

Acetaldehyde hydration by zinc–hydroxo complexes: coordination number expansion during catalysis

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The complexes $[\text{Zn}(\text{tren})(\text{OH})]^+$ and $[\text{Cd}(\text{Me}_6\text{tren})(\text{OH})]^+$ are excellent catalysts for the hydration of acetaldehyde while the closely related complex $[\text{Zn}(\text{Me}_6\text{tren})(\text{OH})]^+$ does not catalyze the reaction, consistent with the notion that zinc ions require the ability to accommodate expanded coordination numbers in hydrolytic reactions such as those catalyzed by the enzyme carbonic anhydrase.

Carbonic anhydrases are widely occurring Zn(II) metalloenzymes that catalyze the interconversion of CO_2 and HCO_3^- . The catalytic zinc ion in human carbonic anhydrase II is ligated by three histidine residues and a water molecule in its resting state. Most detailed mechanisms postulated for carbonic anhydrases¹ require expansion of the coordination sphere of the zinc ion from four to five (or six) during the catalytic cycle for addition of zinc-bound hydroxide to the CO_2 with concomitant polarization of the carbonyl group by zinc, and for associative displacement of the bicarbonate product by water.² Higher coordination number intermediates are suggested by crystallographic analyses,^{3,4} but little functional data is available that bears on the significance of this issue. In this paper, we test the hypothesis that zinc ions require coordination number increases in hydrolysis catalytic cycles, specifically the hydrolysis reaction of acetaldehyde catalyzed by zinc complexes of the synthetic ligands tris(2-aminoethyl)amine (tren) and tris(2-dimethylaminoethyl)amine (Me_6tren).

The hydration of acetaldehyde is catalyzed by carbonic anhydrase and has also been studied extensively with several synthetic catalysts and buffers.^{5,6} A plot of $\log(k_{\text{cat}})$ vs. $\text{p}K_{\text{a}}$ ($k_{\text{cat}} = \text{d}k_{\text{obs}}/\text{d}[\text{ZnL}]$) yields a linear relationship suggesting that the mechanism of the reaction is simple nucleophilic attack upon the carbonyl. A simplified mechanistic scheme for the reaction is shown in Scheme 1, with the zinc-hydroxo form of the

catalyst shown as the catalytically active species. Associative exchange is generally observed for substitution reactions of zinc complexes of these ligands;⁷ formation of intermediate **3** requires an expanded coordination sphere.

The ligands tren and Me_6tren offer an opportunity to examine this question since zinc complexes of tren can form six-coordinate complexes but those of Me_6tren cannot. In five-coordinate complexes, both ligands adopt a C_3 -symmetric conformation [Fig. 1(a)].⁸ However, in six-coordinate complexes, tren adopts a C_0 conformation [Fig. 1(b)] that is sterically inaccessible to Me_6tren due to the steric hindrance caused by the bulky dimethylamino substituents. A search of the Cambridge Structural Database⁹ yielded over 20 structures of tetradentate complexes of Me_6tren , all of which showed the ligand in a C_3 -conformation while tren complexes showed both five-coordinate (C_3) and six-coordinate (C_0) complexes. Thus the zinc ion in $[\text{Zn}(\text{Me}_6\text{tren})(\text{OH})]^+$ should be limited to

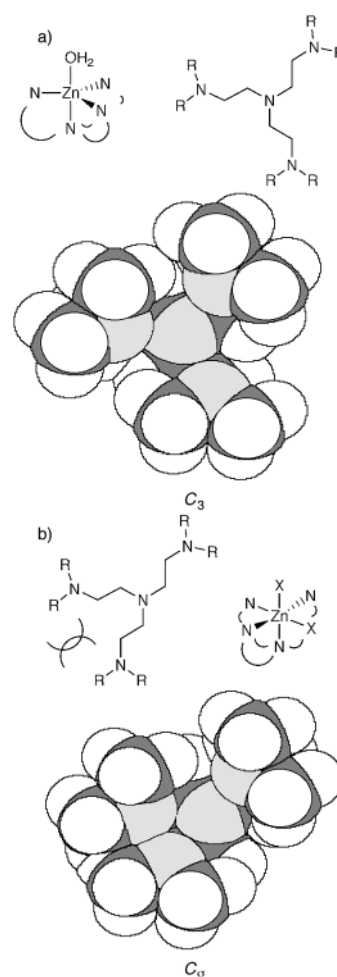
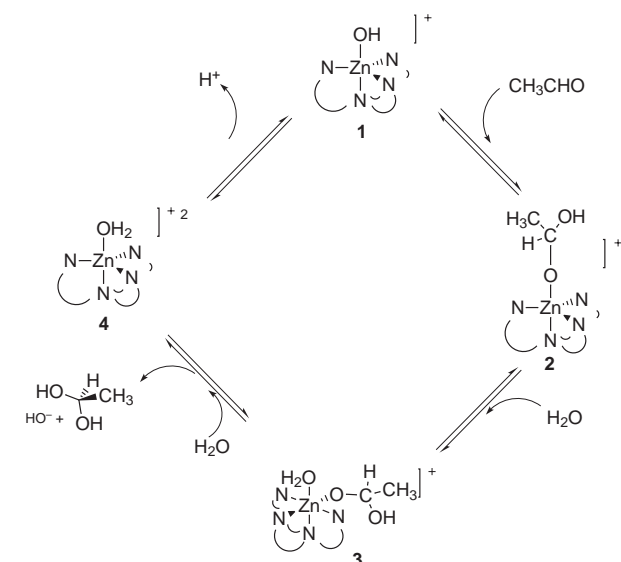


Fig. 1 C_3 (a) and C_0 (b) conformations observed for penta- and hexa-coordination. Steric hindrance in Me_6tren precludes hexa-coordination.



Scheme 1 Proposed mechanism for hydration of acetaldehyde catalyzed by Zn(II) or Cd(II) complexes.

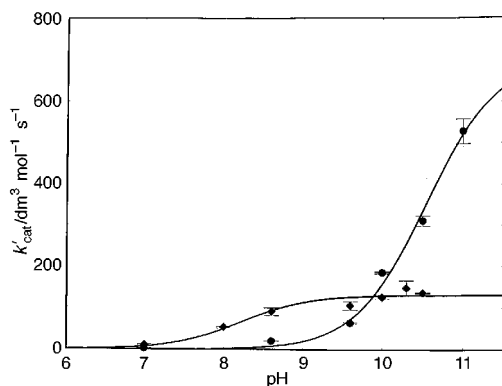


Fig. 2 pH-rate profiles for $[\text{Zn}(\text{tren})(\text{OH})]^+$ (●) and $[\text{Cd}(\text{Me}_6\text{tren})(\text{OH})]^+$ (◆).

maximal five-coordination while in $[\text{Zn}(\text{tren})(\text{OH})]^+$ six-coordination should be readily achievable. Thus, intermediate **3** should be accessible only for the tren complex, and the Me_6tren complex should not catalyze the reaction.

The hydration rate of acetaldehyde in aqueous solution was determined using stopped flow UV-VIS detection, monitoring the decrease of the carbonyl band at 278 nm. The solution used in this work was 10% aq. MeCN {0 °C, 0.1 M NaClO_4 ; $[\text{CH}_3\text{CHO}] = 36 \text{ mM}$, $[[\text{Zn}(\text{L})(\text{OH})]^+] = 0.25\text{--}5 \text{ mM}$ }.^{5,10} The results are shown in Fig. 2, where each data point represents 3–5 experiments at different catalyst concentrations, each run in triplicate; The line is a non-linear least-squares fit of $k_{\text{obs}} = k_{\text{cat}}\chi_{[\text{Zn}(\text{L})(\text{OH})]^+}$ where $\chi_{[\text{Zn}(\text{L})(\text{OH})]^+}$ is the mole fraction of catalytically active species. The observed pH-rate profile for $[\text{Zn}(\text{tren})(\text{OH})]^+$ reveals a point of inflection at pH 10.5 similar to the published $\text{p}K_{\text{a}}$.¹¹ The $\log(k_{\text{cat}})$ is 2.84, consistent with that predicted from the Brønsted analysis (2.9).⁶ However, for the complex $[\text{Zn}(\text{Me}_6\text{tren})(\text{OH})]^+$, no catalysis was observed even though it should be easily seen under these conditions [predicted $\log(k_{\text{cat}}) = 2.0$].

It was reasoned that although the hydration reaction is not catalyzed by $[\text{Zn}(\text{Me}_6\text{tren})(\text{OH})]^+$, it should be catalyzed by $[\text{Cd}(\text{Me}_6\text{tren})(\text{OH})]^+$ complex since the Cd(II) ion may have an expanded coordination number due to its large ionic radius.⁸ The observed pH-rate profile is shown in Fig. 2. The $\text{p}K_{\text{a}}$ value from curve fitting is 8.2 which compares favorably with that determined by pH titration.¹² The $\log(k_{\text{cat}})$ is 2.12 as expected from the Brønsted relationship discussed above.

Based on the available data, the most likely explanation for the inactivity of $[\text{Zn}(\text{Me}_6\text{tren})(\text{OH})]^+$ is the inability of water to displace the hydrated acetaldehyde intermediate due to the steric bulkiness of the ligand that prevents hexa-coordination of the Zn(II) ion. Formation of **2** should not be hindered by the

methyl groups in Me_6tren as indicated by examination of models and calculations of heats of formation (Spartan: MNDO)¹³ of **2** and various similar compounds that failed to show any significant steric interaction between the atoms of the acetaldehyde and the ligand.

It is widely believed that one reason that enzymes maintain low coordination numbers to catalytic zinc ions is to increase the acidity of the zinc-bound water molecule.^{14–16} The implication of the present study is that zinc ions involved in hydrolytic reactions require access to an expanded coordination sphere for catalyst turnover. This requirement may be an additional reason for the frequent observation of four-coordinate zinc ions in crystal structures of resting state enzyme active sites, and higher coordination numbers in corresponding enzyme-inhibitor complexes.

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