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A DFT Study of Model Complexes of Zinc Hydrolases and Their Inhibition by Hydroxamic Acids

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DFT calculations carried out on zinc acetate and zinc hydroxamates using the Hartree–Fock and B3LYP methods with the 6-311+G* basis set give a series of stable pseudotetrahedral chelates (ZnL_2) (L = OAc, FA, AA, NMeAA, GA, SA). Addition of a water molecule to these chelates gives the hydrates, $ZnL_2{\cdot}H_2O$, which in all cases are energetically more stable than the corresponding chelate. Hydrates formed from O,O coordinated hydroxamate species with a five-membered chelate ring contain water molecules occupying vacant coordination sites of the zinc atom. In contrast, those formed from zinc chelates with four-membered chelate rings contain a water molecule inserted into the chelate ring to give a six-membered ring in which one hydrogen of the water molecule is H-bonded to an oxygen atom of the zinc chelate with the water oxygen strongly bonded to the zinc. A slight lengthening of the H-bonded O–H bond suggests incipient hydroxide activation of water by zinc. In contrast, the O,O bonded hydroxamates do not incorporate water into the chelate ring nor activate the water in accordance with the ability of hydroxamic acids to inhibit zinc containing metalloenzymes.

Introduction

Zinc occurs widely in biological systems, and there are now over 200 three-dimensional structures for zinc proteins which have identified three types of zinc sites: structural, catalytic, and cocatalytic[1] commonly employing as ligands the amino acids histidine, glutamic acid, aspartic acid, and cysteine. Water also occurs widely as a ligand. The widespread occurrence of zinc over other first row transition elements has been attributed to a number of factors including its flexible coordination number, fast ligand exchange, and Lewis acidity which can lead to ionization of a bound water molecule to form a nucleophilic hydroxyl.[2] For example, in carboxypeptidase A, it has been suggested that the linkage $Glu\text{-}COO^- - H_2O - ZnL_3$ may be considered as a unit in which the proton shifts easily to form $Glu\text{-}COOH - OH^- - ZnL_3$.[2] The mechanism for water activation by a zinc center is discussed below on the basis of our DFT studies.

Hydroxamic acids are potent inhibitors of many metalloenzymes and matrix metalloproteinases (MMP).[3] The majority of first row transition elements occur in metallohydrolases in dinuclear active sites with bridging carboxylates as in urease (Ni(II)),[4] methionineaminopeptidase (Co(II)),[5] and arginase (Mn(II)).[6] These dinuclear based enzymes are frequently inhibited by hydroxamic acids involving a novel coordination mode with the deprotonated hydroxamate OH group bridging the two metal centers and the carbonyl oxygen bonded to only one metal center as observed in the acetohydroxamate inhibited C319A variant of *Klebsiella aerogenes* urease.[7] This form of hydroxamate inhibition has been modeled in dinuclear complexes such as $[Ni_2(\mu\text{-}OAc)_2(\mu\text{-}AA)(urea)(tmen)_2][OTf]$[8,9] and $[Ni_2(OAc)(Hshi)(H_2shi)(py)_4]$[10] which exhibit the same type of bonding as observed in the above inhibited variant of urease.[7] However, in the

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(8) Abbreviations: OAc, CH_3COO^-; FHA, formohydroxamic acid; FA, formohydroxamate anion; AHA, acetohydroxamic acid; AA, acetohydroxamate anion; NMeAHA, *N*-methylacetohydroxamic acid; NMeAA, *N*-methylacetohydroxamate anion; GHA, glycinehydroxamic acid; GA, glycinehydroxamate anion; SHA, sarcosinehydroxamic acid; SA, sarcosinehydroxamate anion; tmen, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine.
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case of zinc metallohydrolases and in the MMP series, the zinc is frequently in a mononuclear active site and hydroxamate inhibition involves normal O,O chelation of the zinc at this site.[11] There is much activity by the pharmaceutical industry to develop effective inhibitors especially within the MMP series since the overexpression and activation of MMPs have been implicated in a number of diseases including arthritis, periodontal disease, multiple sclerosis, and various cancers.[3]

Because of the widespread occurrence of carboxylate ligands in metallohydrolases and of hydroxamic acids as inhibitors of these hydrolases, we have carried out DFT calculations on various zinc acetate and zinc hydroxamate species with various coordination numbers and coordination modes. Since zinc hydrolases are assumed to activate water molecules, the effect of the addition of a water molecule to these model complexes was also examined to determine whether any stable species are formed.

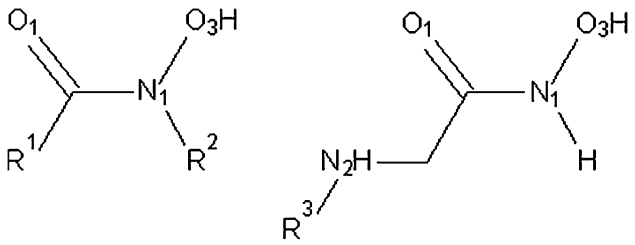
Computational Details

Initial geometries were constructed using the Titan,[12] Jaguar,[13] and GaussView[14] programs. Geometries were fully optimized using Gaussian98.[15] Frequency calculations were performed to characterize stationary points and to obtain the zero-point vibrational energy correction. Hydrated molecules were built either by constructing directly or by adding waters to completely optimized nonhydrated chelates. Waters were added at a number of different angles to the ligands, but in all cases only the lowest energy structure for a given isomer is reported. Geometries were initially optimized using the Hartree−Fock (HF) method and the 6-311+G* basis set, followed by further optimization at the B3LYP[16] level using 6-311+G* once more. All calculations are in the gas phase. Previous calculations on hydroxamic acids have shown that the inclusion of solvent effects does not significantly improve the results.[17]

Results and Discussion

Zinc Acetate and Zinc Hydroxamates (ZnL_2). All of the complexes ZnL_2 (L = OAc, FA, AA, NMeAA, GA, SA) (see Figure 1 for hydroxamic acid structure) gave optimized structures that were bisligated, 4-coordinate, pseudotetrahedral species. Thus, for $Zn(OAc)_2$ (Figure 2; structure **a**), the bond lengths $Zn-O_1$, 2.050 Å; $Zn-O_2$, 2.048 Å; the bond angles $\angle O_1-Zn-O_2$, 64.64°; and $\angle O_1'-Zn-O_2'$, 64.64°; and the dihedral angle between the planes defined by the ligands, 90.35° (Figure 3), give no indication of unsymmetrical bonding in contrast to a structure in which the chelating acetate takes up an η^1 monodentate mode. For formohydroxamic acid (FHA) and acetohydroxamic acid (AHA) ($R^1 = R^2 = H$ and $R^1 = Me$, $R^2 = H$, respectively, Figure 2; structures **e** and **h**), there is the possibility of deprotonation of either the hydroxamate OH group to give O,O coordination and formation of a five-membered chelate ring (Figure 2; structure **e**) or deprotonation of the hydroxamate NH group to give O,N coordination and formation of a four-membered chelate ring (Figure 2; structure **h**). As expected, the latter O,N is much less stable than the former O,O by 32.652 kcal/mol for formohydroxamic acid and by 28.794 kcal/mol for acetohydroxamic acid (Table 1). Again in all cases, the optimized structures are pseudotetrahedral as shown by the bond angles in Figure 3. For species formed from *N*-methylacetohydroxamic acid (NMeAHA, $R^1 = R^2 = Me$) only the O,O isomer is possible (Figure 2; structure **e**).

In the case of species formed from the α-aminohydroxamic acids, glycinehydroxamic acid, $NH_2CH_2CONHOH$ (GHA), and sarcosinehydroxamic acid, $HMeNCH_2CONHOH$ (SHA), two additional coordination modes are possible. The first, N,N coordination, involves deprotonation of the hydroxamate NH group and bonding by the amino-nitrogen lone pair (Figure 2; structure **k**) while the second, O,N_{am}, involves deprotonation of the hydroxamate OH group and bonding by the amino-nitrogen lone pair (Figure 2; structure **m**). These modes involve formation of five-membered and six-membered chelate rings, respectively. For $Zn(GA)_2$, the energies of the four isomers lie in the following sequence of decreasing stability: O,O (**e**) > N,N (**k**) > O,N_{am} (**m**) > O,N_{hy} (**h**) (Table 1). The N,N and O,N_{am} isomers might have been expected to be in the reverse order, but the energy



	R^1	R^2	R^3
FHA	H	H	-
AHA	CH_3	H	-
NMeAHA	CH_3	CH_3	-
GHA	CH_2NH_2	H	H
SHA	CH_2NHCH_3	H	CH_3

Figure 1. General structure of hydroxamic acid ligands showing assignments of R-groups and atom labeling. Structure on right refers to α-aminohydroxamic acids only.

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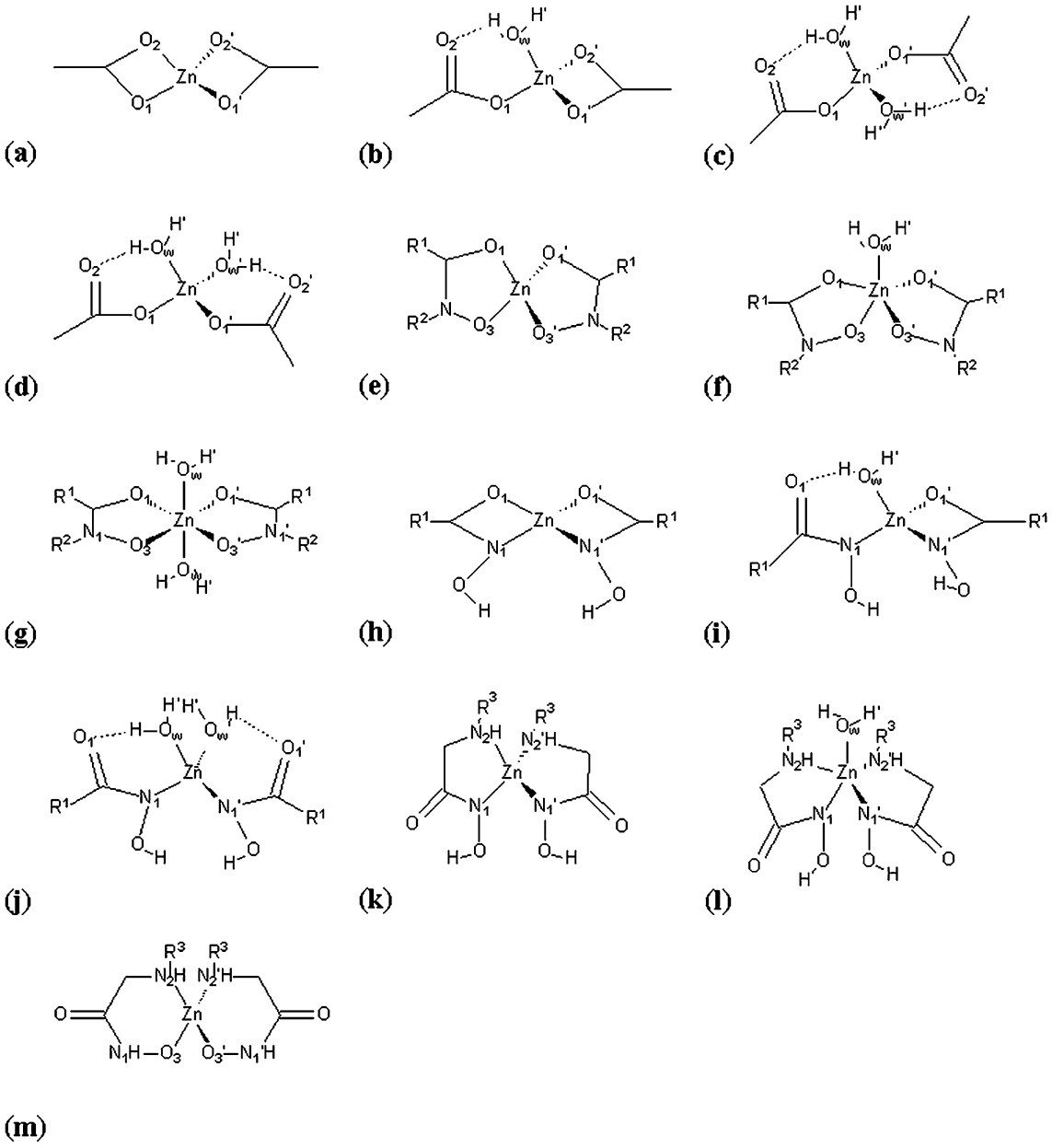

Figure 2. Species studied: (a) $Zn(OAc)_2$; (b) $Zn(OAc)_2 \cdot H_2O$; (c) $Zn(OAc)_2 \cdot 2H_2O$; (d) $Zn(OAc)_2 \cdot 2H_2O$; (e) (O,O) hydroxamate; (f) (O,O) hydrated hydroxamate; (g) (O,O) dihydrated hydroxamate; (h) (O,N) hydroxamate; (i) (O,N) hydrated hydroxamate; (j) (O,N) dihydrated hydroxamate; (k) (N,N) hydroxamate; (l) (N,N) hydrated hydroxamate; (m) alternate (O,N) hydroxamate. See Figure 1 for R-group assignments.

difference between them is quite small (0.965 kcal/mol). It is interesting to note that, for these zinc complexes of the α-aminohydroxamic acids, the O,O isomer is still the most stable of the four possible isomers as in the case of the two isomers of the monohydroxamic acids, FHA and AHA. The first example of N,N coordination was reported for $Ni(GA)_2$ by our group,[18] and subsequently, a number of other examples have been reported[19] but none involving zinc. In one case, $[MoO_2(GA)_2]$, O,O coordination has been observed,[20] but no examples of complexes with the O,N mode have yet been reported.

Hydration of Zinc Chelates. Addition of one or two water molecules to the zinc chelates ZnL_2 (Figure 2; structures **a**, **e**, **h**, **k**) resulted in all cases in the formation of stable structures $ZnL_2 \cdot nH_2O$ ($n = 1,2$) (Figure 2; structures **b**, **f**, **i**, **l**) with lower energies than those of the corresponding chelate ZnL_2; that is, the heats of hydration (ΔE) calculated from eq 1 are positive throughout the series as shown in Table 2.

$$\Delta E = E\{[Zn(L)_2 \cdot n(H_2O)] + mH_2O\} - E\{[Zn(L)_2 \cdot (n+m)(H_2O)]\}; \text{ when } n = 0,\ m = 1,2; \text{ when } n = 1,\ m = 1 \quad (1)$$

The hydrated structures fall into two distinct groups with

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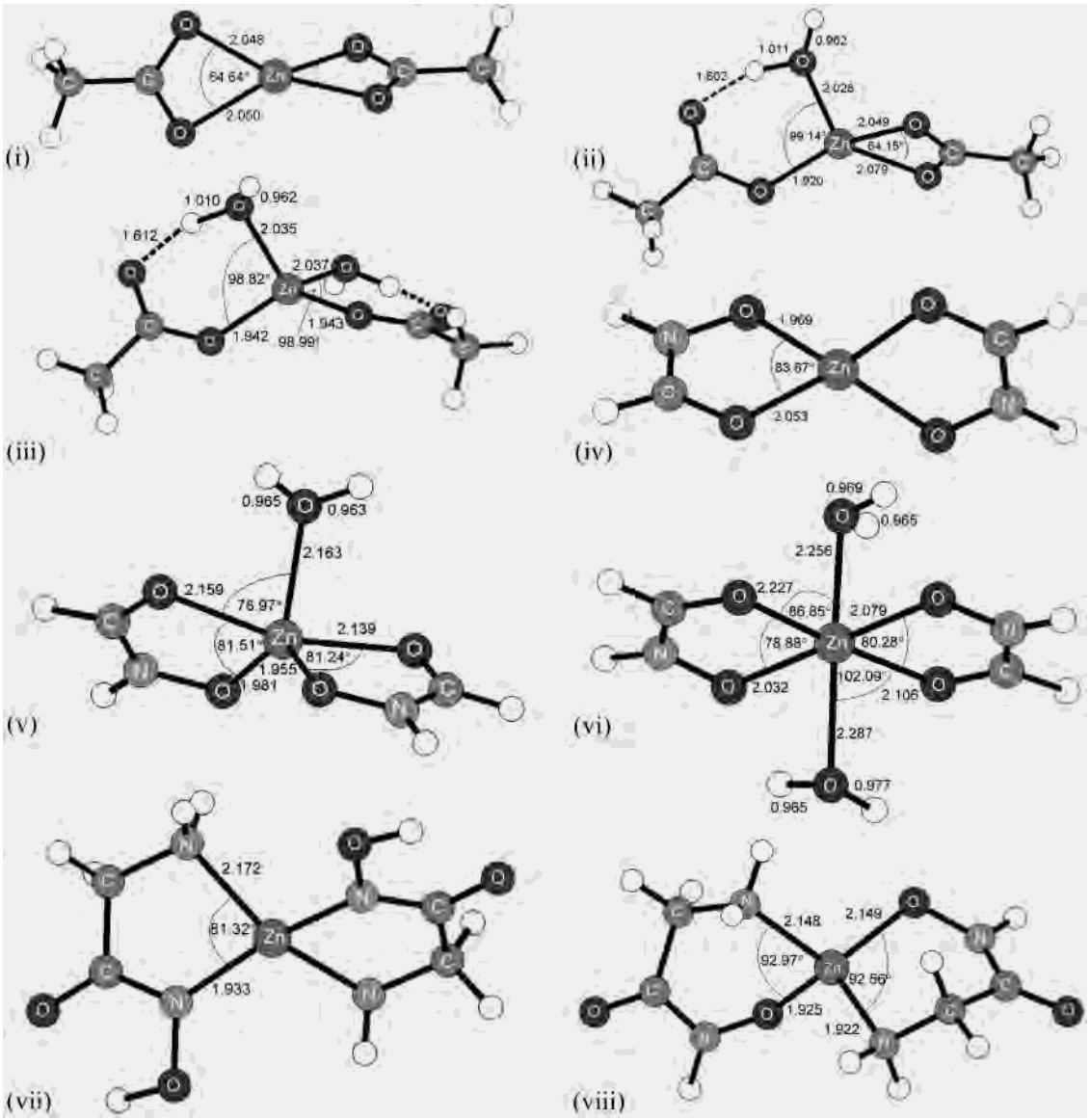

Figure 3. Selected bond lengths (Å) and angles (deg); (i) $Zn(OAc)_2$; (ii) $Zn(OAc)_2 \cdot H_2O$; (iii) $Zn(OAc)_2 \cdot 2H_2O$; (iv) $Zn(FA)_2$ (O,O); (v) $Zn(FA)_2 \cdot H_2O$ (O,O); (vi) $Zn(FA)_2 \cdot 2H_2O$ (O,O); (vii) $Zn(GA)_2$ (N_{hy},N_{am}); (viii) $Zn(GA)_2$ (O,N_{am}).

Table 1. Relative Energies of Zinc Hydroxamates[a]

		bonding mode	relative energy[b,c]
e	$Zn(FA)_2$	O,O	0.000
h	$Zn(FA)_2$	O,N_{hy}[d]	32.652
e	$Zn(AA)_2$	O,O	0.000
h	$Zn(AA)_2$	O,N_{hy}	28.794
e	$Zn(GA)_2$	O,O	0.000
h	$Zn(GA)_2$	O,N_{hy}	31.686
k	$Zn(GA)_2$	N_{hy},N_{am}	7.894
m	$Zn(GA)_2$	O,N_{am}	12.391
e	$Zn(SA)_2$	O,O	0.000
h	$Zn(SA)_2$	O,N_{hy}	29.845
k	$Zn(SA)_2$	N_{hy},N_{am}	8.491
m	$Zn(SA)_2$	O,N_{am}	11.709

[a] Values are in kcal/mol. [b] A lower value for the relative energy indicates the more stable species. [c] Energy comparisons are only made between species of identical chemical formula. [d] N_{hy} refers to the hydroxamate N, and N_{am} refers to the amino N.

quite different structures depending on whether the initial chelate contains a four-membered ring (Figure 2; structures **a**, **h**) or a five-membered ring (Figure 2; structures **e**, **k**).

Hydration of Zinc Chelates Containing Five-Membered Chelate Rings. In the case of zinc chelates containing a five-membered chelate ring (Figure 2; structures **e**, **k**), addition of one water molecule occurs at a vacant coordination site of the zinc atom to give five-coordinate species with retention of the existing five-membered chelate rings (Figure 2; structures **f**, **l**). The $Zn-O_w$ distances in $Zn(FA)_2 \cdot H_2O$ (**f**), 2.163 Å; $Zn(AA)_2 \cdot H_2O$ (**f**), 2.219 Å; $Zn(GA)_2 \cdot H_2O$ (**f**), 2.204 Å; $Zn(SA)_2 \cdot H_2O$ (**f**), 2.193 Å; $Zn(GA)_2 \cdot H_2O$ (**l**), 2.522 Å; and $Zn(SA)_2 \cdot H_2O$ (**l**), 2.392 Å (Table 3) indicate a reasonably strongly bonded water molecule occupying the fifth coordination position with equal O−H bond lengths in the coordinated water molecule in this group of hydrates. The hydration energies of this group lie in the range 4.405−17.761 kcal/mol and are less than those in the second group formed from zinc chelates with four-membered rings which lie in the range 14.167−19.760 kcal/mol (Table 2). Addition of a second water molecule to $Zn(FA)_2 \cdot H_2O$ (Figure 2; structure **f**) occurs at the remaining vacant coordination site

Table 2. Energies of Hydration[a]

system	bonding mode	energy of hydration[b,c]
$Zn(OAc)_2 + H_2O \rightarrow Zn(OAc)_2{\cdot}H_2O$		16.330
$Zn(OAc)_2 + 2H_2O \rightarrow Zn(OAc)_2{\cdot}2H_2O$		32.096
$Zn(OAc)_2{\cdot}H_2O + H_2O \rightarrow Zn(OAc)_2{\cdot}2H_2O$		15.772
$Zn(FA)_2 + H_2O \rightarrow Zn(FA)_2{\cdot}H_2O$	O,O	6.278
$Zn(FA)_2{\cdot}H_2O + H_2O \rightarrow Zn(FA)_2{\cdot}2H_2O$	O,O	4.684
$Zn(FA)_2 + H_2O \rightarrow Zn(FA)_2{\cdot}H_2O$	O,N_{hy}	19.760
$Zn(FA)_2{\cdot}H_2O + H_2O \rightarrow Zn(FA)_2{\cdot}2H_2O$	O,N_{hy}	18.254
$Zn(AA)_2 + H_2O \rightarrow Zn(AA)_2{\cdot}H_2O$	O,O	7.454
$Zn(AA)_2 + H_2O \rightarrow Zn(AA)_2{\cdot}H_2O$	O,N_{hy}	14.167
$Zn(NMeAA)_2 + H_2O \rightarrow Zn(NMeAA)_2{\cdot}H_2O$	O,O	5.273
$Zn(GA)_2 + H_2O \rightarrow Zn(GA)_2{\cdot}H_2O$	O,O	4.405
$Zn(GA)_2 + H_2O \rightarrow Zn(GA)_2{\cdot}H_2O$	O,N_{hy}	19.040
$Zn(GA)_2 + H_2O \rightarrow Zn(GA)_2{\cdot}H_2O$	N_{hy},N_{am}	17.761
$Zn(SA)_2 + H_2O \rightarrow Zn(SA)_2{\cdot}H_2O$	O,O	8.522
$Zn(SA)_2 + H_2O \rightarrow Zn(SA)_2{\cdot}H_2O$	O,N_{hy}	15.175
$Zn(SA)_2 + H_2O \rightarrow Zn(SA)_2{\cdot}H_2O$	N_{hy},N_{am}	16.523

[a] Values are in kcal/mol. [b] In all cases, the hydrated products are lower in energy than their precursors. [c] $\Delta E = E\{[Zn(L)_2{\cdot}n(H_2O)] + mH_2O\} - E\{[Zn(L)_2{\cdot}(n+m)(H_2O)]\}$; when $n = 0$, $m = 1,2$; when $n = 1$, $m = 1$.

on zinc and results in the formation of the octahedral complex $Zn(FA)_2{\cdot}2H_2O$ (Figure 2; structure **g**). The hydration energies of the monohydrates of the zinc complexes containing five-membered chelate rings lie in the range 4.405−17.761 kcal/mol. The O−H bonds of the coordinated water molecule remain close in length, e.g., $Zn(FA)_2{\cdot}H_2O$ (Figure 2; structure **f**), $O_w-H = 0.965$ Å, $O_w-H' = 0.963$ Å and similarly in $Zn(FA)_2{\cdot}2H_2O$ (Figure 2; structure **g**), $O_w-H = 0.965$ Å, $O_w-H' = 0.969$ and 0.977 Å.

Hydration of Zinc Chelates Containing Four-Membered Rings. In the case of zinc chelates containing a four-membered chelate ring (Figure 2; structures **a**, **h**), addition of one water molecule results in incorporation of the water into the chelate ring and expansion to a six-membered ring, as in $Zn(OAc)_2{\cdot}H_2O$ (Figure 2; structure **b**) with formation of a H-bond between the O_2 oxygen of $Zn(OAc)_2$ and a hydrogen of the water molecule accompanied by formation of a strong $Zn-O_w$ bond ($Zn-O_w = 2.028$ Å) in accordance with the larger hydration energy of 16.330 kcal/mol in $Zn(OAc)_2{\cdot}H_2O$ (Figure 2; structure **b**) (Table 2). Addition of a second water molecule to $Zn(OAc)_2{\cdot}H_2O$ (Figure 2; structure **b**) results in similar ring expansion of the second chelating acetate and formation of an identical second six-membered ring in $Zn(OAc)_2{\cdot}2H_2O$ (Figure 2; structure **d)** with a very similar further hydration energy (15.772 kcal/mol) to the monohydrate (i.e., 16.330 kcal/mol). Addition of two waters to $Zn(OAc)_2$ (Figure 2; structure **a**) results in ring expansion of both chelating acetates and formation of two six-membered rings in $Zn(OAc)_2{\cdot}2H_2O$ (Figure 2; structure **c**) with the expected larger hydration energy of 32.096 kcal/mol. $Zn(OAc)_2{\cdot}2H_2O$ (Figure 2; structure **c**) is the stereoisomer of $Zn(OAc)_2{\cdot}2H_2O$ (Figure 2; structure **d**). In contrast to the group of hydrates formed from zinc chelates containing five-membered chelate rings, where the water molecule adds to a vacant coordination site of ZnL_2 and the O−H bonds of the water molecule remain almost equal in length, e.g.,

Table 3. Selected Bond Lengths[a]

	species	bonding mode	Zn−O						Zn−N				Zn−O_w	O_w−H		
			Zn−O_1	Zn−O_2	Zn−O_3	Zn−O_1'	Zn−O_2'	Zn−O_3'	Zn−N_1	Zn−N_2	Zn−N_1'	Zn−N_2'		O_w−H	O_w−H	H−bond
a	$Zn(OAc)_2$		2.048	2.050		2.048	2.050									
b	$Zn(OAc)_2{\cdot}H_2O$		1.920	3.146		2.049	2.079						2.028	1.011	0.962	1.602
c	$Zn(OAc)_2{\cdot}2H_2O$		1.943	3.161		1.943	3.156						2.035	1.011	0.962	1.606
													2.039			1.607
d	$Zn(OAc)_2{\cdot}2H_2O$		1.942	3.165		1.943	3.162						2.035	1.010	0.962	1.612
													2.037			
e	$Zn(FA)_2$	O,O	2.053		1.969	2.053		1.969								
f	$Zn(FA)_2{\cdot}H_2O$	O,O	2.159		1.981	2.139		1.981					2.163	0.965	0.963	
g	$Zn(FA)_2{\cdot}2H_2O$	O,O	2.227		2.032	2.106		2.079					2.256	0.965	0.969	
													2.287	0.965	0.977	
h	$Zn(FA)_2$	O,N	2.088			2.088			2.050		2.050					
i	$Zn(FA)_2{\cdot}H_2O$	O,N	3.223			2.116			1.957		2.054		2.042	1.011	0.962	1.611
j	$Zn(FA)_2{\cdot}2H_2O$	O,N	3.263			3.246			1.972		1.973		2.074	1.016	0.962	1.583
													2.065	1.009	0.963	1.620
e	$Zn(AA)_2$	O,O	2.043		1.967	2.043		1.967								
f	$Zn(AA)_2{\cdot}H_2O$	O,O	2.178		1.972	2.084		2.031					2.219	0.971	0.968	
h	$Zn(AA)_2$	O,N	2.059			2.058			2.052		2.053					
i	$Zn(AA)_2{\cdot}H_2O$	O,N	3.181			2.070			1.961		2.057		2.033	1.017	0.962	1.578
e	$Zn(NMeAA)_2$	O,O	2.030		1.960	2.030		1.960								
f	$Zn(NMeAA)_2{\cdot}H_2O$	O,O	2.088		1.984	2.026		2.018					2.340	0.969	0.966	
e	$Zn(GA)_2$	O,O	2.043		1.968	2.043		1.968								
f	$Zn(GA)_2{\cdot}H_2O$	O,O	2.197		1.971	2.031		2.080					2.204	0.970	0.968	
H	$Zn(GA)_2$	O,N	2.084			2.077			2.035		2.039					
I	$Zn(GA)_2{\cdot}H_2O$	O,N	3.179			2.086			2.036		2.057		2.036	1.013	0.962	1.593
K	$Zn(GA)_2$	N,N							1.933	2.172	1.933	2.172				
L	$Zn(GA)_2{\cdot}H_2O$	N,N							1.955	2.246	1.961	2.146	2.522	0.975	0.963	
M	$Zn(GA)_2$	O,N			1.925			1.922		2.148		2.149				
E	$Zn(SA)_2$	O,O	2.044		1.968	2.040		1.968								
F	$Zn(SA)_2{\cdot}H_2O$	O,O	2.160		1.980	2.109		2.000					2.193	0.967	0.963	
H	$Zn(SA)_2$	O,N	2.085			2.073			2.035		2.042					
I	$Zn(SA)_2{\cdot}H_2O$	O,N	3.181			2.076			1.961		2.062		2.034	1.016	0.962	1.579
K	$Zn(SA)_2$	N,N							1.937	2.181	1.936	2.178				
L	$Zn(SA)_2{\cdot}H_2O$	N,N							1.967	2.276	1.968	2.166	2.392	0.975	0.963	
M	$Zn(SA)_2$	O,N			1.930			1.923		2.153		2.160				

[a] Values are in Å.

$Zn(FA)_2 \cdot H_2O$, (Figure 2; structure **f**), $O_w-H = 0.965$, $O_w-H' = 0.963$ Å, in the second group formed form zinc chelates containing four-membered chelate rings (Figure 2; structures **b**, **c**, **d**, **i**), the water molecule inserts into the four-membered chelate ring in ZnL_2 to give a six-membered ring in which the O_w-H bond which is involved in H-bonding to the $Zn-O_2$ is longer (1.011 Å) than the other O_w-H' bond (0.962 Å) not involved in H-bonding. The weakening of the O_w-H bond is consistent with the concept that the water molecules in these structures (Figure 2; structures **b**, **c**, **d**, **i**) may be regarded as incipient hydroxyls and therefore are a step in the activation of the nucleophilic water as required in zinc hydrolases. The nature of water bonding and water activation in hydrolases is a vexing problem. In some cases, even the number of water molecules in an active site is not clear. They may be present as water molecules, free hydroxide, metal-activated water, or hydroxide. For example, it is suggested that in carboxypeptidase A the linkage Glu-$COO^--H_2O-ZnL_3$ shifts easily to form Glu-$COOH-OH^--ZnL_3$.[2] Moreover, analysis of both small molecule zinc complexes from The Cambridge Structural Database and zinc proteins in the Protein Data Bank showed that zinc carboxylates exhibit a larger range of bonding between the extreme monodentate and bidentate modes than other elements such as Ca, Mg, Mn, Fe, Cu.[21] Finally, molecular dynamics simulations of carboxypeptidase A showed Glu72 coordinating bidentately to Zn in a structure averaged over a 2.0 ns molecular dynamics simulation but only monodentately in 1.0 ps snapshots.[22] We suggest in the light of our DFT results for $Zn(OAc)_2 \cdot nH_2O$, $n = 1,2$, that the flexible nature of the coordinating carboxylate group, as illustrated by the carboxylate shift concept,[23] allows ready formation of zinc activated water molecules in zinc proteins with the activation increasing with the monodentate character of the carboxylate bonding to the zinc atom.

In contrast to the ability of carboxylate to exhibit formally monodentate coordination and thereby, according to the above argument, activate a water molecule in a zinc protein, hydroxamic acids are potent inhibitors of zinc enzymes especially the MMP series. As described above, addition of a water molecule to an O,O bonded zinc hydroxamate results in addition of the water molecule to a vacant coordination site of ZnL_2 (L = AA, FA, NMeAA, GA, SA, Figure 2; structure **e**) and no incorporation of the water molecule into the five-membered ring nor any evidence of unequal OH bond lengths in the water molecule as occurs in the case of ZnL_2 (L = OAc, Figure 2; structure **a**). Inhibition of zinc hydrolases by hydroxamic acids is thus supported by the above DFT results, which illustrate the important consequences of replacement of the flexible carboxylate ligand by the hydroxamate ligand.

In the case of the O,N bonded ZnL_2 chelates (L = FA, AA, GA, SA, Figure 2; structure **h**), addition of waters results in ring expansion (Figure 2; structure **i** and **j**), but to date no metal complexes containing O,N bonded hydroxamates have been observed, in accord with the calculated energies (Table 1). In the case of the α-aminohydroxamic acids, addition of a water molecule causes some changes in relative stabilities of the different coordination isomers, for example $Zn(GA)_2$ (N,N bonded) is less stable than $Zn(GA)_2$ (O,O bonded), but this order is reversed in the monohydrates.

Conclusions

DFT calculations on zinc acetate and zinc hydroxamates give a series of stable pseudotetrahedral chelates (ZnL_2). Addition of water molecules to these chelates gives two groups of hydrates, $ZnL_2 \cdot nH_2O$ $n = 1,2$, which in all cases are more stable than the corresponding chelate. The first group, formed from zinc chelates containing a five-membered chelate ring as present in the O,O zinc hydroxamates, contains water molecules occupying vacant coordination sites on the zinc atom. In contrast, the second group, formed from zinc chelates containing a four-membered chelate ring, contains water molecules inserted into the chelate rings to give six-membered rings in which one hydrogen of the water molecule is H-bonded to the O_2 atom of the zinc chelate with the water oxygen O_w strongly bonded to the zinc. In this structure, the H-bonded water O−H bond is weaker than the other, and so, this structure gives support to the concept of incipient hydroxide activation of water by zinc in the hydrolases. In contrast, the O,O bonded hydroxamates do not incorporate water into the chelate ring nor activate the water in accord with the marked inhibitory power of hydroxamic acids with zinc containing metalloenzymes.

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Supporting Information Available: Listings of dihedral and bond angles and Cartesian coordinates of optimized final geometries. This material is available free of charge via the Internet at http://pubs.acs.org.

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