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The cysteine switch: A principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family

(collagenase/gelatinase/stromelysin/zinc enzyme)

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ABSTRACT **The general applicability of the "cysteine-switch" activation mechanism to the members of the matrix metalloproteinase (MMP) gene family is examined here. All currently known members of the MMP gene family share the characteristic that they are synthesized in a latent, inactive, form. Recent evidence suggests that this latency in human fibroblast collagenase (HFC) is the result of formation of an intramolecular complex between the single cysteine residue in its propeptide domain and the essential zinc atom in the catalytic domain, a complex that blocks the active site. Latent HFC can be activated by multiple means, all of which effect the dissociation of the cysteine residue from the complex. This is referred to as the "cysteine-switch" mechanism of activation. The propeptide domain that contains the critical cysteine residue and the catalytic domain that contains the zinc-binding site are the only two domains common to all of the MMPs. The amino acid sequences surrounding both the critical cysteine residue and a region of the protein chains containing two of the putative histidine zinc-binding ligands are highly conserved in all of the MMPs. A survey of the literature shows that many of the individual MMPs can be activated by the multiple means observed for latent HFC. These observations support the view that the cysteine-switch mechanism is applicable to all members of this gene family. This mechanism is unprecedented in enzymology as far as we know and offers the opportunity for multiple modes of physiological activation of these important enzymes. Since conditions in different cells and tissues may match those necessary to effect one of these activation modes for a given MMP, this may offer metabolic flexibility in the control of MMP activation.**

The matrix metalloproteinases (MMPs) are a family of enzymes secreted by resident and inflammatory cells that are collectively capable of degrading most or all of the constituent macromolecules of the extracellular matrix (1, 2). Although the conserved domains of the MMPs are homologous, these enzymes exhibit considerable diversity in their domain structures and protein substrate specificities. In spite of this diversity, the MMPs share several key characteristics. First, all members of this family are believed to be zinc proteinases (3–6). Second, all of the MMPs are inhibited by tissue inhibitors of metalloproteinase, ubiquitous natural inhibitors which form inactive 1:1 complexes with these enzymes. Third, the MMPs are secreted in a latent form and activated *in situ* by physiological mechanisms that remain to be clarified. In view of the critical role that these enzymes play in the catabolism of both the collagenous and noncollagenous components of the extracellular matrix, it is essential that the mechanisms of activation of these MMPs be understood.

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The latent form of MMPs can be activated by a variety of seemingly disparate means (refs. 7 and 8, and references cited therein). Examples of these activation methods include treatment with proteases (9); conformational perturbants such as sodium dodecyl sulfate (10) and NaSCN (11); heavy metals such as Au(I) compounds (12), Hg(II) (13), and organomercurials (14); oxidants such as NaOCl (15); disulfide compounds such as oxidized glutathione (16); and sulfhydryl-alkylating agents such as *N*-ethylmaleimide (NEM) (13). There have also been numerous reports of spontaneous autoactivation (17). In a recent study of the activation of latent human fibroblast collagenase (HFC), we related the underlying basis for activation by all of these treatments to the disposition of the Cys^{73} residue in the propeptide domain (8). This residue is not freely accessible in the latent enzyme, but it is exposed or modified by the activation treatments. It was proposed that Cys^{73} of the latent enzyme is coordinated to the active-site zinc atom in a fashion that blocks the active site. All modes of activation lead to dissociation of Cys^{73} from the zinc atom with concomitant exposure of the active site. Accordingly, when Cys^{73} is "on" the zinc, the activity of the enzyme is "off." Thus, the dissociation of Cys^{73} from the zinc atom is viewed as the "switch" that leads to activation. In this report, the applicability of this "cysteine-switch" mechanism to the regulation of the entire MMP family is examined.

Domain Structure of the MMP. In humans, the MMP family currently consists of the two interstitial collagenases, HFC (18) and human neutrophil collagenase (HNC) (19, 20), a 72-kDa (21) and a 92-kDa (22) type IV collagenase (also referred to as gelatinases), stromelysin (3), stromelysin 2 (23), and pump 1 (23, 24). Members of this family from other mammalian species include rabbit interstitial collagenase (25), rabbit stromelysin (26), and the rat analogs of the stromelysins referred to as transin (27) and transin 2 (28). Some of the MMPs have been designated by a numerical code, in which HFC is designated MMP-1, HNC is MMP-8, etc. (29).

The protein chains of the MMPs are organized into domains whose boundaries are defined either by autolytic cleavages that occur on activation or degradation or by sections of protein chain that are unique to individual MMP. Matrisian and associates (30) have pointed out that transin (and, by analogy, the stromelysins and interstitial collagenases) consists of an N-terminal latent proteinase and a C-terminal domain that bears a weak sequence resemblance to hemopexin, a heme-binding serum protein. HFC (31), HNC (19), and human fibroblast stromelysin (HFS) (3) all undergo an autolytic cleavage that releases the hemopexin-like do-

Abbreviations: MMP, matrix metalloproteinase; HFC, human fibroblast collagenase; HNC, human neutrophil collagenase; HFS, human fibroblast stromelysin.

main. The remaining N-terminal latent proteinase consists of an N-terminal propeptide domain and a C-terminal catalytic domain. On activation by trypsin or organomercurials, latent HFC (9, 32), HNC (19), HFS (33), and the 72-kDa human type IV collagenase (34) undergo an autolytic cleavage that releases the propeptide domain. Thus, the interstitial collagenases, stromelysins, and transins can be viewed as having a three-domain structure. There is considerable sequence identity in the corresponding domains of the three types of enzymes.

The other MMPs differ from these proteinases primarily by the addition or deletion of domains. Pump 1 consists of only the N-terminal latent proteinase (23). The 72-kDa type IV collagenase contains an additional domain that is inserted into the catalytic domain just before the partial zinc-binding sequence. It consists of 175 amino acids and is composed of three 58 amino acid head-to-tail repeats which are homologous to the type II motif of fibronectin (21). The 92-kDa type IV collagenase has domains that are homologous to the four domains of the 72-kDa type IV collagenase, but it also has a fifth domain inserted close to the border between the catalytic and hemopexin-like domains (22). This domain consists of 54 amino acids and is homologous to a segment of the α2(V) collagen chain. A comparison of the domain organization of these MMPs is shown in Fig. 1.

Conservation of MMP Sequence in the Cysteine-Switch and Zinc-Binding Regions. If the cysteine-switch hypothesis is applicable to all of the MMPs, each must contain both the required cysteine residue and the zinc-binding site. In fact, the *only two domains* that all of the MMPs have in common are the propeptide domain that contains the key cysteine residue and the catalytic domain that contains the zinc-binding site. All 11 of the MMPs for which sequence data of the N-terminal propeptide domain are available (3, 18, 20–23, 25–28, 33) have a cysteine residue located in a highly conserved region that corresponds to residues 71–79 of latent HFC (Table 1), where the numbering of residues assumes that Phe^{11} of the preproenzyme sequenced by Goldberg and associates (18) is the first residue of the secreted form. The consensus sequence for these 11 MMPs is P^{71}R C G V/N P D V/L A/G.

Sanchez-Lopez and coworkers (30) have prepared a series of transin mutants in which the Pro^{71} residue in this sequence has been changed to Leu, Pro^{76} to Asn or Val, and Asp^{77} to Tyr. All of these variants have a markedly increased tendency to undergo spontaneous activation accompanied by the autolytic loss of the propeptide domain. Similar results have been observed with a Cys^{73} to Ser mutant of HFC (35). These mutations apparently impair the ability of the cysteine switch residue to complex with the active-site zinc atom, thus reducing latency and accelerating the autolytic loss of the propeptide domain. The high level of conservation of sequence in the region surrounding this cysteine and the destabilizing effect of substitutions in this region on latency imply that this section of the protein chain adopts a specific conformation that facilitates interaction of the cysteine sulfhydryl group with the active-site zinc atom.

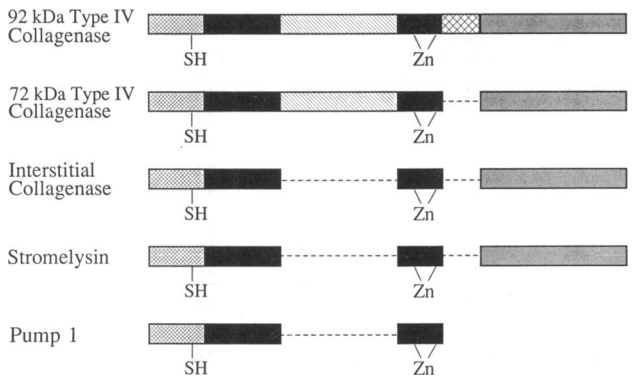

FIG. 1. Schematic representation of the domain structure of five members of the human MMP family. Referring to the 92-kDa type IV collagenase, from left to right, the five domains are the N-terminal propeptide domain, the fibronectin-like domain, the catalytic domain, the α2(V)-like domain, and the hemopexin-like domain. The domains are drawn to scale and the broken lines connecting the domains denote that all these proteins are single polypeptide chains.

The MMPs were originally presumed to be zinc enzymes because they are inhibited by transition-metal-ion-chelating agents (8–10). In spite of this observation, however, none of the MMPs have been *quantitatively* analyzed for zinc, and assumptions regarding its presence and stoichiometry of binding have been based largely on indirect evidence. When the sequences of HFC (18) and HFS (3) were reported, it was immediately recognized (3–6) that they contained a short region of polypeptide chain that was homologous to part of the zinc-binding region of certain bacterial metalloproteinases. Vallee and Auld (36) have analyzed the homologies in this region for the MMP and other zinc proteinases, including thermolysin, whose x-ray structure has been elucidated (37). Residues 139–149 of thermolysin contain His^{142} and His^{146}, which form two of the three protein ligands to the active-site zinc atom (37).

A comparison of the 10 MMPs for which sequence data for the zinc-binding domain are available (3, 18, 21–23, 25–28, 33) shows that they contain a sequence that is highly conserved and homologous to that containing the two histidine ligands in thermolysin (37) and other bacterial metalloproteinases (38–45) (Table 2). With the exception of pump 1, which has a threonine residue inserted between the third and fourth residues, the consensus sequence in this 11-residue region for the MMPs is V/Y^{196}A A H E L/I/F G H S/A L/M G. Thus, the two histidine residues in this sequence are likely candidates as zinc-binding ligands for the MMP. In support of this idea, Sanchez-Lopez and associates (30) have shown that mutation of His^{199} to Leu in transin gives an inactive mutant. Interestingly, the 72- and 92-kDa type IV collagenases differ from the other MMPs in that they have the fibronectin-like domain inserted into the catalytic domain (Fig. 1). This is presumably accomplished without disturbing the cysteine–zinc complex. In summary, while proof that the MMPs are zinc enzymes remains to be confirmed by direct analytical measurement of their metal contents, the existence of a zinc-binding site in all of these enzymes is supported by the inhibitory effect of transition-metal-ion-chelating agents and the sequence data shown in Table 2.

Multiple Modes of Activation of MMPs. If the cysteine-switch activation mechanism proposed for HFC is applicable to the other MMPs, they should also exhibit activation by some or all of the multiple means described earlier (8). The activators can be separated into four groups that include proteases (e.g., trypsin, MMP autolysis), conformational perturbants (e.g., detergents, NaSCN), reversible sulfhydryl-group modifiers [e.g., Hg(II), organomercurials], and irreversible sulfhydryl-group modifiers (e.g., oxidants, alkylating agents). While very little data are available for some of the MMPs, a survey of the literature (3, 8–10, 13–17, 19–21, 24, 30, 33, 34, 46, 47) and recent data (E. B. Springman, S. Netzel-Arnett, W. G. I. Moore, H.B.-H., and H.E.V.W., unpublished results) show that activation by all four classes of reagents is a general phenomenon. This supports the view that the cysteine-switch hypothesis is applicable to all of these MMPs.

Relationship Between the Cysteine-Switch Hypothesis and the History and Current View of MMP Activation. A number of different theories have been advanced to explain the latency and activation of the MMP. The cysteine-switch

Table 1. Sequences surrounding the cysteine switch in the N-terminal propeptide domain of different MMPs

Enzyme	Sequence	Ref.
Human fibroblast interstitial collagenase (HFC)	P^{71} R C G V P D V A	18
Rabbit fibroblast interstitial collagenase	P R C G V P D V A	25
Human neutrophil interstitial collagenase (HNC)	P R C G V P D V A	20
Human fibroblast stromelysin (HFS)	P R C G V P D V G	33
Human stromelysin 2	P R C G V P D V G	23
Rabbit stromelysin	P R C G V P D V G	26
Rat transin	P R C G V P D V G	27
Rat transin 2	P R C G V P D V G	28
Human pump 1	P R C G V P D V A	23
Human 72-kDa type IV collagenase	P R C G N P D V A	21
Human 92-kDa type IV collagenase	P R C G V P D L G	22

mechanism allows the condensation of these theories into a single, integrated mechanism. The early observations that latent HFC could be activated by chaotropic ions and thiol-blocking agents with a concomitant loss in molecular weight led to the proposal that they were enzyme–inhibitor complexes (48). This concept was supported by the observation that inactive collagenase–α_2-macroglobulin complexes could be dissociated to reactivate the collagenase by treatment with trypsin or NaSCN (7). The definitive proof that latent collagenases are not enzyme–inhibitor complexes was the pioneering work that showed that HFC is secreted as a single proenzyme protein chain (18) and that the loss in molecular weight on activation by organomercurials was due to autolysis (9) rather than to release of an inhibitor. Equally important was the observation that treatment with organomercurials *initially* led to activation *without* a decrease in molecular weight, since this was clear evidence that a novel, nonproteolytic, means existed for activation.

Other evidence for nonproteolytic means of activation has come from studies of latent HNC. Macartney and Tschesche (16) made the important observation that latent HNC could be activated by disulfide-containing molecules by a disulfide-exchange mechanism. Their original view of the latent enzyme was that of a disulfide-bonded enzyme–inhibitor complex where activation was believed to release the inhibitor (49). While the activation by disulfide compounds has been confirmed (19), latent HNC is no longer believed to be an enzyme–inhibitor complex (19, 20), and it can be activated without a *requisite* reduction in molecular weight (19). In another series of experiments, Weiss and coworkers have shown that both latent HNC (15) and the latent 92-kDa type IV collagenase (47) released by neutrophils can be activated oxidatively by HOCl that is produced from H_2O_2 and Cl^- by myeloperoxidase during the respiratory burst (15). There have also been reports of the activation of latent MMP by nonenzymic tissue factors (50, 51) by unknown mechanisms. These represent additional examples of MMP activation that are not initiated proteolytically.

While nonproteolytic means of activation exist, it is clear that latent MMP can also be activated by proteases (7, 9). The prototypic mechanism for this activation may be that found for the activation of latent HFC by trypsin (9, 32). The initial event is the hydrolysis of the Arg^{36}-Asn^{37} bond in the propeptide domain of the 52-kDa proenzyme to yield a 46-kDa species that is still inactive. This species subsequently activates autolytically via loss of the propeptide domain that contains the cysteine-switch residue. The autolytic cleavage site in latent HFC is probably immobilized and protected from autolysis in the intact zymogen. Cleavage by trypsin within the propeptide domain apparently triggers the exposure of this site and facilitates autolysis. Similar events are presumably involved in the activation of the other MMP by proteases. The spontaneous autoactivation of the MMP has not yet been studied carefully and the biochemical basis for this remains obscure. However, this could be the consequence of a number of circumstances, ranging from the presence of traces of residual activating proteases, to a slow inherent autolytic activity, or to a slow molecular oxygen-catalyzed oxidation of the sulfhydryl group of the cysteine-switch residue. The activation of the MMP by all of the proteolytic and nonproteolytic means discussed above can be accounted for by the cysteine-switch mechanism.

Table 2. Comparison of partial sequences in the putative zinc binding site region of MMPs and bacterial metalloproteinases

Enzyme	Sequence	Ref.
Human fibroblast interstitial collagenase (HFC)	V^{196} A A H E L G H S L G	18
Rabbit fibroblast interstitial collagenase	V A A H E L G H S L G	25
Human fibroblast stromelysin (HFS)	V A A H E I G H S L G	33
Human stromelysin 2	V A A H E L G H S L G	23
Rabbit stromelysin	V A A H E L G H S L G	26
Rat transin	V A A H E L G H S L G	27
Rat transin 2	V A A H E L G H S L G	28
Human pump 1	Y A A T H E L G H S L G	23
Human 72-kDa type IV collagenase	V A A H E F G H A M G	21
Human 92-kDa type IV collagenase	V A A H E F G H A L G	22
Bacillus thermoproteolyticus protease	V^{139} V A H E L T H A V T	38
Bacillus stearothermophilus protease	V V G H E L T H A V T	39
Bacillus cereus protease	V I G H E L T H A V T	40
Bacillus subtilis protease	V T A H E M T H G V T	41
Bacillus amyloliquefaciens protease	V T A H E M T H G V T	42
Serratia protease	T F T H E I G H A L G	43
Escherichia coli peptidase N	V I G H E Y F H N W T	44
Pseudomonas aeruginosa protease	V A A H E V S H G F T	45

Comparison of MMP Activation with That of Other Zymogens. Many proteinases are synthesized as zymogens (52, 53) and it is of interest to compare some of these with the latent MMP. Dogfish procarboxypeptidase A is a zinc proteinase zymogen whose latency is the result of impaired substrate binding (54). Kinetic studies show that the zymogen has activity, but it exhibits markedly higher K_m values than does carboxypeptidase A. Activation by trypsin in the presence of Ca^{2+} lowers the molecular mass from 44 to 35 kDa and leads to the formation of a more efficient binding site. The structural basis for this activation has not been established. Trypsinogen is a typical pancreatic serine proteinase zymogen that has low inherent activity, in spite of the fact that the disposition of its catalytic residues is almost identical to that in trypsin. Its catalytic inefficiency is due to an incompletely formed substrate-binding site (53, 55). The formation of this site is completed upon proteolytic removal of the N-terminal hexapeptide either by enteropeptidase or by trypsinogen itself. Pepsinogen is an aspartic proteinase zymogen whose latency is due to a segment of the propeptide that occupies the substrate-binding cleft (56). Pepsinogen is activated by stomach acid in a mechanism that involves a H^+-induced conformational change that mobilizes the propeptide, resulting in its autolytic removal to expose the catalytic residues and free the substrate-binding sites.

The MMPs constitute a class of zymogens that share many similarities, but also exhibit key differences, from those discussed above. The latency of the MMPs is due to the complex between the propeptide cysteine and the active-site zinc atom and represents a heretofore unknown mechanism for the latency of a zymogen. The complex simultaneously blocks the active site and probably excludes water from the coordination sphere of the zinc atom. All active zinc hydrolases have water as a fourth ligand that is essential for catalysis (57). All modes of activation of the MMP zymogens involve dissociation of this complex and the probable replacement of the cysteine ligand by water. The cysteine-switch model allows for multiple modes of activation that resemble, but do not duplicate, the activation of other zymogens. At least some of the latent MMPs (e.g., HFC, HNC) have small amounts of residual activity, which is thought to be due to a fraction of molecules in which the cysteine–zinc complex is dissociated at any given time (8). This residual activity may be responsible for the spontaneous autoactivation of these zymogens and resembles the autolytic activation of trypsinogen. Activation of the MMPs may also be accomplished proteolytically in a manner that resembles the activation of trypsinogen by enteropeptidase or of procarboxypeptidase A by trypsin. The nonproteolytic activation of the MMPs by organomercurials and reagents that directly modify the cysteine-switch residue resembles the H^+-induced activation of pepsinogen in that these agents induce a conformational change that leads to activation and autolysis. The activation by proteinases and that by cysteine-modifying reagents share the common feature that they expose the autolytic site and facilitate release of the propeptide. The existence of these multiple modes of activation in the zymogens of a single class of enzymes is unprecedented.

Implications of the Cysteine-Switch Mechanism for Physiological Mechanisms of Activation of MMPs. The *in vitro* activation of the MMPs by exogenous proteinases and the accompanying reduction in molecular weight have prompted most workers to assume that the MMPs are activated like the more "classical" zymogens (i.e., the activation of trypsinogen by enteropeptidase). As a result, most of the physiological mechanisms of activation that have been proposed have involved proteolysis of the N-terminal domain as the key event. Some of these mechanisms involve a proteolytic cascade in which plasminogen activators generate plasmin as the activating protease (58). While proteolysis may well be a physiological mode of activation for individual MMPs under certain circumstances, one important feature of the cysteine-switch mechanism is that the variety of nonproteolytic activation routes discussed above also exists. Therefore, in attempting to establish the true physiological mechanism of activation, the potential role of these nonproteolytic activation routes should not be ignored. One such route is illustrated by the oxidative activation of the latent interstitial and type IV collagenases by the neutrophil (15, 47). By extrapolation, the potential exists for latent MMPs to be activated by other means. For example, they could be activated by specific binding proteins that could conformationally trigger the cysteine switch. Alternatively, specific proteins or metabolites with a propensity to interact with cysteine residues could function as activators. This could be the basis for activation by tissue factors. Another physiological activation mechanism that should be considered involves activation by oxidized glutathione through disulfide exchange, where its concentration is modulated by the glutathione cycle (59).

A second feature of the cysteine-switch model is that it may allow flexibility in the way that an individual MMP is activated. Thus, one MMP may be more susceptible to activation by one mechanism than another. For example, while HFC is efficiently activated by trypsin (9, 32), fibroblast 72-kDa type IV collagenase is poorly activated by this proteolytic route (21). This may have important physiological implications in that it may allow for the selective activation of one or a small number of MMPs at certain sites. Alternatively, since each MMP may be activated by more than one means, this could endow a given cell with flexibility with regard to the way activation is achieved. There may, in fact, be different activation mechanisms for the same MMP in different cells or tissues. The neutrophil is a prime example in that its oxidative burst, a key characteristic of its phagocytic phenotype, is well suited to activate its latent MMP oxidatively. Interestingly, however, the neutrophil apparently has both oxidative and nonoxidative paths for the activation of its 92-kDa type IV collagenase (47). In other cells or tissues, plasminogen activator-dependent pathways may be more appropriate. Until it can be established precisely how a latent MMP is activated under a given set of circumstances, all possible modes of activation should be considered.

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