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# Making a New Turn In Matrix Metalloprotease Inhibition

**The paradigm for matrix metalloprotease inhibition combines active site tailoring and catalytic zinc ligation. But, selectivity has been difficult. Now, Engel et al. [1] present novel compounds, completely selective for MMP-13, with a unique binding mode.**

The enzymes that cleave collagen, gelatin, and other proteins of the extracellular matrix belong to the family of multidomain, zinc-dependent proteins known as matrix metalloproteases (MMPs) [2]. Although the number and nature of subunits can differ, each enzyme contains a homologous catalytic domain with the active site signature sequence HExxHxxGxxH. For 11 members of the family, the catalytic domain crystal structure has been determined.

Involved not only in normal tissue remodeling, but also in diseases such as cancer, arthritis, and periodontal disease, MMPs have become popular targets for drug design. But, to date, no inhibitor of an MMP catalytic domain has become a drug. One major problem seems to be selectivity; although preferred substrates for the various members of the family differ, in some cases substrate specificity has been shown to be dependent on domains other than the catalytic one [3]. Since the catalytic domain itself can thus be promiscuous, severe difficulties arise when designing selective small-molecule inhibitors.

With sequence identity of 50%–60%, MMP catalytic domain structures are highly similar. The basic topology consists of a 5-stranded, curved $\beta$ sheet and three $\alpha$ helices, all on the concave side of the sheet. The first two histidine residues of the signature sequence protrude from adjacent turns of the catalytic helix, which extends across the rear of the active site. The conserved glycine induces a turn, necessary to bring the third histidine in proximity to the other two; all three coordinate the catalytic zinc ion. A methionine, conserved in MMPs and also in members of structurally related families, forms a Met turn directly beneath the zinc, providing the name metzincin to the set of families.

MMPs are expressed with a propeptide of about 80 residues just N-terminal to the catalytic domain. Initially inactive because a prodomain cystine coordinates the zinc, activation occurs when the prodomain is removed by proteolytic cleavage. The mechanism of replacing the cystine with bound water has been denoted the "cystine switch."

The catalytic mechanism is considered to be the same as that of thermolysin [4], involving the zinc ion, the signature glutamic acid, and a zinc bound water molecule. Substrates bind in a groove extending across the face of the protein. Shallow on the unprimed side, it deepens to an extended pocket on the far side of the zinc at S1′; however, beyond S1′, it is again relatively flat. Effective inhibitors have achieved tight binding via extensive van der Waals contacts within the largely hydrophobic interior of S1′ and by strong electrostatic interactions with zinc.

But, compounds selective for a single MMP family member have been hard to achieve [5]. The active sites of MMPs are both homologous and structurally very similar, as is the entrance to S1′. Apart from MMP-1, -7, and -11 (in which pockets are occluded by arginine, tyrosine, or glutamine side chains, respectively), S1′s are long and straight, longer than any naturally occurring amino acid, and may even form a tunnel through the protein core (Figure 1). Although there are differences of both composition and length in the segment on the exterior wall of S1′, denoted the "specificity loop," that part of the protein has been shown to be quite flexible. The conformation of amino acid side chains can vary depending on the nature of the inhibitor with which the protein is crystallized [5, 6]. More dramatic structural changes were found in a complex of MMP-3 with an unprimed side inhibitor, where atoms of the backbone were shifted up to 4 Å from their usual positions, presumably because the pocket was unoccupied [7]. In a few MMP structures, several residues of the loop are disordered [1]. Evidently, the pockets can adapt to accommodate diverse ligands. While S3, S2, S1, S2′, and S3′ sites are not as similar, they are either shallow or solvent exposed, and opportunities for tight binding are limited.

The problem is compounded by the more recently discovered ADAM (a disintegrin and metalloprotease) family [8], also metzincins and of similar topology. These membrane bound enzymes act as sheddases, liberating membrane bound cytokines, cell surface receptors, and growth factors as physiologically active soluble proteins [9]. For some of them, substrates have not yet been identified. The catalytic sites of ADAM family members also manifest the MMP signature sequence HExxHxxGxxH, and small molecules that inhibit MMPs frequently also inhibit ADAM family members. It has even been suggested that musculoskeletal pain associated with broad-spectrum MMP inhibitors may be due to simultaneous inhibition of ADAM proteins [10]. This makes selectivity within and between the families even more important. As expected, the two known structures of ADAM proteins [11, 12] have active sites very similar to those of the MMPs. They are mutually alike, although there are some differences in the distal ends of the S1′ pockets. It will be interesting to evaluate similarity within this family when additional structures have been determined.

Since the first attempts at MMP inhibition, hydroxamic acid has been the preferred zinc-chelating group [5]. Its binding orientation allows linkage to a diverse family of scaffolds, each of which projects P1′ directly into S1′. The geometry is ideal because polar atoms interact simultaneously with zinc, the side chain of the catalytic Glu, and the backbone of a strand lying

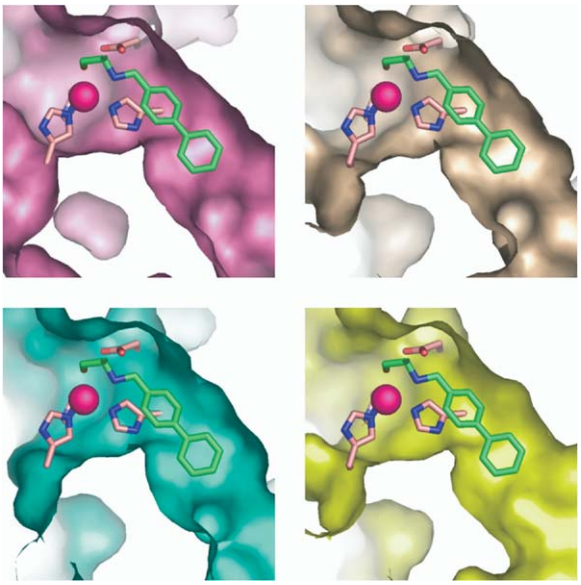

Figure 1. Cross-Sections through the S1′ Pockets of Four Superimposed MMP Crystal Structures

The inhibitor (green) was cocrystallized with MMP-3 but could also fit the other three. The proteins are stromelysin-1 (MMP-3, pink), gelatinase A (MMP-2, beige), collagenase-3 (MMP-13, yellow), and macrophage elastase (MMP-12, cyan).

just above the catalytic site. It has recently been proposed to bind in its neutral form, providing protons for the hydrogen bonds [13]. Carboxylic acid, on the other hand, supposedly binds in a charged form and also has a less favorable geometry; when used in inhibitors, it decreases the affinity 1–2 orders of magnitude. Hydroxamic acids, though, have been associated with metabolic instability [14].

Hydroxamate replacements such as thiols, phosphonates, thiadiazoles, thiadiazines, barbituric acids, and hydantoins have been investigated [15]. For the most part, these suffer from weaker affinity, reduced absorption rates, or toxicity; some are too recent for their potential to have been fully explored. The search for improved, novel zinc binding groups is a continuous one [16].

On page 181 of this issue, Engel et al. [1] present a series of MMP-13 inhibitors that overcome the issues of both selectivity and zinc chelation by binding deep within the S1′ pocket and an additional small region denoted the "S1′ side pocket." The concept is not new; there is probably no group working in the field that has not, at some time, considered the possibility of nonchelating compounds. There are even a few examples in the literature. A weak inhibitor of MMP-13 was found, by NMR, to bind within S1′ well beyond the zinc ion, but it was only after linkage to hydroxamic acid that reasonable affinity was achieved [17]. More recent are crystal structures of nonchelating inhibitors of MMP-12 [18] in a ternary complex with acetohydroxamate (AH), which had been added to improve solubility and is bound to zinc. The presence of AH also improved affinity dramatically, for reasons that are not completely understood.

In the current work [1], a crystal structure with the initial compound indicated its novel binding mode. At its midpoint, the molecule, which appears to be almost completely buried, makes a turn from S1′ into the S1′ side pocket. In this conformation, it embraces a leucyl side chain "like gripping pliers." Rational design then effected improvement to single-digit nanomolar affinity. No inhibition of any other MMP was detected. To our knowledge, these are the first selective, nonchelating inhibitors of high affinity reported.

The authors argue convincingly that complete MMP-13 specificity of these compounds is due to the length and amino acid composition of the inhibitor binding selectivity loop. The interesting question is whether MMP-13 is unique, as the authors suggest, or whether this is the first of a sequence of finely tuned MMP and ADAM inhibitors, each specific to a single enzyme. Developments in this field bear careful watching.

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