Structure-Based Drug Discovery

From Model Complexes to Metalloprotein Inhibition: A Synergistic Approach to Structure-Based Drug Discovery**

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Matrix metalloproteinases (MMPs) are zinc-containing hydrolytic enzymes that are involved in the restructuring of connective tissue. The design of effective inhibitors of matrix metalloproteinases is a significant goal in chemotherapeutic development as a result of the correlation of MMP activity with a variety of illnesses, including cancer, arthritis, and inflammatory disease.[1–3] However, the design of inhibitors for MMPs and other metalloproteins is limited by the ability to predict the interaction of a given inhibitor with the metal ion at the active site. In most cases, the elucidation of a protein structure with the bound inhibitor, by using X-ray diffraction or NMR spectroscopy, is necessary for revealing the metal–inhibitor interactions.[4] Innovative approaches to increasing the efficiency and speed of the drug-discovery process may provide attractive, alternative routes to identifying new drug candidates. For example, the determination of a structure–activity relationship (SAR) by NMR[5] spectroscopy has been used to reveal probable binding modes of metal-ion chelators to facilitate the development of improved MMP inhibitors.[6,7] Despite being a very effective approach, the determination of SARs by NMR spectroscopy still requires substantial amounts of ^{15}N-labeled metalloprotein and is likely to be limited to metalloenzymes that contain diamagnetic metal ions.

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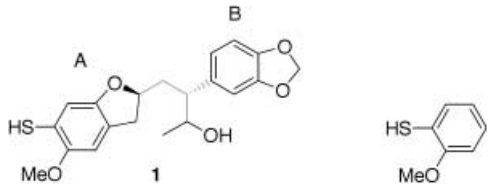
Another approach for metalloprotein drug design is to reproduce the drug–metalloenzyme interactions by using small molecule models that can be readily characterized.[8–10] This strategy is effective for elucidating the interactions of an inhibitor with a metal ion, and we sought to augment this method so that the complete binding of an inhibitor to the active site of a metalloprotein could be predicted. Therefore, an integrated approach to metalloenzyme drug discovery that marries bioinorganic model chemistry with computational methods has been devised. This unique strategy involves the use of simple coordination complexes as active-site models to reveal metal–inhibitor interactions, followed by computational analysis using the model complex structure as a basis for drug docking. The model complex effectively overcomes the computational requirement for parameterization of the metal ion by directly providing the metal–ligand geometry. The result of this combined approach is the elucidation of the complete binding of an inhibitor to a metalloenzyme without the use of additional macromolecular NMR spectroscopic or X-ray crystallographic data.

Futoenones are natural products, derivatives of which are known to interfere with MMP activity;[11] however, the precise mode of binding for these compounds to MMPs has not been determined. Compound **1** was examined (Scheme 1), as it was shown in an earlier study to be the most effective inhibitor against MMP-3 ($IC_{50} = 600$ nM) from



Scheme 1. Futoenone derivative **1** (left, with ring systems labeled) and 2-methoxybenzenethiol (right).

a series of futoenone-derived compounds.[11] Compound **1** was selected because the mode of metal-binding and active-site conformation is unknown, it demonstrates significant selectivity for stromelysin (MMP-3) over other MMPs, and it does not possess a hydroxamate functional group as the metal-ion chelator. The limited number of heteroatoms in this compound indicated that the thiophenol ring moiety (ring system A, Scheme 1) was the most probable zinc-binding group.

2-Methoxybenzenethiol (MBT) was combined with $[(Tp^{Ph,Me})ZnOH]$ ($Tp^{Ph,Me}$ = hydrotris(3,5-phenylmethylpyrazolyl)borate)[10,12] to generate the complex $[(Tp^{Ph,Me})Zn(mbt)]$ as a model for the interaction between **1** and the zinc(II) ion at the MMP active site. The structure of $[(Tp^{Ph,Me})Zn(mbt)]$ was determined by single-crystal X-ray diffraction (Figure 1).[13] The three coordinated pyrazole nitrogen atoms recreate the highly conserved tris(histidine) coordination environment that is found in the active site of all MMPs.[1] The model complex clearly shows that the MBT ligand chelates the zinc ion in a bidentate fashion by utilizing both the sulfur and oxygen donor atoms.

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The modeling of **1** bound to stromelysin was performed in four stages. First, the structure of [(Tp^{Ph,Me})Zn(mbt)] was used to template the conformation of inhibitor **1** in the active site of MMP-3.[14] A portion of the structure (Figure 1) was inserted into the crystal structure of MMP-3[14] by aligning the three pyrazole nitrogen atoms (N2, N4, and N6) with the three histidine (H201, H205, H211) nitrogen atoms of the MMP active site. The overlay was performed in three different orientations because the pyrazole nitrogen donors do not specifically correlate with a particular histidine residue in the protein (Figure 2). Two of the conformations were immediately dismissed on the basis of steric conflicts between the MBT fragment and the protein. The remaining superposition showed no steric clashes with the protein, and was determined to be the most probable binding conformation. These steric evaluations are based only on the X-ray crystallographic data, and the role of protein mobility[15,16] on this analysis will be the subject of further investigations.

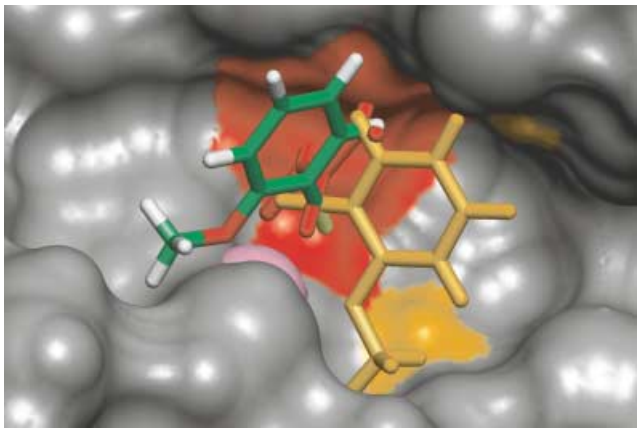

Figure 2. Three superpositions of the [(Tp^{Ph,Me})Zn(mbt)] fragment (Figure 1, bottom right) into the structure of MMP-3. The MBT ligand is shown in stick representations and the catalytic zinc(II) ion is shown as a purple sphere. The red and orange orientations clash with the protein in the areas as shown by the corresponding colors on the protein surface. Only the green orientation is free of steric conflicts.

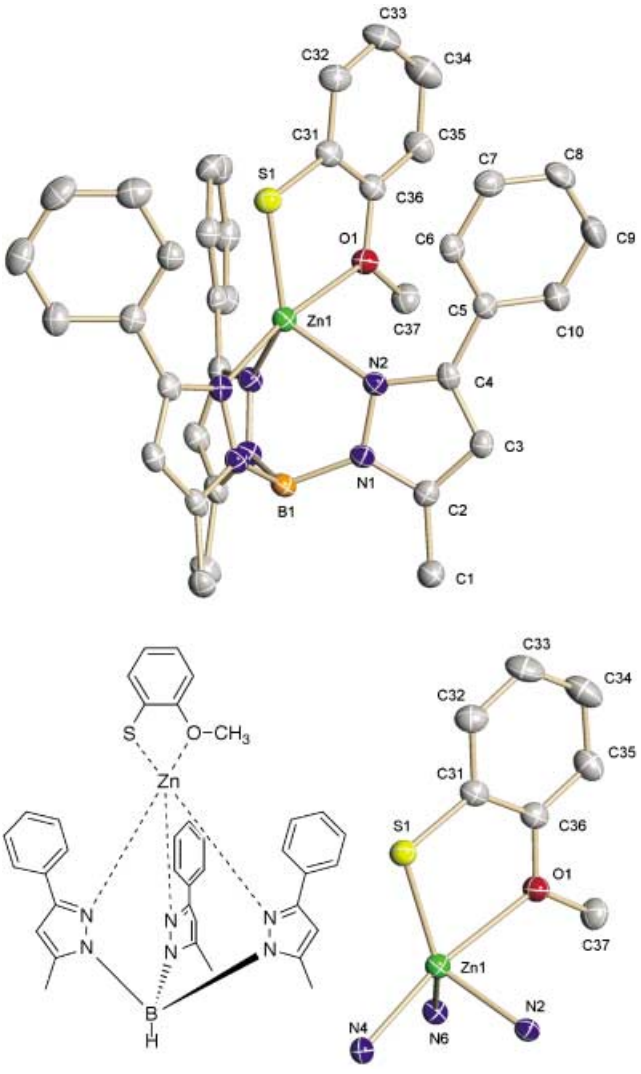

Figure 1. Top: Structural diagram of [(Tp^{Ph,Me})Zn(mbt)] with partial atom numbering schemes (ORTEP, 50% probability ellipsoids). Hydrogen atoms and solvent molecules have been omitted for clarity. Bottom left: A chemical drawing of the complex. Bottom right: A portion of this complex used as a template for computational analysis.

With the orientation of the MBT fragment resolved, the second step was to generate all possible conformers of **1** by varying each of four dihedral angles (Scheme 1, bonds in gray).[17] Of the conformers produced, 14559 were rejected on the basis of internal steric clashes between non-hydrogen atoms. Of the remaining 6177 conformers, every 60th conformation was chosen to obtain a uniform selection of 100 structures. Each structure was placed into the protein by superimposing ring A (Scheme 1) of the calculated conformers with that of the protein-imbedded MBT fragment.

In the third stage, AMBER parm99 and gaff force fields were used to model the protein and inhibitor, respectively.[18,19] Hydrogen atoms were added to the protein using the program WHAT IF.[20] Charges were derived using a Gaussian optimization (HF/6-31G*) and the AMBER 7 module Antechamber.[21] The process was completed by performing minimizations using the SANDER module of AMBER 7.[22] The ligand atoms not shared with the MBT fragment were allowed to move while the remaining atoms (including the protein) were kept rigid. Minimizations were run until the root-mean-square (RMS) deviation of the energy gradient was less than 1×10^{-4} kcal mol^{-1} Å^{-1}.

The strength of ligand binding was assessed according to the minimized energies (see the Supporting Information). A single conformation minimized to the lowest energy of 3.9 kcal mol^{-1}, followed by a cluster of less favorable conformations at 8–9 kcal mol^{-1}. It was apparent that the lowest energy structure relaxed in a unique position relative to the active site cleft (Figure 3). The remaining low-energy conformations minimized outside the protein subsites, thus making contacts predominantly with solvent space (see the Supporting Information).

The lowest energy minimized structure of **1** reveals several unusual features about the binding. Most MMP inhibitors have been designed to occupy the S′ subsites

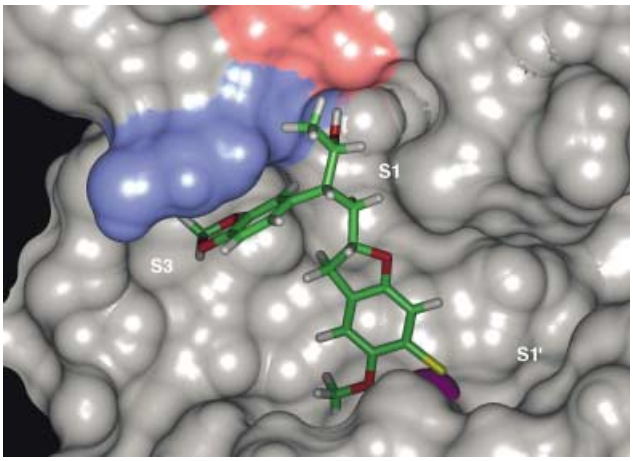

Figure 3. Lowest energy minimized structure of **1** in the active site of stromelysin (MMP-3). Y155 is shown in blue, P156 is shown in pink, and the catalytic zinc(II) ion is shown in purple.

(termed "right-handed" inhibitors), in particular the S1′ pocket (Figure 3).[1] However, the backbone portion of minimized **1** lies in the "left-handed" side of the active site (S subsites). There is a significant interaction in the open S1 and S3 subsites (Figure 3),[14,23] where π stacking is observed (π–π contact, 3.7 Å) between the phenyl group of ring system B of **1** and the side chain of Y155. The known efficacy of **1** against MMP-3 correlates well with other "left-handed" inhibitors that show preferential activity against MMP-3, where interactions with the hydrophobic residue Y155 play an important role.[23] For comparison, the corresponding residue in collagenase (MMP-1) is serine,[1] which thereby eliminates any possible π interactions. Another hydrophobic interaction occurs between the methyl group from the zinc-bound oxygen atom in **1** and a hydrophobic cleft created by the side chains of F86 and F210. Specific hydrogen-bonding interactions is also present between the backbone carbonyl group of P156 and the primary alcohol moiety of **1**.

Earlier attempts to model the binding of **1** relied on comparison to hydroxamate-based inhibitors[11] and failed to distinguish between binding in the S and S′ subsites. By applying a combined bioinorganic–computational technique to this system, we have demonstrated that the interactions of an MMP inhibitor with its target protein can be revealed. This approach is useful not only for known MMP inhibitors,[1] but also new MMP inhibitors that utilize unexplored zinc-binding groups and inhibitors of other medically relevant metalloproteins.

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[13] Further details for the structure of [($Tp^{Ph,Me}$)Zn(mbt)] can be found in the Supporting Information. CCDC 199598 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).
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