Inorg. Chem. **2008**, *47*, 2514−2525

Inorganic Chemistry
Article

Simplified Speciation and Improved Phosphodiesterolytic Activity of Hydroxo Complexes of Trivalent Lanthanides in Aqueous DMSO

Irma Sánchez-Lombardo and Anatoly K. Yatsimirsky*

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

Received September 18, 2007

Potentiometric titrations of La(III), Nd(III), and Eu(III) perchlorates by $Me_4N(OH)$ in 80% vol aq DMSO revealed formation of predominantly mononuclear complexes $M(OH)_n^{3-n}$ ($n = 1$, 2, or 3) and a single binuclear complex $M_2(OH)^{5+}$. Kinetics of the cleavage of two phosphate diesters, bis (4-nitrophenyl) phosphate (BNPP) and 2-hydroxypropyl 4-nitrophenyl phosphate (HPNPP), and a triester, 4-nitrophenyl diethyl phosphate (paraoxon), were studied as a function of metal and $Me_4N(OH)$ concentrations in the same medium. Rate of BNPP cleavage is second-order in metal and is proportional to the product of concentrations of $M(OH)_2^+$ and $M(OH)_3$ species. Rate of HPNPP cleavage is proportional to $[M(OH)_3]^3$ for La(III) and Nd(III) and to $[M(OH)_3]^2$ for Eu(III). Proposed mechanism for BNPP hydrolysis involves formation of $M_2(OH)_5$(diester) intermediate followed by intramolecular nucleophilic attack of hydroxide anion on the phosphoryl group of the substrate. Proposed mechanism for HPNPP cleavage involves formation of $M_3(OH)_9(\text{diester})^-$ or $M_2(OH)_6(\text{diester})^-$ intermediates followed by the general base-assisted intramolecular cyclization of HPNPP. The latter mechanism is supported by observation of the solvent kinetic isotope effect $k_H/k_D = 2.9$ for Eu(III) catalyzed HPNPP cleavage. The efficiency of catalysis in 80% DMSO is much higher than in water. The reaction rate observed in the presence of 1 mM metal in neutral solution surpasses the rate of background hydrolysis by a factor of 10^{12}−10^{13} for BNPP and 10^{10} for HPNPP. The increased catalytic activity is attributed principally to the preferable solvation of lanthanide ions by DMSO, which creates an anhydrous microenvironment favorable for reaction in the coordination sphere of the catalyst. The catalytic activity of lanthanides in paraoxon hydrolysis is much lower with the estimated efficiency of catalysis about 10^5 for 1 mM La(III).

Introduction

There is much current interest in the development of more efficient catalysts for the cleavage of phosphate esters of different types.[1] Recently significant attention has been attracted to study of phosphoesterolytic activity of metal ions in nonaqueous media, mainly anhydrous methanol, as a way to improve the catalytic performance. Greatly enhanced catalytic activity in this solvent, as compared to that observed in water, was reported for trivalent lanthanides[2] and Zn(II) ions and complexes.[3] Also, alkali metal ions lacking catalytic activity in water show significant catalytic effects in anhydrous methanol.[4] A binuclear Cu(II) complex was reported to have sufficient reactivity even for the cleavage of nonactivated dimethyl phosphate substrate in this solvent.[5] In addition, strongly enhanced reactivity was reported in some aprotic solvents: in acetonitrile with Zn(II) and Cu(II) ions[6] and in DMF with a macrocyclic Zn(II) complex bearing an alcohol pendant.[7]

---

* To whom correspondence should be addressed. E-mail: anatoli@servidor.unam.mx. Phone: 55 5622 3813. Fax: 55 5616 2010.

(1) (a) Recent reviews: Mancin, F.; Tecilla, P. *New J. Chem.* **2007**, *31*, 800. (b) Yatsimirsky, A. K. *Coord. Chem. Rev.* **2005**, *249*, 1997. (c) Morrow, J. R.; Iranzo, O. *Curr. Opin. Chem. Biol.* **2004**, *8*, 192. (d) Morales-Rojas, H.; Moss, R. A. *Chem. Rev.* **2002**, *102*, 2497. (e) Liu, C. L.; Wang, M.; Zhang, T. L.; Sun, H. Z. *Coord. Chem. Rev.* **2004**, *248*, 147. (f) Williams, N. H. *Biochim. Biophys. Acta* **2004**, *279*, 1697. (g) Molenveld, P.; Engbersen, J. F. J.; Reinhoudt, D. N. *Chem. Soc. Rev.* **2000**, *75*, 29. (h) Kramer, R. *Coord. Chem. Rev.* **1999**, *182*, 243. (i) Williams, N. H.; Takasaki, B.; Wall, M.; Chin, J. *Acc. Chem. Res.* **1999**, *32*, 485.

(2) (a) Neverov, A. V.; Brown, R. S. *Inorg. Chem.* **2001**, *40*, 3588. (b) Tsang, J. S. W.; Neverov, A. V.; Brown, R. S. *J. Am. Chem. Soc.* **2003**, *125*, 1559. (c) Tsang, J. S. W.; Neverov, A. V.; Brown, R. S. *J. Am. Chem. Soc.* **2003**, *125*, 7602.

(3) Neverov, A. A.; Lu, Z.-L.; Maxwell, C. I.; Mohamed, M. F.; White, C. J.; Tsang, J. S. W.; Brown, R. S. *J. Am. Chem. Soc.* **2006**, *128*, 16398.

(4) (a) Pregel, M. J.; Dunn, E. J.; Nagelkerke, R.; Thatcher, G. R. J.; Buncel, E. *Chem. Soc. Rev.* **1995**, *24*, 449. (b) Buncel, E.; Albright, K.; Onyido, I. *Org. Biomol. Chem.* **2004**, *2*, 601. (c) Onyido, I.; Albright, K.; Buncel, E. *Org. Biomol. Chem.*, **2005**, *3*, 1468.

(5) Jagoda, M.; Warzeska, S.; Pritzkow, H.; Wadepohl, H.; Imhof, P.; Smith, J. C.; Krämer, R. *J. Am. Chem. Soc.* **2005**, *127*, 15061.

10.1021/ic701846e CCC: $40.75

The results for most thoroughly studied lanthanide and Zn(II) based systems for catalytic methanolysis of phosphate esters and related substrates in anhydrous methanol clearly show the advantages of this solvent.[8] First, metal methoxides are soluble in methanol, in contrast to hydroxides, which are insoluble in water. This resolves the problem of concomitant metal precipitation in sufficiently basic solutions required for formation of active species. Second, reduced polarity of this solvent promotes much stronger association of the substrates with metal ions and so enhances the catalytic activity. Third, one may obtain detailed speciation for metal alkoxides in methanol by using precise potentiometric pH titrations with an appropriately calibrated glass electrode and so to identify the reactive species.

We are focusing on the use of mixed aqueous–organic solvents, in particular water−DMSO mixtures, as media for catalytic phosphate ester hydrolysis. At first glance, addition of DMSO should not be favorable for catalysis because this solvent stabilizes metal ions even stronger than water.[9] However, we found that phosphodiesterolytic activity of alkali[10] and alkaline earth[11] cations strongly increased in mixtures containing more than 80% vol DMSO. The development of improved catalytic systems in aqueous–organic mixtures is of interest because these media may be more suitable for practical applications than anhydrous organic solvents. In addition, these systems still use the hydroxide nucleophile bringing about hydrolysis rather than transesterification of the substrate. Therefore, we extended this study to trivalent lanthanides, which are among the most active catalysts for the hydrolysis of phosphate esters in water.[12]

The positive effect of DMSO can be attributed to several factors. The reactivity of anionic nucleophiles, including hydroxide, is strongly enhanced in dipolar aprotic solvents.[13] This effect is due to the removal of hydrogen-bound water molecules from anionic species, which increases their free energies and the availability of lone electron pairs. Apparently this effect can also increase the association of negatively charged phosphodiesters with metal ions. It seems that the preferable solvation of metal ions by DMSO in the mixed solvent also contributes to improved catalytic activity because metal-bound hydroxide and phosphodiester anions appear surrounded by only DMSO molecules in a medium, which still contains a significant amount of water.

There are only rather limited data on metal ion hydrolysis in DMSO−water mixtures. The results of a detailed study of Be(II) hydrolysis in 80% DMSO point however to some interesting features of the system.[14] While the hydrolysis in water leads to predominantly polynuclear species, in the presence of DMSO, the abundance of mononuclear complexes increase greatly because of stronger solvating power of DMSO molecules. Thus one may expect that addition of DMSO may simplify significantly an extremely complicated pattern of lanthanides hydrolysis in water.[15] Another interesting result is that the first $pK_a = 5.25$ of Be(II) in 80% DMSO essentially coincides with that in water, $pK_a = 5.4$. This means that the formation of hydroxo complexes starts in the same pH range both in pure water and 80% DMSO, but because the autoprotolysis constant in this solvent is strongly reduced ($pK_w = 18.4$ in 80% DMSO),[16] the concentration of free hydroxide ions at the same pH will be much smaller and the precipitation of metal hydroxide will occur at a higher metal concentration. This may solve the mentioned above problem of metal precipitation although in a different way than in methanol. The results of this paper show that the behavior of lanthanides in DMSO−water indeed meets these expectations and, in addition, using this medium allows one to increase greatly the observed catalytic activity.

Experimental Section

Materials. Bis(4-nitrophenyl) phosphate (Aldrich) was recrystallized from ethanol–water. Paraoxon (Aldrich) was used as supplied. 2-Hydroxypropyl 4-nitrophenyl phosphate was prepared according to the literature procedure.[17] Sodium perchlorate, reagent grade lanthanide(III) perchlorates as 40 wt % aqueous solutions, and $Me_4N(OH)\cdot 5H_2O$, all from Aldrich, were used as supplied. The concentration of metal ions in stock solutions was determined by titration with EDTA with Xylenol Orange as indicator. Distilled and deionized water (Barnstead Nanopure system) was used. DMSO (Baker) was purified by distillation over CaO.

Potentiometry. Potentiometric titrations were performed in a 30-mL thermostatted cell kept under nitrogen at 25 ± 0.1 °C. Measurements of pH were carried out using an Orion model 710-A research digital pH meter while the titrant (Me_4NOH) solution was added to the system in small increments. The electrode was calibrated in terms of $p[H^+]$ by titrations of 1–3 mM $HClO_4$ as described in ref 18. From these data, the autoprotolysis constant $pK_W = 18.5 \pm 0.1$ was determined in good agreement with published values.[16] The program HYPERQUAD 2003, version 3.0.51,[19] was used to calculate all equilibrium constants. Species distribution diagrams were calculated by using HYSS 2003 software.[19b]

---

(6) Kondo, S.; Yoshida, K.; Yano, Y. *J. Chem. Res. Synop.* **1999**, 106.

(7) Kimura, E.; Kodama, Y.; Koike, T.; Shiro, M. *J. Am. Chem. Soc.* **1995**, *117*, 8304.

(8) Brown, R. S.; Neverov, A. A. *J. Chem. Soc., Perkin Trans. 2* **2002**, 1039.

(9) Kalidas, C.; Hefter, G.; Marcus, Y. *Chem. Rev.* **2000**, *100*, 819.

(10) Gómez-Tagle, P.; Vargas-Zúñiga, I.; Taran, O.; Yatsimirsky, A. K. *J. Org. Chem.* **2006**, *71*, 9713.

(11) Taran, O.; Yatsimirsky, A. K. *Chem. Commun.* **2004**, 1228.

(12) (a) Reviews on lanthanide catalysis: Lim, S.; Franklin, S. J. *Cell. Mol. Life Sci.* **2004**, *61*, 2184. (b) Schneider, H.-J.; Yatsimirsky, A. K. In *Metal Ions in Biological Systems, Vol. 40: The Lanthanides and Their Interrelations with Biosystems*; Sigel, A., Sigel, H., Eds.; Marcel Dekker: New York, 2003; Vol. *40*, p 369. (c) Franklin, S. J. *Curr. Opin. Chem. Biol.* **2001**, *5*, 201. (d) Komiyama, M.; Takeda, N.; Shigekawa, H. *Chem. Commun.* **1999**, 1443. (e) Blasko, A.; Bruice, T. C. *Acc. Chem. Res.* **1999**, *32*, 475.

(13) (a) Parker, A. J. *Chem. Rev.* **1969**, *69*, 1. (b) Buncel, E.; Wilson, H. *Adv. Phys. Org. Chem.* **1977**, *4*, 133.

(14) Chinea, E.; Dominguez, S.; Mederos, A.; Brito, F.; Sanchez, A.; Ienco, A.; Vacca, A. *Main Group Met. Chem.* **1997**, *20*, 11.

(15) (a) Baes, C. F. Jr.; Mesmer, R.E. *The Hydrolysis of Cations*; Wiley: New York, 1976. (b) Rizkalla, E. N.; Choppin, G. R. In *Handbook on the Physics and Chemistry of Rare Earths*; Gschneider, K. A., Jr., Eyring, L., Eds.; Elsevier Science: New York, 1991; Vol. *15*, 393.

(16) (a) Baughman, E. H.; Kreevoy, M. M. *J. Phys. Chem.* **1974**, *78*, 421. (b) Georgieva, M.; Velinov, G.; Budevsky, O. *Anal. Chim. Acta* **1977**, *90*, 83.

(17) Brown, D. M.; Usher, D. A. *J. Chem. Soc.* **1965**, 6558.

(18) Gómez-Tagle, P.; Yatsimirsky, A. K. *Inorg. Chem.* **2001**, *40*, 3786.

(19) (a) Gans, P.; Sabatini, A.; Vacca, A. *Talanta* **1996**, *43*, 1739. (b) Alderlghi, L.; Gans, P.; Ienco, A.; Peters, D.; Sabatini, A.; Vacca, A. *Coord. Chem. Rev.* **1999**, *184*, 311.

With each cation, at least three independent titrations were performed with 0.5–2.0 mM $Ln(ClO_4)_3$ solutions. In more concentrated solutions, the metal hydroxide precipitated in the course of titration. The time required to obtain a stable pH reading increased from 20 to 30 s in acid medium to ca. 5 min at pH above 7. Equal reproducibility and stability of pH readings was observed in titration experiments with background electrolyte (10 mM $NaClO_4$) and without it. The calibration plots of experimental pH readings vs $p[H^+]$ were linear in both media in the range 2.5–16, with the only difference that the plot obtained in the presence of 10 mM $NaClO_4$ was shifted by 0.14 units to lower pH values. Equations for calibration plots were $pH = a + bp[H^+]$ with parameters $a = (1.054 \pm 0.021)$, $b = (0.994 \pm 0.002)$, $R = 0.99994$ in the presence of 10 mM $NaClO_4$ and $a = (0.903 \pm 0.016)$, $b = (0.999 \pm 0.002)$, $R = 0.99997$ in the absence of added electrolyte. The pK_W values determined in both media coincided in limits of experimental errors. A possible variation in activity coefficients of hydrogen ions during titration in the absence of the background electrolyte should be insignificant because the total concentration of anions is kept constant during the titration. Because stock solutions of $Me_4N(OH)$ in 80% DMSO turned yellow after several hours, the aqueous stock solutions of the base were employed and the corresponding amounts of pure DMSO were added to the titration cell after each addition of the titrant to keep the content of DMSO constant.

Spectrophotometry. Spectrophotometric titrations of Nd(III) by $Me_4N(OH)$ were performed with 1–1.5 mM solutions of $Nd(ClO_4)_3$ in 80% DMSO containing 0.1 mM $HClO_4$. Spectra were recorded on a Unicam UV500 spectrometer, and the cuvette path length was 5 cm.

Kinetics. Kinetic measurements were performed on a Hewlett-Packard 8453 diode array spectrophotometer equipped with a thermostatted cell compartment (recirculating water bath at 25 ± 0.1 °C). Reaction solutions were prepared by combining appropriate amounts of metal and tetramethylammonium hydroxide stock solutions to the desired volume. Reactions were initiated by adding an aliquot of the substrate solution.

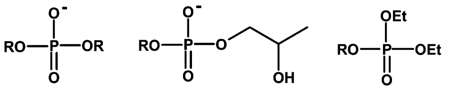
The course of hydrolysis for all substrates was monitored spectrophotometrically by the appearance of 4-nitrophenolate anion at 420 nm. Stock solutions of BNPP and HPNPP were freshly prepared in water. Stock solutions of paraoxon were prepared in DMSO. First-order conditions with base concentrations (0.5–5 mM) at least 50 times greater than the substrate concentration (0.01 mM) were used. Because deprotonation of 4-nitrophenol at lower base concentrations was incomplete, a standard addition of 0.01 mM 4-nitrophenol was applied to each sample after the end of the reaction run to determine the apparent molar absorptivity under given conditions. The observed first-order rate constants (k_{obs}) were calculated by the integral method from at least 90% conversion or, for slow reactions, from initial rates. Rate constants larger than 0.1 s^{-1} (mainly with HPNPP as a substrate) were determined using an Applied Photophysics SX.18MV stopped-flow reaction analyzer thermostatted at 25.0 °C.

NMR Spectroscopy. ^{31}P NMR spectra were recorded on a Varian Gemini 300 NMR spectrometer.

Results and Discussion

Model substrates employed for kinetic studies in this paper are two diesters, bis(4-nitrophenyl) phosphate (BNPP, a model for DNA hydrolysis), 2-hydroxypropyl 4-nitrophenyl phosphate (HPNPP, a model for RNA hydrolysis), and a triester, 4-nitrophenyl diethyl phosphate (Paraoxon), Chart 1.

Chart 1



BNPP **HPNPP** Paraoxon

$R = C_6H_4\text{-}NO_2\text{-}p$

In preliminary experiments, the rate of BNPP hydrolysis was measured in the presence of 1 mM $Eu(ClO_4)_3$ and 1.5–2.5 mM $Me_4N(OH)$ in water−DMSO mixtures of different compositions. The observed rate constants gradually increased on increase in the DMSO content up to 80% vol but became lower again in 90% DMSO. Rate constants in 80% DMSO were ca. 2 orders of magnitude higher than those in pure water. The system stability was sufficiently high. Thus, the incubation of 1 mM $Eu(ClO_4)_3$ with 2.0 mM $Me_4N(OH)$ in 80% DMSO during 90 min did not cause any change in the catalytic activity. On basis of these experiments, 80% vol DMSO was chosen as an optimum medium for further studies.

Composition and Stability of Hydroxo Complexes. The speciation of hydroxo complexes was determined by pH-potentiometric titrations. The 80% of DMSO is sometimes considered as a maximum amount of the organic cosolvent that allows one to obtain reliable pH readings,[20] although reproducible measurements with commercial electrodes were reported in nearly pure and even pure DMSO.[21] In our case, calibration plots were stable and linear in a wide range of pH (defined as $-\log[H^+]$) and pK_w calculated from the results of titration of a strong acid coincided with the reported pK_w values in the same medium (see the Experimental Section). To ensure a complete absence of metal hydroxo species at the beginning of titration, a small exact amount of $HClO_4$ (0.3–1 mM) was added before titration in each experiment. Titration of this excess of the strong acid served also to check the stability of the electrode calibration.

Typical titration curves for 1 mM lanthanides are shown in Figure 1 (points corresponding to titration of the excess $HClO_4$ below pH 5 are eliminated). For smaller and more acid Eu(III) and Nd(III) cations, titrations were possible up to the molar ratio metal ion:hydroxide = 1:3, but for La(III), which was titrated at higher pH values, the maximum attainable ratio was 1:2.5, after which the metal hydroxide started to precipitate. The results of titration experiments were fitted to the set of eq 1 for the formation of hydroxo complexes (the standard p,q,r notation with negative q values for deprotonation equilibria was employed), and the best fit was obtained for a reaction model involving successive formation of mononuclear species $Ln(OH)_n^{3-n}$ with n ranging from 1 to 3 plus one binuclear complex of the composition $Ln_2(OH)^{5+}$. Fitting to a reaction model involving only binuclear complexes $Ln_2(OH)_n^{6-n}$ was statistically poorer

---

(20) Khoo, K. H. *J. Chem. Soc. A* **1971**, 2932.

(21) (a) Ballash, N. M.; Robertson, E. B.; Sokolowski, M. D. *Trans. Faraday Soc.* **1970**, *66*, 2622. (b) Fiordiponti, P.; Rallo, F.; Rodante, F. *Z. Phys. Chem.* **1974**, *88*, 149. (c) Ritchie, C. D.; Uschold, R. E. *J. Am. Chem. Soc.* **1967**, *89*, 1721.

(22) Klungness, G. D.; Byrne, R. H. *Polyhedron* **2000**, *19*, 99.

(23) Kragten, J.; Decnop-Weever, L. G. *Talant,* **1984**, *9*, 731.

(24) Plancque, G.; Moulin, V.; Toulhoat, P.; Moulin, C. *Anal. Chim. Acta* **2003**, *478*, 11.

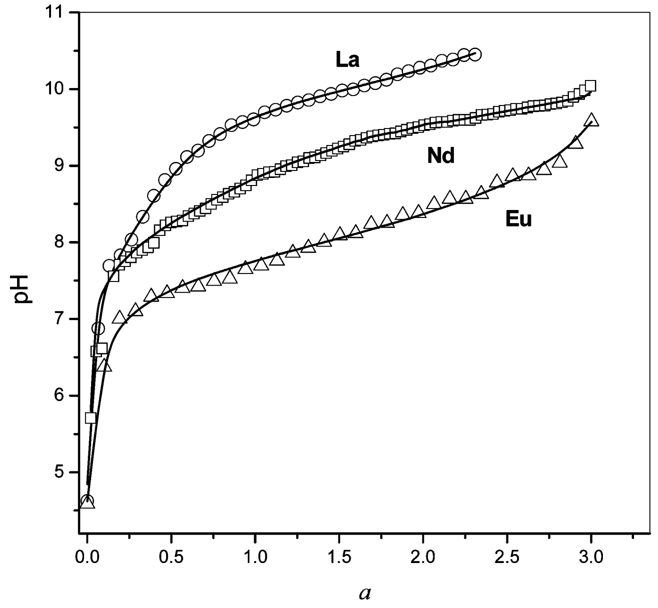


Figure 1. Titration curves for 1 mM lanthanide perchlorates with 0.1 M $Me_4N(OH)$ in 80% vol DMSO; *a* is the number of moles of the base added per 1 mol of the metal ion. Solid lines are the fitting curves generated by HYPERQUAD in accordance with equilibrium constants given in Table 1.

than that for a model with mononuclear complexes (see Table 1S in Supporting Information), and with models involving both mono- and binuclear species only, inclusion of the complex $Ln_2(OH)^{5+}$ led to a statistically significant improvement of the fitting quality. It should be noted, however, that just a statistical criterion is obviously insufficient for a definite discrimination between reaction schemes with mono- or binuclear species in a system with three or more species with overlapping distribution ranges and, in fact, coexistence of species of different nuclearities cannot be excluded. Unfortunately, our attempts to prove the chosen speciation scheme by alternative techniques were unsuccessful (see below).

Because addition of $NaClO_4$ caused unexpectedly strong inhibitory effect on the catalytic hydrolysis (see below), two sets of titration experiments were performed: one in the presence of 10 mM $NaClO_4$ and another one in the absence of added electrolyte (see Experimental Section for details of both procedures). The respective hydrolysis constants defined as in eq 2 are given in Table 1. The results of all titration experiments are given in Table 1S (Supporting Information). In addition, Table 2S in the Supporting Information provides mean values of the hydrolysis constants obtained by joint fitting of all titration plots for each cation and respective sigma values calculated by Hyperquad.

$$p\mathrm{Ln}^{3+} + q\mathrm{H}^{+} \rightleftharpoons \mathrm{Ln}_p(\mathrm{OH})_q^{3p-q} \qquad (1)$$

$$\beta_{pq} = [\mathrm{Ln}_p(\mathrm{OH})_q^{3p-q}]/[\mathrm{Ln}^{3+}]^p[\mathrm{H}^+]^q \qquad (2)$$

We attempted to support the speciation scheme derived from potentiometric results by conductometric titrations and FAB experiments, but both techniques gave inconclusive results. More informative were results of the spectrophotometric titration of Nd(III). We chose Nd(III) for spectroscopic studies because this cation possesses a so-called hypersensitive absorption band in the range 570–595 nm, which undergoes relatively strong changes on coordination with different donor atoms.[25–27] Figure 2A shows the spectral course of titration in the hypersensitive region. The absorbance in the maximum at 582 nm initially increases, but then monotonically goes down, Figure 2B. The maximum in absorbance corresponds to the molar ratio $[\mathrm{OH}^-]/[\mathrm{Nd(III)}] = 1{:}2$, and its observation is indicative of formation of $Nd_2(OH)^{5+}$ complex. A smaller and opposite change in absorbance is observed at 575 nm. The concentration profile of the absorbance at this wavelength, Figure 2B, shows an inflection above the molar ratio 0.5, again in agreement with formation of $Nd_2(OH)^{5+}$ complex. Both profiles generally agree with the speciation scheme derived from potentiometric data. The solid lines in Figure 2B are the theoretical profiles calculated by summation of contributions of all $\mathrm{Nd}_p(\mathrm{OH})_q^{3p-q}$ species to the observed molar absorptivity as $\epsilon_{\mathrm{obs}} = \sum \epsilon_i f_i$ where f_i are the molar fractions of the complexes calculated with hydrolysis constants given in Table 1 and ϵ_i are the respective molar absorptivities. The molar absorptivities of Nd^{3+} and $Nd(OH)_3$ were taken from initial and final spectra, respectively, and molar absorptivities of intermediate hydroxo complexes were estimated by a multiple regression of the polynomial expression for ϵ_{obs}.

In aqueous solutions, the substitution of coordinated water molecules by different donor atoms usually leads to increase in the oscillator strength (*f*) of the hypersensitive band calculated by integration of the absorption band.[25,26,28] This effect is not observed in 80% DMSO, where hydroxide ions substitute coordinated DMSO molecules: spectral changes shown in Figure 2A are accompanied by a very small and irregular changes in *f* in limits of 5%.

The principal effect of added $NaClO_4$ is the disappearance of dihydroxo species $Ln(OH)_2^+$, apparently because of the shift in the disproportionation equilibrium (eq 3) toward the mixture of mono and trihydroxo complexes.

$$2\mathrm{Ln}(\mathrm{OH})_2^+ \xrightleftharpoons{K_{\mathrm{disp}}} \mathrm{Ln}(\mathrm{OH})_3\mathrm{Ln}(\mathrm{OH})^{2+} \qquad (3)$$

In fact, these species hardly disappear completely. The equilibrium constant K_{disp} for the reaction (3) can be estimated from β_{pq} values given in Table 1 as $K_{\mathrm{disp}} = \beta_{1-3}\beta_{1-1}/(\beta_{1-2})^2 = 0.5$ for La(III) and 4 for Eu(III) in the absence of added sodium perchlorate. Under these conditions, the fraction of $Ln(OH)_2^+$ species reaches as a maximum 30–40% of total metal. An increase in K_{disp} by, e.g., 1 order of magnitude, which may result from a stronger ion pairing of the dicationic species than the monocationic species with perchlorate, may reduce the fraction of dihydroxo complexes

---

(25) Henrie, D. E.; Fellows, R. L.; Choppin, R. G. *Coord. Chem. Rev.* **1976**, *18*, 199.

(26) (a) Stephens, E. M.; Schoene, K.; Richardson, F. S. *Inorg. Chem.* **1984**, *23*, 1641. (b) Stephens, E. M.; Davis, S.; Reid, M. F.; Richardson, F. S. *Inorg. Chem.* **1984**, *23*, 4607.

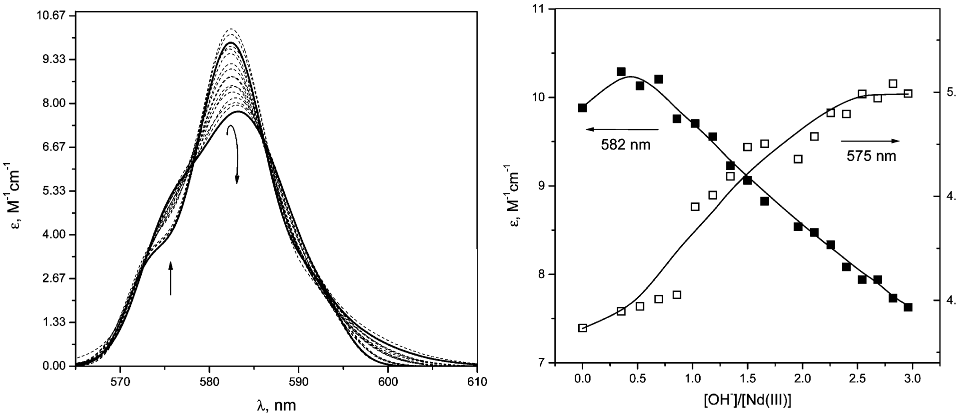
(27) (a) Hancock, R. D.; Jackson, G.; Evers, A. *J. Chem. Soc., Dalton Trans.* **1979**, 1384. (b) Birnbaum, E. R.; Darnall, D. W. *Bioinorg. Chem.* **1973**, *3*, 15.

(28) Yang, W.; Gao, J.; Kang, J.; Yang, W. *J. Solution Chem.* **1997**, *26*, 105.

Table 1. Hydrolysis Constants of Trivalent Lanthanides in 80% vol DMSO at 25 °C[a]

	log β_{pq}							
	$[NaClO_4] = 0$			$[NaClO_4] = 10$ mM		in water		
species	La(III)	Nd(III)	Eu(III)	La(III)	Eu(III)	La(III)	Nd(III)	Eu(III)
$Ln_2(OH)^{5+}$	-5.7 ± 0.2	-5.5 ± 0.1		-5.65 ± 0.09	-4.9 ± 0.1			
$Ln(OH)^{2+}$	-9.1 ± 0.1	-8.5 ± 0.1	-7.6 ± 0.1	-8.59 ± 0.09	-8.21 ± 0.03	-8.81^b	-8.18^b	-7.76^b
$Ln(OH)_2^{+}$	-19.18 ± 0.09	-17.9 ± 0.3	-15.8 ± 0.2				-16.2^c	-14.9^d
$Ln(OH)_3$	-29.55 ± 0.07	-27.64 ± 0.06	-24.2 ± 0.2	-28.4 ± 0.2	-25.1 ± 0.3		-24.3^c	-24.1^d

[a] The mean values of log β_{pq} are given with standard errors calculated by averaging of β values obtained in at least three independent titration experiments. Errors in log β_{pq} values as fitting parameters in each titration experiment were less than 0.05. [b] Ref 22 at $I = 0$. [c] Ref 23 at $I = 1$. [d] Ref 24 at $I = 0$.



A B

Figure 2. (A) Absorption spectra of 1.5 mM Nd(III) perchlorate in the hypersensitive region at increased amounts of added $Me_4N(OH)$ in 80% vol DMSO. Arrows show the direction of spectral changes on increased concentration of hydroxide. Solid lines are the initial and final spectra. (B) Plots of molar absorbance at 582 nm (solid squares, left scale) and at 575 nm (open squares, right scale) vs molar ratio of added hydroxide to metal ion. Solid lines are simulated profiles calculated with potentiometrically determined hydrolysis constants (see the text).

below 3–4% of total metal, and so these species may be easily overlooked in the titration experiment.

For comparison, Table 1 lists also the available β_{pq} values in water, which are remarkably close to those in 80% DMSO. On the other hand, considering formation of hydroxo complexes as an association process, reaction 4, one observes a very strong difference between formation constants in water and in 80% DMSO. The K_{OH} constants are related to β_{1-1} in accordance with eq 5, where K_W is the autoprotolysis constant.

$$Ln^{3+} + OH^- \overset{K_{OH}}{\rightleftharpoons} Ln(OH)^{2+} \tag{4}$$

$$\log K_{OH} = pK_W - pK_a = pK_W + \log \beta_{1-1} \tag{5}$$

Obviously, if pK_W increases from 14 in water to 18.5 in 80% DMSO, but log β_{1-1} values remain practically the same, this means that log K_{OH} will increase by 4.5 units on going from water to the mixed solvent. Thus, if in water log K_{OH} values are in the range 5.2–6.2, in 80% DMSO, they are in the range 9.3–10.9, and this allows formation of significant amounts of hydroxo complexes already in neutral solutions (pH of neutrality in 80% DMSO is 9.25). This effect can be attributed to the well-known phenomenon of destabilization of anions in dipolar aprotic solvents, the same as that responsible for increased reactivity of anionic nucleophiles.[13]

In contrast to hydrolysis of lanthanide cations in water, dominated by formation of polynuclear hydroxo species of irreproducible and practically unidentifiable composition followed by precipitation of metal hydroxides from millimolar metal solutions already at pH close to 8, hydrolysis in 80% DMSO is a much more simple process dominated by formation of mononuclear complexes. The reason for this is stronger solvation of cations by DMSO than by water, reflected in large negative transfer free energies of cations from water to DMSO[9] and strong preferable solvation of lanthanides by DMSO in mixtures with water.[33] Apparently, this strong solvation induces depolymerization of polynuclear species. At the same time, transfer free energies of anions to DMSO are also large but positive,[29] reflecting more weak solvation of anions in DMSO, and these weakly solvated anions successfully compete with DMSO molecules for the coordination spheres of metal ions forming complexes of even higher stability than that in water.

Kinetics of Phosphate Ester Hydrolyses. The alkaline hydrolysis of both phosphodiesters and phosphotriesters is accelerated by addition of DMSO to water, but in 80% vol of DMSO, which is still a rather aqueous medium (mol fraction of water is 50%), the effect is very modest.[10] We found the second-order rate constant for the alkaline hydrolysis of BNPP in this medium to be practically the same as in water, see Table 3. The rate constants for HPNPP and paraoxon (see Table 3) are, respectively, 3.5 and 10 times larger than those in water.

The rate of catalytic hydrolysis appeared to be very sensitive to the presence of neutral electrolytes, Table 2. Already, addition of 1 mM $NaClO_4$ reduces the rate by 50%

---

(29) Cox, B. G.; Parker, A. J.; Waghorne, W. E. *J. Phys. Chem.* **1974**, *78*, 1731.

Table 2. Salt Effects on the Rate of BNPP Hydrolysis in the Presence of 1 mM La(III) and 2 mM Me_4NOH in 80% DMSO

La(III) salt	added salt	k_{obs}, s^{-1}
$La(ClO_4)_3$	none	0.058
	1 mM $NaClO_4$	0.027
	5 mM $NaClO_4$	0.018
	10 mM $NaClO_4$	0.012
	10 mM $NaNO_3$	0.0050
	10 mM NaCl	0.0040
$La(Tfl)_3$	none	0.037

and 10 mM salt causes a 5-fold inhibition. Nitrate and chloride salts cause a 10-fold rate reduction. The catalytic activity of La(III) triflate (last line in Table 2) is a little bit lower than that of perchlorate.

A possible reason for these effects is the ion pairing of anions with cationic species, which may change the distribution of active species and/or block the interaction with anionic substrate. In the case of $NaClO_4$, we observed a significant decrease in the fraction of $Ln(OH)_2^+$ complexes (see above), which, as will be shown below, are the active species in BNPP hydrolysis.

In line with these observations, buffer components also caused a significant inhibitory effect. Figure 4 shows the observed first-order rate constants for BNPP hydrolysis in the presence of 1 mM $Eu(ClO_4)_3$ in 80% DMSO measured at increasing concentrations of TRIS added as a free base to the solution containing also 1 mM $HClO_4$. Like in unbuffered solutions (see below), the reaction rate passes though a maximum on increase in the base concentration, but the k_{obs} value at the optimum base concentration is 5 times smaller than the maximum k_{obs} value observed with $Me_4N(OH)$ as a base (see Figure 3).

On the basis of these preliminary results, we chose for the kinetic studies unbuffered solutions containing 0.5–2 mM metal perchlorate and up to 5 mM tetramethylammonium hydroxide as a base, for which the speciation of metal hydroxo complexes was established by potentiometric titrations in the same concentration range of components.

Parts A and B of Figure 4 show the observed first-order rate constants for the hydrolysis of BNPP and HPNPP in the presence of 1 mM $La(ClO_4)_3$ at variable concentration of added $Me_4N(OH)$ superimposed with the species distribution diagram.

An inspection of these profiles shows that both of them do not follow exactly the distribution curve for any single hydroxo complex. Obviously lower hydroxo complexes $La_2(OH)^{5+}$ and $La(OH)^{2+}$ do not contribute to the reactivity. The BNPP hydrolysis apparently can be attributed to the catalysis by a combination of $La(OH)_2^+$ and $La(OH)_3$ species, and the cleavage of HPNPP proceeds with participation of the highest hydroxo complex only.

Additional information was obtained by studying the effect of variable metal ion concentration under conditions of a constant $[La(III)]:[OH^-]$ ratio. Figure 5 illustrates in logarithmic coordinates such dependence obtained at the constant ratio $[La(III)]:[OH^-] = 1:2$. Species distribution calculations show that although $La(OH)^{2+}$, $La(OH)_2^+$, and $La(OH)_3$ all coexist under these conditions contributing 24%, 39%, and 32% to total La(III), respectively, their relative abundances remain practically constant on variation in total concentrations in employed limits. Therefore, the concentration of each species is a linear function of total concentration and the slope of such plot gives one the reaction order in the active species. Slopes of the lines in Figure 5 equal 4.2 ± 0.2 for HPNPP and 2.5 ± 0.4 for BNPP.

Combining results of both series of experiments, one concludes that for BNPP hydrolysis, the most probable rate law involves the product of concentrations of $La(OH)_2^+$ and $La(OH)_3$ in the form of eq 6. This explains why a maximum is observed in Figure 4A and is consistent with the second-order kinetics in La(III), which follows from results in Figure 5. For HPNPP, the only active species is $La(OH)_3$ and the rate law involves its concentration in a high degree, probably up to 4. In parts A and B of Figure 6, results from both series of experiments for each substrate are analyzed in terms of these suppositions. For BNPP, one observes in agreement with eq 6 a satisfactory linear correlation between k_{obs} and the product $[La(OH)_2^+][La(OH)_3]$ (Figure 6A) with the slope $k_3 = (4.3 \pm 0.1) \times 10^5\ M^{-2}\ s^{-1}$. For HPNPP, correlation between k_{obs} and $[La(OH)_3]$ taken from all data, Figure 6B, shows the third-order in metal kinetics, eq 7, with $k_4 = (2.5 \pm 0.6) \times 10^{10}\ M^{-3}\ s^{-1}$.

$$k_{obs} = k_3[Ln(OH)_2][Ln(OH)_3] \qquad (6)$$

$$k_{obs} = k_4[Ln(OH)_3]^3 \qquad (7)$$

Similar kinetics was observed with Nd(III). Parts A and B of Figure 7 show the profiles of k_{obs} vs concentration of added $Me_4N(OH)$, which have the same characteristics as those with La(III). Slopes of the plots of log k_{obs} vs log[Nd(III)], obtained at the constant ratio $[Nd(III)]:[OH^-] = 1:2$ (Figure S1, Supporting Information), equal 2.3 ± 0.2 for BNPP and 3.4 ± 0.2 for HPNPP. Analysis of all data (see Figure S2A,B, Supporting Information) confirm the validity of eqs 6 and 7 also for Nd(III) with rate constants given in Table 3.

Behavior of Eu(III) was qualitatively similar, but the reaction kinetics was of a lower order. As one can see from Figure 8A, the rate constants for BNPP hydrolysis roughly follow the concentration of a single species $Eu(OH)_2^+$. The slope of the plot of log k_{obs} vs log[Eu(III)] obtained at the constant ratio $[Eu(III)]:[OH^-] = 1:2$ (Figure S3, Supporting Information) equals 1.00 ± 0.05 for BNPP, and when we analyze together all data, both from variation in hydroxide and in metal, k_{obs} values show somewhat scattered, but generally acceptable linear correlation with $[Eu(OH)_2^+]$ (Figure S4A, Supporting Information). A better fit can be obtained if an additional term proportional to the product $[Eu(OH)_2^+][Eu(OH)_3]$ is included so that the final rate equation takes the form of eq 8, with rate constants given in Table 3. The solid line in Figure 8A is drawn in accordance with eq 8:

$$k_{obs} = k_2[Eu(OH)_2] + k_3[Eu(OH)_2][Eu(OH)_3] \qquad (8)$$

The profile observed for HPNPP, Figure 8B, also resembles those observed with La(III) and Nd(III), but the rate

Table 3. Summary of Rate Constants for Phosphate Ester Hydrolysis by Lanthanide Cations in 80% DMSO at 25 °C

phosphate ester	k_{OH}[a] M^{-1} s^{-1}	rate law	rate constant[b] La(III)	 Nd(III)	 Eu(III)
BNPP	2×10^{-5}	$k_3[Ln(OH)_2^+][Ln(OH)_3]$	$(4.3 \pm 0.1) \times 10^5$	$(6.3 \pm 0.3) \times 10^5$	$(6.5 \pm 0.1) \times 10^4$
		$k_2[Ln(OH)_2^+]$			21.2 ± 0.6
HPNPP	0.34	$k_4[Ln(OH)_3]^3$	$(2.5 \pm 0.6) \times 10^{10}$	$(5.8 \pm 0.5) \times 10^9$	
		$k_3[Ln(OH)_3]^2$			$(1.93 \pm 0.06) \times 10^6$
paraoxon	0.19	$k_2[Ln(OH)^{2+}]$	0.105 ± 0.005		
		$k_2'[Ln(OH)_2^+]$	0.086 ± 0.005		

[a] Rate constant for alkaline hydrolysis. [b] k_2 in M^{-1} s^{-1}, k_3 in M^{-2} s^{-1}, k_4 in M^{-3} s^{-1}.

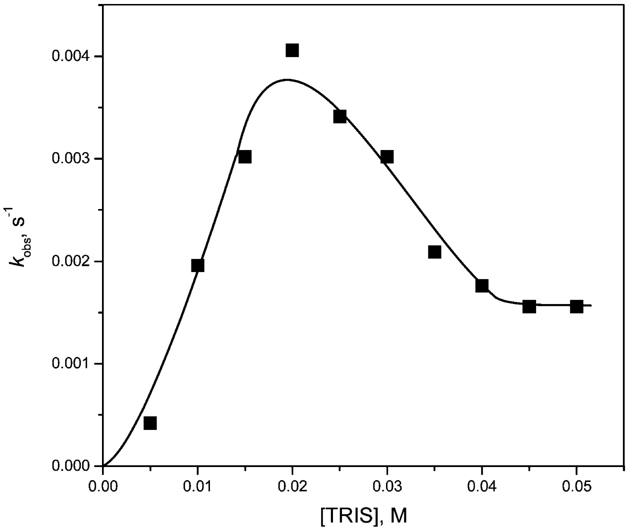


Figure 3. Observed rate constants for BNPP hydrolysis in the presence of 1 mM $Eu(ClO_4)_3$ in 80% DMSO vs concentration of TRIS. The reaction mixture contains 1 mM of $HClO_4$.

increases less sharply. The slope of the plot of log k_{obs} vs log[Eu(III)] obtained at the constant ratio $[Eu(III)]:[OH^-] = 1:2$ (Figure S3, Supporting Information) equals 2.6 ± 0.3 for HPNPP, and k_{obs} values from all data show good linear correlation with $[Eu(OH)_3]^2$ (Figure S4B, Supporting Information). Thus, the rate law for HPNPP hydrolysis by Eu(III) takes the form of eq 9, with k_3 values given in Table 3.

$$k_{obs} = k_3[Eu(OH)_3]^2 \tag{9}$$

With both phosphodiester substrates and all three lanthanides, the rate of hydrolysis observed in presence of the mixture of metal ion and tetramethylammonium hydroxide is much higher than that observed in the presence of tetramethylammonium hydroxide alone at the same total concentration. For example, in the presence of 2 mM tetramethylammonium hydroxide alone, k_{obs} for BNPP hydrolysis is 4×10^{-8} s^{-1} and k_{obs} for HPNPP hydrolysis is 7×10^{-4} s^{-1}, but addition of 1 mM $La(ClO_4)_3$ raises k_{obs} for BNPP to 0.06 s^{-1} and k_{obs} for HPNPP to 1 s^{-1} (see Figure 4A,B), that is, by factors of 10^6 and 10^3, respectively. The "catalytic effect" discussed in the next section in terms of the acceleration factor over the rate of hydrolysis in the presence and in the absence of metal at the same pH value is even higher. In contrast to this, we observed that with a triester paraoxon addition of lanthanides to tetramethylammonium hydroxide caused a significant retardation of the hydrolysis. The inhibitory effect of metal ions increased in the series of lanthanides on going from La(III) to Eu(III). Kinetics of the hydrolysis of paraoxon was studied in some detail only with La(III).

Figure 9 shows the effect of $La(ClO_4)_3$ on the rate of alkaline hydrolysis of paraoxon in the presence of 3 mM tetramethylammonium hydroxide superimposed with the species distribution diagram. Evidently, at the beginning, k_{obs} drops following a decrease in the concentration of free hydroxide ions, but then it starts to increase again following distribution curves for lower hydroxo complexes $La(OH)_2^+$ and $La(OH)^{2+}$. The results were fitted to the rate eq 10a, and the respective rate constants are given in Table 3. Rate constants for both complexes are lower than the rate constant of alkaline hydrolysis.

$$k_{obs} = k_{OH}[OH^-] + k_2[La(OH)^{2+}] + k_2[La(OH)_2] \tag{10a}$$

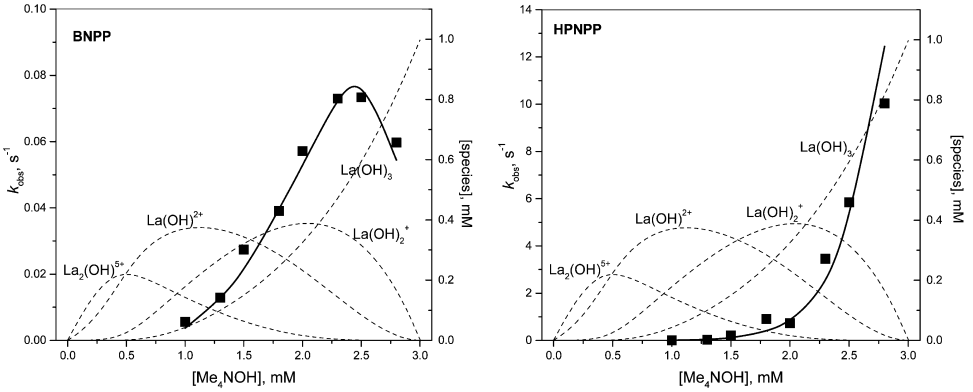
Efficiency and Mechanism of Catalysis. The observed rates of phosphodiester cleavage by lanthanides in 80% DMSO are much higher than those in water. Different rate laws observed in water and in DMSO do not allow us to compare the individual rate constants in both media, but a comparison of k_{obs} at the same, e.g., 1 mM total concentration of metal ion under optimum conditions of pH shows that the reactivity in 80% DMSO (k_{obs} in the range 0.02–0.1 s^{-1} for BNPP and in the range 1–10 s^{-1} for HPNPP) is at least 10^2-fold higher than for most active lanthanide catalysts in water with both substrates.

Second- and third-order in metal ion kinetics of the catalytic hydrolysis of phosphodiesters most probably reflects the fact that actual active species are binuclear or trinuclear hydroxocomplexes reversibly formed at low concentrations from the predominantly existing mononuclear complexes. Binuclear lanthanide complexes stabilized by appropriate organic ligands were identified previously as the catalytically active forms in phosphodiester hydrolysis in water, where the aggregation is more favorable than in DMSO.[18,30,31] Also binuclear lanthanide complexes bridged by methoxide anion are the active forms in transesterefication of phosphodiesters in methanol.[2] The reactivity of hydroxo species in water is bigger for higher hydroxo complexes, with OH:M ratio 2 or larger like, e.g., $M_2L_2(OH)_4$ or $M_2L(OH)_5$, and a similar tendency is observed in 80% DMSO. Another similarity is

---

(30) Medrano, F.; Calderon, A.; Yatsimirsky, A. K. *Chem. Commun.* **2003**, 1968.

(31) Aguilar-Pérez, F.; Gómez-Tagle, P.; Collado-Fregoso, E.; Yatsimirsky, A. K. *Inorg. Chem.* **2006**, *45*, 9502.



A B

Figure 4. Observed first-order rate constants for the hydrolysis of BNPP (A) and HPNPP (B) by La(ClO$_4$)$_3$ at variable concentration of added Me$_4$N(OH) in 80% vol DMSO at 25 °C. Dash curves show the species concentrations given on the right axis. Solid lines are theoretical profiles for k_{obs} calculated in accordance with rate eqs 6 and 7 for BNPP and HPNPP, respectively.

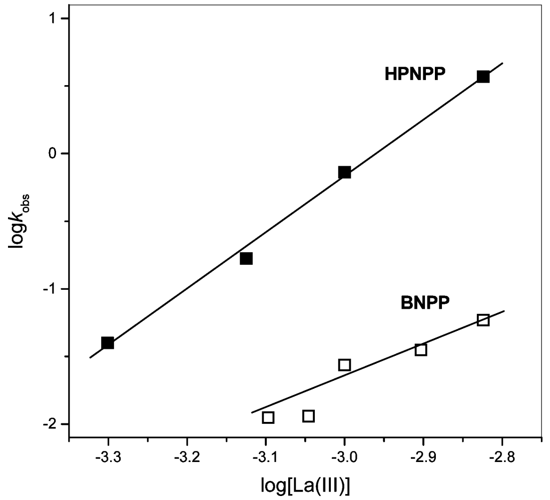


Figure 5. Observed first-order rate constants for the hydrolysis of BNPP (open squares) and HPNPP (solid squares) by La(ClO$_4$)$_3$ at variable concentrations of La(ClO$_4$)$_3$ and Me$_4$NOH taken at the constant ratio [La(III)]:[OH$^-$] = 1:2 in 80% vol DMSO at 25 °C.

low catalytic activity of lanthanides in triester hydrolysis in both media. However, in contrast to the present system, in water, the same species are active toward hydrolysis of both BNPP and HPNPP,[31] while in 80% DMSO, hydrolysis of HPNPP involves complexes with higher OH:M ratio and of higher nuclearity than BNPP hydrolysis.

We shall discuss first the mechanism of BNPP hydrolysis. For all three lanthanides, there is a contribution proportional to the product of concentrations of $\mathrm{Ln(OH)_2^+}$ and $\mathrm{Ln(OH)_3}$ species, most probably reflecting the reaction involving the binuclear complex $\mathrm{Ln_2(OH)_5^+}$ as the active species, which is formed via reaction 10b

$$\mathrm{Ln(OH)_2 + Ln(OH)_3 \rightleftarrows Ln_2(OH)_5} \qquad (10b)$$

with the association constant $K_{assoc} = [\mathrm{Ln_2(OH)_5^+}]/[\mathrm{Ln(OH)_2^+}][\mathrm{Ln(OH)_3}]$. From this point of view, eq 6 may be rearranged as follows.

$$k_{obs} = k_3[\mathrm{Ln(OH)_2 + Ln(OH)_3}] = (k_3/K_{assoc})[\mathrm{Ln_2(OH)_5}] = k_2[\mathrm{Ln_2(OH)_5}] \qquad (11)$$

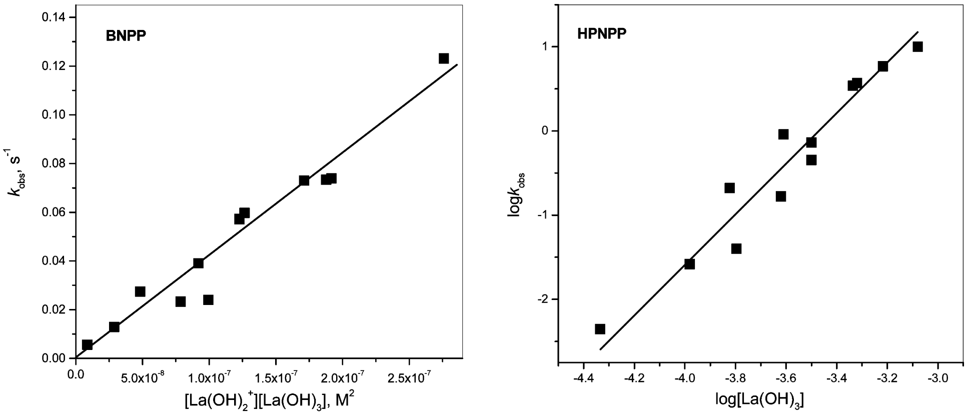
In eq 11, the second-order rate constant $k_2 = (k_3/K_{assoc})$ corresponds to the reaction between BNPP and $\mathrm{Ln_2(OH)_5^+}$. We can estimate only the upper limit of K_{assoc} and consequently only the lower limit of k_2. Because potentiometric titrations do not detect $\mathrm{Ln_2(OH)_5^+}$, its contribution to the total metal concentration should be less than ca. 5%. With total metal concentration about 1 mM and with abundances of $\mathrm{Ln(OH)_2^+}$ and $\mathrm{Ln(OH)_3}$ complexes ca. 30% in basic solutions, this is possible if K_{assoc} is less than ca. 300 M^{-1}. With this estimate and given above k_3 values, the respective k_2 values should be larger or equal to 1.4 × 10^3 M^{-1} s^{-1} for La(III), 2 × 10^3 M^{-1} s^{-1} for Nd(III), and 2.2 × 10^2 M^{-1} s^{-1} for Eu(III). Comparison with second-order rate constants for BNPP cleavage by $\mathrm{Ln_2(OH)_5L}$ (L = 4-imidazolecarboxylate) in water, which equals 1.5 and 0.84 M^{-1}s^{-1} for Nd(III) and Eu(III), respectively (for La(III), the active species has a different composition),[31] shows that the reactivity of $\mathrm{Ln_2(OH)_5^+}$ in 80% DMSO is at least 10^2−10^3 times higher.

A stronger association of the anionic phosphodiester substrate with lanthanide cations in 80% DMSO as compared to water may contribute to this effect. To estimate this contribution, we determined the binding constants of diphenylphosphate with La^{3+} by ^{31}P NMR titration in water and in 80% DMSO. Addition of La(ClO$_4$)$_3$ in both solvents induced shifts of ^{31}P NMR signals to more negative δ values, as it was observed with different phosphodiesters including diphenylphosphate in methanol.[2] Figure 10 shows the change in δ values as a function of La(III) concentration in 80% DMSO and in water (inset).

Fitting of the results in Figure 10 to the theoretical equation for the formation of a 1:1 complex under conditions of fast exchange,[32] which takes in this particular case the form of eq 12, gives the binding constant K = 65 ± 8 M^{-1} in water and 170 ± 40 M^{-1} in 80% DMSO. Thus, binding of the phosphodiester to La(III) in 80% DMSO is stronger than in water, but the effect is less than 1 order of magnitude.

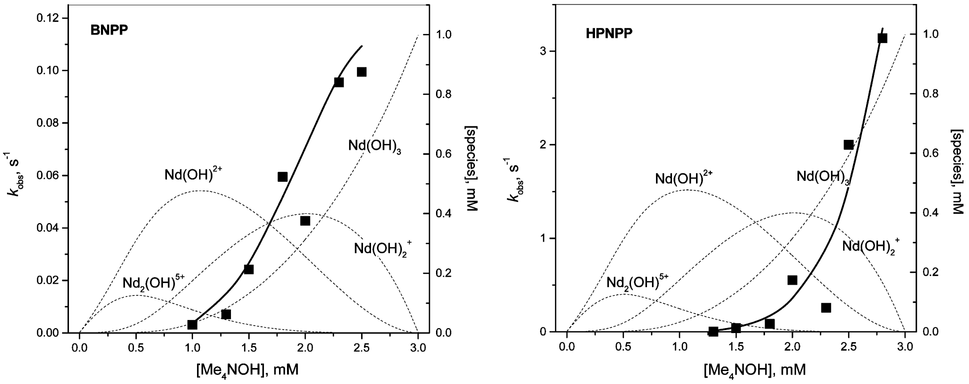
$$\Delta\delta = (\delta_{bound} - \delta_{free})K[\mathrm{La(III)}]/(1 + K[\mathrm{La(III)}]) \qquad (12)$$

It seems that the principle effect of DMSO is due to preferable solvation of lanthanide complexes by the organic cosolvent in aqueous−DMSO mixtures. It has been shown by measurements of the luminescence lifetimes of Eu(III) that in 50% mol DMSO−water (80% DMSO by volume),



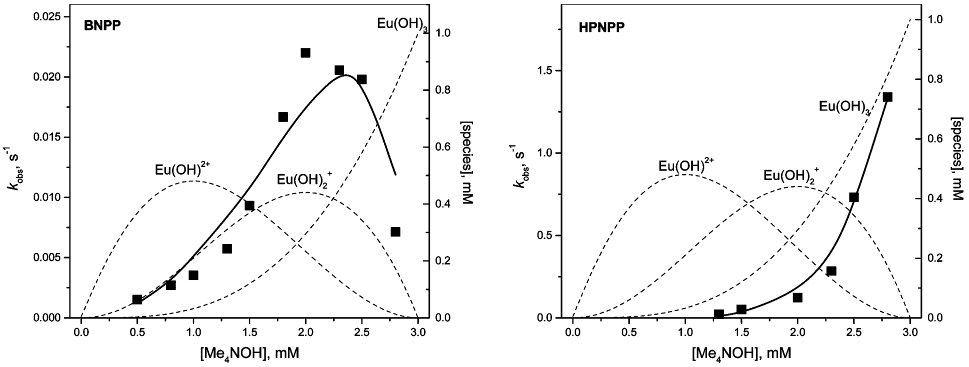
A B

Figure 6. Observed first-order rate constants for the hydrolysis of BNPP (A) and HPNPP (B) by $La(ClO_4)_3$ in 80% vol DMSO at 25 °C as a function of calculated concentrations of $La(OH)_2^+$ and $La(OH)_3$ in accordance with kinetic eqs 6 and 7, respectively. The slope of the line in (A) equals k_2 and the slope of the line in (B) equals 3.



A B

Figure 7. Observed first-order rate constants for the hydrolysis of BNPP (A) and HPNPP (B) by $Nd(ClO_4)_3$ at variable concentration of added $Me_4N(OH)$ in 80% vol DMSO at 25 °C. Dash curves show the species concentrations given on the right axis. Solid lines are theoretical profiles for k_{obs} calculated in accordance with rate eqs 6 and 7 for BNPP and HPNPP, respectively.



A B

Figure 8. Observed first-order rate constants for the hydrolysis of BNPP (A) and HPNPP (B) by $Eu(ClO_4)_3$ at variable concentration of added $Me_4N(OH)$ in 80% vol DMSO at 25 °C. Dash curves show the species concentrations given on the right axis. Solid lines are theoretical profiles for k_{obs} calculated in accordance with rate eqs 8 and 9 for BNPP and HPNPP, respectively.

the coordination sphere of the metal ion contains just one water molecule.[33] Therefore the metal-bound hydroxo anion should be surrounded by only rather voluminous DMSO molecules and should attack the metal-bound substrate in a practically nonaqueous microscopic environment where its reactivity is greatly increased due to dehydration. In other words, the simultaneous coordination of the phosphodiester and hydroxo anions to the metal ion transfers them from the medium of 80% DMSO, where the rate of alkaline hydrolysis is still very close to that in pure water, to the medium of nearly pure DMSO, where the rate of alkaline hydrolysis increases by 2–3 orders of magnitude.[10] The situation is illustrated by structures **1** and **2** for second-order reactions with La(III) and Nd(III) and **3** for the first-order reaction with $Eu(OH)_2^+$, respectively (charges on all noninteracting atoms and groups are omitted).

In line with this interpretation is the fact that addition of DMSO does not improve the efficiency of catalysis by

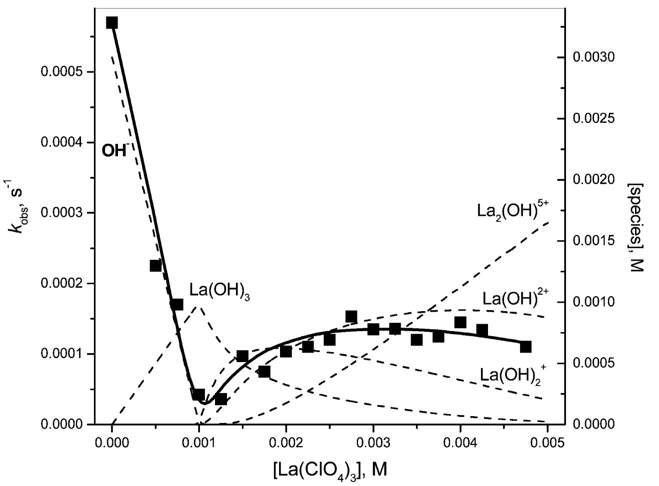


Figure 9. Observed first-order rate constants for the hydrolysis of paraoxon by 3 mM Me$_4$N(OH) at variable concentration of added La(ClO$_4$)$_3$ in 80% vol DMSO at 25 °C. Dashed curves show the species concentrations given on the right axis. The solid line is the theoretical profile for k_{obs} calculated in accordance with the rate eq 10a.

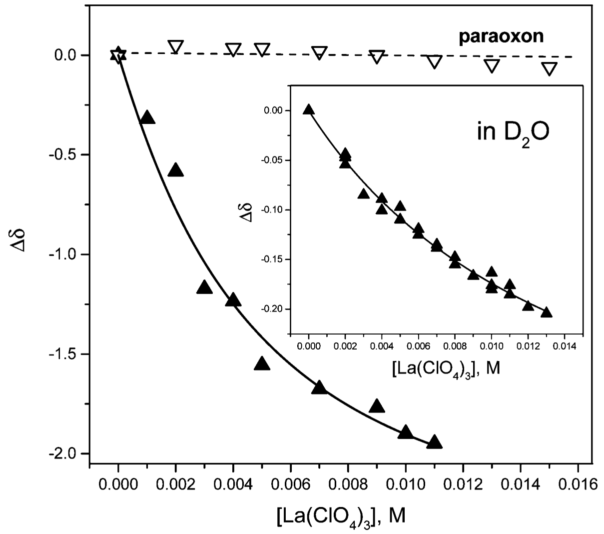
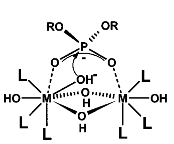
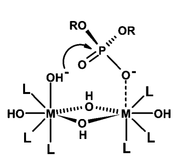


Figure 10. ^{31}P NMR titrations of diphenylphosphate (solid triangles) and paraoxon (open triangles) in 80% d_6-DMSO, 20% D$_2$O, and in pure D$_2$O (inset). Solid lines are the fitting curves to eq 12.

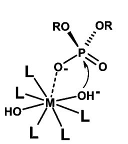


1

or



2

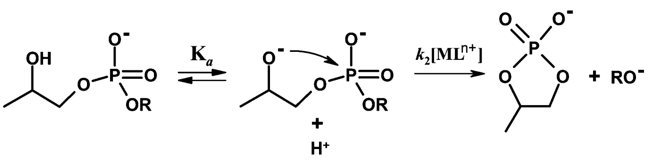


3

L = DMSO

lanthanides in the hydrolysis of a neutral triester paraoxon. In water with a similar substrate, 4-nitrophenyl diphenylphosphate, the catalytic second-order rate constant for a binuclear La(III) hydroxo complex is 2-fold larger than k_{OH},[31] but in 80% DMSO, rate constants k_2 and k_2' for the hydrolysis of paraoxon by hydroxo complexes of La(III) are 2-fold smaller than k_{OH} (see Table 3). Paraoxon is a neutral ligand and in contrast to what happens with anionic phosphodiesters, addition of DMSO does not make it a better ligand. The results of ^{31}P NMR titrations of paraoxon with La(ClO$_4$)$_3$ shown in Figure 9 indicate very weak if any complex formation in 80% DMSO. Therefore, paraoxon should react with DMSO solvated hydroxo complexes either intermolecularly or via unfavorable ligand substitution,[34] which costs a significant energy compensating a possible gain from the discussed above microenvironment effect. Interestingly, in less donor methanol solvent, lanthanides are highly efficient catalysts for methanolysis of both BNPP and paraoxon.[2a,c]

Scheme 1



Kinetics of HPNPP cleavage, which proceeds as an intramolecular transesterification, is more complicated. The reaction mechanism established for aqueous solutions is outlined in Scheme 1.[35] It involves fast reversible deprotonation of the β-hydroxyl and subsequent cyclization catalyzed by a metal complex in its aquo rather than hydroxo form. This leads to the same rate-pH sigmoid profile as in a case of BNPP hydrolysis, where it corresponds to kinetically indistinguishable mechanism with participation of hydroxo complexes as the active species providing metal-bound "internal" hydroxo anion as the nucleophile. However, in 80% DMSO rate vs [Me$_4$N(OH)] profiles, equivalent to rate-pH profiles, are shifted to more basic solutions in the case of HPNPP as a substrate (see Figures 4, 7, and 8). In addition, cleavage of HPNPP proceeds via kinetics of higher order in metal. Thus, in this case, instead of typical ambiguity of kinetically equivalent mechanisms with "external" or "internal" hydroxide we clearly see the participation of different reactive species for two substrates.

An important question is whether or not the mechanism outlined in Scheme 1 can still operate in 80% DMSO. The problem is that, in this medium, pK_a of the HPNPP hydroxyl group should be strongly increased as compared to that in water and the concentration of the deprotonated form may be too low to allow the reaction to proceed with the observed rate. The value of pK_a of HPNPP is unknown, but it can be estimated as follows. One can start from the known statistically corrected pK_a 15.1 of a hydroxyl group of ethylene glycol in water[36] and correct it for the effect of substitution of ROP(=O)(O$^-$)−group for hydrogen atom at the second hydroxyl group. In this way, one obtains pK_a of the hydroxyl group of ROP(=O)(O$^-$)−OCH$_2$CH$_2$OH, which should be very close to pK_a of HPNPP (the nature of group R is rather insignificant because it is separated from OH by 5 bonds

---

(32) Schneider, H.-J.; Yatsimirsky, A. K. *Principles and Methods in Supramolecular Chemistry*; John Wiley & Sons: Chichester, UK, 2000; Chapter D.

(33) (a) Tanaka, F.; Kawasaki, Y.; Yamashita, S. *J. Chem. Soc., Faraday Trans. 1* **1988**, *84*, 1083. (b) Kimura, T.; Nagaishi, R.; Kato, Y.; Yoshida, Z. *J. Alloys Compd.* **2001**, *323–324*, 164.

(34) On basis of known donor numbers (DN), DMSO (DN = 30) should be a better ligand for any metal ion than a phosphate triester (DN = 23) for trimethyl phosphate.

(35) (a) Yang, M.-Y.; Iranzo, O.; Richard, J. P.; Morrow, J. R. *J. Am. Chem. Soc.* **2005**, *127*, 1064. (b) Feng, G.; Mareque-Rivas, J. C.; Torres Martýn de Rosales, R.; Williams, N. H. *J. Am. Chem. Soc.* **2005**, *127*, 13470.

(36) Ballinger, P.; Long, F. A. *J. Am. Chem. Soc.* **1960**, *82*, 795.

and the methyl group has only a small inductive effect). An estimate of the effect of the phosphoryl group can be done by comparison of pK_a values of, e.g., adenosine (12.5)[37] and adenosine 3′-ethyl phosphate (12.51)[38] or a series of compounds of the structure $HOCH_2CH(NH_3)^+-X$ (X = Gly, pK_a 7.33, or Leu, pK_a 7.45) and compounds $PhOP(=O)(O^-)-OCH_2CH(NH_3)^+-X$ (X = Gly, pK_a 6.95, or Leu, pK_a 7.12).[39] Obviously, the effect is small, and on average, one may expect a reduction in pK_a by ca. 0.2 units, which leads to the expected pK_a 14.9 for HPNPP in water. Next, one needs to take into account the effect of DMSO. It has been shown that pK_a values of ROH acids in 80% DMSO correlate linearly with their pK_a in water with the slope 1.4.[16] The complete regression equation through the data given in ref 16 has the form pK_a(80% DMSO) = 1.42 pK_a(water)− 1.37. Therefore, the expected pK_a for HPNPP in 80% DMSO is 19.8.

For the mechanism in Scheme 1, the observed second-order rate constant for the catalytic reaction is given by the eq 13 where k_2 is the second-order rate constant for the reaction between deprotonated HPNPP and the active form of the metal complex catalyst.

$$k_{2obs} = k_2K_a/[H^+] \qquad (13)$$

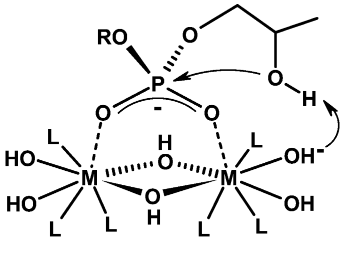
Let us consider the results for Eu(III), which shows the simplest kinetics and the lowest reaction rate. In the presence of 1 mM $Eu(ClO_4)_3$ under conditions of complete transformation of Eu(III) into $Eu(OH)_3$, the observed first-order rate constant equals 1.9 s^{-1} in accordance with the k_3 value given in Table 3. The second kinetic order in respect of $Eu(OH)_3$ most probably reflects the participation of a binuclear complex $Eu_2(OH)_6$ as the active species. Although the association constant for the formation of $Eu_2(OH)_6$ is unknown, one may estimate the lower limit of k_{2obs}, which is sufficient for the purpose of this analysis, by assuming the complete transformation of $Eu(OH)_3$ into the binuclear active complex. Therefore $k_{2obs} = k_{obs}/[Eu_2(OH)_6] \geq 1.9/0.0005 \approx 4 \times 10^3\ M^{-1}\ s^{-1}$ in the solution containing 1 mM Eu(III) and 3 mM $Me_4N(OH)$. In this solution, pH = 10.2, and one obtains by using eq 13 an estimate for the lower limit of k_2 as follows.

$$k_2 = k_{2obs}[H^+]/K_a \geq 4 \times 10^3 \times 10^{-10.2}/10^{-19.8} = 1.6 \times 10^{13}\ M^{-1}\ s^{-1} \qquad (14)$$

Obviously, the second-order rate constant for the catalytic reaction required to explain the observed reaction rate surpasses by several orders of magnitude the diffusion-controlled limit even for the less active catalyst and therefore the mechanism in Scheme 1 cannot be valid in this case. Recently, a similar conclusion was reached for the HPNPP cleavage in methanol in the presence of binuclear Zn(II) and Cu(II) complexes.[40]

An alternative mechanism involves the transition-state proton transfer from the β-hydroxyl group of HPNPP to a metal-bound hydroxide anion acting as a general base. A characteristic feature of such a mechanism is the observation of a significant solvent H/D kinetic isotope effect.[41] Because the isotope effect may also exist in β_{pq} values defined by eq 2, substitution of D_2O for H_2O may also affect the distribution of hydroxo complexes and in this way affect indirectly the reaction rate. To exclude this effect, the rates of HPNPP hydrolysis were measured in D_2O and H_2O at a given fixed metal concentration but at variable metal:hydroxide molar ratios close to 1:3 in order to extrapolate the observed isotope effect to a condition of complete conversion of the metal ion into $M(OH)_3$ form. The experiments were performed with 0.5 mM $Eu(ClO_4)_3$ in the presence of 1.3, 1.4, and 1.5 mM Me_4NOH (molar ratios 1:2.6, 1:2.8, and 1:3) in DMSO containing 20% vol H_2O or D_2O. The observed k_H/k_D ratios were 0.8, 1.6, and 2.9, respectively. We conclude therefore that there is an isotope effect in β_{pq} values that opposes the kinetic isotope effect and that the latter is close to 3. This value of the solvent isotope effect is in the range typically observed for the general base catalysis.[41] It should be noted that the solvent isotope effect in β values most probably is indirect and reflects the isotope effect in water autoprotolysis.

On basis of the above discussion, we propose that the cleavage of HPNPP involves trinuclear (La and Nd) of binuclear (Eu) active complexes, reversibly assembled from $Ln(OH)_3$ species, and proceeds via a mechanism with the general base assistance by the metal-bound hydroxo anion, as shown schematically in structure **4** for the $M_2(OH)_6$ species. It is difficult to propose a possible arrangement of $Ln(OH)_3$ units into a trinuclear complex; however, it is worth noting that the third-order in metal kinetics is not unprecedented and was observed earlier in the BNPP hydrolysis by a macrocyclic La(III) complex in 75% ethanol[42] as well as in the BNPP[43] and ribonucleotide[44] hydrolyses by peroxide complexes of lanthanides in water.



4

---

(37) Izatt, R. M.; Rytting, J. H.; Hansen, L. D.; Christinsen, J. J. *J. Am. Chem. Soc.* **1966**, *88*, 2641.

(38) Acharya, S.; Földesi, A.; Chattopadhyaya, J. *J. Org. Chem.* **2003**, *68*, 1906.

(39) Fölsch, G.; Österberg, R. *J. Biol. Chem.* **1959**, *234*, 2298.

(40) Lu, Z.; Liu, C. T.; Neverov, A. A.; Brown, R. S. *J. Am. Chem. Soc.* **2007**, *129*, 11642.

(41) Jencks W. P. *Catalysis in Chemistry and Enzymology*; Dover: New York, 1987.

(42) Jurek, P. E.; Jurek, A. M.; Martell, A. E. *Inorg. Chem.* **2000**, *39*, 1016.

(43) Mejía-Radillo, Y.; Yatsimirsky, A. K. *Inorg. Chim. Acta* **2003**, *351*, 97.

(44) Kamitani, J.; Sumaoka, J.; Asanuma, H.; Komiyama, M. *J. Chem. Soc. Perkin Trans. 2* **1998**, 523.

The reason why the reaction with HPNPP involves a complex containing a larger number of bound hydroxo ligands than in the case of reaction with BNPP is not clear. Apparently, the coordinated hydroxo anion may act as a stronger base in neutral $M_2(OH)_6$ than in cationic $M_2(OH)_5^+$, but the difference hardly is very significant. It seems more probable that neutral $M_n(OH)_{3n}$ species have a better geometrical fit to the transition state of a general base catalyzed transesterification reaction, while $M_2(OH)_5^+$ species fit better to the transition state for BNPP hydrolysis via nucleophilic attack by a metal-bound hydroxo anion. There is also an uncertainty in the mode of phosphodiester coordination to polynuclear active species. The bridging coordination as in **1** or **4** can provide a stronger binding and electrophilic activation of the substrate, but the observation of the first-order reaction with mononuclear $Eu(OH)_2^+$ shows that nonbridging coordination as in the complex **2** also can be efficient at least for BNPP hydrolysis. Given the above estimate of the lower limit of $k_2 \geq 2.2 \times 10^2$ M^{-1} s^{-1} for $Eu_2(OH)_5^+$ shows that the reaction involving a binuclear complex (**1** or **2**) proceeds at least 1 order of magnitude faster than the reaction involving the mononuclear complex $Eu(OH)_2^+$ (**3**), for which $k_2 = 22.2$ M^{-1} s^{-1} (Table 3). Therefore, a higher efficiency of catalysis by polynuclear species is quite evident but still cannot be clearly interpreted. Currently, we are attempting to get more information on these aspects by theoretical calculations of possible structures of DMSO-solvated binuclear hydroxo complexes.

A measure of the catalytic efficiency often discussed in the literature is the so-called catalytic effect calculated as the ratio of the observed rate constant measured in the presence of catalyst to the rate constant of the background hydrolysis (k_{un}) measured or estimated at the same pH in the absence of catalyst. For such an analysis, we shall take the optimum conditions for BNPP hydrolysis, which correspond to the mixture of lanthanide perchlorate with 2.5 mol equiv of $Me_4N(OH)$. These conditions are also close to optimum for HPNPP cleavage. In solutions containing 1 mM metal and 2.5 mM $Me_4N(OH)$, the values of pH equal 10.7 for La(III), 9.8 for Nd(III), and 8.8 for Eu(III). Assuming that the background hydrolysis proceeds as a reaction with free hydroxo anions, one obtains with (given in Table 3) values of k_{OH} and $pK_W = 18.5$ in 80% DMSO the values of k_{un} for BNPP hydrolysis, which range from 1×10^{-15} s^{-1} for the solution containing Eu(III) to 1.3×10^{-13} s^{-1} for the solution containing La(III), and k_{un} for HPNPP transesterification, which range from 1.7×10^{-11} to 2.2×10^{-9} s^{-1} in the same solutions. Comparison with k_{obs} measured for these substrates at 1 mM metal and 2.5 mM hydroxide (Figures 4, 7, and 8) shows that the k_{obs}/k_{un} values are between 5.4×10^{11} and 2×10^{13} for BNPP and between 2.7×10^9 and 4.4×10^{10} for HPNPP. Note that pH of neutrality in 80% DMSO is 9.2 and therefore estimated catalytic efficiencies refer to essentially neutral solutions. These catalytic efficiencies surpass by far those reported for the best phosphodiesterase catalysts in water with model substrates and are close to those measured in anhydrous methanol.[2,3] Actually, this is the level of efficiency of the enzyme catalysis. Indeed, k_{cat} reported for bimetallic phosphodiesterases with BNPP as a substrate are about 10 s^{-1},[45] which corresponds to the catalytic efficiency of 10^{12} at "saturation".

It is worth noting that in terms of this approach, there is also a significant catalysis by $La(ClO_4)_4$ in the paraoxon hydrolysis in 80% DMSO in spite of the fact that addition of the salt to $Me_4N(OH)$ solutions leads to an inhibitory effect, see Figure 9. Indeed, after addition of 3 mM La(III) to 3 mM $Me_4N(OH)$, the solution pH is 9.8 and estimated k_{un} equals 4.6×10^{-10} s^{-1}, while $k_{obs} = 1.4 \times 10^{-4}$ s^{-1}. This corresponds to a decent catalytic effect of 3×10^5, which could be really observed if the reaction kinetics would be studied in buffered solutions.

In conclusion, using 80% vol. DMSO as a reaction medium allowed us to greatly simplify the speciation of lanthanide hydroxo complexes thanks to elimination of polymeric aggregates of indefinite and irreproducible composition dominating in aqueous solutions. This made possible a kinetic study of phosphoesterolytic activity of simple mononuclear hydroxo complexes without stabilizing ligands. Results for phosphodiester substrates BNPP and HPNPP showed participation of bi- and even trinuclear active complexes reversibly assembled from $Ln(OH)_2^+$ and $Ln(OH)_3$. The efficiency of catalysis is much higher than in water and approaches that reported in anhydrous methanol although the reaction medium is still fairly "aqueous" with 50% mol of water. It is proposed that the increased reactivity is due to the preferable solvation of lanthanide ions by DMSO, which creates an anhydrous microenvironment favorable for reaction in the coordination sphere of the catalyst. A similar effect can contribute to increased reactivity typical for the active sites of natural hydrolytic metalloenzymes.

Acknowledgment. The financial support of DGAPA-UNAM (project IN 204805) is gratefully acknowledged.

Supporting Information Available: Summary of results of all potentiometric titrations, plots of k_{obs} for hydrolysis of BNPP and HPNPP by $Nd(ClO_4)_3$ and $Eu(ClO_4)_3$ at variable concentrations of $Ln(ClO_4)_3$ and $Me_4N(OH)$ taken at the constant ratio [Ln(III)]:$[OH^-]$ = 1:2; plots of k_{obs} for hydrolysis of BNPP and HPNPP by $Nd(ClO_4)_3$ at $Eu(ClO_4)_3$ vs calculated concentrations of hydroxo complexes in accordance with kinetics eqs (6−9). This material is available free of charge via the Internet at http://pubs.acs.org.

IC701846E

---

(45) (a) Vogel, A.; Schilling, O.; Niecke, M.; Bettmer, J.; Meyer-Klaucke, W. *J. Biol. Chem.* **2002**, *277*, 29078. (b) Keppetipola, N.; Shuman, S. *J. Biol. Chem.* **2006**, *281*, 19251.