

## MATRIX METALLOPROTEINASES AND THEIR FUNCTION IN MYOCARDIUM

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A significant number of myocardial diseases are accompanied by increased synthesis and degradation of the extracellular matrix (ECM) as well as by changed maturation and incorporation of ECM components. Important groups of enzymes responsible for both normal and pathological processes in ECM remodeling are matrix metalloproteinases (MMPs). These enzymes share a relatively conserved structure with a number of identifiable modules linked to their specific functions. The most important function of MMPs is the ability to cleave various ECM components; including such rigid molecules as fibrillar collagen molecules. The amount and activity of MMPs in cardiac tissue are regulated by a range of activating and inhibiting processes. Although MMPs play multifarious roles in many myocardial diseases, here we have focused on their function in ischemic cardiac tissue, dilated cardiomyopathy and hypertrophied cardiac tissue. The inhibition of MMPs by means of synthetic inhibitors seems to be a promising strategy in cardiac disease treatment. Their effects on diseased cardiac tissue have been successfully tested in several experimental studies.

### INTRODUCTION

Matrix metalloproteinases or matrixins (MMPs) represent a family of structurally related enzymes that belong to the large group of zinc-dependent metalloproteinases, sometimes called metzincins. Metzincins can be divided into 4 groups according to their mode of zinc binding – astacins, reprolysins (ADAMs enzymes), serralyins and matrixins. MMPs have been found in both vertebrates, and invertebrates, and even in plants. Evolutionarily, however, they developed from far lower organisms as protein sequence homology shows that amino acid sequence for metalloproteinase toxin-2 of *Bacteroides fragilis* has 59% homology with 27 amino acid chain of human interstitial collagenase (MMP-1)(ref.<sup>1</sup>). These enzymes not only take part in biological processes, such as degradation of connective tissue, and ontogenesis (morphogenesis, angiogenesis, growth), but also processes associated with wound healing<sup>2-4</sup>. Changes in MMP expression and activity can be observed in most inflammatory, degenerative, and especially malign processes associated with increased synthesis, degradation or impaired maturation and organization of extracellular matrix (ECM)(ref.<sup>5-8</sup>). There are also other enzymes, such as cystyl- and seryl-proteases group and other metalloproteinases participating in ECM degradation. 24 human matrix metalloproteinases were known at the time of preparing this review (abbreviated MMP plus an Arabic numeral). The overview of MMPs is listed in Table 1.

### STRUCTURE OF MATRIX METALLOPROTEINASES

Matrix Metalloproteinases are homologous proteins which can be divided into 6 groups according to their common features: collagenases, stromelysins, matrilysins, gelatinases, membrane-type metalloproteinases (MT-MMPs) and other MMPs, namely zinc- and calcium-dependent endopeptidases. While the classification to 6 basic groups is probably the most known among sciences, they have been published other classifications e.g. *a) classification based on solubility*: soluble MMPs, membrane-associated MMPs and CA (cystein array) MMP; *b) classification into 9 subgroups* on the basis of their structures and substrate specificities: collagenases, gelatinases, stromelysins, matrilysins, furin-activated secreted MMPs, transmembrane-type MMPs, GPI (glycophosphatidyl inositol)-anchored MT-MMPs, type II transmembrane MMPs and other MMPs<sup>9-11</sup>.

MMPs are synthesized in the form of proenzymes. They are usually secreted from the cell as inactive proenzymes (beside the membrane MMPs). A proenzyme molecule is organized into the 3 basic structural domains: N-terminal propeptide, catalytic domain, and the C-terminal part of the molecule (Fig. 1)(ref.<sup>1</sup>). *N-terminal propeptide* consists of approximately 80–90 amino acids containing cysteine residue which interacts with catalytic zinc *via* its side chain thiol group (so called cysteine switch), and this ensures the enzymatic latency of the proenzyme. Elimination of a highly conserved sequence (...PRCGXPD...) presented in the propeptide causes activation of zymogene<sup>12,13</sup>. The *catalytic domain* (approximately 170 amino acids) consists of the zinc-

binding motive HEXXHXXGXXH and anchored methionine, which forms a unique structure, called Met-turn. Incidentally, this very structure is typical for the other metzincins to which MMPs belong<sup>14</sup>. This domain consists of five-stranded  $\beta$ -sheet, three  $\alpha$ -helices, and bridging loops. The catalytic domain consists of two zinc ( $\text{II}^+$ ) ions and 2–3  $\text{Ca}^{2+}$  ions. One  $\text{Zn}^{2+}$  appears in the active site and participates directly in the catalytic processes, the second  $\text{Zn}^{2+}$  (structural) together with  $\text{Ca}^{2+}$  are approximately 12 Å distant from  $\text{Zn}^{2+}$  in the catalytic site<sup>15</sup>.

The C-terminal domain shows structural similarity to proteins of the hemopexin family, including e.g. hemopexin, vitronectin or placental protein II (it has about 210 amino acid residues). An ellipsoidal disk shape with a four bladed  $\beta$ -propeller structure is typical for the C-terminal domain structure. Each blade consists of 4 antiparallel  $\beta$ -strands and an  $\alpha$ -helix. The first and second blades are connected by a disulfide bond. The catalytic and C-terminal domains, as shown in collagenase-1, are covered as entities in crystal with a connecting flexible peptide linker or a hinge region, which is freely bound<sup>16</sup>. The length of this hinge is extremely variable: 16 residues in collagenases or by contrast, 65 residues in MMP-15. The function of the linker, which contains a lot of prolines and connects catalytic and hemopexin-like domains, is not completely known.

The hemopexin-like domain (Fig. 1), essential to collagenases, breaks down the interstitial collagen triplehelix, allowing the catalytic domain to manifest its

proteolytic activity on other substrates<sup>17</sup>. By contrast, matrilysins do not have this domain. There is an additional transmembrane domain in the membrane-type matrix metalloproteinases (MT-MMPs) that binds (anchors) the enzyme to the cell surface. This domain consists of a hydrophobic chain containing approximately 25 amino acids with a recognizing motive RXXR for the furin-like convertases at the end of the propeptide chain (with the exception of MT4-MMP and MT6-MMP). MT4-MMP and MT6-MMP are tethered to the cell surface *via* a glycosylphosphatidylinositol (GPI) membrane anchor<sup>1, 18–20</sup>. Gelatinases consist of a domain having structural similarity to the matrix proteins. Three tandems of replicas of domain (58 amino acids) with the sequence fibronectin type II – like module of collagen bonding domain are found in all gelatinases, while a collagen type V – like module occurs only in longer gelatinases<sup>21</sup>.

On the other hand, matrilysins (MMP-7, MMP-26) have no hemopexin-like domain, making them the smallest MMP molecules. MMP-23 contains, in addition to the structure of matrilysins, a hydrophobic signal N-terminal anchor and cystein array<sup>22, 23</sup>. MMP-19, MMP-20, MMP-27 and MMP-12 can be classified as a new MMP group because it has no of the significant structural features of existing groups<sup>24–27</sup>. Some other MMPs contain a recognizing motive for the protein convertase RXXRKR, e.g. MT-MMPs and stromelysine-3 in their molecules<sup>28</sup>.

## MECHANISM OF EFFECT AND SUBSTRATE SPECIFICITY OF MMPs

Most of MMPs are able to degrade both majority and minority components of extracellular matrix; therefore, a lot of MMPs have wide substrate specificity. MMP-11 and MMP-23, whose ability to cleave the components of ECM is very low, are remarkable exceptions. This, however, does not exclude their ability to cleave other molecules (e.g. MMP-11 cleaves serpin)<sup>29, 30</sup>. MMPs also cleave or activate molecules which are not components of ECM (e.g. MMP-2, 3, 7 cleave decorin that is an important reservoir of  $\text{TGF-}\beta_1$ ). Cleavage of decorin initiates a release of the growth factor into the tissue which activates processes which are not necessarily directly connected with the extracellular matrix<sup>31</sup>.

### Collagenases

Collagenases, compared to other MMPs, break down the native helix of collagen without previous denaturation of the molecule at neutral pH and include MMP-1, -8, -13 (see Tab. 1). They break down the  $\alpha$  chain of the interstitial collagen (types I, II, III) into 2 specific parts, 1/4 and 3/4 fragments, in the site of the molecule that has a relaxed triplehelix and which contains a more hydrophobic group. There is a sequence Gly<sup>775</sup>/Ile<sup>776</sup> in the  $\alpha_1$  chain of collagen type I, and Gly<sup>775</sup>/Leu<sup>776</sup> in  $\alpha_2$  chain of collagen type I<sup>32, 33</sup>. Enzymes of this group markedly differ in their substrate specificity, e.g. neutrofil collagenase (MMP-8)

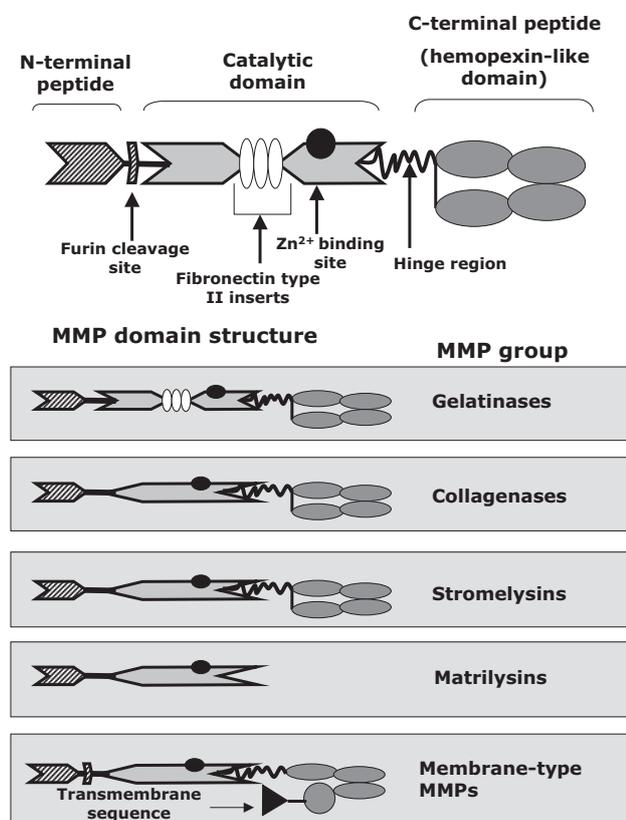


Fig. 1. MMP Domain Structure

prefers type I, while interstitial collagenase (MMP-1) collagen, type III. MMP-3 binds collagen type I, but it does not break down its triplehelix<sup>34-36</sup>. MMP-13 breaks down collagen type I and III, but slower than type II<sup>37</sup>.

Collagenases exhibit 2 different activities – *catalytic and substrate binding*<sup>37</sup>. De Souza and others suggest that the interstitial collagenases „hinge-domain“, rich in proline, simulates the collagen-like conformation<sup>38</sup>. With this domain, the enzyme is able to destroy the quaternary structure of the collagen triplehelix. Both similar structures of collagen and collagenase interact together forming a so called proline zipper. This destabilizing step leads to the following cleavage. The catalytic domain then breaks down only one fiber of collagen triplehelix in the relaxed structure. After this cleavage other MMPs can also participate in the following cleavage. The hemopexin-like domain also plays a certain role in stabilizing indirectly the procollagen-like domain of collagenase. Another possibility is that this domain ensures a nonfunctional bond of the substrate and then the “hinge-domain” ensures correct orientation of the substrate before its cleavage<sup>39</sup>. *Membrane-type MMPs* manifest approximately the same substrate specificity as analogous free MMPs. Collagen is not only the substrate for MT-MMPs. They also differ in activity (e.g. MMP-14 is 5-7 times less effective in cleavage of type I hydrolyzed collagen than analogous MMP-1 but in contrast manifests an 8 times higher gelatinolytic activity than MMP-1<sup>40</sup>). MT4-MMP breaks down gelatin and synthetic substrates but it is intact to collagen type I and IV, fibronectin or laminin<sup>41</sup>. Others are able to break down proMMPs and thus activate them (in detail see the MMPs activation and inhibition).

#### *Gelatinases*

This category includes MMP-2 and MMP-9 (see Table 1). In particular *gelatinases* break down denatured collagen, type IV intact collagen of basal membranes and also non-denatured collagens type V, VII, X, XIV, fibronectin, aggrecan, and elastin. MMP-2 is even considered to break down type I native collagen<sup>34</sup>. Further studies showed that it only binds intact collagen to prevent its autolytic inactivation<sup>42</sup>. Besides gelatin and other forms of denatured collagen, MMP-9 breaks down a whole range of other physiological substrates<sup>43,44</sup>. Although the cleaved sequences are not yet fully identified, the main one which was cleaved by a majority of MMPs (not only MMP-9), contains a Pro-X-X-Hy-(Ser/Thr) motive where X is any amino acid residue and Hy is hydrophobic amino acid residue in position P<sub>3</sub> to P<sub>2</sub>. Another group contained Gly-Leu-(Lys/Arg) motive in a position between P<sub>1</sub> and P<sub>2</sub>. The last group of substrates contained arginine residues both in P<sub>1</sub> and P<sub>2</sub> and these substrates were explicitly preferred by MMP-9(ref.<sup>45</sup>).

#### *Stromelysins*

The substrate specificity of stromelysins is relatively wide. They break down all non-collagen ECM proteins (proteoglycans, glycoproteins, fibronectin, and laminin). Type IV collagen is cleaved by stromelysins only in globu-

lar but not in helical conformation. Stromelysins include MMP-3, MMP-10, MMP-11 (see Table 1).

#### *Macrophagous elastase and other MMPs*

This category includes MMPs with the ability to break down other molecules. Macrophagous elastase (MMP-12) shares its ability to break down elastin with only a few MMPs (gelatinases and matrilysins) and is also able to break down fibronectin, laminin, basal membrane collagen, entactin, chondroitin sulfate and others<sup>46</sup>. This enables macrophages to penetrate basal membranes and thus subsequently remodel the tissue affected by inflammation. However, basal membranes are also degraded by MMP-19<sup>27</sup>. Components of enamel (mainly majority amelogenin) are specifically degraded by MMP-20(ref.<sup>47</sup>). Many MMPs (MMP-3, MMP-13 and MMP-8, MT1-MMP) break down *in vitro* IGD aggrecan bond in the position Asn<sup>341</sup>-Phe<sup>342</sup> (ref.<sup>15</sup>).

## MATRIX METALLOPROTEINASES ACTIVATION AND INHIBITION

Regulation of MMPs activity is a complex process. It includes various levels of activation of latent MMPs, inhibition, secretion of the enzyme molecule and the regulation of gene transcription or inhibition of MMPs activity by tissue inhibitors of metalloproteinases (TIMPs). The selective expression of genes for tissue-specific MMPs and their inducibility by biologically active molecules (growth factors, cytokines, oncogenes, tumor promoters) are presently being studied.

#### *a) Regulation of MMPs gene expression*

Most genes for MMPs are inducible; besides biologically active molecules, genes can be induced by various chemical agents such as phorbol esters<sup>48</sup>. Gene expression can be inhibited by some suppressive factors (TGF- $\beta$ , glucocorticoids, retinoic acid)(ref.<sup>49,50</sup>). Genes for MMPs are expressed only if the tissue, whether under physiological or pathological conditions, is remodeled. MMP-2 is an exception; regulation is realized at the level of activation and inhibition of enzyme activity<sup>51,52</sup>. Promoters for most MMP genes include the same AP-1 element in position – 70 and one or two replicas of PEA-3 element in position between –140 and –200. The first one reacts with Fos and Jun proto-oncogene family. PEA-3 element reacts with Ets family of transcription factors. Mutation analyses showed that the AP-1 sequence itself or in combination with PEA-3 regulate the basic stages and inducibility of these genes by means of various molecules. Transcription factors which recognize these sites are the already mentioned proto-oncogenes in the *cis* conformation (*cis*-elements). For example, stromelysin-1 PDGF responsive element (SPRE). One of these transcription factors, recognizes the site –1573 on the gene for MMP-3, but it has also been identified on other promoters of MMP genes; therefore it generally results in regulation of MMP gene expression<sup>20</sup>. Natural sequence variations of MMP genes promoters can

**Table 1.** Classification of Matrix MetalloproteinasesRASI I: Name of gene encoding MMP 19, IL-1 $\beta$ : Interleukin -1 $\beta$ , PI : protein inhibitor, MBP: myelin basic protein, MT-MMP = membrane-type MMP

MMP	Alternative Names	Group	EC Number	Chromosome	Substrates
MMP-1	Collagenase (Type I, interstitial)	Collagenases	EC3.4.24.7	11q22-q23	Collagens (I,II,III,VIII a X); gelatin; aggrecan; L-selectin; IL-1 $\beta$ proteoglycans, entactin; ovostatin; MMP-2; MMP-9
MMP-2	Gelatinase A 72 kDa Gelatinase Type IV Collagenase	Gelatinases	EC3.4.24.24	16q13	Collagens (I,IV,V,VII,X,XI a XIV);gelatin; elastin;fibronectin;aggrecan; MBP; osteonectin; laminin-1; MMP-1; MMP-9; MMP-13
MMP-3	Stromelysin-1 Proteoglycanase	Stromelysins	EC3.4.24.17	11q23	Collagens (III,IV,V, a IX); gelatin; aggrecan; perlecan; decorin; laminin; elastin; casein; osteonectin; ovostatin; antactin; plasminogen; MBP; IL-1 $\beta$ ; MMP-2/TIMP-2; MMP-7; MMP-8; MMP-9; MMP-13
MMP-7	Matrilysin Putative MMP	Matrilysins	EC3.4.24.23	11q21-q22	Collagens (IV a X); gelatin; aggrecan; decorin; fibronectin; laminin; entactin; elastin; casein; transferrin; plasminogen; MBP; $\beta$ 4-integrin; MMP-1; MMP-2; MMP-9; MMP-9/TIMP-1
MMP-8	Neutrophil collagenase	Collagenases	EC3.4.24.34	11q21-q22	Collagens (I,II,III,V,VII,VIII a X); gelatin; aggrecan; fibronectin
MMP-9	Gelatinase B	Gelatinases	EC3.4.24.35	20q11.2-q13.1	Collagens (IV,V,VII,X a XIV); gelatin; entactin; aggrecan; elastin; fibronectin; osteonectin; plasminogen; MBP; IL-1 $\beta$
MMP-10	Stromelysin-2	Stromelysins	EC3.4.2.22	11q22.3-q23	Collagens ( III-V); gelatin; casein; aggrecan; elastin; MMP-1; MMP-8
MMP-11	Stromelysin-3	Stromelysins	not assigned	22q11.2	Unknown (casein)
MMP-12	Macrophage metal- loelastase		EC3.4.24.65	11q22.2-q22.3	Collagen IV; gelatin; elastin; casein; fibronectin; vitronectin; laminin; entactin; MBP; fibrinogen; fibrin; plasminogen
MMP-13	Collagenase-3	Collagenases	not assigned	11q22.3	Collagens (I,II,III,IV,IX,X,a XIV); gelatin; plasminogen; aggrecan; perlecan; fibronectin; osteonectin; MMP-9
MMP-14	MT1-MMP	Membrane MMP	not assigned	14q11-q12	Collagens (I-III); gelatin; casein; fibronectin; laminin; vitronectin; entactin; proteoglycans; MMP-2; MMP-13
MMP-15	MT2-MMP	Membrane MMP	not assigned	16q12.2-q21	Fibronectin; entactin; laminin; perlecan; MMP-2
MMP-16	MT3-MMP	Membrane MMP	not assigned	8q21	Collagen III; gelatin; casein; fibronectin; MMP-2
MMP-17	MT4-MMP	Membrane MMP	not assigned	12q24	Unknown
MMP-18	Collagenase-4	Collagenases	not assigned	Unknown	Unknown
MMP-19	RASI-1		not assigned	12q14	Gelatin; aggrecan; fibronectin
MMP-20	Enamelysin		not assigned	11q22	Amelogenin; aggrecan
MMP-21			not assigned	1p36.3	Unknown
MMP-22			not assigned	1p36.3	Unknown
MMP-23			not assigned	11q22	Amelogenin
MMP-24	MT5-MMP	Membrane MMP	not assigned	20q11.2	Pro-MMP 2
MMP-25	Leukolysin/MT6- MMP	Membrane MMP	not assigned	16p/3.3	Pro-gelatinase A; fibrin; fibronectin; collagen IV; gelatin
MMP-26	Endometase, matrilysin-2	Matrilysins	not assigned	Unknown	Gelatin I $\alpha$ ; P1; fibrinogen; fibronectin; vitronectin; $\beta$ -casein
MMP-27			not assigned	Unknown	Unknown
MMP-28	Epilysin		not assigned	17q11.2	Casein

modify MMP gene expression in many organisms and in a variety of ways.

There are a number of described signaling pathways resulting in the MMPs expression. Inflammatory cytokines, TNF- $\alpha$  and IL-1 trigger ceramide signaling. Ceramide-dependent expression of MMP-1 in human dermal fibroblast is influenced by the three distinct MAP kinase pathways, ERK1/2, stress-activated protein kinase (SAPK)/JNK and p38(ref.<sup>53, 54</sup>). One inducible factor is also ultraviolet B irradiation, which increases expression of MMP-1, MMP-3 and MMP-9 in human dermal fibroblasts. Protein kinases JNK-2 are activated by stress *via* reactive free radicals and *via* lipid peroxidation<sup>55, 56</sup>.

Gene expression is influenced also by the ECM-cell and cell-cell interactions. As an example, we can also mention glycoprotein EMMPRIN (extracellular matrix metalloproteinase inducer) that was first identified on the surface of human tumor cells. It stimulates MMP-1,-2 and -3 production in human fibroblasts and increases mRNA MMP-1 expression; this stimulating effect requires activity of p38<sup>57, 58</sup>. MMPs or EMMPRIN synthesis can also be influenced by the changed hemodynamic overload<sup>59</sup>. MMP-9 is induced by  $\alpha_5\beta_1$  integrin-fibronectin interaction during the differentiation of macrophages<sup>60</sup>. MT1-MMP expression is triggered by the cells of endothelium, fibroblasts and also neoplastic cells via interaction by  $\alpha_2\beta_1$  integrin<sup>61</sup>.

#### b) Regulation of MMPs enzyme activity by cystein switch mechanism

MMPs are expressed as zymogens. There is a certain reserve of inactive MMP bonded to various components of ECM in the extracellular space. Certain stimuli lead to fast MMPs activation. MMP-2 binds to the ECM structures containing elastin, MMP-3 to basal membranes and occasionally to collagen fibrils and MMP-13 to proteoglycans, collagen and elastin<sup>62</sup>. Not all MMPs are stored in latent forms. MMP-8 is in specific granules, MMP-1 and MMP-3 are constitutively produced after the release of cytokines and inflammatory mediators<sup>1</sup>.

After propeptide cleavage, the MMP zymogens are changed into *active enzymes*. Propeptide contains a conservative sequence PRG[V/N]PD, which maintains the enzyme in latent form by the chelating action of cystein residuum to Zn<sup>2+</sup> ions at the catalytic site of the enzyme. PRCG[V/N]PD motive is usually found in propeptides of matrixins, but -SH group of this sequence are only found in native propeptides. ProMMPs do not react easily with -SH reactants until Zn<sup>2+</sup> in proMMPs is eliminated by some chelating molecules<sup>13, 63</sup>. SH cystein group in PRCG[V/N]PD interacts with Zn<sup>2+</sup> in enzymatic active sites. This interaction [Zn<sup>2+</sup>-cystein] maintains zymogene inactivity. Activation of the enzyme is explained by a so called mechanism of a cystein switch<sup>64, 13</sup>. The key step is dissociation of zinc-cystein interaction. Then zinc interacts with water. Extracellular activation of the enzyme includes 2 steps. The first is initial cleavage of the MMP propeptide by protease, destabilization of propeptide binding interactions, and break of coordination bond of cystein

and Zn<sup>2+</sup> ions. The second step is the final cleavage of the propeptide, that is usually ensured by another MMP. The result is a matured enzyme. In many cases, MMPs cannot participate in reaction until the last part of the propeptide is removed. In MT-MMPs and stromelysine-3 the active site is formed directly after the previous interaction of 11 residues containing consensus RXKR (where X can be any amino acid) with the furine family of enzymes - with endopeptidases associated with the Golgi apparatus. Intracellular cleavage of MT-MMPs and stromelysine-3 by the furine-like enzyme activates the enzyme<sup>65-67</sup>.

There are also *alternative ways of MMPs activation*. MT1-MMP is as an inactive zymogene located on the cell membranes. Gelatinase A (MMP-2) and collagenase-3 can be activated extracellularly on the surface of cells by the active, membrane bound MMP which, in this case, plays the role of enzyme and also membrane receptor. This activation process requires active MT-1-MMP and TIMP-2 bond to MT-1-MMP(ref.<sup>68-70</sup>).

#### c) Inhibition of MMPs by means of TIMPs

Tissue inhibitors of matrix metalloproteinases (TIMPs) are the main endogenous regulators of MMPs in the tissue. They are proteins of size 21-30 kDa. Four homologous molecules termed TIMP-1,-2,-3,-4 have been described. They bind to MMPs in a ratio 1:1 forming binary non-covalent complexes with very high K<sub>d</sub> (10<sup>-9</sup>-10<sup>-10</sup>). In this way they protect binding sites for the substrates being split off<sup>71</sup>. *TIMP-1* forms the complex preferentially with MMP-9, and *TIMP-2* preferentially with MMP-2. The crystalline structure of the complex formed between TIMP-1 and catalytic domain MMP-3 has already been described: critical sites of the whole inhibition process are localized around a disulfide bond between Cys<sup>1</sup> and Cys<sup>70</sup>(ref.<sup>72</sup>). The N-terminal  $\alpha$ -amino and carbonyl groups of Cys<sup>1</sup> bidentately coordinate the Zn<sup>2+</sup>. Thus TIMPs prevent autocatalytic cleavage of the MMP molecule leading to the formation of the active molecule (mechanism of cystein switch). Active membrane bound MMPs can be inhibited with TIMPs in complexes with other molecules. A catalytic amount of MMP-3 considerably activate proMMP-9 in the absence of TIMP-1, but not the [proMMP-9-TIMP-1] complex. Thus MMP-3 is inhibited by TIMP-1(ref.<sup>73, 74</sup>). If the ternary complex [proMMP-9-TIMP-1-MMP-3] is formed, interaction between proMMP-9 and TIMP-1 weakens and partially dissociates into free proMMP-9 and [TIMP-1-MMP-3] complex. Thus, a higher concentration of MMP-3 is needed for the activation of proMMP-9, which is inhibited by TIMP-1, which must be saturated by another matrixin<sup>75</sup>. This explains what impairs MMPs inhibition mediated by TIMPs. TIMP-1 expression is regulated by cytokines (TNF- $\alpha$ ) which can modify TIMP-1 expression *via* induction of nuclear transcription factors, TIMP-2 expression by cytokine stimulation does not take place<sup>76, 77</sup>.

*TIMP-3* has been identified in the myocardium of the mouse embryo, where it probably takes part in ECM embryonal remodeling and heart development<sup>78</sup>. It differs from other TIMPs by direct binding to the ECM compo-

nents (TIMP-1 and TIMP-2 are freely diffusible in the interstitial space). Therefore, TIMP-3 models MMP activity better than other TIMPs(ref.<sup>79</sup>). *TIMP-4* has been detected in low concentrations in kidneys and the colon. It is not in lungs, liver or the brain. By contrast, high TIMP-4 expression has been identified in the heart<sup>80</sup>. TIMPs also show other biological functions. TIMP-1 and TIMP-2 have mitogenic activity in various cell types, TIMP-2 influences interaction/adhesion between substrates and the cell<sup>81,82</sup>. TIMPs overproduction slows down the growth of tumor cells. Decrease in TIMPs concentration during the healing of damaged tissue increases collagenolytic activity. Reduced TIMPs activity also enables tumor cells to impair the surrounding extracellular matrix, and thus they migrate to the adjacent tissues. Therefore it is clear that regulation of the balance between collagenases and their inhibitors is necessary for remodeling of tissue whose impairment can significantly change the tissue functional characteristics<sup>83</sup>.

## MMPS AND CARDIAC TISSUE

### *Structure and Function of Myocardial ECM*

Cardiac tissue consists of the two basic components – cellular and extracellular. Two thirds of the total tissue content comprises myocytes, one third of cellular matrix is occupied by other cells – fibroblasts, endothelial cells, other cells and acellular components – extracellular tissue fluid and extracellular matrix. Pathological changes at the molecular level are characterized by the change in the qualitative and quantitative constitution of ECM and the constitution of myocytes. These changes are the result of tissue remodeling, as well as the changed synthesis, degradation, maturation and incorporation of individual ECM components that form a highly organized structure with not only structural but also regulating and communicative functions<sup>84,85</sup>. Slow turnover of extracellular matrix is characteristic of healthy cardiac tissue: synthesis and degradation are in a dynamic balance. The structural backbone myocardial ECM is formed by a fibrillar collagenous network consisting, above all, of collagen type I and III that are extremely resistant to proteolytic cleavage. However, myocardial ECM also contains components in lower quantity, which connect individual components, communicate with myocytes, modulate transport and organization of ECM or individual components, influencing cellular signaling. Besides various minor collagens (type IV, V, VI, VII, VIII, IX, X, XV, XVIII and others)(ref.<sup>86,87</sup>) there is a whole range of glycoproteins, glycosaminoglycans (heparan sulfate and chondroitin sulfate), microfibrillar proteins (fibrillin and fibulin), laminin, fibronectin, elastin, integrins, tenascins and others<sup>88-90</sup>. There are also various groups of enzymes which take part in degradation of ECM components in myocardium, such as the above discussed MMPs and ADAM metalloproteinases, serlyte-proteases (plasmin, neutrophil elastase, cathepsin G), cysteine proteases (cathepsin B,L and S), aspartyl proteases (cathepsin D)(ref.<sup>91</sup>).

## MMPS AND DISEASE OF MYOCARDIUM

*Myocardial remodeling* in various pathological situations is characterized by several different processes of cardiac tissue turnover. *The first*, which is characteristic of myocardial infarct, is very similar to the process of any damaged tissue healing. The previous ischemic injury of the original tissue, is followed by a repair processes which removes necrotic tissue and replaces it with a new one. *The second* is a result of long-term reaction of cardiac tissue under volume- or pressure-overload. Thus, tissue turnover enables the maintenance of heart function even at changed pressure and volume conditions and leads to right-ventricular or left-ventricular heart failure. This is a qualitative and quantitative change of ECM components.

### *MMPs Function in Ischemic Cardiac Tissue*

An acute ischemic phase (proteolytic and redox insult) activates MMPs and this results in the impairment of the ECM at the site of the ischemic injury; TIMP concentration is also changed. Later on, concentrations of MMP inhibitors are lowered, hence degradation of ECM components continues. This leads to dilatation and systolic failure. Continuous ECM synthesis at sites distal from infarcted area leads to fibrosis and diastolic failure. Structural remodeling of tissue after the myocardial infarct leads to development of hypertrophy and tissue stretching. These processes are then accompanied by development of fibrosis in the non-infarcted part of the heart and dilatation of the infarcted part. This together results in cardiac failure<sup>92,93</sup>.

After a myocardial infarct, MMP-1 in cardiac tissue is activated by the proteinases of inflammatory cells and at the same time considerable reduction in number of present TIMPs occurs<sup>94,95</sup>. In ischemic left ventricle in rats, increased collagenolytic activity was found 2 days after coronary artery ligation, a maximum was reached after 7 days, and then a decrease in MMP-1 activity and a parallel increase in MMP-2 and MMP-9 activities were observed. An increase in mRNA MMP-1 expression was not detected earlier than 7 days after coronary artery ligation. TIMP mRNA transcription is already observed after 6 hours, a maximum is reached after 2 days of infarct. Post-translational activation of latent MMP-1 is, in the process of injured myocardial tissue healing, more important than transcription of appropriate mRNA proMMP-1. Transcription of MMP-1 occurs after depletion of latent enzyme amount. TIMP mRNA synthesis is regulated by activation of matrix metalloproteinases. Balance between collagenase activation and TIMP inhibition determines the stage of collagenolysis in infarcted tissue<sup>96</sup>. Hence, MMP-1 is not the enzyme which is fully responsible for collagen degradation in ischemic myocardium. Therefore, other enzymes from the group of cysteine proteases (e.g. plasmin) or lysosomal cysteine proteases (e.g. cathepsin G) are at work here<sup>91</sup>. Delayed reperfusion changes activity of MMPs in the infarcted focus of the rat's heart, as well as the lowering of activities of MMP-1, MMP-2

(50% to 60% after 7 days) and MMP-9 (up to 84% after 48 hours)<sup>97</sup>. On the other hand, in early reperfusion, by means of initiated inflammatory processes, the secretion of active MMP-9 part by infiltrating neutrophils, probably activates proteolysis of other MMPs and the mechanism of the infarcted focus turnover process is different. By contrast, inhibition of the inflammatory process is associated with an increased risk of ventricular aneurysms, cardiac ruptures and death<sup>98</sup>. MMP-2 release during reperfusion after ischemia contributes to the cardiac mechanical dysfunction. Specific inhibition of MMPs may be a new strategy in the treatment of ischemia-reperfusion injury<sup>99</sup>. For example, an early left ventricle hypertrophy was avoided by administration of broad spectrum MMP inhibitor (CP-471, 474) to mice with experimentally induced myocardial infarct<sup>100</sup>.

#### *MMPs Function in Remodeling of Hypertrophic Cardiac Tissue*

Pressure or volume overload of heart muscle leads to hypertrophy and consequent cardiac tissue remodeling.

In spontaneously hypertensive rats, the increase in MMPs activity (e.g. MMP-2, 9) appears during heart failure. TGF- $\beta$  is a known inducer of collagens and TIMPs expression and inhibitor of MMPs (activity or expression). In spite of this, profibrotic components are increased during hypertrophic remodeling which leads to development of cardiovascular fibrosis and diastolic dysfunction<sup>92</sup>. In experimentally induced myocardial fibrosis in hypertensive rats, the increase in MMP-2 activity was detected after 2 months, while activity for MMP-1 remained unchanged<sup>101</sup>.

Some studies also showed that the total MMPs activity in patients in the last stages of cardiomyopathies was increased<sup>102,103</sup>. There is considerable selective reduction of tissue MMP inhibitor expression and independent increase in gelatinolytic activity presented by MMP-9 activity in the failing human heart<sup>102</sup>. In some hypertrophic cardiomyopathies, increase in tissue inhibitor concentration was detected, compared to controls, up to 100 times higher<sup>104</sup>, showing the exceptional position of TIMPs in the process of tissue remodeling.

We demonstrated increased expression of TIMP-1 mRNA (more than 3.5 times) and at the same time a 2.5 times increase in MMP-2 expression in hypertrophied right ventricle after the 14 days of hypoxic hypoxia (10% O<sub>2</sub>) in rats<sup>105</sup>. By contrast, the decrease in TIMP-1 and MMP-2 expression occurred after a 4-day hypoxic stimulus<sup>106</sup>.

#### *MMPs and Dilated Cardiomyopathy*

In dilated cardiomyopathies (DCM), ventricular wall weakening occurs, myocytes are hypertrophied and disruptions in ECM appear. The DCM result is systolic heart failure. It was demonstrated in hamster DCM models that MMPs activity was increased after 180 days and did not change within 310 days. This increased MMPs activity was associated with reduction in TIMPs expression<sup>107</sup>. Similarly in humans, MMP-1 is specifically and differentially induced, while TIMS expression is re-

duced (inhibited)(ref.<sup>107</sup>). There are two possible explanations: it is either mutation, affecting the propeptide part of MMP-1, which leads to production of already active MMP-1 without the need for posttranslational activation or a dramatic reduction in the presence of MMP tissue inhibitors<sup>104</sup>. Beside MMP-1, MMP-3 and MMP-9 activities are also increased. Increased MMP-3 activity was detected during left ventricular dilatation in animals and humans<sup>108,103</sup>. Pigs with congestive heart failure had MMP-1 activity higher by up to 319% in the left ventricle, MMP-2 activity increased by 194% and MMP-3 by 493% in comparison with healthy animals<sup>109</sup>. The reduction of cross-links between collagenous fibrils also occurs during dilatation. Newly formed collagen is not connected by cross-links; collagenous depositions are made and they succumb to the increased turnover as a result of increased MMPs activity in the process of cardiac tissue dilatation. An interesting finding was that higher activity of neutrophil collagenase (MMP-8) was detected in human cardiac tissue at DCM<sup>110</sup>. In rats with symptomatic congestive heart failure, significant increase in MMPs activity occurs. Some authors further showed that ventricular dilatation can develop even when synthesis of collagen considerably exceeds its degradation. It is clear that the concentration of the total collagen is not responsible for the process of ventricular compliance. However, decreased interaction between collagen and integrin receptors, changes in collagen cross-linking or inner changes in architecture of cardiomyocytes play important roles in ventricular remodeling<sup>111</sup>.

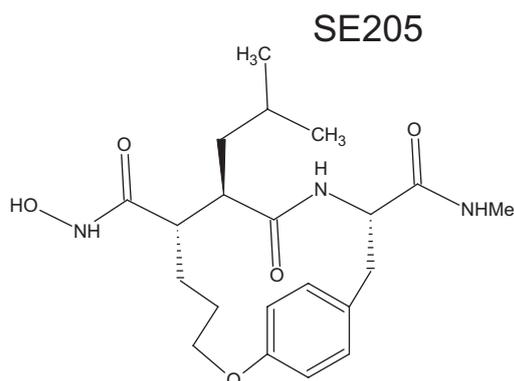
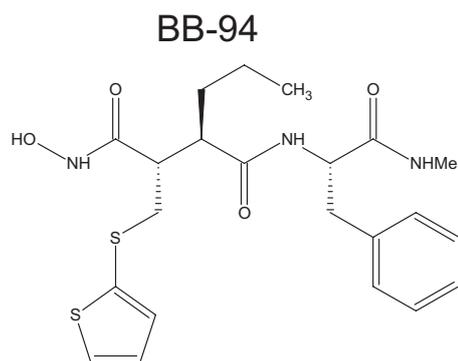
#### SYNTHETIC INHIBITORS OF MMPs

Several chemotherapeutic agents, antibiotics and synthetic peptides modulated production or activity of different classes of MMPs, and were tested with varying success in animals and patients above all those with malignant diseases. Disruption of tissue organization is a hallmark of malignant growth, it creates space for local tumor invasion and allows the tumor cells to gain access to the vascular and lymphatic systems. Lately, great effort has been focused on the development of effective specific inhibitors of MMPs and their use in therapy of non-malignant diseases<sup>8</sup>. MMPs inhibitors can be divided into 4 groups: a) peptidomimetic inhibitors of MMPs, b) non-peptidomimetic inhibitors of MMPs, c) tetracycline derivatives, d) biphosphonates. The structures of some of these are shown in Fig. 2.

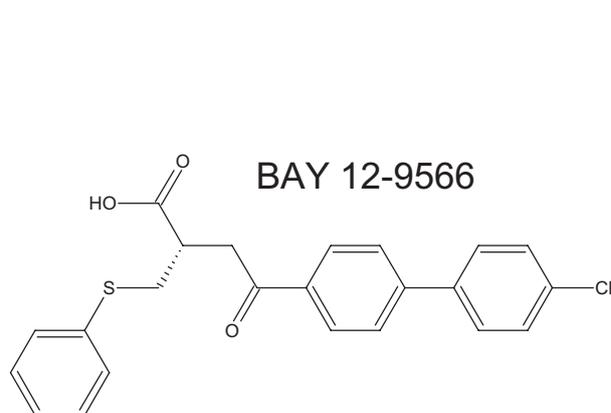
##### *a) Peptidomimetic Inhibitors of MMPs*

The first synthetic inhibitors of MMPs (peptidomimetic) were developed on the basis of our knowledge of the sequence of sites where collagenous molecules are cleaved by MMP-1. These inhibitors contain a chelating group which binds zinc in the MMP active site. BB-94 (Batimastat)(ref.<sup>112</sup>) and this inhibits the right side of the cleavage site is a typical example of such inhibitor. Inhibitors based on the sequence of the left side of the

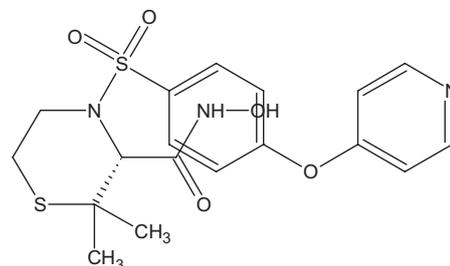
### Peptidomimetic inhibitors



### Non-peptidomimetic inhibitors

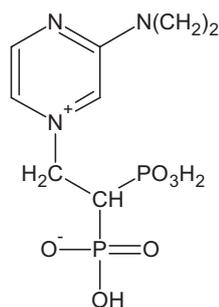


### AG3340

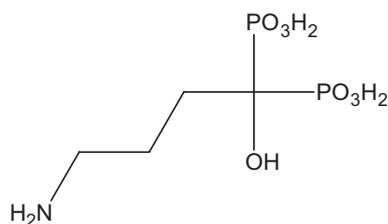


### Bisphosphonates

#### VS-6

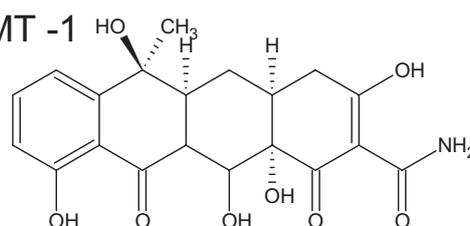


#### Alendronate



### Modified tetracyclines

#### CMT -1



**Fig. 2.** Synthetic Inhibitors of MMPs.

MMP cleavage site were much weaker<sup>113</sup>. To maintain the constant structure of these inhibitors, a so called hydroxamate structure was chosen. The hydroxamate serves as an effective ligand of  $Zn^{2+}$  ion in the MMP active site. Modification of the side chain of the hydroxamate (e.g. by the succinate) increases the inhibiting effect of these compounds. A group of these inhibitors (e.g. BB-94 or BB-1101) has broad substrate specificity. Further effort was made to develop a compound that would inhibit the

right side as well as the left side of the cleavage site of the enzyme (e.g. SE205)(ref.<sup>114</sup>).

Peptidomimetic inhibitors have been successfully used in experimental cardiology. For example, administration of Batimastat prevented heart failure which would have been caused by myocardial hypertrophy development or by diastolic dysfunctions in young transgenic mice with enhanced expression of  $TNF-\alpha$  (restricted to myocardium)(ref.<sup>115</sup>).

### b) Non-Peptidomimetic Inhibitors of MMP

The group of nonpeptidic (non-peptidomimetic) inhibitors with broad spectrum of inhibition include, for example, N-sulfonyl amino acid hydroxymates that have been orally-available such as CGS27023A and AG3340. Newly developed arylhydroxymate compounds are still being tested for their contingent mutagenic effects. Other non-hydroxymate alternatives of synthetic inhibitors include carboxylic acids and thiols that are able to bind to zinc in the catalytic center of MMP(ref.<sup>116</sup>). BAY 12-9566 as an example of such an inhibitor is a biphenyl derivate of the anti-inflammatory drug Fenbufen<sup>117</sup>. From carboxylates, inhibitor PD166793 has been successfully used in experimental studies. Administration of this inhibitor to pigs with congestive heart failure helped to diminish dilatation and remodeling of the left ventricle<sup>118</sup>. The same inhibitor prevented the loss of heart mechanical function caused by peroxynitrite<sup>119</sup>. Failing heart in spontaneously hypertensive rats treated by PD166793 had reduced volume of the left ventricle compared to untreated animals where the value of peak +dP/dt was comparable with control animals<sup>120</sup>. In case of rats with spontaneous hypertension, activity of inhibitors reduced left-ventricle dilatation, maintained systolic function, normalized MMP/TIMP expression and reduced the volume of collagenous fraction<sup>121</sup>. Thiol groups, due to their ability to bind zinc at the active site, are incorporated into the molecules of MMPs inhibitors. In spite of the fact that the real affinity of „monodentate“ thiol groups is less than „bidentate“ carboxylates and hydroxamates, easier solubility and ionization balance their final inhibiting potential<sup>122</sup>.

### c) Derivatives of Tetracyclines

Some agents of the group of antibiotics – derivatives of tetracyclines (doxycycline, minocycline and chemically modified tetracyclines (CMT)) are inhibitors of MMPs. These agents inhibit not only activity but also production of MMPs. The inhibiting effect is ensured by both blocking the activity of the enzyme by chelated zinc at the bond site and interference with proteolytic activation of proMMPs; MMPs expression is also reduced<sup>123,124</sup>. CMT-3 (metastat), which is not toxic and has a long half-life is especially effective<sup>122</sup>.

### d) Biphosphonates

Biphosphonates are synthetic analogues of pyrophosphate with high affinity for hydroxyapatite crystals of bone tissue; they prevent bone re-adsorption. They are successfully used for the treatment of osteoporosis and other bone diseases. However, in the treatment of cardiovascular diseases they have not been used yet. Mechanisms of MMP activity have not been sufficiently investigated. It has been suggested that their ability to induce production of cytokines results in MMP inhibition<sup>125-127</sup>.

## CONCLUSION AND FUTURE PROSPECTS

This review summarizes some of the recent discoveries that have shed new light on the role of MMPs in remodeling of cardiac tissue. In the past, the role of the extracellular matrix of myocardium was considered to be passive. Development of a whole range of analytic and biochemical approaches has significantly contributed to the clarification of the structure and function of this compartment of heart muscle. Matrix metalloproteinases take part in degradation of extracellular matrix. In mature tissue they have the primary function of repair and protection, more so than in the process of morphogenesis, where their function is completely different. While the discovery that MMPs have a broad range of substrates other than ECM proteins providing new insight into the complexity of their role in tissue remodeling, it also raises numerous questions as to the mechanisms whereby the net effect of their activity is determined. Changed MMP activity is observed above all in impaired tissue. Knowledge of mechanisms of different MMP activation and inhibition may contribute to the treatment of heart diseases. MMPs and their selective synthetic inhibitors will be used not only in the treatment of various oncological diseases but also in diseases of the cardiovascular system.

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