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Unusually high phosphodiesterolytic activity of La(III) hydroxide complexes stabilized by glycine derivatives†

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Glycine and *N*,*N*-dimethylglycine stabilize La(III) hydroxide complexes of the type $La_2L_2(OH)_4$ which possess phosphodiesterolytic activity close to that observed with most active tetravalent cations like Ce(IV).

Phosphodiester hydrolysis by lanthanide ions and complexes is an area of intensive current research aimed at the development of artificial nucleases.[1] Typically, catalytically active hydroxide or alkoxide Ln(III) complexes stabilized by an organic ligand are generated in solution by deprotonation of coordinated water or alcohol groups at sufficiently high pH values.[1a,2] Although the nature of the stabilizing ligand is of an obvious importance, there is still little understanding of what kind of ligands are optimum and how the ligand structure affects the type and reactivity of active species. Aminocarboxylates constitute an important group of possible ligands and in several instances glycinate containing ligands were successfully used to prepare catalytically active metal complexes.[3] On the other hand, extensive use of simple α-amino acids for the ligand-controlled preparation of Ln(III) hydroxide complexes revealed an ability of these ligands to stabilize different types of polynuclear hydroxide species.[4] These findings prompted us to try to generate a series of hydroxide complexes of La(III) stabilized by simple glycine derivatives of different structures and to explore their possible catalytic activities.

Although complexes of trivalent lanthanides reported at the moment are generally more active than complexes of transition metal ions, they still possess rather low activity in phosphodiester hydrolysis and are used mainly for the cleavage of highly activated bonds in RNA and its synthetic analogues.[1b,c] Activities *ca.* 2 orders of magnitude higher were reported for more electrophilic tetravalent cations *e.g.* Ce(IV),[5] but their use poses serious problems of easy polymerization and precipitation of metal hydroxides in neutral solutions. Therefore, an important question is whether or not the catalytic activity of trivalent lanthanides may be increased significantly by properly chosen ligands. As will be shown below, some ligands of glycinate type do allow reaching activities typical for tetravalent cations.

The ligands employed range from weakly coordinating essentially monodentate N-acetylglycine (**1**) to strongly coordinating tridentate iminodiacetate (**5**), Chart 1. Potentiometric titrations of the ligands **1–5** at concentrations of 5–50 mM alone and in the presence of 2–10 mM $La(ClO_4)_3$ with 0.01 M $NaClO_4$ as a background electrolyte at different metal-to-ligand ratios revealed formation of a series of mixed hydroxide mono- and binuclear complexes in addition to simple mononuclear binary complexes in accordance with the following equilibria (charges omitted):‡

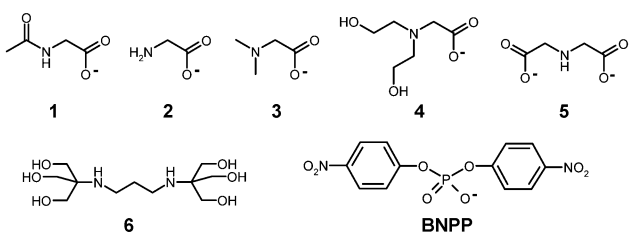

Chart 1

$$La + L \leftrightarrows LaL \quad (1)$$

$$La + 2L \leftrightarrows LaL_2 \quad (2)$$

$$La + L \leftrightarrows LaL(OH) + H^+ \quad (3)$$

$$2La + 2L \leftrightarrows La_2L_2(OH)_4 + 4H^+ \quad (4)$$

$$2La + L \leftrightarrows La_2L(OH)_4 + 4H^+ \quad (5)$$

Fig. 1 illustrates a typical titration experiment for the case of **3**. The titration curve for the ligand–metal mixture (open squares) is shifted to lower pH values respective to the curve for the free ligand (solid squares) indicating the competition of metal ions with protons for the ligand and, consequently, the complex formation. Such a shift is observed even when no hydroxide complexes are formed and therefore at the first step the results were fitted to a model which involved only reactions (1) and (2). The dashed line "*a*" shows the best fit to such a model. Evidently, the steepness of the experimental curve is much smaller indicating that added hydroxide ions are consumed by the system. Inclusion of reaction (3) significantly improves the fitting quality (dash line "*b*"), but still a higher hydroxide complex, reaction (4), is required to obtain a satisfactory fitting shown by the solid line. Discrimination between binuclear and mononuclear complexes $La_2L_2(OH)_4$ and $LaL(OH)_2$ was made on the basis of statistical criteria in favour of the former. The equilibrium (3) is significant only for L = **2** and the equilibrium (5) is observed only for L = **4**, but the formation of binuclear tetrahydroxo complexes $La_2L_2(OH)_4$

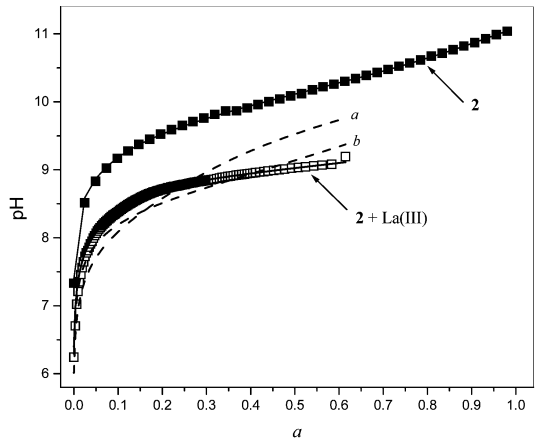

Fig. 1 Titration curves for 10 mM **3** alone (solid squares) and in the presence of 3.0 mM $La(ClO_4)_3$; *a* is the number of equivalents of NaOH per 1 equiv. of **3**. Solid lines are the fitting curves generated by HYPERQUAD 2000 with the equilibrium constants for reactions (1)–(4) given in Table 1. Dash lines "*a*" and "*b*" are the best fitting curves obtained taking into account only reactions (1)–(2) and (1)–(3) respectively.

† Electronic supplementary information (ESI) available: observed rate constants for the BNPP hydrolysis and species distribution diagrams for ligands **1**–**4**. See http://www.rsc.org/suppdata/cc/b3/b304719a/

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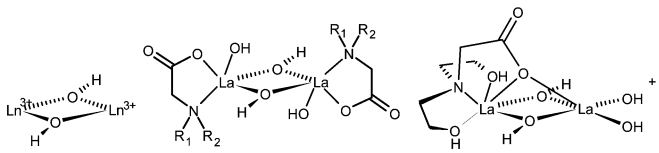
Table 1 Logarithms of the overall formation constants of La(III) complexes for ligands **1–6** and the second-order rate constants (k_2) for the cleavage of BNPP by $La_2L_2(OH)_4$ complexes at 25 °C[a]

	1	**2**	**3**	**4**	**5**	**6**[b]
LaL	2.11 ± 0.06	3.1 ± 0.1	2.77 ± 0.03	4.8 ± 0.1	6.01 ± 0.07	2.30
LaL_2		5.22 ± 0.02	6.08 ± 0.02	8.4 ± 0.1	11.6 ± 0.1	
LaL(OH)		−6.0 ± 0.2				
$La_2L_2(OH)_4$	−27.9 ± 0.6	−27.5 ± 0.7	−26.63 ± 0.01	−23.9 ± 0.2	−19.33 ± 0.05	−26.24
$La_2L(OH)_4$				−28.0 ± 0.6		
$k_2/M^{-1}\ s^{-1}$	1.7	10.9	12.8	0 (0.42)[c]	0	0.195

[a] The constants are mean values from three titrations at different metal and ligand total concentrations. [b] Data from ref. 6. [c] The second-order rate constant for the BNPP cleavage by $La_2L(OH)_4$ complex.



a b c

Chart 2

is observed for all ligands. No evidence was found for formation of tetranuclear hydroxide complexes of the type $La_4(OH)_4L_n$ isolated by precipitation from basic solutions of lanthanides in the presence of amino acids.[4] Stability constants for all complexes are collected in Table 1.

The structure of $La_2L_2(OH)_4$ species may involve both bridged and terminal hydroxides. Probably often observed[4] binuclear fragment "a" (Chart 2) is involved and on this assumption a hypothetic structure "b" may be proposed.

Kinetic studies were performed with a commonly used model substrate bis(4-nitrophenyl) phosphate (BNPP, Chart 1).§ Fig. 2 shows the pH-profiles of the observed first-order rate constants (k_{obs}) of the BNPP hydrolysis in the presence of La(III) and ligands **1** (the inset) and **2** superimposed with the distribution curves for the hydroxide complexes.¶ In the case of **2** two hydroxide complexes co-exist in solution in comparable concentrations: mononuclear $LaL(OH)^+$ and binuclear $La_2L_2(OH)_4$. As one can see from the results shown in Fig. 2, the observed reactivity with this ligand is entirely due to the binuclear species. Similarly, for complexes with ligands **1** and **3** (rate–pH profiles together with complete distribution curves for all ligands are given in the ESI†) one observes a clear correlation of k_{obs} with the fraction of $La_2L_2(OH)_4$ species, which therefore may be considered as a reactive form of the catalyst. Measurements with ligands **2** and **3** were limited by precipitation of La(III) hydroxide at pH ≥ 9.5 even in the presence of a 5-fold excess of the ligand. The maximum attainable formation degree with these ligands is only 20–30%, as is seen from the results in Fig. 2 for the ligand **2**. It is worth noting that even with active complexes present at these low yields one observes unusually high k_{obs} values of *ca.* 0.004 s^{-1} (half-life 3 min) with 1 mM total La(III) at room temperature.

The rate *vs.* metal concentration profiles studied at pH 9.2 also agree with $La_2L_2(OH)_4$ active species. With ligand **1** k_{obs} was a linear function of total metal concentration, but for **2** and **3** the dependences are approximately quadratic, Fig. 3 (solid points). However, when the same values of k_{obs} are plotted *vs.* the concentration of $La_2L_2(OH)_4$ species, calculated in accordance with the stability constants given in Table 1, one observes a good linear dependence indicating first-order kinetics with respect to $La_2L_2(OH)_4$ (Fig. 3, open points). The second-order rate constants (k_2) for BNPP cleavage by

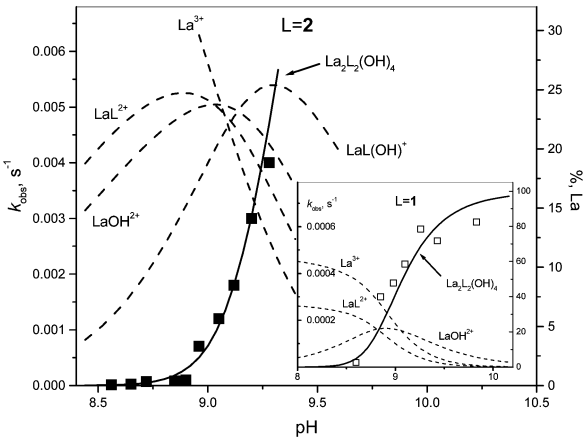


Fig. 2 Observed rate constants for the BNPP hydrolysis at 25 °C *vs.* pH and species distribution diagrams for 1.5 mM La(III) and 5 mM **2** (solid squares) or **1** (inset, open squares).

$La_2L_2(OH)_4$ complexes with L = **1–3** are given in the last row of Table 1. Active species of the composition $Ln_2L_2(OH)_4$ were reported previously with a neutral amino alcohol ligand L = **6**[6] and the respective data are included in Table 1 for comparison.

Due to the low stabilities of simple glycinate complexes with **1–3** we turned our attention to better ligands of a similar type, *i.e.* **4** and **5**. No catalytic activity was observed with dianionic ligand **5**. The situation with **4** was more complicated. The pH-profile for k_{obs} obtained with 1 : 1 La(III) to ligand mixture (Fig. S4, see ESI†) shows a better correlation with the fraction of $La_2L(OH)_4$ complexes than with $La_2L_2(OH)_4$ complexes. The reaction kinetics were also studied under conditions of an excess of metal over the ligand when the $La_2L(OH)_4$ complex should be the predominant species. The results for a 2 : 1 La(III) to ligand mixture with the same total La(III) concentration (Fig. S5, see ESI†) showed *ca.* 5-fold increased k_{obs}, which correlates well with the increased fraction of $La_2L(OH)_4$ complexes. The second-order rate constant for the $La_2L(OH)_4$ complex is given in Table 1.

Let us discuss first the reactivity trend in the series of complexes $La_2L_2(OH)_4$ with glycinate derivatives **1–5**, which follows the order **1** < **2** ≈ **3** >> **4**, **5**. The stability of complexes increases monotonically (4th row in Table 1) within the series. One may expect that more tight ligand binding will decrease the effective positive charge on the metal ion thus decreasing its ability for electrophilic substrate activation. The absence of reactivity with **5** can be attributed to this effect. In the

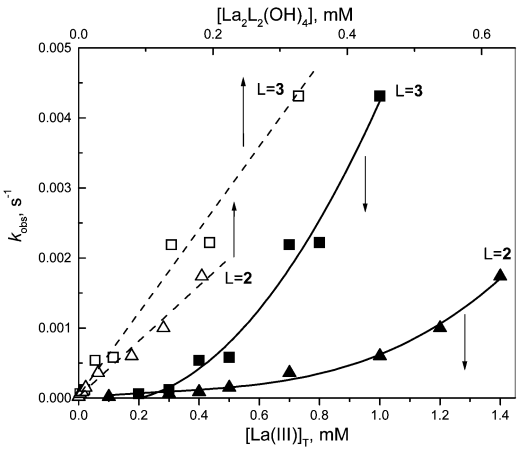


Fig. 3 Observed first-order rate constants for the BNPP hydrolysis at pH 9.2 *vs.* total concentration of La(III) (solid symbols) and concentration of $La_2L_2(OH)_4$ complexes (open symbols, top axes) calculated for the same conditions in the presence of ligands **2** and **3**. The molar ratios La(III):**2** and La(III):**3** are kept constant at 1 : 3.3 and 1 : 5 respectively.

case of **4** the stronger binding results from the presence of two additional neutral hydroxyethyl groups, which should not significantly decrease the positive charge on the metal ion. Ligand **4** is tetradentate, however, and therefore may leave too little space for substrate binding even with the high coordination number 9 typical for La(III). Interestingly, the reactivity is restored on going to the complex with only one ligand **4** bound to two metal cations. It is difficult to explain why such species are observed only with **4** but not with other ligands. One possibility is that the binding of two hydroxyethyl groups to one of the La(III) cations induces a shift of the terminal hydroxide to another cation affording the hypothetical structure shown in Chart 2c. Thus one of the La(III) cations becomes free of the ligand and now can bind the substrate, but the reactivity of this species is still lower than that for symmetrical binuclear complexes with ligands **1–3**.

Dimethylation of the amino group of glycine practically does not affect the intrinsic reactivity of the complex (k_2), but acetylation of the amino group (ligand **1**) leads to a significant decrease in the k_2 value (Table 1). On the other hand, with the neutral ligand **6** possessing a secondary amino group but lacking the carboxylic group the reactivity of the binuclear complex is even smaller (Table 1). It is worth noting that attachment of the carboxylic group as a "cofactor" to an azamacrocyclic ligand was found to increase the reactivity of the respective lanthanide complexes although their reactive forms and the actual role of carboxylate were not identified.[3a]

Since all six ligands stabilize hydroxide complexes of the same composition and probably the same structure, the observed variation in the reactivity does not result from a change in the type of the active species. The highest activity is observed with bidentate monoanionic ligands while complexes with both neutral and dianionic ligands are less active. The latter is easy to explain by increased repulsion with anionic substrate and decreased positive charge on the metal ion (see above), but the higher reactivity of complexes with anionic ligands as compared to neutral requires a more complex explanation, in particular, in light of previous observations of inhibitory effects of anionic ligands.[7] It seems rather improbable that the carboxylate group may participate directly in the transition state stabilization *via e.g.* a general-base mechanism, but it may have an indirect effect on the reactivity of the bound hydroxide nucleophile. Indeed, hydroxide ions in positively charged complexes $La_2L_2(OH)_4^{2+}$ with neutral ligands L should be less basic and therefore less nucleophilic than in neutral complexes with monoanionic ligands. In line with this assumption we found that glycinate complexes with heavier and more acidic lanthanides Nd(III) and Eu(III) were progressively less reactive than with La(III) again indicating the importance of sufficiently high basicity of bound hydroxide.

The most important aspect of the present study is the unusually high phosphodiesterolytic activity of glycinate and *N*,*N*-dimethylglycinate complexes that surpass all currently known catalytic systems based on trivalent lanthanides. Normally addition of typically 1–2 mM of Ln(III) ions or complexes allows one to reach a k_{obs} for BNPP hydrolysis of the order 10^{-4} s^{-1} at temperatures of 37–50 °C.[1a,2,6,7] However, with just 0.2 mM $La_2L_2(OH)_4$ (L = **2** or **3**) we get a k_{obs} above 10^{-3} s^{-1} at 25 °C, see Fig. 3. In fact, the reactivity of these species is similar to that reported for the most efficient Ce(IV),[5] Th(IV)[8] and Zr(IV)[9] catalysts: *e.g.* a reported $k_{obs} = 2.6 \times 10^{-2}$ s^{-1} in the presence of 2 mM Ce(IV) at 37 °C[5] corresponds to a second-order rate constant of 13 M^{-1} s^{-1}, similar to k_2 for L = **3** at 25 °C (Table 1). The factors which contribute to this high reactivity are the binuclear nature of the complexes (in accordance with the expected superiority of binuclear over mononuclear catalytic centers)[10] and a proper balance of the coordination strength and charge of the ligand. It seems from the above results that while trivalent lanthanides certainly are inferior to tetravalent cations in their ability to carry out electrophilic phosphate activation this can be compensated by a higher basicity/nucleophilicity of metal bound hydroxide ions. Further studies aimed at characterization of the binuclear complexes with a series of Ln(III) cations by NMR and electronic spectroscopy are in progress.

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Notes and references

‡ Potentiometric titrations and the electrode calibration were performed as described in ref. 6. Measurements of pH were taken on an Orion Model 710-A research digital pH meter as carbonate-free NaOH solution was added to the system in small increments. Careful removal of carbonate and exclusion of CO_2 absorption during the titration are extremely important for obtaining reproducible titration curves. The program Hyperquad[11] 2000 Version 2.1 NT was used to calculate all equilibrium constants. At the first step the titrations of free ligands were performed and the resulting pK_a values, which agreed well with published data, were used as fixed parameters in the titrations of ligand–La(III) mixtures.

§ The course of BNPP cleavage was monitored spectrophotometrically by the appearance of the 4-nitrophenolate anion at 400 nm. Kinetic measurements used 20 μM substrate and varied concentrations of $La(ClO_4)_3$ and a ligand in the range 0.1–5 mM at 25 °C. The mixture of La(III) and a ligand provided sufficient buffer capacity in the pH range 8–10 so no extra buffer was added. Solution pH was measured after each run and all kinetic runs in which pH variation was larger than 0.1 were excluded. The observed first-order rate constants (k_{obs}) were calculated by the integral method or, for slow reactions, from initial rates.

¶ Species distribution diagrams were calculated by using Species Ver. 0.8 Academic Software 1999 by L. D. Pettit.

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