Carboxylate- and Phosphodiester-Bridged Dinuclear Magnesium(II) Complexes

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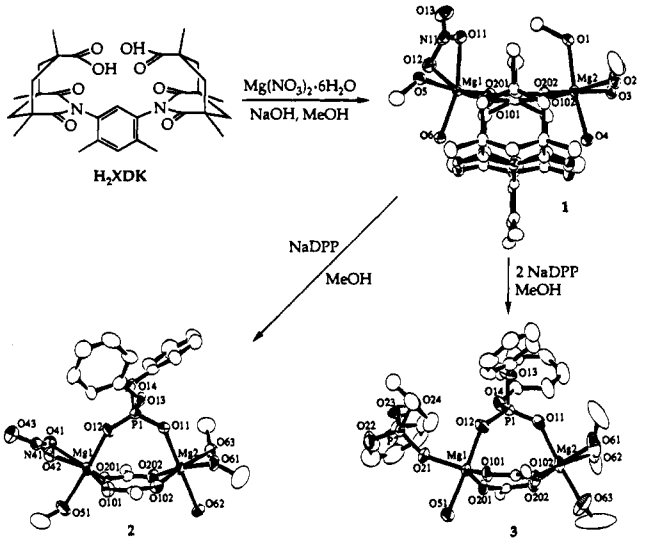
Magnesium is an essential cofactor in biology. A frequently encountered form of magnesium is the carboxylate-bridged dimagnesium(II) unit which occurs in the active sites of phosphate ester processing enzymes, including the proteolytic Klenow fragment of DNA polymerase I from *Escherichia coli*,[1] ribonuclease H of HIV-1 reverse transcriptase,[2] rat DNA polymerase β,[3] inositol monophosphatase,[4] and inositol polyphosphate 1-phosphatase.[5] Moreover, studies of catalytic RNA systems have revealed that divalent metal ions such as Mg^{2+} and Mn^{2+} are required for their function,[6] and a general two-metal-ion mechanism has been proposed for the cleavage of phosphate ester bonds in ribozymes.[7] Despite the ubiquity of Mg^{2+} in nature, its biomimetic chemistry has received relatively little attention, by comparison to that of the transition metals, and is generally less well understood.[8] We have therefore initiated a program to synthesize, characterize structurally, and investigate the fundamental chemistry of carboxylate- and phosphate ester-bridged dimagnesium(II) complexes as potential models for magnesium-activated phosphatase enzymes. Reported here are the first results of this work in which the dinucleating ligand XDK, where H_2XDK is *m*-xylenediamine bis(Kemp's triacid imide),[9] has been used to stabilize the $\{Mg(\mu\text{-}O_2CR)\}_2^{2+}$ moiety. Three carboxylate-bridged dimagnesium(II) complexes are described, two of which also contain phosphate ester bridges. The phosphate ester ligand exchange properties of these latter complexes are quantitatively assessed by ^{31}P NMR spectroscopy and compared to those of a corresponding dizinc(II) complex.

The reaction of 2 equiv of $Mg(NO_3)_2{\cdot}6H_2O$ with Na_2XDK in methanol gave the dimagnesium complex $[Mg_2(XDK)(CH_3OH)_4(H_2O)_2(NO_3)](NO_3)$, **1**$(NO_3)$ (Scheme 1).[10] Its structure was determined in an X-ray crystallographic analysis, which revealed a carboxylate-bridged dinuclear magnesium(II) center. The metal−metal distance is 4.783(2) Å, and the remaining octahedral coordination spheres of the two magnesium ions are filled by labile solvent and nitrate ligands. XDK thus appears to be efficient in assembling a discrete dimagnesium(II) center.

Scheme 1



Reaction of **1** with 1 equiv of NaDPP, where HDPP is diphenyl phosphate, afforded the complex $[Mg_2(XDK)(DPP)(CH_3OH)_3(H_2O)(NO_3)]\cdot 3CH_3OH$ (**2**·$3CH_3OH$).[10] As shown by X-ray diffraction, compound **2** retains the $\{Mg_2(XDK)\}^{2+}$ core but has an additional, bridging diphenyl phosphate ligand, which reduces the Mg···Mg distance to 4.240(5) Å (Scheme 1). When another equivalent of NaDPP was added, the complex $[Mg_2(XDK)(DPP)_2(CH_3OH)_3(H_2O)]\cdot CH_3OH$ (**3**·CH_3OH) crystallized.[10] Its structure contains both bridging and terminal diphenyl phosphate ligands (Scheme 1). One magnesium ion in **3** is octahedrally coordinated, and the other has trigonal bipyramidal stereochemistry. The metal−metal distance of 4.108(3) Å in **3** is comparable to similar distances in the Klenow fragment of *E. coli* DNA polymerase I (3.9 Å),[1] ribonuclease H of HIV-1 reverse transcriptase (4 Å),[2] rat DNA polymerase β (4 Å),[3] inositol monophosphatase (3.8 Å),[4] inositol polyphosphate 1-phosphatase (3.88 Å),[5] and enolase (4.05 Å).[11] The flexibility of the bridging carboxylates in the XDK ligand is manifested by the ≈0.75 Å range of Mg···Mg distances in **1**−**3**, which can readily adjust to changes in the metal coordination environment. This feature may facilitate substrate binding and product release at similar carboxylate-bridged dimagnesium(II) centers in the enzymes.

Conductivity measurements of **3** in methanol solution revealed it to be a 1:1 electrolyte, indicating dissociation of one DPP^- ligand. Consistent with this observation was the $^{31}P\{^1H\}$ NMR spectrum of **3** at room temperature in d_4-methanol, which revealed two phosphorus signals of equal intensities at −15.45 and −9.14 ppm, corresponding to bound and free phosphodiester groups, respectively. This assignment was made by comparison to the $^{31}P\{^1H\}$ NMR spectrum of **2** (−15.46 ppm). The addition of 1 equiv of $(Me_4N)DPP$ to **3** gave a $^{31}P\{^1H\}$ NMR spectrum which integrated for a 2:1 ratio of free-to-bound phosphodiester groups. This result indicates that the bridging phosphate ester ligand is more stable to dissociation than the terminal one.

A variable temperature $^{31}P\{^1H\}$ NMR study of **3** in d_4-methanol over the range −85 °C < T < 60 °C indicated that the bridging phosphodiester ligand can exchange with free diphenyl phosphate at elevated temperatures. A line shape

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analysis of the $^{31}P\{^1H\}$ NMR spectral changes revealed the free energy of activation for phosphodiester exchange to be 60 kJ mol^{-1}. This value may be compared with that obtained by similar means for the structurally related dinuclear zinc complex, $[Zn_2(XDK)(DPP)_2(CH_3OH)_2(H_2O)]\cdot CH_3OH$ (**4**·CH_3OH),[12] 45 kJ mol^{-1}. The phosphodiester exchange rate of the dimagnesium(II) compound (1.9×10^2 s^{-1}, 25 °C) is $\approx 10^2$ times slower than that of the dizinc(II) analogue (7.5×10^4 s^{-1}, 25 °C), which is similar to the difference in H_2O exchange rates of hydrated magnesium and zinc ions (10^5 and 3×10^7 s^{-1}, respectively).[13] The intrinsic difference in phosphodiester exchange rates for **3** and **4** may help to explain the metal ion preferences of phosphate ester hydrolyzing enzymes which employ a carboxylate-bridged dimetallic center.

The Klenow fragment of *E. coli* DNA polymerase I is an example of an enzyme which can function with either 2 Mg^{2+}, 2 Zn^{2+}, or 1 Mg^{2+} and 1 Zn^{2+} in the active site.[14] The X-ray crystal structure of this enzyme complexed with a deoxynucleoside monophosphate product molecule revealed a dimetallic center similar to that in **3**, with a pentacoordinate Zn^{2+} in one site (site A) and an octahedral Mg^{2+} in the other (site B).[1] A two-metal-ion phosphoryl transfer mechanism was proposed, in which the site A metal generates the attacking hydroxyl ion and the site B metal stabilizes the pentavalent phosphorus transition state.[1] Our finding that carboxylate-bridged magnesium ions provide a kinetically more stable binding site for diphenyl phosphate esters than zinc ions is consistent with this model. Nature may thus have optimized a system which takes advantage of the intrinsic capabilities of these two metal ions.

In conclusion, we have synthesized and characterized structurally three novel carboxylate-bridged dimagnesium(II) complexes which may serve as useful bioinorganic models of the active sites in enzymes used to hydrolyze phosphate esters. Since the current X-ray structural resolution of many dimagnesium-dependent metalloenzymes is too low to reveal detailed geometries around the metal centers, the present dimagnesium complexes should be valuable as models for fitting electron density in protein crystal structures. The observed differences in phosphate ester exchange rates for the dimagnesium and dizinc complexes may also be useful information for biochemists investigating phosphatase enzyme mechanisms. Studies of $\{Mg_2(XDK)\}^{2+}$ complexes with biologically more relevant phosphate esters are currently in progress.

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Supplementary Material Available: Experimental details of the preparation, characterization, and crystallographic analysis of **1**–**3**, including tables of atomic positional and anisotropic thermal parameters and ORTEP diagrams (15 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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