Preparation and Characterization of Hydroxo-zinc(II) Complex Surrounded with Hydrogen Bonding and Hydrophobic Interaction Groups.
A Structural/Functional Model of Carbonic Anhydrases

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A novel Zn(II)–OH complex surrounded with hydrogen bonding and hydrophobic interaction groups has been prepared as a structural/functional model of carbonic anhydrases, and characterized by X-ray structure analysis and ESIMS and ^{1}H- and ^{13}C-NMR spectroscopies. The complex has been found to exhibit a reversible binding of CO_2.

Studies on the hydrolytic reactions for Zn(II)-containing enzymes, such as carbonic anhydrases (CA), carboxypeptidases, and phosphatases, are extensively interested from the viewpoint of bioinorganic subjects.[1] Especially, CA plays physiologically important function to catalyze the interconversion of CO_2 and HCO_3^- in biological system.[2] The crystal structures of human CAs I and II have revealed that each Zn^{2+} ion is a pseudo-tetrahedrally coordinated by three histidine side chains and an H_2O/OH molecule, depending upon pH.[3,4] In this system, the H_2O molecule is polarized by the Zn^{2+} ion to the OH^- ion which can nucleophilically attack the nearby enzymatically-bound CO_2 thereby converting it to HCO_3^-.[2] The catalytic activity is characterized by a pK_a value of ca. 7, which is crucial pK_a for that of the coordinated water molecule on the Zn(II) center in CA; hydration of CO_2 is dominant above pH 7, while dehydration of HCO_3^- is observed below pH 7. Although some studies on the structural and/or functional models of an active site for CA have been reported hitherto using pyrazolylborate,[5] pyrazoylmethylamine,[6] macrocyclic amine ligands,[7] or other ligands,[8] the functional model that has a hydrogen-bonding site and can reversibly bind CO_2 molecule has not been reported. Recently we have succeeded in the preparation of some structural models of the active sites for physiologically-important metalloenzymes.[9,10] The ligands employed in these studies contain noncovalent interaction sites, such as hydrogen bonding NH and hydrophobic *tert*-butyl groups, in the polypyridylamine ligand, which have been mimicked from the local structures around the active centers of metalloenzymes.[9,10] Using the tripodal polypyridylamine ligand which has been substituted by the noncovalent interaction groups, tris(6-neopentylamino-2-pyridylmethyl) amine (TNPA),[10] novel functional model that can reversibly bind CO_2 molecule, $[Zn(tnpa)(OH)]ClO_4$ (**1**), has been prepared. Here we report the preparation and characterization of **1** regulated with hydrogen-bonding interaction and the interesting behavior of reversible binding of CO_2 molecule.

Reaction of $Zn(ClO_4)_2 \cdot 6H_2O$ and an equimolar amount of TNPA in methanol solution gave $[Zn(tnpa)(OH)]ClO_4$ (**1**), whose colorless single crystal was obtained by standing the resultant solution for a few days.[11] The crystal structure of **1** revealed that the Zn(II) ion has a trigonal-bipyramidal geometry with three pyridine nitrogen atoms in equatorial plane (Zn–N(2) = 2.1015(7), Zn–N(3) = 2.1601(9), Zn–N(4) = 2.1828(8) Å) and tertiary amine nitrogen and hydroxide oxygen atoms in axial positions (Zn–N(1) = 2.1495(9) and Zn–O(1) = 1.9315(8) Å), as shown in Figure 1. The Zn–O(1) bond is similar to those observed in the active sites of native CAs I (1.9 Å) and II (2.05 Å) that have been obtained from X-ray diffraction study of the crystals, although it is slightly shorter than that of CA II.[3,4] The trigonal-bipyramidal geometry is also understandable from τ value of 0.90 for the Zn(II) site estimated from the crystal structure.[12] As expected from the design concept of the ligand TNPA, the hydroxide ion is surrounded by the bulky *tert*-butyl groups of the neopentylamino substituents and hydrogen-bonded intramolecularly with the NH groups of the ligand; $O(1)\cdots N(5) = 2.805(1)$, $O(1)\cdots N(6) = 2.827(2)$ and $O(1)\cdots N(7) = 3.003(1)$ Å (Figure 1). The hydrogen bonding interactions in complx **1** are also very similar to those in CA; the zinc-binding hydroxide oxygen is hydrogen bonded with two water molecules (2.60, 2.79 Å) and threonine OH (2.83 Å).[4]

Interestingly, alternate bubbling of CO_2 and Ar into a DMSO-d_6 solution of complex **1** exhibited a reversible spectral change at room temperature, as examined by ^{1}H-NMR. The N–H proton peak observed at 9.21 ppm showed definitive up-field shift to 7.71 ppm by addition of dry CO_2 for 20 min, although the other proton peaks also exhibited slight changes, which were returned to the initial position by bubbling of Ar for 30 min.[13] The reversible binding of CO_2 has also been characterized from ^{13}C-NMR spectroscopy in CD_3OD solution of **1** (Figure 2): A new

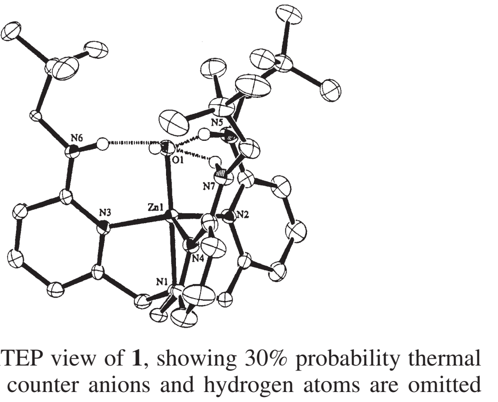


Figure 1. ORTEP view of **1**, showing 30% probability thermal elipsoids. The counter anions and hydrogen atoms are omitted except for NH and OH hydrogens for clarity. Selected bond lengths (Å) and angles (°): Zn(1)–O(1) 1.9483(5), Zn(1)–N(1) 2.132(1), Zn(1)–N(2) 2.111(1), Zn(1)–N(3) 2.1451(6), Zn(1)–N(4) 2.174(1), O(1)–Zn(1)–N(1) 177.86(2), O(1)–Zn(1)–N(2) 101.36(1), O(1)–Zn(1)–N(3) 100.67(2), O(1)–Zn(1)–N(4) 101.36(2), N(1)–Zn(1)–N(2) 80.71(3), N(1)–Zn(1)–N(3) 78.94(5), N(1)–Zn(1)–N(4) 77.23(4), N(2)–Zn(1)–N(3) 112.45(2), N(2)–Zn(1)–N(4) 112.93(5), N(3)–Zn(1)–N(4) 123.64(3).

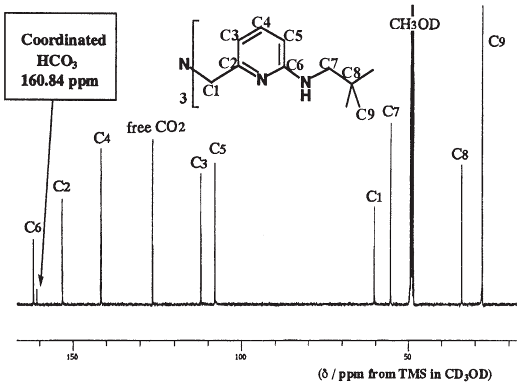
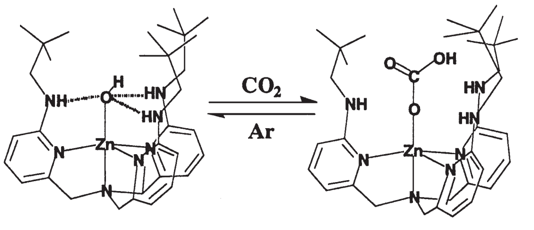


Figure 2. ^{13}C-NMR spectrum of CD_3OD solution of complex **1** after bubbling CO_2 gas (for 20 min) at ambient temperature. The carbon peak at 160.84 ppm disappeared by addition of Ar gas (for 30 min).

peak was detected at 160.84 ppm, assignable to an sp^2 carbon of the coordinated HCO_3^-, because it was detected at different position from those of CO_2 (126.34 ppm) and free HCO_3^- (158 pm).[6a] The peak has disappeared by bubbling of Ar gas. It is also clear from the ESIMS spectroscopic study that the addition of CO_2 to **1** gave the feature isotope clusters at m/z 670.3, corresponding to the formula $[Zn(tnpa)(HCO_3)]^+$, which was replaced by those of $[Zn(tnpa)(OH)]^+$ by bubbling of Ar.



Scheme 1.

It is clear that the coordination mode of HCO_3^- to Zn(II) ion in solution is not a bidentate fashion but a symmetric unidentate one (Scheme 1), because both of the ^{1}H- and ^{13}C-NMR spectra showed higher symmetric spectral patterns and did not exhibit any significant spectral changes except for the large upfield-shift of N$\underline{H}$ peak. Furthermore, the up-field shift of N$\underline{H}$ proton peak of complex **1** by bubbling of CO_2 indicates the losing of hydrogen bonds with the coordinated HCO_3^- from the steric demand between the ligand and bulky *tert*-butyl groups of the TNPA ligand.

In order to estimate a pK_a value of the Zn(II)-coordinated hydroxide oxygen, the pH titration ($Zn–OH^- + H^+ \rightleftarrows Zn–OH_2$) of complex **1** was examined by ^{1}H-NMR spectroscopy following the chemical shift of pyridine H_4-proton peak. The pK_a values of zinc-coordinated hydroxide oxygen, when the titration was carried out in CD_3CN/D_2O (with a 1:1 ratio) and in THF-d_8/H_2O (with a 1:1 ratio) because of low solubility of the complex in water, has been estimated to be 7.88 and 7.60, respectively. They are comparable to that of native CA (p*K*a 7.0 up to 7.5).[2,14] The good agreement in pK_a values between complex **1** and CA may attribute to similarlity in the intramolecular hydrogen bonds as well as that in the active site structures for both cases.[14]

In conclusion, we succeeded in preparation and structural characterization of novel Zn(II)–OH complex with TNPA ligand, in which the labile hydroxo species has been stabilized by intramolecular hydrogen bonding and hydrophobic interaction groups of the ligand. The complex **1** exhibited a reversible binding of CO_2, which was identified by ^{1}H- and ^{13}C-NMR and ESIMS spectroscopies. The estimated pK_a value of the Zn(II)-coordinated hydroxide oxygen was in good agreement with that of native CAs.[2,14] On the basis of these findings, we can suggest that the intramolecular hydrogen bonds with the hydroxide species may regulate the reversible binding of CO_2 for not only $[Zn(tnpa)(OH)]^+$ but also CA.[2]

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11 Crystal data for compound **1**: $C_{33}H_{52}N_7O_5ClZn$, $M_r = 727.65$, Orthorhombic, space group $Pna2_1$ (No. 33), $a = 20.7743(8)$, $b = 15.1716(6)$, $c = 11.8066(4)$ Å, $V = 3721.2(2)$ Å^3, $Z = 4$, $D_{calcd} = 1.299\,g\,cm^{-3}$, $\mu = 7.79\,cm^{-1}$, Mo Kα radiation ($\lambda = 0.71070$ Å), $T = 173$ K, $R_1 = 0.029$, $R_w = 0.090$, and GOF = 1.23 for 25638 independent reflections with $I > 3.0\sigma(I)$ and 428 variables. Elemental analysis: Calcd for **1** ($C_{33}H_{52}N_7O_5ClZn$) C, 54.47; H, 7.20; N, 13.47%. Found: C, 54.41; H, 7.16; N, 13.46%.

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