Matrix Metalloproteinases

X-ray Structures of Binary and Ternary Enzyme-Product-Inhibitor Complexes of Matrix Metalloproteinases**

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Matrix metalloproteinases (MMP) are a class of extracellular zinc-containing peptidases that are involved in a variety of tissue-remodeling activities, and whose misfunction/misregulation is implicated in several pathologies ranging from arthritis rheumatoides to metastatic processes.[1–5] Such proteins, which may be bound to the outer cell membrane or

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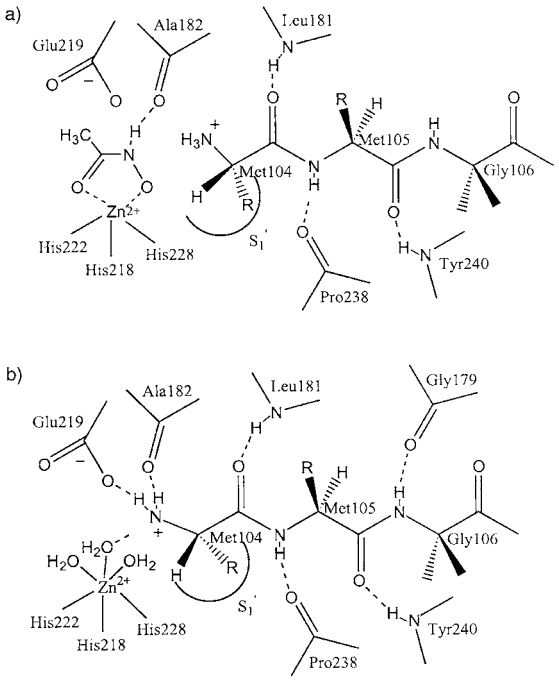
released into the matrix as soluble molecules, are composed of a prodomain, a catalytic domain, and a hemopexin-like (or substrate-recognition) domain.[3,6–8] The prodomain is folded back into the active-site crevice and acts as an inhibitor until it is cleaved by a protease (or another MMP) to produce the active enzyme form.[7,9,10] The catalytic mechanism of MMP has been proposed[11,12] to parallel that of thermolysin.[13,14] The peptide substrate is believed to enter the active-site crevice and interact with the catalytic zinc ion through the carbonyl group of the scissile peptide bond, while the peptide NH group is hydrogen bonded to the carbonyl carbon atom of Ala 182 (MMP-12 numbering). The zinc-bound water molecule is activated by hydrogen bonding to the catalytically essential Glu 219 residue. The coordinated water molecule then transfers the proton to Glu 219 while attacking the peptide carbonyl carbon atom, and the proton is finally transferred to the terminal nitrogen atom of the amino leaving group when the peptide bond is broken.[11,12] While structures mimicking transition-state analogues are available to support this mechanism,[15] none of the approximately 80 MMP structures available to date can be taken as representative of enzyme-product complexes after the hydrolytic event.

In the case of MMP-1 (fibroblast collagenase), a catalytic domain that was three residues longer in the N-terminal end showed a filamentous structure with the N-terminal residues 102–104 entering the active-site cavity of an adjacent molecule.[11] However, the NH_2 and CO groups of Leu 102 were found to chelate the catalytic zinc ion in an unproductive self-inhibitory adduct.[11] Metal chelation by the N-terminal residue is also typical of tissue inhibitors of metalloproteinases (TIMPs), proteins that act as physiological inhibitors of MMPs.[16–18]

We have expressed the F171D mutant of the catalytic domain of MMP-12 bearing two additional N-terminal methionine residues, Met 104–Met 105, before the catalytic Glu 106–Asn 258 domain. The F171D mutation has been designed to enhance solubility without altering the catalytic activity and the reaction mechanism.[19] We report here the structures of the above MMP-12 constructions obtained from crystals grown in the presence of acetohydroxamic acid (AHA), a weak zinc-directed inhibitor. In these structures, the N-terminal Met 104–Met 105 residues of one domain are hosted into the active cleft of the following domain, with an arrangement similar to that described above for MMP-1.[11] One of these structures is the first example of a non-inhibited zinc site, while in the other the small AHA ligand blocks the catalytic zinc site. In both cases, there is room for the amino terminal group to assume product-like arrangements.

Crystals of MMP-12 catalytic domains can be obtained under a relatively wide range of solute conditions, and the structures are solved to resolutions ranging from 2.15 to 1.5 Å. In all cases, the catalytic domains are chained to one another forming double helices whose arrangement will be described in detail elsewhere. The asymmetric unit of the $P3_1$ cell always comprises six different molecules, three from one chain and three from an adjacent chain. In all cases, the structure of the MMP-12 domain is almost identical to the recently published structures of strongly inhibited forms.[20,21] The six independent molecules are labeled A–F. Molecule B hosts the N-terminal methionine residues of A, C hosts those of B, and A those of C, crystallographically related to C through two successive 3_1 symmetry operations. Likewise, in the adjacent chain, E hosts the N terminals of D, F hosts those of E, and D hosts those of F′. As the six molecules in the unit cell are inequivalent, the relative orientations of the relevant species and fragments in the active-site crevice are not necessarily identical. However, it appears that the A-B-C chain is virtually identical to the D-E-F chain, while differences are seen within each chain, that is, $A \approx D \neq B \approx E \neq C \approx F$. In all cases, the N-terminal Met residue of an adjacent MMP molecule is positioned in such a way that the hydrophobic *S*-CH_3 terminus points towards the inner part of the main hydrophobic S_1' site (see Scheme 1 a,b) and is close to the



Scheme 1.

Tyr 240 ring. The Met 104 carbonyl group is hydrogen-bonded to the NH group of Leu 181, the NH group of Met 105 is hydrogen-bonded to the carbonyl group of Pro 238, and the carbonyl group of Met 105 is hydrogen-bonded to the NH group of Tyr 240. However, there are differences in the zinc ligand(s) and in the position of the N-terminal NH_3^+ group, which is the part of the molecule which is closest to the catalytic zinc ion. These different arrangements are best illustrated by molecules B and E of a crystal diffracting at 1.85 Å (**1**) and by molecules A and D of another crystal diffracting at 2.15 Å (**2**). The detailed crystallization conditions of **1** and **2** are reported in the Supporting Information.

In molecules A and D of **2**, an AHA molecule is bound to the zinc ion (Figure 1 a). AHA binds in a bidentate fashion, as is typical for MMPs, with the oxygen atom of the O-N-H moiety pointing towards the metal center, and with the hydrogen atom involved in a hydrogen bond with the

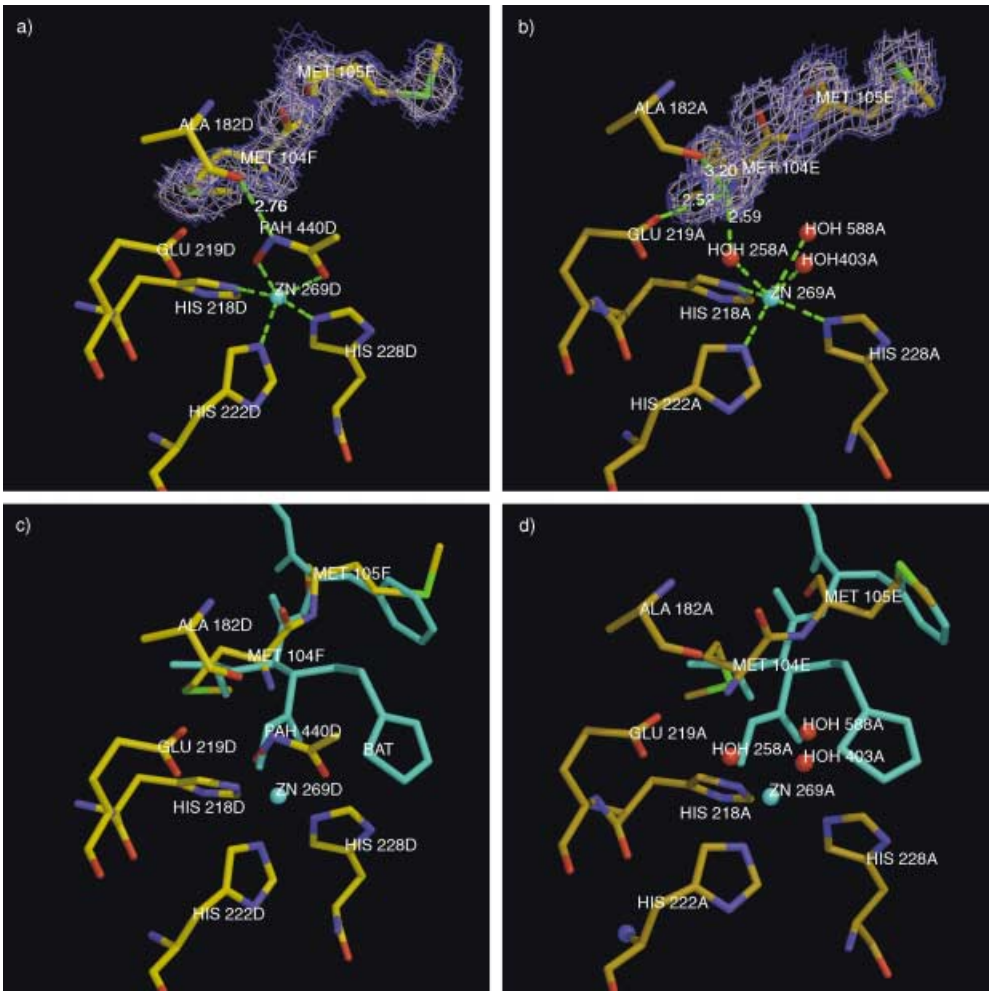


Figure 1. $2F_o–F_c$ density map (blue) contoured at 1σ and $F_o–F_c$ omit map (pink) contoured at 3σ around the Met 104, Met 105, and Gly 106 residues of molecules C′ in A (a) and molecules D in E (b), which shows two different arrangements of the N-terminal residues within the cavity. Panels c) and d) show the superposition of batimastat to the N-terminal residues in the arrangements described in a) and b), respectively.

backbone carbonyl group of Ala 182 (Figure 2a, Supporting Information). The CO moiety of AHA is also bound to the metal center, and oriented towards the His 222 ring. The structural features of the active site occupied by the N-terminal and AHA molecule are summarized in Scheme 1a.

Conversely, in molecules B and E of **1** there is no electron density from the solute ligand. The zinc ion appears to be coordinated by three water molecules (Figure 2b, Supporting Information) in a reasonably regular octahedral fashion (Figure 1b). The lack of the AHA ligand in this case allows the N-terminal NH_3^+ group to move by about 1 Å towards the metal center and to drastically change its hydrogen-bonding pattern. All three N-terminal hydrogen atoms are now engaged in hydrogen bonds; the first is formed with one of the metal-coordinated water molecules, another with the carbonyl group of Ala 182, and the third with the carboxylate group of the catalytically essential Glu 219 residue. Finally, a further hydrogen bond (besides those with the NH group of Leu 181, the carbonyl group of Pro 238 and the NH group of Tyr 240, also observed in molecules A and D of **2**) is formed between the NH group of Gly 106 and the carbonyl group of Gly 179. Overall, this arrangement of the N-terminal Met–Met residue (Scheme 1b) would correspond beautifully to the amine product after hydrolysis and before its release, in accordance with the widely accepted proposals for the mechanism of MMP and of other endopeptidases.[11–14] Recent calculations[22,23] confirm that the arrangement of the amine product observed here is the most energetically plausible one along the catalytic pathway.

These results are, therefore, the first examples of MMP structures demonstrating possible arrangements of enzyme–amine product complexes; the present findings are even more valuable when compared with the X-ray structure of the catalytic domain of MMP-1,[11] as well as with a variety of MMP complexes with strong inhibitors. Besides the unique and catalytically competent arrangement of the N-terminal NH_3^+ group of Met 104, all the other hydrogen-bonding interactions of the N-terminal residues 104–106 are also observed in the self-inhibited MMP-1 structure.[11] In short, and neglecting the zinc-bound Leu 102 residue in MMP-1, there is a perfect match between Thr 103, Glu 104, and Gly 105 in the MMP-1 cavity, and Met 104, Met 105, and Gly 106 in the MMP-12 cavity of molecules B and E of **1**. Four hydrogen-bonding interactions are maintained, and are

indeed observed in several complexes that the catalytic domain forms with TIMPs[24] and the prodomain.[25] These interactions are among the crucial interactions for the proper positioning of the substrate, and are apparently maintained in the amine product. In this light, the position of the N-terminal Met residue in molecules A and D of **2**, pushed away from its hydrogen-bonding interactions in the immediate neighborhood of the metal center by the bulkier metal-coordinated AHA molecule (Scheme 1a), could be representative of a further detachment step of the amine product. Interestingly, strong chemical inhibitors, such as batimastat,[21] match the arrangement of the N-terminal Met residue in molecules A and D of **2** better than that in molecules B and E of **1** (compare Figure 1c and 1d). Therefore, the binding modes observed here for N-terminal peptides suggest that improved S_1'-directed drugs could mimic the present Gly-Met-Met tripeptide by actually exploiting as many as seven of its eight hydrogen bonds, and be optimally positioned to carry a metal ligand. The latter could actually be attached to the terminal amino group, which could still maintain two of its three hydrogen bonds (with the CO group of Ala 182 and the carboxylate group of Glu 219) and replace the third hydrogen bond by a covalent link with the metal-binding moiety.

Experimental Section

The cDNA of the F171D mutant of the MMP12 catalytic domain was obtained using a "quick-change" site-directed mutagenesis kit. The protein was expressed as an inclusion body, purified by FPLC, and refolded, as has been widely reported for MMPs.[19,26–30]

Crystals of the catalytic domain of MMP-12 belonging to the trigonal space group $P3_1$ ($a = b = 125.44$, $c = 72.34$ Å for **1**; $a = b = 123.84$, $c = 69.73$ Å for **2**) were obtained from a solution containing PEG 6000 and 200 mM AHA in Tris-HCl buffer at pH 8.0. Structural details are available in the Supporting Information.

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