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Supplementary Material Available: Tables for $[PPh_3Me]^+_2[Au_6Ni_{12}(CO)_{24}]^{2-}$ listing atomic coordinates, interatomic distances, bond angles, anisotropic displacement coefficients for the non-hydrogen atoms, and fixed positional and isotropic thermal parameters for the hydrogen atoms (13 pages). Ordering information is given on any current masthead page.

Ribonuclease H Activation by Inert Transition-Metal Complexes. Mechanistic Probes for Metallocofactors: Insights on the Metallobiochemistry of Divalent Magnesium Ion

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Our laboratory is studying the metallobiochemistry of the alkali and alkaline-earth metals.[1-4] Divalent magnesium frequently serves as a metal cofactor for enzymes catalyzing phosphoryl transfer reactions and phosphate ester hydrolysis. In this regard, two mechanistic roles are commonly proposed: (1) reduction of the pK_a for bound H_2O to form an activated nucleophile [$Mg(OH)^+$] and (2) a combined template/Lewis acid catalyst (I and II in Figure 1, respectively).[5,6] These inner-sphere coordination modes have been successfully adopted in the design of coordination complexes for metal-catalyzed hydrolysis of RNA;[7,8] however, the generality of these pathways for enzymatic nuclease activity has not yet been established.[9,10] Herein, we report the use of inert transition-metal complexes as probes of reaction mechanism and show that both of the above coordination states are ineffective for RNase H, a low molecular weight, magnesium-dependent restriction nuclease that cleaves the RNA strand of RNA·DNA hybrids.[11,17] This is the first demonstration of nuclease activation by formation of an outer-sphere complex with an inert metal cofactor and is of added relevance in view of a recent crystallographic analysis of the structurally related HIV RNase H domain.[18]

We have used cobalt(III) hexaammine as a probe for outer-sphere chemistry of $Mg^{2+}(aq)$, based on the following criteria: (1) $Co(NH_3)_6^{3+}$ is similar in size to $Mg(H_2O)_6^{2+}$; (2) Co^{3+} is inert, and so exchange of the inner-sphere ligands is slow; and (3) $Co(NH_3)_6^{3+}$ can form an outer-sphere H-bond network similar to that formed by $Mg(H_2O)_6^{2+}$. RNase H was found to be fully active with $Co(NH_3)_6^{3+}$ and $Co(en)_3^{3+}$ as replacements for $Mg(aq)^{2+}$. Kinetic parameters were determined from the digestion of $([^3H]\text{-A}\cdot\text{dT})_{20}$.[12] These are listed in Table I, which compares the reactivity of RNase H with $Mg^{2+}(aq)$, $Mn^{2+}(aq)$, $Ca^{2+}(aq)$, $Co(NH_3)_6^{3+}$, and $Co(en)_3^{3+}$, as active-site cofactors. By agarose gel electrophoresis, these reactions show varying rates of digestion of the RNA strand of poly(rA)·poly(dT) to give residual ss DNA.[14] Control experiments with each complex ion and substrate mixture alone gave no reaction.

Examination of the catalytic parameters in Table I demonstrates that both $Co(NH_3)_6^{3+}$ and $Co(en)_3^{3+}$ show significant reactivity as metal cofactors. It is unlikely that the enzyme can effect a facile pathway for nucleophilic substitution of these complexes by the phosphodiester backbone, thereby activating the diester to hydrolysis,[15] and so $Mg(H_2O)_6^{2+}$ is presumably the active complex in the native reaction.[16] The metal cation may serve several functions: (1) provision of electrostatic relief between the negatively charged polynucleotide chain and the catalytic carboxylates at the active site (III, Figure 1); (2) assistance in binding the substrate by formation of bridging hydrogen bonds; (3) stabilization of the active structure of the enzyme; (4) promotion of nucleophilic or base catalysis by an outer-sphere mechanism. Neither a Lewis acid complex with the phosphodiester backbone nor an activated nucleophile [$Mg(OH)^+$] is a viable intermediate. The latter has been suggested by comparison with the 3′-5′ exonucleolytic activity of DNA polymerase I, which possesses two divalent metal ions and four carboxylates at the active site.[9,18] We conclude that hydrolysis of the RNA backbone by RNase H (*Escherichia coli*) does not result from attack by metal-bound nucleophiles to form an inner-sphere metal-stabilized pentacovalent phosphorus intermediate.[11] Direct attack by solvent H_2O (or carboxylate with subsequent hydrolysis) appears most likely. Reaction rates also depend on the ionic radius of the metal cofactor [$V_{max}(Mg^{2+}) > V_{max}(Mn^{2+}) > V_{max}(Ca^{2+})$ and $V_{max}[Co(NH_3)_6^{3+}] > V_{max}[Co(en)_3^{3+}]$, Table I; where $r(Mg^{2+}) = 0.86$ Å, $r(Mn^{2+}) = 0.97$ Å, $r(Ca^{2+}) = 1.14$ Å].

Recent crystallographic analyses have demonstrated a close structural relationship between *E. coli* RNase H and the HIV RNase H domain.[11,17,18] These are also structurally related to DNase I, a Ca^{2+}-dependent enzyme.[10,17] In contrast to RNase

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(15) Ethylenediammine (en) is a chelating ligand, and so the probability of attack by the poor phosphodiester nucleophile is greatly reduced.
(16) It is possible that one of the carboxylates at the catalytic site may bind directly to Mg^{2+}. The Asp10 to Mg^{2+} distance is 2.0 Å,[11,17] although this may be induced by crystal packing forces. However, $Co(NH_3)_6^{3+}$ activates RNase H, and so *coordination by the enzyme has no bearing on the catalytic role of the metal ion.*
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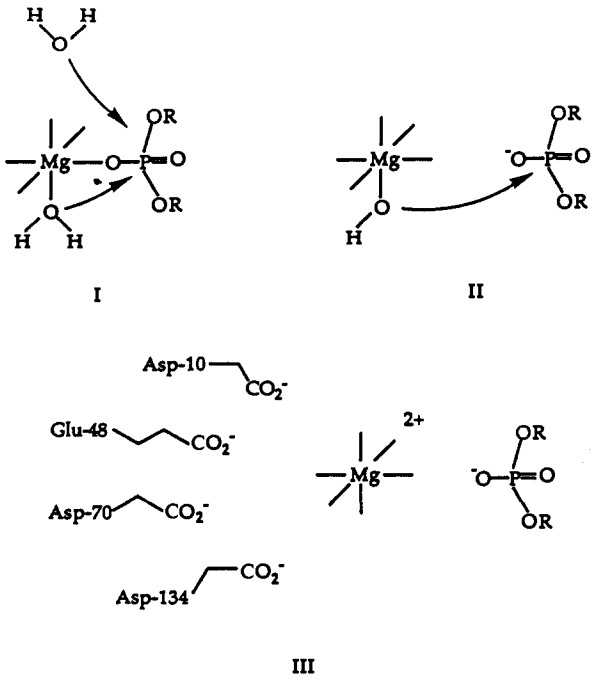

Figure 1. Schematic representation of three possible mechanistic roles for $Mg^{2+}(aq)$ as a RNase H cofactor: (I) Lewis acid/template activation of the phosphodiester backbone toward nucleophilic attack; (II) activation of nucleophilic water [$Mg(OH)^+$]; (III) outer-sphere-complex formation [Asp10 (2.0 Å), Glu48 (4.2 Å), Asp70 (4.5 Å), Asp134 (4.9 Å); distances from active-site carboxylates to Mg^{2+} are noted in parentheses[17]]. Possible roles for the bound cation are described in the text.

Table I. Kinetic Parameters for RNase H Digestion of $([^3H]\text{-A}\cdot\text{dT})_{20}$ with a Variety of Metallocofactors[a,b]

M^{n+}	V_{max}, $\mu M\ s^{-1}$	K_m, μM
$Co(NH_3)_6^{3+}$	0.31 ± 0.02	1.0 ± 0.2
$Co(en)_3^{3+}$	0.19 ± 0.01	0.57 ± 0.08
$Mg(aq)^{2+}$	0.33 ± 0.01	1.0 ± 0.1
$Mn(aq)^{2+}$	0.13 ± 0.01	0.36 ± 0.08
$Ca(aq)^{2+}$	0.047 ± 0.009	0.2 ± 0.1

[a] Each result is the average of at least three experiments. [b] The reaction mixture (20 μL) contained the following: 10 mM M^{n+}, 40 mM Tris (pH 7.5), 100 mM KCl, 5% (w/v) sucrose, 0.1 mM DTT, $([^3H]\text{-A}\cdot\text{dT}_{20}$ (2×10^4 cpm), RNase H (1 unit). After incubation (37 °C, 30 min), the reaction mixture was stored on ice and quenched with 7% $HClO_4$ (20 μL, 0 °C, 30 min). The mixture was then centrifuged (14 000 rpm, 30 min) and the supernatant (10 μL) added to scintillation fluid (10 mL). Substrate concentration varied from 94 nM to 4.4 μM.

H, $Co(NH_3)_6^{3+}$ inhibits the reaction of DNase I. Presumably $Co(NH_3)_6^{3+}$ binds at the active site but cannot coordinate directly to the phosphate backbone to activate the diester to attack by a Glu-His-OH relay, as previously proposed for this enzyme.[10]

In this paper we have described a simple test to identify the active coordination state of the metal cofactor for metallonuclease enzymes. This will be of value in the design of inhibitor complexes for HIV RNase H activity. Comparisons of enzyme activity when using metal cofactors with well-defined coordination states offers considerable insight on the mechanistic role of the metal cation.[19]

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Supplementary Material Available: A figure displaying the Michaelis-Menten data used to generate Table I (1 page). Ordering information is given on any current masthead page.

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Selective γ C-H Bond Cleavage in Alkoxides: 2-Propanol on Mo(110)

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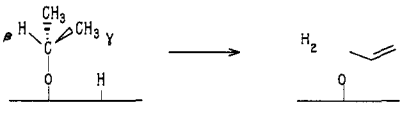
The kinetic stability of alkoxides adsorbed on Cu(110) and Ag(110) has been correlated with the strength of the C-H bond adjacent to the oxygen atom. The presence of the electronegative oxygen atom renders the C-H bond for the carbon bound to oxygen 4-7 kcal/mol weaker than the other C-H bonds in the complex. The radical formed from homolytic C-H bond cleavage is stabilized by the electronegative oxygen; hence, the bond dissociation energy is lowered.[1] The weaker C-H bond for the position β to the metal center in alkoxides bound to metals results in a propensity for β-hydrogen elimination. Indeed, when the C-O bond is retained and a product of selective dehydrogenation is isolated, β C-H bonds are preferentially cleaved in alkoxides bound either to metal surfaces[1-8] or to metal clusters.[9-12] The reactions of alkoxides on metal surfaces have been studied more extensively on the later transition-metal surfaces with relatively fewer reports on earlier transition metals, i.e., Mo. In addition, the absence of β-hydrogens in the alkoxide increases its kinetic stability with respect to dehydrogenation, as seen with *tert*-butoxide on Cu(110) and Ag(110).[1,2] In fact, the alkoxide C-O bond is retained on most transition-metal surfaces. For example, CO and H_2 are the primary products from methoxide decomposition on Mo(100).[13,14] Some exceptions are seen, however, in the reactions of methanol on W(100)[15] and Ti(0001),[16] methanol and ethanol on Fe(100),[17] and methanol on Pt(110)-(1×1).[18] C-O bond retention is also seen during the reaction of 2-propanol on MoO_3;[19] acetone is a primary product.

Alkoxide reactivity on Mo(110) is distinctly different from that on most other transition-metal surfaces. Importantly, the C-O bonds of alkoxides are cleaved on Mo. In addition, we report here that the C-H bond in 2-propoxide γ to Mo is cleaved, while the β C-H bonds remain *intact* (eq 1). Reactions of selectively



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