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Carboxy and Diphosphate Ester Hydrolysis Promoted by Dinuclear Zinc(II) Macrocyclic Complexes. Role of Zn(II)-Bound Hydroxide as the Nucleophilic Function

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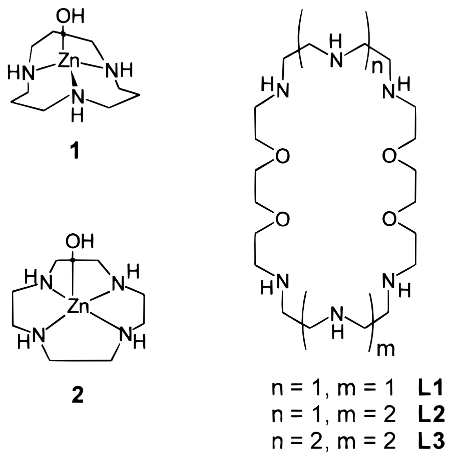
Introduction

Many metalloenzymes with hydrolytic properties use two metals in a bifunctional catalytic mechanism. In particular, dizinc enzymes are responsible for the hydrolysis of the phosphate ester bond in a variety of low molecular weight metabolites and nucleic acids.[1−9] The catalytic role of zinc is ascribed to two main functions: (i) binding and activation of the substrates and (ii) deprotonation of Zn(II)-coordinated water molecules to give Zn−OH functions, which can act as nucleophiles in the hydrolytic mechanism.

A number of mononuclear synthetic Zn(II) complexes has been used as model systems for the above enzymes.[10−20] Among these, the Zn(II) complexes with [12]aneN_3 (**1**)[11] and [12]aneN_4 (**2**)[11] (Chart 1) have been shown to promote activated carboxy and phosphate ester hydrolysis. Dinuclear Zn(II) complexes able to hydrolyze phosphate esters are less common.[21−25] Recently, we found that the dizinc complex with L1 (Chart 1) is able to promote the hydrolysis of activated carboxy and phosphate diesters in aqueous solutions.[24] Therefore, we decided to extend this study to dinucleating ligands containing different polyamine units as metal-binding sites, such as L2 and L3 (Chart 1). L2 and L3 present an overall molecular architecture similar to L1, but contain respectively one and two additional amine groups. The present work analyzes the effects of different coordination environments of Zn(II) on the acidic properties of the Zn(II)-bound water molecules and the role of the generated Zn(II)−OH function as nucleophile in *p*-nitrophenyl acetate (NA) and bis(*p*-nitrophenyl) phosphate (BNP) hydrolysis.

Chart 1



Experimental Section

Synthesis of the Compounds. Ligand L3 was synthesized with a procedure similar to that previously reported for L1 and L2.[26] The experimental details concerning the synthesis of L3 and its dizinc complex are reported in the Supporting Information.

Emf Measurements. All potentiometric measurements (pH = −log [H^+]) were carried out with 0.1 mol dm^{-3} NMe_4NO_3 (pK_w = 13.83 and 13.40 at 298.1 and 308.1 K, respectively) at 298.1 or 308.1 ± 0.1 K, by using equipment[27] and procedures[24] that have been already described. The computer program HYPERQUAD[28] was used to calculate both protonation and stability constants from emf data.

Kinetics of *p*-Nitrophenyl Acetate (NA) and Bis(*p*-nitrophenyl) Phosphate (BNP) Hydrolysis. The hydrolysis rate of NA in the presence of the Zn−L2 and Zn−L3 complexes was measured by an initial-slope method following the increase in the 403 nm absorption of the released *p*-nitrophenate at 298.1 ± 0.1 K as already described.[24] UV spectra were recorded on a Shimadzu UV-2101PC spectrophotometer. The UV absorption decay was followed generally until 2% decay of *p*-nitrophenyl acetate. MOPS (pH 7−7.8), TAPS (pH 7.8−8.9), and CHES (pH 8.9−9.5) buffers were used (50 mM), and the ionic strength was adjusted to 0.1 with NMe_4NO_3. The concentrations of the binuclear Zn(II) complexes were varied in the range 0.1−1 mM. Two species, $[Zn_2L(OH)]^{3+}$ and $[Zn_2L(OH)_2]^{2+}$, promote NA hydrolysis

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Table 1. Logarithms of the Equilibrium Constants for the Complexation Reactions of Zn(II) with L1−L3

	log K					
	L1[a]		L2		L3	
	298.1 K	308.1 K	298.1 K	308.1 K	298.1 K	308.1 K
$2Zn^{2+} + L = Zn_2L^{4+}$ [b]	13.33	13.4	16.52(2)	16.21(3)	19.41(2)	19.12(4)
pK_{a1}[c]	7.08	7.01	7.52(2)	7.53(3)	7.85(2)	7.83(3)
pK_{a2}[d]	8.64	9.1	9.07(2)	9.38(3)	9.36(2)	9.88(4)

[a] From ref 24. [b] The stability constants of the mononuclear Zn(II) complexes are reported in the Supporting Information. [c] K_{a1} is relative to the equilibria $Zn_2L^{4+} + H_2O = Zn_2L(OH)^{3+} + H^+$ (L = L1−L3). [d] K_{a2} is relative to the equilibria $Zn_2L(OH)^{3+} + H_2O = Zn_2L(OH)_2^{2+} + H^+$ (L = L1−L3).

Table 2. Second-Order Rate Constants k'_{NA} ($M^{-1}\ s^{-1}$) for Hydrolysis of *p*-Nitrophenyl Acetate at 298.1 K

nucleophile	k'_{NA}	pK_a	nucleophile	k'_{NA}	pK_a
$[Zn_2L1(OH)]^{3+}$ [a]	0.094	7.08	$[Zn_2L1(OH)_2]^{2+}$ [a]	1.3	8.64
$[Zn_2L2(OH)]^{3+}$	0.16 ± 0.02	7.52	$[Zn_2L2(OH)_2]^{2+}$	2.0 ± 0.2	9.07
$[Zn_2L3(OH)]^{3+}$	0.35 ± 0.04	7.85	$[Zn_2L3(OH)_2]^{2+}$	3.5 ± 0.3	9.36
1[b]	0.041	7.2	**2**[b]	0.11	7.9

[a] From ref 24. [b] From ref 11.

(L = L2 and L3); second-order rate constants (k_{NA}) were calculated for each species with the procedure in ref 24. The hydrolysis rate of BNP to give mono(*p*-nitrophenyl) phosphate (MNP) and *p*-nitrophenate was measured in aqueous solution at 308.1 ± 0.1 K by using methods and procedures similar to those reported for NA hydrolysis. NMR spectra (200 MHz, ^1H; 81.01 MHz, ^{31}P) showed MNP and *p*-nitrophenate as the unique reaction products. Errors on k_{BNP} values were about 5%.

Results and Discussion

Zn(II) Binding by L1−L3 in Aqueous Solution. All ligands show similar binding features with regard to Zn(II), forming both mono- and dinuclear complexes in aqueous solutions. Dinuclear complexes, however, are largely prevalent in aqueous solutions containing the ligands and Zn(II) in 1:2 molar ratios. The stability constants of the binuclear species are reported in Table 1, in comparison with the previously reported constants of L1.[24] The formation of the dinuclear $[Zn_2L]^{4+}$ complex at pH >6 is followed at neutral or slightly alkaline pH by deprotonation of a Zn(II)-coordinated water to give the monohydroxo complexes $[Zn_2L(OH)]^{3+}$ (L = L1−L3), denoting facile deprotonation of the coordinated water with pK_{a1} values (Table 1) of 7.08, 7.52, and 7.85 at 298.1 K for $[Zn_2L1]^{4+}$, $[Zn_2L2]^{4+}$, and $[Zn_2L3]^{4+}$, respectively. These rather low pK_a values are often attributed to a bridging coordination of hydroxide to the two metal centers.[23−26,29] Actually, the crystal structure of $[Zn_2(\mu-OH)L1]^{3+}$ showed that each metal is coordinated by a polyamine moiety and the bridging OH^-.[26] A similar coordination mode for hydroxide can be also proposed for the monohydroxo complexes $[Zn_2L2(OH)]^{3+}$ and $[Zn_2L3(OH)]^{3+}$. For all three ligands, the formation of dihydroxo species is observed at alkaline pH's, with pK_{a2} values at 298.1 K of 8.64, 9.07, and 9.36 for L1−L3, respectively. These pK_a values are higher than those usually found for bridging hydroxide groups, suggesting that the second hydroxide anion binds to a single metal.

These characteristics make these dinuclear zinc complexes promising models for hydrolytic dizinc enzymes, where Zn−OH functions usually act as nucleophiles in the catalytic process. They form hydroxo complexes at neutral or slight alkaline pH, where two Zn(II) ions are kept at close distance by a macrocyclic framework and may act cooperatively in substrate activation, while a Zn−OH function may behave as a nucleophile toward the substrate.

On the other hand, the pK_{a1} and pK_{a2} values of the metal-coordinated water molecules in the L1−L3 dinuclear complexes increase from L1 to L3, and thus, also the nucleophilicities of the generated Zn−OH functions increase in the same order. Therefore, with the aim to test the effective hydrolytic activity of these functions, we have undertaken a study on activated carboxy and phosphate ester hydrolysis promoted by the present dizinc complexes.

Kinetics of *p*-Nitrophenyl Acetate (NA) Hydrolysis. As previously observed for the dizinc complexes of L1,[24] both the dinuclear complexes $[Zn_2L(OH)]^{3+}$ and $[Zn_2L(OH)_2]^{2+}$ (L = L2 and L3) promote NA hydrolysis, and second-order kinetics is followed at different pH values. In all cases, plots of the k_{NA} values as a function of the percentages of $[Zn_2L(OH)]^{3+}$ and $[Zn_2L(OH)_2]^{2+}$ (L = L2 and L3) give rise to straight lines. No effect is observed below pH 6.5, where such species are absent in solutions. Since the $[Zn_2L(OH)]^{3+}$ and $[Zn_2L(OH)_2]^{2+}$ complexes are formed in at most 70% amounts in the pH ranges used in the kinetic measurements, second-order rate constants k'_{NA} have been determined from the maximum k_{NA} values by using the equation

$$v = k_{NA}[\text{total Zn(II) complex}][NA] = k'_{NA}[Zn_2L(OH)^{3+}][NA]$$

The k'_{NA} values for the present complex are listed in Table 2, together with the rate constants found for the mononuclear Zn(II) complexes **1** and **2**.[11]

Table 2 clearly shows that for all three ligands, the monohydroxo $[Zn_2L(OH)]^{3+}$ complexes exhibit remarkably lower rate constants than the corresponding dihydroxo $[Zn_2L(OH)_2]^{2+}$ ones. As already proposed for the L1 complex, the much lower hydrolytic properties of the $[Zn_2L(OH)]^{3+}$ complex can be ascribed to the bridging coordination of hydroxide to two electrophilic metal centers, which reduces the nucleophilicity of the dizinc-bound hydroxide. Furthermore, the crystal structure of the $[Zn_2L1(\mu-OH)](ClO_4)_3$ salt[26] showed that the $[Zn_2(\mu-OH)]$ unit is tightly encapsulated within the macrocyclic framework, making the dizinc hydroxo function only slightly available for the hydrolytic process. Addition of a second hydroxide anion to a Zn(II) ion may lead to partial detachment of the bridging OH^- from one of the metals, giving a more "opened" conformation of the macrocycle, which can leave catalytic sites accessible on the two Zn(II) ions, as sketched in

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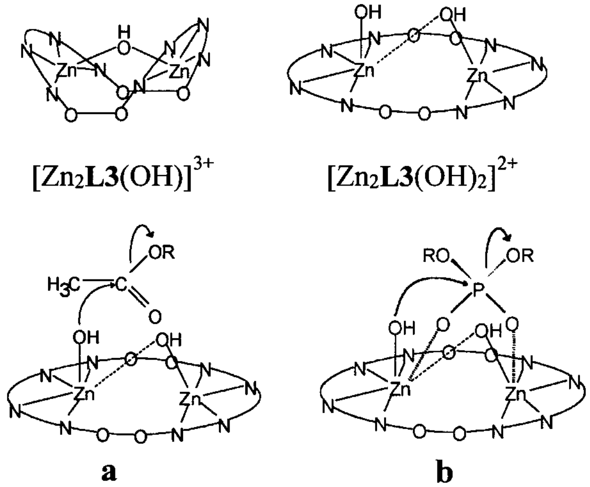
Chart 2



$[Zn_2\mathbf{L3}(OH)]^{3+}$ $[Zn_2\mathbf{L3}(OH)_2]^{2+}$

a **b**

Chart 2 for the L3 complexes. As a matter of fact, the present dizinc complexes give also much higher rate constants in NA hydrolysis than the mononuclear complexes **1** and **2**, indicating an enhanced nucleophilicity of the Zn−OH functions in $[Zn_2L(OH)_2]^{2+}$. Actually, the bridging hydroxide in $[Zn_2L(OH)]^{3+}$ reduces the positive charge on the metal ions. This determines the rather high pK_{a2} values found for deprotonation of a Zn(II)-bound water molecule to give the $[Zn_2L(OH)_2]^{2+}$ complexes and increases the nucleophilic character of the resulting Zn−OH functions.

The most interesting result in Table 2 is the fact that, for both the monohydroxo $[Zn_2L(OH)]^{3+}$ and the dihydroxo $[Zn_2L(OH)_2]^{2+}$ complexes, the k'_{NA} values increase from L1 to L3, i.e., with the pK_a of the coordinated water molecules, as already observed by Kimura and co-workers in the case of the monohydroxo complexes **1** and **2**.[11,12] This indicates that NA hydrolysis occurs via a bimolecular mechanism, which involves the nucleophilic attack of the metal-bound hydroxide on the carbonyl group of the ester and release of *p*-nitrophenate. The two Zn(II) ions do not play any cooperative role in substrate binding, and a simple nucleophilic mechanism is predominant (Chart 2, mechanism a).

Kinetics of Bis(*p*-nitrophenyl) Phosphate (BNP) Hydrolysis. Only the dihydroxo complexes $[Zn_2L(OH)_2]^{2+}$ promote BNP hydrolysis in aqueous solution at 308.1 K, to give mono(*p*-nitrophenyl) phosphate (MNP) and *p*-nitrophenate, while the monohydroxo species $[Zn_2L(OH)]^{3+}$ do not promote this process, in accord with the very low activity in NA hydrolysis found for this species. A weak interaction of MNP and the $[Zn_2L1]^{4+}$ and $[Zn_2L2]^{4+}$ complexes was found by means of potentiometric measurements (log $K = 2.7$ and 2.4 for the equilibrium $[Zn_2L]^{4+} + MNP = [Zn_2L(MNP)]^{2+}$ with L = L1 and L2, respectively), while in the case of $[Zn_2L3]^{4+}$, the interaction was not detected. Binding and successive hydrolysis of MNP were recently achieved by Kimura using a dinuclear Zn(II) complex with a propanol-bridged octaazacryptand.[23] In this case, however, the nucleophilic attack on the substrate was executed by an amine group weakly bound to the metal. In the present case, ^1H and ^{31}P NMR measurements showed that the MNP formed is not further hydrolyzed.

Table 3. Second-Order Rate Constants k'_{BNP} ($M^{-1}\ s^{-1}$) for Hydrolysis of Bis(4-nitrophenyl) Phosphate and pK_{a2} Values at 308.1 K

nucleophile	k'_{BNP}	pK_{a2}
$[Zn_2L1(OH)_2]^{2+}$ [a]	1.15×10^{-4}	9.1
$[Zn_2L2(OH)_2]^{2+}$ [b]	$(9.08 \pm 0.04) \times 10^{-5}$	9.38
$[Zn_2L3(OH)_2]^{2+}$ [c]	$(5.40 \pm 0.03) \times 10^{-5}$	9.88

[a] From ref 24; pH 10.5. [b] This work; pH 10.9. [c] This work; pH 11.5.

Plots of the second-order rate constants k_{BNP} for the Zn/L2 and Zn/L3 complexes as a function of pH fit the distribution curves of the $[Zn_2L2(OH)_2]^{2+}$ and $[Zn_2L3(OH)_2]^{2+}$ species at 308.1 K (Figure S4, Supporting Information), indicating that these dihydroxo complexes are the kinetically active species. Table 3 reports the k'_{BNP} values for the $[Zn_2L(OH)_2]^{2+}$ complexes, in comparison with the corresponding pK_{a2} values. The most interesting finding is the fact that the activity of these complexes in BNP hydrolysis decreases from L1 to L3; i.e., the hydrolytic properties decrease as the pK_a values of the complexes increase, that is the opposite of the behavior found in NA cleavage. Thus, in BNP hydrolysis, the hydrolytic properties of these complexes are determined not only by the nucleophilicity of the Zn−OH functions. This observation leads us to propose an "associative" mechanism, in which the substrate approaches the $[Zn_2L(OH)_2]^{2+}$ complexes and two oxygens of BNP start associating with the two electrophilic metal Zn(II) ions, through a bridging coordination (Chart 2, mechanism b). Simultaneously, a zinc-bound hydroxide nucleophilically attacks the substrate. Among the three dinuclear $[Zn_2L(OH)_2]^{2+}$ complexes, the L1 complex, which contains the less nucleophilic Zn(II)−OH functions, gives the higher rate acceleration. On the other hand, in the L1 complex, both metals are coordinated to three nitrogen donors and present a less coordinately saturated coordination sphere than those in the L2 and L3 complexes. This allows a better accommodation of the phosphate ester and a stronger interaction of this ester with the two Zn(II) ions during the hydrolytic process, leading to an enhanced substrate activation and to stronger hydrolytic properties of the L1 complex with respect to the L2 and L3 complexes.

These results point out that the rate of phosphate ester hydrolysis is mainly determined by substrate activation due to a bridging interaction with the two electrophilic metal centers, while the nucleophilicity of the Zn−OH functions plays a minor role.

Supporting Information Available: Text presenting experimental details for the L3 synthesis and for potentiometric and kinetic measurements, tables of protonation constants for L1−L3 and stability constants for their Zn(II) complexes, distribution diagrams for the systems Zn/L2, Zn/L3, Zn(II)/L1/MNP, and Zn(II)/L2/MNP (Figures S1 and S3), plots of the k_{NA} values as a function of the percentages of hydroxo complexes with L2 and L3 (Figure S2), and plots of the k_{BNP} values as a function of pH (Figure S4). This material is available free of charge via the Internet at http://pubs.acs.org.

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