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Hydroxamate-based peptide inhibitors of matrix metalloprotease 2

Márton Jani, Hedvig Tordai, Mária Trexler, László Bányai, László Patthy *

Institute of Enzymology, Biological Research Center, Hungarian Academy of Sciences, Budapest, P.O. Box 7, 1518, Hungary

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Abstract

There is major interest in designing inhibitors for matrix metalloproteinase 2 (MMP-2, gelatinase A) since this enzyme is known to be involved in pathological processes such as tumor invasion or rheumatoid arthritis. The majority of MMP-2 inhibitor candidate drugs block the active site of MMP-2 by binding to its catalytic Zn^{2+} ion through a chelating (hydroxamate, sulphonate etc.) group. Despite the general interest in designing MMP-2 inhibitors, the results with many of the drug candidates were disappointing, their failure was usually explained by cross-reactions with other MMPs. One way to enhance MMP-2 selectivity is to design inhibitors that interact with both the active site and exosites such as the fibronectin type II (FN2) domains of the enzyme. In the present work, we have examined the inhibitory potential and MMP-2 selectivity of hydroxamates of three groups of peptides known to bind to the collagen-binding FN2 domains of MMP-2. The first type of peptides consisted of collagen-like $(Pro\text{-}Pro\text{-}Gly)_n$ repeats, peptides of the second group were identified from a random 15-mer phage display library based on their binding to immobilized FN2 domains of MMP-2. A hydroxamate of peptide p33–42, known to bind to the third FN2 domain of MMP-2 has also been tested. Our studies have shown that these compounds inhibited MMP-2 with IC_{50} values of 10–100 μM. The fact that their inhibitory potential was nearly identical for MMP-2del, a recombinant version of MMP-2 that lacks the FN2 domains, suggests that inhibition is not mediated by their binding to FN2 domains. It seems likely that the failure to exploit interaction with the FN2 domains is due to the fact that the FN2 domains and the catalytic domain of MMP-2 tumble independently, therefore only a tiny fraction of the conformational isomers can bind peptide hydroxamates via both the active site and the FN2 domain(s).

Keywords: Matrix metalloproteinases; Gelatinase A; Tumor metastasis; Drug design

1. Introduction

1.1. Matrix metalloproteinases as drug targets

Matrix metalloproteinases (MMPs) are zinc-endopeptidases that mediate the breakdown of various constituents of the extracellular matrix. The activity of these enzymes is closely controlled through expression as proenzymes that require activation for activity, and also through direct inhibition of the enzymes by tissue inhibitors of metalloproteinases [1].

MMPs were shown to participate in a variety of physiological and pathological processes requiring tissue remodeling: they play important roles in embryonic development, wound healing, inflammation, rheumatoid arthritis, osteoarthritis, tumor invasion and metastasis, multiple sclerosis and Alzheimer's disease. Increased plasma/serum levels of different MMPs were observed in cancer, rheumatoid arthritis, osteoarthritis, systemic lupus erythematosus, multiple scle-

Abbreviations: Abu, L-α-aminobutyryl; Abz, 2-aminobenzoyl; APMA, *p*-aminophenylmercuric acetate; Cha, β-cyclohexylalanyl; DMC, 7-dimethylaminocoumarin-4-yl; DMSO, Dimethyl sulfoxide; DNP, 2,4-dinitrophenyl; Dpa, *N*-3-(2,4-dinitrophenyl)- L-2,3-diaminopropionyl; DTE, 1,4-dithioerythriol; EDTA, ethylenediaminetetraacetic acid; Fmoc, 9-fluorenylmethoxycarbonyl; HEPES, *N*-(2-hydroxyethyl) piperazine-*N*′-(2-ethanesulfonic acid); MCA, (7-methoxycoumarin-4-yl)-acetyl; MMP, Matrix metalloproteinase; MMP-2del, The proenzyme form of MMP-2 lacking both the FN2 domains and the pexin domain; Nva, L-norvaline; PCR, Polymerase chain reaction; p33–42, Peptide PIIKFPGDVA corresponding to residues 33–42 of the pro-domain of MMP-2; SDS-PAGE, Sodium dodecyl sulfate polyacrylamide gelelectrophoresis; THS, Peptide THSHQWRHHQFPAPT; WHW, Peptide WHWRHRIPLQLAAGR.

* Corresponding author. Institute of Enzymology, Biological Research Center, Hungarian Academy of Sciences, Karolina út 29, H-1113, Budapest, P.O. Box 7, H-1518, Hungary. Tel.: +361-2093-537; fax: +361-4665-465.

E-mail address: patthy@enzim.hu (L. Patthy).

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rosis, polycystic kidney disease, abdominal aortic aneurism, unstable angina and acute myocardial infarction [2]. Variants of human MMP genes have been shown to be associated with susceptibility and/or progression of atherosclerosis, aneurysms and cancer [3,4].

Matrix metalloproteinases are secreted or membrane-associated enzymes, the membrane-type (MT)-MMPs being anchored to the cell membrane by a C-terminal transmembrane domain or a glycosylphosphatidylinositol-anchor. Most members of the matrix metalloproteinase family contain an amino-terminal propeptide, the catalytic domain and (except MMP-7, MMP-23 and MMP-26) a hemopexin-like domain at the C-terminal end [5]. MMPs are produced as inactive proenzymes that are activated by matrix metalloproteinases or serine proteinases, which remove the propeptide region.

In view of their involvement in various diseases, inhibition of specific MMPs may prove to be clinically effective in halting the advance of these diseases. In the last decades numerous MMP inhibitors were tested in different clinical trials, however, they often displayed serious side effects and only a few molecules allowed to attend phase III clinical trials [6].

Some of the serious side effects may be inevitable in as much as the targeted MMPs implicated in the disease process are also essential for various physiological functions. Another source of the observed side-effects could be that the MMP inhibitors tested inhibited a rather broad spectrum of MMPs, not only the one(s) involved in the disease process. The latter type of side effects could be eliminated with the development of inhibitors that are more specific for the MMP(s) involved in the disease.

The rather broad specificity of many active site inhibitors of MMPs is most probably due to the fact that the active site geometry of the catalytic domains of different matrix metalloproteinases is very similar, although they differ quite significantly in their overall domain architectures. Accordingly, specificity of an inhibitor may be improved if it interacts both with the active site and with exosites unique to individual MMPs. In the present work, we wished to explore this possibility in the case of MMP-2.

1.2. Matrix metalloproteinase 2 and 9 as drug targets

MMP-2 and MMP-9 are unique among matrix metalloproteinases in as much as they are the only ones, which contain type II fibronectin-like domains or FN2 domains, inserted into their catalytic domains [7,8].

The FN2 domains endow MMP-2 and MMP-9 with high affinity for various constituents of the extracellular matrix, such as type IV and type V collagen, all types of denatured collagens and elastin [9,10]. Recombinant proteins corresponding to the fibronectin-related regions of gelatinase A and B were found to have high affinity for gelatin [9,11,12]. Recombinant MMP-2 lacking the FN2 domains was shown to be devoid of affinity for gelatin or type I and type IV collagens, indicating that the fibronectin-like domain is the sole site of collagen-binding [13,14].

It has been shown that the FN2 domains of MMP-9 are required for the type V and XI collagenolytic and gelatinolytic activity; however, MMP-9 lacking these modules retained its ability to cleave small synthetic peptide substrates [15]. The crucial importance of FN2 domains is further illustrated by the fact that the addition of the fibronectin-like domain of MMP-9 to fibroblast collagenase is sufficient to endow the enzyme with the ability to cleave type V collagen [16].

The functional significance of the gelatin-binding site is suggested by the observation that although deletion of this domain does not affect the catalytic properties of MMP-2 on small synthetic substrates [17], activity on gelatin is drastically reduced and the cleavage pattern of type IV collagen is altered [13,17]. On the basis of these observations, it has been proposed that the fibronectin-like domain of MMP-2 specifically orientates the enzyme on type I gelatin or type IV collagen thus enhancing the rate of substrate-cleavage [13].

Since MMP-2 and MMP-9 (previously referred to as ‘type IV collagenases’) are capable of degrading type IV collagen (a major constituent of basement membranes which has to be penetrated during migration of tumor cells) they have long been thought to be essential for metastasis. Significantly, secretion of MMP-2 and MMP-9 is well correlated with metastasis and transformation [18–20]. The importance of MMP-2 and MMP-9 in tumor metastasis is strongly supported by the fact that mice lacking MMP-2 or MMP-9 display markedly reduced rates of angiogenesis, experimental tumor growth and metastasis after intradermal implantation of cancer cells [21,22]. Targeted gene disruption of MMP-2 and/or MMP-9 suppresses development of experimental abdominal aortic aneurysms [23,24]. Thus, MMP-2 and MMP-9 represent key proteinases in a variety of processes of biological and clinical relevance.

1.3. Structure and function of FN2 domains of MMP-2

To clarify the role of the FN2 domains of MMP-2 we have initiated structure–function studies on these domains to define their structure and their interaction with substrates. First, we have shown that although each of the three FN2 domains bind gelatin, recombinant proteins containing all three tandem FN2 domains of MMP-2 have significantly higher affinity than any of the individual units [12]. Second, we have localized the gelatin-binding site on an FN2 domain of MMP-2 by site-directed mutagenesis [25]. Third, we have determined the NMR solution structure of all three FN2 domains and their binding sites for synthetic collagen-like peptides with consensus sequence $(Pro\text{-}Pro\text{-}Gly)_n$ were also identified. These studies have revealed that the gelatin-binding sites of the three FN2 modules fall at similar locations within the FN2 domains [26–28].

Based on our findings we have proposed a model for active MMP-2 in which the three tandem FN2 domains are