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MINIREVIEW

Possible Role of Matrix Metalloproteinases in Reconstruction of Peripheral Pulmonary Arteries Induced by Hypoxia

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Summary

Exposure to chronic hypoxia results in hypoxic pulmonary hypertension characterized by structural remodeling of peripheral pulmonary vasculature. An important part of this remodeling is an increase of collagen turnover and deposition of newly formed collagen fibrils in the vascular walls. The activity of collagenolytic metalloproteinases in the lung tissue is notably increased in the first days of exposure to hypoxia. The increased collagenolytic activity results in the appearance of collagen cleavages, which may be implied in the triggering of mesenchymal proliferation in peripheral pulmonary arteries. We hypothesize that radical injury to pulmonary vascular walls is involved in collagenolytic metalloproteinase activation.

Key words
Chronic hypoxia • Hypoxic pulmonary hypertension • Vascular remodeling • Collagen • Metalloproteinases

Remodeling of the walls of peripheral pulmonary vasculature is an important pathogenic mechanism in the development of all forms of pulmonary hypertension. Most of the information concerning the structural remodeling of pulmonary blood vessels comes from studies in man and animals exposed to chronic hypoxia (for review see Herget *et al.* 1978, Reid 1986, Reeves and Herget 1984). Sojourn in hypoxia results in reversible structural reconstruction of peripheral pulmonary arteries which is characterized by muscularization of prealveolar arteries, increased turnover of collagen in arterial walls (Nissen *et al.* 1978) and increased deposition of connective tissue proteins and their qualitative changes (Laurent *et al.* 1990, Tozzi *et al.* 1994, Novotná and Herget 1998, Tozzi *et al.* 1998).

Arterial extracellular matrix wall composition

Collagen and elastin are the major structural components of blood vessel walls. To date, nineteen different collagen types have been found. In the vessel wall, types I, III, IV, V, VI, VIII, XII, XIII and XIV were detected (Kehrel 1995; Lethias *et al.* 1996). Collagen types I and III, however, represent 80-90 % . The cross-banded fibrils of collagens I and III, visible by electron microscopy, provide the tensile strength throughout the extracellular matrix of the tunica media and tunica adventitia (Mayne 1986). Fibril-forming collagens constitute major cross-striated fibrils and all share a large triple-helical segment of about 1000 amino acid residues. The basic structure unit of fibrilar forming collagens is

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the α-chain. This α-chain contains a repeat sequence - **Gly** - **X** (amino acid) - **Y** (amino acid) - over its entire length. The molecule of collagen type I comprises two separate α1 (I) and one α2 (I) chains. Collagen type III consists of three identical α1 (III) chains. Newly synthesized collagen α chains are translocated into the rough endoplasmatic reticulum. There the individual α chains undergo posttranslation modifications including hydroxylation of several prolyne residues to 4-hydroxyproline and lysine residues to hydroxylysine. Then certain hydroxylated lysine residues are glycosylated. (Kivirikko and Myllyla 1984). The presence of 4-hydroxyproline is crucial for achieving thermal stability of collagen triple helices so that their melting points are above body temperature. The newly synthesized procollagen molecule is secreted out of the cell. The most common forms of supramolecular assembly of fibrilar collagens are cross-striated fibrils. Fibril formation is preceded extracellularly by a proteolytic removal of terminal domains (including the C-terminal globular module and N-terminal globular module) from the procollagen molecule by specific procollagen proteases (Fig. 1). Collagen molecules within cross-striated fibrils are stabilized by covalent cross-links based on lysine and hydroxylysine aldehydes generated extracellularly by the action of lysyl oxidase.

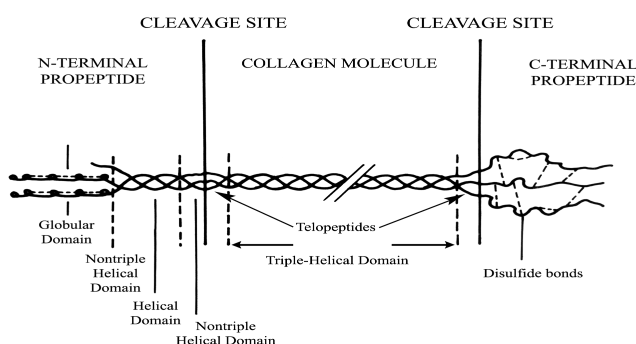

Fig. 1. *Schematic representation of the structure of procollagen and collagen molecule.*

In addition to type I and III, the non-fibrilar collagen types are present in the blood vessel wall. The non-fibrilar collagens associate with fibril-forming forms or form separate structures including microfibrilar meshworks (collagen VI), short filaments (collagen VII) or microfibrils. Their triple helical segments are of variable length (Brown and Timpl 1995). Collagen type IV is present in basement membranes (basal lamina), surrounds vascular smooth muscle cells (VSMCs) and underlies the endothelium. Collagen type V forms small fibrils associated with type I and III. Collagen type VI provides functional support and an organizational network (microfibrils) for larger collagen fibrils (type I and III) (Brown and Timpl 1995). Collagen type VIII synthesized by endothelial cells and VSMCs is present in both the intima and media. It is considered a constitutive product of VSMCs of normal vessels (Macbeath *et al.* 1996).

The extracellular matrix (ECM) is a biologically active and dynamic composition of structural, adhesive and counteradhesive fibrous proteins embedded in a hydrated ground substance of glycosaminoglycans and proteoglycans. The ability of cells to detect small differences in the specific combination, concentration and distribution of matrix components suggests that perturbations of the matrix homeostasis can lead to remodeling of the vascular wall. It occurs after vascular injury and a variety of pathological conditions such as atherosclerosis and hypertension (Coats and Faxon 1997, Faxon and Borst 1997).

Specific and unique proteolytic enzymes, matrix metalloproteinases (MMPs), regulate the integrity of the ECM structure. Many other enzymes (endopeptidases) can degrade various extracellular matrix components with different degrees of specificity. Serine-proteinases, including plasminogen activators, plasmin, elastase, thrombin, trypsin, chymotrypsin and cathepsins G and E, degrade various structural glycoproteins of ECM and can activate some latent MMPs (Emonard and Grimoud 1990).

ECM proteins (collagens and elastin) and other components (proteoglycans and structural glycoproteins) are some of best characterized substrates for MMPs. MMPs are a family of structurally related enzymes engaged in physiological processes such as development, growth or wound healing. MMP activity controls signals produced by ECM molecules involved in cell proliferation, differentiation and cell death (Matrisian and Hogan 1990). Under pathological conditions, an increase of MMPs activity is associated with cancer propagation (Fang *et al.* 2000) and acute and chronic inflammations.

Characteristics of matrix metalloproteinases

The MMPs are zinc-dependent endopeptidases capable of cleaving one or more ECM components (Emonard and Grimoud 1990, Massova *et al.* 1998). The main structure of MMPs is organized into three basic and well-conserved domains: propeptide domain in an

aminoterminal site of the polypeptide chain, a catalytic domain; and a hemopexin-like domain at the carboxyterminal polypeptide chain which shares structural similarities with hemopexin and vitronectin (Massova *et al.* 1998).

MMPs differ from other metalloproteinases in several aspects:

1) They have a highly conserved sequence motif PRCGXPD (X = V or N mainly) present in the propeptide.

2) The zinc-binding motif in the catalytic domain, in which three histidines bind to the catalytic zinc atom, is conserved among all MMPs. The catalytic zinc ion is essential for proteolytic activity of MMPs.

3) The hemopexin-like domain present in most MMPs provides substrate binding specificity, inhibitor binding, matrix binding and cell surface localization (Murphy and Knauper 1997) (Fig. 2).

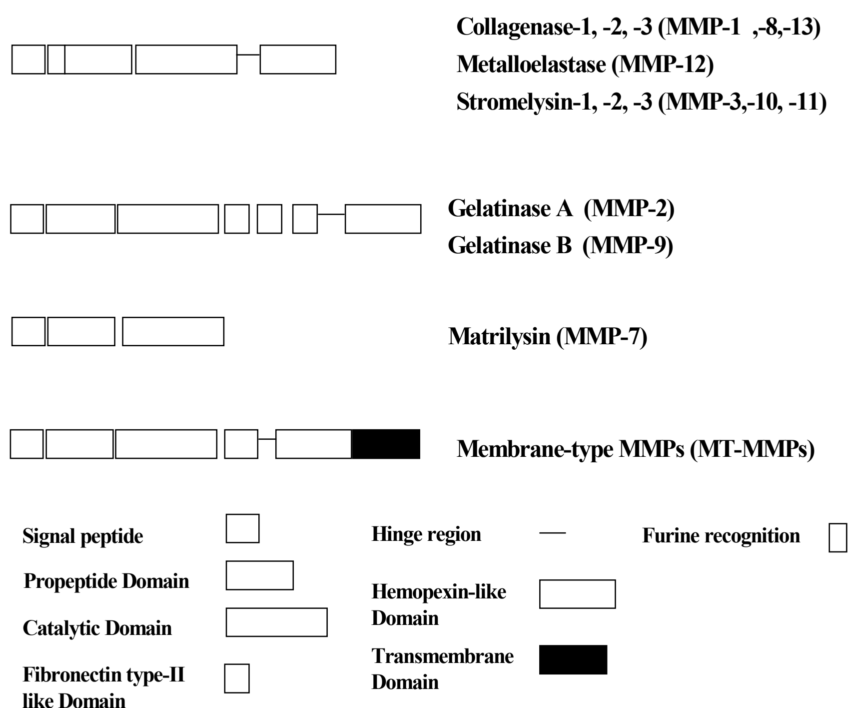

Fig. 2. *Schematics of the domain structures of the MMPs.*

In addition to these basic domains, individual MMPs incorporated or deleted domains typical for individual subgroups of MMPs. For example, gelatinases MMP-2 and MMP-9 have three repeat domains in their catalytic domain homologous to type-II module of fibronectin (known as gelatin binding domain or fibronectin type-II-like domain). It binds to denatured collagen or gelatine (Murphy *et al.* 1994). Some MMPs bind to the cell membrane. This transmembrane domain family, membrane-type MMPs (MT-MMPs), contains a transmembrane hydrophobic domain of 25 amino acids, which is incorporated into the cell membrane. Table 1 shows members of vertebrate MMPs and Table 2 lists of MMPs substrates.

Altogether 66 different MMPs have been sequenced to date, of which 21 are from human tissues. These human enzymes have counterparts in other vertebrates, some of which have closely similar (for example, the rodent type of interstitial collagenase share the highest degree of homology with human collagenase 3 – also called MMP-13) (Massova *et al.* 1998, Nagase and Woessner 1999, Pei 1999, Kojima *et al.* 2000, Marchenko *et al.* 2001).

Most of MMPs are not present in high concentrations under physiological conditions. They appear under pathological conditions, and growth factors and cytokines precisely regulate their expression. A high expression of MMPs is associated with cancer

malignancy and tumor ability to metastasize. An important expression of MMPs is also related to the processes of angiogenesis.

The MMP enzymatic activity is regulated at four levels: gene transcription, secretion, proenzyme activation and enzyme inhibition which control enzyme activity. The MMPs undergo activation by plasmin, cytokines or MT-MMP. The molecular mechanism involves protein-kinase C activation (Chen *et al.* 2001). Whereas the mechanism of activation of MMPs has been described in detail, information concerning the primary signal which activates MMP expression is more fragmented.

The MMPs expression is regulated by the cytoskeleton. Organization of cytoskeleton depends on the effects of ECM matrix components, such as fibronectin, tanescin and laminin. Cytokines (TNF-α, IL1-β, EGF, FGF) are involved in the regulation of MMPs expression during embryonic development (Murphy *et al.* 1999). Cytokines, notably TNF-α, induce a marked increase in MMP-9 (gelatinase B) activity and mRNA level in rat carotid blood VSMCs (Cho *et al.* 2000). Fibronectin upregulates MMP-2 (gelatinase A). Increases in MMP-2 expression and activation may be signalled by different integrin receptors according to the cell type. A number of reports have shown that MT1-MMP or MT2-MMP are receptors and their expression on the cell surface is associated with binding of MMP-2 (Sato and Seiki 1996, Belkhiri *et al*. 1997).

Table 1. Common names of matrix metalloproteinases

MMP No.	Common name	MMP No.	Common name
1	Collagenase 1 Fibroblast collagenase Interstitial collagenase	16	MT3-MMP
2	Gelatinase A	17	MT4-MMP
3	Stromelysin 1	18	Collagenase 4 (*Xenopus*)
7	Matrilysin	19	No trivial name
8	Collagenase 2	20	Enamelysin
	Neutrophil collagenase		
	Gelatinase B elastinase	21	XMMP (*Xenopus*)[1]
	92-kDa Gelatinase		
10	Stromelysin 2	22	CMMP (chicken)[1]
11	Stromelysin 3	23	(No trivial name)[1]
12	Macrophage elastase	24	MT5-MMP[2]
13	Collagenase 3	25	MT6-MMP[3]
	Rat osteoblast collagenase	26	Matrilysin 2[4]
14	MT1-MMP	27	Stromelysin-homologous[5]
15	MT2-MMP	28	Epilysin[6]

[1]Greenwald and Woessner (1999), Nagase and Woessner (1999), [2]Pei (1999), [3]Kojima *et al.* (2000), [4]Marchenko *et al.* (2001), [5]Benoit de Coignac *et al.* (2001), [6]Illman *et al.* (2001)

MMPs are secreted from the connective tissue cells of mesenchymal origin (such as fibroblasts, myoblasts, chondrocytes, osteoblasts, synovial cells, keratinocytes, hepatocytes, gingival cells, endothelial cells and cells of inflammatory origin, including macrophages, monocytes, neutrophils and mast cells) (Turto *et al.* 1977, Emonard and Grimoud 1990, Tozzi *et al.* 1998, Johnson *et al.* 1998). Cells secrete all MMPs as proenzymes and the activation of these proenzymes is the critical step that initiates ECM breakdown. MMP enzymes, which are generally active at neutral pH, require Ca^{2+} ions for full activity and are inhibited by Ca^{2+} chelating agents. This property can be used for their differentiation (Fig. 3). Inhibitors for serine, cysteine, or aspartic proteinases do not change MMPs activity.

Most proMMPs are activated extracellularly. Intracellular activation by furine-like proteinase was shown in stromelysin 3 (MMP-11) and MT- MMPs, since

they contain a furin recognition site (Pei and Weiss 1995, Sato *et al.* 1996). The activation of proMMPs is a sequential proteolytic process and occurs at the cell surface by several mechanisms. The first is the urokinase-like plasminogen activator (uPA), uPA receptor (uPAR) and the plasminogen cascade for plasmin generation (Murphy and Knauper 1997, Murphy *et al.* 1999). Cell surface associated plasmin is a key initiator of MMP activation. Plasmin activates particularly proMMP-3 (prostromelysin 1) and proMMP-10 (prostromelysin 2) and plasmin and stromelysins then activate other MMPs. We believe the second mechanism is important in serious vascular injury: latent MMPs are activated by non-proteolytic compounds such as SH reactive agents (4-aminophenylmercuric acetate – APMA, HOCl, oxidized glutathione), denaturants (urea, sodium dodecylsulfate - SDS) (Nagase 1997) and reactive oxygen species (ROS) (Fig. 4). The sources of ROS in the vascular wall are VSMCs, endothelial cells and macrophages (Rajagopalan *et al.* 1996a).

Table 2. Some of the best characterized substrates for matrix metalloproteinases

MMP No.	ECM substrates
MMP-1	Fibril collagens (I, II, III), collagens VII, X, gelatins, entactin, aggrecan
MMP-2	Gelatins of fibril collagens (denatured form), collagens IV, V, VII, X, and XI, fibronectin, laminin, aggrecan, insoluble elastin
MMP-3	Collagens type IV, V, IX, gelatins, fibronectin, laminin, aggrecan, vitronectin
MMP-7	Gelatins, collagen IV, fibronectin, laminin, aggrecan, insoluble elastin, vitronectin
MMP-8	Fibril collagens (I, II, III)
MMP-9	Gelatins of fibril forming collagens, collagens type IV, V, XIV
MMP-10	Collagen type IV, aggrecan, fibronectin
MMP-11	Weak activity on collagen IV, gelatins, fibronectin, laminin, aggrecan
MMP-12	Insoluble elastin, gelatin, aggrecan, fibronectin
MMP-13	Fibril forming collagen I, II and III and their N-telopeptides, gelatins of fibril collagens, collagen type IV, IX, X, aggrecan, fibronectin, laminin, tanescin
MMP-14	Fibril forming collagens, gelatin, fibronectin, laminin, activated MMP-2 and MMP-13
MMP-15	Activates proMMP-2
MMP-16	Collagen III, fibronectin, gelatin
MMP-17	Activates proMMP-2

According to Murphy and Knauper (1997), Nagase (1997)

The more recently discovered membrane-type MMPs (MT-MMPs) support another concept of pericellular activation cascade for MMPs. It has been shown that MT1-MMP activates proMMP-2 (progelatinase A). MMP-2 activates proMMP-13 (procollagenase 3) to active MMP-13 (Knauper *et al.* 1996b). MMP-13 than activates proMMP-9 (progelatinase B) (Knauper *et al.* 1997b). The MMPs activation cascade based on MT1-MMP was confirmed by Cowell *et al.* (1998). Hence the regulation of MT-MMPs themselves becomes of physiological importance as a determinant of MMP activity. This suggests that activation of proMMPs prevails mainly in the pericellular space. The generation of partially active or active MMPs triggers a cascade of cleavages, which generates fully active enzymes (Murphy *et al.* 1999). To date six MT-MMPs have been identified (Kojima *et al.* 2000).

Inhibitory regulation of MMPs activity is provided by a non-specific inhibitor (α2-macroglobulin) or by specific inhibitors, the family of tissue inhibitors of metalloproteinases (TIMPs). α2-macroglobulin and related general inhibitors, regulate MMPs primarily in the plasma, whereas TIMPs are key inhibitors of MMPs activity in tissues. TIMPs presently include four proteins: TIMP-1, TIMP-2, TIMP-3, and TIMP-4 (Willenbrock *et al.* 1993, Sang 1998, Kranzhofer *et al.* 1999). TIMPs inhibit MMP activity by non-covalent binding to the catalytic domain of MMPs. MMPs associate with TIMPs and form high affinity non-covalent 1:1 complexes. TIMPs show a high degree of sequence similarity (40 % identity in sequence), including 12 conserved cysteine

residues that are disulfide bonded to form a six-loop structure. Amino acid residues 1-127 of human TIMP-2 (N-terminal region) is a discrete protein domain which is necessary for MMPs inhibition of interaction with the catalytic N-terminal domain of active MMPs (Butler *et al.* 1999, Williamson *et al.* 1999). Disruption of TIMP to MMP balance results in diseases associated with uncontrolled proteolysis of connective tissue (arthritis, tumor cell invasion and metastasis, atherosclerosis and fibrosis) (Gomez *et al.* 1997).

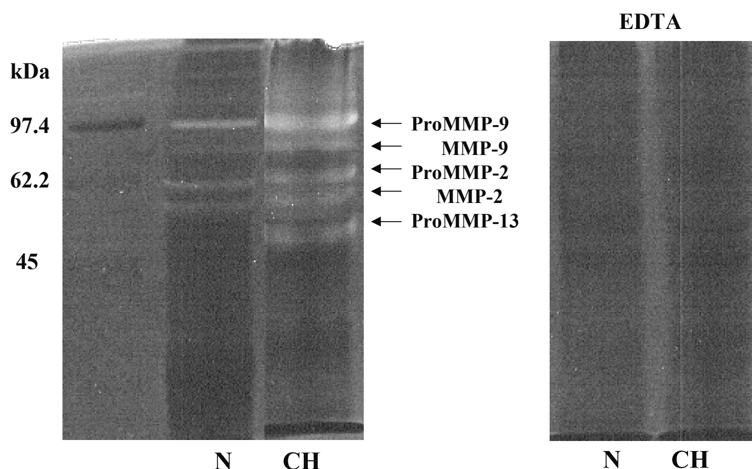

Fig. 3. *Zymographic analysis of MMPs extracted from normal rat lung tissue (N) and from lung tissue of rat exposed to chronic hypoxia (CH) with Ca^{2+} in the incubation media and in the incubation media containing Ca^{2+} chelating agents EDTA. In the first line M.W. markers are indicated.*

The role of MMPs in vascular wall development and remodeling

Remodeling of the vessel wall in response to injury involves expression of MMPs. Rat artery cells produce MMP-2 constitutively. MMP-2 and MMP-9 are involved in VSMCs activation and neointimal formation that characterize arterial tissue remodeling after injury. Inhibition of gelatinases activity reduced VSMCs migration (Bendeck *et al.* 1994, Zempo *et al.* 1994). Batimastat (a collagen peptide based hydroxamic acid), which is a selective synthetic inhibitor of MMPs, inhibits MMP-2 and MMP-9 activation and decreases VSMCs migration in primate arterial explants (Kenagy *et al.* 1996). Overexpression of MMP-9 enhances VSMC migration *in vitro* (Mason *et al.* 1999).

MMPs are involved in angiogenesis. Secretion of MMPs by microvascular endothelial cells of new blood vessels is an essential step of angiogenesis. Angiogenesis occurs in physiological processes such as wound healing as well as in pathological situations, such as solid tumors, tumor metastasis, diabetes or arthritis. MMPs secretion is an important initial step enabling degradation of the basement membrane and allowing endothelial cells to migrate through the connective tissue. MMP-9 was demonstrated in the cytosol of microvascular endothelial cells as a free active enzyme. As endothelial cells migrate, the active form of MMP-9 is secreted from cells in short bursts to locally degrade the basement membrane (Nguyen *et al.* 1998). MMP-1 and MMP-2 secreted by endothelial cells may also be involved in this process (Jackson and Nguyen 1997, Sang 1998). TIMPs inhibit neovascularization (Sang 1998). Also MMP-7 (matrilysin), MMP-9 and MMP-12 (metalloelastase) may block angiogenesis by converting plasminogen to angiostatin, the most potent angiogenesis antagonist (Sang 1998).

The increase of metalloproteinase activity in pulmonary blood vessels in chronic hypoxia.

The collagen molecule, except for the telopeptide domain, is resistant to proteolysis. Only interstitial (MMP-1) and neutrophil (MMP-8) collagenases can cleave the native collagen molecule. The enzyme is able to disrupt the quaternary organization of triple helix in the collagenase susceptible site (types I, II or III collagens) (de Souza *et al.* 1996) between the 775

– 776 residue (Gly – Ile/Leu) and produce three quarters (75 kDa) and one quater (25 kDa) collagen primary degradation products which are then much less resistant to proteolysis (Weingarten and Feder 1986). The only nonvertebrate enzyme capable of cleaving collagen triple helix is bacterial collagenase.

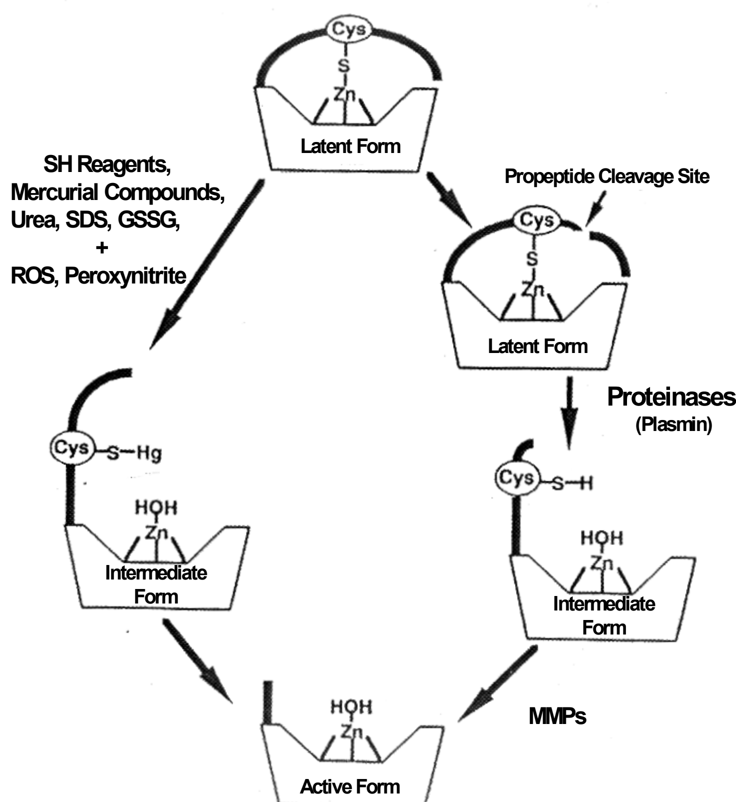

Fig. 4. *Scheme of stepwise activation of proMMPs by a cysteine switch mechanism. Proteolytic enzyme cleavage of the propeptide to generate the intermediate form. In a second step, the intermediate form can be cleaved by the action of some MMPs (MMP-3, MMP-1) to generate fully active MMP. Alternatively, reagents that react with the SH group can also activate proMMP [modified according to Sato and Saki (1996)].*

Using SDS slab gel electrophoresis, we identified a low molecular weight peptide (about 76 kDa) in extracts from peripheral pulmonary arteries isolated from chronically hypoxic rats (F_{iO2} = 0.1, 3 weeks). Such a peptide was not present in vessels obtained from normoxic rats (Novotná and Herget 1998). Using immunoblotting (Novotná and Herget 1998) and N-terminal amino acid sequencing, we proved that the peptide originates from the collagen type I (Deyl *et al.* 1998). Because the molecular weight of the peptide corresponded to three quarters of the molecular weight of the collagen molecule, we assumed that it is a result of the rodent-type interstitial collagenase (MMP-13) degradation product of vascular matrix collagen in hypoxia. In a subsequent study, we found that the presence of collagen cleavages is more pronounced in early phases (4 days) of the exposure to hypoxia. At four days, we detected not only the 3/4, but also the 1/4 collagen type I collagenase cleavages (Novotná and Herget 2001).

Using zymography, we measured collagenolytic activity in extracts from peripheral pulmonary arteries of normoxic and hypoxic rats. It was significantly increased in rats exposed to hypoxia as compared to normoxic controls (Novotná and Herget 1998).

Tozzi and co-workers (Thakker-Varia *et al.* 1998) reported an increase in collagenolytic activity in conduit pulmonary blood vessels during recovery from chronic exposure to hypoxia. Unlike us, however, they did not find any similar changes at the end of the sojourn to hypoxia. This controversy seems to imply that chronic hypoxia has different effects on collagen metabolism in

big conduit and in small prealveolar pulmonary arteries. As we shall explain later, the changes in peripheral pulmonary arteries reflect radical injury to the vascular walls. In the conduit part of the pulmonary vasculature, the collagen metabolism reflects changes of transmural pressure (Riley and Gullo 1988). The increase of collagenolysis in these vessels during recovery may therefore be the result of a decrease in pulmonary arterial blood pressure. We reported earlier that the pulmonary arterial blood pressure recovers rather rapidly when chronic hypoxia is discontinued (Herget *et al.* 1978). The wall thickness of the main pulmonary vascular branches reflects the changes of pulmonary arterial blood pressure (Heath and Kay 1967). The collagen changes in peripheral pulmonary arteries during recovery from hypoxia are, according to our observations, similar to those found in exposure to mild hyperoxia (Novotná *et al.* 2001). Rat interstitial collagenase MMP-13 can cleave the collagen molecule by triple helicase activity, resulting in the characteristic 3/4 and 1/4 fragment size (Knauper *et al.* 1996a). Unlike other collagenases, the MMP-13 can cleave native fibrilar forming collagens I, II and III also at the amino-terminal telopeptide site (telopeptidase activity) (Krane *et al.* 1996, Knauper *et al.* 1997a). Under certain conditions (hyperoxia, reoxygenation from hypoxic exposure) telopeptidase activity of MMP-13 prevails (Novotná *et al.* 2001).

The mechanism of collagenolysis activation in exposure to hypoxia

We hypothesize that the hypoxic remodeling of peripheral pulmonary arteries is a healing process from radical injury to their walls in the early phases of exposure to hypoxic environment (for review see Herget *et al.* 2000, Hampl and Herget 2000). An important part of this process, similar to other forms of healing, is an increase in the turnover of matrix proteins. Although both degradation and neosynthesis of matrix proteins are increased, the formation of new collagen fibrils prevails. This results in fibrotisation and decreased compliance of the walls of peripheral pulmonary arteries and, consequently, in pulmonary hypertension. The presence of collagen degradation products may be one of the triggering stimuli for fibroblast and vascular smooth muscle cell proliferation (Gardi *et al.* 1990, 1994, Bačáková *et al.* 1997, 1999, 2002).

The activation of collagenolysis in the early phases of hypoxic exposure is, according to our hypothesis, related to the production of radical oxygen species (ROS) and other radicals. Evidence is accumulating that lung tissue hypoxia results in oxidative stress (Herget *et al.* 2000, Wilhelm and Herget 1999, Wilhelm *et al.* 2002). Hypoxic pulmonary hyper-tension is accompanied by an increase in lung nitric oxide production (for review see Hampl and Herget 2000). A very reactive substance, peroxynitrite and its derivatives, originate from superoxide and nitric oxide interaction (Muijsers *et al.* 1997). Plasma concentration of nitrotyrosine, a marker of peroxynitrite production (Beckman and Koppenol 1996) increases in the first days of exposure to hypoxia (Mrázková *et al.* 2000). The radicals superoxide, hydrogen peroxide, nitric oxide and peroxynitrite react with the catalytic site of the metalloproteinases (Rajagopalan *et al.* 1996b). It has been shown experimentally that peroxynitrite activates MMP-8 (Okamoto *et al.* 1997a), proMMP-1 and proMMP-9 (Okamoto *et al.* 1997b) and MMP-2 and MMP-9 (Rajagopalan *et al.* 1996b). Activation of metallo-proteinases by peroxynitrite was potentiated by reduced glutathione (Okamoto *et al.* 1997b). Another possibility for peroxynitrite to activate collagenolysis is its interaction with TIMPs (Frears *et al.* 1996) and their inactivation.

In response to increased collagenolysis, fibroblasts and VSMCs start to proliferate and synthesize new extracellular matrix components. This initiates remodeling of the peripheral pulmonary vascular wall, which leads to hypoxic pulmonary hypertension.

The presented data support our hypothesis that hypoxic injury to the pulmonary vascular wall, which induces matrix protein breakdown, is one of the important pathogenic mechanisms of vascular remodeling in hypoxic pulmonary hypertension. This concept is supported by our recent finding that a synthetic inhibitor of MMPs, Batimastat, effectively prevents the development of hypoxic pulmonary hypertension in rats (Novotná *et al.* 2000, Tomášová *et al.* 2001).

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