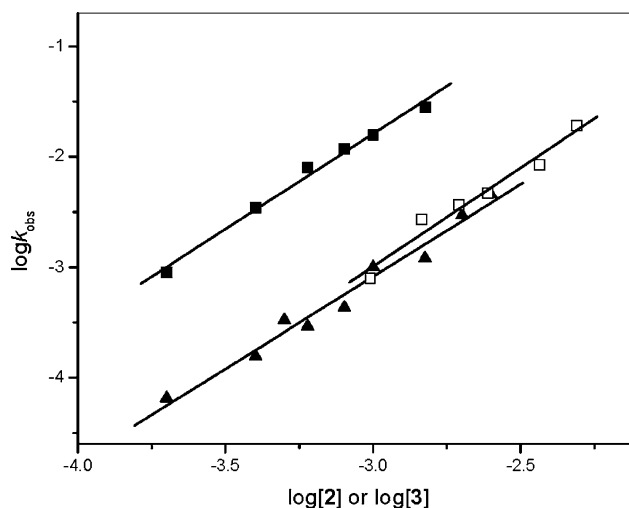
Fig. 9 The logarithmic plots of observed first-order rate constants for the BNPP hydrolysis at 25 °C *vs.* total concentration of **2** at pH 7.9 (open squares) and pH 5.8 (solid squares) and **3** at pH 8.3 (solid triangles).

The fractional kinetic order is often observed as an approximation to a rate law, which in fact corresponds to a variable reaction order, changing from, for example, one to two on variation in the total reactant concentration. Looking more carefully at the concentration profiles at lower and higher pH values one can see that at pH 5.8 the slope of the profile tends to decrease at higher Ce(IV) concentrations, but at pH 7.9 the opposite tendency is observed (see the ESI; Fig. 8S shows expanded plots together with lines drawn having slopes of one and two so that these trends can be seen more clearly). The plots of apparent second-order rate constants $k_{2app} = k_{obs}/[\mathbf{2}]$ shown in Fig. 10 were used for the quantitative analysis of the kinetic results. At pH 7.9 the plot (open squares) is linear with a positive intercept in accordance with eqn. (3) where $k_1 = 0.17$ M^{-1} s^{-1} and $k_2 = 630$ M^{-2} s^{-1} are the respective second- and third-order rate constants for the cleavage of BNPP by $Ce_4(OH)_{15}^+$.

$$k_{2app} = k_{obs}/[\mathbf{2}] = k_1 + k_2[\mathbf{2}] \qquad (3)$$

Fig. 7 Titration curves for 1 mM **2** by NaOH (A) and HCl (B); *a* is the number of mol equivalents of base or acid per one mol of **2**. The solid lines are the curve fits in accordance with equilibria (1) and (2) and protonation of BTP. The dashed line is the titration profile expected if BTP were the sole titrating component.
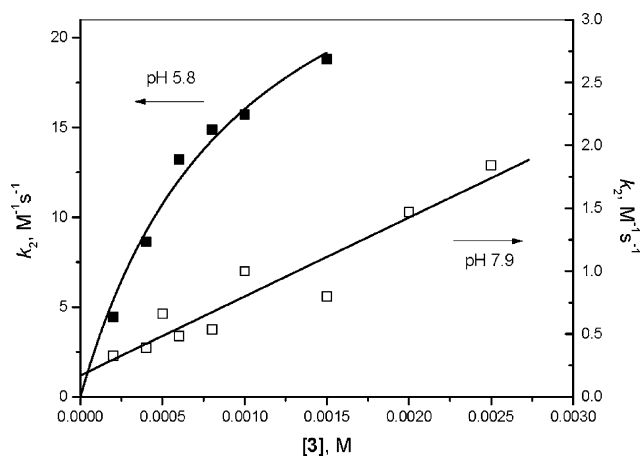
The kinetics of BNPP cleavage by **2** was studied in the pH range 5–9 in 5% DMSO. Above pH 7, the reaction proceeded with simultaneous liberation of both nitrophenyl groups of BNPP *via* first-order kinetics, but in more acidic solutions two distinct consecutive steps for the cleavage of the starting diester and the intermediate monoester were observed. The first-order rate constants observed for the phosphodiester cleavage determined in the presence of 1 mM **2** are plotted *vs.* pH in Fig. 8. The species distribution diagram calculated in accordance with eqns. (1) and (2) is superimposed on the kinetic results in the same figure.

The results in Fig. 8 show that the catalytic activity in the pH range 7.5–9 is independent of pH and may be attributed to $Ce_4(OH)_{15}^+$ species, which in this pH range constitute 100% of total Ce(IV). The average value of the observed rate constant in the presence of 1 mM **2** is (6.9 ± 0.8) × 10^{-4} s^{-1}, representing a reasonably high activity in comparison to other Ce(IV) based systems (see Table 1). A decrease in pH to below this range causes a very strong increase in k_{obs} which correlates with the degree of formation of the complexes $Ce_4(OH)_{14}^{2+}$ and $Ce_4(OH)_{13}^{3+}$ possessing higher positive charges. The observed rate constants reach values close to 0.1 s^{-1} demonstrating a very large catalytic effect of 10^{10} (for the spontaneous BNPP hydrolysis at 25 °C $k_{obs} = 10^{-11}$ s^{-1}).[19,25]

Fig. 10 Apparent second-order rate constants for the BNPP hydrolysis at 25 °C vs. total concentration of **2** at pH 7.9 (open squares) and pH 5.8 (solid squares).

Fig. 11 Observed first-order rate constants for the BNPP hydrolysis at 25 °C vs. pH in the presence of 1.0 mM **3** at 25 °C in 10% DMSO. Crosses show representative data points for 1.0 mM **2** (cf. Fig. 8).

At pH 5.8 the plot (solid squares) is of a "saturation" type. This may be due to different factors. One possibility is that this saturation results from the auto-association of the reactive species, $Ce_4(OH)_{14}^{2+}$ in this case, affording a dimer, which reacts with BNPP via first-order catalyst kinetics while the reaction with $Ce_4(OH)_{14}^{2+}$ is second-order with respect to the catalyst. However, it seems difficult to explain why a species with higher positive charge undergoes auto-association while, in the same concentration range, the monocationic species $Ce_4(OH)_{15}^+$ does not. Another possibility is that "saturation" reflects the complex formation between BNPP and the catalyst. Such complexation is often observed for trivalent lanthanides having binding constants in the range 100–500 M^{-1}.[26] Of course, one may expect BNPP complexation with the more electrophilic Ce(IV) to be more efficient and, in addition, the binding to a dicationic species should be stronger than to a monocationic one, explaining why there is no "saturation" at pH 7.9. A possible reaction scheme is presented in eqns. (4) and (5), where C is the reactive catalyst species.

$$BNPP + C \xrightleftharpoons{K} C(BNPP) \qquad (4)$$

$$C(BNPP) + C \xrightarrow{k_C} \text{reaction products} \qquad (5)$$
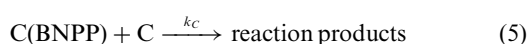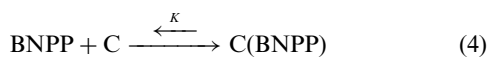
The respective rate equation takes the form

$$k_{2app} = k_{obs}/[\mathbf{2}] = k_C K[\mathbf{2}]/(1 + K[\mathbf{2}]) \qquad (6)$$

and the fitting of the results in Fig. 10 to this equation gives the values $k_C = 35 \pm 4$ M^{-1} s^{-1} and $K = (1.03 \pm 0.25) \times 10^3$ M^{-1}. Since at pH 5.8 only 20% of the total Ce(IV) exists as $Ce_4(OH)_{14}^{2+}$ in accordance with protonation constants given above (eqns. (1) and (2)), the binding constant for this species must be five times larger, assuming that there is no complexation of BNPP with $Ce_4(OH)_{15}^+$. So the corrected value of K equals 5×10^3 M^{-1}. Thus, this model requires a binding constant for Ce(IV) one order of magnitude higher than is typically observed for trivalent lanthanides and this seems reasonable from general considerations.

The kinetic results for the third pre-synthesized complex, [Ce(tris)O(NO₃)(OH)] (**3**), with the tris ligand were very similar to those for **2**. Fig. 11 shows the pH-profile for **3** which is essentially the same, both in shape and absolute rate constant values, as seen for **2** (cf. Fig. 8). The concentration dependence of **3** obtained in the pH-independent region is shown in Fig. 9 (solid triangles) and it also nearly coincides with that for **2**. The reaction order in this case is 1.8 ± 0.2. We believe, therefore, that both precursor complexes generate similar reactive Ce(IV) hydroxo species in water.
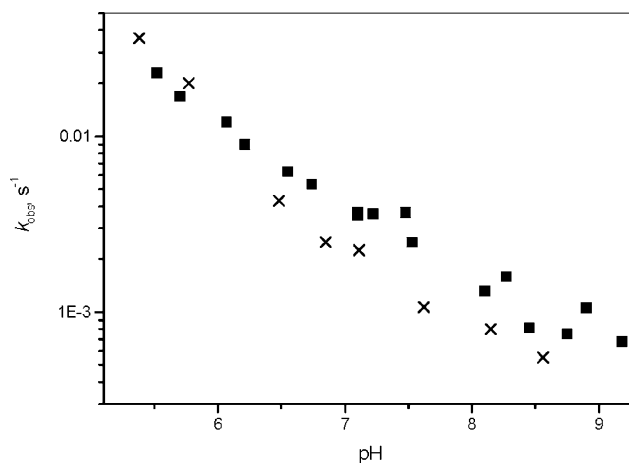
It is worth noting that the systems obtained from precursor complexes also show, in contrast to cerium ammonium nitrate, the normal behavior with variation in temperature. Fig. 5 shows the Arrhenius plots for complexes **2** and **3**, which are linear as expected, and give the activation energies 76.2 ± 3.9 kJ mol^{-1} for **2** and 60.9 ± 4.9 kJ mol^{-1} for **3** (the activation energy for the spontaneous hydrolysis of BNPP equals 106.2 kJ mol^{-1}).[25]

A common feature of all the systems studied above, mentioned already in connection with the cerium ammonium nitrate system, is the observation of higher than first-order kinetics with respect to the catalyst. This reflects the participation of more than one metal complex in the transition state of the catalytic reaction. The reasons for this are probably the same as those proposed to explain well the documented superiority of binuclear over mononuclear catalytic centers.[27,28]

In contrast to what is typically observed with other metal complex catalysts, in particular those with complexes of trivalent lanthanides,[26] the catalytic activity of **2** and **3** decreases rather than increases on going to higher pH values when hydroxo complexes, usually considered as catalytically active forms, are generated. The most widely accepted mechanism for catalytic phosphodiester hydrolysis involves nucleophilic attack of the coordinated hydroxo anion on the metal bound substrate; this mechanism was also postulated for Ce(IV) compounds.[1,2] However, the hydroxo anion bound to highly electrophilic Ce(IV) dramatically loses its basicity and may also lose its nucleophilic reactivity to the level observed for neutral water molecules. Since water is present at a much higher concentration, it may be possible that in the presence of Ce(IV) the phosphodiester undergoes nucleophilic attack by water molecules and bound hydroxo anions only reduce the positive charge on metal ion and consequently the degree of Lewis acid activation.[29] In support of this, a significant solvent isotope effect, $k_H/k_D = 3.1$, is observed with 1 mM **2** in H₂O at pH 6 vs. D₂O at pD 6. A positive solvent isotope effect, $k_H/k_D = 2.4$, was also reported for cyclic nucleotide cleavage by cerium ammonium nitrate in an acidic medium and was attributed to proton transfer to the leaving group.[2c] In the case of a BNPP substrate, such an explanation is implausible because the basicity of nitrophenyl groups leaving this substrate is too low to require the protonation in the transition state. On the other hand, the "water" reaction often involves proton transfer from the attacking water molecule to e.g. another water molecule or a buffer component in the transition state and proceeds more slowly in D₂O than in H₂O with isotope effects k_H/k_D in the range from three to six.[30]

Conclusions

Representative rate constants for the cleavage of BNPP in the systems described in this study are included in Table 1 (entries

six–nine) together with the literature values for other Ce(IV) based catalysts. In terms of observed catalytic activity, these new systems are close to that reported for a micellar medium at a somewhat higher temperature and catalyst concentration (Table 1, entry two). A possible contribution of micellar catalytic effects[31] to the reactivity of the latter system was not discussed, but can be expected since the metal ion is bound to a hydrophobic ligand and BNPP also possesses hydrophobic fragments. An approximately 100-fold positive effect of a similar nature is observed in the presence of cyclodextrins attached to the cerium bound ligand (compare entries four and five in Table 1). Taking into account these supramolecular contributions, we suppose that by their intrinsic reactivity described in this study these systems surpass previously reported Ce(IV) systems by at least one or two orders of magnitude.

Of course, it is much more desirable to obtain a well characterized highly active Ce(IV) complex, stable in water and possessing a definite structure in solution, rather than just a set of metal hydroxo species. The results of this study indicate that, in principle, this should be possible because we observe very high reactivity for species of low total charge. Therefore, the use of strongly coordinating anionic ligands should not be too harmful just because they reduce the positive charge on metal. We see, on the other hand, that in all cases the catalytic reaction is second-order or higher with respect to the catalyst, indicating the importance of the polynuclearity of the active catalyst form. From this point of view, the inhibitory effect of strong ligands may be attributed to the fact that they prevent the formation of the required polynuclear species stabilizing mononuclear forms and the future direction of research in this area should be the design of ligands capable of stabilizing polynuclear complexes.

Experimental

Materials

The compounds $(NH_4)_2Ce(NO_3)_6$, BTP (1,3-bis[tris(hydroxymethyl)methylamino]propane), imidazole, and phenanthroline were purchased from Aldrich. Tris(tris[hydroxymethyl]-aminomethane) was purchased from Sigma. The compound studied as the substrate, BNPP (bis(p-nitrophenyl) phosphate) (Aldrich) was recrystallized from water. All solvents were purchased from J. T. Baker.

All solutions for NMR studies were prepared in purified (Milli-Q Reagent Water System) water or in D_2O or DMSO-d_6 purchased from Aldrich.

Syntheses

[Ce(Phen)₂O(NO₃)₂] (1). Phenanthroline (0.43 g, 2.16 mmol) was dissolved in 5 mL of acetonitrile and added to a solution of $(NH_4)_2Ce(NO_3)_6$ (0.29 g, 0.54 mmol) in acetonitrile (5 mL). The mixture was stirred for 2 h at room temperature. The solid formed was filtered off and washed twice with acetonitrile and then with ether. Yield: 0.25 g (72.3%). Anal. calcd. for $C_{24}H_{16}N_4O_7Ce$ (640.54 g mol^{-1}): C, 45.00; H, 2.52; N, 13.12. Found C, 45.02; H, 2.55; N, 13.21. ^{1}H-NMR (DMSO-d_6, 300 MHz): $\delta = 7.79$ (q, 2H); 8.03 (s, 2H); 8.54 (q, 2H); 9.08 (s, 2H). IR (KBr, cm^{-1}): 3036 m, 1624 m, 1517 m, 1460 m, 1383 s, 1363 sh, 842 m, 728 m.

[Ce(BTP)₂(NO₃)₄]·2H₂O (2). BTP (2 g, 7.08 mmol) was dissolved in water (4 ml) and then mixed with 16 ml of acetonitrile. Then a solution of $(NH_4)_2Ce(NO_3)_6$ (1.94 g, 3.54 mmol) in acetonitrile (15 ml) was added and the mixture was stirred for 2 h at room temperature. The dense orange oil was separated by decantation and treated with 50 ml of dry acetone. After 1 h stirring it was transformed into a yellow powder, which was filtered off and washed twice with acetonitrile and then with ether. Yield: 3.30 g (94.3%). Anal. calcd. for $C_{22}H_{56}N_8O_{26}Ce$ (988.83 g mol^{-1}): C, 26.72; H, 5.71; N, 11.33. Found C, 26.72;

H, 5.73; N, 11.51. ^{1}H-NMR (DMSO-d_6, 300 MHz): $\delta = 1.99$ (m, 2H); 3.02 (t, 4H); 3.37 (s, 4H); 3.55 (s, 12H); 5.29 (s, 6H); 8.23 (s, 2H). After addition of 10% vol. of D_2O (99.9% D) the signals at 8.23 and 5.29 ppm disappeared and the signal at 3.37 became more intense indicating that the two downfield signals belong to exchangeable (NH and OH) protons and the signal at 3.37 belongs to water protons. IR (KBr, cm^{-1}): 3361 sb, 3082 sh, 2891 m, 2395 w, 1762 w, 1584 m, 1384 s, 1049 m, 825 w, 756 w, 573 wb.

[Ce(tris)O(NO₃)(OH)] (3). Tris (0.5 g, 4.13 mmol) was dissolved in 4 ml of methanol and added to a solution of $(NH_4)_2Ce(NO_3)_6$ (0.56 g, 1.03 mmol) in acetonitrile (10 ml). The mixture was stirred for 1 h at room temperature. The solid formed was filtered and washed with ice-cold acetonitrile, methanol, and then with ether. Yield: 0.29 g (75.2%). Anal. calcd. for $C_4H_{12}N_2O_8Ce$ (374.28 g mol^{-1}): C, 13.49; H, 3.40 N, 7.86. Found C, 13.52; H, 3.12; N, 7.86. ^{1}H-NMR (DMSO-d_6, 300 MHz): $\delta = 7.43$ (broad s, 2H); 5.14 (s, 3H); 3.47 (s, 6H). IR (KBr, cm^{-1}): 3382 sb, 2921 sh, 1614 m, 1458 w, 1383 s, 1352 s, 1059 m, 834 w, 700 w.

Instrumentation

Ultraviolet–visible spectra were obtained with a Hewlett Packard 8453 spectrophotometer.

^{1}H NMR spectra were recorded on 300 MHz Varian Unity INOVA spectrometer.

IR spectra were obtained with a Perkin-Elmer 1320 spectrophotometer.

Measurements of pH were taken on an Orion Model 710-A research digital pH meter.

Methodology

Stock solutions of $(NH_4)_2Ce(NO_3)_6$ were prepared in acetonitrile, stock solutions of complexes **1–3** were freshly prepared in dimethylsulfoxide, and stock solutions of BNPP were prepared in water.

In experiments with $(NH_4)_2Ce(NO_3)_6$ the final amount of acetonitrile in the reaction mixture was between 0.1 and 1% v/v (in the majority of experiments 0.4% v/v) and variation in pH was achieved by adjusting the pH of the imidazole buffer, typically 10 mM, before mixing with the other reaction components. Reaction solutions for kinetic measurements with complexes **1–3** were prepared by adding the required volumes of stock solution of reactants to an aqueous buffer containing DMSO in the amounts required to obtain 10% v/v DMSO for complexes **1** and **3** or 5% v/v DMSO for complex **2** in the final solution, and pH was adjusted by adding small volumes of strong acid or base as necessary. Reactions were initiated by adding an aliquot of the substrate solution. The solution pH was measured before and after each run and experiments where the pH variation was larger than 0.1 were excluded. The following 10 mM buffers were employed: pH 5–6.5 MES, pH 6.5–8 imidazole and pH 7–9 BTP.

In experiments with $(NH_4)_2Ce(NO_3)_6$ measurements repeated immediately produced rate constants reproducible within ±10%, however, measurements made on different days produced rate constants which varied by a factor of two or three, e.g. with 0.2 mM Ce(IV) in 10 mM imidazole at pH 7.5 the observed rate constants varied from $(1.1 \pm 0.1) \times 10^{-3}$ to $(2.7 \pm 0.3) \times 10^{-3}$ s^{-1} in different series of experiments. Therefore, to obtain reliable trends in k_{obs}, such as the effects of pH, concentrations of reactants, or temperature, measurements were performed on one day with the same cells, buffer and stock solutions.

The course of BNPP cleavage was monitored spectrophotometrically by the appearance of the 4-nitrophenolate anion at 400 nm. Kinetic measurements used 20–30 μM BNPP at a pH > 6.5, and 40–100 μM BNPP at lower pH values. In the majority of experiments two moles of p-nitrophenol per one mol of

BNPP were liberated and the observed first-order rate constants were calculated by the integral method from the kinetic curves followed to 95% or higher conversion of the substrate. The fast hydrolysis of the intermediate monoester was confirmed in a separate experiment. In experiments with $(NH_4)_2Ce(NO_3)_6$, rate constants lower than 0.002 s^{-1} were estimated from the initial rates in order to eliminate the effect of inactivation of the catalyst (see the text). For BNPP hydrolysis by complex **2** at pH > 6, biphasic kinetic curves were observed with fast liberation of first nitrophenol followed by slower liberation of the second nitrophenol molecule. These results were fitted to the respective two-exponential equation derived for the mechanism involving two consecutive steps of hydrolysis of BNPP to mono(p-nitrophenyl) phosphate and then of the monoester to inorganic phosphate.[16] A typical kinetic curve and the fitting procedure are shown in the ESI †, Fig. 9S.

Potentiometric titrations were performed and analyzed as described previously.[16]

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