Comparison of Zinc and Cadmium Coordination Environments in Synthetic Analogues of Carbonic Anhydrase: Synthesis and Structure of {[Pim$^{Pr^i,Bu^t}$]Cd(OH$_2$)(OClO$_3$)}(ClO$_4$)

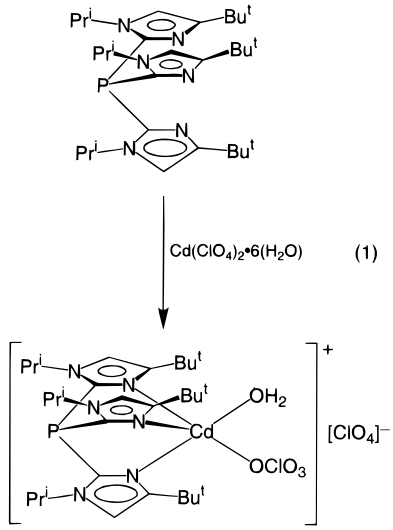
Clare Kimblin and Gerard Parkin*

Department of Chemistry, Columbia University, New York, New York 10027

Received August 19, 1996

Carbonic anhydrase (CA) is the zinc enzyme responsible for equilibrating bicarbonate with carbon dioxide and water in both plants and animals.[1] It is, therefore, a most extensively studied enzyme, with considerable attention having been given to metal-substituted derivatives in order to provide insight into both active-site structure and mechanism of action.[1−3] In part, such studies are a consequence of the lack of a useful spectroscopic probe for the Zn^{II} center of the native enzyme; for example, cadmium-substituted carbonic anhydrase[4] has been employed since it is amenable to study by both ^{111}Cd and ^{113}Cd NMR spectroscopy.[5,6] In this paper, we compare the structures of two related zinc and cadmium complexes that are supported by a common coordination environment which closely resembles that in carbonic anhydrase.

We recently described the use of the tris(1-isopropyl-4-*tert*-butyl-2-imidazolyl)phosphine ligand,[7] Pim$^{Pr^i,Bu^t}$, to model the three histidine donors that bind zinc to the peptide backbone in carbonic anhydrase.[8] As part of these studies, we have synthesized {[Pim$^{Pr^i,Bu^t}$]ZnOH}(ClO$_4$) and have identified it as an excellent synthetic analogue of the active site in carbonic anhydrase, since it is the first structurally-characterized monomeric zinc hydroxide complex coordinated to three imidazolyl groups. The Pim$^{Pr^i,Bu^t}$ ligand, therefore, has the potential to provide a useful system that may allow one to evaluate how the coordination environment of the active site of carbonic anhydrase would be modified or influenced by metal ion substitution. Such a study of a synthetic analogue system is important because the insight to be gleaned from an investigation of metal-substituted enzymes depends intimately on our knowledge of the coordination chemistry of the respective metals. For this reason, the cadmium counterpart {[Pim$^{Pr^i,Bu^t}$]Cd(OH$_2$)(OClO$_3$)}(ClO$_4$) was synthesized by the reaction of Cd(ClO$_4$)$_2$•6H$_2$O with Pim$^{Pr^i,Bu^t}$ (eq 1) and was structurally-characterized



by X-ray diffraction (Figure 1).[9] Significantly, the diffraction study demonstrates that the cadmium is coordinated to the oxygen atoms of both a water molecule and a perchlorate anion, in a distorted trigonal bipyramidal array with axial H_2O and imidazolyl groups. The five-coordinate environment of {[Pim$^{Pr^i,Bu^t}$]Cd(OH$_2$)(OClO$_3$)}(ClO$_4$) provides a marked contrast to the tetrahedral coordination observed for zinc in {[Pim$^{Pr^i,Bu^t}$]-ZnOH}(ClO$_4$).

Although commonly viewed as a "noncoordinating" anion,[10] perchlorate is nonetheless known to bind to certain metal centers,[11] including cadmium.[12−17] Interestingly, however, the

(1) (a) Bertini, I.; Luchinat, C. In *Bioinorganic Chemistry*; Bertini, I., Gray, H. B., Lippard, S. J., Valentine, J., Eds.; University Science Books: Mill Valley, CA, 1994. (b) Botre, F., Gros, G., Storey, B. T., Eds. *Carbonic Anhydrase*; VCH: New York, 1991. (c) Christianson, D. W. *Adv. Protein Chem.* **1991**, *42*, 281−355. (d) Silverman, D. N.; Lindskog, S. *Acc. Chem. Res.* **1988**, *21*, 30−36. (e) *The Carbonic Anhydrases*; Dodgson, S. J., Tashian, R. E., Gros, G., Carter, N. D., Eds.; Plenum: New York, 1991.

(2) (a) Williams, R. J. P. *J. Mol. Catal.* **1985**, *30*, 1−26. (b) Bertini, I.; Luchinat, C.; Viezzoli, M. S. In *Zinc Enzymes*; Bertini, I., Luchinat, C., Maret, W., Zeppezauer, M., Eds.; Progress in Inorganic Biochemistry and Biophysics, Vol. 1; Birkhäuser: Boston, 1986; Chapter 3.

(3) (a) Håkansson, K.; Wehnert, A.; Liljas, A. *Acta Crystallogr.* **1994**, *D50*, 93−100. (b) Håkansson, K.; Wehnert, A. *J. Mol. Biol.* **1992**, *228*, 1212−1218.

(4) (a) Bauer, R.; Limkilde, P.; Johansen, J. T. *Biochemistry* **1976**, *15*, 334−342. (b) Závodsky, P.; Johansen, J. T.; Hvidt, A. *Eur. J. Biochem.* **1975**, *56*, 67−72. (c) Coleman, J. E. *Biochemistry* **1965**, *4*, 2644−2655. (d) Coleman, J. E. *Nature* **1967**, *214*, 193−194.

(5) (a) Jonsson, N. B.-H.; Tibell, L. A. E.; Evelhoch, J. L.; Bell, S. J.; Sudmeier, J. L. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 3269−3272. (b) Armitage, I. M.; Pajer, R. T.; Schoot Uiterkamp, A. J. M.; Chlebowski, J. F.; Coleman, J. E. *J. Am. Chem. Soc.* **1976**, *98*, 5710−5712. (c) Armitage, I. M.; Schoot Uiterkamp, A. J. M.; Chlebowski, J. F.; Coleman, J. E. *J. Magn. Reson.* **1978**, *29*, 375−392. (d) Sudmeier, J. L.; Bell, S. J. *J. Am. Chem. Soc.* **1977**, *99*, 4499−4500. (e) Evelhoch, J. L.; Bocian, D. F.; Sudmeier, J. L. *Biochemistry* **1981**, *20*, 4951−4954. (f) Blackburn, G. M.; Mann, B. E.; Taylor, B. F.; Worrall, A. F. *Eur. J. Biochem.* **1985**, *153*, 553−558.

(6) For ^{113}Cd NMR in other systems see: (a) Summers, M. F. *Coord. Chem. Rev.* **1988**, *86*, 43−134. (b) Rivera, E.; Kennedy, Ellis, P. D. *Adv. Magn. Reson.* **1989**, *13*, 257−273. (c) Kennedy, M. A.; Ellis, P. D. *J. Am. Chem. Soc.* **1989**, *111*, 3195−3203. (d) Ellis, P. D. *J. Biol. Chem.* **1989**, *264*, 3108−3110. (e) Munakata, M.; Kitagawa, S.; Yagi, F. *Inorg. Chem.* **1986**, *25*, 964−970.

(7) Sorrell, T. N.; Allen, W. E.; White, P. S. *Inorg. Chem.* **1995**, *34*, 952−960.

(8) Kimblin, C.; Allen, W. E.; Parkin, G. *J. Chem. Soc., Chem. Commun.* **1995**, 1813−1815.

(9) {[Pim$^{Pr^i,Bu^t}$]Cd(OH$_2$)(OClO$_3$)}(ClO$_4$) is monoclinic, $P2_1/c$ (No. 14); a = 11.088(3) Å, b = 24.067(6) Å, c = 16.547(3) Å, β = 106.83(2)°, V = 4226(2) Å^3, Z = 4.

(10) *Chemistry of The Elements*; Greenwood, N. N., Earnshaw, A. Pergamon: New York, 1984; p 1017.

(11) Gowda, N. M. N.; Naikar, S. B.; Reddy, G. K. N. *Adv. Inorg. Chem. Radiochem.* **1984**, *28*, 255−299.

(12) Setzer, W. N.; Tang, Y.; Grant, G. J.; VanDerveer, D. G. *Inorg. Chem.* **1992**, *31*, 1116−1118.

(13) Drew, M. G. B.; Cabral, J. d. O.; Cabral, M. F.; Esho, F. S.; Nelson, S. M. *J. Chem. Soc., Chem. Commun.* **1979**, 1033−1035.

(14) Adams, H.; Bailey, N. A.; Fenton, D. E.; Ford, I. G.; Kitchen, S. J.; Williams, M. G.; Tasker, P. A.; Leong, A. J.; Lindoy, L. F. *J. Chem. Soc., Dalton Trans.* **1991**, 1665−1674.

(15) Adam, K. R.; Arshad, S. P. H.; Baldwin, D. S.; Duckworth, P. A.; Leong, A. J.; Lindoy, L. F.; McCool, B. J.; McPartlin, M.; Tailor, B. A.; Tasker, P. A. *Inorg. Chem.* **1994**, *33*, 1194−1200.

(16) Dean, P. A. W.; Payne, N. C.; Vittal, J. J.; Wu, Y. *Inorg. Chem.* **1993**, *32*, 4632−4639.

S0020-1669(96)01007-5 CCC: $12.00

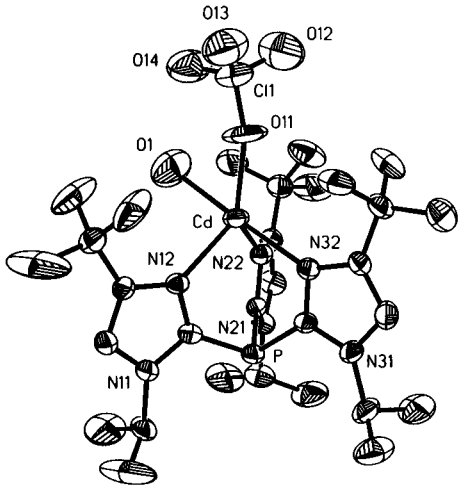


Figure 1. Molecular structure of $\{[Pim^{Pr^i,Bu^t}]Cd(OH_2)(OClO_3)\}(ClO_4)$ (only cation shown for clarity; 20% thermal ellipsoids).

$Cd-OClO_3$ bond length of 2.203(8) Å in $\{[Pim^{Pr^i,Bu^t}]Cd(OH_2)(OClO_3)\}^+$ is close to the sum of the covalent radii of Cd and O (2.14 Å)[18] and is considerably shorter than the range previously established for cadmium complexes listed in the Cambridge Structural Database (2.33 Å−2.65 Å).[12−17] For example, the $Cd-OClO_3$ interaction in the related *cis* aqua−perchlorate complex $\{[\eta^5\text{-}(C_2H_4S)_5]Cd(OH_2)(OClO_3)\}^+$ is sufficiently long [2.653(5) Å] that it may be considered to be effectively nonbonding.[12,19] In contrast to the different $Cd-OClO_3$ interactions in the above complexes, however, the $Cd-OH_2$ bond lengths in $\{[Pim^{Pr^i,Bu^t}]Cd(OH_2)(OClO_3)\}^+$ [2.297(9) Å] and $\{[\eta^5\text{-}(C_2H_4S)_5]Cd(OH_2)(OClO_3)\}^+$ [2.253(4) Å][12] are comparable.

The higher coordination number of the metal center in $\{[Pim^{Pr^i,Bu^t}]Cd(OH_2)(OClO_3)\}^+$ compared to that in $\{[Pim^{Pr^i,Bu^t}]ZnOH\}^+$ is undoubtedly a reflection of the larger size of cadmium,[18] which enables it to bind an additional ligand. Because the acidity of a coordinated water molecule is reduced considerably upon the binding of an anionic ligand to a metal center,[20] the pK_a of the monocation $\{[Pim^{Pr^i,Bu^t}]Cd(OH_2)(OClO_3)\}^+$ would be expected to be greater than that of dicationic $\{[Pim^{Pr^i,Bu^t}]Zn(OH_2)\}^{2+}$.[21] Consequently, deprotonation of the coordinated water molecule in $\{[Pim^{Pr^i,Bu^t}]Zn(OH_2)\}^{2+}$ would be more facile than that of the water molecule in $\{[Pim^{Pr^i,Bu^t}]Cd(OH_2)(OClO_3)\}^+$, with the result that a hydroxide complex is isolated for the zinc system whereas an aqua complex is isolated for the cadmium system, *i.e.* $[Zn^{II}-OH]$ *versus* $[Cd^{II}-OH_2]$.

A key step of the proposed mechanism of action of carbonic anhydrase involves the generation of the active zinc−hydroxide intermediate by deprotonation of a zinc-bound water molecule.[1] As such, the isolation of a zinc−hydroxide complex, under conditions similar to those which give a cadmium−aqua derivative, is consistent with the considerably lower activity of Cd−CA compared to that of the native zinc enzyme at *ca.* pH 7.[4a,d] Activity of Cd−CA is, however, induced at higher pH, with an activity profile corresponding to the ionization of a cadmium-bound water molecule with a pK_a of *ca.* 9,[4a] 2 units greater than that for the zinc enzyme with a pK_a of *ca.* 7. Furthermore, pK_a values in the range 9.2−9.7 for Cd−CA have also been determined by ^{113}Cd NMR studies as a function of pH.[5a] Interestingly, the difference in pK_a values for the zinc and cadmium enzymes is greater than that for the hydrated ions, Zn^{2+}(aq) (pK_a = 9.0) and Cd^{2+}(aq) (pK_a = 10.1).[21] Thus, coordination of the apoenzyme to M^{2+}(aq) appears to exert less influence on the acidity of the metal-bound water molecules for Cd^{2+}(aq) than for Zn^{2+}(aq). A possible rationalization for this observation may be that the active sites of the zinc and cadmium enzymes are not, in fact, isostructural. Although the active site of the zinc enzyme has been determined to be four-coordinate by X-ray diffraction, Cd−CA has not been structurally-characterized;[22] nevertheless, four-coordinate structures have been invoked in interpreting ^{113}Cd NMR studies.[5c] However, the fact that the cadmium center of $\{[Pim^{Pr^i,Bu^t}]Cd(OH_2)\}^{2+}$ binds perchlorate, a ligand regarded as "noncoordinating" by conventional standards, indicates that cadmium in a tetrahedral environment surrounded by water and three imidazole groups shows a strong tendency to bind additional ligands. Consequently, the coordination mode of cadmium in Cd−CA may not be tetrahedral. Since pK_a values are influenced by both the coordination number and the charge on a metal center,[20] the origin of the relatively high pK_a value for the cadmium-substituted enzyme may be the result of a nontetrahedral active site.

In summary, comparison of $\{[Pim^{Pr^i,Bu^t}]ZnOH\}^+$, a synthetic analogue of the active site of carbonic anhydrase, with its cadmium counterpart $\{[Pim^{Pr^i,Bu^t}]Cd(OH_2)(OClO_3)\}^+$ suggests that the active site of Cd−CA may not be isostructural with that of the native zinc enzyme. Furthermore, the difference in coordination environments provides a rationale for the observation that, by comparison with the zinc system, the pK_a of Cd−CA is not as significantly reduced from that of Cd^{2+}(aq).

Supporting Information Available: Text giving the synthetic procedure, spectroscopic data, and X-ray experimental details, tables giving crystal data, intensity collection parameters, atomic coordinates, bond lengths, bond angles, and thermal parameters, a fully labeled ORTEP diagram, and an additional view of the molecule for $\{[Pim^{Pr^i,Bu^t}]Cd(OH_2)(OClO_3)\}(ClO_4)$ (12 pages). Ordering information is given on any current masthead page.

IC961007D

(17) Setzer, W. N.; Tang, Y.; Grant, G. J.; VanDerveer, D. G. *Inorg. Chem.* **1991**, *30*, 3652−3656.

(18) $r_{cov}(O) = 0.66$ Å; $r_{cov}(Cd) = 1.48$ Å; $r_{cov}(Zn) = 1.31$ Å. See: Pauling, L. *The Nature of The Chemical Bond*, 3rd ed.; Cornell University Press: Ithaca, NY, 1960; p 246.

(19) The $Cd-OClO_3$ [2.33(3) Å] and $Cd-OH_2$ [2.24(3) Å] bond lengths in a hexagonal bipyramidal $[(L_{N_6})Cd(OH_2)(OClO_3)]^+$ *trans* aqua−perchlorate complex are, however, comparable to those in $\{[Pim^{Pr^i,Bu^t}]Cd(OH_2)(OClO_3)\}^+$. See ref 13.

(20) For some theoretical calculations addressing the variation of pK_a values of some zinc complexes, see: (a) Bertini, I.; Luchinat, C.; Rosi, M.; Sgamellotti, A.; Tarantelli, F. *Inorg. Chem.* **1990**, *29*, 1460−1463. (b) Åqvist, J.; Warshel, A. *Chem. Rev.* **1993**, *93*, 2523−2544. (c) Åqvist, J.; Warshel, A. *J. Mol. Biol.* **1992**, *224*, 7−14. (d) Åqvist, J. *THEOCHEM* **1992**, *256*, 135−152.

(21) Furthermore, such an effect would be enhanced because zinc is smaller than cadmium. Thus, the pK_a of Cd^{2+}(aq) is greater than that of Zn^{2+}(aq). See: Smith, R. M.; Martell, A. E. *Critical Stability Constants*; Plenum: New York, 1976; Vol. 4.

(22) For the structures of some other metal-substituted carbonic anhydrases, see ref 3a.