Kinetics of phosphodiester hydrolysis by lanthanide ions in weakly basic solutions

Paola Gómez-Tagle and Anatoly K. Yatsimirsky *,†

Facultad de Química, Universidad Nacional Autónoma de México, 04510 México D.F., México

Lanthanide(III) cations in aqueous Bis-tris propane buffer remained stable at elevated pH values and at pH 9.0 catalyzed the hydrolysis of several phosphodiester substrates 10^3 times more efficiently than in neutral solutions.

Much attention is currently focused on lanthanide (Ln) catalysis in phosphodiester hydrolysis.[1] Although in the majority of known systems the active forms of Ln catalysts were not identified, most probably these are hydroxo complexes of Ln(III) cations, as one can conclude from available pH-dependencies of rates of metal-promoted phosphate ester hydrolysis reactions [RNA hydrolysis by LaCl$_3$,[1j] phosphate tri-ester hydrolysis by a La(III) macrocyle,[2] phosphodiester hydrolysis by complexes of Co(III),[3] Zn(II),[4] Ni(II)[5] and Cu(II),[5] phosphonate ester hydrolysis by La(III)[6]]. There is, however, a certain difficulty in generation and stabilization of such complexes in solution. From one side, Ln(III) aqua-cations possess high pK_a values of about 9[7] and appreciable amounts of their hydroxo complexes are formed only in basic solutions. On the other hand, Ln(III) hydroxides have small solubility products of about 10^{-20}–10^{-22} mol^4 dm^{-12} and readily co-precipitate with simple inorganic ions like nitrate or chloride that further reduce their solubilities.[7a] As a result, solutions of Ln(III) salts become unstable at pH slightly above 7 and the hydroxo complexes can be produced only at very low concentrations.

A possible solution to this problem would be the use of appropriate ligands, which would protect a Ln(III) cation from precipitation, but would not decrease too much its electrophilicity, necessary to generate coordinated OH anions and to activate the substrate. Hay and Govan[2] reported a 100-fold increase in activity of a La(III) macrocyclic complex in the hydrolysis of 2,4-dinitrophenyl diethyl phosphate on going from pH 7 to 9.5; no hydroxide precipitation was observed in basic solutions, but the substrate employed was highly activated. Schneider and co-workers[1e,g] tested effects of various ligands on the hydrolytic reactivity of Eu^{3+} towards bis(p-nitrophenyl)phosphate (BNPP) (a typical phosphodiester substrate with very low intrinsic reactivity) at pH 7.0, and found that amines, aminoethers and polyols produced minor effects, but carboxylic acid ligands strongly inhibited the hydrolysis. It seems from these results that neutral ligands, *e.g.* amino-alcohols, could function as non-deactivating stabilizers of Ln(III) cations at elevated pH values.

We tested several ligands for their ability to prevent precipitation of 2 mM Ln(III) cations (Ln = La, Nd, Pr or Eu) in the range pH 7–9 including ethylendiamine, 1,3-diaminopropane, polyamines, Bis-tris [2,2-bis(hydroxymethyl)-2,2′,2″-nitrilotriethanol], TRIS [tris(hydroxymethyl)aminomethane] and Bistris propane {BTP, 1,3-bis[tris(hydroxymethyl)methylamino]propane}; and obtained satisfactory results with the latter. A minimum concentration of BTP sufficient to prevent Ln(III) precipitation over the whole pH range was 10 mM. The pro-

† E-mail: anatoli@servidor.unam.mx

cedure of solution preparation was as follows: to the required amount of BTP solution of pH slightly higher than the final one, an aliquot of a Ln(III) stock solution was added and the pH of the solution, which was still *ca.* 0.2 higher than desired, was adjusted with perchloric acid. We found it important to always follow this method of solution preparation in order to get reproducible results. Perchlorate salts of Ln(III) cations (Strem) were employed. The stock solutions of lanthanides were standardized by volumetric titration with H$_4$EDTA.[8]

Kinetics of hydrolysis was followed spectrophotometrically by the appearance of p-nitrophenolate at 400 nm in the cases of BNPP and p-nitrophenylphosphate (NPP) as substrates and by the appearance of phenol at 280, 285 and 290 nm in the case of diphenylphosphate (DPP). The kinetics of hydrolysis of NPP and DPP was strictly first-order and the respective rate constants (k_{obs}) were calculated by the integral method or (for very slow reactions) from initial rates. During the BNPP hydrolysis the liberation of 2 equivalents of p-nitrophenol per 1 substrate equivalent was observed, but the reaction kinetics deviated noticeably from the simple first-order rate law due to insufficiently fast hydrolysis of the intermediate NPP. The kinetic curves were fitted to a respective two-exponential equation derived from the scheme of two successive pseudo-first-order steps of hydrolysis of BNPP to NPP and further to an inorganic phosphate with liberation of p-nitrophenolate at each step.‡ The correctness of the scheme was confirmed by comparison of calculated rate constants with those found from the initial rates (k_{obs} of the first step) and from the kinetics of NPP hydrolysis (k_{obs} of the second step). The sole phosphate product of BNPP hydrolysis was the inorganic phosphate quantitatively determined in the reaction mixture by a standard molybdate/hydrazine colorimetric method[9] [no color was developed with unhydrolyzed BNPP; analysis of a mixture containing 0.04 mM BNPP, 2 mM La(ClO$_4$)$_3$ and 20 mM BTP after 24 h at 25 °C showed the presence of 0.046 ± 0.005 mM H$_3$PO$_4$ and 0.083 ± 0.002 mM p-nitrophenol].

Fig. 1 shows the plots of k_{obs} of the first step of BNPP hydrolysis at room temperature *vs.* BTP concentration in the presence of 2 mM La(III) at two different pH values. The shape of the plots most probably reflects the formation of higher unreactive BTP/La(III) complexes on going to increased ligand concentrations (see below). At a fixed ligand concentration k_{obs} was directly proportional to the La(III) concentration in the range 1–14 mM and increased on going to higher pH values.

Fig. 2 shows the pH-dependence of k_{obs} of the first step of BNPP hydrolysis at fixed ligand and metal concentrations. A rapid increase in the reaction rate in the range pH 8–9 is indicative of formation of the reactive hydroxo complexes of the type [La(BTP)(OH)$_n$]$^{3-n}$. The plot in Fig. 2 fits eqn. (1)

$$k_{obs} = k_{obs}^0/(1 + [H^+]^2/K_a) \qquad (1)$$

‡ The equation has the form $A_t = \varepsilon_{NP}[\text{BNPP}]_0\{2+(k_{obs,1}e^{-k_{obs,2}t} + (k_{obs,1} - 2k_{obs,2})e^{-k_{obs,1}t}/(k_{obs,2} - k_{obs,1}))\}$ where A_t is the absorbance at time t, ε_{NP} and [BNPP]$_0$ are the molar absorptivity of p-nitrophenolate and initial substrate concentration respectively, $k_{obs,1}$ and $k_{obs,2}$ are the rate constants of the cleavage of BNPP and NPP respectively.

Table 1 Kinetics of phosphate ester hydrolysis by Ln(III)/BTP at 25 °C and pH 9.0

Substrate	Metal	$k_2{}^a$/dm³ mol⁻¹ s⁻¹	E_a/kJ mol⁻¹	$k_{obs}{}^b$/s⁻¹
Bis(*p*-nitrophenyl)phosphate	None			$6.9 \times 10^{-11\,c}$
(BNPP)	La	0.22	55 ± 6	2.2×10^{-3}
	Nd	0.061		6.1×10^{-4}
	Pr	0.087		8.7×10^{-4}
	Eu	0.030		3.0×10^{-4}
p-Nitrophenyl phosphate	None			$1.7 \times 10^{-9\,d}$
(NPP)	La		50 ± 6	4.1×10^{-3}
Diphenyl phosphate	None			$5 \times 10^{-15\,e}$
(DPP)	La	1.4×10^{-3}	97 ± 2	1.4×10^{-5}

a Second-order rate constants based on total metal concentration at fixed 20 mM BTP concentration; relative error ±5%. *b* At 10 mM Ln(III) and 20 mM BPT. *c* The sum of pseudo-first-order rate constants of spontaneous hydrolysis (1.1×10^{-11} s⁻¹)[1i] and of alkaline hydrolysis ($k_{OH} = 5.8 \times 10^{-6}$ dm³ mol⁻¹ s⁻¹ at 25 °C)[10] of BNPP at pH 9.0 and 25 °C. *d* First-order rate constant of the hydrolysis of NPP dianion at 25 °C.[11] *e* Calculated for 25 °C as described in ref. 1*e*.
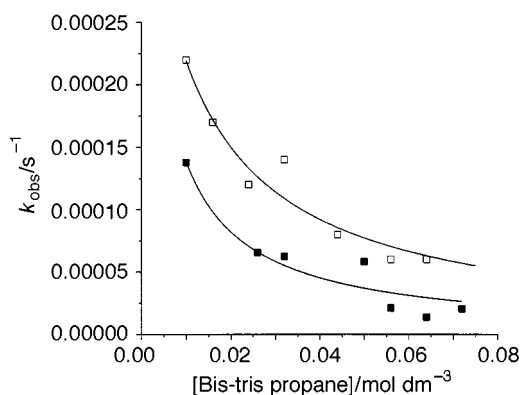

Fig. 1 Pseudo-first-order rate constants of the hydrolysis of BNPP at 25 °C in the presence of 2 mM La(ClO₄)₃ as a function of BTP concentration. Solid squares, pH 8.5; open squares, pH 9.0.
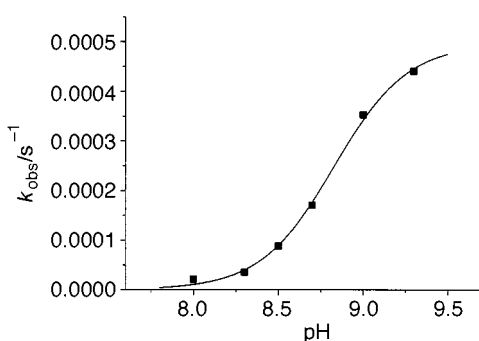

Fig. 2 Pseudo-first-order rate constants of the hydrolysis of BNPP at 25 °C in the presence of 2 mM La(ClO₄)₃ and 10 mM BTP as a function of pH. Solid line is the theoretical fit to eqn. (1).

which corresponds to a reaction scheme where the reactive form is a dihydroxo complex ($n = 2$)§ with $k_{obs}{}^0 = (5.0 \pm 0.1) \times 10^{-4}$ s⁻¹ [the pseudo-first-order rate constant of hydrolysis of BTPP by a dihydroxo complex of La(III)] and $pK_a = 17.66 \pm 0.03$ [K_a corresponds to dissociation of two aqua ligands: $M(H_2O)_2 \rightleftharpoons M(OH)_2 + 2H^+$]. A negligible contribution of an aqua La(III)/BTP complex under given conditions is quite expected. Indeed, reported[1i] for 2 mM La(III) at 25 °C and pH 7.0 a value of $k_{obs} = 1.4 \times 10^{-7}$ s⁻¹ is 4×10^3 smaller than $k_{obs}{}^0$. Mononuclear dihydroxo complexes of La(III) were not reported,[7a] but the strict first-order in La(III) indicates that most probably the reactive species is a mononuclear BTP/La(III) complex rather than one of known polyhydroxo polynuclear complexes.[1j,7a]

§ Fitting of the results in Fig. 2 to a complete equation which corresponds to a reaction scheme where the aqua and monohydroxo complexes also contribute to the observed reactivity gives statistically insignificant values for the rate constants of these forms.

Another possibility is that the reactive form is a coordinated alcoholate of the ligand, but this is not consistent with the formation of inorganic phosphate as the reaction product.

The determination of stoichiometry and stability constants of Ln(III)/BTP complexes is in progress. Some conclusions can be inferred, however, from the kinetic results. As mentioned above, kinetic first-order in La(III) and pH-dependence represented by eqn. (1) indicate the reactive species to be a mononuclear dihydroxo complex. The inhibitory effect of an excess of BTP, Fig. 1, should reflect the coordination of at least a second ligand molecule since 2 mM lanthanide(III) perchlorate alone precipitated at pH > 8. Inspection of molecular models shows that BTP can function as a hexadentate ligand through two N and four O atoms. Therefore, it probably forms only 1:1 and 1:2 metal–ligand complexes, the first of which is the reactive species. In this case plots in Fig. 1 should be bell-shaped, but we can observe only their decreasing hyperbolic parts due to the necessity of adding enough ligand to prevent precipitation of La(OH)₃. Fitting of these plots to the respective theoretical equation shows that the first stepwise stability constant must be ≥10³ dm³ mol⁻¹ and the second stability constants equal 220 and 85 dm³ mol⁻¹ at pH 8.5 and 9.0 respectively. A decrease in stability constant on going to higher pH probably reflects the competition with hydroxo ligands.

Similar results were obtained for other lanthanides studied (Nd, Pr or Eu). Table 1 collects the second-order rate constants (k_2) for all metals found from the slopes of k_{obs} *vs.* total metal concentration profiles (ranging from 1 to 10 mM) at pH 9.0 (the highest pH value at which all metals did not precipitate in the presence of BTP) and 25 °C. Also the pseudo-first-order constants at 10 mM Ln concentration are given for comparison with related published results. In the case of La(III) the activation energy was determined from the Arrenius plot of k_{obs} in the range 25–50 °C.

The kinetics of the hydrolysis of NPP was studied where the only purpose was to confirm the reaction scheme used for analysis of the integral kinetics of BNPP (see above). One can see from the last column in Table 1 that NPP hydrolysis proceeds two times faster than BNNP hydrolysis and has practically the same activation energy, that is, the relative reactivity of these two substrates is independent of temperature.

With the most active metal La(III) the kinetics of hydrolysis of a less reactive substrate DPP was studied. Similar kinetic behavior was observed, but the rate constant decreased *ca.* 200 times and the activation energy was considerably higher with this substrate, Table 1.

The kinetics of phosphate diester hydrolysis by the Ln(III)/BTP system is substantially different from that by Ln(III) aqua ions. First, no "saturation" at metal concentrations at about 10 mM as reported for all Ln(III) aqua ions[1e–h] is observed. Most probably this is due to the lower charge of the metal species (+1 instead of +3) since phosphate binding to the metal is predominantly electrostatic.[1h] In agreement with this we observed a

very weak dependence of k_{obs} on the ionic strength in contrast to a strong dependence reported for Ln(III) aqua ions.[1h] Secondly, the order of activities of lanthanides (La > Nd ≈ Pr > Eu) in the Ln(III)/BTP system is opposite to that (La < Pr < Eu) for Ln(III) aqua ions.[1h] In the case of the Ln(III)/BTP system the activity trend parallels the trend in pK_a values of Ln(III) aqua ions, which increase in the order Eu < Nd < Pr < La.[7b] The same trend is typical for esterolytic activity of hydroxo cations towards p-nitrophenyl acetate as the substrate.[12] Evidently, in both cases the dominant factor is the nucleophilic reactivity of the metal-coordinated OH anion, while for aqua cations of Ln(III) the dominant factor is electrophilic assistance.[1h]

Finally, the catalytic activity of Ln(III)/BTP complexes in a weakly basic media is much higher than that of Ln(III) ions in neutral solutions. Comparisons of k_{obs} values given in the last column of Table 1 with those published in the literature show that although the activity of the Ln(III)/BTP system for Ln = La does not reach that of Th(IV) (2.82×10^{-2} s^{-1} at 37 °C),[13] it is essentially similar to that of La(III)/H$_2$O$_2$ (4.8×10^{-3} s^{-1} at 25 °C)[1i] which is the most active at the moment among all Ln(III)-based systems. The value of k_{obs} for La(III) extrapolated to 50 °C is calculated to be 1.25×10^{-2} s^{-1} by using the activation energy given in Table 1; the activation energy is 10^3 times higher than that in neutral solutions "saturated" with La(III) and 15 times higher than with most active Er(III) species at the same temperature.[1h] Comparison with spontaneous hydrolysis shows acceleration factors of *ca.* 10^8 for BNPP and DPP and *ca.* 10^6 for NPP, Table 1, which make the system practically useful for applications at room temperature. It is also worth noting that the fairly simple kinetic behavior of the Ln(III)/BTP system makes it suitable for further mechanistic study.

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