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The Ligand Effect on the Hydrolytic Reactivity of Zn(II) Complexes toward Phosphate Diesters

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The catalytic effects of the Zn(II) complexes of a series of poliaminic ligands in the hydrolysis of the activated phosphodiesters bis-*p*-nitrophenyl phosphate (BNP) and 2-hydroxypropyl-*p*-nitrophenyl phosphate (HPNP) have been investigated. The reactions show first-order rate dependency on both substrate and metal ion complex and a pH dependence which is diagnostic of the acid dissociation of the reactive species. The mechanism of the metal catalyzed transesterification of HPNP has been assessed by solvent isotopic kinetic effect studies and involves the intramolecular nucleophilic attack of the substrate alcoholic group, activated by metal ion coordination. The intrinsic reactivity of the different complexes is controlled by the nature and structure of the ligand: complexes of tridentate ligands, particularly if characterized by a facial coordination mode, are more reactive than those of tetradentate ligands which can hardly allow binding sites for the substrate. In the case of tridentate ligands that form complexes with a facial coordination mode, a linear Brønsted correlation between the reaction rate (log k) and the pK_a of the active nucleophile is obtained. The β_{nuc} values are 0.75 for the HPNP transesterification and 0.20 for the BNP hydrolysis. These values are indicated as the result of the combination of two opposite Lewis acid effects of the Zn(II) ion: the activation of the substrate and the efficiency of the metal coordinated nucleophile. The latter factor apparently prevails in determining the intrinsic reactivity of the Zn(II) complexes.

Introduction

Phosphodiesters have been chosen by nature as the building blocks of the structural backbone of nucleic acids, with the role to preserve the genetic information. The reason for this choice is their tremendous resistance to hydrolytic cleavage: thus, in water at pH 7 and 25 °C, the estimated half-life of RNA is 110 years and that of DNA is in the range of 10−100 billion years.[1] On the other hand, nature can obtain the hydrolysis of nucleic acids making use of the proper enzymes: natural nucleases can hydrolyze DNA in a few seconds, achieving accelerations up to 10^{17} times. Most nucleases contain in their active site metal ions, typically Zn(II), Ca(II), Mg(II), and Fe(II), which can play fundamental catalytic roles.[2]

The different mechanisms by which a metal ion can accelerate the hydrolysis of phosphate esters have been investigated and partially exploited in the realization of simple artificial nucleases based on metal ion complexes.[3] The interest on such biomimetic models is not only restricted to the elucidation of the enzymatic mechanisms. In fact, several important applications can be envisaged: from the development of artificial restriction enzymes for molecular biology, to the realization of antiviral and antitumor drugs.[3c] Despite the numerous efforts, none of the several models so far described approaches the enormous catalytic efficiency of natural enzymes.

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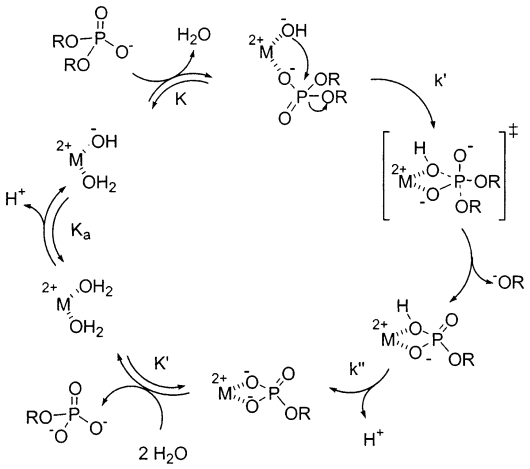
Scheme 1. Mechanism of the Hydrolysis of Activated Phosphate Diesters Catalyzed by Metal Complexes



Clearly, a more accurate comprehension of the mechanisms of the metal catalyzed phosphodiester hydrolysis is an important prerequisite to the realization of really effective artificial metal-based nucleases. Detailed quantitative studies have been performed on substitutional inert Ir(III)[4] and Co(III)[5] complexes and on labile Cu(II)[6] and Zn(II)[7] complexes in the hydrolysis of activated phosphate esters.

The general mechanism, stemming out from these studies and now generally accepted, involves (Scheme 1) the acid dissociation of a metal coordinated water molecule, the coordination of the substrate to the metal ion, and the nucleophilic attack of the metal bound hydroxide on the substrate. However, the relative importance of the different factors at play (nucleophile activation, substrate activation, general base catalysis, template effect) is still largely undefined as is the role of the ligand in modulating the properties of the metal ion. In their studies on Co(III) complexes of a series of macrocyclic tetraamines, Chin and co-workers have reported that the rate of phosphodiester hydrolysis increases with the increase of the N−Co−N angles in the complexes, which leads to the stabilization of the four-membered-ring transition state.[5] Changing to labile metal complexes, Burstyn and co-workers investigated the reactivity of the Cu(II) complexes of a series of macrocyclic triamines of increasing size and found a similar intrinsic reactivity for all the complexes, the only important difference being the different tendency to form unreactive μ-hydroxo dimers.[6b] On the contrary, Fujii and co-workers, studying the Cu(II) complexes of triazacyclonane, triazacyclododecane, and triaminocyclohexane, did not observe any dimerization of the complexes and found a linear relationship between their hydrolytic reactivity and the pK_a of the metal coordinated water molecule.[6e] Such different behaviors can be partly ascribed to the different conditions and to the different features of the metal ions employed, but they clearly indicate the need for further investigations.

Zn(II) is probably the best suited metal ion for the development of artificial metallonucleases. In fact, being a strong Lewis acid and exchanging ligands very rapidly, it is an ideal candidate to play the role of hydrolytic catalysts.[3c] Furthermore, it is characterized by a well-defined coordination chemistry, which allows detailed studies of the reactivity of its complexes, and by the absence of a relevant redox chemistry that rules out competing oxidative cleavage pathways. However, its reactivity is somewhat lower than that of the other commonly employed transition metals, such as Cu(II) or lanthanide ions, and,[3c] for this reason, the realization of efficient Zn(II)-based artificial metallonucleases requires an even more precise knowledge of the factors that influence its catalytic activity. Fujii and co-workers have shown, in the case of the metal catalyzed hydrolysis of phosphotriesters, an interesting inverse correlation between the activity of the complex and the basicity of the metal coordinated water molecule.[7a,b] To the best of our knowledge, no detailed structure−reactivity studies have been performed on the phosphodiester hydrolysis promoted by mononuclear Zn(II) complexes, with the only exception of a comparative investigation of the reactivity of the Zn(II) complexes of 1,4,7-triazacyclododecane and 1,4,7,10-tetraazacyclododecane toward the phosphodiester bis-*p*-nitrophenyl phosphate (BNP) by Kimura and co-workers.[7c]

In this paper, we describe the results obtained in the study of the Zn(II) complexes of the macrocyclic and linear polyamine ligands reported in Figure 1 as catalysts of the hydrolysis of two phosphodiesters, the bis-*p*-nitrophenyl phosphate (BNP) as a DNA model, and the 2-hydroxypropyl-*p*-nitrophenyl phosphate (HPNP) as an RNA model (Figure 1). Particular attention has been devoted the metal catalyzed transesterification of HPNP, extensively employed as an RNA model substrate, whose mechanism, rather surprisingly, has never been investigated in detail. The reactivity data obtained for the different complexes have been related to their thermodynamic parameters as essential means to evaluate the factors which control the Zn(II) catalyzed phosphodiester hydrolysis.

Experimental Section

General Methods and Materials. UV−vis spectra and kinetic traces were recorded on a Perkin-Elmer Lambda 16 spectrophotometer equipped with a thermostated cell holder. NMR spectra were recorded using a Bruker AV300 (300 MHz) spectrometer; the operating frequency for ^{31}P experiments is 121.5 MHz. Potentiometric titrations were performed using a Metrohom 716 DMS Titrino dynamic titrator. $Zn(NO_3)_2$ was an analytical grade product. Metal ion stock solutions were titrated against EDTA following standard procedures. The buffer components were used

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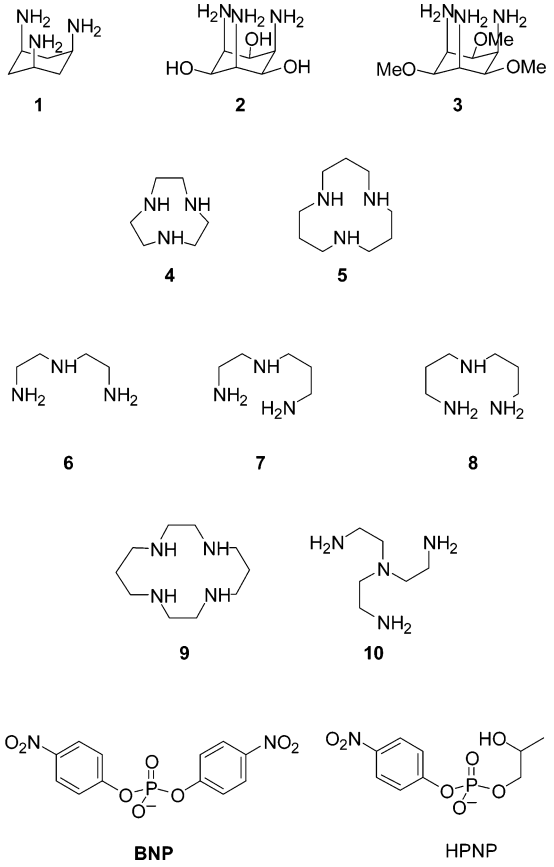

Figure 1. Ligands and substrates.

as supplied by the manufacturers: acetic acid (Aldrich), 2-morpholinoethanesulfonic acid (MES, Fluka), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, Sigma), 4-(2-hydroxyethyl)-1-piperazinepropanesulfonic acid (EPPES, Sigma), 2-[*N*-cyclohexylamino]ethanesulfonic acid (CHES, Sigma), and 3-[cyclohexylamino]1-propanesulfonic acid (CAPS, Sigma). The bis-*p*-nitrophenyl phosphate sodium salt (BNP) was an Aldrich product, used as received, and 2-hydroxypropyl-*p*-nitrophenyl phosphate (HPNP) was prepared and purified as a barium salt following literature methods.[8] Ligands **4**−**10** are commercial products (Aldrich) and were used as supplied. Ligands **1**,[9] **2**,[10] and **3**[11] were synthesized as reported.

Potentiometric Titrations. Protonation constants and Zn^{II} complex formation constants for ligands **1**−**3** were determined by pH potentiometric titrations (25 °C, 0.10 M NaCl). Solutions containing approximately 1×10^{-3} M of the hydrochloride (**1**, **3**) or sulfate (**2**) salts of the ligands and, when necessary, $Zn(NO_3)_2$ were titrated using a 0.1 M sodium hydroxide solution. The electrode system was calibrated by titrating a 0.01 M solution of HCl so that the pK_w value was 13.78. The pH and the volume of added NaOH data were fitted with the computer program BEST[12] to obtain the desired protonation and complex formation constants.

Kinetic Measurements. The kinetic traces were recorded on a Perkin-Elmer Lambda 16 spectrophotometer equipped with a thermostated cell holder. Reaction temperature was maintained at 25 ± 0.1 °C. For the studies of HPNP transesterification, the reactions were started by adding 20 μL of a $(1-2) \times 10^{-3}$ M solution of HPNP to a 2-mL solution of metal complex in the appropriate buffer and monitored by following the absorption of *p*-nitrophenoxide at 400 nm. The initial concentration of substrate was $(1-2) \times 10^{-5}$ M, and the kinetics were in each case first order up to 90% of the reaction. The pseudo-first-order rate constants were obtained by nonlinear regression analysis of the absorbance versus time data, and the fit error on the rate constant was always less than 1%. Kinetic K''_a values were obtained by nonlinear regression analysis of the pseudo-first-order rate constants versus pH data according to the following equation: $k_\psi = kK''_aC_t/(K''_a + [H^+])$, where C_t is the total complex concentration and k the apparent second-order rate constant of the reaction. Cleavage rate versus metal complex concentration profiles were obtained at pH 10.0 (ligands **1**, **4**, **5**, **8**), 10.5 (ligands **2**, **3**, **7**), and 11.0 (ligands **6**, **8**). At these pH values, the rates of the uncatalyzed transesterification of HPNP were found to be, respectively, 9.9×10^{-6}, 2.7×10^{-5}, and 8.7×10^{-5} s^{-1} (see Supporting Information for the whole pH profile in the interval 8.0−11.0).

In the case of the experiments of BNP hydrolysis, the reactions were started by adding 30 μL of a 2.57×10^{-2} M solution of substrate to a 2-mL solution of metal complex in CAPS buffer at pH 11.0 and monitored by following the absorption of *p*-nitrophenoxide. Reactions were followed up to about 1% of substrate hydrolysis (ca. 15 h). The pseudo-first-order rate constants were obtained from the slope of the absorbance versus time data (the fit error was always less than 1%) divided by the absorbance of the *p*-nitrophenoxide and the concentration of substrate. The rate of the uncatalyzed hydrolysis of BNP in these conditions was found to be 7.3×10^{-9} s^{-1}.

The solvent deuterium isotope effect for the transesterification of HPNP in buffer alone (pH 9) and in the presence of **2**·Zn(II) (pH 10.5) was determined by kinetic experiments performed using the same procedure. Simultaneous reactions were performed in water and 99.9% D_2O under identical conditions. For the reactions in D_2O the correction $pD = pH_{measured} + 0.4$[13] was applied to the pH meter readings of the buffers.

Results

Ligands and Zn(II) Complexes. Ligands **4**−**10** are commercially available, and ligands **1**−**3** were synthesized as described.[9−11] The relevant thermodynamic data, particularly the complex formation constants and the deprotonation constants of the metal coordinated water molecule, are available in the literature for most of the Zn(II)−polyamine complexes employed, so that only the pK_a values of the metal coordinated water molecule for complexes of the ligands **2**, **3**, and **4** were measured.

Potentiometric titrations were performed for ligands **2** and **3** as well as for **1 (**for comparison purposes). In the case of ligand **4**, the determination of the pK_a of the coordinated water was not accessible due to the limited solubility of the Zn(II) complex at pH values over 7.[15] The results obtained are reported in Table 1 and, when available, agree very well

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Table 1. Formation Constants (log K_f's) for the Zn(II) Complexes of the Polyaminic Ligand, Acid Dissociation Constants of the Coordinated Water Molecule (pK'_a's) and of the Acidic Hydroxyl (the Reactive Nucleophile) in the HPNP Transesterification (pK''_a), and Apparent Second-Order Rate Constants (k's) for the HPNP Transesterification and BNP Hydrolysis, in Water at 25 °C

ligand	log K_f	pK'_a [a]	pK''_a [b]	k (HPNP) M^{-1} s^{-1}	k (BNP) M^{-1} s^{-1}
1	6.95[c] (6.95)[d]	8.13[c] (7.95)[d]	8.22	0.12	9.7×10^{-5}
2	8.39[c] (8.40)[e]	8.56[c]	8.87	0.23	1.2×10^{-4}
3	10.23[c] (10.80)[f]	8.51[c]	8.61	0.17	1.1×10^{-4}
4	11.62[g]		8.1[h]	0.065	6.8×10^{-5}
5	8.25[d]	7.44[d]	7.39	0.018	
6	8.92[d]	8.93[d]	8.84	0.12	2.3×10^{-5}
7	8.41[d]	8.90[d]	9.18	0.13	6.4×10^{-5}
8	7.94[d]	8.58[d]	8.13	0.020	2.3×10^{-5}
9	14.5[d]	9.87[d]			3.9×10^{-6}
10	12.1[d]	9.8[d]			1.1×10^{-5}

[a] Measured by potentiometric titrations. [b] Evaluated from pH dependent kinetic experiments. [c] This work. [NaCl] = 0.1 M. [d] Reference 7a. [$NaClO_4$] = 0.1 M. [e] Reference 14. [KNO_3] = 0.1 M. [f] Reference 11. [KNO_3] = 0.1 M. [g] Reference 15. [KNO_3] = 0.1 M. [h] Extrapolated at 25 °C from ref 16.

with literature data. As described,[11] the presence of the 2,4,6-hydroxy and methoxy groups in ligands **2** and **3** has a dramatic effect on the complex formation constant, which increases by more than 3 orders of magnitude from **1** to **3**, due to different energy requirements of the conformer with the three binding amino groups in axial position. On the other hand, the effect of the same groups on the acidity of the metal coordinated water molecule is much less important, the increase of the pK_a values being of 0.5 and 0.4 logarithmic units, respectively, on changing from **1** to **2** and **3**. All the selected ligands have a sufficiently high affinity for the Zn(II) ion to ensure the stability of the complexes at the pH values employed in the kinetic experiments.

Transesterification of Hydroxypropyl-*p*-nitrophenyl Phosphate (HPNP). Preliminary experiments were carried out to establish the reaction pathway for the cleavage of hydroxypropyl-*p*-nitrophenyl phosphate (HPNP) promoted by the Zn(II) complexes of the selected ligands under the conditions used for the kinetic study. The products of the reaction, identified by ^1H and ^{31}P NMR spectroscopy, are the cyclic phosphate and the *p*-nitrophenoxide resulting from a transesterification process.

The kinetic study was performed to measure the pseudo-first-order rate constants of the reactions by following the formation of *p*-nitrophenoxide.

The pH dependence of the cleavage rate constants was first assessed. In all cases, except those of the complexes of the ligands **9** and **10**, which showed no accessible reactivity, and of the complex of the ligand **4**, which is scarcely soluble above pH 7, the plots of the pseudo-first-order rate constants versus the solution pH gave a sigmoidal curve, as exemplified in Figure 2 for the complex **2**·Zn(II) (the pH profiles for the other complexes are reported in the Supporting Information). Such behavior is diagnostic of the involvement of an acidic group in the reaction. The kinetic pK_a values (pK''_a) obtained by the fitting of data (see Experimental Section) are reported in Table 1 and are generally close to the pK_a value (pK'_a) of the metal coordinated water molecule. A kinetic pK_a of 8.1 was estimated for complex **4**·Zn(II) on the basis of literature data obtained at higher temperature.[16]

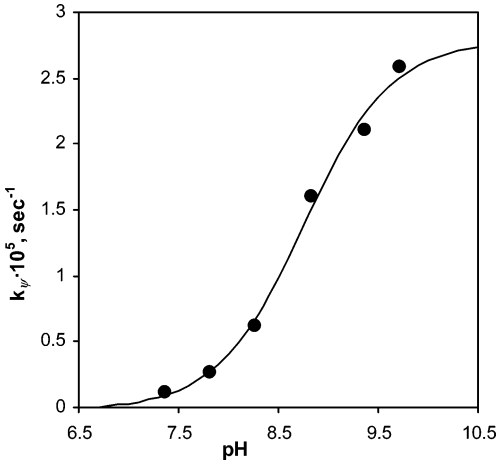

Figure 2. pH dependence of the pseudo-first-order rate constants (k_ψ) for the transesterification of HPNP catalyzed by the complex **2**·Zn(II) at 25 °C.

The effect of the concentration of the Zn(II) complexes on the reaction rate was then investigated at a pH value high enough (see Experimental Section) to ensure complete deprotonation of the metal complexes. In each case, within the concentration range explored (1×10^{-4} to 1×10^{-3} M),[17] the reaction rates exhibit a first-order dependence on the complex concentration. Again, the complexes of ligands **9** and **10** did not react at any detectable extent in reasonable time. The values of the second-order rate constants (k) obtained for the reaction between the deprotonated complex and HPNP are reported in Table 1. The behavior of the different systems can be conveniently analyzed in terms of a Brønsted plot, relating the logarithms of the second-order rate constants and the kinetic pK''_a values obtained for the different complexes (Figure 3). Inspection of Figure 3 shows that an excellent linear correlation is obtained in the case of the Zn(II) complexes of cyclic ligands **1**−**5** whereas the rate constants for the linear ligands fall below the line. On the whole, in each series of complexes the reactivity cleanly increases with increasing basicity of the active nucleophile, but the overall catalytic efficiency of the complexes of linear ligands **6**−**8** is smaller than that of the complexes of cyclic ligands **1**−**5**.

The commonly accepted mechanism for the hydrolysis of RNA dinucleotides or of model substrates, such as HPNP, involves the participation of a metal coordinated hydroxide as a general base catalyst.[18] However, scarce evidence has been reported against the kinetically equivalent mode of

(16) Rossi, P.; Felluga, F.; Tecilla, P.; Formaggio, F.; Crisma, M.; Toniolo, C.; Scrimin, P. *J. Am. Chem. Soc.* **1999**, *121*, 6948−6949. A kinetic pK_a value of 7.8 at 40 °C in the hydrolysis of HPNP promoted by **4**·Zn(II) is reported by the authors; from this value, a pK_a value of 8.1 at 25 °C can be estimated on the basis of the temperature dependence of similar systems (see refs 7b and 19b).

(17) In the case of complex **4**·Zn(II), the concentration range explored was limited to 1×10^{-5} to 1×10^{-4} due to the solubility problem of the system.

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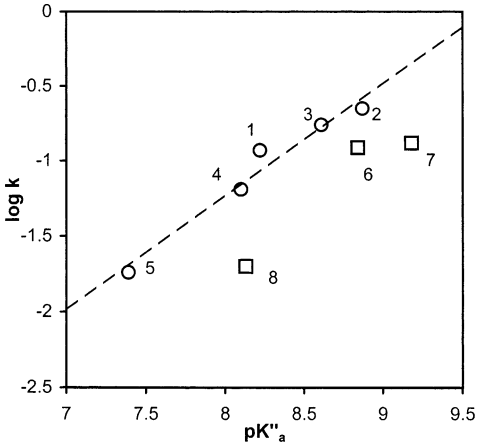


Figure 3. Plot of log k vs pK''_a for the transesterification of HPNP catalyzed by Zn(II) complexes in water at 25 °C. The dashed line shows the linear fit of the reactivity data for the complexes of cyclic ligands **1**−**5**.

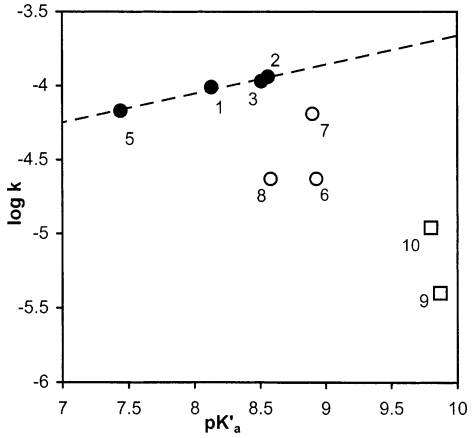


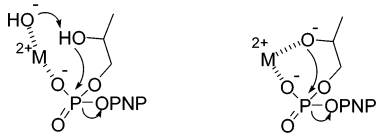
Figure 4. Plot of log k vs pK'_a for the hydrolysis of BNP catalyzed by Zn(II) complexes in water at 25 °C. The dashed line shows the linear fit of the reactivity data for the complexes of cyclic ligands **1**−**3** and **5**.

reaction involving nucleophilic attack by the deprotonated hydroxy group of the substrate, coordinated to the metal ion.[18b] In order to obtain further insight on the reaction mechanism, the solvent deuterium isotopic effect (^Dk) was determined to distinguish between a general base and nucleophilic reaction path. The solvent deuterium isotope effect (^Dk) observed for the transesterification of HPNP in buffer alone at pH 9 is 4.01, while the value obtained for the reaction promoted by the complex **2**·Zn(II) at pH 10.5 is 1.43. The different values are quite significant, as it will be discussed in following paragraphs.

Hydrolysis of Bis-*p*-nitrophenyl Phosphate (BNP). The hydrolysis of bis-*p*-nitrophenyl phosphate (BNP) promoted by the Zn(II) complexes of the selected ligands was investigated in water at 25 °C. The kinetic studies were carried out by following the absorption increase due to the formation of *p*-nitrophenoxide, using the initial rates method. The very slow rate of the reactions did not allow the investigation of the pH effect on the reaction rate, so that only the effect of the metal complex concentration was determined at a pH value of 11.0, so as to ensure complete deprotonation of the complexes. As in the case of HPNP, the rates of the reaction in the presence of each complex show a linear dependence from the complex concentration. Reactivity data for the complex **4**·Zn(II) could not be obtained due to its low solubility.

The values of the second-order rate constant (k) obtained for the reaction between the deprontonated complex and BNP are reported in Table 1 and visualized in the Brønsted plot of Figure 4. In this diagram, the log k values are plotted against the potentiometrically determined pK'_a of the metal bound water molecule, which is assumed to be the active nucleophile in the cleavage of BNP. The plot shows that the reactivity differences between the complexes of the different classes of ligand are rather remarkable. Here again, as observed for the hydrolysis of HPNP (Figure 3), the systems **1**−**5**·Zn(II) show the highest reactivity and follow a linear correlation, although the increase of the reactivity of the complexes increasing their pK_a is much smaller than in the case of HPNP. On the other hand, the complexes of the tetradentate ligands **9** and **10** show a very low activity, despite their high pK_a, while those of the linear tridentate ligands **6**−**8** show an intermediate efficiency without any clear trend.

Scheme 2. Possible Mechanism of the Transesterification of HPNP Catalyzed by Metal Complexes.



Discussion

As previously mentioned, the mechanism of the metal catalyzed hydrolysis of phosphate diesters with good leaving groups has been investigated in great detail (Scheme 1).[4−7] The catalytic cycle involves the following: deprotonation of a metal bound water molecule (K_a), binding of the substrate to the metal complex (K), intracomplex nucleophilic attack of the hydroxide (k') with the simultaneous departure of the leaving group, and, eventually, deprotonation and decomplexation of the substrate to restore the catalyst. This reaction path implies a pH dependence of the reaction rate, as observed (Figure 2), and, at least in principle, a saturation behavior of the metal complex concentration profiles, as a result of the formation of the substrate−catalyst complex before reacting. However, the affinities of phosphate diesters to Zn(II) complexes are very low ($K < 0.5\ M^{-1}$),[7c] and hence, the curvature of the plot is not detectable in the exploitable concentration range. For this reason, only the apparent second-order rate constants (k), comprising the contributions of both K and k', can be determined.

The suggested pathway of the metal catalyzed transesterification of HPNP involves deprotonation of the substrate hydroxy group by a metal bound hydroxide (Scheme 2A), which acts as a general base.[18] However, the available

Engbersen, J. F. J.; Kooijman, H.; Spek. A. L.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1998**, *120*, 6726−6737. (d) Liu, S. Hamilton, A. D. *Tetrahedron Lett.* **1997**, *38*, 1107−1110. (e) Kalesse, M.; Loos, A. *Liebigs Ann. Chem.* **1996**, 935−939.

evidence for this mechanism is quite limited. As a matter of fact, an alternative mechanism which implies (Scheme 2B) the direct coordination of the substrate alcoholic function to the metal ion and the subsequent nucleophilic attack cannot be ruled out.[18b] Also, the finding that the pH versus reaction rate profiles (Figure 2) indicate the participation of an acidic function with a pK_a very close to that of the metal coordinated water molecule (see Table 1) is not conclusive. In fact, several studies have shown that the pK_a values for water molecules and alcoholic hydroxy groups when bound to metal ions are almost identical.[19]

The solvent deuterium isotope effect (^Dk) is a classical method to distinguish a general base from a nucleophilic mechanism.[20] In the case of carboxylic esters, extensive studies have shown that a solvent deuterium isotope effect lower than 1.5 is diagnostic of a nucleophilic mechanism, where no proton is in flight during the rate limiting step, while a ^Dk value greater than 2 probes a general base mechanism.[20] The same criteria apparently apply also in the case of phosphate ester hydrolysis. In fact, in the case of the hydroxide promoted transesterification of HPNP, which can occur only by a general base mechanism, the ^Dk value measured is 4.01. On the other side, the ^Dk value of 1.55 reported for the hydroxide promoted hydrolysis of bis(2,4-dinitrophenyl) phosphate is consistent with a nucleophilic mechanism.[21] Furthermore, solvent deuterium isotopic effects, respectively, of 1.14 and 1.20 have been reported for the hydrolysis of *p*-nitrophenyl phosphate by a Co(II) complex[22] and for the hydrolysis of BNP by a Cu(II) complex,[6d] and in both the cases, a nucleophilic mechanism was established. On these premises, the measured ^Dk value of 1.43 for the **2**·Zn(II) catalyzed transesterification of HPNP suggests that the mechanism involves the nucleophilic attack by the metal coordinate substrate alkoxide as depicted in Scheme 2B.[23]

Despite the bidentate binding mode predicted by the mode of action of Scheme 2B, the affinity of the substrate for the metal complex does not increase to a detectable level so that no saturation behavior is observed (see preceding paragraphs), and again, only apparent second-order rate constants (k), containing both the terms for the complexation and the nucleophilic attack, can be obtained from the complex concentration versus reaction rate experiments.

So, there are substantial similarities in the mechanism of the hydrolysis of the two substrates of choice if one takes into account that a nucleophilic mechanism, involving a metal bound hydroxide (BNP) or alkoxide (HPNP), is involved. In each case, the metal ion plays a triple role: (i) it brings in close proximity the nucleophile and the substrate, acting as a template; (ii) it increases the reactivity of the substrate toward the nucleophilic attack, acting as a Lewis acid; and (iii) again as such, it decreases the pK_a of the metal bound water or hydroxyl, allowing the presence of large amounts of a reactive anionic nucleophile also at pH values close to neutrality. On the other hand, the decrease of the pK_a is expected to bring about a decrease of the nucleophilicity of the metal bound species. On the whole, there are two opposite effects [(ii), (iii) see previous text] of the metal ion to be considered, and it is not easy to predict which prevails: the increase of the substrate reactivity or the decrease of the nucleophile reactivity. In the case of phosphate triester hydrolysis, Fujii and co-workers have demonstrated that the substrate activation is more important than the decrease of the hydroxide nucleophilicity.[7a,b] Analogous indications come from the study by Kimura and co-workers, by comparing the reactivity of Zn(II) complexes of 1,4,7-triazacyclododecane and 1,4,7,10-tetraazacyclododecane in the cleavage of the phosphate diester BNP.[7c]

The Lewis acidity of the Zn(II) ion is modulated by the ligand nature. The present results indicate that the effect of the ligand structure on the reactivity of the Zn(II) complexes follows a similar trend for both the substrate studied.

The first feature which emerges from the analysis of the reactivity of the Zn(II) complexes with HPNP and BNP is the significant difference between the tetradentate and tridentate ligands. The tetradentate ligands reduce the Lewis acidity of the metal ion much more than the tridentate ones, as suggested by the higher pK_a values reported for the metal bound water molecule (see Table 1), but they show a sensibly lower reactivity which cannot be explained simply on the basis of the decreased activation of the substrate toward the nucleophilic attack and the increased nucleophilicity of the metal bound nucleophile. The crucial point, as indicated by Kimura and co-workers, is the occupation of four binding sites of the metal by the ligand which allows little room for the interaction with the substrate.[7c] This is further supported by the different reactivity of the complexes of ligands **9** and **10** toward BNP. The Zn(II) ions in both complexes have almost the same Lewis acidity (the pK_a values for the metal bound water molecule are very close), but **10**·Zn(II) is about 3 times more reactive than **9**·Zn(II). In complex **10**·Zn(II), the ligand has a facial coordination mode, and molecular models show that there is room enough (on the other face of the metal ion) to bind the substrate as a further ligand. Such coordination geometry of the complex including the substrate is much more difficult in the case of ligand **9**, which wraps around the metal ion in a bipyramidal trigonal geometry.

The coordination geometry is suggested to play an important role also among the tridentate ligands to explain the observed difference in reactivity between the linear and the cyclic ligands. In fact, such difference cannot be simply ascribed to the Lewis acidity of the metal ion as can be

(19) (a) Young, M. J.; Wahnon, D.; Hynes, R. C.; Chin, J. *J. Am. Chem. Soc.* **1995**, *117*, 9441−9447. (b) Kimura, E.; Nakamura, I.; Koike, T.; Shionoya, M.; Kodama, Y.; Ikeda, T.; Shiro, M. *J. Am. Chem. Soc.* **1994**, *116*, 4764−4771.

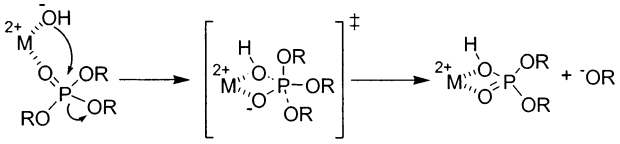
(20) *Advances in Physical Organic Chemistry*; Gold, V., Ed.; Academic Press: New York, 1967.

(21) Kirby, A. J.; Younas, M. J. *J. Chem. Soc. B* **1970**, 510−513. The value was assigned by the authors to a general base mechanism, but it is considerably lower than that usually found for general base reactions, see ref 20.

(22) Jones, D. R.; Lindoy, L. F.; Sargeson, A. M. *J. Am. Chem. Soc.* **1983**, *105*, 7327−7336.

(23) A possible different explanation for the small deuterium kinetic solvent isotope effect has been suggested by one of the reviewers: it involves the mutually opposite effects of a better general base ability of the metal bound OD and a lower acidity of the substrate OD group in a general base catalysis mechanism.

Scheme 3. Mechanism of the Hydrolysis of Activated Phosphate Triesters Catalyzed by Metal Complexes.



argued from the comparison of the pK_a values for the two series of complexes. Taking into account that the estimated energy barrier which the Zn(II) ion opposes to the conformational changes of its complexes is low, the phosphate diester and the hydroxide, both negatively charged, will try to maximize their distance on the complex to minimize the electrostatic repulsion. In the case of the linear ligands, an equatorial coordination mode is possible and may allow some conformational freedom to the complex and hence a larger distance between the two species. This is not possible in the case of the cyclic ligands which can only undergo a facial coordination mode. As a result, the reactivity of the complexes of the cyclic ligand series is favored over that of linear ones.

The complexes of cyclic tridentate ligands **1**−**5** make up a sufficiently large and homogeneous group that shows a definite reactivity trend. Both in the transesterification of HPNP and in the hydrolysis of BNP, very good linear Brønsted plots (Figures 3 and 4) are obtained using the pK_a values of the active nucleophile. The β_{nuc} values are 0.75 in the case of HPNP transesterification and 0.20 in the case of BNP hydrolysis. These values reflect the combination of the two opposite effects due to the Zn(II) ion already discussed: activation of the substrate and loss of efficiency of the nucleophile. The latter appears to be the dominant parameter which controls the reactivity of the Zn(II) complex. This observation is at variance with the kinetic results reported for the hydrolysis of phosphate triesters which led to negative β_{nuc} values: −0.15 in the case of Zn(II) complexes that catalyzed hydrolysis of 2,4-dinitrophenyl diethyl phosphate.[7a] In the hydrolysis of phosphate triesters, activation of the substrate is thus the most important effect at play, and the finding can be explained assuming that the developing negative charge in the transition state benefits from the Lewis acidity of the Zn(II) ion (Scheme 3).[1]

In the case of phosphate diester hydrolysis or transesterification (Scheme 1), the developing negative charge is localized on a substrate oxygen atom which is not coordinated to the metal ion, and by consequence, its stabilization by the positive charge of the metal ion is less effective. The importance of the Lewis acid activation is hence minor compared to that in phosphate triester hydrolysis,[1] and the nucleophilicity of the metal bound hydroxide assumes a major role. This is particularly true in the case of HPNP transesterification characterized by a notably larger β_{nuc} value. Such behavior is probably related to the greater reactivity of the metal bound alkoxides, which are known to be better nucleophiles than metal bound hydroxides probably because of the lesser solvatation of the oxygen atom due to the steric hindrance from the lipophilic alkyl moiety.[19b]

Conclusions

The results reported in this paper highlight the important role played by the ligand in modulating the ability of the complexed Zn(II) ion in promoting the hydrolytic cleavage of phosphate diesters. Not only the number of coordinating atoms on the ligand but also his favored coordination geometry are important parameters, so that the most reactive complexes are those of the triamino ligands with a facial or tripodal coordination mode. Incidentally, this is the same coordination geometry adopted by nature in the reactive site of Zn(II) based hydrolytic enzymes.

Within the family of ligands capable of adopting this coordination mode, the intrinsic reactivity of the system appears to be controlled by the nucleophilicity, and hence the basicity, of the metal coordinated water molecule. The conventional wisdom coming out from the previous studies, which pointed the attention on the enhanced Lewis acidity of the metal center as source of greater reactivity, is therefore to be revised. On the other hand, when the target is the realization of biomimetic systems or catalysts for biological applications, it must be taken into account that the system should operate at pH values as close as possible to the physiological ones. Under these conditions, only a fraction of the metal complex present will be available in a deprotonated form. As a consequence, a greater Lewis acidity of the metal ion and subsequently a lower pK_a value for the metal bound nucleophile will ensure the presence of a greater amount of the reactive species. This is particularly important in the case of BNP hydrolysis, where the differences in the intrinsic reactivity of the different complexes are very small. For instance, at pH 7, the complex **5**·Zn(II) becomes about 6 times more reactive than complex **2**·Zn(II) despite its lower reactivity but thanks to a smaller pK_a value (see Table 1).

Although it will not be an easy task, it would be important to verify if the information obtained from the *p*-nitrophenyl phosphate esters has a general validity also for substrates with poorer leaving groups, which could follow different reaction pathways toward the hydrolytic cleavage or transesterification. Further investigations are needed to clarify this point.

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Supporting Information Available: Listing of pH versus reaction rate profiles for HPNP transesterification. This material is available free of charge via the Internet at http://pubs.acs.org.

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