CO_2 Hydration by Mimic Zinc Complex for Active Site of Carbonic Anhydrase

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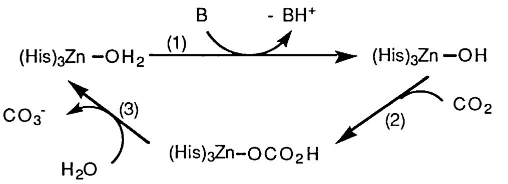
The deprotonation of the zinc-bound water and the zinc-bound bicarbonate which arose from hydration of CO_2 have been simulated *in vitro* according to the proposed mechanism for *carbonic anhydrase,* with the model complex of its own active site, $[LZn(OH_2)]^{2+}$, where L=tris(2-benzimidazolylmethy)amine.

Carbonic anhydrase (*CA*) containing zinc ion in its active site has a catalytic function of CO_2 hydration. In the active site of *CA*, zinc ion has tetrahedral geometry and coordinated by three imidazoles of histidines and H_2O (or OH^-).[1] It is suggested that the CO_2 hydration of *CA* consists of the three processes[2,3] as follows :



A number of studies on the syntheses, structures and reaction of the model complexes as the active site of *CA* have reported many zinc complexes which consist of pyrazolylborate / pyrazolylmethylamine ligands or macrocyclic amine ligands.[4] The coordinated OH^- in these model complexes was reacted with CO_2 to produce dimer and trimer complexes[5] bridged by CO_3^{2-}. Since the polymerization does not take place *in vivo*, the products are not enzyme mimicking model. We designed the model complex for the active site of *CA* providing (i) imidazole ligand which corresponds to histidine imidazole, (ii) coordinated water molecule and (iii) hydrophobic pocket which specifies the direction for substrate to approach the active site. This paper reports the *in vitro* simulation of CO_2 hydration with the zinc model complex and the characterization of zinc-bound water molecule.

The mimic model complexes, $[LZn(OH_2)]^{2+}$ **1** [6,7] and $[LZn(OD_2)]^{2+}$ **1'**, were synthesized from its chloride derivative, $[LZnCl]^+$ **2**[8] by substituting H_2O, D_2O for Cl^- with Ag^+ in acetone/H_2O, D_2O (20:1 v/v). Here, the ligand, tris(2-benzimidazolylmethyl)amine, **L**[8], can reproduce the tetrahedral geometry which is identical with the active site of *CA* because of its own steric hindrance. Since the result of X-ray crystallography for **2**[9] indicated the tetrahedral coordination geometry around zinc ion, as shown in Figure 1, the tetrahedral geometry around zinc ion in **1/1'** was unambiguously assigned on the basis of EXAFS analysis and ^{13}C / ^2H / ^1H NMR. Zinc ion is four-coordinate with three Zn-N(benzimidazolyl) distances of 2.05 Å and one Zn-O (H_2O) distances of 2.00 Å, determined by EXAFS analysis. Now, the result of EXAFS analysis for **2**·PF_6 (Zn-N = 2.00 Å, Zn-Cl =2.25 Å) was in good agreement with that of X-ray crystallography (Figure 1).

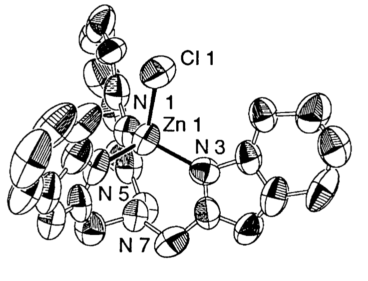
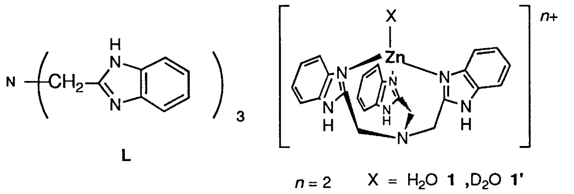


Figure 1. ORTEP drawing of the structure of **2**. Ellipsoids are depicted at the 40% probability level. Selected bond distances (Å) : Zn1-Cl1 2.260(5), Zn1-N1 2.00(2), Zn1-N3 2.02(1), Zn1-N5 2.01(1), Zn1 ··· N7 2.55(2).

Deprotonation from zinc-bound water molecule. In human *CA* II, zinc-bound water molecule may be deprotonated by a couple of water molecules connected to His 64 and Thr 199 through hydrogen bonds.[10,11] In this work, **1'** was deprotonated by weak base (pK_a = 6 ~ 11) and reacted with CO_2 gas. The evidence of deprotonation was obtained from ^2H NMR shown in Figure 2. Both **1'**+base and D_2O+base showed one signal because of the rapid exchange of D^+. The **1'** +base showed the shift to downfield by formation of conjugate acid, baseD^+. The deprotonation of **1'** led to the production of $[LZn(OD)]^+$. On the other hand, ^2H NMR chemical shifts for D_2O+base were almost unchanged. The p*K*a value of the zinc-bound water molecule became lower by coordination bond, compared with that of free water.



n = 2 X = H_2O **1** ,D_2O **1'**
n = 1 X = Cl **2**

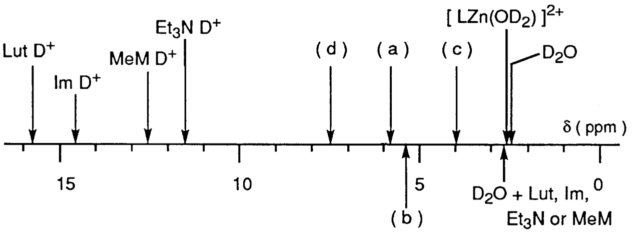


Figure 2. The evidence of deprotonation of the zinc-bound water molecule by ^2H NMR chemical shifts in DMF. The concentrations of **1'**, D_2O, and base are 50 mM. Et_3N : triethylamine, MeM : N-methylmorpholine, Lut : 2,6-lutidine, Im : imidazole. (a) **1'** + Et_3N, (b) **1'** + MeM, (c) **1'** + Lut, (d) **1'** + Im.

The coordinated water molecule of **1/1'** was characterized by ^2H NMR in DMF solution and far IR in CH_3CN solution. ^2H NMR spectra of **1'** showed a single broad profile even at -50°C. The rapid exchange between free D_2O and coordinated D_2O gave rise to a coalescence of both signals. The broad signal of ^2H NMR for D_2O observed at 35 °C was simulated using two site exchange model[12], as shown in Figure 3. The calculated

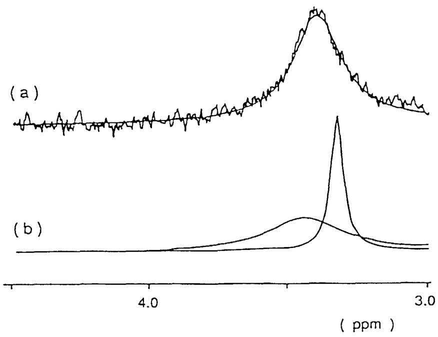


Figure 3. (a) Observed ^2H NMR spectrum of $1'\cdot(PF_6)_2$ in DMF at 35 °C and its simulated ^2H NMR spectrum (solid curve). (b) The calculation of each spectrum for coordinated D_2O (broad signal) and free D_2O (sharp signal) was carried out by using the chemical shifts and transverse relaxation rates obtained from the successful simulation of (a) under the condition of $\tau_c=\tau_f=\infty$ for each life time.

half width of the coordinate D_2O is six times larger than that of free D_2O. The broadening of ^2H NMR spectrum for the zinc-bound D_2O may be induced by ^2H quadrupole relaxation under the effect of Zn(II) ion. The life time for the coordinated D_2O obtained from successful simulation is ca.10^{-5} s which is somewhat longer than the life time obtained from H_2O exchange reaction of $[Zn(OH_2)_6]^{2+}$ (*ca.* 10^{-7} s or k = *ca.* 10^7 s^{-1}).[13] Thus, the water molecule coordinated to Zn(II) in **1/1'** has the kinetic property different from those of water molecules in $[Zn(OH_2)_6]^{2+}$.

The Zn-N, Zn-O and Zn-Cl stretching bands for **1/1'** and **2** were observed at 412 cm^{-1}, 283 cm^{-1} and 280 cm^{-1}, respectively for far IR measurements. The simple harmonic oscillator model for Zn-N in the coordinated benzimidazole, Zn-O in the coordinated water molecule and Zn-Cl gave the force constants of 1.1×10^{-2}, 0.6×10^{-2} and 1.0×10^{-2} N m^{-1}. The largest force constant of Zn-N brings about the stable complex of the three coordinated benzimidazoles around zinc ion. The smallest value of Zn-O may give a chance of ligand exchange reaction, such as a substitution of water molecule for the zinc-bound bicarbonate.

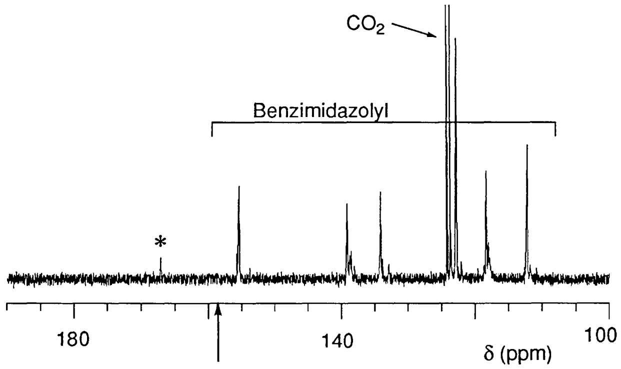


Figure 4. ^{13}C NMR spectrum of $1'\cdot(PF_6)_2$+Et_3N solution after bubbling CO_2 for 5h. The peak with * was assigned to zinc-bound bicarbonate and an arrow points out the position of HCO_3^- for $((Ph_3P)_2N)HCO_3$ solution.

In vitro simulation of enzymatic CO_2 hydration. The bicarbonate complex $[LZn(OCO_2D)]^+$ produced by the reaction of $[LZn(OD)]^+$ with CO_2 gas has been identified by ^{13}C NMR and IR studies. The ^{13}C NMR spectrum (Figure 4) indicated the evidence of CO_2 hydration. The new signal at 167 ppm observed after bubbling CO_2 for 3-5 hours into DMSO solution of $1'\cdot(PF_6)_2$+Et_3N has been attributed to the production of zinc-bound bicarbonate and shifted to downfield from 158 ppm of HCO_3^- in DMSO solution of$((Ph_3P)_2N)HCO_3$. The IR spectrum of the product after bubbling CO_2 showed the new bands at 1675 cm^{-1} and ca. 1440 cm^{-1}, which were not observed for $1'\cdot(PF_6)_2$. Since these two bands were assigned to the symmetric and asymmetric CO stretching mode, the IR spectrum revealed the production of coordinated bicarbonate. The separation of these two bands, $\Delta\nu$ = 235 cm^{-1}, demonstrates unidentate complexation for the zinc-bound bicarbonate.

In this work we used polar aprotic organic solvents, such as DMSO and DMF. Further studies using polar protic solvent of aqueous solution are now in progress.

References and Notes

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6 Anal. Found: C, 37.19 ; H, 3.60 ; N, 13.09%. Calcd for $C_{24}H_{23}N_7F_{12}OP_2Zn$: C, 36.78 ; H, 3.34 ; N, 12.51%. ^1H NMR(acetone-d_6): 4.78(6H, s, CH_2), 7.36(6H, m, benzimidazolyl), 7.58(3H, m, benzimidazolyl), 8.86(3H, m, benzimidazolyl). Gregorzik *et al.* synthesized **1** by different method.[7] Here, coordinated water was never analyzed and characterized.

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9 The structure of $\mathbf{2}\cdot BF_4$ was solved with Rigaku AFC7R diffractometer(Cu K_α=1.54178 Å). The non-hydrogen atoms except for BF_4^- were refined anisotropically. Crystal Data: $\mathbf{2}\cdot BF_4\cdot 0.5H_2O$, $C_{24}H_{22}BClF_4N_7O_{0.5}Zn$, FW = 604.12, triclinic, $P\overline{1}$, a = 15.182(4) Å, b = 18.713(9) Å, c = 13.557(4) Å, α = 100.69(3) °, β = 112.27(2) °, γ = 69.21(3) °,V=3326(2) $Å^3$, Z = 4, R = 0.107.

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