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In the past several years, we have been developing a unified mechanistic approach to designing artificial hydrolytic metalloenzymes. The unified mechanistic approach led us to develop some of the most reactive *cis*-diaqua metal complexes for hydrolyzing not only phosphate esters with good or poor leaving groups but also for hydrolyzing carboxyl esters, amides, and nitriles.

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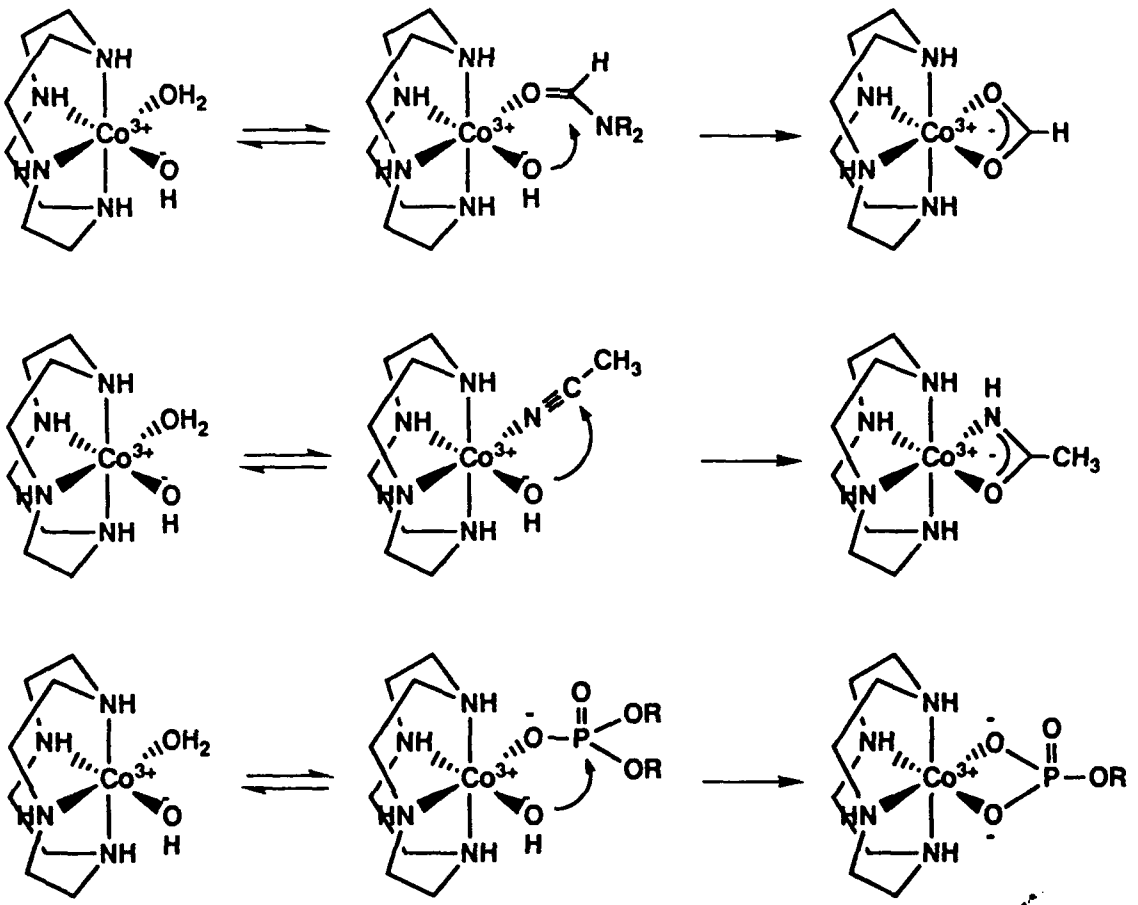
FINAL REPORT

Our main goal is to develop highly efficient artificial hydrolytic metalloenzymes that cleave phosphate esters (including di and triesters). Some phosphate esters are reactive hydrolytically because they have good leaving groups (eg ATP, Parathion, DIPF, Sarin, and Soman) while other phosphate esters are stable because they have poor leaving groups (eg DNA, VX, Tabun, and cyclic AMP). It has been shown that catalysts that are highly efficient at hydrolyzing esters with good leaving groups may not be reactive at all for hydrolyzing esters with poor leaving groups.[1] Hence it is important to develop a unified mechanistic approach to hydrolyzing both esters with good and poor leaving groups.

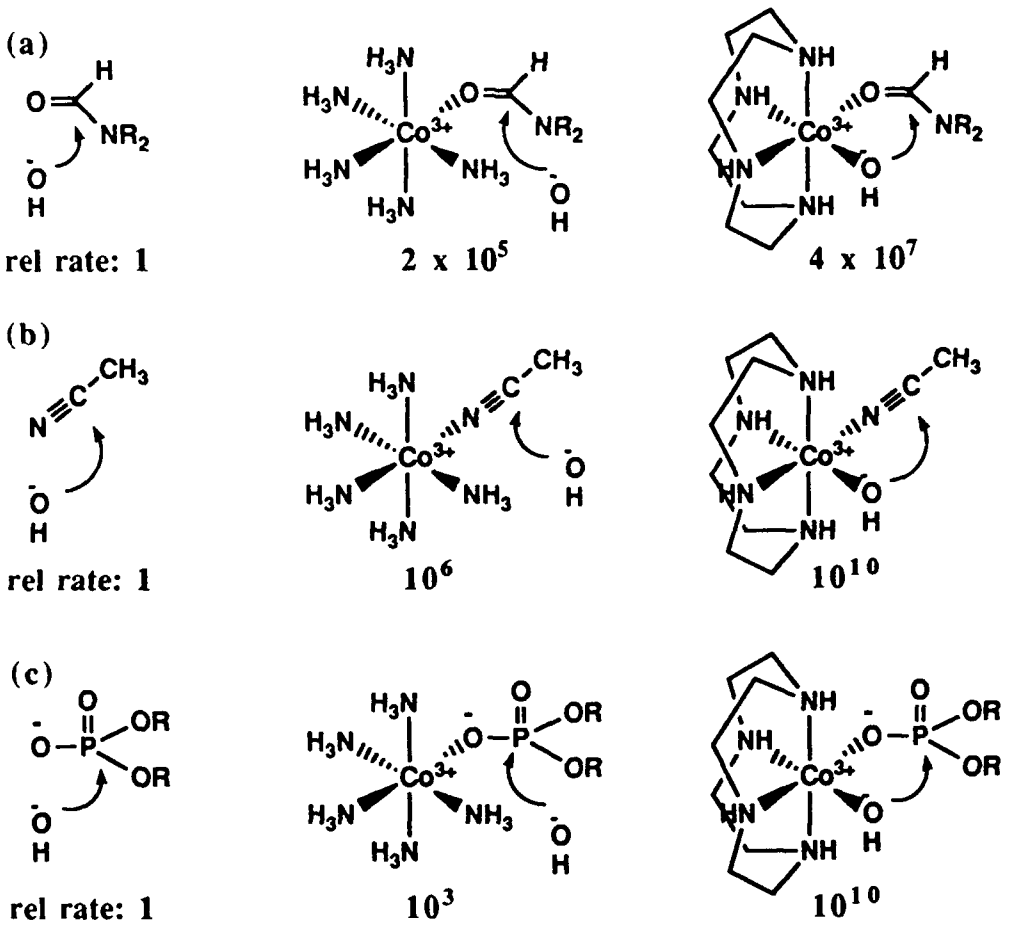
In the past several years, we have been developing a unified mechanistic approach to designing artificial hydrolytic metalloenzymes.[2] The unified mechanistic approach led us to develop some of the most reactive *cis*-diaqua metal complexes for hydrolyzing not only phosphate esters with good or poor leaving groups[3] but also for hydrolyzing carboxyl esters,[4] amides,[5] and nitriles (**Figure 1**).[6]

Figure 1



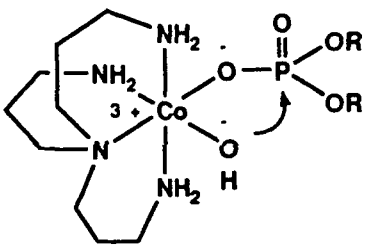
Cis-diaqua metal complex catalyzed hydrolysis of amides, nitriles and phosphate diesters involves *joint Lewis acid activation and intramolecular metal-hydroxide activation* resulting in formation of key *four-membered ring* intermediates (**Figure 1**). Our most recent findings include the following: (1) the strained four-membered ring intermediates in the hydrolysis of esters,[2] amides,[7] nitriles[6] and phosphate esters[2] have been isolated and their crystal structures determined providing strong support for the unified catalytic mechanism. (2) Equilibrium constants for coordination of amides,[6] nitriles[6a] and phosphate esters[3a,b] to Co(III) complexes in water have been directly measured. Interestingly, the substrates bind much more tightly than the solvent water molecules to the metal complexes even though some of the substrates (eg. nitriles) are up to ten orders of magnitude less basic than water. (3) Determination of the equilibrium constants for complexation of substrates (amides, nitriles, and phosphate esters) to Co(III) complexes has allowed us to *dissect the overall rate-accelerations* for the hydrolyses reactions into those due to *Lewis acid activation* and *intramolecular metal-hydroxide activation* (**Figure 2**).

Figure 2

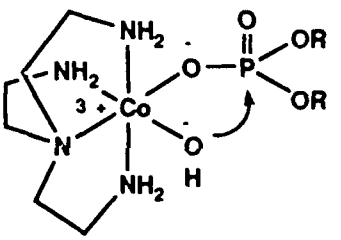


For example, Lewis acid activation alone gives a 10^3 fold rate acceleration for phosphate diester hydrolysis at neutral pH. By comparison, joint Lewis acid activation and intramolecular metal-hydroxide activation can provide a 10^{10} fold rate-acceleration for the same reaction at pH 7 (**Figure 2c**).

The reactivity of *cis*-diaqua metal complexes for hydrolyzing phosphate diesters is highly sensitive to its structure in a predictable way: the reactivity increases dramatically with decrease in the O-M-O bond angle (where M represents the metal). For example, the phosphate diester bond in **1** is hydrolyzed over 300 times more rapidly than that in **2**. The O-M-O bond angle can be decreased by increasing the N-M-N bond angle directly opposite the O-M-O bond angle. The N-M-N bond angle in **1** is larger than that in **2** since the two nitrogens in **1** are connected by three carbons while those in **2** are connected by two carbons. It is interesting to note that such subtle changes in the catalyst structure can result in enormous changes in the catalytic activity. The effective concentration[8] of the metal-hydroxide in **1** is over 300 times greater than that in **2**.



1



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We now have a firm foundation upon which to build highly efficient catalysts for hydrolyzing amides, nitriles and phosphate esters. A distinct advantage of taking a unified mechanistic approach to designing the catalyst is that *what is learned from studying the catalytic hydrolysis of one substrate can be applied to the catalytic hydrolyses of other substrates.* Indeed our catalysts that are the most reactive for hydrating nitriles[6] are also the ones that are highly reactive for hydrolyzing amides[5a] and phosphate esters.[3a] Reactivity trend of our catalysts for nitrile hydration parallel that for amide and phosphate ester hydrolysis. The *cis*-diaqua metal complexes have served us well for hydrolyzing amides, nitrile and phosphate esters.

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