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Investigation of the identity of the nucleophile initiating the hydrolysis of phosphate esters catalyzed by dinuclear mimics of metallohydrolases☆

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ABSTRACT

di-Zinc(II) complexes of the ligands 2,6-bis((bis(2-methoxyethyl)amino)methyl)-4-methylphenol (HL1), 2,6-bis(bis(hydroxyethyl)aminomethyl)-4-methylphenol (HL2) and 2,6-bis((hydroxyethyl)(methoxyethyl)-aminomethyl)-4-methylphenol (HL3) have been prepared and characterized. The three ligands differ in their donor types, having ether donors (HL1), alkoxido donors (HL2) and both ether and alkoxido donors (HL3). These differences allowed an investigation into the role of the potential nucleophiles in the hydrolysis reaction with the phosphodiester substrate bis(2,4-dinitrophenyl)phosphate (BDNPP). In addition, the di-Mg(II) complex of ligand HL2 was prepared in order to examine the potential for Mg(II) to replace Zn(II) in these biomimetic systems. Kinetically relevant pK_a values for the three di-Zn(II) complexes were determined to be 7.14 and 9.21 for $[Zn_2(L1)(CH_3COO)_2](PF_6)$, 7.90 and 10.21 for $[Zn_2(L2)(CH_3COO)_2](BPh_4)$ and 8.43 and 10.69 for $[Zn_2(L3)(CH_3COO)_2](BPh_4)$. At the respective pH optima the relevant catalytic parameters are $k_{cat} = 5.44(0.11) \times 10^{-5}$ s^{-1} ($K_m = 5.13(0.92)$ mM), $2.60(0.87) \times 10^{-4}$ s^{-1} ($K_m = 5.49(1.51)$ mM) and $1.53(0.27) \times 10^{-4}$ s^{-1} ($K_m = 2.14(0.50)$ mM) for $[Zn_2(L1)(CH_3COO)_2](PF_6)$, $[Zn_2(L2)(CH_3COO)_2](BPh_4)$ or $[Zn_2(L3)(CH_3COO)_2](BPh_4)$, respectively. The di-Mg(II) complex was found to be unreactive in the hydrolysis reaction with BDNPP under the conditions employed. Computational methods using the $[Zn_2(L2)(CH_3COO)_2](BPh_4)$ complex were used to discriminate between different possible mechanistic pathways. The DFT calculations indicate that an alkoxido-mediated pathway in the complexes formed with ligands L2 or L3 is unlikely, because it induces significant distortion of the $Zn_2(L)$ unit; a direct attack by a coordinated hydroxide is preferred in each of the three systems studied here. The calculations also revealed the important role of ligand structural rigidity.

1. Introduction

We and others, have explored extensively the functional roles that model complexes can play in reproducing the electronic, structural and reactivity characteristics of organophosphate-hydrolyzing metalloenzyme systems [1–26]. One of the prominent issues targeted in these studies is the identity of the nucleophilic agent(s) in the models and in the corresponding metalloenzymes [27]. It is generally accepted that in the key chemical step in hydrolytic enzymes a hydroxide acts as the pertinent nucleophile [27]. This hydroxide may be coordinated directly to a metal ion in the active site, or be located in the second coordination sphere, activated through hydrogen bonding interactions with amino acid side chains and/or water molecules lining the active site of the enzyme [27–29]. Exogenous moieties (*e.g.* serine residues) have also been implicated as potential nucleophiles in enzymes such as alkaline phosphatases [27]. Among the various described model systems there are examples of both complexes that use a metal ion-coordinated hydroxide as nucleophile, and those that use an alkoxide moiety for this role [1,30]. In the case of a number of zinc(II) complexes it has been proposed that a coordinated alcohol group is a stronger nucleophile than a coordinated hydroxide [1,30,31]; in addition, it has been suggested that the coordinated alcohol is deprotonated at or below the same pH as a coordinated water molecule [31]. However, it should be pointed out that in metallohydrolases, enzymes and corresponding model complexes, the unambiguous identification of relevant nucleophiles and hence the elucidation of the mechanistic pathway is difficult [25,30].

Here, in order to address the issue of identifying relevant nucleophiles, we describe the synthesis and characterization of the di-zinc(II) complexes of three closely related ligands, 2,6-bis((bis(2-methoxyethyl)amino)methyl)-4-methylphenol (HL1), 2,6-bis(bis(hydroxyethyl)aminomethyl)-4-methylphenol (HL2), and 2,6-bis((hydroxyethyl)(methoxyethyl)-aminomethyl)-4-methylphenol (HL3) (Chart 1) [20,32–40]. These ligands provide ether donor (HL1), or alkoxido donor ligands (HL2), or an asymmetric combination (HL3) with both ether and alkoxido ligands. The catalytic potential of these complexes has been explored using the phosphodiester substrate

☆ In memory of Professor Graeme Hanson and his contributions to bioinorganic chemistry and electron paramagnetic resonance.

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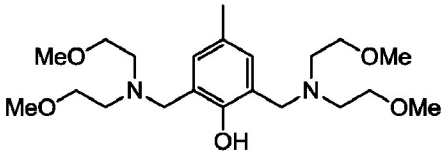

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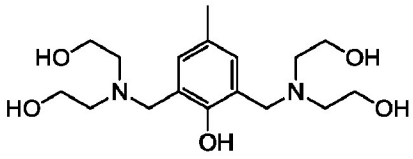

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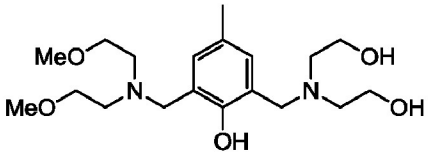

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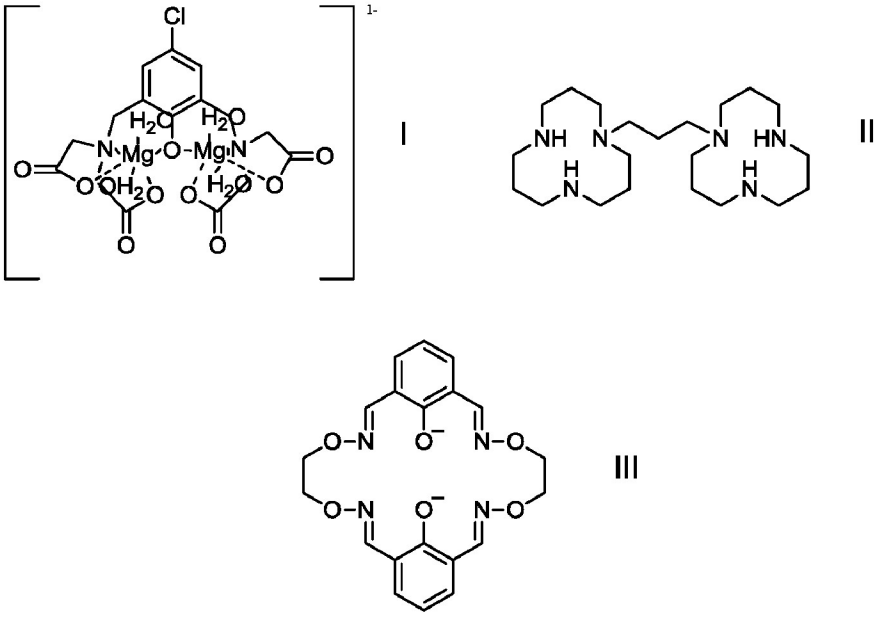

Chart 1. Ligands discussed in this work.

bis(2,4-dinitrophenyl)phosphate (BDNPP). In addition, we have employed computational methods in an attempt to discriminate between different mechanistic pathways possible for these systems. As part of this study the di-Mg(II) complex of ligand HL2 has also been prepared with a view to examining a previous suggestion that Mg(II) is able to replace Zn(II) in these hydrolytic enzyme model systems [41–43].

2. Experimental

2.1. General methods and materials

Elemental microanalyses (C, H, N, S) were performed by the Micro-analytical service at the School of Chemistry and Molecular Biosciences, the University of Queensland. UV–vis spectroscopy was recorded with an Agilent 8453 UV–visible Spectrophotometer, IR spectra were recorded with a Perkin-Elmer Frontier FT-IR/NIR spectrometer SPECTRUM 400 (mode: MIR) with a Smiths DuraSamplIR II ATR diamond window. The software used for data processing was Perkin Elmer Spectrum version 10.03.09. ^{1}H NMR spectra were recorded at room temperature with a 300 MHz Bruker AV 300/400 spectrometer. The abbreviations s = singlet, d = doublet, t = triplet, m = multiplet have been employed. ^{13}C NMR spectra were recorded at room temperature with a 100 MHz Bruker AV 400 spectrometer. Chemical shifts are reported relative to d^{4}-MeOD (δ_C = 49.0(7)). The software used for data processing was TOPSPIN 3.0 from Bruker. For column chromatography silica gel (SiO_2, grain size 0.04–0.06 nm) produced by Scharlan was used. Solvent mixtures of methanol and ethyl acetate mobile phases were used.

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