

# Enthalpic Control of Zinc–Water Acidity in Complexes of Tris(2-aminoethyl)amine and Tris(2-(dimethylamino)ethyl)amine

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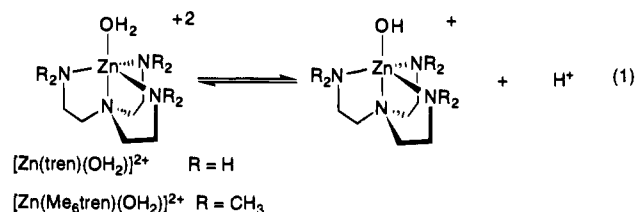
**Abstract:** The enthalpies of ionization ( $\Delta H_a$ ) of zinc-bound water in the complexes  $[\text{Zn}(\text{tren})(\text{OH}_2)]^{2+}$  and  $[\text{Zn}(\text{Me}_6\text{tren})(\text{OH}_2)]^{2+}$  have been determined by isothermal titration calorimetry and potentiometric methods in 1 M  $\text{NaClO}_4$ . The  $\Delta G^\circ$  (14.6 kcal/mol) for  $[\text{Zn}(\text{tren})(\text{OH}_2)]^{2+}$  is mainly due to large positive  $\Delta H$  (11.0 kcal/mol), while that for  $[\text{Zn}(\text{Me}_6\text{tren})(\text{OH}_2)]^{2+}$  (12.1 kcal/mol) is attributable equally to positive  $\Delta H$  (6.1 kcal/mol) and negative  $\Delta S$  at 298 K. The increase in acidity of  $[\text{Zn}(\text{Me}_6\text{tren})(\text{OH}_2)]^{2+}$  relative to  $[\text{Zn}(\text{tren})(\text{OH}_2)]^{2+}$  corresponds to a  $\Delta\Delta G^\circ$  of  $-2.5$  kcal/mol. The  $\Delta\Delta H$  is  $-4.9$  kcal/mol, and it is partially offset by a 2.4 kcal/mol contribution from  $\Delta(T\Delta S)$ . These values differ from ones previously reported, particularly in that the thermodynamic basis of the increased acidity of the latter complex is not related to entropic effects, as was previously believed. Several possible origins of the enthalpic difference in the two ionization reactions are discussed. The results of the study invalidate the original data that led to the view that solvent structure mediates the  $\text{p}K_a$  of zinc-bound water in zinc hydrolytic enzymes.

## Introduction

The structure and function of the matrix metalloproteinases<sup>1</sup> (e.g., collagenases,<sup>2</sup> gelatinases,<sup>3</sup> and stromelysins<sup>4</sup>), astacins,<sup>5</sup> snake venom metalloproteinases,<sup>6</sup> and serralyisins<sup>7</sup> is currently an area of active investigation. Enzymes of this class contain the amino acid consensus sequence HEXXHXXGXXH, presenting three histidine ligands to a catalytic zinc ion that also ligates a water molecule. Due to active site structural similarities, the mechanisms of the endopeptidases are thought to be similar to the much better characterized enzyme thermolysin.<sup>8</sup> The acidity of zinc-bound water is crucial to the function of zinc hydrolytic enzymes.<sup>9</sup> The nucleophilic water molecule is activated by both ligation to the zinc ion and interactions with various active site residues. While the effect of the zinc ion and its coordination sphere are at least partially understood, the magnitude and physical basis of secondary interactions with active site residues remain unclear. Site-directed mutagenesis studies, for example, indicate that such effects can be very large.<sup>10–12</sup>

Hydrophobic surface area in the proximity of metal-bound

water tends to enhance the acidity of the water molecule.<sup>11,13–15</sup> A traditional rationalization of this effect can be attributed largely to a classic paper in which it was reported that metal aquo complexes of tris(2-(dimethylamino)ethyl)amine ( $\text{Me}_6\text{tren}$ ) were more acidic by 1–2  $\text{p}K_a$  units than similar complexes of tris(2-aminoethyl)amine ( $\text{tren}$ ), eq 1.<sup>13</sup> Temperature dependence



of the acid dissociation constants ( $K_a$ ) indicated that the effect was predominantly entropic in nature. It was postulated that solvent structure near the water molecule in the  $\text{Me}_6\text{tren}$  complex was more highly structured than in the corresponding  $\text{tren}$  complex, and that protolysis resulted in greater increase in solvent disorder. We have measured directly the heats of reactions shown in eq 1 using titration calorimetry and together with potentiometric methods find that the effect is entirely due to enthalpy changes.

## Materials and Methods

**Materials.**  $\text{Me}_6\text{tren}$  was prepared by published procedures.<sup>27</sup> Hydrochloride salts of the ligands were prepared and recrystallized twice from methanol/water; elemental combustion analysis yielded satisfactory results ( $<0.4\%$ ). Solutions of  $[\text{Zn}(\text{tren})](\text{ClO}_4)_2$  and  $[\text{Zn}(\text{Me}_6\text{tren})](\text{ClO}_4)_2$  were prepared from the ligand hydrochlorides and  $\text{Zn}(\text{ClO}_4)_2$  (standardized with EDTA) or from preformed  $[\text{Zn}(\text{L})](\text{ClO}_4)_2$  complexes of satisfactory elemental analysis. All measurements were done with sample solutions containing 1 M  $\text{NaClO}_4$  as the ionic strength adjuster. Additional chemicals were of the highest purity commercially available.

**Potentiometry.** Potentiometric studies were conducted with an Orion 801A digital pH meter and a Radiometer combined pH electrode ( $\text{Ag}/\text{AgCl}$ ) with a double liquid junction using saturated  $\text{LiCl}$  solution

- <sup>⊗</sup> Abstract published in *Advance ACS Abstracts*, November 1, 1995.
- (1) Willenbrock, F.; Murphy, G.; Phillips, I. R.; Brocklehurst, K. *FEBS Lett.* **1995**, *358*, 189.
- (2) Lovejoy, B. et al. *Science* **1994**, *263*, 375.
- (3) Sato, H.; Takino, T.; Okada, Y.; Cao, J.; Shinagawa, A.; Yamamoto, E.; Seiki, M. *Nature* **1994**, *370*, 61.
- (4) Gooley, P. R.; O'Connell, J. F.; Marcy, A. I.; Cuca, G. C.; Salowe, S. P.; Bush, B. L.; Hermes, J. D.; Esser, C. K.; Hagmann, W. K.; Springer, J. P.; Johnson, B. A. *Nature Struct. Biol.* **1994**, *1*, 111.
- (5) Bode, W.; Gomis-Rüth, F. X.; Huber, R.; Zwilling, R.; Stöcker, W. *Nature* **1992**, *358*, 164.
- (6) Gomis-Rüth, F. X.; Kress, L. F.; Kellermann, J.; Mayr, I.; Lee, X.; Huber, R.; Bode, W. *J. Mol. Biol.* **1994**, *239*, 513.
- (7) Baumann, U.; Wu, S.; Flaherty, K. M.; McKay, D. B. *EMBO J.* **1993**, *12*, 3357.
- (8) Matthews, B. W. *Acc. Chem. Res.* **1988**, *21*, 333.
- (9) Vallee, B. L.; Auld, D. S. *Acc. Chem. Res.* **1993**, *26*, 543.
- (10) Krebs, J. F.; Ippolito, J. A.; Christianson, D. W.; Fierke, C. A. *J. Biol. Chem.* **1993**, *268*, 27458.
- (11) Fierke, C. A.; Krebs, J. F.; Venters, R. A. In *Carbonic Anhydrase*; Botre, F., Gros, G., Storey, B. T., Eds.; VCH Publisher, Inc.: Weinheim, 1991; p 22.
- (12) Chen, X.; Tu, C.; LoGrasso, P. V.; Laipis, P. J.; Silverman, D. N. *Biochemistry* **1993**, *32*, 7861.

- (13) Coates, J. H.; Gentle, G. J.; Lincoln, S. F. *Nature* **1974**, *249*, 773.
- (14) Woolley, P. *Nature* **1975**, *258*, 677.
- (15) Groves, J. T.; Baron, L. A. *J. Am. Chem. Soc.* **1989**, *111*, 5442.

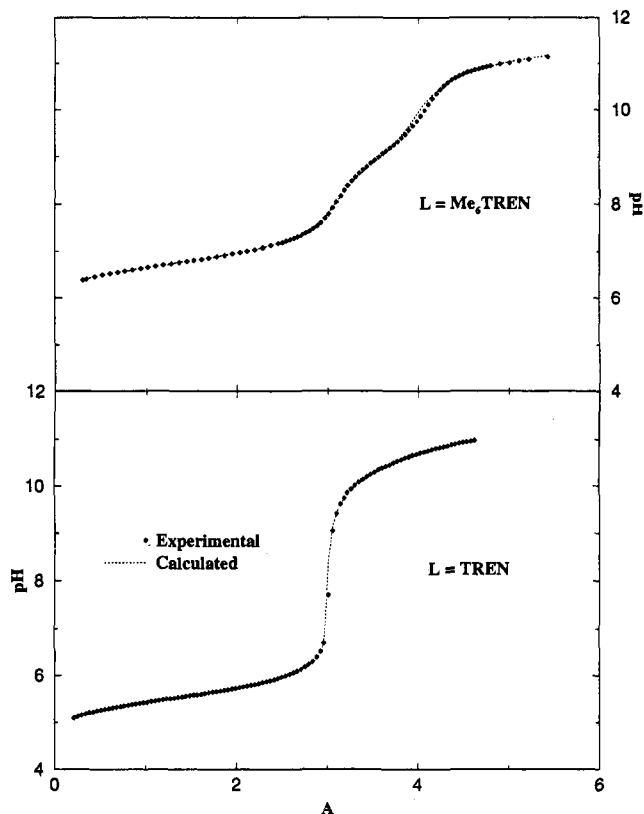


Figure 1. Plot of pH vs  $A = [-OH]/([Zn(L)OH_2]^{2+})_{initial}$ .

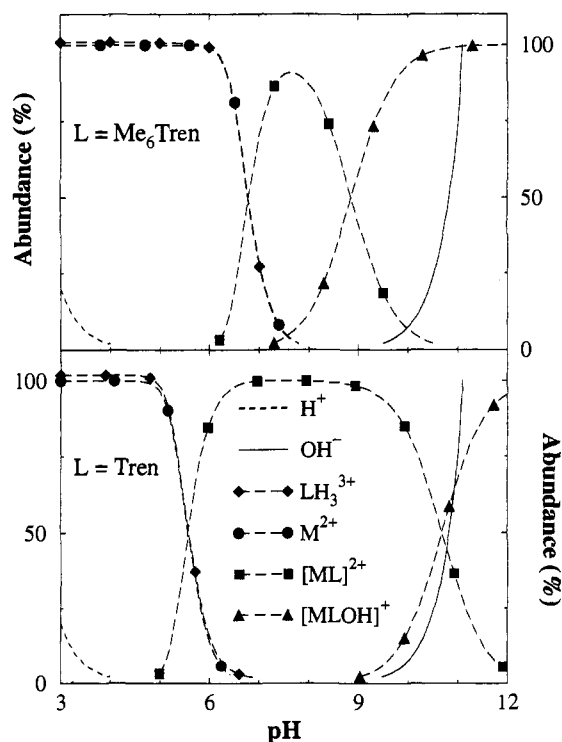


Figure 2. Species distribution as a function of pH, determined by potentiometric titration of ligands in the presence and absence of  $Zn(ClO_4)_2$ . Species  $LH_2^{2+}$ ,  $LH^+$ , and  $L$  were each less than 1% over the pH range studied.

for the salt bridge. The system was calibrated with dilute standard acid and alkali solutions, thus defining  $pH = -\log [H^+]$ .<sup>18</sup>  $K_w$  was determined in 1 M  $NaClO_4$  at each temperature studied. Solutions (5 mM) of  $tren \cdot 3HCl$  and  $Me_6tren \cdot 3.5HCl$  were titrated to  $pH > 11$ . The  $HCl$  stoichiometry was confirmed by pH titration. The dissociation constants were calculated from the data using the program BEST.<sup>18</sup> Species distributions were calculated using the program SPECS<sup>18</sup> and

Table 1. Dissociation Constants As Determined by Potentiometry

temp, °C	$H_3tren^{3+}$			$Zn(tren)(OH_2)^{2+}$	
	log $K_1$	log $K_2$	log $K_3$	$pK_{H_2O}$	log $K_{ML}$
25	$10.43 \pm 0.03$	$9.87 \pm 0.01$	$9.01 \pm 0.01$	$10.68 \pm 0.04$	$15.20 \pm 0.03$
35	$10.24 \pm 0.01$	$9.61 \pm 0.01$	$8.73 \pm 0.01$	$10.48 \pm 0.01$	$14.96 \pm 0.04$
45	$10.11 \pm 0.01$	$9.39 \pm 0.01$	$8.49 \pm 0.01$	$10.27 \pm 0.01$	$14.86 \pm 0.01$

temp, °C	$H_3Me_6tren^{3+}$			$Zn(Me_6tren)(OH_2)^{2+}$	
	log $K_1$	log $K_2$	log $K_3$	$pK_{H_2O}$	log $K_{ML}$
25	$9.98 \pm 0.01$	$9.31 \pm 0.01$	$8.11 \pm 0.02$	$8.86 \pm 0.01$	$9.68 \pm 0.01$
35	$9.91 \pm 0.02$	$9.14 \pm 0.02$	$7.95 \pm 0.05$	$8.74 \pm 0.01$	$9.70 \pm 0.01$
45	$9.87 \pm 0.02$	$8.99 \pm 0.02$	$7.87 \pm 0.05$	$8.58 \pm 0.01$	$9.97 \pm 0.02$

are shown in Figure 2. All data represent the average of at least two independent experiments.

**High Precision Isothermal Titration Calorimetry (ITC).** The heat of reaction ( $\Delta H_b$ ) of base with metal-bound water was measured using the Omega titration calorimeter from Microcal, Inc. (Northampton, MA).<sup>17</sup> In a typical titration and at a particular temperature, 1.4 mL of a complex solution ( $[Zn(tren)](ClO_4)_2 \cdot 3HCl$  or  $[Zn(Me_6tren)](ClO_4)_2 \cdot 3.5HCl$ ) with 1 mM concentration is titrated with a 0.06 M NaOH solution using a 100- $\mu$ L syringe by 26 injections of 4  $\mu$ L each. This syringe also serves to mix the reactants completely in the sample cell by rotating it at 400 rpm. The reference cell of the calorimeter was filled with water and the instrument is calibrated by means of a known standard electrical pulse. For each injection, the area under the resulting peak is proportional to the heat of reaction. Once this heat is corrected for the titrant dilution at the proper pH and normalized by the titrant solution concentration it becomes equal to the reaction enthalpy,  $\Delta H_b$ . The precision of the heat of each injection is about 1  $\mu$ cal, which is only 0.1% of the actual measured heats. The resulting calorimetric profile, heat vs volume, was compared with pH vs volume of the potentiometric data, generated by titrating 3.5 mL of complex solution with 10- $\mu$ L injections of 0.06 M NaOH to maintain equivalence of states. The  $\Delta H_a$  was determined at three temperatures from the observed heat of reaction after a correction for the heat of neutralization,  $\Delta H_w$ . This latter enthalpy was determined experimentally at each temperature in calorimetric titrations of  $HClO_4$  with NaOH, both solutions in 1 M  $NaClO_4$ .

## Results and Discussion

**Potentiometry.** Typical pH titrations (Figure 1) and the derived species distribution functions are shown in Figure 2. The dissociation constants for each of the three acid species of each molecule, as well as the  $K_{H_2O}$  and  $K_{ML}$  of the zinc-water complexes, are presented in Table 1. The potentiometric data indicate that tren forms a much more stable complex with  $Zn(II)$  (log  $K_{ML} = 15.2$ ) than does  $Me_6tren$  (log  $K_{ML} = 9.68$ ).<sup>19</sup> The lower log  $K_{ML}$  in the  $Me_6tren$  complex correlates with the lower  $pK_a$  of each of the three basic nitrogen atoms in  $Me_6tren$  (9.98, 9.31, and 8.11) than in  $tren$  (10.43, 9.87, 9.01). The lower acidity of the tertiary ( $Me_6tren$ ) vs primary amines ( $tren$ ) closely parallels the observed acidities of trimethylammonium (9.80) and methylammonium (10.6) ions.<sup>20</sup> The  $pK_{H_2O}$  data correlate well with the originally reported data<sup>13</sup> and with other published data at 25 °C.<sup>19</sup>

**Isothermal Titration Calorimetry.** Typical heat vs pH profiles for each complex are shown in Figure 3; the reaction heats and the heats of neutralization are presented in Table 2. Both acid dissociation reactions are endothermic. The observed

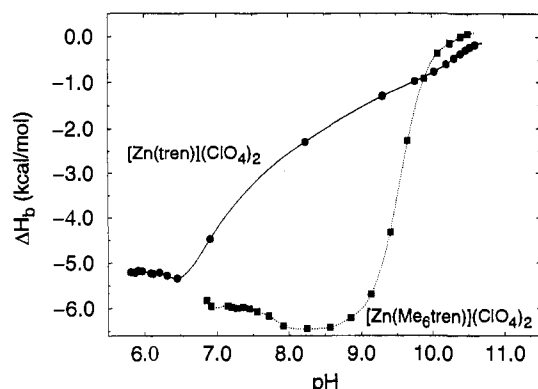
(16) (a) Marky, L. A.; Breslauer, K. J. *Biopolymers* **1987**, *26*, 1601. (b) van Holde, K. E. *Physical Biochemistry*, 2nd ed.; Prentice-Hall, Inc.: Englewood Cliffs, NJ, 1971; p 57.

(17) Wiseman, T.; Williston, S.; Brandts, J. F.; Lin, L.-N. *Anal. Biochem.* **1989**, *179*, 131.

(18) Martell, A. E.; Motekaitis, R. J. *Determination and Use of Stability Constants*, 2nd ed.; VCH Publishers, Inc.: New York, 1992.

(19) Anderegg, G.; Gramlich, V. *Helv. Chim. Acta* **1994**, *77*, 685.

(20) Jones, I. F. M.; Arnett, E. M. *Prog. Phys. Org. Chem.* **1974**, *11*, 263.



**Figure 3.** Plot of  $\Delta H_b$  vs pH. Obtained from the experimental curves ( $q$  and pH vs volume of titrant) as described in the text.

**Table 2.** Observed  $\Delta H$  and Extrapolated  $\Delta C_p$  for the Reaction of  $\text{OH}^-$  with  $[\text{Zn}(\text{tren})(\text{H}_2\text{O})]^{2+}$  and  $[\text{Zn}(\text{Me}_6\text{tren})(\text{H}_2\text{O})]^{2+}$

	[Zn(tren)(OH <sub>2</sub> )] <sup>2+</sup> at given pH <sup>a</sup>			[Zn(Me <sub>6</sub> tren)(OH <sub>2</sub> )] <sup>2+</sup> at given pH <sup>a</sup>			$\Delta H_w$
	7.5	8.0	8.5	8.5	9.0	9.5	
25.0 °C	-2.00	1.52	-1.16	-6.04	-6.40	-6.42	12.48
35.0 °C	-2.70	-1.93	-1.20	-5.77	-5.87	-5.73	12.49
45.0 °C	-2.00	-1.29	-0.69	-5.34	-5.35	-5.28	12.34
$\Delta C_p$	-7.25	4.32	15.9	28.0	45.4	49.6	

<sup>a</sup> kcal/mol.

**Table 3.** Thermodynamic Parameters Determined for the Acid Dissociation of Water in  $[\text{Zn}(\text{tren})(\text{H}_2\text{O})]^{2+}$  and  $[\text{Zn}(\text{Me}_6\text{tren})(\text{H}_2\text{O})]^{2+}$  at 298 K (1 M NaClO<sub>4</sub>)

	[Zn(tren)OH <sub>2</sub> ] <sup>2+</sup>		[Zn(Me <sub>6</sub> tren)OH <sub>2</sub> ] <sup>2+</sup>		difference	
	this work	lit <sup>b</sup>	this work	lit <sup>b</sup>	this work	lit <sup>b</sup>
pK <sub>a</sub>	10.7	10.3	8.9	9.0	-1.8	-1.3
$\Delta G^\circ$ (kcal/mol)	14.6		12.1		-2.5	
$\Delta H$ (kcal/mol)	11.0 <sup>a,c</sup>	2.8	6.1 <sup>a,e</sup>	11.8	-4.9	9.0
$-T\Delta S$ (kcal/mol) <sup>f</sup>	3.6 <sup>a,c</sup>	11.1	6.0 <sup>a,e</sup>	0.5	2.4	-10.6
$\Delta C_p$ (cal/(°C·mol)) <sup>d</sup>	4 <sup>g</sup>		45 <sup>g</sup>			g

<sup>a</sup> Calorimetry; 3% estimated error. <sup>b</sup> From ref 13. <sup>c</sup> pH = 9.0. <sup>d</sup> Slope from the plot of  $\Delta H_{\text{cal}} + \Delta H_w$  vs  $T$ . <sup>e</sup> pH = 8.0. <sup>f</sup> Calculated from Gibbs equation using  $K$  and  $\Delta H$ . <sup>g</sup> Values are small compared to experimental error. 1 cal = 4.18 J.

$\Delta H$  varied less than 6% within  $\pm 0.5$  pH units. We have measured a soft dependence of the enthalpies on temperature; i.e., the measured heat capacities are small in magnitude, which is consistent with a slightly more hydrophobic character for the complex with Me<sub>6</sub>tren.

**Thermodynamics of Zinc–Water Acidity.** Standard thermodynamic profiles at 25 °C are shown in Table 3. The heats of reaction correspond to the pH at which mainly  $[\text{Zn}(\text{L})\text{OH}]^+$  is formed during the titration, as indicated by the potentiometric species distribution profiles of Figure 2. The  $\Delta G^\circ$  for  $[\text{Zn}(\text{tren})(\text{OH}_2)]^{2+}$  is primarily due to a large endothermic  $\Delta H$ , while that for  $[\text{Zn}(\text{Me}_6\text{tren})(\text{OH}_2)]^{2+}$  is attributable equally to both unfavorable  $\Delta H$  and  $\Delta S$  at this temperature. The increase in acidity of  $[\text{Zn}(\text{Me}_6\text{tren})(\text{OH}_2)]^{2+}$  relative to  $[\text{Zn}(\text{tren})(\text{OH}_2)]^{2+}$  corresponds to a  $\Delta\Delta G^\circ$  of  $-2.5$  kcal/mol. The  $\Delta\Delta H$  is  $-4.9$  kcal/mol, and is partially compensated by a 2.4 kcal/mol entropy contribution. These results show that the difference in acidity is primarily due to enthalpic effects. Application of the van't Hoff equation to our potentiometric data leads to a  $\Delta\Delta H$  of  $-3.0$  kcal/mol, which is in qualitative agreement with the calorimetric results. Thus, the reason for the discrepancy with the literature value is unclear.

The physical basis of the enthalpic effect is not easily ascribed to any single phenomenon. However, the simplest explanation

is that the methyl groups reduce the overall capacity of the Me<sub>6</sub>tren complex to stabilize charge in the conjugate acid (2+) form, favoring proton dissociation and formation of zinc complex with lower overall charge (1+, eq 1). Whether the detailed mechanism involves solvation effects (e.g., hydrophobic hydration) or simply shielding of the charge from solvent dielectric by the methyl substituents is not discernible from the present data.

A related series of observations can be seen in thermodynamic studies of the anomalous order in alkylamine acidity<sup>20</sup> (that solution acidities are opposite to those observed in the gas phase for the series  $\text{NH}_4^+$ ,  $\text{RNH}_3^+$ ,  $\text{R}_2\text{NH}_2^+$ , and  $\text{R}_3\text{NH}^+$ ).<sup>20</sup> These studies showed that the absence of  $\text{N}-\text{H}\cdots\text{OH}_2$  hydrogen bond interactions between the cation and water results in reduced solvation of the cation and thus greater acidity.<sup>20</sup> Perhaps  $\text{N}-\text{H}\cdots\text{OH}_2$  hydrogen bond interactions may influence the pK<sub>a</sub> of  $\text{Zn}-\text{OH}_2$  since ligand  $\text{N}-\text{H}\cdots\text{OH}_2$  hydrogen bonding would disperse charge and thus stabilize the conjugate acid. Indeed, the enthalpies and heat capacities associated with  $\text{RNH}_3^+$  vs  $\text{R}_3\text{NH}^+$  acid dissociation also parallel the present data.<sup>20</sup> Similar effects are believed to play a role in biology; e.g., hydrogen bonding to the distal imidazole nitrogen atoms is commonly observed in X-ray crystal structures of zinc enzymes, and such interactions are known to influence metalloprotein properties.<sup>21</sup>

Steric factors may also affect  $\text{N}-\text{Zn}^{\text{II}}$  bond energies and thus may indirectly influence  $\text{Zn}-\text{OH}_2$  acidity.<sup>19</sup> However, the available X-ray data<sup>22–24</sup> do not support this argument, but indicate that the average  $\text{Zn}-\text{N}$  bond distance is approximately the same in each reported complex. In order to examine this question further, we utilized established methods<sup>25</sup> to calculate the  $\Delta H_a^\circ$  of the complexes (MNDO, as implemented on SPARTAN),<sup>26</sup> The results indicate that in the gas phase the  $[\text{Zn}(\text{tren})\text{OH}_2]^{2+}$  complex is *more* acidic than  $([\text{Zn}(\text{Me}_6\text{tren})\text{OH}_2]^{2+})$  ( $\Delta\Delta H^\circ = -3.8$  kcal/mol), consistent with Me<sub>6</sub>tren acting as a better ligand in the absence of solvent.

The possibility that the complexes differ in coordination number cannot be completely ruled out.<sup>19</sup> It is probable that zinc is five-coordinate in solution in both  $[\text{Zn}(\text{Me}_6\text{tren})\text{OH}_2]^{2+}$  and  $[\text{Zn}(\text{Me}_6\text{tren})\text{OH}]^+$ .<sup>27</sup> Liquid-phase X-ray diffraction data indicate that  $[\text{Zn}(\text{tren})\text{Cl}]^+$  maintains five-coordination in aqueous solution,<sup>28</sup> but to our knowledge definitive data on the solution structure of the present complexes are not available.

In summary, the difference in the chemical behavior between the complexes  $[\text{Zn}(\text{tren})\text{OH}_2]^{2+}$  and  $[\text{Zn}(\text{Me}_6\text{tren})\text{OH}_2]^{2+}$  does not provide evidence for entropic hydrophobic effects, as previously believed. Rather, the observed enthalpic control correlates with the relative capacity of the coordination complexes to stabilize charge. This study underscores the need to exercise caution in the application of the van't Hoff equation to the determination of enthalpies.<sup>16</sup>

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(21) Luchinat, C.; Sola, M. In *Encyclopedia of Inorganic Chemistry*; Klug, R. B., Ed.; John Wiley & Sons: Chichester, 1994; p 4406.

(22) Sime, R. J.; Dodge, R. P.; Zalkin, A.; Templeton, D. H. *Inorg. Chem.* **1971**, *10*, 537.

(23) Jain, P. C.; Lingafelter, E. C. *J. Am. Chem. Soc.* **1969**, *91*, 4112.

(24) Di Vaira, M.; Orioli, P. L. *Acta Crystallogr.* **1968**, *B24*, 1269.

(25) Dewar, M. J. S.; Dieter, K. M. *J. Am. Chem. Soc.* **1986**, *108*, 8075.

(26) Spartan v. 3.0; Wavefunction, Inc., Irvine, CA.

(27) Ciampolini, M.; Nardi, N. *Inorg. Chem.* **1966**, *5*, 41.

(28) Dagnall, S. P.; Hague, D. N.; Towl, A. D. C. *J. Chem. Soc., Faraday Trans.* **1983**, *2*, 1817.