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Reactivity of Coordinated Nucleophiles. A Comparison of Metal Bound Imidazolate and Hydroxide Ions as Models for Carbonic Anhydrase

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Abstract: The cleavage of 4-nitrophenyl acetate by the simple metal complexes $(NH_3)_5CoOH^{2+}$ and $(NH_3)_5CoIm^{2+}$ (Im = N-deprotonated imidazole) has been studied in water and dimethyl sulfoxide (Me_2SO) solvents. In both solvents for both complexes the reactions are exclusively nucleophilic, as demonstrated by the detection of the acetylated reactants, $(NH_3)_5CoO_2CCH_3^{2+}$ and $(NH_3)_5CoImCOCH_3^{3+}$. The pK_a determined titrimetrically for $(NH_3)_5CoImH^{3+}$ in water (25 °C, μ = 1.0, $NaClO_4$) is 10.0 and the large difference in nucleophilic capacity towards 4-nitrophenyl acetate between $(NH_3)_5CoIm^{2+}$ ($k_N = 9$ M^{-1} s^{-1}, 25 °C, $\mu = 1.0$, $NaClO_4$) and $(NH_3)_5CoCH^{2+}$ ($k_N = 1.5 \times 10^{-3}$ M^{-1} s^{-1}) is closely parallel to the difference in basicity (pK_a $(NH_3)_5CoOH_2^{3+} = 6.4$, 25 °C, $\mu = 1.0$, $NaClO_4$). In Me_2SO the complexes are of similar activity towards the ester ($k_{Im} = 30$ M^{-1} s^{-1}, $k_{OH} = 0.72$ M^{-1} s^{-1}, 25 °C) and it is found that this may be largely attributed to a marked increase in the basicity of $(NH_3)_5CoOH^{2+}$ relative to that of $(NH_3)_5CoIm^{2+}$ in this dipolar, aprotic solvent. Similar trends for dimethylformamide are indicated and mechanistic and kinetic aspects of this study are discussed in relation to the esterase properties of the zinc metalloenzyme, carbonic anhydrase.

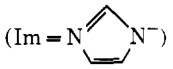
One of the most thoroughly investigated metalloenzymes is carbonic anhydrase,[1] a highly efficient catalyst for the equilibrium

$$CO_2 + H_2O \rightleftharpoons H^+ + HCO_3^-$$

and a control for the CO_2, HCO_3^-, and H^+ levels in biological systems. The hydration-dehydration reaction is exceptionally fast ($k_{cat} \sim 10^7$ M^{-1} s^{-1} at 25 °C), but at much slower rates the enzyme has been shown to catalyze several analogous reactions, notably the hydrolysis of esters and sultones and the hydration of carbonyl groups.[2]

X-Ray crystallographic analyses of several enzyme forms[1,3,4] indicate that the Zn^{2+} ion is bound by three histidine imidazole residues. Two bonded through N(3′) and one through N(1′), Figure 1. A water molecule or an hydroxide ion appears to be coordinated as a fourth ligand to give overall a distorted tetrahedral geometry of the bonded atoms about the metal ion. Numerous publications[1,4-12] have described enzyme mechanisms and several elementary models for the active site have been advanced.[13-17] The so-called "zinc-hydroxide" mechanism (Figure 1) (N denotes the N donor atom of a histidine imidazole ring) has usually been the object of assessment in these model studies, and both positive attributes and deficiencies have been noted. Recently, detailed proton magnetic resonance studies of the enzyme[18,19] have provided support for a different mechanism involving deprotonation not of water but of histidine imidazole coordinated to Zn(II), Figure 2.

While the indicated general base function for the metal-coordinated nucleophile has been preferred by Pesando et al.[18a,c] little justification presently exists for exclusion of the direct analogue of the "zinc-hydroxide" mechanism in which the "zinc-imidazolate" nucleophile captures CO_2,[20] Figure 3. As the facile generation of coordinated imidazolate anions has been described several times,[21] a number of systems are available for testing as models for carbonic anhydrase. The present work reports an attempt to establish both the nucleophilic and general base capacities of the simple complexes imidazolatopentaamminecobalt(III) ion, $[(NH_3)_5CoIm]^{2+}$



and $[(NH_3)_5CoOH]^+$ towards 4-nitrophenyl acetate. These ions are kinetically robust and the parent ligands do not leave the metal center in the lifetime of the reactions to be discussed. With such models the kinetic results are not complicated by equilibria involving dissociation of the ligands from the metal center.

Experimental Section

Instrumentation. Spectrophotometric measurements were made on Cary 16K and 118C and Gilford 2100 instruments. Rates up to a half-life of ~1 s were followed by use of a simple rapid mixing device. pH-stat titrations were made under nitrogen by use of a Radiometer Model 26 pH meter and SBR2 automatic burette with a G202B glass electrode and a saturated calomel reference electrode (connected to reaction mixtures via an $NaNO_3$ salt bridge). The glass electrode was standardized with phosphate (pH 6.86, 25 °C) and borate (pH 9.18, 25 °C) buffers and checked in the range 9.5–12.0 pH with various carbonate and phosphate buffers. Final pH readings on all reaction media were also taken under nitrogen on the same equipment. Proton magnetic resonance spectra were recorded at 100 MHz on a JEOL MH-100 "Minimar" spectrometer.

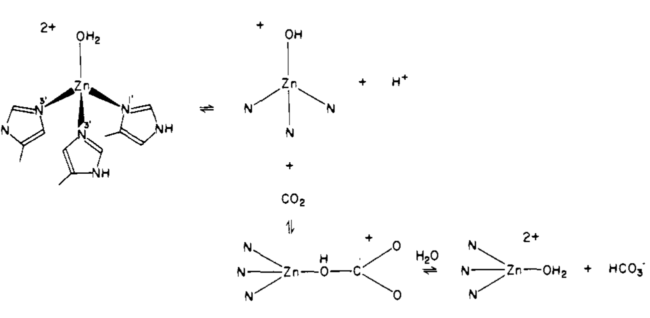


Figure 1. The nucleophilic "zinc-hydroxide" mechanism for hydration of carbon dioxide by carbonic anhydrase.

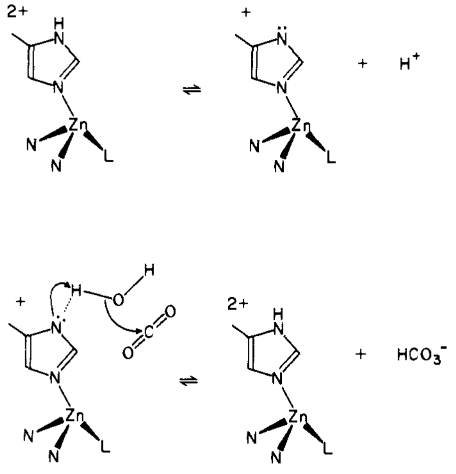


Figure 2. A general base "zinc-imidazolate" mechanism for hydration of carbon dioxide by carbonic anhydrase. Possible features involving incipient CO_2 coordination[18b] or a general acid role for Zn^{II} coordinated H_2O[18c] have been omitted for clarity.

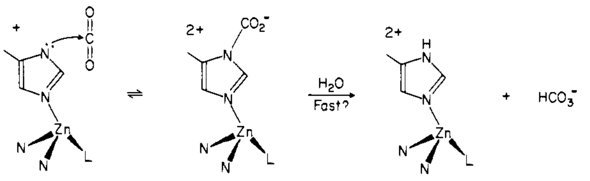


Figure 3. The nucleophilic "zinc-imidazolate" mechanism for hydration of carbon dioxide by carbonic anhydrase.

Chemicals. All chemicals used in the preparation of reaction media were of analytical reagent grade and solutions were freshly prepared immediately prior to use. 4-Nitrophenyl acetate was prepared by reaction between sodium 4-nitrophenolate and acetic anhydride in water at 0 °C and was recrystallized twice from ethanol by addition of water immediately prior to any usage.

$[(NH_3)_5CoImH](ClO_4)_3$. A solution of $[(NH_3)_5CoMe_2SO](ClO_4)_3$[22] (20 g) and imidazole (5 g, ~ × 2 M amount) in Me_2SO (100 ml) was heated at 80 °C for 45 min. The resulting yellow-brown solution was cooled, diluted to 1 l. with H_2O, filtered, and absorbed on H^+ form Dowex 50WX2 ion exchange resin. The resin was washed with H_2O, then eluted with 1 M HCl to remove a trace of Co^{2+}. Subsequent elution with 3 M HCl readily removed the bulk yellow material and the eluate was taken to dryness under reduced pressure. The residue was dissolved in the minimum volume of water and the complex(es) rapidly reprecipitated by adding excess $HClO_4$ and cooling the solution on ice for 30 min. The solid (11 g) was collected and washed with ethanol and ether. Small amounts of $[(NH_3)_6Co](ClO_4)_3$ were invariably present in this material, but were readily removed as follows: the solid was extracted with acetone (15 ml/g) by stirring at room temperature for 10 min. The undissolved powder was filtered out and discarded and complex precipitated from the extract by the addition of several volumes of ether. The extraction, filtration, and precipitation were then repeated. The solid finally obtained was twice recrystallized from water by the addition of $HClO_4$. With pure complex no immediate precipitation occurred but large, thin, golden flakes formed on standing and their deposition was then completed by cooling on ice.

Anal. Calcd for $CoC_3H_{19}N_7Cl_3O_{12}$: Co, 11.54; C, 7.06; H, 3.75; N, 19.21; Cl, 20.83. Found: Co, 11.63; C, 7.26; H, 4.04; N, 18.95; Cl, 20.74. Visible spectrum (λ_{max}, ϵ_{max}, 1 M HCl) 472 nm, 61.5 M^{-1} cm^{-1}; 334 nm, 70.7 M^{-1} cm^{-1}. ^1H NMR spectrum (δ, ppm, at 100 MHz in Me_2SO-d_6 vs. sodium trimethylsilylpropanesulfonate as internal reference) 3.59 (15 H, ammine ligand protons), 7.31, 7.69, 8.28 (each 1 H, imidazole CH protons), 13.2 (1 H, imidazole NH proton) (NB chemical shifts were found to be detectably sensitive to H_2O in the Me_2SO-d_6 and hence are reported for solutions dried 3 h over molecular sieves).

$[(NH_3)_5CoIm](ClO_4)_2 \cdot 0.5H_2O$. $[(NH_3)_5CoImH](ClO_4)_3$ (3.2 g) was dissolved in excess aqueous NaOH (1 M, 20 ml) and filtered and $NaClO_4$ (10 g) dissolved in the filtrate. Rapid precipitation of yellow-brown crystals occurred and, after 15 min at 0 °C, the solid was collected and washed with ethanol and ether (yield 2.1 g). The complex was recrystallized from water containing a few drops of 1 M NaOH by adding excess $NaClO_4$ and cooling on ice. Large yellow-brown blocks were thereby obtained.

Anal. Calcd for $CoC_3H_{18}N_7Cl_2O_8 \cdot 0.5H_2O$: Co, 14.06; C, 8.60; H, 4.57; N, 23.40; Cl, 16.92. Found: Co, 14.06; C, 8.85; H, 4.96; N, 23.30; Cl, 16.93. Visible spectrum (λ_{max}, ϵ_{max}, 1 M NaOH) 478 nm, 69.7 M^{-1} cm^{-1}; 344 nm, 107.9 M^{-1} cm^{-1}. ^1H NMR spectrum (δ, ppm at 100 MHz in dried Me_2SO-d_6 vs. sodium trimethylsilylpropanesulfonate as internal reference) 3.38 (15 H, ammine ligand protons), 6.99, 7.15, 7.42 (each 1 H, imidazole CH protons).

$[(NH_3)_5CoImCOCH_3](ClO_4)_3$. Finely ground $[(NH_3)_5CoIm](ClO_4)_2$ (2.0 g) was added to acetic anhydride (25 ml). The initially slightly soluble solid transformed under vigorous stirring into an insoluble yellow powder which, after 10 min, was filtered off and washed thoroughly with ether. It was quickly dissolved in the minimum volume of ice cold 0.1 M $HClO_4$, filtered, and immediately reprecipitated by the addition of a large excess of $NaClO_4$. The clusters of fine, yellow needles obtained were then recrystallized from acetone by the addition of ether (yield 1.7 g).

Anal. Calcd for $CoC_5H_{21}N_7Cl_3O_{13}$: Co, 10.67, C, 10.87; H, 3.83; N, 17.75; Cl, 19.25. Found: Co, 10.68; C, 10.56; H, 4.19; N, 17.32; Cl, 19.33.

Between 600 and 350 nm the spectrum of the complex is essentially identical with that of $[(NH_3)_5CoImH](ClO_4)_3$. Major differences occur only below 260 nm, though maxima for both complexes lie below 220 nm. ^1H NMR spectrum (δ, ppm, at 100 MHz in Me_2SO-d_6 vs. sodium trimethylsilylpropanesulfonate as internal reference) 2.77 (3 H, acetyl methyl protons), 3.87 (15 H, ammine ligand protons), 7.46, 8.16, 9.16 (each 1 H, imidazole CH protons).

$[(NH_3)_5CoOH](ClO_4)_2$. $[(NH_3)_5CoOH_2](ClO_4)_3$ was dissolved in water (25 °C), treated with NaOH (~10% excess) and the pink-violet hydroxo complex precipitated by the addition of $NaClO_4$. It was recrystallized from dilute NaOH (~0.01 M, 25 °C) by adding $NaClO_4$ and cooling on ice. The small, pink-violet, hexagonal plates obtained were washed with ethanol and ether.

Anal. Calcd for $CoH_{16}N_5Cl_2O_9$: Co, 16.37; H, 4.48; N, 19.45; Cl, 19.70. Found: Co, 16.14; H, 4.31; N, 19.61; Cl, 19.72.

$[(NH_3)_5CoOAcyl](ClO_4)_2$. When concentrated (~0.1 M) solutions of $[(NH_3)_5CoOH](ClO_4)_2$ and slightly more than an equimolar amount of various activated carbonyl compounds in Me_2SO were mixed and allowed react for periods between 30 s and 10 min, essentially quantitative yields of the corresponding O-acylated complexes were obtained. Thus, 4-nitrophenyl acetate gave acetatopentaamminecobalt(III); chloral hydrate, formatopentaamminecobalt(III); dimethyl (and diethyl) oxalate, methyl(ethyl)oxalatopentaamminecobalt(III); and succinic and maleic anhydrides the succinato and maleatopentaamminecobalt(III) complexes, respectively. The complexes were isolated by pouring the reaction mixtures into ethanol and recrystallizing the crude precipitate obtained from water.

Anal. Calcd for $CoC_2H_{18}N_5Cl_2O_{10}$: Co, 14.66; C, 5.98; H, 4.51; N, 17.42; Cl, 17.64. Found: Co, 14.74; C, 6.11; H, 4.57; N, 17.39; C, 17.64. Calcd for $CoCH_{16}N_5Cl_2O_{10}$: Co, 15.19; C, 3.10; H, 4.16; N, 18.05; Cl, 18.27. Found: Co, 14.60; C, 3.48; H, 4.05; N, 17.88; Cl, 18.59. Calcd for $CoC_3H_{18}N_5Cl_2O_{12}$: Co, 13.21; C, 8.08; H, 4.07; N, 15.70; Cl, 15.90. Found: Co, 13.70; C, 7.62; H, 3.83; N, 15.49; Cl, 16.09. Calcd for $CoC_4H_{20}N_5Cl_2O_{12}$: Co, 12.81; C, 10.44; H, 4.38; N, 15.22; Cl, 15.41. Found: Co, 13.73; C, 10.40; H, 4.41; N, 14.98;

Cl, 15.62. (for ethyloxalato complex). Found: Co, 12.28; C, 10.41; H, 4.47; N, 15.42 (succinato complex). Calcd for $CoC_4H_{18}N_5Cl_2O_{12}$: Co, 12.87; C, 10.49; H, 3.96; N, 15.29; Cl, 15.48. Found: Co, 12.58; C, 10.43; H, 3.86; N, 14.93.

Kinetic Measurements. To follow the hydrolysis of 4-nitrophenyl acetate in the presence of aqueous $(NH_3)_5CoImH^{3+}/(NH_3)_5CoIm^{2+}$ buffers the increase in absorbance at 400 nm due to released 4-nitrophenolate was measured following rapid mixing of separate solutions (both of $\mu = 1.0$ made up with $NaClO_4$) of ester and complex buffer. Ester concentrations in the final reaction media were between 5×10^{-5} and 10^{-4} M, and the complex was never in less than 40-fold excess. The ester solution was prepared by dissolving the amount required for 1 l. of solution in the minimum volume of acetone or methanol (1-2 ml), adding this to the appropriate mass of $NaClO_4$ dissolved in ~900 ml of H_2O then making the mixture up to 1 l. For study of the reaction in 99.75% D_2O, anhydrous $NaClO_4$ was prepared by heating the monohydrate at 120 °C for 30 h, and appropriate amounts were then weighed out with the greatest possible alacrity.

By use of low complex concentrations the cleavage of 4NPA by $(NH_3)_5CoIm^{2+}$ in Me_2SO was followed without resort to use of a rapid mixing device. A solution of $[(NH_3)_5CoIm](ClO_4)_2$ and $[(NH_3)_5CoIm](ClO_4)_3$ in freshly distilled, dry Me_2SO was dried over molecular sieves for 2 h. The solution was then filtered and 3 ml was quickly transferred to a stoppered 1-cm cell and brought to the desired temperature in the cell block of the spectrophotometer. A volume (0.05 ml) of a solution of 4NPA in Me_2SO of such concentration that the final ratio complex:ester was >20 was added, mixed in well, and observation of the absorbance at 400 nm commenced. An identical procedure was used to follow cleavage of 4NPA in Me_2SO buffers of $(NH_3)_5Co\ OH^{2+}$ and $(NH_3)_5Co\ OH_2{}^{3+}$.

Increase in 4-nitrophenolate absorption was also used to follow 4NPA hydrolysis in the presence of aqueous $(NH_3)_5CoOH^{3+}$. Reaction was examined using both a dilute solution of the complex in "Tris" buffer (pH 8.62) and a buffer of the complex alone plus $(NH_3)_5CoOH_2{}^{3+}$. Under the latter conditions the buffer ratio was chosen so as to give a pH above the pK_a of 4-nitrophenol, and complex concentrations close to saturation were used to provide a convenient reaction rate. Ester solutions were prepared as above, but because reaction was much slower with the hydroxo complex, ester and complex solutions were simply mixed in a constant temperature bath and a portion of the mixture was withdrawn to fill a cell of appropriate path length, which was then placed in the temperature controlled block of the spectrophotometer.

Procedures used to follow the hydrolysis of $[(NH_3)_5CoImCOCH_3]^{3+}$ were complicated by the lability of this species. Thus, it was necessary to prepare all media and bring them to temperature (25 °C) before adding and dissolving the complex. In strong acid solutions, a 1-cm cell containing solution was brought to temperature in the cell compartment of the spectrophotometer, then an amount of complex required to give an initial concentration between 10^{-4} and 10^{-3} M was added, quickly dissolved, and monitoring of absorbance at a fixed wavelength immediately began. In solutions of near neutral pH where reaction was followed by pH-stat titration with NaOH, 1 M $NaClO_4$ (50 ml) was firstly brought to the chosen temperature and pH, then the weighed amount of complex quickly dissolved in the very minimum volume of water (~0.1 ml) was added and vigorously mixed in. Recording of titrant volume was begun as soon as the pH returned to its initial value. To follow the very rapid reactions in carbonate/bicarbonate and $(NH_3)_5CoImH^{3+}/(NH_3)_5CoIm^{2+}$ buffers, solutions of 1 M $NaClO_4$ and buffer were brought to temperature in the separate reservoirs of a rapid mixing device. $[(NH_3)_5CoImCOCH_3](ClO_4)_3$ was then quickly dissolved in the 1 M $NaClO_4$, the solutions were mixed, and reaction was followed by the measurement of absorbance at a fixed wavelength. Reaction in 1 M $NaClO_4$ was sufficiently slow for up to seven runs to be made with the one stock solution before changes in initial absorbance were detectable.

Results

Synthesis and Properties of the Imidazole Complex. The imidazolepentaamminecobalt(III) ion was readily prepared by allowing either the (dimethyl sulfoxide)pentaamminecobalt(III) or the nitratopentaamminInecobalt(III) complex and imidazole to react in Me_2SO. The 100-MHz ^1H NMR spectrum of the bound imidazole shows three C-H signals of equal intensity, indicating bonding through a nitrogen atom (not through carbon[23]). The visible spectrum is also consistent with a hexaammine chromophore, maxima being observed at 472 nm (ϵ 61.5 $M^{-1}\ cm^{-1}$) and 334 nm (ϵ 70.7 $M^{-1}\ cm^{-1}$). In 1 M DCl, the ^1H NMR spectrum shows a broad resonance at δ 4.14 due to the combined cis and trans NH_3 groups, but under such conditions the imidazole NH proton (δ 13.2 in Me_2SO-d_6) exchanges too rapidly ($k_{ex} > 10^{-1}\ s^{-1}$ at 30 °C) to be observed. In $DCO_3{}^-/CO_3{}^{2-}$ buffer (D_2O, pD 11.1) all ammine protons exchange for D^+ very rapidly but no imidazole carbon proton exchange is observed over 44 h at 30 °C.

Table I. pK Determination Using $(NH_3)_5CoImH^{3+}/(NH_3)_5CoIm^{2+}$ Buffers (25 °C)

$[(NH_3)_5CoIm^{2+}]$,[a] M	Buffer ratio[b]	pH[c]	pK_a[d]
1.00×10^{-3} [e]	9:1	9.04	10.00
2.54×10^{-3} [f]	2.95:1	9.58	10.07
5.00×10^{-3} [e]	1:1	9.98	10.01
5.02×10^{-3} [f]	1:1	10.03	10.06
7.55×10^{-3} [f]	1:2.92	10.39	10.03
9.00×10^{-3} [e]	1:9	10.62	9.94

[a] Formal concentration based on weight of solid or volume of NaOH solution added. [b] Ratio of formal concentrations acid/base (a/b). [c] Measured under N_2 on N_2 saturated solutions. [d] Calculated from the approximation $K_a = ((b[H^+] - K_w)/(a[H^+] + K_w))[H^+]$. [e] Formed by addition of NaOH. [f] Based on mass of solid $[(NH_3)_5CoIm](ClO_4)_2$.

The Acidity of Coordinated Imidazole. The acidity constant of $[(NH_3)_5CoImH]^{3+}$ was determined from pH measurements on buffers of the complex and its conjugate base prepared both by addition of NaOH to solutions of $[(NH_3)_5CoImH](ClO_4)_3$ and by mixing solutions containing $[(NH_3)_5CoImH](ClO_4)_3$ and $[(NH_3)_5CoIm](ClO_4)_2$. For a total cobalt(III) concentration of 10^{-2} M and an ionic strength of 1.0 made up with $NaClO_4$, the data obtained are given in Table I. Measurements of pH on various reaction media (see below) are in agreement with the mean value for the pK_a of 10.02 ± 0.04 obtained from the data of Table I.

Equilibria in Me_2SO. The molar absorptivities of cobalt(III) pentaamminehydroxo, imidazole, aqua, and imidazolato perchlorates in dry Me_2SO were, 570 nm, 37.7, 1.7, 10.6, 5.0; 560 nm, 46.9, 2.7, 16.1, 8.2; 480 nm, 60.8, 67.9, 53.5, 80.1; 460 nm, 40.5, 64.6, 44.7, 67.7; 410 nm, 63.4, 14.8, 12.0, 33.8; 400 nm, 78.5, 10.0, 11.4, 42.5; and 350 nm, 62.2, 81.9, 52.9, 125 $M^{-1}\ cm^{-1}$, respectively. The ratio of acidity constants $K_a(ImH)/K_a(OH_2)$ was calculated from these data and the spectra of equimolar mixtures (10^{-2} M) of the pairs of complexes in dry Me_2SO as 0.14 ± 0.01. The pK_a values for the imidazole and aqua complexes are now comparable in Me_2SO whereas in water there is a difference of 3.6 log units. Moreover, acetic acid and 4-nitrophenol protonated $[(NH_3)_5CoOH](ClO_4)_2$ in Me_2SO, whereas phenol was unable to do so. Using recent values of the dissociation constants of these acids in Me_2SO[24] we estimated the pK_a of the $[(NH_3)_5CoOH_2]^{2+}$ ion to be between 14 and 16 in this solvent and the imidazole complex therefore must have a similar value.

Pathways for the Reaction between $(NH_3)_5CoIm^{2+}$ and 4-Nitrophenyl Acetate (4NPA). The low solubility of 4-nitrophenyl acetate in water obviated the characterization of any species other than the intensely colored 4-nitrophenolate anion as the immediate product of reaction in essentially aqueous media. Incorporation of a considerable proportion (~50% by volume) of an organic solvent such as acetone or dimethyl sulfoxide, however, enabled sufficiently high concentrations of 4NPA to be used to allow establishment of the initial reaction stoichiometry under conditions where the ester concentration exceeded that of the complex. (Rate studies could not,

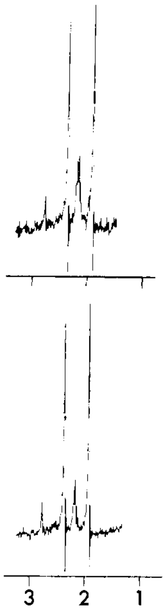


Figure 4. (a) (Lower trace). Methyl resonances observed ca. 20 s after mixture of $(NH_3)_5CoIm^{2+}$ in D_2O with an equimolar amount of 4NPA in an equal volume of acetone-d_6. The peaks seen (chemical shifts relative to internal Me_4Si at 100 MHz, ~30 °C) are δ 1.94, free acetate; 2.2, residual protons of acetone-d_6; 2.38, 4NPA; 2.77, $(NH_3)_5CoImCOCH_3^{3+}$. (b) (Upper trace). Methyl resonances observed ca. 20 s after mixture of $(NH_3)_5CoImCOCH_3^{3+}$ in acetone-d_6 with an equimolar amount of sodium 4-nitrophenolate in an equal volume of D_2O.

of course, be made under such conditions without the incorporation of another buffer). Thus, ^1H NMR spectroscopy was a convenient means of identifying both organic and inorganic products when an organic solution of 4NPA was rapidly mixed with an aqueous solution of $(NH_3)_5CoIm^{2+}$. For a molar ratio of ester to complex <1, the only resonances detectable within 20 s of mixing were those of acetate, nitrophenol, and reactant complex (or, more precisely, those of the mixture of acid and conjugate base forms of each appropriate to the medium pH). When this ratio became >1, however, a set of transient resonances was detected (Figure 4a). When the reaction was conducted for any ratio of reactants in purely organic solvent (Me_2SO-d_6) the immediate, relatively stable product[25] solution showed these same resonances. Subsequent synthesis of $(NH_3)_5CoImCOCH_3^{3+}$ established that this compound was the source of these new resonances. Neither in pure organic nor aqueous/organic solvent was evidence obtained (from ^1H NMR) for any intermediate involved in the formation of $(NH_3)_5CoImCOCH_3^{3+}$ from $(NH_3)_5CoIm^{2+}$ and 4NPA. Unfortunately, the method of initial product characterization was too crude to establish that in mixed solvents reaction through $(NH_3)_5CoImCOCH_3^{3+}$ was the sole pathway. However, for a mole fraction of acetone as low as ~0.2, the initially observed distribution of products (including 4NPA) for reaction of equimolar amounts of $(NH_3)_5CoIm^{2+}$ and 4NPA was essentially identical with that for reaction of $(NH_3)_5CoImCOCH_3^{3+}$ with 4-nitrophenolate (compare Figure 4a and 4b). Thus, in a predominantly aqueous medium no evidence was obtained for a role other than that of a nucleophile for $(NH_3)_5CoIm^{2+}$.

Kinetics of the Hydrolysis of 4-Nitrophenyl Acetate Catalyzed by $(NH_3)_5CoIm^{2+}$. Under conditions where the complex buffer was in large (>40-fold) excess in aqueous medium the release of 4-nitrophenolate anion followed first-order kinetics, all rate plots being linear for at least three half-lives. Rate constants obtained for a range of buffer concentrations, though predominantly at a buffer ratio of 1.00, are given in Table II.

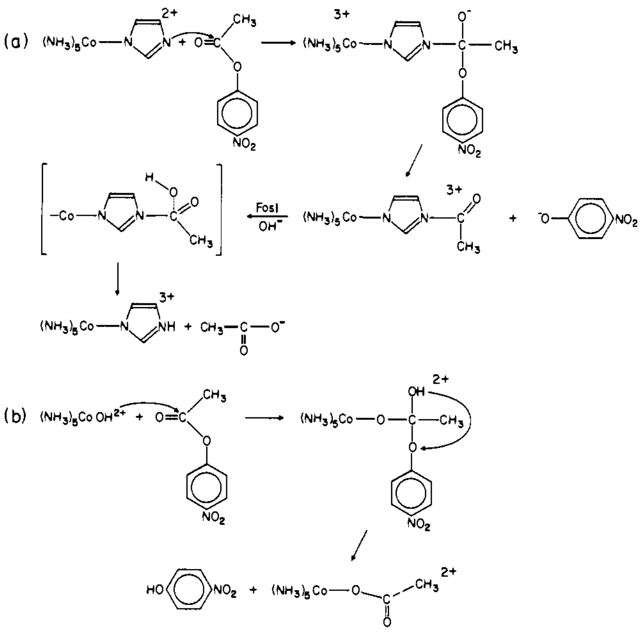


Figure 5. Proposed nucleophilic mechanisms for cleavage of 4-nitrophenyl acetate by (a) $(NH_3)_5CoIm^{2+}$, (b) $(NH_3)_5CoOH^{2+}$.

Table II. Pseudo-First-Order Rate Constants for Hydrolysis of 4-Nitrophenyl Acetate in $(NH_3)_5CoImH^{3+}/(NH_3)_5CoIm^{2+}$ Buffers (25 °C, μ = 1.0 ($NaClO_4$))

$[(NH_3)_5CoIm^{2+}]$, M	Buffer ratio[a]	pH[b]	k_{obsd} (s^{-1})
5.00×10^{-2}	1:1[c]	10.03	0.450[d]
2.50×10^{-2}	1:1[c]	10.03	0.240[d]
1.25×10^{-2}	1:1[c]	10.04	0.112[d]
6.25×10^{-3}	1:1[c]	10.03	0.0506[d]
3.13×10^{-3}	1:1[c]	9.90	0.0239[d]
1.56×10^{-3}	1:1[c]	9.90	0.0105[d]
1.67×10^{-3}	5:1	9.42	0.138×10^{-1}[e]
3.33×10^{-3}	2:1	9.69	0.275×10^{-1}[e]
5.00×10^{-3}	1:1	10.00	0.412×10^{-1}[e]
6.67×10^{-3}	1:2	10.14	0.544×10^{-1}[e]
2.50×10^{-3}[f]	1:1		0.0240[d]

[a] Acid/base. [b] Determined for the reaction mixture on completion of a set of runs and hence after some exposure of the solutions to atmospheric CO_2. [c] Buffers prepared by successive dilutions of the most concentrated, prepared from $(NH_3)_5CoImH^{3+}$ and NaOH. [d] Mean of quintuplicates. [e] mean.of triplicates. [f] Solvent 99.8% D_2O.

When a plot of k_{obsd} vs. $[(NH_3)_5CoIm^{2+}]$ was constructed for media of constant buffer ratio a straight line passing through the origin of the graph was obtained, demonstrating that the rate dependence on buffer was first order and that buffer independent rate terms were negligible. A similar plot was obtained for media of various buffer ratios, demonstrating that the base component of the buffer was the active species in promoting hydrolysis of the ester. Assuming the simplest rate law consistent with these facts,

$$k_{obsd} = k_N[(NH_3)_5CoIm^{2+}]$$

the weighted mean value of k_N calculated from the data of Table II was 8.7 ± 0.3 $M^{-1}\ s^{-1}$.

Direct spectroscopic detection of possible changes in complex ion absorption (due to formation and decay of intermediates) during 4NPA hydrolysis was precluded by the low solubility of 4NPA in water and hence the low concentration of complex involved in reaction, among other experimental difficulties. However, as an index of mechanism applicable to

Table III. Hydrolysis of $[(NH_3)_5CoImCOCH_3]^{3+}$ 25 °C, μ 1.0 as a Function of pH and Medium

Medium	pH	$10^3\ k_{obsd}$ (s^{-1})
1 M $HClO_4$	0^a	$1.64^{b,g,i}$
0.1 M $HClO_4$ 0.9 M $NaClO_4$	1.0^a	$1.11^{b,g,i}$
1 M $NaClO_4$	5.50	$1.13^{c,g}$
1 M $NaClO_4$	6.50	$1.59^{c,g}$
1 M $NaClO_4$	7.50	$4.04^{c,g,j}$
1 M $NaClO_4$	8.00	$12.8^{c,g,j}$
1 M $NaClO_4$	8.50	$39.2^{c,g,j}$
$[HCO_3^-] + [CO_3^{2-}] = 0.05$ M,^{e} $\mu = 1.0$ ($NaClO_4$)	9.46	$475^{b,h,i}$
$[HCO_3^-] + [CO_3^{2-}] = 0.025$ M,^{e} $\mu = 1.0$ ($NaClO_4$)	9.46	$405^{b,h,i}$
$[HCO_3^-] + [CO_3^{2-}] = 0.050$ M,^{e} $[A_5CoImH^{3+}] + [A_5CoIm^{2+}] = 3 \times 10^{-3}$ M, $\mu = 1.0$ ($NaClO_4$)	9.47	$550^{b,h,i}$
$[HCO_3^-] + [CO_3^{2-}] = 0.025$ M,^{e} $[A_5CoImH^{3+}] + [A_5CoIm^{2+}] = 1 \times 10^{-3}$, $\mu = 1.0$ ($NaClO_4$)	9.48	$450^{b,h,i}$
$[HCO_3^-] + [CO_3^{2-}] = 0.025$ M,^{f} $\mu = 1.0$ ($NaClO_4$)	9.63	$597^{b,h,i}$
$[HCO_3^-] + [CO_3^{2-}] = 0.025$ M,^{d} $\mu = 1.0$ ($NaClO_4$)	9.11	$200^{b,h,i}$

[a] Calculated from pH $-\log [H^+]$. [b] Rates measured spectrophotometrically at λ 250 nm. [c] Rates measured by pH-stat titration. [d] Buffer ratio 7:3. [e] Buffer ratio 1:1. [f] Buffer ratio 2:3. [g] Mean of duplicate measurements. [h] Mean of between five and seven measurements. [i] Initial reactant concentration between 1 and 5×10^{-4} M. [j] Initial reactant concentration 10^{-2} M.

aqueous reaction media, the deuterium isotope effect[26] on the reaction rate was determined. As is seen from the final entry in Table II, the rate of 4-nitrophenolate anion production in 99.8% D_2O was essentially the same as the rate in H_2O, viz., within experimental error no isotope effect was detected.

The effect of transferral from water to dipolar aprotic solvents on the reactivity of $(NH_3)_5CoIm^{2+}$ towards 4NPA was found to be relatively small. Rate constants for 4-nitrophenol release from 4NPA in Me_2SO in the presence of $(NH_3)_5CoIm^{2+}$ are given in Table IV.

Kinetics of the Hydrolysis of $(NH_3)_5CoImCOCH_3^{3+}$. To establish whether or not the complex $(NH_3)_5CoImCOCH_3^{3+}$ could be involved as an intermediate in the hydrolysis of 4NPA by $(NH_3)_5CoIm^{2+}$ in aqueous media, determination of the rate of its cleavage to $(NH_3)_5CoImH^{3+}$ and acetate ion was required. Further, examination of the reaction (if any) between $(NH_3)_5CoImCOCH_3^{3+}$ and $(NH_3)_5CoIm^{2+}$ offered the prospect of an unambiguous estimate of the general base activity of the latter species. Kinetic data for the hydrolysis of $(NH_3)_5CoImCOCH_3^{3+}$ in various media, including some not of direct relevance but yielding a more complete characterization of the reactivity of the complex, are given in Table III. Under all conditions the reaction was first order in complex, rate plots being linear over at least four half lives. (In carbonate/bicarbonate buffers of pH >9.6 intrusion of a second process causing a small absorbance change at 250 nm was detected. Since the contribution of this process was dependent on the buffer concentration, it was assumed to be due to the reaction involving nucleophilic attack of CO_3^{2-} on $(NH_3)_5CoImCOCH_3^{3+}$. Since rates above pH 9.6 were at the limit of instrumental capacities and analysis of the biphasic kinetics was therefore rather approximate, the data were ignored.)

For buffer independent reaction pathways the data are well represented by the expression

$$k_{obsd}(s^{-1}) = 1.1 \times 10^{-3} + 5 \times 10^{-4}[H^+] + 6860[OH^-]$$

(provided $[OH^-]$ is estimated from the expression $-\log [OH^-] = pK_w - pH$, with $pK_w = 13.77$ for 1 M $NaClO_4$).

While the dependence of the rate on HCO_3^-/CO_3^{2-} buffer concentration was readily detected, though not extensively investigated, experimental difficulties frustrated a precise determination of the dependence of the rate on imidazole complex buffer concentration. Thus, the close spectral similarities of reactant and buffer components required the use of reaction media of high absorbance and the relatively high pH at which significant amounts of $(NH_3)_5CoIm^{2+}$ could be generated led to rates near the limit of instrumental capabilities. Note that the reaction in the absence of added $(NH_3)_5CoImH^{3+}$ and $(NH_3)_5CoIm^{2+}$ was not detectably autocatalytic for initial reactant concentrations in the range 10^{-4}–10^{-3} M. Assuming that the rate constant increases observed when $(NH_3)_5CoImH^{3+}$ and $(NH_3)_5CoIm^{2+}$ were added to HCO_3^-/CO_3^{2-} buffered media represented the contribution of a term $k_{GB}[(NH_3)_5CoIm^{2+}]$ to the total reaction rate, a very approximate value of $k_{GB} = 160 \pm 40\ M^{-1}\ s^{-1}$ was calculated (based on the concentration of $(NH_3)_5CoIm^{2+}$ calculated from the observed pH and the pK_a of 10.02).

Kinetics and Mechanism of the Hydrolysis of 4-Nitrophenyl Acetate by $(NH_3)_5CoOH^{2+}$. Under conditions comparable to those used in study of $(NH_3)_5CoIm^{2+}$ the effect of $(NH_3)_5CoOH^{3+}$ on the rate of hydrolysis of 4NPA could not be detected. Thus, in "Tris' buffer at pH 8.62 the observed pseudo-first-order rate constant for hydrolysis of 4NPA was $1.18 \times 10^{-4}\ s^{-1}$ (25 °C). In the same buffer made 10^{-2} M with respect to $(NH_3)_5CoOH^{2+}$, the observed rate constant was $1.13 \times 10^{-4}\ s^{-1}$. For these conditions the complex ion is totally in the hydroxo form ($pK_a^{25} = 6.4$ at $\mu = 1.0$).[32] In a buffer of 0.10 M $(NH_3)_5CoOH^{2+}$, 0.010 M $(NH_3)_5CoOH_2^{3+}$ (pH 7.45, 25 °C, $\mu = 1.0$, $NaClO_4$), however, reaction involving the complex was readily detected. Thus, water and hydroxide dependent rates at pH 7.45 are negligible[27] compared to the observed rate which gave $k_{obsd} = 1.52 \times 10^{-4}\ s^{-1}$.

To establish the pathway for 4-nitrophenolate release from 4NPA in the presence of $(NH_3)_5CoOH^{2+}$ some of the complex buffer was mixed with a solution of ~0.3 mmol of 4NPA in acetone (2 ml) and the mixture stood, with occasional shaking, at 25 °C for 24 h. The solution was then diluted to 500 ml, acidified to pH 4, and absorbed on H^+ from Dowex 50W × 2 cation exchange resin. The resin was washed with water (500 ml), then eluted with 1 M $NaClO_4$ (pH 3). 4-Nitrophenol, which was held back quite strongly by the resin, was present in both the water wash and $NaClO_4$ eluate and slightly contaminated the red complex eluted by the $NaClO_4$. The red complex was shown to be $(NH_3)_5CoO_2C{\cdot}CH_3^{2+}$ both from the visible spectrum of the eluate (λ_{max} 500 nm) and from the properties (visible and ^1H NMR spectrum) of the solid precipitated when the eluate volume was decreased by evaporation under reduced pressure. The total amounts of both 4-nitrophenol and $(NH_3)_5CoO_2C{\cdot}CH_3^{2+}$ eluted from the resin were determined spectrophotometrically. For a yield of 2.35×10^{-4}

Table IV. Cleavage of 4-Nitrophenyl Acetate by $(NH_3)_5CoIm^{2+}$ and $(NH_3)_5CoOH^{2+}$ in Me_2SO at 25 °C

$[(NH_3)_5CoIm^{2+}]$ (M)	$[(NH_3)_5CoImH^{3+}]$ (M)	k_{obsd} (s^{-1})	k_N[a] ($M^{-1}\ s^{-1}$)
1×10^{-3}	1×10^{-3}	3.03×10^{-2}	30
5×10^{-3}	1×10^{-3}	1.43×10^{-1}	29
$[(NH_3)_5CoOH^{2+}]$ (M)	$[(NH_3)_5CoOH_2^{3+}]$ (M)		
5×10^{-3}	5×10^{-3}	3.57×10^{-3}	0.71
10×10^{-3}	1×10^{-3}	7.29×10^{-3}	0.73

[a] $k_N = k_{obsd}/[(NH_3)_5CoOH^{2+}]$ or $k_{obsd}/[(NH_3)_5CoIm^{2+}]$.

mol of 4-nitrophenol a yield of 2.40×10^{-4} mol of $(NH_3)_5CoO_2C{\cdot}CH_3^{2+}$ was obtained. Within experimental error, therefore, all 4-nitrophenol released from 4NPA in the presence of $(NH_3)_5CoOH^{2+}$ arose from direct nucleophilic attack of the complex on the ester. The observed first-order rate constant thus may be converted into a second-order nucleophilic rate constant of $1.5 \times 10^{-3}\ M^{-1}\ s^{-1}$ (25 °C, μ = 1.0).

The enhanced activity of $(NH_3)_5CoOH^{2+}$ in dipolar, aprotic solvents observed in various preparative reactions was established quantitatively by observation of the release of 4-nitrophenol from 4NPA in dimethyl sulfoxide in the presence of excess $(NH_3)_5CoOH^{2+}$. First-order rate plots based on 400-nm absorbance changes showed some curvature due to a slow process involving a small absorbance increase. This process was observed for the $(NH_3)_5CoOH^{2+}/(NH_3)_5CoOH_2^{3+}$ solution in the absence of 4NPA and was therefore assumed due to formation of $(NH_3)_5CoMe_2SO^{3+}$. Analysis of the curved plots by conventional procedures[16] provided the rate constants for nitrophenol release given in Table IV. Preliminary data obtained for the same reaction in dimethylformamide solvent show a similar enhancement of the reactivity of $(NH_3)_5CoOH^{2+}$, the calculated second-order rate constant being ~0.26 $M^{-1}\ s^{-1}$.

Discussion

In the presence of excess imidazolatopentaamminecobalt(III) ion in aqueous medium, 4-nitrophenyl acetate was rapidly cleaved to 4-nitrophenolate and acetate ions. The *N*-acetylimidazolepentaamminecobalt(III) complex is of sufficient lability for it to be involved as an undetected intermediate under the reaction conditions employed, though in aqueous–organic solvent mixtures it has been observed as a transient species. For the latter conditions the concentration of the ester was substantially larger than for the solely aqueous system and the opportunity to observe the intermediate was thereby enhanced. Also, for mole fractions of organic solvents varying between ~0.2 and 1.0 there was no evidence for a reaction pathway not involving $(NH_3)_5CoImCOCH_3^{3+}$. It is suggested therefore that in purely aqueous medium the reaction proceeds through the nucleophilic path shown in Figure 5a. This proposition is supported by the observation of a negligible solvent deuterium isotope effect. Indeed the mechanism is that anticipated on the basis of the pK_a of the base complex and the correlation between mechanism and pK_a observed by Jencks and Gilchrist[27] for the hydrolysis of 4-nitrophenyl acetate catalyzed by a wide variety of bases. Towards nitrophenyl acetate therefore, imidazolate anion coordinated to cobalt(III) is a nucleophile of activity comparable to free hydroxide ion. It is also of comparable activity to tertiary amine bases of similar pK_a. This implies that incorporation of the nucleophile into a complex with an overall positive charge has no marked effect on its capacities towards a neutral substrate, other than that arising from the change in base strength.

A small effect on reactivity due to coordination in a positively charged complex is seen in the rate of hydrolysis of complexed *N*-acetylimidazole in $(NH_3)_5CoImCOCH_3^{3+}$. Thus, attack of both water and hydroxide ion is accelerated by a factor ~20 relative to that on the free (uncharged) organic molecule.[28] In acid media the reactivity of *N*-acetylimidazole[28] is strongly inhibited by coordination to Co(III), reflecting a familiar difference in the capacities of metal ions and the proton as catalysts.

Though the cleavage of 4-nitrophenyl acetate in water is promoted far more effectively by $(NH_3)_5CoIm^{2+}$ than by $(NH_3)_5CoOH^{2+}$, conditions are readily obtained where all reaction of the ester is carried by a path involving the latter complex. The reaction products then observed are 4-nitrophenol and acetatopentaamminecobalt(III) ion only. As the acetato complex is hydrolytically stable under the reaction conditions[29] but is not formed in detectable amounts from $(NH_3)_5CoOH^{2+}$ (plus $(NH_3)_5CoOH_2^{3+}$) and acetate ion at the concentrations produced by ester hydrolysis, its presence demonstrates that $(NH_3)_5CoOH^{2+}$ acts solely as a nucleophile towards 4-nitrophenyl acetate.

While it is concluded that both coordinated imidazolate and hydroxide anions act as nucleophiles towards 4-nitrophenyl acetate, it is obvious that in detail there must be considerable differences in the processes involving the two species. The acetylated complexes differ greatly in their reactivity (particularly towards 4-nitrophenolate anion) owing to the facile loss of a proton from the coordinated hydroxide after addition to the electrophile. Intramolecular proton transfer, which may only occur for the hydroxo complex reaction (Figure 5b), may greatly facilitate breakdown of the addition intermediate to products and it is conceivable, therefore, that the rate determining steps in the processes involving the two nucleophiles may not be the same. No kinetic evidence was obtained for significant buildup of the addition intermediate in either reaction nor for any general acid function for the acid form of the complexes. The two reactions do differ markedly, however, in the sensitivity of the rates to solvent. The large rate enhancement observed for $(NH_3)_5CoOH^{2+}$ (~200-fold in DMF, ~500-fold in Me_2SO) contrasts strongly with the small effects for $(NH_3)_5CoIm^{2+}$ (~onefold and ~threefold increases, respectively). For free hydroxide ion an increase in reactivity of very large magnitude is observed on transferral from water to dipolar, aprotic solvents and has been ascribed to the inhibitory effect of the very strong solvation of hydroxide ion by water.[24] Simple acidoamine complexes of cobalt(III), however, show much stronger solvation by dipolar, aprotic solvents (such as Me_2SO) than by water,[30] and on a superficial basis, therefore, it would be expected that coordinated nucleophiles (in a cationic complex) would be more effective in water than, say, Me_2SO. Since $[(NH_3)_5CoOH](ClO_4)_2$ is more soluble (and therefore presumably better solvated) in Me_2SO than in water, the observation of its enhanced nucleophilic character in Me_2SO can be rationalized only by the assumption that solvation specifically of the hydroxide ligand is weaker in Me_2SO than in water. This is borne out by the observation that $(NH_3)_5CoOH^{2+}$ is a much stronger base in Me_2SO than in H_2O (pK_a = 14–16). The increased reactivity of $(NH_3)_5CoOH^{2+}$ parallels the increase in basic strength of this ion in Me_2SO relative to water. Moreover the relative rate changes for the two complexes closely parallel the relative basic strength changes in the solvents.

Any extrapolation of the present characterization of coordinated imidazolate and hydroxide nucleophiles to the active site of carbonic anhydrase involves numerous complications. Since we can find no evidence that the esterase activity of the enzyme is a biological function, it is possible that the esterase and hydrase functions involve different active groups, e.g., CO_2 hydration may occur via a $Zn-OH^+$ species and ester hydrolysis via $Zn-Im^+$. It may be of significance that the pH-rate profile for bovine carbonic anhydrase catalyzed hydrolysis of 4-nitrophenyl acetate shows evidence for two different ionizable catalytic groups differing in pK by ~4.[1,3] Given that the Zn^{2+} ion of carbonic anhydrase would show similar relative effects on the acidity of water and (histidine) imidazole to Zn^{2+} and first-row transition metal dipositive ions in simple model systems,[21] a simple interpretation of the esterase properties of the enzyme would be that the species active at lower pH is $ZnOH^+$, at higher pH is $Zn-Im^+$. This in turn would suggest that the active species in CO_2 hydration is $Zn-OH^+$. A major inadequacy in this interpretation is the enormous rate difference observed between the rate of attack of carbonic anhydrase and of, for example, $(NH_3)_5CoOH^{2+}$ on CO_2.[15] (Table V). Though the rate at which $(NH_3)_5CoIm^{2+}$ catalyzes the hydration of CO_2 is unknown, the present data suggest that it could exceed, even in a general base role, the rate observed for $(NH_3)_5CoOH^{2+}$. The present data also suggest, however, that some of the inadequacy of coordinated hydroxide as a model for carbonic anhydrase may be lost if the active site environment of the enzyme approximates more closely a dipolar aprotic medium than it does water. Though water must be present within the active site cleft, if its concentration is insufficient for any extended structuring to be possible, then the coordinated nucleophile may well be poorly solvated relative to a true aqueous environment.

Table V. Rate Constants for Hydration of CO_2 and Hydrolysis of 4-Nitrophenyl Acetate by Various Bases

	k ($M^{-1}\,s^{-1}$, 25 °C)	pK_a
CO_2 Hydration		
OH^-	8500 (15)[a]	15.5
Carbonic anhydrase	10^7–10^8 (3)	~8 (3)
$(NH_3)_5CoOH^{2+}$	220 (15)	6.4 (32)
H_2O	6.7×10^{-4} (15)	−1.7 (15)
$(NH_3)_5CrOH^{2+}$	$\lesssim 10$ (15)	5.2 (15)
$(NH_3)_5RhOH^{2+}$	470 (15)	6.78 (15)
$(NH_3)_5IrOH^{2+}$	590 (15)	6.70 (15)
4NPA Hydrolysis		
OH^-	9.5 (27)	15.5
Imidazole	0.58 (27)	14 (21)
$(NH_3)_5CoIm^{2+}$	9	10.02
Carbonic anhydrase	460 (2)	7.5 (2)
$(NH_3)_5CoOH^{2+}$	1.52×10^{-3}	6.4 (32)

[a] References in parentheses.

An important feature of the present work in relation to the "zinc-imidazolate" mechanism of Pesando[18] is that no effective general base activity has been found for $(NH_3)_5CoIm^{2+}$. This is also true for $(NH_3)_5CoOH^{2+}$ (towards 4-nitrophenyl acetate), though the most frequently proposed "zinc-hydroxyl" mechanism does not involve a general base role for the $Zn-OH^+$ moiety. Towards the natural enzyme substrate, CO_2, the general base activity of simple complexes in hydration is as yet poorly characterized, and may, of course, be quite different to that towards a substrate such as 4-nitrophenyl acetate. Though one imidazole N atom of the enzyme appears especially well placed[1] to act as a nucleophile at the entrance to the active site cavity and the prospect of it capturing CO_2 to form a carbamate is real enough, the evidence from studies of the decomposition of carbamates[20,31] is for heterolytic N–C fission rather than H_2O addition followed by elimination of HCO_3^- or H_2CO_3. Clearly such a fission would not catalyze the hydration and the enzyme would need to modify the chemistry if such a mechanism was valid. Thus, although the present results do not resolve confusion as to the chemical nature of the active site functional group in carbonic anhydrase (except by exclusion of the possible involvement of an imidazole carbanion), they do strongly imply a purely nucleophilic role for this group. It remains plausible that the enzyme may employ dual catalytic groups,[1] viz., the $ZnOH^+$ site may act to hydrate CO_2 and a $ZnIm^+$ site may be involved in esterase activity. Properties of the enzyme, such as its inhibition by the binding of sulfonamides and inorganic anions and by alkylation of histidine residues[1,3] may be rationalized for either group. Clearly, ligand binding could modify the acidity of coordinated imidazole as well as exclude hydroxide ion. Similarly, histidine alkylation could influence the acidity of $ZnOH_2^{2+}$ as well as preclude imidazole N deprotonation. Superficially, the deprotonated imidazole complex is quantitatively (within a factor of 100) a far more adequate model to mimic the esterase activity of carbonic anhydrase (in aqueous medium) than is the corresponding hydroxo complex. However, if the basic strength of the two coordinated bases pK 10.02 and 6.4,[32] respectively, are compared, the factor of $10^{3.6}$ between them largely accommodates the rate difference observed. Such an effect transposed to the enzyme could make either nucleophile effective ca. pH 7.

Some comment about the pK_a difference in H_2O seems warranted. The free acids have pK_a values of approximately 14 and 15.5 for imidazole and water (if the concentration of H_2O is taken into account). The smaller change for coordinated imidazole may reflect the distance of the basic atom from the positive metal center in keeping with effects observed with other coordinated ligands for example CO_3^{2-} and PO_4^{3-} [33] bound to first-row transition metal ions.[21,33] Note that the introduction of another imidazole into the complex, e.g., *cis*-$[Co(en)_2(ImH)_2]^{3+}$, does not substantially alter the pK_a for deprotonation of the first imidazole. Despite the problems in relating the results to the enzymic function these coordinated nucleophiles have some utility. They allow access to much higher concentrations of anionic nucleophiles such as the imidazolato ion than those accessible in aqueous solutions at low pH. The coordinated nucleophile ion is probably not as effective as the free ion, but most metal ions do not modify ligand properties as much as a proton. The ligand can therefore retain much of its basicity and nucleophilic capacity, more than the free protonated ligand for example.

The considerable residual basicity and nucleophilic character of coordinated hydroxide has been exploited in preparative reactions.[15,16,31,34] The especial efficacy of this nucleophile in Me_2SO (and other dipolar aprotic solvents) has not, however, been well recognized. Clearly its use under such conditions has much synthetic utility in the attachment of sensitive ligands to kinetically inert metal complexes provided an electrophilically active derivative of the ligand is available (e.g., a nitrophenyl ester of carboxylic acid). Several syntheses based on these principles have been described in the experimental section. Attack at a variety of unsaturated carbon residues by coordinated amide, imide, thiolate, and carbon nucleophiles as well as coordinated hydroxide have been observed[35] and numerous synthetic applications are envisaged.

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The Reduction of Molecular Nitrogen, Organic Substrates, and Protons by Vanadium(II)[1]

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Abstract: The reaction of molecular nitrogen with alkaline suspensions of $V(OH)_2$ in $Mg(OH)_2$ is shown to yield hydrazine via diimide, N_2H_2, as the intermediate. The yields of hydrazine depend critically on the V^{2+}/Mg^{2+} ratio in the hydroxide gels. At higher than optimal V^{2+} concentrations the yields of N_2H_4 decline and hydrogen is evolved instead. Coprecipitation of $V(OH)_2$ with ZrO_2·aq also produces gels which also reduce nitrogen to hydrazine, although less efficiently than $V(OH)_2$-$Mg(OH)_2$ systems. In the absence of a host lattice, only traces of hydrazine are formed by the reaction of nitrogen with $V(OH)_2$ while hydrogen production occurs to a greater extent. The reduction of acetylene to ethylene, or of ethylene to ethane by $V(OH)_2$ does not require a host lattice. Studies of the reaction stoichiometries reveal that $V(OH)_2$ acts as a two-electron reductant. Reduction of acetylene, ethylene, and, to a slight extent, of nitrogen was also found to occur in acidic solutions of V^{2+} (aq) on irradiation with uv light.

In part 1 of this series we reported on the evolution of H_2 from suspensions of $Fe(OH)_2$ and also described the reduction of a number of organic substrates as well as of molecular nitrogen.[2] We subsequently directed our attention to the reactions of $V(OH)_2$, a powerful reducing agent, which is known to undergo spontaneous decomposition with H_2 evolution.[3] Moreover, Shilov et al.[4,5] demonstrated that $V(OH)_2$, coprecipitated with $Mg(OH)_2$, reduces not only C_2H_2 to C_2H_4, but also N_2 to N_2H_4. The latter reaction has been the subject of extensive investigations, but its mechanism is as yet unknown. It has been suggested[6] that V^{2+} acts as a one-electron reductant and that its incorporation into the $Mg(OH)_2$ lattice favors an aggregation of four V^{2+}-ions. These "quadrupoles" were assumed to reduce N_2 directly to N_2H_4. A reaction of such high order should have very low statistical probability and is experimentally not well supported. Recent studies[7] with isotopically labeled N_2 revealed that the original N–N bond of the substrate remains intact during its reduction to N_2H_4. Attempts to detect the intermediacy of diimide, N_2H_2, did not produce conclusive results.[7] Since N_2H_2 has been shown to be