Notes

Ab Initio Comparison of the Bonding Modes of Nitrate and Bicarbonate in Model Carbonic Anhydrase Systems

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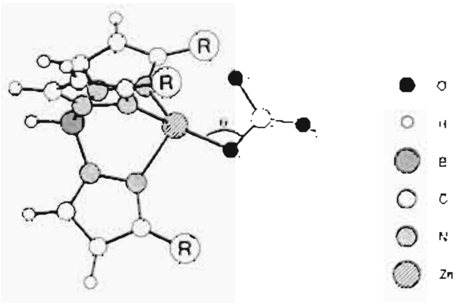
Introduction

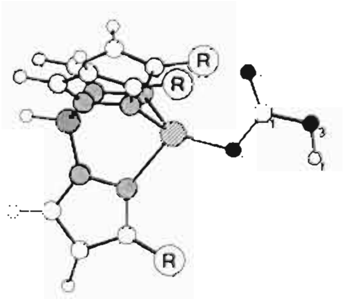
Carbonic anhydrase[1] is a zinc-containing metalloenzyme which catalyzes the reversible hydration of CO_2 to HCO_3^- and H^+. Various studies using the metal-substituted enzyme have shown that the Zn(II) enzyme has the highest catalytic activity, followed by Co(II). Neither Ni(II)- nor Cu(II)-containing enzymes exhibit significant activity.[2] Though there are several studies available, the mechanism by which the enzyme catalyzes the reaction is still controversial. One suggested mechanism[1c] has the following steps: (1) The initial complex contains a tetracoordinate zinc, with three histidine nitrogens and one water molecule completing the coordination sphere around Zn. (2) This complex is deprotonated to form (histidine)$_3$Zn–OH. (3) CO_2 attacks the zinc–hydroxide complex, leading to a Zn–bicarbonate complex. (4) HCO_3^- is displaced by another water molecule, and the original catalyst is regenerated. Several attempts have been made to mimic the enzyme by model systems which replace the three histidine moieties around the metal with triazacyclononane,[3a] tris(aminomethyl)methane,[3b] triaminocyclohexane,[3c] tris(pyrazolyl)hydroborato, η^3-HB(3-Rpz)$_3^-$ (Rpz = substituted pyrazole),[3d,e] and imidazolyl ligands.[3f,g] On the basis of the coordination of the isoelectronic nitrate anion (NO_3^-) to various metals in [η^3-HB(3-*t*-Bupz)$_3$]M(NO_3) (M = Zn(II), Co(II), Ni(II), Cu(II)), Han and Parkin suggested that the activity of the enzyme may depend on the coordination of the bicarbonate to the metal.[4a] According to this hypothesis, if the bicarbonate coordinates in a monodentate fashion, the enzyme's activity is at its maximum, but bidentate coordination reduces the activity to zero. Crystallographically, nitrate is found to exhibit monodentate coordination with Zn, whereas both Ni and Cu bind NO_3^- in a bidentate fashion in [η^3-HB(3-*t*-Bupz)$_3$]M(NO_3).

In this note, we report ab initio potential energy surfaces for [η^3-HB(3-Rpz)$_3$]Zn(NO_3) (**1**) and [η^3-HB(3-Rpz)$_3$]Zn(HCO_3) (**2**) (R = H, *t*-Bu) to compare the bonding capabilities of the two ligands NO_3^- and HCO_3^- and thus assess the viability of modeling the bicarbonate-containing biological system with nitrate-containing species. To maintain four-coordination at zinc and to inhibit oligomerization, the experimental studies have employed *tert*-butyl groups at the 3-position of the pyrazolyl ring. Therefore we studied both the parent (R = H) and the *t*-Bu derivatives of **1** and **2**. While numerous theoretical studies on the mechanism of carbonic anhydrase have appeared,[5] as far as we know this is the first report comparing the ligand capabilities of NO_3^- and HCO_3^- in model carbonic anhydrase systems.



1

a R = H
b R = *t*-Bu



2

a R = H
b R = *t*-Bu

Results and Discussion

Since geometry optimizations on systems of this size using ab initio methods are computationally prohibitive, all geometries were optimized with the PRDDO (partial retention of diatomic differential overlap) method,[6,7] and single-point energies were calculated for these geometries at several values of θ (= ZnO_1N_1 or ZnO_1C_1 angle; also see **1** for the definition of θ) using the ab initio program GAMESS.[8] The potential energy surface connects the presumed bidentate ($\theta \sim 90°$) and monodentate ($\theta > 115°$) coordinations for zinc with the NO_3^- (Figure 1) and HCO_3^-

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(1) (a) Lindskog, S. In *Zinc Enzymes*; Spiro, T. G., Ed.; Wiley: New York, 1983; p 77. (b) Sigel, H., Ed.; *Metal Ions In Biological Systems*; Marcel Dekker: New York, 1983; Vol. 15. (c) Silverman, D. N.; Lindskog, S. *Acc. Chem. Res.* **1988**, *21*, 30. (d) Silverman, D. N.; Vincent, S. H. *CRC Crit. Rev. Biochem.* **1983**, *14*, 207. (e) Lipscomb, W. N. *Annu. Rev. Biochem.* **1983**, *52*, 17. (f) Woolley, P. *Nature* **1975**, *258*, 677. (g) Bertini, I.; Luchinat, C.; Scozzafava, A. *Struct. Bonding (Berlin)* **1982**, *48*, 45. (h) Erikkson, E. A.; Jones, T. A.; Liljas, A. *Prog. Inorg. Biochem. Biophys.* **1986**, *1*, 317. This paper suggests that Thr 199 stabilizes the monodentate binding of bicarbonate to zinc in carbonic anhydrase.

(2) (a) Lindskog, S.; Malmstrom, B. G. *J. Biol. Chem.* **1962**, *237*, 1129. (b) Coleman, J. E. *Nature* **1967**, *214*, 193. (c) Thorslund, A.; Lindskog, S. *Eur. J. Biochem.* 1967, *3*, 117. (d) Williams, R. J. P. *J. Mol. Catal.* **1985**, *30*, 1.

(3) (a) Yang, R.; Zompa, L. J. *Inorg. Chem.* **1976**, *15*, 1499. (b) Sabatini, A.; Vacca, A. *J. Chem. Soc., Dalton Trans.* **1980**, 519. (c) Wentworth, R. A. D. *Inorg. Chem.* **1968**, *7*, 1030. (d) Trofimenko, S. *Chem. Rev.* **1972**, *72*, 497. (e) Trofimenko, S. *Prog. Inorg. Chem.* **1986**, *34*, 115. (f) Tang, C. C.; Davalian, D.; Huang, P.; Breslow, R. *J. Am. Chem. Soc.* **1978**, *100*, 3918. (g) Brown, R. S.; Salmon, D.; Curtis, N. J.; Kusuma, S. *J. Am. Chem. Soc.* **1982**, *104*, 3188.

(4) (a) Han, R.; Parkin, G. *J. Am. Chem. Soc.* **1991**, *113*, 9707. (b) Alsfasser, R.; Powell, A. K.; Vahrenkamp, H. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 898.

(5) (a) Merz, K. M., Jr.; Hoffmann, R.; Dewar, M. J. S. *J. Am. Chem. Soc.* **1989**, *111*, 5636. (b) Jacob, O.; Cardenas, R.; Tapia, O. *J. Am. Chem. Soc.* **1990**, *112*, 8692. (c) Liang, J.-Y.; Lipscomb, W. N. *Biochemistry* **1987**, *26*, 5293. (d) Liang, J.-Y.; Lipscomb, W. N. *Int. J. Quantum Chem.* **1989**, *36*, 299. (e) Pullman, A. *Ann. N.Y. Acad. Sci.* **1981**, *367*, 340. (f) Allen, L. C. *Ann. N.Y. Acad. Sci.* **1981**, *367*, 383. (g) Cook, C. M.; Allen, L. C. *Ann. N.Y. Acad. Sci.* **1984**, *429*, 84. (h) Sola, M.; Lledos, A.; Duran, M.; Bertran, J. *J. Am. Chem. Soc.* **1992**, *114*, 869 and references cited therein.

(6) (a) Marynick, D. S.; Lipscomb, W. N. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 1341. (b) Halgren, T. A.; Lipscomb, W. N. *J. Chem. Phys.* **1973**, *58*, 1569.

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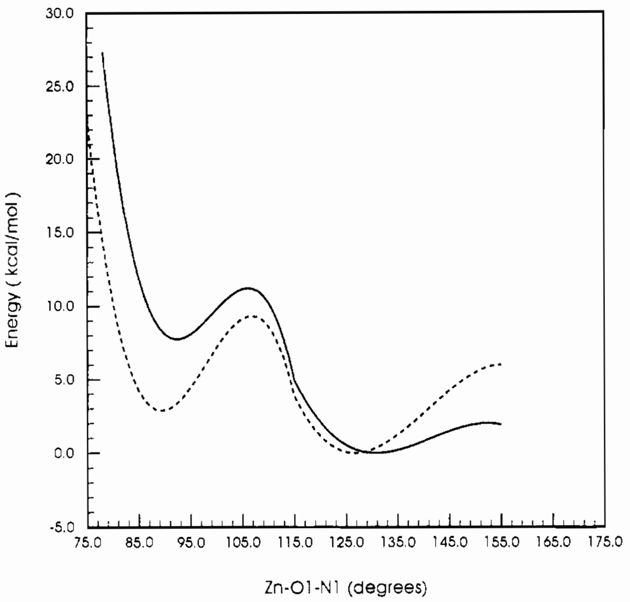

Figure 1. Ab initio potential energy surface for $[\eta^3\text{-HB(3-Rpz)}_3]\text{Zn(NO}_3)$ (R = H, *t*-Bu) (**1**) as a function of $\text{Zn–O}_1\text{–N}_1$ angle (θ): solid line, R = *t*-Bu; dashed line, R = H.

(Figure 2) ligands. Initially, we generated the potential energy surface for **1a** using a 4-31G basis set[9] for the ligand $\eta^3\text{-HB(3-Hpz)}_3{}^-$ and the $NO_3{}^-$ group and an extended basis set[10] for Zn. Since the surface generated was essentially identical to the one in which the $\eta^3\text{-HB(3-Hpz)}_3{}^-$ ligand was described by a minimal (STO-3G) basis set,[12] we decided to use a minimal basis set to describe this ligand (R = H, *t*-Bu) in all other calculations.

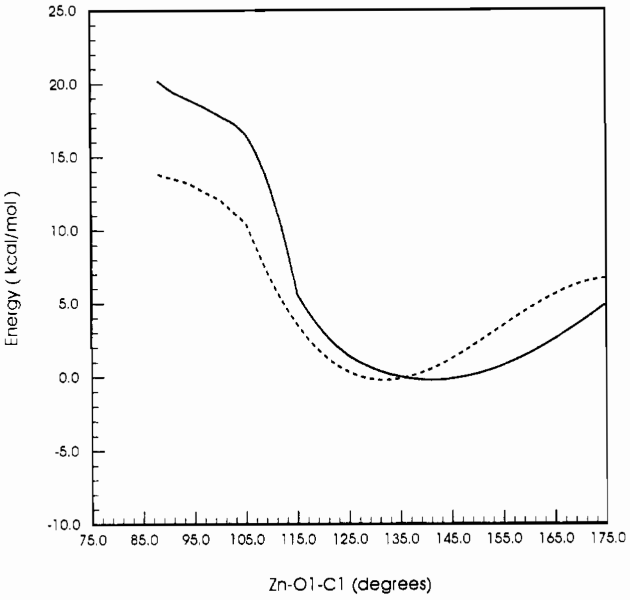

Figure 2. Ab initio potential energy surface for $[\eta^3\text{-HB(3-Rpz)}_3]\text{Zn(HCO}_3)$ (R = H, *t*-Bu) (**2**) as a function of $\text{Zn–O}_1\text{–C}_1$ angle (θ): solid line, R = *t*-Bu; dashed line, R = H.

During the PRDDO optimizations on the parent systems (R = H), only the Zn–NO_3 and Zn–HCO_3 groups were optimized while the rest of the ligand was left unchanged. For the *tert*-butyl derivatives, all dihedral angles involving *tert*-butyl groups were also independently optimized along with Zn–NO_3 and Zn–HCO_3 groups.

The potential energy surface for **1a** is shown in Figure 1 (dashed line). It has a double-well shape, with the structure exhibiting monodentate coordination ($\theta \sim 125°$) being the most stable (ΔE (energy difference between monodentate and bidentate structures) = 3 kcal/mol). The barrier upon going from the bidentate to the monodentate minimum is found to be ~6 kcal/mol. Since the experimental system has *tert*-butyl groups at the 3-position of the pyrazolyl ring, we repeated the ab initio energy surface using **1b** (R = *t*-Bu). The surface is shown in Figure 1 (solid line). It also has two minima, but the monodentate form is more stable than the bidentate form (ΔE = 8 kcal/mol), as expected from steric considerations. The barrier between the monodentate and bidentate forms is also reduced to only 3 kcal/mol. The monodentate minimum appears around the same value of θ as seen in the parent system (R = H). A comparison to the experimental structure[4] shows that our prediction of a monodentate structure for the global minimum of **1b** is in qualitative agreement with experiment, although the experimental structure exhibits a somewhat smaller value of θ (~110°).

Is nitrate a good model for bicarbonate in this system? To answer this question, we repeated the calculations on $[\eta^3\text{-HB(3-Rpz)}_3]\text{Zn(HCO}_3)$ (**2a,b**) as described above and generated the energy surfaces.

The ab initio energy surface for **2a** (R = H) shows a well-defined single minimum corresponding to the monodentate form with $\theta \sim 130°$ (Figure 2, dashed line).[13] As we did for **1b**, we also calculated the ab initio energies for **2b** (R = *t*-Bu; Figure 2, solid line). Again, this surface has only one minimum corresponding to the monodentate form, but the potential well is even deeper than that found for **2a**. A recent experimental study by Vahrenkamp et al. suggested that **2b** should exhibit a monodentate coordination of bicarbonate to Zn.[14] Our results on **2a,b** are again in tune with the experimental trends.

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(7) The PRDDO method is known to give reasonable geometries for transition metal complexes: (a) Marynick, D. S.; Axe, F. U.; Hansen, L. M.; Jolly, C. A. In *Topics in Physical Organometallic Chemistry*; Gielen, M., Ed.; Freund Publishing House Ltd.: London, 1989; Vol. 3, p 43. (b) Lawless, M. S.; Marynick, D. S. *J. Am. Chem. Soc.* **1991**, *113*, 7513. (c) Lawless, M. S.; Marynick, D. S. *Organometallics* **1991**, *10*, 543. (d) Rogers, J. R.; Kwon, O.; Marynick, D. S. *Organometallics* **1991**, *10*, 2816.

(8) General atomic and molecular electronic structure system (GAMESS): Dupuis, M.; Spangler, D.; Wendolski, J. J. *National Resources for Computations in Chemistry Software Catalog*; University of California: Berkeley, CA, 1980; Program QG01. QCPE version: Schmidt, M. W.; Baldridge, K. K.; Boatz, J. A.; Jensen, J. H.; Koseki, S.; Gordon, M. S.; Nguyen, K. A.; Windus, T. L.; Elbert, S. T. *QCPE Bull.* **1990**, *10*, 52.

(9) Ditchfield, R.; Hehre, W. J.; Pople, J. A. *J. Chem. Phys.* **1971**, *54*, 724.

(10) This is basis set A for the ^1S atomic configuration of Zn in: Hansen, L. M.; Marynick, D. S. *J. Phys. Chem.* **1988**, *92*, 4588. These basis sets are designed to utilize the sixth Cartesian Gaussian d function to describe the 3s orbital on the metal. Each basis function is a Gaussian expansion of a Slater type orbital (STO). The 1s, 2s, and 2p orbitals are described by a three-Gaussian (3G) expansion of single-ζ STOs. The 3p and 4sp orbitals are described by a two-term Gaussian expansion of a double-ζ STO basis. Starting values for the 4sp exponents were taken from the optimized values for the ^1S atomic state of Zn. These values were then scaled by energy optimizing a common scaling factor in $\text{Zn(NH}_3)_4{}^{2+}$. The final 4sp exponents were 2.8824 and 1.7073. The 3d orbital is a 2G expansion of a triple-ζ STO. Ab initio studies were performed on PRDDO-optimized geometries of **1** and **2** at various values of θ.[11] These were performed using GAMESS on a CONVEX C-220 computer at the University of Texas at Arlington. A typical calculation on the *tert*-butyl derivative of **1** or **2** required 6-7 h of CPU time. The Zn atom has a total of 37 basis functions, which gives grand totals of 163 basis functions for **1a**, 165 for **2a**, 247 for **1b**, and 249 for **2b**.

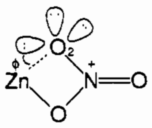
(11) Some important geometric parameters used in the ab initio calculations (distances in angstroms and angles in degrees; see **1** and **2** for numbering): **1a** (monodentate form) Zn–O_1 = 1.783, $\text{O}_1\text{–N}_1$ = 1.360, $\text{N}_1\text{–O}_2$ = 1.290, $\text{N}_1\text{–O}_3$ = 1.265, $\text{Zn–O}_1\text{–N}_1$ = 125; **1a** (bidentate form) Zn–O_1 = 1.947, $\text{O}_1\text{–N}_1$ = 1.337, $\text{N}_1\text{–O}_2$ = 1.345, $\text{N}_1\text{–O}_3$ = 1.245, $\text{Zn–O}_1\text{–N}_1$ = 90.8; **1b** (monodentate form) Zn–O_1 = 1.770, $\text{O}_1\text{–N}_1$ = 1.362, $\text{N}_1\text{–O}_2$ = 1.284, $\text{N}_1\text{–O}_3$ = 1.264, $\text{Zn–O}_1\text{–N}_1$ = 135; **1b** (bidentate coordination) Zn–O_1 = 2.003, $\text{O}_1\text{–N}_1$ = 1.341, $\text{N}_1\text{–O}_2$ = 1.345, $\text{N}_1\text{–O}_3$ = 1.242, $\text{Zn–O}_1\text{–N}_1$ = 89.6; **2a** (monodentate form) Zn–O_1 = 1.751, $\text{O}_1\text{–C}_1$ = 1.323, $\text{C}_1\text{–O}_2$ = 1.228, $\text{C}_1\text{–O}_3$ = 1.379, $\text{Zn–O}_1\text{–C}_1$ = 135; **2b** (monodentate coordination) Zn–O_1 = 1.759, $\text{O}_1\text{–C}_1$ = 1.321, $\text{C}_1\text{–O}_2$ = 1.226, $\text{C}_1\text{–O}_3$ = 1.379, $\text{Zn–O}_1\text{–C}_1$ = 135.

(12) Hehre, W. J.; Stewart, R. F.; Pople, J. A. *J. Chem. Phys.* **1969**, *51*, 2657.

(13) We repeated the entire potential energy surface calculations on $[\eta^3\text{-HB(3-Hpz)}_3]\text{Zn(HCO}_3)$, where the zinc binds the other oxygen (O_3) atom. The potential energy surface has only one minimum ($\theta \sim 132°$). Therefore we considered only **2b** when R = *t*-Bu.

(14) Looney, A.; Parkin, G.; Alsfasser, R.; Ruf, M.; Vahrenkamp, H. *Angew. Chem. Int., Ed. Engl.* **1992**, *31*, 92.

A comparison of Figure 1 and 2 reveals that **2** differs from **1** in one fundamental respect: it has no second minimum corresponding to the bidentate form. The difference between these two surfaces can be understood by comparing the localized valence structures **3** and **4**, obtained by applying the Boys localization method[15] to PRDDO wave functions at the bidentate geometries (θ = 90.8 and 89.1°) for **1a** and **2a**, respectively.[16] The relevant quantity is the angle ϕ between the hybrid of the lone pair on O_2 coordinating to Zn and the Zn–O_2 axis. For **1a**, ϕ is 11°, while, for **2a**, it is 18°. This difference is expected on the basis of the formal hybridizations of O_2 in **1a** (sp^3) and **2a** (sp^2).[17] The larger value of ϕ for **2a** leads directly to a reduced overlap population[18] and a destabilization of bidentate coordination. Binding through the other oxygen (O_3) in **2** would alleviate this problem, since this oxygen is sp^3 hybridized; however, a single monodentate minimum[13] still results, probably because O_3 is slightly less basic than O_2.[19]



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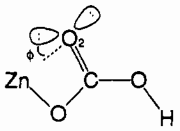

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From the above results, we conclude two points: (1) In agreement with experiment, [η^3-HB(3-Rpz)$_3$]Zn(HCO_3) (**2**) has a monodentate minimum. (2) [η^3-HB(3-Rpz)$_3$]Zn(NO_3) (**1**) has a lowest energy minimum corresponding to monodentate coordination but also has a bidentate minimum. Thus, when compared to bicarbonate, NO_3^- exhibits an enhanced capability to bind in a bidentate fashion. This suggests that comparison of nitrate to bicarbonate should be done with some degree of caution.

Acknowledgment. We thank the Robert A. Welch Foundation (Grant No. Y-743), the University of Texas Center for High Performance Computing, and the Texas Advanced Technology Program for support of this work.

Note Added in Proof. After acceptance of this paper, two related theoretical studies have appeared: Zheng, Y.-J.; Merz, K. M. *J. Am. Chem. Soc.* **1992**, *114*, 10498. Aqvist, J.; Fothergill, M.; Warshel, A. *J. Am. Chem. Soc.* **1993**, *115*, 631.

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(15) Boys, S. F. In *Quantum Theory of Atoms, Molecules, and the Solid State*; Lōwdin, P.-O., Ed.; Academic Press: New York, 1966.

(16) These angles result in Zn–O_2 distances which are exactly the same in the two complexes being compared, thus facilitating the comparison of LMO's and population analyses.

(17) The calculated average hybridizations for the O_2 lone-pair orbitals exhibit the same trends: 2.1 for **1a** and 1.3 for **2a**.

(18) The calculated overlap populations for Zn–O_2 also exhibit the same trends: 0.225 (**1a**) and 0.207 (**2a**).

(19) Compare, for instance, the gas-phase basicities of H_2O and H_2CO: Lias, S. G.; Liebman, J. F.; Levin, R. D. *J. Phys. Chem. Ref. Data* **1984**, *13*, 695.