Tris(4,5-diisopropylimidazol-2-yl)phosphine:Zinc(2+). A Catalytically Active Model for Carbonic Anhydrase

R. S. Brown,* N. J. Curtis, and J. Huguet

Contribution from the Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2. *Received February 3, 1981. Revised Manuscript Received June 29, 1981*

Abstract: The title compound (**2c**:Zn^{2+}) displays many features in common with the Zn^{2+} metalloenzyme carbonic anhydrase (CA). Several physicochemical studies with **2c**:Zn^{2+} such as metal-binding affinity, Co^{II} spectral studies, and catalysis of $CO_2 \rightleftharpoons HCO_3^-$ interconversion indicate that it can be considered a reasonable, but deficient model for the active site of CA. On the basis of catalysis studies and anionic inhibition of catalysis, a mechanism for activity is proposed.

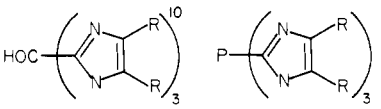
Carbonic anhydrase (CA) is a ubiquitous Zn^{2+}-containing metalloenzyme,[1] its only known physiological function being to catalyze the interconversion of CO_2 and HCO_3^-[1e] (eq 1). It has

$$CO_2 + H_2O \overset{CA}{\rightleftharpoons} H^+ + HCO_3^- \qquad (1)$$

however been shown to catalyze other processes in which nucleophilic attack of HO^- (H_2O) at an electrophilic center occurs[1g] such as hydration of aldehydes,[2a] pyruvic acid,[2b] and alkyl pyruvates[2c] and hydrolysis of some carboxylic, carbonic, sulfonic, and phosphoric esters.[3] Because of its fundamental importance to both plant and animal processes involving photosynthesis, calcification, pH maintenance, ion transport, and CO_2 exchange,[4] various forms of CA have been intensively studied and a variety of mechanisms of action has been presented[1,3d,5] which are compatible with most if not all of the available physicochemical data.[1]

Complete X-ray crystallographic structures[5f,6] have been determined for the human B and C isozymes (M_r = ~30000) and show the active site to be comprised of a Zn^{2+} ion coordinated in a distorted tetrahedral fashion by three histidine imidazoles: the fourth ligand site is said to be occupied by a coordinated H_2O or OH^- group which may be important for the catalytic process (Figure 1). This special environment around the metal is manifested in extremely powerful catalysis, the turnover numbers for CA being among the highest known. A number of investigators have studied smaller M^{2+} and M^{3+}-coordinating systems in order to cast some light on various enzyme functions such as catalysis,[7] known spectral,[8] and assorted physicochemical properties.[9] However, with the exception of a few studies,[8a,9a-c] no small systems have been reported which attempt to mimic the known Zn^{2+} binding site in CA.

Recently we reported[9a,b] the synthesis and physical studies of a variety of tridentate ligands consisting of three imidazole residues anchored (via C-2) to a central carbinol[9a,10] or phosphine[9b] center (**1** and **2**, respectively) which appeared from molecular models



1 2

a, R = H; b, R = CH_3; c, R = $CH(CH_3)_2$

to be reasonable mimics for the binding cavity in CA. It became evident from NMR studies that for **1a**, deleterious 2:1 (**1a**:Zn^{2+}) complex formation occurred in solution, but this could be prevented by placing large buttressing groups at the 4 and 5 ring positions. Unfortunately, the corresponding carbinol system (**1c**) was quite labile under basic conditions when complexed to metal, leading to highly colored materials which were likely formed by dehydration of the somewhat strained **1c**:Zn^{2+} complex. It appeared that the corresponding phosphine[11] (**2c**) would circumvent both the dehydration and complex strain problems, the latter being aleviated by the fact that the P-C_2 bond length in **2** is longer than the HOC-C_2 bond length in **1** (~1.8 Å vs. 1.5 Å), allowing a

(1) For reviews of carbonic anhydrase see: (a) Lindskog, S.; Henderson, L. E.; Kannan, K. K.; Liljas, A.; Nyman, P. O.; Strandberg, B. "The Enzymes"; Boyer, P. D., Ed.; Academic Press: New York; 1971, Vol. 5, pp 587-665. (b) Coleman, J. E. In "Progress in Bioorganic Chemistry"; Kaiser, E. T.; Kézdy, F. J., Eds.; Wiley-Interscience: New York; 1971; Vol. 1, pp 159-344. (c) Coleman, J. E. In "Inorganic Biochemistry"; Eichorn, G. L., Ed.; Elsevier: 1973; Vol. 1, pp 488-548. (d) Dunn, M. F. *Struct. Bonding (Berlin)* **1975**, *23*, 61-122. (e) Pocker, Y.; Sarkanen, S. In "Advances in Enzymology"; Meister, A., Ed.; Wiley: New York; 1978; Vol. 47, pp 149-274. (f) Wyeth, P.; Prince, R. H. *Inorg. Perspect. Biol. Med.* **1977**, *1*, 37-71. (g) Prince, R. H. In "Advances in Inorganic Chemistry and Radiochemistry"; Emeléus, H. J., Sharpe, A. G., Eds.; Academic Press: New York, 1979; Vol. 2, pp 349-440. (h) Golding, B. T.; Leigh, G. J. *Inorg. Biochem.* **1979**, *1*, 50. (i) Galdes, A.; Hill, H. A. O. *Ibid.* **1979**, *1*, 319. (j) Bundy, H. F. *Comp. Biochem. Physiol. B* **1977**, *57B*, 1-7.

(2) (a) Pocker, Y.; Meany, J. E. *J. Am. Chem. Soc.* **1965**, *87*, 1809-1811. (b) Pocker, Y.; Meany, J. E. *J. Phys. Chem.* **1970**, *74*, 1486-1492. (c) Pocker, Y.; Meany, J. E.; Davis, B. C. *Biochemistry* **1974**, *13*, 1411-1416.

(3) (a) Tashian, R. E.; Plato, C. C.; Shows, T. B. *Science (Washington, D.C.)* **1963**, *140*, 53-54. (b) Pocker, Y.; Bjorkquist, L. C.; Bjorkquist, D. W. *Biochemistry* **1977**, *16*, 3967-3973. (c) Malmström, B. G.; Nyman, P. O.; Strandberg, B.; Tilander, B. In "Structure and Activity of Enzymes" FEBS-*Symp.* **1964**, *No. 1*, 121. (d) Lo, K. W.; Kaiser, E. T. *J. Am. Chem. Soc.* **1969**, *91*, 4912-4918.

(4) Tashian, R. E.; Goodman, M.; Tanis, R. J.; Ferre, R. E.; Osborne, W. R. A. In "The Isozymes"; Markert, C. L., Ed.; Academic Press: New York; 1975; Vol. 4, pp 207-223.

(5) (a) Cambell, I. D.; Lindskog, S.; White, A. I. *Biochim. Biophys. Acta* **1977**, *484*, 443-452. (b) Coleman, J. E. *J. Biol. Chem.* **1967**, *242*, 5212-5219. (c) Khalifah, R. G. *Ibid.* **1971**, *246*, 2561-2573. (d) Wang, J. H. *Proc. Natl. Acad. Sci. U.S.A.* **1970**, *66*, 874-881. (e) Riepe, M. E.; Wang, J. H. *J. Biol. Chem.* **1968**, *243*, 2779-2787. (f) Kannan, K. K.; Petef, M.; C-d-Dresdner, H.; Lövgren, S. *FEBS Lett.* **1977**, *73*, 115-119. (g) Pesando, J. M. *Biochemistry* **1975**, *14*, 681-688. (h) Gupta, R. K.; Pesando, J. M. *J. Biol. Chem.* **1975**, *250*, 2630-2634. (i) Pocker, Y.; Storm, D. R. *Biochemistry* **1968**, *7*, 1202-1214.

(6) (a) Notstrand, B.; Vaara, I.; Kannan, K. K. In "The Isozymes"; Markert, C. L. Ed.; Academic Press: New York; 1975; Vol. 1, pp 575-599. (b) Kannan, K. K.; Notstrand, B.; Fridborg, K.; Lövgren, S.; Ohlssen, A.; Petef, M. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 51-55. (c) Liljas, A. et al. *Nature (London), New Biol.* **1972**, *235*, 131-137. (d) Kannan, K. K. et al. *Cold Spring Harbour Symp. Quant. Biol.* **1971**, *36*, 221-231.

(7) (a) Tabushi, I.; Kuroda, Y.; Mochizuki, A. *J. Am. Chem. Soc.* **1980**, *102*, 1152-1153. (b) Wooley, P. *Nature (London)* **1975**, *258*, 677-682. (c) Pocker, Y.; Meany, J. E. *J. Am. Chem. Soc.* **1967**, *89*, 631-636. (d) Chaffee, E.; Dasgupta, T. P.; Harris, G. M. *Ibid.* **1973**, *95*, 4169-4173. (e) Palmer, D. A.; Harris, G. M. *Inorg. Chem.* **1974**, *13*, 965-969. (f) Harrowfield, J. McB.; Norris, V.; Sargeson, A. M. *J. Am. Chem. Soc.* **1976**, *98*, 7282-7289.

(8) (a) Bertini, I.; Canti, G.; Luchinat, C.; Mani, F. *Inorg. Chim. Acta* **1980**, *46*, 91-92. (b) Bertini, I.; Canti, G.; Luchinat, C.; Scozzafava, A. *J. Am. Chem. Soc.* **1978**, *100*, 4873-4877. (c) Navon, G.; Shinar, H. *Inorg. Chim. Acta* **1980**, *46*, 51-55. (d) For a review of the reporter properties of Co^{II} as a function of ligation see: Ciampolini, M. *Struct. Bonding (Berlin)* **1969**, *6*, 52-93 and ref 1d.

(9) (a) Brown, R. S.; Huguet, J. *Can. J. Chem.* **1980**, *58*, 889-901. (b) Brown, R. S.; Huguet, J. *J. Am. Chem. Soc.* **1980**, *102*, 7471-7572. (c) Tang, C. C.; Davalian, D.; Huang, P.; Breslow, R. *Ibid.* **1978**, *100*, 3918-3922. (d) Appleton, D. W.; Sarkar, B. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 1686-1690.

(10) The tris imidazolyl carbinols were initially reported by Breslow et al.[9c]

(11) Curtis, N. J.; Brown, R. S. *J. Org. Chem.* **1980**, *45*, 4038-4040.

0002-7863/81/1503-6953$01.25/0

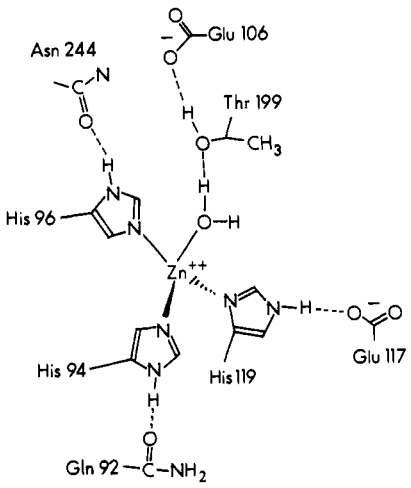


Figure 1. Schematic representation of the active site of carbonic anhydrase showing the Zn^{2+} binding site and H-bonding network.[5a]

nearly strain-free insertion of the Zn^{2+} into the cavity. Preliminary studies[9b] with **2c** indicated not only did stable 1:1 (**2c**:M^{2+}) complexes form but also that they showed many features in common with CA including the ability to catalyze the interconversion of CO_2 and HCO_3^-. Herein we report more complete studies which show that **2c**:Zn^{2+} can be considered a reasonable (albeit imperfect) synthetic enzyme specific for catalyzing the process in eq 1.

Experimental Section

Phosphine **2c** was prepared and purified as previously described.[11] Ligand pK_a and p$K_{M^{2+}}$ values were determined by potentiometric titration using methods and equipment described[9a] except for reasons of solubility a solvent of 80% ethanol/H_2O was used throughout. pH values were simply those read from a Radiometer Model GK2322C combination electrode directly immersed in the mixture and are uncorrected for the high organic content of the medium. Metal solutions were made from their perchlorate salts and were standardized by EDTA titration.[12] Ionic strength was maintained at 0.2 M with $NaClO_4$ or NaCl. UV-visible spectra were recorded with a Cary Model 210 spectrophotometer.

Kinetic Studies. Kinetic determinations of the reaction rates for establishing the equilibrium between CO_2 and HCO_3^- were performed on an Aminco-Morrow stopped-flow system under pseudo-first-order conditions, the data being analyzed by an analogue comparison technique. Absorbance vs. time curves were stored in a Tracor NS-570 signal averager and then output to a dual trace oscilloscope for comparison with a synthetic exponential curve, the time constant of which could be changed by adjusting a variable resistance in the circuit. Since neither CO_2 nor HCO_3^- have easily monitored spectral properties, well-established indicator techniques[5c,13] are used whereby the change in $[H^+]$ accompanying CO_2 hydration or HCO_3^- dehydration is monitored by observing changes in absorbance of an indicator (A^-) whose response to $\Delta[H^+]$ is rapid relative to the reaction in question (eq 2). Generally CO_2

$$A^- + CO_2 + H_2O \overset{\text{slow}}{\rightleftharpoons} HCO_3^- + H^+ + A^- \overset{\text{fast}}{\rightleftharpoons} HCO_3^- + HA \quad (2)$$

hydration or HCO_3^- dehydration has been studied by an initial rate method whereby the course of the reaction is monitored for the first few percent to record only the initial linear portion, and then plotting absorbance vs. time in a zero-order fashion to yield the rate constants in one or the other direction. For the present work, HEPES or MES buffers held $[H^+]$ essentially constant so that the reactions are run under pseudo-first-order conditions. Of course $[H^+]$ must vary to some minor extent since this provides the observable for the kinetic analysis, but under appropriate buffering conditions $\Delta[H^+]$ can be held to less than 0.04 pH unit (8%). Under these conditions, eq 1 reduces to a typical first-order equilibrium reaction (eq 3), where x and x_e denote the concentration of

$$CO_2 \underset{k_{debyd}}{\overset{k_{byd}}{\rightleftharpoons}} HCO_3^- \quad (3)$$

$$\frac{d_x}{d_t} = k_{obsd}(x_e - x) \quad (3a)$$

the product of the reaction (HCO_3^- for hydration and CO_2 for dehydration) at time t and at equilibrium, respectively. Thus $k_{obsd} = (k_{byd} + k_{debyd})$, the sum of all the forward and reverse rate constants which includes those dependent upon the various forms of the buffer[13d,14] and other species present in the medium.

A typical experiment was performed as follows. Into one drive syringe was placed a solution consisting of 10^{-4} M indicator (bromocresol purple BCP), 5×10^{-2} M buffer (HEPES pH 7.3–6.4; MES pH 6.4–6.1) adjusted to the desired pH reading by addition of 1 M NaOH, and the appropriate amount of $NaClO_4$ to bring the ionic strength to 0.2 M. The second drive syringe contained a solution of 10^{-3} M $NaHCO_3$ (prepared from a stock 1 M solution) or CO_2 (prepared by ~5-fold dilution of a saturated 80% ethanol/H_2O solution), ionic strength being maintained at 0.2 M with $NaClO_4$. The solvents in both syringes consisted of 80% ethanol/H_2O (21.05 mL of 95% ethanol, 3.95 mL of triply distilled H_2O). Hydration or dehydration reactions were monitored by observing the disappearance or appearance, respectively, of the BCP anion at 600 nm after rapid mixing at 25.0 ± 0.2 °C. Control experiments performed as above but by using no $NaHCO_3$ or CO_2 in the second syringe showed no absorbance change other than that attributable to rapid dilution, thereby demonstrating that the various acid–base equilibria were established rapidly relative to the reaction in question.

In order to assess the effect of added catalyst, we used solutions as those above except that between 5 and 15×10^{-4} M in each of phosphine **2c** and $Zn(ClO_4)_2$ were added to syringe 1 maintaining the ionic strength at 0.2 M ($NaClO_4$). Catalyst solutions were made up immediately prior to use since it was observed that k_{cat}^{obsd} values diminished if the solutions were allowed to stand overnight. Control experiments using catalyst but no $NaHCO_3$ or CO_2 in the second syringe again showed no absorbance changes at 600 nm other than those attributable to dilution. Values of second-order rate constants (k_{cat}^{obsd}) were obtained as in eq 4, where k_{obsd}

$$k_{cat}^{obsd} = \frac{k'_{obsd} - k_{obsd}}{[cat]} \quad [cat] = [\mathbf{2c}{:}Zn^{2+}] \quad (4)$$

and k'_{obsd} are the pseudo-first-order rate constants ($k_{byd} + k_{debyd}$) in the absence and presence of catalyst, respectively, and [cat] is taken to be that in syringe 1 corrected for a dilution factor of 2 on mixing, assuming that [**2c**:Zn^{2+}] is completely formed.[15]

Inhibition studies were performed at a measured pH of 6.4 in the dehydration direction, by placing known concentrations of inhibitor into syringe 1, and the data analyzed as before.

Results and Discussion

CA exhibits a number of unusual physicochemical properties which can be compared with those exhibited by the models. If a model can be shown to mimic to a reasonable degree known properties of the enzyme, then extrapolation of new results obtained from the model to unknown or ambiguous properties of the enzyme might be considered to be on safer grounds. We have therefore investigated such features as metal binding, Co^{II} absorption spectra as a function of pH and anions present, and the ability to catalyze the interconversion of CO_2 and HCO_3^-.

(a) Metal-Binding Studies. To be considered a good model for the active site of CA, **2c** must be shown to bind Zn^{2+} (or Co^{2+}) strongly in a tridentate fashion. pK_a and p$K_{M^{2+}}$ values of **2c** obtained by potentiometric titration methods[9a] under the same initial conditions used for subsequent kinetic measurements are presented in Table I. Clearly under all conditions, **2c** binds Zn^{2+} more strongly than Co^{2+} paralleling the situation known for the enzyme,[16] which may be a result of enforced four-coordinate geometry.[9a] Both Zn^{2+} and Co^{2+}-binding affinity of the enzyme

(12) Flaschka, H. A. In "EDTA Titrations", 2nd ed.; Pergamon Press: New York, 1964.

(13) (a) Kernohan, J. C. *Biochim. Biophys. Acta* **1965**, *96*, 304–317. (b) Gibbons, B. H.; Edsall, J. T. *J. Biol. Chem.* **1963**, *238*, 3502–3507. (c) Ho, C.; Sturtevant, J. M. *Ibid.* **1963**, *238*, 3499–3501. (d) Pocker, Y.; Bjorkquist, D. W. *J. Am. Chem. Soc.* **1976**, *98*, 6357–6353. (e) Khalifah, R. G.; Edsall, J. T. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 172–176. (f) DeVoe, H.; Kistiakowsky, G. B. *J. Am. Chem. Soc.* **1961**, *83*, 274–279.

(14) Roughton, F. J. W.; Boothe, V. H. *Biochem. J.* **1938**, *32*, 2049.

(15) Since our **2c**:Zn^{2+} binding constants determined by potentiometric titration are $10^{6.0}$ (Table I), under the experimental condition >98% of **2c** should be bound as its 1:1 (**2c**:Zn^{2+}) complex. Since k_{cat}^{obsd} is independent of [cat] for the range of concentrations studied, we feel that the supposition is justified.

(16) (a) Lindskog, S. J. *Biol. Chem.* **1963**, *238*, 945–951: p$K_{Zn^{2+}}$ = 10.5; p$K_{Co^{2+}}$ = 7.2. (b) Lindskog, S.; Nyman, P. O. *Biochim. Biophys. Acta* **1964**, *85*, 462–474.

(17) Read, R.; James, M. N. G., preceding paper in this issue.

Table I. pK_a and $pK_{M^{2+}}$ Values for **2c** Determined by Potentiometric Titration[a,b]

	pK_{a_1}	pK_{a_2}	pK_{a_3}	$pK_{Zn^{2+}}$	$pK_{Co^{2+}}$
0.2 M $NaClO_4$	6.66 ± 0.07	4.36 ± 0.01	2.55 ± 0.03	6.00 ± 0.04	3.48 ± 0.08
0.2 M NaCl	6.45 ± 0.05	4.43 ± 0.04	3.12 ± 0.02	7.71 ± 0.06	4.30 ± 0.15

[a] pK values determined in 80% ethanol/H_2O as in ref 9a. [b] 2c:Zn $\rightleftarrows$ 2c + Zn^{2+} ($K_{Zn^{2+}}$).

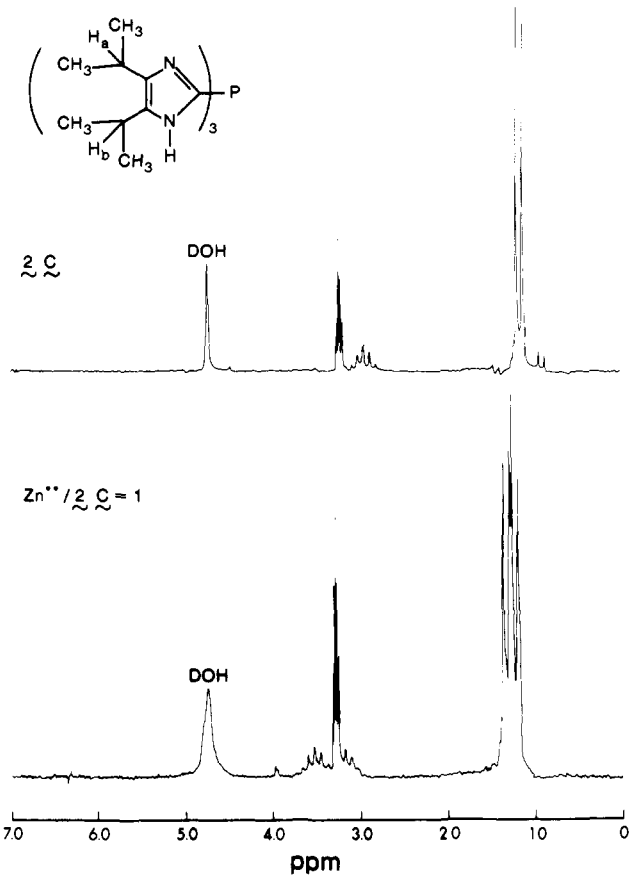
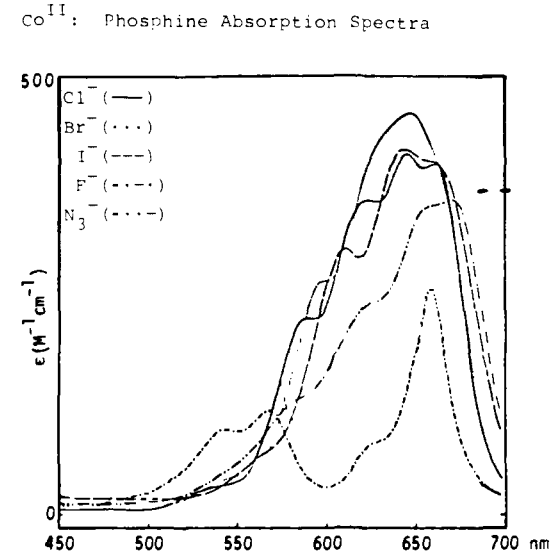


Figure 2. 100-MHz ^1H NMR spectra of **2c** (toptrace) and its 1:1 complex (**2c**:Zn^{2+}), solvent system methanol-d_4/D_2O.



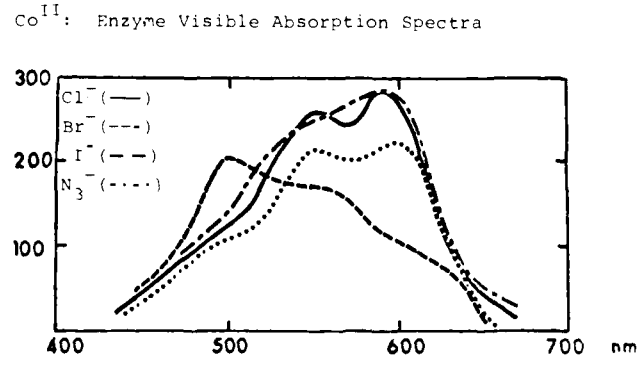


Figure 3. Anion-dependent visible spectra of **2c**:Co^{II} determined in 80% ethanol/H_2O solution saturated with NaX (X = I, Br, Cl, F), compared with that of Co^{II}:CA.

is substantially greater than that of **2c** ($pK_{Zn^{2+}}{}^{E}$ = 10.5, $pK_{Co^{2+}}{}^{E}$ = 7.2),[16] although strict numerical comparison is tenuous since a highly organic solvent was used for measurements here. With use of ClO_4^- as a supporting counterion, the $pK_{M^{2+}}$ values are lower than when Cl^- is used. Curiously in the presence of ClO_4^-, no characteristic blue color was observed during the course of Co^{2+} titration, indicating little formation of four- or five-coordinate species. On the other hand, with Cl^- as a counterion the situation was markedly different with deep blue colors being observed above pH 5.5. These observations as well as the fact that the pK_M values are larger with Cl^- than ClO_4^- indicate that Cl^- somehow stabilizes complex formation of **2c**:M –Cl, a fact that will be important when inhibition studies are discussed (vide infra).

^1H NMR analysis of 0.026 M **2c** in methanol-d_4/D_2O shows that the spectrum changes as a function of added $Zn(Br)_2$, signals attributable to 1:1 (**2c**:Zn^{2+}) appearing at the expense of those for free **2c** as is shown in Figure 2. Further additions of $Zn(Br)_2$ caused no additional changes in the spectrum.

The observation that there are two equal intensity but chemical shift inequivalent sets of isopropyl groups in the 1:1 complex indicates that rapid debinding and rebinding of the imidazoles is not occurring since this should give rise to tautomerically equivalent isopropyls as in the upper trace. Similarly, the symmetry displayed in the lower trace rules out binding situations involving two imidazoles and the phosphine center. Molecular models suggest that the isopropyl groups encapsulate the Zn^{2+} sufficiently to prevent 2:1 (**2c**:Zn^{2+}) complexation, and the NMR spectrum provides no evidence for such species. Finally we note that X-ray crystallographic analysis of **2c**:Zn^{2+}–Cl^- indicates that the metal geometry is indeed distorted tetrahedral with N–Zn^{2+}–N angles of 94–96°.[17]

(b) Co^{II} Absorption Spectra. The Zn^{2+} of native CA can be removed by dialysis of the enzyme against 1,10-phenanthroline in acetate buffer (pH 5),[18a] or more rapidly by EDTA.[18b] Other metals can be inserted into the apoenzyme[16] but significant restoration of catalytic activity is only observed with Co^{II} (~45% of the native enzyme[18a]). Co^{II}CA appears as a reddish blue derivative and displays a complex absorption spectrum characteristic of four- and/or five-coordination about the metal.[8b,16a,19] One general feature of Co^{II}CA is the anion dependence of the absorption spectra[16a,8b] again analyzed in terms of equilibrium distributions of pseudotetrahedral and five-coordinate species,[8b] the latter situation being evidenced by the presence of a low intensity band between 710 and 850 nm as well as low to moderate intensity bands ($40 < \epsilon < 200$ M^{-1} cm^{-1}) between 480 and 650 nm. Bertinis' analysis[8b] of the pH dependence of the Co^{II} spectra

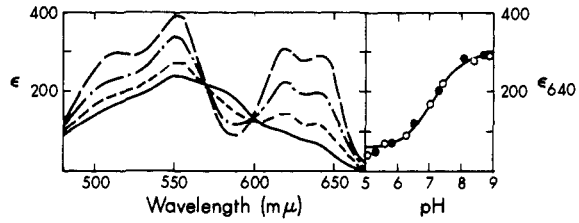
(18) (a) Lindskog, S.; Malström, B. G. *J. Biol. Chem.* **1962**, *237*, 1129–1137. (b) Hunt, J. B.; Rhee, M.-J.; Storm, C. B. *Anal. Biochem.* **1977**, *79*, 614–617.

(19) (a) Bertini, I.; Luchinat, C.; Scozzafava, A. *Inorg. Chim. Acta* **1980**, *46*, 85–89. (b) Jacob, G. S.; Brown, R. D.; Koenig, S. H. *Biochemistry* **1980**, *19*, 3754–3765.

Table II. Pseudo-First-Order Rate Constants for $CO_2 + H_2O \rightarrow$ Hydration[a-c]

pH	k_{obsd}, s^{-1} [0][e]	[2.5][e]	[5.0][e]	k_{cat}^{obsd},[d] M^{-1} s^{-1}
7.30 (HEPES)	0.235 ± 0.002	0.380 ± 0.002	0.548 ± 0.003	626 ± 10
7.00 (HEPES)	0.446 ± 0.003	0.635 ± 0.006	0.818 ± 0.014	744 ± 34
6.70 (HEPES)	0.733 ± 0.008	0.917 ± 0.004	1.118 ± 0.012	770 ± 40
6.40 (HEPES)	1.263 ± 0.028	1.539 ± 0.026	1.712 ± 0.031	898 ± 118

[a] Followed by observing change in absorbance of bromocresol purple at 600 nm, $T = 25 \pm 0.2$ °C; ionic strength = 0.2 M ($NaClO_4$), 80% EtOH/H_2O (v/v). [b] pH changes <0.04 unit; 2.5×10^{-2} M buffer. [c] CO_2 solutions made by dilution of a stock saturated CO_2 solution. k_{obsd} is independent of variations in $[CO_2]$. [d] $k_{cat}^{obsd} = (k'_{obsd} - k_{obsd})/[cat]$. [e] [Catalyst] $\times 10^4$ M.



Co" CA Visible Absorption Spectrum at Different pH's.
S. Lindskog, J. Biol. Chem. 238, 945 (1963)

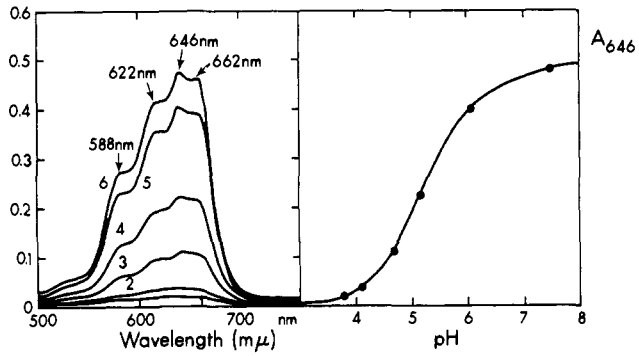


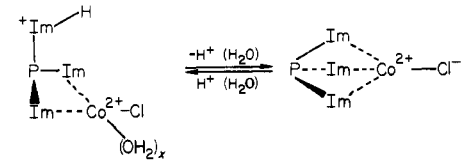
Figure 4. pH-dependent UV-vis spectra of $Co^{II}CA$[16a] (adapted from ref 16a) and **2c**:$Co^{II}Cl$.

indicates that the alkaline form is predominantly distorted pseudotetrahedral while the acidic form is apparently in equilibrium between four- and five-coordinate.

Preliminary results[9b] concerning the absorption spectra of **2c**:Co^{II} demonstrated a marked anion dependence (Figure 3) of what appears to be predominantly four-coordinate species, based on the relatively large ϵ_{max} values observed. In the presence of NO_3^- of ClO_4^- counterions, the spectra are not indicative of four-coordination but are more consistent with five- and/or six-coordinate Co^{II} although due to instrumental limitations we do not know if bands are present above 700 nm. This may indicate that the cavity of **2c**:Zn^{2+} is flexible enough to allow access to one, two, or three additional ligands other than phosphine depending on the counterion, a fact which may be important if the transition state for CO_2 hydration (bicarbonate dehydration) is five-coordinate. However, since Co^{II} does not appear to bind to **2c** as strongly as does Zn^{2+} and both $pK_{M^{2+}}$ values appear to be anion dependent (Table I), one cannot be certain how much **2c**:Co^{II} complex is present under these conditions.

Originally, Lindskog[16a] looked at the spectral properties of Co^{II}:CA as a function of pH and reported that the pH vs. absorbance profile (Figure 4a) depended upon a group with pK_a = ~7.1. Later work by Bertini et al.[19a] showed in the presence of noninteracting buffers or in ion-free solutions the absorbance vs. pH profile is not as simple as that expected for a single dissociating group. The spectrophotometric pK_a of $Co^{II}CA$ appears to be markedly dependent upon anion concentration, changing from 5.6 to 7.9 as $[Cl^-]$ increases from 0 to 0.6 M.[19b] These results suggest that the pK_a of the group controlling the characteristic Co^{II} blue color increases as the amount of negative charge in the active site increases, although ionic strength factors or mass action effects cannot be specifically ruled out.

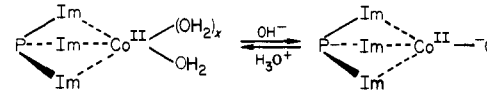
In Figure 4b is presented the reversible absorbance vs. pH profile for **2c**:$Co^{II}Cl^-$ (generated from $CoCl_2$) in an unbuffered medium, pH being adjusted by aliquots of 1 N NaOH. The profile suggests that the formation of the four-coordinate complex is in this case tied to the dissociation of a single group (pK_a = 5.6) as shown in eq 5. At progressively lower pH, protonation of an



(5)

$x = 2, 3$

imidazole causes its debinding, opening up additional ligand sites on the metal allowing five- or six-coordination depending upon the affinity of the now protonated phosphine for metal. The fact that one sees no analogous pH absorbance profile when $Co(ClO_4)_2$ is the source of metal again indicates that the counterion is responsible for the observed four-coordination with **2c**:Co^{II}. Space-filling models suggest that ClO_4^- is too large to fit into the fourth ligand site without severe buttressing with the isopropyl groups. Hence it is unlikely that the appearance of four-coordinate species with **2c**:Co^{II} is simply related to titration of a Co^{II} bound H_2O as in eq 6, although that eventuality is not ruled out for the enzyme.



(6)

$x = 0, 1$

Further studies on the pH dependence of the $Co^{II}CA$ absorption spectrum in the presence of various anions should shed light on this question.[19b]

(c) Catalytic Studies. As stated above, although the only known function of CA is to assist interconversion of CO_2 and HCO_3^-, it will at a reduced rate catalyze the hydration of some aldehydes and hydrolysis of esters such as *p*-nitrophenyl acetate (pNPA). We have as yet been unable to demonstrate that **2c**:Zn^{2+} will catalyze the hydrolysis of pNPA[20] likely due to steric restrictions which prevent the ester carbonyl from closely approaching the Zn^{2+}. However, **2c**:Zn^{2+} will efficiently catalyze the reaction in eq 1 approaching equilibrium from either direction between measured pHs of 6.1 and 7.3.

CO_2 hydration itself is a relatively difficult process to monitor at pH values <7; however, the sum of the pseudo-first-order hydration and dehydration rate constants (k_{obsd}) can be evaluated at various initial pHs as described in the Experimental Section and are presented in Table II. Values of k_{obsd} are averages of at least six determinations and are seen to increase as the pH drops from 7.3 to 6.4, showing some acid catalysis. Differences between k_{obsd} and k'_{obsd} (the observed sum of rate constants in the presence of catalyst) are used to evaluate k_{cat}^{obsd}. Since k'_{obsd} is seen to be linearly dependent upon [cat] some bimolecular process involving cat and CO_2/HCO_3^- is indicated.

In Table III are presented the analogous data approaching equilibrium from the HCO_3^- dehydration direction between initial

(20) Huguet, J.; Brown, R. S., unpublished results.

Table III. Psdueo-First-Order Rate Constants for $HCO_3^- + H^+ \rightarrow$ Dehydration[a,b]

pH	k_{obsd}, s^{-1} [0][d]	[2.5][d]	[5.0][d]	[7.5][d]	k_{cat}^{obsd},[c] M^{-1} s^{-1}
7.30 (HEPES)	0.190 ± 0.001	0.328 ± 0.004	0.426 ± 0.006	0.546 ± 0.009	472 ± 14
7.00 (HEPES)	0.295 ± 0.006	0.419 ± 0.003	0.578 ± 0.004	0.737 ± 0.005	566 ± 20
6.70 (HEPES)	0.455 ± 0.006	0.615 ± 0.012	0.778 ± 0.009	0.918 ± 0.008	646 ± 30
6.40 (HEPES)	0.822 ± 0.007	1.019 ± 0.012	1.272 ± 0.025	1.506 ± 0.019	900 ± 64
6.40 (MES)	0.903 ± 0.012	1.103 ± 0.016	1.295 ± 0.021		784 ± 66
6.20 (MES)	1.274 ± 0.014	1.434 ± 0.030	1.585 ± 0.022		622 ± 72
6.10 (MES)	1.480 ± 0.014	1.581 ± 0.024	1.708 ± 0.035		456 ± 98

[a] 80% EtOH/H_2O (v/v); 25° ± 0.2 °C; 5×10^{-4} M $NaHCO_3$; 2.5×10^{-2} M HEPES or MES buffer; ionic strength = 0.2 M ($NaClO_4$). [b] Followed by monitoring the increase in absorbance at 600 nm for bromocresol purple indicator (5×10^{-5} M), pH determined directly from electrode immersed in solution. pH changes held to be <0.04 unit. [c] $k_{cat}^{obsd} = (k'_{obsd} - k_{obsd})/[cat]$. [d] [Catalyst] $\times 10^4$ M.

Table IV. Control Experiments for $HCO_3^- + H^+ \rightarrow$ Dehydration Reaction,[a] pH 6.40 (HEPES)[a]

run no.	[indicator],[b] M	[phosphine],[c] M	[Zn^{2+}],[d] M	k_{obsd}, s^{-1}
1	5×10^{-5}	0	0	0.822 ± 0.007
2	5×10^{-5}	5×10^{-4}	0	0.833 ± 0.006
3	5×10^{-5}	0	5×10^{-4}	0.831 ± 0.004
4	5×10^{-5}	5×10^{-4}	5×10^{-4}	1.272 ± 0.025
5	2×10^{-4}	5×10^{-4}	5×10^{-4}	1.277 ± 0.008

[a] $T = 25 \pm 0.2$ °C; pH change <0.04 unit, monitored at 600 nm. Ionic strength = 0.2 M ($NaClO_4$); pH 6.40 (HEPES). [b] Bromocresol purple. [c] Tris(4,5-diisopropylimidazol-2-yl)phosphine. [d] $Zn(ClO_4)_2$.

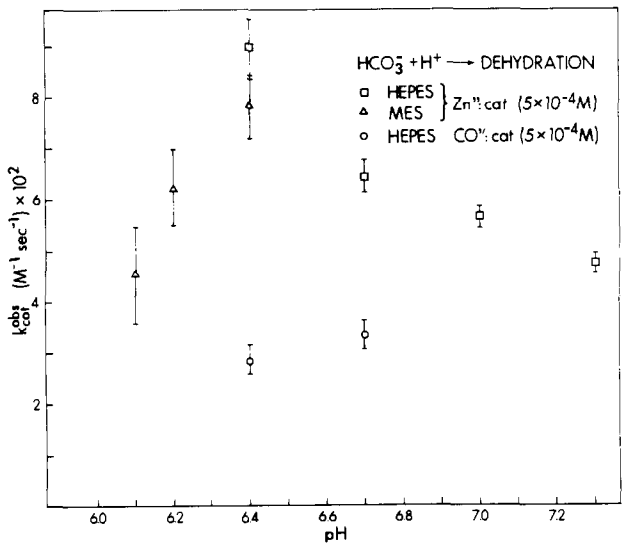


Figure 5. Second-order k_{cat}^{obsd} values for **2c**:Zn^{2+} catalysis of $HCO_3^- \rightleftharpoons CO_2$ under pseudo-first-order conditions (T = 25 ± 0.2 °C; μ = 0.2 M ($NaClO_4$)): HEPES buffers (□); MES buffers (△); **2c**:Co^{II}(HEPES) (□).

pHs of 7.4 and 6.1. The fact that k_{obsd} values (pH 6.4) for MES and HEPES are slightly different indicates that some buffer catalysis may be present under these conditions although the k_{cat}^{obsd} values are within experimental error. Second-order rate constants

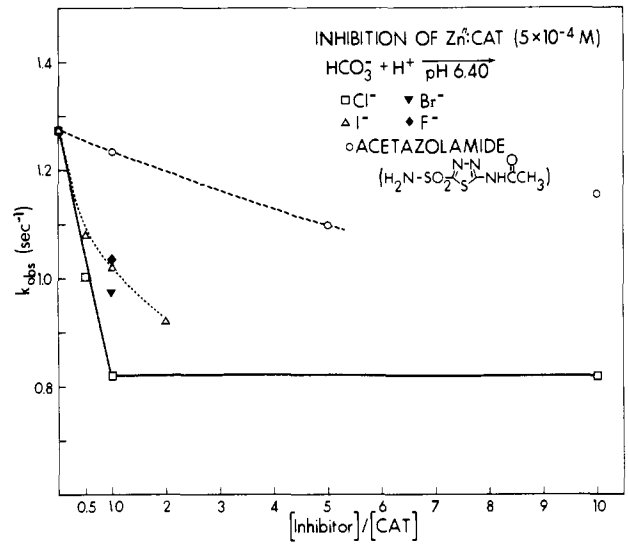


Figure 6. Effect of inhibitors on k'_{obsd} as assessed from $HCO_3^- \rightarrow$ dehydration (pH 6.40, HEPES; data of Table V).

(k_{cat}^{obsd}) are seen to maximize at initial pH 6.40, the effect being graphically illustrated in Figure 5.

A few points are worthy of consideration. In principle the k_{obsd} values in Tables II and III should be the same approaching equilibrium from either direction since in both cases $k_{obsd} = (k_{hyd} + k_{dehyd})$. In fact, the data indicate that k_{obsd} in the dehydration direction is always slightly less than that in the hydration direction. The anomaly can be resolved if one considers that during the hydration reaction H^+ is being released, while in the reverse direction the net [H^+] diminishes slightly. Since k_{obsd} appears to be dependent upon [H^+], then although the initial pHs may be identical, the final values diverge by as much as 0.08 unit depending upon whether the course of reaction is followed in the forward (e.g., pH 6.40 → 6.36) or reverse (pH 6.40 → 6.44) directions, leading to the disparity in k_{obsd} between Tables II and III. The effects are also augmented when one considers that the various [B^-] and [BH] components of the buffer can catalyze the process and are themselves changing in response to Δ[H^+]. Hence a reasonable approximation to the "true" k_{obsd} values which attempts to correct for the pH divergence might be the average of

Table V. Inhibitor Effects on k_{obsd} for $HCO_3^- + H^+ \rightarrow$ Dehydration[a]

inhibitor	k_{obsd}, s^{-1} 0[c]	0.5[c]	1.0[c]	2.0[c]	5.0[c]	10.0[c]	20.0[c]
NaCl	1.272 ± 0.025	1.002 ± 0.007	0.820 ± 0.004			0.818 ± 0.006	0.829 ± 0.009
NaBr	1.272 ± 0.025		0.977 ± 0.006				
NaI	1.272 ± 0.025	1.081 ± 0.007	1.018 ± 0.006	0.918 ± 0.016			
NaF	1.272 ± 0.025		1.029 ± 0.006				
acetazolamide[b]	1.272 ± 0.025		1.234 ± 0.013	1.097 ± 0.007	1.116 ± 0.014		

[a] $T = 25.0 \pm 0.2$ °C; ionic strength = 0.2 M ($NaClO_4$); bromocresol purple indicator, 600 nm, 80% EtOH/H_2O; [5×10^{-4} M, Zn:Cat]; maximal pH change <0.04 unit; initial pH 6.40 (HEPES). [b] At high [acetazolamide], kinetic plots differed markedly from first order. [c] [Inhibitor]/[catalyst].

Table VI. Inhibitor Effects of Cl^- on k_{obsd} for $HCO_3^- + H^+ \rightarrow$ Dehydration Catalyzed by 2c:Co^{II} (pH 6.40)[a]

	[Cl^-]/[catalyst]			
	0	1	5	no catalyst
k_{obsd}, s^{-1}	0.622 ± 0.009	0.594 ± 0.005	0.542 ± 0.005	0.455 ± 0.006

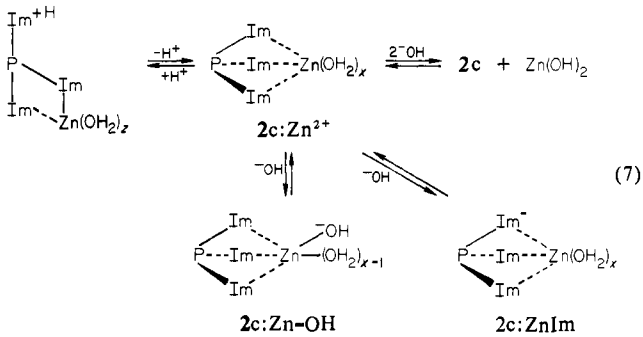
[a] HEPES buffer, [2c:Co^{II}] = 5×10^{-4} M, BCP indicator; $T = 25 \pm 0.2$ °C.

those in Tables II and III at a given pH.

Control experiments (Table IV) for the bicarbonate dehydration show that the catalytic effect is due to cooperative effect between **2c** and Zn^{2+} since k_{obsd} with either phosphine or Zn^{2+} alone produce no rate enhancement over the situation when neither component is present. Changes in [BCP indicator] also do not affect k_{obsd}.

As stated previously, reconstitution of the Zn^{2+}-deficient apoenzyme with Co^{II} (and no other metal) produces a catalytically viable metalloenzyme. We have performed some preliminary catalytic studies at pH 6.40 and 6.70 (Figure 5) by using 5×10^{-4} M in each of **2c** and $Co(ClO_4)_2$. Some net catalysis is observed, although since Co^{II} is only poorly bound to **2c** (Table I), it is difficult to know the actual [2c:Co^{II}]: the graphed points represent the second-order k_{cat}^{obsd}, assuming complete binding ([2c:Co^{II}] = 5×10^{-4} M).

The observation that k_{cat}^{obsd} appears to follow a somewhat distorted bell-shaped pH profile for 2c:Zn^{2+} maximizing at pH 6.40 indicates a certain deficiency in this model system which we believe is attributable to the processes schematized in eq 7 (Zn^{2+} charges being omitted for simplicity).



(7)

As a function of pH, 2c:Zn^{2+} can suffer a number of consequences which reduce the concentration of catalytically active species. Although we have no specific evidence from the pH rate profile which (or whether any) of 2c:Zn^{2+}, 2c:ZnOH, or 2c:ZnIm is the catalytically active species, the observation that increasing [H^+] reduces the net catalysis implies that on protonation of the ligand, the integrity of the four- or five-coordinate environment is disrupted, leading to an inert species. Some evidence for this possibility, at least for 2c:$Co^{II}Cl$ comes from its pH spectral profile which indicates that tetrahedral ligation of Co^{II} is destroyed at reduced pH. Conversely as pH is increased (above pH 6.4), OH^- effectively sequesters Zn^{II} away from the complex again, giving rise to a catalytically inert species. Visible precipitation of what we believe is $Zn(OH)_2$ occurs above pH 7.5 which supports this analysis. Clearly future catalyst designs will require large enough metal affinities to circumvent these problems.

(d) Inhibition Studies. It has long been known that the activity of CA can be inhibited by monovalent anions[16a,13a,21] but not by divalent anions[13a,16a] (see, however, ref 1g), noncompetitively for CO_2 hydration[20] and competitively for HCO_3^- dehydration.[1a] UV studies with the Co^{II} enzyme show the inhibiting anions bind near the metal[16a] and indicate its environment to be four- and/or five-coordinate.[8b] Crystallographic measurements show that the anions also bind in the vicinity of the Zn^{2+} in the native enzyme.[6d] Aromatic sulfonamides are the most potent and selective inhibitors of animal and bacterial CA,[1a,f,23] the sulfonamide N or O being bound to the Zn^{2+},[6c] but since the apoenzyme still retains significant affinity for sulfonamides, van der Waals' and H-bonding interactions may play a significant part in stabilizing the holoenzyme complexes.[1f]

Anions also strongly inhibit the activity of 2c:Zn^{++} as is shown by the data of Table 5 which is depicted in Figure 6. While for the enzyme, the order of inhibition is $I^- > Br^- > Cl^- > F^-$, inversely following their solvation energies,[24] the situation for 2c:Zn^{2+} is $Cl^- > Br^- > I^- > F^-$. In fact Cl^- is such a potent inhibitor that all catalytic activity is lost when [2c:Zn^{2+}] = [Cl^-] = 5×10^{-4} M.

Without knowing the actual mechanism of anion inhibition, it is difficult to rationalize the observed ordering, although it might be tempting to suggest it results from a combination of steric access to the cavity, and energy required to desolvate the anion. While it is clear that Cl^- can and does fit readily into the fourth Zn^{2+} coordination site[17] and the other halides can be positioned at their preferred distances from the Zn^{2+} without severe steric buttressing,[17] such is not the case for ClO_4^-. The 2c:Co^{II} spectral variations in the presence of anions clearly indicate that the halides do interact with the Co^{II}, occupying one of its sites although ClO_4^- does not.

It is clear from the above that small anions cannot be used as counterions for the kinetic measurements. While SO_4^{2-} apparently does not inhibit the enzyme, we were unable to use it in this medium for solubility reasons. Under the belief that increased anion size would diminish inhibition, we adopted ClO_4^- as a "compromise" although it is distinctly possible that there is some inhibition under these conditions of large excess of [ClO_4^-] relative to [2c:Zn^{2+}].

Acetazolamide, an aromatic sulfonamide which is one of the most powerful inhibitors for CA, was also tested with 2c:Zn^{2+} and shown to have some small effect. Likely its reduced inhibitor effect in this case is due to both fit of the $-SO_2NH_2$ portion into the cavity and the fact no interaction between the aromatic sulfonamide ring and catalyst (comparable to that in the enzyme) can occur.

Finally we tested 2c:Co^{II} for anionic inhibition by Cl^- at pH 6.70 in the dehydration direction, and the results are shown in Table VI. Apparently inhibition occurs here as well, but as stated previously there are attendant problems in analysis relating to poor binding of **2c** by Co^{II}.

(e) Mechanistic Conclusions. Although one can never be certain that extrapolations to enzymes made on the basis of model studies are accurate, we believe that some of the properties of 2c:M^{2+} at least set small molecule precedent for conclusions drawn from enzyme studies. In particular, observation of catalytic activity provides evidence that cooperative interaction between a suitably constructed imidazole-containing tridentate ligand and Zn^{2+} can lead to reasonably large rate enhancements[25] provided that certain minimum requirements are met, namely, the ability of the ligand system to provide a distorted four- (five-) coordinate environment and to limit the number of H_2O molecules on the Zn^{2+}.

(21) (a) Roughton, F. J. W.; Booth, V. H. *Biochem. J.* **1946**, *40*, 319. (b) Meldrum, N. U.; Roughton, F. J. W. *J. Physiol.* **1933**, *80*, 113. (c) King, R. W.; Burgen, A. S. V. *Proc. R. Soc. London, Ser. B* **1976**, *193*, 107.

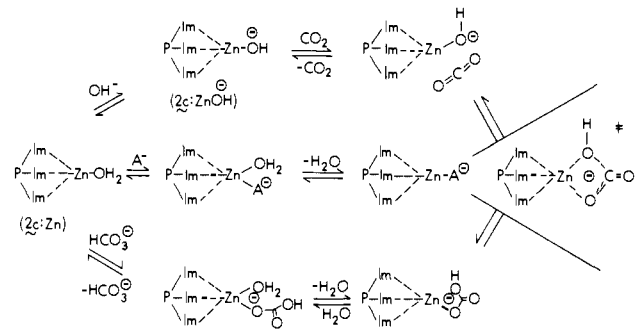
(22) (a) Verpoorte, J. A.; Mehta, S.; Edsall, J. T. *J. Biol. Chem.* **1967**, *242*, 4221–4229. (b) Lindskog, S. *Biochemistry* **1966**, *5*, 2641–2646.

(23) (a) Mann, T.; Keilin, D. *Nature (London)* **1940**, *146*, 164. (b) Maren, T. *Physiol. Rev.* **1967**, *47*, 595.

(24) (a) Cotton, F. A.; Wilkinson, G. "Advanced Inorganic Chemistry"; Interscience: New York; 1966; Vol. 2, p 43. (b) Haliwell, H. F.; Nyburg, S. C. *Trans. Faraday Soc.* **1963**, *59*, 1126–1140.

(25) In the earlier report,[9b] catalysis exhibited by 2c:Zn^{2+} is significantly reduced from that reported here. While we have no definite explanation for this, the indicator and solvent system is slightly different. In addition we have noted that solutions of 2c:Zn^{2+} lose their activity after being left standing overnight.

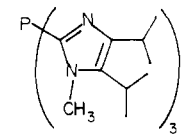
Scheme I



Basically mechanisms for CA can be categorized into two main types. In the first category the primary purpose of the metal is to reduce the pK_a of an attached group (OH_2 or imidazole)[26,27] such that it becomes an effective nucleophile (or general base) toward CO_2. The metal may also serve an additional role as Lewis acid which sufficiently polarizes the CO_2 to render it more susceptable to nucleophilic attack.

Alternatively, for the second category, attendant to the above metal function, an additional required function of the protein is to provide catalytically important groups to assist in proton transfer from the metal-bound H_2O or to act as general bases to assist in delivering free H_2O to CO_2.[1d,5d,e,i] Since **2c**:Zn^{2+} shows catalytic behavior but mimics only the metal-binding site, it may be tempting in the most generous extrapolation to favor the first mechanistic category. Of course since the catalysis afforded by **2c**:Zn^{2+} is modest relative to the enzyme,[28] protein interactions could be fine tuning its catalysis. In fact, it now seems certain enzymically, the rate-determining step is not the actual metal-catalyzed CO_2 hydration, but a subsequent proton transfer involving two or more equivalent hydrogens in the transition state.[29]

In the case of **2c**:Zn^{2+}, one must propose either a $Zn^{2+}OH^-$ mechanism[1] or a Zn^{2+} imidazolate mechanism,[26,27] neither being distinguished by the above observations. In principle, these could be distinguished if it were observed that the corresponding *N*-methyl derivative **3** could be catalytically viable. However we



3

are convinced that **3** would not bind Zn^{2+} as well as **2c** since preliminary work in related systems[9a] shows each N-H to N-CH_3 substitution reduces a ligand's $pK_{M^{2+}}$ by 1-1.5 units. Additionally, **3** is very difficult to purify, most operations leading to the easily formed phosphine oxide.

For reasons of simplicity we favor a mechanism as in Scheme I, akin to that proposed by Lövgren et al.[6b] for the enzyme, the difference here being that the H-bonding network between Zn-OH_2 and the protein is absent. In this mechanism, the transition state (‡) involves a five-coordinate Zn^{2+}, the purpose of the metal being to serve as a template for delivery of a bound ^-OH and stabilizing the incipient change on CO_2. That the low coordination numbers for Zn^{2+} are required for activity is supported by observations[9b] which indicate that neither **2a**.Zn^{2+} nor **2b**:Zn^{2+} appear to catalyze the $CO_2 \rightleftharpoons HCO_3^-$ interconversion,[30] and their corresponding CO^{II} complexes do not show evidence of four- (five-) coordination. The inhibition of activity by anions is most easily rationalized in Scheme I as a nonproductive association of A^- with the Zn^{2+} which blocks further access of CO_2 (HCO_3^-). The above rationalization indirectly supports the Zn hydroxide mechanism[1] since it is less satisfying (but not impossible if one invokes a severe reduction of the Zn^{2+} electropositive character on its association with anion) to explain the effect of anionic inhibition in terms of the Zn imidazolate mechanism.[26]

Acknowledgment. We thank the University of Alberta and National Science and Engineering Council of Canada for support of this work. In addition we acknowledge Professor H. B. Dunford, Professor M. N. G. James, and Mr. R. Read for helpful discussions and Professor R. B. Jordan for the use of his stopped-flow machine.

(26) For evidence supporting this mechanism see ref 1g,h and 9d.

(27) For criticisms of this mechanism see: Martin, R. B. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 4346-4347.

(28) We have avoided strict numerical comparison of catalysis by **2c**:Zn^{2+} and CA for several reasons. First, the medium used in this study is very different from those employed for enzyme studies[13] as is the present kinetic procedure which monitors $k_{obsd} = k_{hyd} + k_{dehyd}$. Also, there is an obvious problem of units in comparing the present second-order catalytic process, with the enzymic process which involves Michaelis-Mentin kinetics. Previous work in model CA systems[7a,9b] has attempted such comparison by using $k_{cat}/K_M = 10^7$ and 8×10^7 M^{-1} s^{-1} for human CA-B and CA-C, respectively.

(29) (a) Venkatasubban, K. S.; Silverman, D. N. *Biochemistry* **1980**, *19*, 4984-4991. (b) Silverman, D. N.; Tu, C. K.; Lindskog, S.; Wynns, G. C. *J. Am. Chem. Soc.* **1979**, *101*, 6734-6740. (c) Steiner, H.; Jonsson, B. H.; Lindskog, S. *Eur. J. Biochem.* **1975**, *59*, 253.

(30) Our preliminary studies with **2a-c** showed the former two Zn^{2+}-bound species to be completely inactive toward $CO_2 \rightleftarrows HCO_3^-$ interconversion.[9b] While suggestive of the importance of reduced coordination of Zn^{2+}, these experiments are not strictly comparable to those with **2c**:Zn^{2+} since the former species required solvent systems of a more highly aqueous content.[9b]

Communications to the Editor

Carbon Monoxide Activation by Organoactinides. Migratory CO Insertion into Metal-Hydrogen Bonds to Produce Mononuclear Formyls

Paul J. Fagan, Kenneth G. Moloy, and Tobin J. Marks*

Department of Chemistry, Northwestern University Evanston, Illinois 60201

Received July 6, 1981

Revised Manuscript Received September 1, 1981

Although the migratory insertion of CO into a metal-hydrogen bond to produce a formyl[1,2] (A, eq 1) has been much discussed as a primary event in the heterogeneous catalytic hydrogenation of carbon monoxide,[3] there is little unambiguous evidence for this

$$\underset{}{M\text{-}H} + CO \rightarrow \underset{A}{M(CHO)} \qquad (1)$$

(1) (a) Steinmetz, G. R.; Geoffroy, G. L. *J. Am. Chem. Soc.* **1981**, *103*, 1278-1279. (b) Thorn, D. L. *Ibid.* **1980**, *102*, 7109-7110. (c) Casey, C. P.; Andrews, M. A.; McAlister, D. R.; Rinz, J. E. *Ibid.* **1980**, *102*, 1927-1933. (d) Wong, W.-K.; Tam, W.; Gladysz, J. A. *Ibid.* **1979**, *101*, 5440-5442. (e) Brown, K. L.; Clark, G. R.; Headford, C. E. L.; Marsden, K.; Roper, W. R. *Ibid.* **1979**, *101*, 503-505. (f) Collman, J. P.; Winter, S. R. *J. Am. Chem. Soc.* **1973**, *95*, 4089-4090. (g) Pearson, R. G.; Walker, H. W.; Mauermann, H.; Ford, P. C. *Inorg. Chem.* **1981**, *20*, 2741-2743. (h) To our knowledge, the first suggestion of the possibility of such an insertion process was in: Basolo, F.; Pearson, R. G. "Mechanisms of Inorganic Reactions"; 2nd ed.; John Wiley: New York, 1968; Chapter 7. (i) **Note Added in Proof**: For the formation of an unstable formyl via carbonylation of a rhodium porphyrin hydride, see: Wayland, B. B.; Woods, B. A. *J. Chem. Soc., Chem. Commun.* **1981**, 700-701.

0002-7863/81/1503-6959$01.25/0