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Regulation of the Hydrolytic Activity of Mg^{2+}-Dependent Phosphatase Models by Intramolecular NH···O Hydrogen Bonds

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Supporting Information

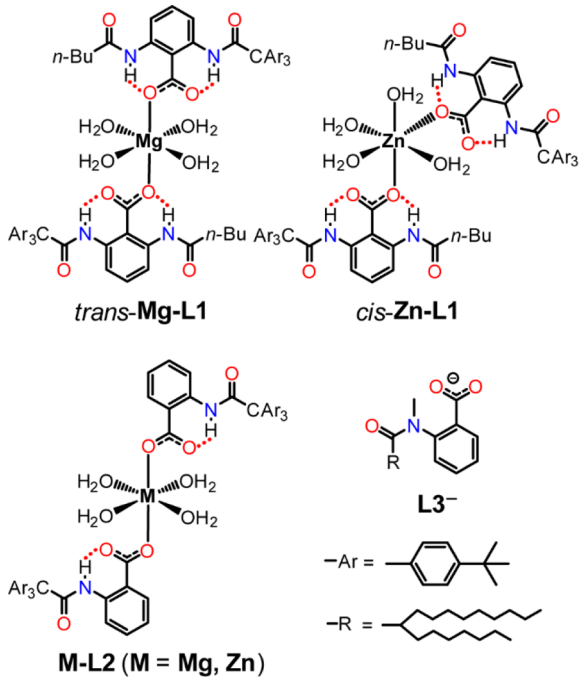
ABSTRACT: Magnesium-dependent phosphatase models containing intramolecular NH···O hydrogen bonds were synthesized and structurally characterized by X-ray analysis. The Mg−O bond distances varied with the mode of the hydrogen bonds. ^{1}H NMR spectra in nonpolar solvents revealed that the acidity of the coordinated water molecule was regulated by the hydrogen bonds. Further, stoichiometric hydrolysis of phosphoric ester significantly depended on the hydrogen bonds. Zinc analogues showed similar but smaller dependencies, which suggest the indispensable role of Mg^{2+} ion in the activation of the enzymes.

Magnesium is present in most living organisms and related to phosphorylated compounds used in the storage, replication, and expression of genetic information.[1,2] Many phosphatase enzymes, are known as Mg^{2+}-dependent phosphatases and require Mg^{2+} or a similar divalent metal ion (normally Mn^{2+}) to be activated. Because Mg^{2+} ions weakly bind to the enzyme ($K_a < 10^5$ M^{-1}), high intracellular concentrations of Mg^{2+} ion *in vivo* are necessary to sufficiently activate the enzyme.[3] Consequently, the labile Mg−O bond limits the direct observation of the active site using spectroscopic techniques (e.g., NMR) in solution.[4] Therefore, reaction mechanisms involving inner-sphere/outer-sphere pathways and the coordination chemistry of the active sites have been proposed from the following: kinetic measurements of enzyme activity (which is dependent on the concentration of Mg^{2+} or experimental conditions), studies of enzymes modified by substitution of inert metal ions such as Cr^{3+}, and theoretical approaches based on the crystal structures of enzymes.[2,3,5−11] Magnesium carboxylates are regarded as the most likely candidates for suitable models, and many have been reported and structurally characterized.[10,12] However, most magnesium carboxylates are hardly soluble in nonpolar solvents, while ligand dissociation was found to occur in polar solvents.[13]

Recently, we synthesized a series of magnesium carboxylates containing intramolecular NH···O hydrogen bonds, and revealed that the hydrogen bonds influence the Mg−O distances depending on the mode by X-ray analysis and theoretical calculations. Moreover, a new switching mechanism that controls the acidity of the coordinated water molecule was proposed.[14] However, the insolubility of these complexes in nonpolar solvents makes it difficult to elucidate their structures in solution, and the hydrolytic reactivity could not be evaluated experimentally.

Herein, we present new readily soluble magnesium carboxylates containing intramolecular NH···O hydrogen bonds in nonpolar solvents in order to inhibit dissociation of the labile Mg−O bond. Such low dielectric media also simulate the buried metal-binding cavity in the native protein matrix.[15] The model complexes are simplified mononuclear complexes to estimate the effect clearly, although the importance of a multinuclear metal site has been argued.[7,9] These complexes are similar to previously reported ones forming secure hydrogen bonds, as illustrated in Chart 1.[14] The sterically encumbering $(4\text{-}t\text{-}BuC_6H_4)_3CCONH$ group[16,17] effectively protected the ionic parts and surprisingly improved the solubility of the ligand and the complexes in nonpolar solvents like toluene. Analogous zinc complexes, which should exhibit stronger Zn−O bonds than Mg−O bonds,[15] were synthesized for comparison. For example, **L1** contains double NH···O hydrogen bonds between the amide NH and both carbonyl oxygen atoms. **L2** forms a single hydrogen bond to an

Chart 1. Model Compounds with and without NH···O Hydrogen Bonds



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uncoordinated carbonyl oxygen atom. **L3** was designed without any hydrogen bond but with a branched long hydrocarbon to achieve better solubility in toluene.

The molecular structures of **M-L1** and **M-L2** (**M** = Mg, Zn) were determined by X-ray analysis (see Supporting Information (SI), Table S1, Figure S1). **Mg-L1**, **Mg-L2**, and **Zn-L2** crystallized in the normal *trans* configuration, which is typically observed for many magnesium dicarboxylates;[12] however, **Zn-L1** crystallized in the *cis* form. Such a *cis* configuration is scarcely known in nonchelating analogous complexes, except for the previously reported *cis*-[Mg[O_2C-2,6-(*n*-Bu-CONH)$_2$$C_6H_3$]$_2$($H_2O$)$_2$($CH_3OH$)$_2$].[14] A fast *trans*/*cis* isomerization should occur in solution as described below. The selected geometrical parameters are listed in Table 1. Intra- and intermolecular hydrogen bonds are shown in Figure S1 with distances. The contributions of NH···O hydrogen bonds to M−O bonds are illustrated in Figure 1 and are essentially similar to the reported ones.[14] The direct hydrogen bond to the coordinated oxygen atom in **M-L1** elongates the M−O (carboxylate) bond and shortens the M−OH_2 bonds. Meanwhile, the hydrogen bond to the uncoordinated oxygen atom in **M-L2** stabilizes the M−O (carboxylate) bonds and weakens the M−OH_2 bonds. The direct hydrogen bond is essential for the elongation of the M−O bond, while the other hydrogen bond in **M-L1** has little effect.[14] It is noteworthy that switching the mode of the hydrogen bond between direct and indirect results in exactly the opposite effect on the M−O bond. These structural perturbations at the Mg−O bonds reflect the distribution of electrons. The long or ionic Mg−O (carboxylate) bond indicates a localized negative charge on the oxygen atom, which should form a stronger NH···O hydrogen bond. The stronger Mg−OH_2 bond means the decrease in electron density on the oxygen atom, which results in an increase in the acidity of the coordinated water.

Table 1. Selected X-ray and ^{1}H NMR Data for M-L*n*

	Mg-L1	Mg-L2	Zn-L1	Zn-L2
	Bond Distances (Å)			
M−O(Ln)	2.053(2)	2.037(3)	2.069(2)	2.040(3)
M−OH_2	2.058(2)	2.113(3)	2.132(2)	2.151(4)
	2.083(2)[a]	2.095(3)[a]	2.101(2)[a]	2.100(5)[a]
	2.070[b]	2.104[b]	2.117[b]	2.126[b]
	Chemical Shifts (ppm)[c]			
NH[d]	11.28	10.72	11.09, 10.76	10.58
H_2O[d]	2.85	2.61	2.86	2.35

[a]Forming intramolecular OH···O=C hydrogen bond. [b]Mean value. [c]In $CDCl_3$. [d]Broad signals.

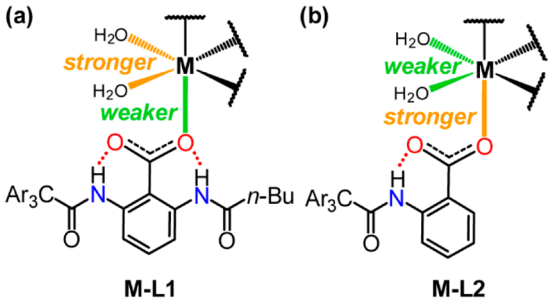
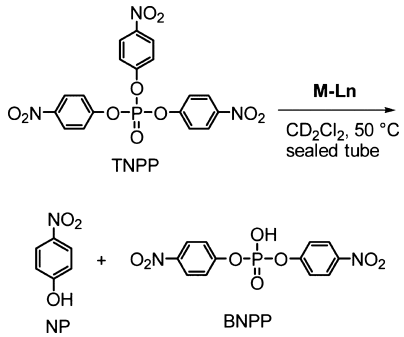


Figure 1. Contribution of the intramolecular NH···O hydrogen bond to M−O bonds.

^{1}H NMR measurements in nonpolar solvents provided insight into the solution structure, which is the same as that in the solid state and contains labile Mg−O bonds although a fast conformational change was observed. ^{1}H NMR spectra of **M-L*n*** (**M** = Mg, Zn; ***n*** = 1,2) in $CDCl_3$ are shown in Figure S2 in the SI. Because **Mg-L1** and **Zn-L1** were crystallized in *trans* and *cis* forms, respectively, the similarity of the spectra between the two complexes suggests the presence of a fast *trans*/*cis* isomerization in the NMR time scale. The chemical shifts of the amide NH and the coordinated water molecule for **M-L*n*** are summarized in Table 1. The data indicate that the strength of the NH···O hydrogen bond and the acidity of the coordinated water molecule fall in the order of **M-L1** > **M-L2**; that is, the strengths of M−O (carboxylate) and M−OH_2 are **M-L1** < **M-L2** and **M-L1** > **M-L2**, respectively. These data in solution agreed with the crystal structures (Figure 1).

The hydrolytic activities of these complexes were evaluated by stoichiometric reactions with tris(4-nitrophenyl) phosphate (TNPP) to afford bis(4-nitrophenyl) phosphate (BNPP) and 4-nitrophenol (NP) in dichloromethane (Scheme 1). The hydrolysis of TNPP (5 mM) by **M-L*n*** (5 mM) to afford NP in dichlorometane-d_2 was monitored by ^{1}H NMR spectral measurements (SI, Figure S3). The yield of NP was plotted against time for each complex (Figure 2). When metal ion was absent, NP was not detected (control). It is apparent that the rate of hydrolysis by **Mg-L2** is 2−3 times faster than that by **Mg-L1**. Interestingly, the plot for **Mg-L3** without NH···O hydrogen bond was found between the two complexes. This pertains to the order of the strength of the Mg−O (carboxylate) bond, not the

Scheme 1. Hydrolysis of TNPP by M-L*n*



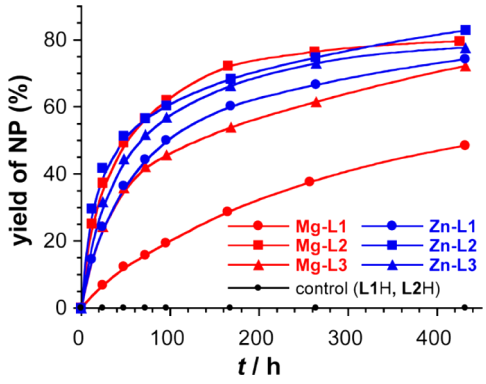


Figure 2. Time course for the hydrolysis of TNPP by **M-L*n*** in CD_2Cl_2 monitored by ^{1}H NMR spectroscopy.

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number of hydrogen bonds. The differences among **Zn-L1**, **Zn-L2**, and **Zn-L3** were significantly smaller than that found in **Mg-L*n***, but the rates were the same order of magnitude. The results suggest that the coordinated water molecule is activated by the metal ion, depending on the strength of the M−O (carboxylate) bond.

A proposed mechanism is shown in Figure 3. The acidity of the coordinated water molecule is increased due to the presence of

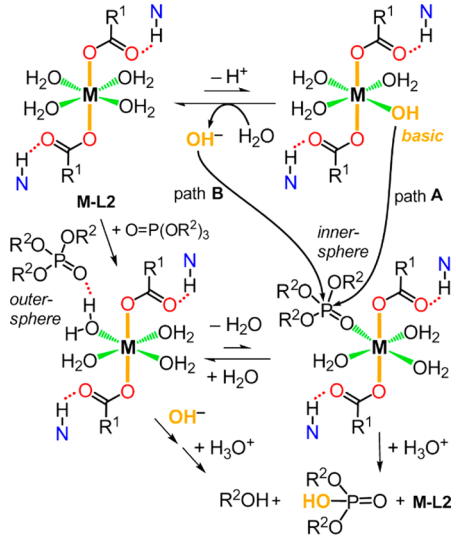

Figure 3. Proposed mechanism for the hydrolysis of TNPP by **M-L2**. R^1 and NH represent aromatic and the adjacent NH groups in **L2**. R^2 denotes 4-nitrophenyl group. The intermediate is an outer-sphere (left) or inner-sphere (right) complex. In this figure, the arrows showing the attack of OH^- (paths A, B) via outer-sphere mechanism are omitted.

the metal ion (Lewis acid). The deprotonated OH^- group directly (path A) or indirectly (path B) attacks the phosphorus atom of the phosphoric ester. The substrate likely interacts with another complex via the coordinated water molecule (outer-sphere) or by the direct P═O−M bond (inner-sphere). An outer-sphere mechanism is sterically favorable in the *trans* configuration, but an inner-sphere mechanism is acceptable in the *cis* configuration. We tried to detect such an intermediate by ^{31}P NMR spectroscopy but the difference is negligibly small between the presence and absence of **M-L2**. Unfortunately, we could not determine which pathway (A or B and outer- or inner-sphere) is most likely to occur experimentally. In both cases, the amount of the intermediate is probably small.

Our results present some insights into the related enzymes, although the present model reaction proceeds slowly. The reaction rates are on the order of **M-L2** > **M-L3** > **M-L1**, which corresponds to the strength of the M−O (carboxylate) bonds. Because the acidity of he coordinated water molecule is considered to follow the opposite trend, the key to understanding the reaction is the basicity of the OH^- group rather than the deprotonation process. If the mode of the hydrogen bond is switched during the reaction, in other words, if facile deprotonation and basic OH^- are compatible, the reaction should be efficiently accelerated. In enzymes with flexible peptide chains, the modes are most likely switchable. The magnesium complexes exhibit significant dependency on the hydrogen bonds; however, the corresponding zinc complexes are less sensitive to the perturbation of the hydrogen bonds. The magnesium ion is widely used as a divalent metal ion to activate enzymes, but the zinc ion can deactivate the enzymes because of an induced change in the coordination geometry or conformational change of the protein.[2,15] The present work indicates that the reactivity of our magnesium complexes are presumably regulated by the hydrogen bonds. This suggests the indispensable role of Mg^{2+} ions in the activation of the enzymes that is coupled with the mode of the hydrogen bond, which must be precisely regulated by the conformational change of protein. It is needless to say that the electrostatically heterogeneous environment in the enzymes is necessary to realize the excellent reactivity.[18,19]

■ ASSOCIATED CONTENT

Supporting Information

X-ray crystallographic data for **Mg-L1**, **Mg-L2**, **Zn-L1**, and **Zn-L2** in CIF format and details (Table S1, Figure S1); ^{1}H NMR spectra of the complexes (Figure S2) and for the hydrolysis (Figure S3); and experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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