Crystal Structures of *Staphylococcus aureus* Methionine Aminopeptidase Complexed with Keto Heterocycle and Aminoketone Inhibitors Reveal the Formation of a Tetrahedral Intermediate

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Received September 10, 2003

Abstract: High-resolution crystal structures of *Staphylococcus aureus* methionine aminopeptidase I in complex with various keto heterocycles and aminoketones were determined, and the intermolecular ligand interactions with the enzyme are reported. The compounds are effective inhibitors of the *S. aureus* enzyme because of the formation of an uncleavable tetrahedral intermediate upon binding. The electron densities unequivocally show the enzyme-catalyzed transition-state analogue mimicking that for amide bond hydrolysis of substrates.

Methionine aminopeptidases (MetAPs) are ubiquitous enzymes found in both eukaryotic and prokaryotic cells and play a critical role in the maturation of proteins for proper function, targeting, and degradation.[1] MetAP removes the N-terminal methionine residue from newly synthesized polypeptide chains that contain a small, uncharged amino acid in the penultimate position.[2,3] In prokaryotes, mitochondria, and chloroplasts, translation of proteins is initiated with *N*-formylmethionine, whereas in the cytosol of eukaryotes protein biosynthesis begins with methionine. However, the majority of mature proteins do not retain this residue.[4] The physiological importance of MetAP is underscored by the lethality of its absence in *Escherichia coli*, *Salmonella typhimurium*, and *Saccharomyces cerevisiae*.[5−7] Moreover, MetAPs have been identified biochemically to be the molecular target of the antiangiogenesis agent fumagillin and its derivatives.[8−10] Thus, as a result of their importance for cell growth and proliferation, MetAPs are promising antifungal, antibacterial, and antiangiogenesis targets.

Several crystal structures of the catalytic domain of type I *E. coli* and *S. aureus* MetAP[11−13] indicate an identical fold and topology of the enzyme. The active site, located near the center of a central β-sheet, constitutes a dinuclear metal center formed by highly conserved acidic residues. Several metal ions Fe(II), Zn(II), Mn(II), Co(II), and Ni(II) can be substituted in vitro and retain the enzymatic activity.[12,14] In vivo studies

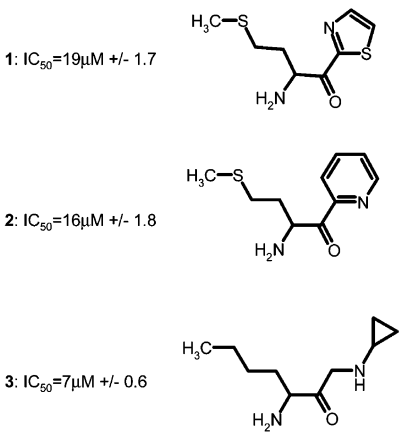

Figure 1. Chemical structures of *Sa*MetAP inhibitors. IC_{50} values are the average of three or more determinations

indicate a higher activity of the yeast MetAP I in the presence of Zn^{2+}, while the *E.coli* enzyme requires Mn^{2+} or Fe^{2+}.[15−18]

Aldehydes, fluoroketones, keto esters, diketones, ketoamides as well as α-keto heterocycles have been shown to be potent inhibitors for proteases. In all cases, the scissile amide bond of the protease substrate is replaced by an electron-deficient carbonyl group that can readily hydrate. These molecules are expected to be competitive protease inhibitors because the sp^3-hybridized hydrate resembles the transition state for amide bond hydrolysis.[19−23]

In an attempt to design novel *Staphylococcus aureus* methionine aminopeptidase (*Sa*MetAP) I inhibitors, we have identified two series of molecules, the keto heterocycles and the aminoketones, that inhibit the target enzyme (Figure 1). The α-keto heterocycles and aminoketones are potent inhibitors of the Co^{2+}-loaded staphylococcal enzyme with IC_{50} values in the low micromolar range, employing an assay previously described.[12] The inhibition pattern of the compounds was typical for a competitive inhibitor when analyzed using Dixon and Cornish-Bowden plots[26] (data not shown). To determine the mode of enzyme inhibition, we have solved the crystal structures of the binary complexes in the presence of cobalt at atomic resolution. Crystallographic data and refinement statistics are presented in the Supporting Information. Atomic coordinates have been deposited with the Protein Data Bank (1QXW, 1QXY, 1QXZ).

The three-dimensional structure of the *S. aureus* enzyme has been described and resembles the conserved pita bread fold, common to all known MetAPs.[11−13,27−29] Briefly, the *S. aureus* ensyme is similar to the *E. coli* enzyme; two cobalt ions are located in the active site and are coordinated by the side chains of the conserved residues D97, D108, H171, E204, and E235. The residues interact in a monodentate or bidentate manner similar to that observed for the *E. coli* enzyme.[11,13] The amino acid side chains of P59, E63, F65, C70, H79, L177, and W221 form the S1 subsite of the enzyme on one site of the metal center as a gorge, which has largely hydrophobic character. It recognizes the N-terminal

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10.1021/jm034188j CCC: $27.50
Published on Web 02/07/2004

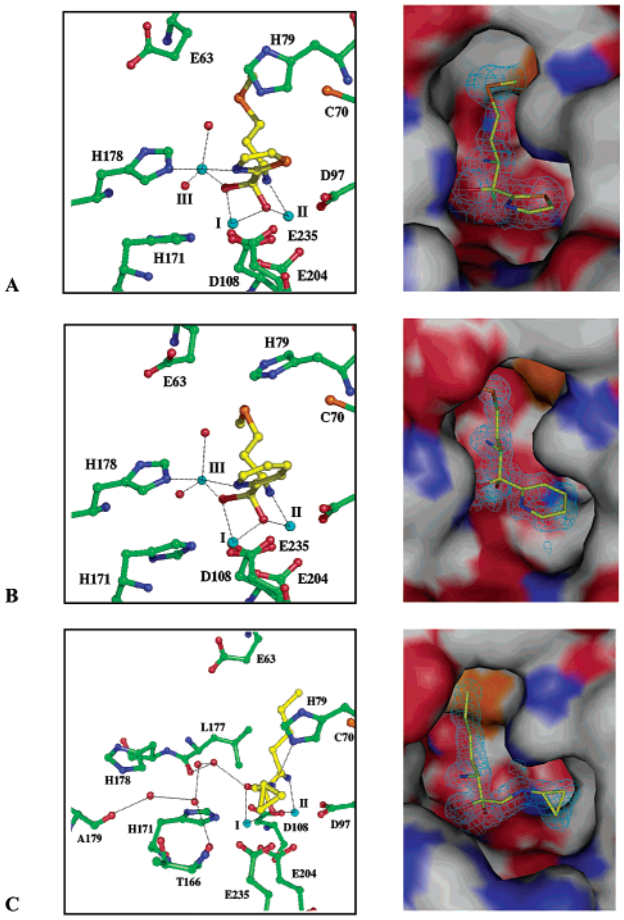

Figure 2. Inhibitor binding to the active site of *S. aureus* MetAP I. The left column shows compounds **1** (A), **2** (B), and **3** (C) in ball-and-stick form. Electrostatic interactions are indicated by dashed lines. The right column shows the molecular surface of the active site of the *S. aureus* enzyme superimposed with inhibitors and their final, experimentally determined $2F_o - F_c$ electron density contoured at 1.0σ. The figures were generated using PyMOL.[28]

methionine residue of the substrate, whose binding includes direct interactions with both metals.[11] A second, shallower binding pocket, located toward the protein surface on the other site of the central metal binding site, is composed of the residues A78, H79, and F206 and the β-sheet residues L168, T169, and G170 and forms the S1′ subsite of the enzyme. It is believed to accommodate the small, uncharged residue of the substrate at the penultimate position.[11]

All three inhibitors show a common mode of inhibition. The high-resolution structures of the binary complexes clearly indicate that the α-keto heterocycle and aminoketone inhibitors of *S. aureus* MetAP I bind as transition-state analogues. The sp^2 carbonyl carbon of all inhibitors undergoes a conversion to an sp^3 carbon in the tetrahedral intermediate upon binding (Figure 2). The transition-state analogues coordinate two cobalt metal ions with one of the geminal hydroxyl groups deriving from the activated solvent molecule, which bridges the two cobalt ions in the apo structure.[12] The second hydroxyl function of the gem-diol provides the sixth coordination to CoI. The common N-terminal amino group of the inhibitors coordinates CoII, whereas the methionyl side chain of the keto heterocycles and the alkyl moiety of the aminoketone are located in the hydrophobic S1 subsite. Spatial rearrangements occur in this subsite upon inhibitor binding. The side chain of E63 becomes ordered and adopts a conformation that enlarges the S1 pocket. This allows the methyl group of each compound to adopt different orientations corresponding to the possible rotamers (Figure 2). The heterocyclic ring systems and the cyclopropyl moiety form the P1′ residues. Compared to the thiazole and pyridine groups of compounds **1** and **2**, the cyclopropyl substituent is rotated by approximately 80°, orienting it toward the inner side of the S1′ pocket (Figure 2).

Surprisingly, in both binary α-ketoheterocycle complexes, a third cobalt ion (CoIII) is pentacoordinated in the active site (Figure 2A,B). The formation of the tetrahedral intermediate results in a favorable metal binding environment formed by the hydroxyl group of the gem-diol, the NE2 of H178, the nitrogen in the keto heterocycle ring moiety, and two solvent molecules and thus allows the trapping of a cobalt ion. In turn, this metal ion helps in the stabilization of the anion derived from the geminal diol, in a similar way as does the oxyanion hole in serine proteases. The position of the nitrogen atom in the heterocyclic thiazole and pyridine moiety plays a critical role in the activity of the ketoheterocycle inhibitors **1** and **2**. Displacement of the ring nitrogen to a site different from the β-position leads to a complete loss of activity (SAR data will be published elsewhere), which is in agreement with our crystallographic data. The importance of a nitrogen atom at the β-position in α-ketoheterocycles has also been observed for inhibitors of serine proteases where this atom plays a critical role in hydrogen-bond formation with the histidine residue of the catalytic triad.[24,25,31]

The aminoketone inhibitor **3** interacts with the enzyme in a way that the secondary amine is oriented toward H79 to provide a strong hydrogen bond. Moreover, H178 is flipped away from the metal ion center (Figure 2C). In this case, no third cobalt ion is observed. Instead, a network of water molecules connects the hydroxyl group of the gem-diol to the carbonyl group of L177 as well as to the carbonyl groups of T166 and A179 at the surface of the molecule with distances from 2.6 to 2.9 Å (Figure 2C). Similar stabilization of α-ketoamide transition-state analogues by a network of water molecules has been reported for thrombin inhibitors.[33] Histidines 178 and 79, which are involved in the key interactions with compounds **1**−**3**, are strictly conserved residues in all MetAPs sequenced to date. An H178A mutant of the *Ec*MetAP I enzyme has been shown to exhibit a 50-fold lower activity than the wild-type enzyme, supporting that H178 is not a catalytically required residue.[11] Recent kinetic and spectroscopic analyses of H178A *Ec*MetAP I suggests the implication of H178 in regulating the pK_a of the metal-bound nucleophile.[34] In contrast, the mutation of H79 to alanine leads to a nearly complete loss of activity.[11] This result, combined with the structural information provided by the X-ray structures of *Ec*MetAP I with phorphorous-based transition-state analogues,[13] supports the idea that H79 interacts with the nitrogen atom of the scissile bond. This interaction seems to be crucial for catalysis. Despite the loss of direct interactions with H178, the aminoketone inhibitor **3** has an IC_{50} similar to that of the ketoheterocycles **1** and **2**. The loss of the

interaction with H178 is sufficiently compensated by a strong hydrogen bond with the side chain of H79.

In our search for inhibitors of *Sa*MetAP I, we have identified two series of compounds containing a keto group, which are the keto heterocycle and the aminoketone inhibitors. Trifluoroketones, α-keto esters, and α-diketones, which are potent inhibitors of several proteases, were reported to hydrate in aqueous DMSO solution because of the electron-withdrawing effect of the group neighboring the ketone moiety.[19−23] Here, the ketone group of the inhibitors **1** and **2** is not hydrated in an aqueous DMSO solution[31] (data not shown); however, the compounds form an enzyme-catalyzed transition-state analogue, mimicking that for amide bond hydrolysis of substrates.

The activated water (or hydroxide ion) located between both metal ions attacks the keto group of the inhibitors, which is further transformed into a tetrahedral intermediate. Although isothermal titration calorimetry studies on *Ec*MetAP has indicated that the active site can accommodate three cobalt ions, one with high and two with low affinity,[34] the stabilization of the keto heterocycle transition-state analogues by a third metal ion is questionnable because our experimental conditions include 10 mM Co^{2+}, a concentration that certainly differs from the biological conditions.[16,32] This stabilization might as well occur through a network of solvent molecules as observed for the aminoketone inhibitor **3**.

Finally, the structural data provide the foundation for further optimization of these classes of inhibitors. For example, the inhibitors could be optimized by exploiting the S1 subsite, which can accommodate a larger substituent, as observed with the triazole series of inhibitors.[12] Moreover, the cyclopropyl group allows for extensions toward the S1′ subsite.

Acknowledgment. We gratefully acknowledge the excellent technical assistance of S. Haefeli and Daniel Mareque, and we thank Dr. C. Schulze-Briese at the SLS protein crystallography beamline X06SA, Paul Scherrer Institute, Villigen, Switzerland, for his experimental help and advice.

Supporting Information Available: Crystallographic details and synthesis of the discussed compounds and their spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM034188J