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Effect of Donor Atom Identity on Metal Binding Pharmacophore Coordination

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Abstract

The inhibition and binding of three metal-binding pharmacophores (MBPs), 2-hydroxycyclohepta-2,4,6-trien-1-one (tropolone), 2-mercaptopyridine-*N*-oxide (1,2-HOPTO), and 2-hydroxycyclohepta-2,4,6-triene-1-thione (thiotropolone) to human carbonic anhydrase II (hCAII) and a mutant protein hCAII L198G were investigated. These MBPs displayed bidentate coordination to the active site Zn(II) metal ion, but the MBPs respond to the mutation of L198G differently, as characterized by inhibition activity assays and X-ray crystallography. The L198G mutation increases the active site volume thereby decreasing the steric pressure exerted on MBPs upon binding, allowing changes in MBP coordination to be observed. When comparing the binding mode of tropolone to thiotropolone or 1,2-HOPTO (*O,O* versus *O,S* donor sets), structural modifications of the hCAII active site were shown to have a stronger effect on MBPs with an *O,O* versus *O,S* donor set. These findings were corroborated with density functional theory (DFT) calculations of model coordination complexes. These results suggest that the MBP binding geometry is a malleable interaction, particularly for certain ligands, and that the identity of the donor atoms still play a significant role in ligand coordination. Understanding underlying interactions between a MBP and a metalloenzyme active site may aid in the design and development of potent metalloenzyme inhibitors.

Introduction

Metalloenzymes comprise a significant portion of known enzymes [1] and are promising as therapeutic targets [2] due to their roles in a variety of diseases and infections, including glaucoma [3], cancer [4], and HIV [5]. Metalloenzymes can be inhibited by small molecules possessing metal-binding pharmacophores (MBPs) that coordinate to the active site metal

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ion, resulting in a bioinorganic complex [6]. Historically, MBPs such as carboxylic acids, phosphates, thiols, and hydroxamic acids have been utilized, but in recent years a broader repertoire of pharmacophores has been investigated [2, 7–10]. However, a detailed understanding of how the coordination chemistry of these MBPs is influenced and altered by the metalloenzyme active site is still lacking. We and others have found that the interactions of MBPs with the enzyme active site, including both the primary [11] and secondary [12] coordination spheres around the metal ion, can have a profound influence on their binding geometries and affinities. These interactions and their influence on MBP binding have important implications for the design of metalloenzyme inhibitors, as little is understood how specific MBPs respond to changes in metalloprotein active site structure.

Model complexes have often been used to interrogate the influence of the active site environment on MBP coordination [13, 14]. Despite major advances in their development in mimicking the coordinating residues and the first coordination sphere of an active site [15], model complexes still struggle to mimic the nuanced details of the second coordination sphere of a metalloenzyme active site. These key second coordination sphere interactions, which are usually absent in model complexes, can have a significant impact on inhibitor binding and selectivity and should not be neglected. Important second coordination sphere interactions that control selectivity include sterics, hydrogen bonding, and electrostatics. These interactions may force MBPs to coordinate to the metal center in a non-idealized, distorted geometries relative to model complexes.

Our group previously highlighted the differences between predicted and observed MBP binding based on model complexes and MBP binding in a metalloenzyme active site. This was achieved by comparing the binding of various MBPs to human carbonic anhydrase II (hCAII) and a Zn(II) model complex that employed a (hydrotris(5,3-methylphenylpyrazolyl)borate) ligand ($\text{Tp}^{\text{Ph,Me}}$) [12, 13, 16]. Although the primary coordination spheres provided by hCAII and $\text{Tp}^{\text{Ph,Me}}$ are similar, the $\text{Tp}^{\text{Ph,Me}}$ model lacks the second coordination sphere nuances of hCAII. The influences of the second coordination sphere were made apparent by analyzing the structure of the MBPs 1-hydroxypyridine-2(1*H*)-thione (1,2-HOPTO) and 3-hydroxy-2-methyl-4*H*-pyran-4-thione (thiomaltol) when bound to $[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(\text{MBP})]$ versus hCAII-MBP. The $[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(\text{MBP})]$ complexes showed bidentate coordination with coordination dihedral angles near 180° (Figure 1). In contrast, the complex of 1,2-HOPTO with hCAII showed bidentate binding as well, but with a highly distorted, non-ideal dihedral angle of $\sim 130^\circ$ (Figure 1). The thiomaltol model complex $[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(\text{thiomaltol})]$ was similar to that observed with 1,2-HOPTO, but when bound to hCAII, the thiomaltol complex deviated even more dramatically, displaying monodentate coordination to the Zn(II) ion via only the sulfur donor atom, as well as a compressed dihedral angle $< 180^\circ$. From the differences in MBP binding to the $\text{Tp}^{\text{Ph,Me}}$ complex and hCAII it was determined that the active site pocket and steric influences had a major influence on MBP binding. To understand the observed deviations between the MBP-model complexes and the corresponding hCAII-MBP complexes, density functional theory (DFT) calculations were performed utilizing the $[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(\text{MBP})]$ complexes. The DFT calculations demonstrated that MBP coordination with a larger dihedral angle was energetically favorable as was bidentate coordination (as opposed to the monodentate coordination observed with thiomaltol-hCAII).

The computational results provided further support for the significant influence the second coordination sphere has on the coordination geometries of MBPs to metalloenzymes.

In the present work, to further probe the influence of protein active sites on observed binding geometry, particularly steric influences, a mutation that decreases the steric influence of the hCAII active site was investigated. A previous study on L198 mutants of hCAII revealed that the polarity of residue 198 modulated the inhibitory activities of the known hCAII inhibitors, acetazolamide and dansylamide [17]. From the results of this study and the location of residue 198 in the active site, it was hypothesized that a L198G mutation would decrease the steric congestion of the active, allowing MBPs to form less distorted complexes with hCAII L198G. Therefore, using the hCAII L198G mutant, we probed the effects of decreased steric pressure on the selectivity and binding mode of several MBPs to hCAII versus hCAII L198G by screening a library of MBP fragments and crystallizing select hCAII-MBP complexes to determine their mode of binding. The results reveal that the binding of an MBP in a metalloenzyme active site depends on the size and shape of the active site, the active site residues, and on the identity of the donor atoms of the MBP that are coordinated to the active site metal ion. Additionally, DFT calculations were performed to estimate the energetic costs for differences in dihedral angle and coordination geometry for the observed complexes.

Materials and Methods

Chemicals

The MBP 2-hydroxycyclohepta-2,4,6-trien-1-one (tropolone) and 1-hydroxypyridine-2(1*H*)-thione (1,2-HOPTO) were purchased from commercial suppliers and were used without further purification. 2-Hydroxycyclohepta-2,4,6-triene-1-thione (thiotropolone) was synthesized as previously reported [18]. The hydrotris(5,3-methylphenylpyrazolyl)borate ($\text{Tp}^{\text{Ph,Me}}$) ligand was purchased from Strem Chemical and the $[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(\text{OH})]$ complex was prepared as previously reported [8]. All other chemicals utilized in buffers or assays were purchased from chemical suppliers and used without further purification or modification.

Synthesis and Crystallization of $[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(\text{thiotropolone})]$

To a round bottom flask, $[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(\text{OH})]$ (100 mg, 0.18 mmol) was dissolved in 15 mL of CH_2Cl_2 and the resulting solution was added to a stirring solution of 1.0 equiv (25 mg, 0.18 mmol) thiotropolone dissolved in 10 mL of MeOH. The mixture was stirred overnight under nitrogen at room temperature. The reaction mixture was then evaporated to dryness under vacuum resulting in a yellow-white solid. The resulting solid was dissolved in benzene (3 mL) and filtered to remove any insoluble materials. The filtrate solution was directly used for crystallization via vapor diffusion with pentane, resulting in pale yellow blocks suitable for single-crystal X-ray diffraction (SXRD). Data collection and refinement statistics details for $[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(\text{thiotropolone})]$ can be found in the Supporting Information.

CA Activity Assay

The plasmids encoding hCAII and hCAII L198G with a T7 RNA polymerase promotor and an ampicillin resistance gene (pACA) were generous gifts from Thomas R. Ward (U. Basel, Switzerland) and Carol A. Fierke (University of Michigan). Both hCAII and hCAII L198G were expressed and purified using previously reported methods [19]. Assays were performed in 50 mM HEPES pH 8.0 containing no more than 5% DMSO. *p*-Nitrophenyl acetate was used as the substrate at a concentration of 1 mM with a final protein concentration of 100 nM. MBP fragments were added to protein solutions in 96-well plates, followed by incubation at room temperature for 15 min before addition of substrate. Absorbance at 405 nm was monitored for 20 min at 1 min intervals over a linear range. Half maximal inhibitory concentration (IC₅₀) values were determined by comparing activity of reaction wells containing fragments to the activity of a control well containing no inhibitor. Inhibitor binding constants (K_i) were derived using the Cheng-Prusoff equation [20] and verified by comparison to isothermal titration calorimetry (ITC, Figure S1, S2) measurements (Supporting Information). Absorbance data for all assays were performed using a BioTek Synergy HT plate reader.

CA Crystallization

Crystals of hCAII and hCAII L198G were obtained by sitting drop vapor diffusion method. The concentration of the protein solution was 20 mg/mL of hCAII or hCAII L198G with 1 mM *p*-chloromercuri-benzoic acid in 50 mM Tris-SO₄ (pH 8.0). The precipitation solution for hCAII was 2.7–3.0 M (NH₄)₂SO₄ in 50 mM Tris-SO₄ (pH 8.15). For hCAII L198G the precipitation solution was 3.0–3.1 M (NH₄)₂SO₄ in 50 mM Tris-SO₄ (pH 8.15). For growing hCAII and hCAII L198G crystals, protein and precipitant solution were added in ratios ranging from 0.5:1 to 1:0.5 to sitting drop wells and equilibrated against precipitant solution in a reservoir well. Crystals appeared within 2 weeks with an average size of 0.3 × 0.3 × 0.3 mm. MBP fragments were soaked into crystals by placing crystals for 2–3 days in 15–20 μL of a solution containing the MBP at high concentrations (typically between 1–10 mM), 1.5 M sodium citrate, 5% glycerol, and no more than 5% DMSO in 50 mM HEPES (pH 8.15).

Protein Crystal Structure Determination

X-ray diffraction experiments on protein crystals were performed using a Bruker D8 Smart 6000 CCD detector at 100K utilizing Cu K α radiation ($\lambda = 1.5478 \text{ \AA}$) from a Bruker-Nonius FR-591 rotating anode generator. Collected data was integrated and scaled using the Bruker APEX software suite. All diffracted hCAII and hCAII L198G crystals were in the $P2_1$ space group. The phase problem was solved via molecular replacement using a previously solved hCAII structure (PDB: 3KS3) [21] with Zn occupancy set to 0 and all water molecules deleted. Model refinement was performed by refining with REFMAC5[22] followed by manual refinement using Coot [23]. All ligands and MBP were generated via PRODRG server [24]. Data collection and refinement statistics details for hCAII and hCAII L198G crystal structures can be found in the Supporting Information.

Density Functional Theory Calculations

Both linear transit computations and free geometry optimizations were performed using the Gaussian 09 suite of programs [25]. All calculations were performed with Becke's three-parameter hybrid method with the Lee, Yang, and Parr correlation functional (B3LYP) [26–29] and the 6-311+G(d,p) basis set with CPCM solvation (CH_2Cl_2 , $\epsilon = 8.93$) [30–32]. Ligand coordination of models of Zn(II) metalloenzyme active sites have successfully been studied using the B3LYP functional previously [33] as well as free energies of water-chloride exchange in zinc chloride complexes [34]. Calculations were performed with the phenyl rings omitted to decrease computational cost; the truncated system is referred to as $\text{Zn}(\text{Tp}^{\text{Me}})$.

Results

hCAII and hCAII L198G Inhibition

To determine inhibition activity of MBPs against hCAII and hCAII L198G, both enzymes were screened against a library of approximately 250 MBP fragments, which was an expansion of a previous compound library [35]. Percent inhibition values from a MBP library screen at fragment concentration of 500 μM revealed that most MBPs exhibited greater activity against the L198G mutant when compared to wild type hCAII. Although the tropolone derivative, β -thujaplicin (4-isopropyltropolone) has been previously studied as an inhibitor of hCAII, the unsubstituted tropolone examined here has not been previously described [36]. The MBP tropolone (Table 1) exhibited about 3- to 4-fold greater percent inhibition against hCAII L198G compared to hCAII. In contrast, both thiotropolone and 1,2-HOPTO (Table 1) did not exhibit significant differences in percent inhibition against hCAII and the hCAII L198G mutant, suggesting that the sulfur heteroatom in both 1,2-HOPTO and thiotropolone might play a role in retaining activity against both forms of the metalloenzyme. To confirm these observations, the inhibitor binding constants (K_i) values for the three MBPs were determined against hCAII and hCAII L198G (Table 1). The two sulfur-containing ligands, 1,2-HOPTO and thiotropolone, showed small differences (0.4 and 1.6-fold) in activity against the wild type and mutant metalloenzymes. The inhibitory activity of thiotropolone is significantly greater toward either form of the enzyme than that of 1,2-HOPTO (about 6- to 23-fold). Unlike the sulfur-containing ligands, tropolone is less active than either 1,2-HOPTO or thiotropolone against hCAII and displays a greater difference in activity (5.5-fold) against hCAII L198G and hCAII. In order to validate that the K_i values, isothermal titration calorimetry (ITC) experiments with tropolone and wild type hCAII were performed. A K_d of 1.68 mM, which was close to the determined K_i of 1.35 mM (Figure S1). To further validate these measurements, 4-(trifluoromethyl)-benzene sulfonamide was assayed and analyzed by ITC against hCAII. The results with this sulfonamide inhibitor showed a close agreement between the K_i and K_d values (~ 230 nM and ~ 70 nM, respectively, Figure S2). To better understand the origin of the similarities and differences in these K_i values, crystallographic and computational studies were performed.

Crystallography of hCAII and hCAII L198G

The crystal structure of ligand-free hCAII L198G was obtained and compared to hCAII (PDB: 3KS3) (Figure 2). The L198G mutation increases the volume of the hydrophobic

pocket adjacent to the active site by $74 \text{ \AA}^3$ (the approximate volume of a leucine side chain). In the crystal structure of the mutant, this free space is occupied by two water molecules. The larger size of the active site as shown in Figure 2 has the potential to decrease the steric congestion exerted by the active site on bound MBPs.

The crystal structures of both hCAII and hCAII L198G with tropolone bound were obtained by soaking the tropolone MBP into pre-formed crystals. The resolution for both tropolone-bound structures was obtained to $1.5 \text{ \AA}$. As seen in Figure 3, the binding mode and coordination geometry of tropolone differs substantially in the two enzymes. In both structures, tropolone displays the expected bidentate coordination to the active site Zn(II) ion with Zn-O bond distances ranging from $\sim 1.9 \text{ \AA}$ to $\sim 2.4 \text{ \AA}$ (Table S1). However, the coordination geometry of tropolone is much more distorted from its ideal trigonal bipyramidal binding geometry in the wild type enzyme than in the L198G mutant (Figure 3). In the structure of tropolone bound to hCAII, the aromatic ring of tropolone makes considerable contact with the hydrophobic wall of the active site, including residue L198 in the case of tropolone binding to hCAII. As shown in Figure 3, in hCAII L198G the tropolone ring partially occupies the space vacated by the L198G mutation, while still maintaining considerable contacts with the hydrophobic wall. Two waters (not shown) occupy the residual freed space. Therefore, the measurable difference (~ 5.5 -fold) in K_i values of tropolone toward hCAII versus hCAII L198G (Table 1) can be attributed to the difference in binding geometry.

Tropolone binding to the L198G active site adopts a binding geometry that is closer to the binding geometry exhibited in the $[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(\text{tropolone})]$ model complex [16]. Along with the change in coordination geometry tropolone binds with different dihedral angles ϕ (Zn(II)-O-O-C) of around 152° in hCAII and 161° in hCAII L198G (Table S1). The dihedral angle ϕ (Figure 1) for hCAII L198G is closer to ideal head-on geometry (180°), which has been shown to be energetically favorable [12]. The more favorable coordination geometry in the mutant structure correlates well with the increased inhibitory activity of tropolone against hCAII L198G compared to the wild type hCAII.

The structures of hCAII and hCAII L198G with thiotropolone bound were obtained by soaking the MBP into pre-formed crystals. The structure of hCAII was obtained to a resolution of $1.33 \text{ \AA}$ and the structure of hCAII L198G was obtained to a resolution of $1.42 \text{ \AA}$. The binding of thiotropolone to both enzymes was nearly identical (Figure 4) with Zn-S and Zn-O bond distances of $\sim 2.4 \text{ \AA}$ and $\sim 2.5 \text{ \AA}$, respectively. The resulting complexes are 5-coordinate species with almost identical trigonal bipyramidal coordination geometries. The dihedral angles ϕ (Zn(II)-S-O-C) of the complexes are 132° for hCAII and 137° for hCAII L198G, which are much smaller than those exhibited by tropolone. In both structures the MBP ring makes significant contacts with the hydrophobic wall of the active site, which likely contributes to the high inhibitory activity of thiotropolone. While thiotropolone makes contacts with residue L198, this MBP maintains a similar binding affinity the L198G mutant indicating the contacts made with this residue in the wild type are not critical for strong binding. Indeed, the free volume that is created in the L198G mutation is occupied by a DMSO molecule.

To facilitate comparison to our earlier studies [12], the MBP 1,2-HOPTO was soaked into crystals of the hCAII L198G mutant. The crystal structure of hCAII L198G with 1,2-HOPTO bound (1.42 Å resolution) shows the MBP does not change its binding geometry when compared to the same compound bound in wild type hCAII (Figure 5, PDB:3M1K) [37]. In both enzymes, 1,2-HOPTO is bound in a bidentate fashion to the Zn(II) ion, with Zn-S and Zn-O bond distances of ~2.4 Å to ~2.6 Å creating a 5-coordinate species at the metal center with a trigonal bipyramidal geometry. The dihedral angles observed for this MBP in hCAII and hCAII L198G are almost identical at 128° and 127°, respectively. The ring of 1,2-HOPTO makes some contacts with the hydrophobic wall of hCAII and hCAII L198G, including residue L198 in hCAII. The small decrease in the K_i of 1,2-HOPTO for hCAII L198G (Table 1), while maintaining nearly identical modes of binding to both hCAII and hCAII L198G, is consistent with the contacts between 1,2-HOPTO and residue L198 contributing to the binding of this ligand. In an almost exact fashion as was observed in the thiotropolone-hCAII L198G structure a DMSO molecule occupies the space created by in the L198G.

[(Tp^{Ph,Me})Zn (thiotropolone)] Model Complex

In order to understand the ‘ideal’ binding of thiotropolone to a tris(histidine) metal active site, a model complex of this active site was synthesized and the structure was determined by SXRD. The structure of [(Tp^{Ph,Me})Zn (thiotropolone)] shows thiotropolone bound in a bidentate fashion resulting in a 5-coordinate complex (Figure 6). The complex exhibits trigonal bipyramidal coordination geometry, with a dihedral angle ϕ (Zn(II)–S–O–C) of 179° and Zn-S and Z-O bond distances of ~2.3 Å and ~2.1 Å, respectively. The coordination geometry is generally similar to that found for tropolone in the same model system, except for the Zn-S bond being longer and causing the coordination of thiotropolone to be slightly asymmetric when compared to tropolone, to accommodate the longer bond (Figure 6).

Density Functional Theory Analysis

To better understand the observed differences in MBP binding in the tropolone and thiotropolone hCAII L198G mutant, linear transit computations utilizing DFT were performed on truncated [(Tp^{Me})Zn(MBP)] model complexes varying ϕ from 180° to 105°, as defined as the Zn(II)–(S/O)–O–N dihedral angle (Figure 1). Free geometry optimizations were performed with the truncated model complexes using both tropolone and thiotropolone as the MBP and the resulting structures had no significant deviations from the crystallographically observed structures (Figure 5, and supporting information).

According to the linear transition computations, the donor atom bond distances increased slightly as ϕ decreased from an initial value of 180° until the coordination of both tropolone and thiotropolone “irreversibly” switched from a bidentate to monodentate mode of coordination. This change in coordination occurred at dihedral angles of $\phi = 115^\circ$ and $\phi = 120^\circ$ for [(Tp^{Me})Zn(tropolone)] and [(Tp^{Me})Zn(thiotropolone)], respectively and was clearly apparent due to a sharp increase in distance between Zn(II) ion and the equatorial oxygen donor atom to values >3.3 Å and >3.0 Å for [(Tp^{Me})Zn(tropolone)] and [(Tp^{Me})Zn(thiotropolone)], respectively (Figure 7).

Both optimized trigonal bipyramidal $[(\text{Tp}^{\text{Me}})\text{Zn}(\text{tropolone})]$ and $[(\text{Tp}^{\text{Me}})\text{Zn}(\text{thiotropolone})]$ complexes possessed ϕ of approximately 180° , permitting ideal head-on binding. Decreasing ϕ of these complexes caused parabolic increases in their respective energies until a change in coordination occurred. Interestingly, the parabolic increase in energy for tropolone was much greater than that of thiotropolone, with the two complexes differing in energy by approximately $3.5 \text{ kcal mol}^{-1}$ at $\phi = 125^\circ$.

Discussion

Based on X-ray crystallography, thiotropolone binds to hCAII and hCAII L198G in nearly identical trigonal bipyramidal geometries. The $[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(\text{thiotropolone})]$ model complex and the enzyme-thiotropolone complexes exhibited a large difference in ϕ , as was observed previously with 1,2-HOPTH [12]. In contrast, tropolone bound to the enzyme with a ϕ dihedral angle (ca. 152° and 161° for the wild type hCAII and hCAII L198G, respectively) more in line with that of the model complex, $[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(\text{tropolone})]$. The hCAII L198G mutation cavity allows tropolone to bind to the enzyme with a larger (more ideal) ϕ , and the change in geometry is quite pronounced in the tropolone-hCAII L198G complex and essentially absent in the thiotropolone-hCAII L198G complex. These observations indicate that steric congestion plays a significant role in the coordination geometry of tropolone to the hCAII active site.

The large dihedral angles observed when tropolone binds to hCAII and hCAII L198G indicates that the head-on binding observed in the $[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(\text{tropolone})]$ model complex is important for maintaining strong binding in the tropolone-protein complex. This importance of a large dihedral angle for binding, does not appear to be the same for thiotropolone binding. The far from ideal dihedral angle observed with thiotropolone bound to hCAII and the minimal change in inhibition (Table 1), coordination geometry, and dihedral angle in response to the L198G mutation, strongly suggests that the sulfur donor atom enables the MBP to bind with a non-ideal geometry (dihedral angle). The DFT computations demonstrate that distortion of tropolone from head-on binding (i.e. $\phi = 180^\circ$, Figure 1) to the metal center is energetically disfavored compared to the corresponding distortion of thiotropolone (Figure 7). These results demonstrate sulfur donor atoms can enable MBPs to adopt 'non-ideal' dihedral angles without significant penalties in binding affinity. This is consistent with both 1,2-HOPTH and thiotropolone as they did not as significantly change coordination geometry or binding affinity between the hCAII and hCAII L198G enzymes.

The lack of change observed in dihedral angle and coordination geometry with thiotropolone and 1,2-HOPTH (*O,S* donor MBPs) when binding to hCAII and hCAII L198G is consistent with previous studies, where changes in coordination geometry and binding mode for 1,2-HOPTH were only observed when unfavorable interactions were introduced by derivatization of the 1,2-HOPTH MBP [38]. On the other hand, MBPs that bind only with *O,O* donor atoms, such as tropolone, are found to be quite sensitive to perturbations from the ideal coordination geometry, as highlighted by changes in the dihedral angle upon binding. This is manifest by tropolone being a significantly more potent inhibitor of hCAII L198G (Figure 2), where it exhibits a more idealized coordination geometry and a larger dihedral

angle than when it binds hCAII. These difference in *O,S* and *O,O* donor MBPs to changes in protein active site composition will likely have important implications for their development into metalloenzyme inhibitors.

In order to improve the design of metalloenzyme inhibitors, an understanding of how the inhibitors interact with active site metal ions and the active site environment is needed. Sulfur donor atom MBPs often show higher activity against Zn(II)-dependent metalloenzymes than their oxygen-containing analogs, due to the thiophilicity of Zn(II) [39]. The results here demonstrate that strong coordination by sulfur may permit inhibitors to adopt more distorted dihedral angles while preserving strong bidentate coordination, without a substantial loss in binding affinity for the metal center. This may allow for better predictability in binding mode for these MBPs across different metalloenzymes. In contrast, the affinity of *O,O* donor set MBPs are more sensitive to deviations in coordination dihedral angle, which can cause their mode of binding to be more variable in order to preserve a favorable dihedral angle. This variability, as seen with the MBP tropolone, is dependent on the protein active site structure and second coordination sphere and may allow for these MBPs to better adapt to mutational changes in the metalloprotein active site. This study highlights that the choice of MBP for metalloenzyme inhibitors merits serious consideration, as donor atom identity can have effect on the introduction of other protein-inhibitor interactions, constraining the inhibitor to a specific binding geometry, and make the mode of binding more or less predictable and tolerant of active site mutations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Andreini C, Bertini I, Cavallaro G, Holliday GL, Thornton JM. Journal of biological inorganic chemistry. 2008; 13:1205–1218. [PubMed: 18604568]
2. Rouffet M, Cohen SM. Dalton Transactions. 2011; 40:3445–3454. [PubMed: 21290034]
3. Supuran CT, Scozzafava A, Casini A. Medicinal Research Reviews. 2003; 23:146–189. [PubMed: 12500287]
4. Paris M, Porcelloni M, Binaschi M, Fattori D. Journal of Medicinal Chemistry. 2008; 51:1505–1529. [PubMed: 18247554]
5. Fernández-Montero JV, Vispo E, Soriano V. Expert Opinion on Pharmacotherapy. 2014; 15:211–219. [PubMed: 24289800]
6. Jacobsen FE, Lewis JA, Cohen SM. ChemMedChem. 2007; 2:152–171. [PubMed: 17163561]

7. Mutule I, Borovika D, Rozenberga E, Romanchikova N, Zalubovskis R, Shestakova I, Trapencieris P. *Journal of Enzyme Inhibition and Medicinal Chemistry*. 2015; 30:216–223. [PubMed: 24939099]
8. Puerta DT, Lewis JA, Cohen SM. *Journal of the American Chemical Society*. 2004; 126:8388–8389. [PubMed: 15237990]
9. Göçer H, Akincio lu A, Göksu S, Gülçin , Supuran CT. *Journal of Enzyme Inhibition and Medicinal Chemistry*. 2015; 30:316–320. [PubMed: 24964347]
10. Moradei O, Vaisburg A, Martell RE. *Current topics in medicinal chemistry*. 2008; 8:841–858. [PubMed: 18673170]
11. Martin DP, Hann ZS, Cohen SM. *Inorganic Chemistry*. 2013; 52:12207–12215. [PubMed: 23706138]
12. Martin DP, Blachly PG, Marts AR, Woodruff TM, de Oliveira FCA, McCammon JA, Tierney DL, Cohen SM. *Journal of the American Chemical Society*. 2014; 136:5400–5406. [PubMed: 24635441]
13. Puerta DT, Cohen SM. *Inorganic chemistry*. 2003; 42:3423–3430. [PubMed: 12767177]
14. Puerta DT, Schames JR, Henchman RH, McCammon JA, Cohen SM. *Angewandte Chemie, International Edition in English*. 2003; 42:3772–3774.
15. Cook SA, Hill EA, Borovik AS. *Biochemistry*. 2015; 54:4167–4180. [PubMed: 26079379]
16. Jacobsen FE, Lewis JA, Heroux KJ, Cohen SM. *Inorganica Chimica Acta*. 2007; 360:264–272.
17. Nair SK, Krebs JF, Christianson DW, Fierke CA. *Biochemistry*. 1995; 34:3981–3989. [PubMed: 7696263]
18. Machiguchi T, Hasegawa T, Kano Y. *Bulletin of the Chemical Society of Japan*. 1993; 66:3699–3706.
19. Monnard FW, Heinisch T, Nogueira ES, Schirmer T, Ward TR. *Chemical Communications (Cambridge)*. 2011; 47:8238–8240.
20. Yung-Chi C, Prusoff WH. *Biochemical Pharmacology*. 1973; 22:3099–3108. [PubMed: 4202581]
21. Avvaru BS, Kim CU, Sippel KH, Gruner SM, Agbandje-McKenna M, Silverman DN, McKenna R. *Biochemistry*. 2010; 49:249–251. [PubMed: 20000378]
22. Murshudov GN, Vagin AA, Dodson EJ. *Acta crystallographica. Section D, Biological crystallography*. 1997; 53:240–255. [PubMed: 15299926]
23. Emsley P, Cowtan K. *Acta crystallographica. Section D, Biological crystallography*. 2004; 60:2126–2132. [PubMed: 15572765]
24. Schüttelkopf AW, van Aalten DMF. *Acta crystallographica. Section D, Biological crystallography*. 2004; 60:1355–1363. [PubMed: 15272157]
25. Frisch, MJ., Trucks, GW., Schlegel, HB., Scuseria, GE., Robb, MA., Cheeseman, JR., Scalmani, G., Barone, V., Mennucci, B., Petersson, GA., Nakatsuji, H., Caricato, M., Li, X., Hratchian, HP., Izmaylov, AF., Bloino, J., Zheng, G., Sonnenberg, JL., Hada, M., Ehara, M., Toyota, K., Fukuda, R., Hasegawa, J., Ishida, M., Nakajima, T., Honda, Y., Kitao, O., Nakai, H., Vreven, T., Montgomery, JA., Jr, Peralta, JE., Ogliaro, F., Bearpark, MJ., Heyd, J., Brothers, EN., Kudin, KN., Staroverov, VN., Kobayashi, R., Normand, J., Raghavachari, K., Rendell, AP., Burant, JC., Iyengar, SS., Tomasi, J., Cossi, M., Rega, N., Millam, NJ., Klene, M., Knox, JE., Cross, JB., Bakken, V., Adamo, C., Jaramillo, J., Gomperts, R., Stratmann, RE., Yazyev, O., Austin, AJ., Cammi, R., Pomelli, C., Ochterski, JW., Martin, RL., Morokuma, K., Zakrzewski, VG., Voth, GA., Salvador, P., Dannenberg, JJ., Dapprich, S., Daniels, AD., Farkas, Ö., Foresman, JB., Ortiz, JV., Cioslowski, J., Fox, DJ. *Gaussian 09. Gaussian, Inc; Wallingford, CT, USA*: 2009.
26. Becke AD. *Journal of Chemical Physics*. 1993; 98:5648.
27. Lee C, Yang W, Parr RG. *Physical Review B*. 1988; 37:785–789.
28. Stephens PJ, Devlin FJ, Chabalowski CF, Frisch MJ. *Journal of Physical Chemistry*. 1994; 98:11623–11627.
29. Vosko SH, Wilk L, Nusair M. *Canadian Journal of Physics*. 1980; 58:1200–1211.
30. Barone V, Cossi M. *Journal of Physical Chemistry A*. 1998; 102:1995–2001.
31. Cossi M, Rega N, Scalmani G, Barone V. *Journal of Computational Chemistry*. 2003; 24:669–681. [PubMed: 12666158]
32. Klamt A, Schüürmann G. *J Chem Soc, Perkin Trans*. 1993; 2:799–805.

33. Ryde U. *Biophysical Journal*. 1999; 77:2777–2787. [PubMed: 10545376]
34. Dudev T, Lim C. *Journal of the American Chemical Society*. 2000; 122:11146–11153.
35. Jacobsen JA, Fullagar JL, Miller MT, Cohen SM. *Journal of Medicinal Chemistry*. 2011; 54:591–602. [PubMed: 21189019]
36. Costa G, Gidaro MC, Vullo D, Supuran CT, Alcaro S. *Journal of Agricultural and Food Chemistry*. 2016; 64:5295–5300. [PubMed: 27268752]
37. Schulze Wischeler J, Innocenti A, Vullo D, Agrawal A, Cohen SM, Heine A, Supuran CT, Klebe G. *ChemMedChem*. 2010; 5:1609–1615. [PubMed: 20629007]
38. Martin DP, Blachly PG, McCammon JA, Cohen SM. *Journal of Medicinal Chemistry*. 2014; 57:7126–7135. [PubMed: 25116076]
39. Sigel H, McCormick DB. *Accounts of Chemical Research*. 1970; 3:201–208.

Figure 1.

The angle ϕ is illustrated in this image with 1,2-HOPTO binding to Zn(II) (*left*), as the Zn(II)-S-O-N dihedral angle, which represents the degree of ring canting observed when MBP binds the active site Zn(II). The differences in MBP binding mode between a [(Tp^{Ph,Me})Zn(1,2-HOPTO)] model complex (*middle*) and the protein hCAII-1,2-HOPTO (*right*) is apparent for this MBP. Adapted from reference [12].

Figure 2. Structures of hCAII (*top*, molecular surface rendered with carbon in cyan, oxygen in red, and nitrogen in blue) (PDB: 3KS3) and hCAII L198G (*bottom*, molecular surface rendered with carbon in green, oxygen in red, and nitrogen in blue) with residue 198 highlighted in yellow and the two water molecules that replace L198 side chain shown as small, red spheres.

Figure 3.

The binding mode of tropolone in hCAII (gray) and hCAII L198G (pink), shown in two different orientations (*left* and *middle*). Surface structure of hCAII showing tropolone (pink) occupying significant free space created by mutating the L198 sidechain shown as the yellow mesh (*right*).

Figure 4. The binding mode of thiotropolone in hCAII (gray) and hCAII L198G (pink) shown in two different orientations. Surface structure of hCAII showing thiotropolone (pink) and free space created by mutating the L198 sidechain shown as the yellow mesh (*right*).

Figure 5.

The binding mode of 1,2-HOPTH in hCAII (gray) and hCAII L198G (pink) shown in two different orientations (*left* and *middle*). Surface structure of hCAII showing 1,2-HOPTH (pink) and free space created by mutating the L198 sidechain shown as the yellow mesh (*right*).

Figure 6.

Crystal structure of [(Tp^{Ph,Me})Zn(thiotropolone)] (*left*) (hydrogen atoms removed for clarity, 50% probability ellipsoids) along with an overlay of [(Tp^{Ph,Me})Zn(thiotropolone)] and [(Tp^{Ph,Me})Zn(tropolone)] complexes viewed from different orientations (*middle* and *right*) (with hydrogen, phenyl, and methyl groups removed for clarity in overlay). Color scheme: carbon = gray, sulfur = yellow, oxygen = red, nitrogen = blue, boron = pink, thiotropolone carbon = teal, tropolone carbon = green, and zinc = black.

Figure 7. Relative binding energies (top) and Zn-O bond distances (bottom) as a function of the Zn(II)-X-O-C dihedral.

Table 1

Structure of the MBPs examined in this study and K_i values against hCAII and hCAII L198G, with relative differences in activity against hCAII and hCAII L198G shown.

MBP	K_i hCAII (μ M)	K_i hCAII L198G (μ M)	Difference in Activity (K_i WT/ K_i L198G)
1,2-HOPTO	567 $\pm$ 85	1420 $\pm$ 30	0.4-fold
Tropolone	1350 $\pm$ 40	245 $\pm$ 14	5.5-fold
Thiotropolone	96 $\pm$ 13	61 $\pm$ 5	1.6-fold