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Zinc Binding in HDAC Inhibitors: A DFT Study

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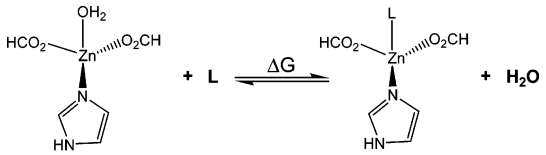

L= H_2O, CH_2O, $HCONH_2$, BMD, FMA, MeSH, HCO_2H, HCONHOH, PYR, MeS^-, $HCONHO^-$, PYR^-

Histone deacetylases (HDACs) are attractive targets for the treatment of cancers and a variety of other diseases. Most currently studied HDAC inhibitors contain hydroxamic acids, which are potentially problematic in the development of practical drugs. DFT calculations of the binding modes and free energies of binding for a variety of other functionalities in a model active site of HDAC are described. The protonation state of hydroxamic acids in the active site and the origin of the high affinity are discussed. These results emphasize the importance of a carefully chosen pK_a for zinc binding and provide guidance for the design of novel, non-hydroxamic acid HDAC inhibitors.

Histone deacetylases (HDACs) control the acetylation state of lysines on the surface of histones and are key enzymes in the regulation of gene expression.[1] It has also been found that they are involved in the regulation of the acetylation state of a number of other important proteins, most notably the tumor supressor gene p53.[2] A number of HDAC inhibitors were found to be promising anti-cancer reagents. The first such compound was recently approved for treatment of T-cell lymphoma and several others are now in clinical development. The vast majority of the currently available HDAC inhibitors, including the recently FDA-approved vorinostat, contain a hydroxamic acid functionality that binds to a zinc ion in the active site. On the basis of the experience with hydroxamic acids as inhibitors of matrix metallo proteases (MMPs), and the fact that this functional group strongly binds to a wide range of metals, there is widespread interest in non-hydroxamic acid HDAC inhibitors.[3] However, most of them were found to be weaker binders by several orders of magnitude compared to their hydroxamic acid counterparts. There are a number of oligopeptide natural products such as apicidin or depsipeptide that combine surface binding moieties with weaker metal binders such as carbonyls or thiols and are potent HDAC inhibitors. To design and synthesize potent non-hydroxamic acid HDAC inhibitors, a method to evaluate the relative zinc binding ability of different functional groups and to understand the requirements for efficient zinc binding would be desirable.

On the basis of their earlier computational studies of zinc binding in HDACs,[4] Vanommeslaeghe et al. studied a series of closely related metal binding groups for the purpose of deriving molecular descriptors for QSAR.[5] Zhang et al. showed in a QM/MM study that the charge distribution in the active site has a profound effect on the metal coordination and the overall reaction mechanism.[6] These results also suggest that a correlation of HDAC inihibition with the binding constants of different functional groups in aqueous solution is not likely to be of predictive value. Both studies proposed mechanisms different from the one proposed by Finnin et al. in their influential study of the first X-ray structure of a histone-deacetylase like protein (HDLP).[7]

As part of our ongoing computational studies of HDAC inhibitors,[8] we present the results of a DFT study of the binding modes and free energies of binding of a series of structurally diverse metal binders to an active site model of HDACs. Similar to previous computational studies,[4−6] the model active site consisted of one imidazole and two formates, representing the first coordination sphere of the zinc by His^{170}, Asp^{168}, and Asp^{258} (using the HDLP numbering scheme).[7] This binding site model is smaller than the ones used earlier[4−6] in order to focus on the zinc−ligand interaction and minimize secondary effects such as changes in the interactions of model residues among themselves. A water molecule bound to the zinc that was located in the active site of HDLP in the available high-resolution X-ray structures was chosen as the reference state. Several starting conformations (mainly differing in the dihedral angles around O−Zn^{2+} bonds) were tested, but either converged on the structures shown here or were higher in energy. All structures were fully optimized by using the B3LYP hybrid functional with a 6-311G* basis set on zinc and the 6-31+G* basis set on all other atoms. Thermochemical corrections were obtained from harmonic frequency analysis of the optimized structures for standard conditions. Several of the ligands discussed are anionic,

(1) For recent overviews, compare: (a) Bolden, J. E.; Pearts, M. J.; Johnstone, R. W. *Nat. Rev. Drug Design* **2006**, *5*, 769−784. (b) Miller, T. A.; Witter, D. J.; Belvedere, S. *J. Med. Chem.* **2003**, *46*, 5096−5115. (c) Yoshida, M.; Matsuyama, A.; Komatsu, Y.; Nishino, N. *Curr. Med. Chem.* **2003**, *10*, 2351−2358.

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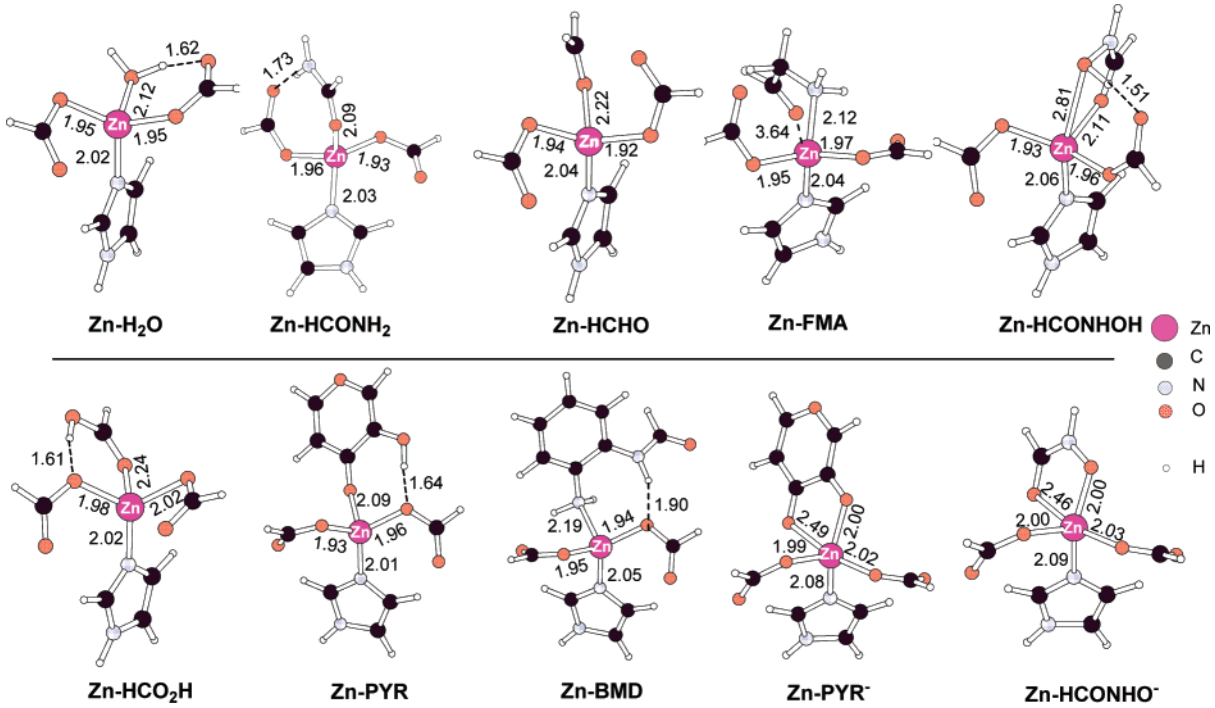

FIGURE 1. Selected B3LYP optimized structures of selected metal binders to the active site model.

and they will be subject to a significant desolvation penalty upon transfer from aqueous solution to the active site. The desolvation penalty was thus estimated by calculating the solvation energies by using CPCM single-point calculations on the gas-phase optimized geometries. Because the biggest portion of this solvent correction will be the desolvation of the ligand in water and because of the highly charged nature of both the actual and the model active sites (a Zn^{2+} ion, two formates, and, in case of the actual active site, a presumably protonated histidine), the CPCM parameters for water ($\epsilon = 78.4$) have been chosen to represent these polar environments in a consistent manner. For comparison purposes, the results from CPCM calculations in ether ($\epsilon = 4.34$) are also included. All calculations were performed with the G03 series of programs.[9]

Figure 1 shows the calculated structures of selected metal binders in the model active site. It is well-known that the zinc-(II) ion is a soft metal ion that can sustain different coordination numbers, the most common being tetracoordination, although penta- and hexacoordination of zinc(II) ion have also been observed in X-ray structures. Like many other zinc proteases, HDAC has three residues that bind to the zinc(II) ion, while the remaining coordination site is occupied by the exogenous ligand. Unlike most other zinc proteases, the 2+ charge is neutralized by two aspartate anions, making the overall charge neutral and increasing the accuracy of these small model system calculations compared to a positively charged system such as thermolysine. For the reference water ligand, the coordination model has two carboxylates binding with the zinc ion in a monodentate fashion. The Zn−H_2O complex is stabilized by a short hydrogen bond (1.62 Å) between the nonbinding oxygen in one carboxylate and the in-plane water hydrogen. A similar result was obtained previously for a thermolysin active site model[10] but could be replaced by hydrogen bonding to other amino acid residues in the full active site. For $HCONH_2$ and HCHO, models of the natural substrate and the binding unit in apicidin, respectively, the Zn−O distances are quite different at 2.09 and 2.22 Å. The difference is indicative of the increased negative charge on the amide carbonyl carbon due to the donation of electron density from the lone pair electrons on the nitrogen atom to the attached oxygen. This is supported by the result that formyl methyl amine (FMA), another well-known metal binder,[11] is calculated to bind in a monodentate fashion via the amino group. The nitrogen acts as the Lewis base, while the coordination to the carbonyl oxygen is with an oxygen−zinc distance of 3.64 Å weak.

It is noteworthy that, although hydroxamic acids (here represented as HCONHOH) are typically thought of as bidentate ligands, our calculations indicate that the carbonyl oxygen is much more strongly coordinated with a bond length of 2.11 Å than the hydroxyl oxygen with a bond length of 2.81 Å. This is consistent with several other computational studies,[4−6] but not with the available high-resolution structures of hydroxamic acids bound to HDACs.[12] In contrast, the O-deprotonated hydroxamic acid is calculated to act as a bidentate ligand. In agreement with the available X-ray structures, the bond from the carbonyl carbon to the zinc ion is calculated to be longer than the bond from the deprotonated hydroxyl oxygen. Computational studies by Duca and co-workers indicate that the acidity of hydroxamic acids increases by ~3.3 pK_a units upon complexation to zinc

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in the active site of TACE.[13] This suggests that the pK_a of 9.4[14] for aliphatic hydroxamic acids in aqueous solution is sufficently lowered by zinc complexation in the active site for them to be deprotonated by the histidine, leading to an active site structure similar to the one proposed by Vanommeslaeghe et al.[4,5] The deprotonation of a hydroxamic acid in the active site rather than in solution could explain the unique inhibition properties of hydroxamic acids for zinc metalloproteases. Because they are present in the neutral form in aqueous solution, the desolvation penalty upon binding to the protein is small. Once they are bound in the active site, they are deprotonated, presumably by the adjacent conserved His[129] residue.[15] The hydroxamate anion interacts strongly with the zinc ion in a bidentate fashion and leads to the experimentally observed[7,11] pentaccordinate zinc whereas a hydroxamic acid has an additional hydrogen bond of the acidic proton to one of the Asp residues. Although previous computational studies indicated the presence of a hydroxamate and a protonated histidine in the active site,[4,5] most experimental studies, including several X-ray studies, assume a neutral hydroxamic acid to be present.[7,12]

Two different structural elements, the 3-hydroxy-4-pyrone (PYR) and the benzamidine (BMD) moieties, have been recently explored as zinc binding moieties for the inhibition of MMP-3[16] and HDACs,[17] respectively. In analogy to the results for hydroxamic acid, both ligands bind in a monodentate fashion but with an additional hydrogen bond to one of the carboxylates representing an Asp residue in the active site. The very short distances for the hydrogen bond indicate that these are very favorable interactions. Even though PYR could act alternatively as a bidentate ligand, the monodentate arrangement is more favorable than the bidentate binding mode. Deprotonation of PYR, which is with a pK_a of ~7.9 more acidic than a hydroxamic acid, then leads to a bidentate mode where, again in analogy to a hydroxamic acid, the bond to the enolate oxygen is shorter than the one to the carbonyl oxygen.

The calculated binding energies relative to water are summarized in Table 1. The entropy contribution to the free energy of binding of ~1 kcal/mol is, unlike for metal binding in solution where multiple waters are released from the metal ion,[18] relatively small. The free energies of binding for the thiol, aldehyde, amide, and carboxylic acid ligands are endothermic compared to water. This not only emphasizes the importance of the secondary hydrogen bonding interactions to the carboxylates, but also indicates that the majority of the binding interactions in non-hydroxamic acid HDAC inhibitors such as apicidin and the acetylated lysine substrate itself are on the surface of the protein rather than in the active site. On the basis of the difference between the aldehyde and carboxylic acid ligands, the contribution of the secondary hydrogen bond to the free energy of binding can be estimated to be ~6 kcal/mol. However, this carboxylic acid is likely to be deprotonated in known HDAC inhibitors such as valproic acid. Binding of the thiol ligand, a model of depsipeptide, in its protonated form is particularly unfavorable, suggesting that zinc binding might lower the pK_a of ~10.6[19] sufficiently to allow the formation and binding of the thiolate. Correction of the calculated free energy of binding for solvation by the CPCM method yields free energies of binding that are ~3 kcal/mol less endothermic due to the increased polarization of the carbonyl bond by zinc complexation compared to water in the aldehyde. This effect is not obtained for the carboxylic acid, possibly because of the strong interaction of the acid with water. Consistent with this hypothesis, the effect of the CPCM salvation is small in the case of the amide.

TABLE 1. Calculated Binding Energies for 11 Ligands (in kcal/mol) Relative to Water Ligand

Ligand	ΔE	ΔG	ΔG(water)	ΔG(ether)
CH_3SH	10.7	10.6	11.1	11.9
CH_2O	10.9	9.8	5.5	7.3
$HCONH_2$	0.9	2.1	2.8	3.0
HCO_2H	2.6	3.5	5.7	5.2
 BMD	0.6	3.3	8.9	9.3
 PYR	0.6	1.5	5.4	5.7
 FMA	-1.3	-0.1	-1.0	-0.3
	-3.1	-1.4	0.0	0.3
CH_3S^-	-24.3	-22.9	-4.5	-8.5
 PYR^-	-21.1	-20.3	-4.6	-6.6
	-25.0	-25.0	-6.7	-9.6

The results obtained for the structures of the potentially bidentate ligands BMD, PYR, FMA, and hydroxamic acid shown in Figure 1 are consistent with the results for the energies summarized in Table 1. The BMD and PYR ligands, which show very similar binding motifs, have also very similar free energies of binding that are more favorable than the ones discussed earlier due to the secondary hydrogen bonding interaction. While the solvent correction for the PYR ligand is similar to the one for the structurally related carboxylic acid, it makes binding of the BMD less favorable by 5.6 kcal/mol. This surprising finding can be rationalized by the decrease in the polarization of the amide unit due to the secondary hydrogen bond, which decreases the solvation stabilization of the bonded form. The structural similarities of the FMA and hydroxamic acid ligands also lead to similar favorable binding energies that make FMA a promising ligand for HDAC inhibition. These results also show that hydroxamic acids are good zinc binding units despite the differences in calculated and experimental structures discussed above.

The energies and free energies of binding calculated for the anionic ligands are, as expected, much higher than those for any of the neutral ligands due to the strong electrostatic interaction with the zinc ion even in an overall neutral active site model. However, inclusion of an estimate of the desolvation penalty for these charged species provides a more realistic estimate of the binding energies. As can be expected, the difference in the results from the CPCM calculations in water

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and ether is largest for these ligands. Closer analysis of the individual terms reveals that this is mainly due to the differences in the solvation energy of the free anionic ligands. It is therefore expected that the CPCM results in water are the most reliable ones because they better represent the reference state of the anionic ligands dissolved in water. These results identify, in agreement with the available experimental data, hydroxamates as the best zinc binders, with the thiolate and PYR^- ligands predicted to be approximately 1 order of magnitude lower in binding.

In summary, the results presented here indicate that the hydroxamic acid that constitutes the zinc binding unit of the most common HDAC inhibitors is, in contrast to the assumptions in most experimental and computational papers, deprotonated in the acitive site. This suggests some possibly general design principles for the selection of zinc binding moieties in HDAC inhibitors. First, a matching of the pK_a value of the zinc binding moiety to the values in the active site allows for strong binding while minimizing the desolvation penalty of a charged species. Second, an additional hydrogen bond to the zinc-binding Asp residue can increase the binding constant. Third, metal binding groups such as 3-hydroxy pyrones or β-amino ketones are promising zinc binding units, but have to the best of our knowledge not yet been exploited as HDAC inhibitors. Finally, calculations of the active site model system described here provide a rapid and easy method to estimate the binding ability of new zinc binding moieties before they are synthesized.

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Supporting Information Available: Cartesian coordinates, SCF and CPCM energies, as well as thermodynamic corrections for all structures discussed. This material is available free of charge via the Internet at http://pubs.acs.org.

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