Matrix metalloproteinases: useful and deleterious

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Abstract

MMPs (matrix metalloproteinases) are zinc-dependent endopeptidases that degrade both matrix and non-matrix proteins. They play an important role in morphogenesis, and in a wide range of processes including tissue repair and remodelling. Their abnormal expression contributes to pathological processes including arthritis, cancer, and cardiac and central nervous system diseases, which explains the large interest in finding specific MMP inhibitors for therapeutic use. In this review we describe the structural features of MMPs, with special emphasis on their interaction with specific inhibitors. The effect of new, hydroxamate-based inhibitors on MMP isolated from bovine brain is evaluated.

Introduction

MMPs (matrix metalloproteinases) are zinc-dependent endopeptidases that play a key role in the degradation of ECM (extracellular matrix). They are extracellular proteins, but recently it has been reported the presence of some MMPs also intracellularly [1]; in addition, a number of non-matrix proteins have been identified as possible MMP substrates [2]. Therefore it has been accepted that they have many other functions besides ECM degradation, and they are associated with a variety of normal and pathological conditions. MMPs play a role in intercellular communication, and can influence cell migration and tumour progression. Thus studies on the newest member of the MMP family, epilysin (MMP-28), demonstrated a role for this enzyme in the regulation of epithelial cell function and in the induction of carcinogenesis [3]. According to their structure and substrate specificities (although the true physiological substrates are not entirely known yet), the MMP family members are classified into subgroups, such as: collagenases, gelatinases, stromelysins, matrilysins, MT-MMPs (membrane-type MMPs) and ‘others’, including seven MMPs not classified in the five mentioned subgroups [4].

Structural aspects of MMPS

From a structural point of view, most MMPs are organized into basic, well-conserved domains: an N-terminal signal sequence (‘pre’ domain), followed by an N-terminal propeptide, a catalytic domain and a C-terminal Hpx (haemopexin-like) domain [1,5,6].

The N-terminal propeptide contains a conserved cysteine, in the ‘cysteine switch’ motif PRCGXP, which chelates the catalytic Zn$^{2+}$ ion, keeping proMMP inactive. All the MMPs are synthesized as zymogens, and the propeptide has to be removed during the activation, either by other MMPs or other proteases.

The catalytic domain contains the zinc-binding motif HEXXHXXGXXH, where three histidine residues coordinate a zinc ion, and also a conserved methionine residue, forming a ‘Met-turn’, which contributes to protect the catalytic zinc [1]. Unlike other MMPs, MMP-2 and MMP-9 contain fibronectin type II domains inserted in the middle of the catalytic domain, which may enhance the substrate binding.

The Hpx domain, present in all MMPs, but MMP7 and MMP26, is a regulatory subunit, supposed to control MMPs substrate specificity; a variable hinge region separates this domain from the catalytic domain. The hinge region also contributes to the specificity of MMPs, either by direct binding of the substrate, or by influencing the orientation of haemopexin and the catalytic domain [7].

This basic structure evolved into different subgroups by the addition or deletion of structural or functional domains. For example, incorporation of a hydrophobic peptide of approx. 25 amino acids at the C-terminal end of the propeptide introduces a furin cleavage site, which is a characteristic of MT-MMPs, except that MMP-14, -15, -16 and -24 have transmembrane and cytosolic domains, whereas MMP-17 and -25 have C-terminal hydrophobic extensions that act as a glycosylphosphatidylinositol-anchoring signal [6].

The three-dimensional structure of a number of MMPs has been determined by X-ray crystallography and NMR; although the domains’ primary structure showed little homology, the polypeptide folds of MMP catalytic domains are almost superimposable. The catalytic domain consists of 5-stranded $\beta$-pleated sheets, three $\alpha$-helices and connecting loops, two zinc ions (structural and catalytic) and three calcium ions which stabilize the structure. The substrate-binding site includes the hydrophobic 'S1 pocket', variable in depth, contributing to MMPs substrate specificity [1]. The propeptide domain consists of three $\alpha$-chains and connecting loops; the ‘cysteine switch’ lies in the substrate-binding pocket, the cysteine sulfhydryl group interacting with the catalytic zinc ion. In the case of crystallized recombinant
human proMMP-1 it has been found that the prodomain interacts with the Hpx domain, resulting in a ‘closed’ configuration, in contrast with the ‘open’ configuration of the active MMP-1, which suggests the mechanism of collagen–collagenase interaction [8].

**MMPs, friends and enemies**

MMPs are proteolytic enzymes that play a key role in the degradation of ECM and basement membrane, and are implicated in both physiological and pathological processes.

They are very important for morphogenesis, wound healing, tissue repair and remodelling in response to injury, e.g. after myocardial infarction, but also in progression of diseases such as rheumatoid arthritis, and in tumour growth, invasion and metastasis. MMPs can enhance leucocyte invasion and regulate the inflammatory process, but enzymes such as MMP-2 and -9 can favour either anti- or pro-inflammatory action [9].

MMPs are associated with the blood–brain barrier opening after brain injury, oedema formation and the demyelinating process, and are generally considered detrimental to the CNS (central nervous system). However, they may play an important role in neural plasticity, including remodelling of synaptic connections. Moreover, it has been demonstrated recently that at a certain disease phase they may have an important regenerative role [10].

**MMPIs (MMP inhibitors)**

**Endogenous inhibitors**

The *in vivo* activity of MMPs is controlled both by activation of the latent pro-enzymes, and by inhibition induced by endogenous inhibitors, such as α₂-macroglobulin or other protease inhibitors, and by specific TIMPs (tissue inhibitors of metalloproteinases); currently four TIMPs are known in humans [1]. Unbalanced activities of MMPs and TIMPs are associated with pathological conditions. Based on the multitude of data resulting from the studies on structures and functions of MMPs as well as of their inhibitors, a great number of inhibitors have been designed and synthesized, and some of them were clinically tested. Unfortunately, the therapeutic use of TIMPs is limited; they are proteins of 21–29 kDa, and besides their inhibitory activity they possess other biological capacities as well [11]. As a consequence, the therapeutic application of TIMPs in cancer or cardiovascular disease through gene therapy or directly, as a protein, is still in an early phase.

**Synthetic inhibitors**

There is an increasing interest nowadays in designing and synthesizing MMPIs. Most MMPIs have been identified by a structure-based approach of inhibitor design, namely the amino acid sequence of the cleavage site in a collagen molecule; the presence of a ZBG (zinc-binding group) in the MMP molecule is essential for chelating the catalytic Zn(II) ion. Peptide or peptide-like compounds, most of them containing hydroxamic acid derivatives as zinc chelators [12], as well as other classes of compounds, such as carboxylate, organoborate and dithiolate, have been identified as MMPIs [13]. Hydroxamic acid (R-CO-NH-OH) compounds proved to be stronger inhibitors than corresponding carboxylates (R-CO-OH), although many carboxylates are effective inhibitors. In a group of MMP-1 identical inhibitors, differing only by ZBG, the inhibitory activity decreased from hydroxamate to carboxylate, with formyl hydroxylamine, thiol, phosphate, aminocarboxylate as intermediate [14]. Binding of a hydroxamate group to MMP involves the coordination of the two hydroxamate oxygens to the catalytic zinc ion and stable hydrogen bonds between hydroxamate nitrogen and the carbonyl of the enzyme backbone (Figure 1); van der Waals interactions and hydrophobic contacts stabilize the inhibitor–enzyme complex.

N-Sulfonyl amino acid hydroxamates have been identified as MMPIs and some of them are orally available, broad-spectrum inhibitors. Recently identified MMPIs are aryl hydroxamates and the corresponding heterocyclic analogues. Unfortunately, the progress of these compounds through the clinical trial is uncertain, because of their mutagenic properties. Another promising group of inhibitors are tetracycline derivatives, which not only inhibit MMPs, but also decrease their production, inhibit activation and increase their degradation.
In spite of the great number of MMPIs synthesized and pursued as clinical candidates especially for cancer, arthritis and cardiovascular disease, most of them have been discontinued (82%), and from the seven compounds remaining in trial, only one has been approved (Periostat®). The lack of success could be explained by their low specificity, high toxicity, or the stage of the disease. The MMPs’ ability to adapt the binding pocket to the inhibitor shape [13], besides their intrinsic similarity, could contribute to the lack of specificity.

This is why the identification of new inhibitory compounds is necessary. Our own research programme led to discovery of heterocyclic hydroxamate-based compounds, which are benzimidazole derivatives substituted at CS with various groups, as broad-spectrum MMPIs. These compounds, denoted C1, C2 and C3, had an inhibitory effect on all MMP fractions isolated from bovine brain (Figure 2), the amount of inhibition decreasing in the following order: C1 > C2 > C3. The selective inhibition by C1 of MMP-2, the enzyme implicated in CNS pathology [15], suggests the possible therapeutic application of this compound. Future studies should prove these findings by testing the inhibitor effect on both extracellular and cytoplasmic zinc proteinases.

**Conclusions**

Considerable progress has been made in understanding the structure and function of MMPs, but the biological role of some members of this family is not clear yet.

The role of MMPs in pathology has been extensively studied and these enzymes are generally found to be detrimental to the CNS. However, their ‘dual’ nature should be carefully considered, due to some of their positive effects, such as degradation of amyloid fibrils by MMP-9.

The development of synthetic inhibitors for therapeutic use should continue, in spite of the lack of success so far, provided very complex preclinical studies are carried out before the clinical trial.

**References**


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