# The recombinant catalytic domain of membrane-type matrix metalloproteinase-1 (MT1-MMP) induces activation of progelatinase A and progelatinase A complexed with TIMP-2

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**Abstract A truncated form of the membrane-type matrix metalloproteinase-1 [(Ala$^{21}$–Ile$^{318}$)proMT1-MMP] lacking the hemopexin-like and trans-membrane domain was produced in *E. coli*. We demonstrate that the recombinant proenzyme was autoproteolytically processed to a fully active catalytic domain with N-terminal Ile$^{114}$. The catalytic domain of MT1-MMP initiated the activation of progelatinase A and progelatinase A complexed with tissue inhibitor of metalloproteinases-2 (TIMP-2). As a typical soluble metalloproteinase it was able to cleave physiologic as well as synthetic substrates. Our kinetic data demonstrate that TIMP-2 is a potent inhibitor for the recombinant enzyme.**

*Key words:* Matrix metalloproteinase; Gelatinase A; Type IV collagenase activation; Membrane-type matrix metalloproteinase, TIMP-2

## 1. Introduction

The matrix metalloproteinases (MMPs, matrixins) comprise a family of zinc proteinases capable of remodeling, repairing and destroying connective tissue via hydrolysis of matrix proteins. The matrixin family is divided into six subgroups, namely the collagenases, gelatinases, stromelysins, matrilysin, metalloelastase and membrane-type matrix metalloproteinases [1–3]. Members of the matrix metalloproteinase family share several structural and functional properties, but they differ to some extent with regard to substrate specificity. There is increasing evidence that a disregulated expression of MMPs leads to many pathophysiological events connected to extracellular matrix destruction, cancer invasion and metastasis [4–6]. Type IV collagen is the major structural component of basement membranes [7,8]. The close correlation between the type IV collagenolytic activities of MMP-2 (gelatinase A) and MMP-9 (gelatinase B) and the metastatic progression of various tumors suggests that their activator is the key enzyme which triggers the metastatic process. ProMMPs can be activated in vitro by organomercury compounds and individual species by certain serine proteinases such as trypsin, plasmin, plasma kallikrein, leukocyte elastase or cathepsin G [9]. Many investigators have reported that in vivo a 'plasma membrane dependent' mechanism is responsible for proMMP activation. Like other MMPs, progelatinase A is synthesized and secreted from the cells as an inactive precursor. In vitro it is readily activated by 4-aminophenylmercuric acetate [10], but progelatinase A is unique among MMPs in that it has proved to be resistant to proteolytic activation by both serine proteinases and other MMPs [10–12]. Sato et al. recently isolated a cDNA from a human placenta cDNA library which coded for a novel MMP family member with a transmembrane domain. Membrane-type MMP-1 (MT1-MMP) was shown to be localized on cell surfaces [13], and converted progelatinase A to the 62 kDa fully active form, indicating that MT1-MMP is a physiological activator of gelatinase A. Cao and co-workers reported that the transmembrane domain (TM) at the C-terminus of MT1-MMP is essential for progelatinase A activation because the activity was abolished by truncation of the membrane linker [14]. The resulting MT1-MMP fragment did not activate proMMP-2. In this article we describe the bacterial expression of MT1-MMP without the hemopexin-like domain and a simple procedure for in vitro folding and generation of the active enzyme. In contrast to the above mentioned work we report that the catalytic domain of MT1-MMP (cdMT1-MMP) is an efficient activator of progelatinase A as well as of the progelatinase A/TIMP-2 complex. Thus, neither the hemopexin-like domain nor the TM domain is required to activate the proenzyme. Furthermore, our study indicates that the truncated proform of MT1-MMP in vitro may be processed by autocleavage of two amino acids C-terminal of the described processing site for the full-length enzyme [15]. Our kinetic study shows that proteolytic activity of cdMT1-MMP is specifically effected by TIMP-2.

## 2. Materials and methods

### 2.1. PCR amplification, bacterial expression and purification of (Ala$^{21}$-Ile$^{318}$)proMT1-MMP

The cDNA for the full-length enzyme was generously provided by Dr. Bartnik (Hoechst AG, Wiesbaden). The cDNA fragment encoding Ala$^{21}$–Ile$^{318}$ of proMT1-MMP (numbering includes the signal peptide sequence) was amplified as one product from cDNA by PCR using sense primers with a unique *Nde*I restriction site and antisense primers with a unique *Bam*HI site. The *Nde*I-*Bam*HI fragment encod-

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*Abbreviations:* A, absorbance; Amp, ampicillin; APMA, 4-aminophenylmercuric acetate; BPB, bromophenol blue; cDNA, copy DNA; DMSO, dimethyl sulfoxide; ib, inclusion body; LB, Luria-Bertani (broth); Mca peptide, (7-methoxycoumarin-4-yl)acetyl-Pro-Leu-Gly-Leu-(3-[2,4-dinitrophenyl]-L-diaminopropionyl)-Ala-Arg-$NH_2$; MMPs, matrix metalloproteinases; MT-MMP, membrane-type matrix metalloproteinase; cdMT-MMP, catalytic domain of MT-MMP; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate buffered saline; PCR, polymerase chain reaction; proMMP, proenzyme form of MMP; SDS, sodium dodecyl sulfate; TIMP, tissue inhibitor of metalloproteinases; TM, transmembrane

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ing ($Ala^{21}$-$Ile^{318}$)proMT1-MMP was subcloned between the *Nde*I and *Bam*HI sites of the T7 expression vector pET11a (Novagen) and was used to transfect *E. coli* strain BL21[DE3] [16]. ($Ala^{21}$–$Ile^{318}$)proMT1-MMP was expressed as insoluble inclusion body material. Solubilization was achieved by resuspending the inclusion bodies in buffer (50 mM Tris-HCl pH 8.5, 6 M urea, 1 mM EDTA, 150 mM 2-mercaptoethanol) with Ultra-Turrax treatment. The enzyme was purified using a Sephacryl S-200 column (Pharmacia) equilibrated with S-200 buffer (50 mM Tris-HCl pH 8.5, 4 M urea, 200 mM NaCl, 80 mM 2-mercaptoethanol). The eluted protein was dialyzed twice against 5 l PBS buffer containing 100 μM $ZnCl_2$. Under these conditions, the recombinant protein remained soluble and was correctly folded.

### 2.2. Gelatin zymography

Zymography (substrate gel electrophoresis) was conducted with 0.1% gelatin polymerized into standard Laemmli SDS gels (10% acrylamide) and samples were applied without reduction, as previously described [17,18]. SDS gels were run at 20 mA. The SDS was removed after electrophoresis by washing the gels in 2.5% Triton X-100 for 30 min with one change of washing. The gelatin and casein gels were incubated at 37°C for 2 h in incubation buffer (5 mM Tris-HCl pH 7.5, 200 mM NaCl, 10 mM $CaCl_2$ and 100 μM $ZnCl_2$). The gelatinolytic and caseinolytic activities were visualized by staining the gels with 0.01% Coomassie brilliant blue R 250 in ethanol/acetic acid/water (3:1:6, v:v:v) and destaining in water overnight. Zones of proteinase activity were detected as negatively stained bands. Gelatinolytic activity was quantitated by densitometric measurement of the bands seen on the gels.

### 2.3. Activation of progelatinase A and progelatinase A/TIMP-2 complex

Progelatinase A (proMMP-2, 72kDa type IV gelatinase, EC 3.4.24.24) and progelatinase A/TIMP-2 complex were prepared according to an established procedure [19]. The assay buffer (used in all assays, unless otherwise noted) was 20 mM Tris-HCl pH 7.3, 100 mM NaCl, 5 mM $CaCl_2$, 100 μM $ZnCl_2$, 0.02% $NaN_3$. Maximum gelatinase A activity was generated by the incubation of 7.5 μl progelatinase A (15.6 pmol) with 1.5 μl cdMT1-MMP (4.2 pmol) at 37°C for 90 min. The activation of progelatinase A complexed with TIMP-2 was performed with an excess of cdMT1-MMP.

### 2.4. Kinetic studies

Bovine TIMP-2 was kindly provided by Dr. Calvete (Medizinische Hochschule Hannover, Hannover, Germany). The (7-methoxycoumarin-4-yl)acetyl-Pro-Leu-Gly-Leu-(3-[2,4-dinitrophenyl]-L-2,3-diamino-propionyl)-Ala-Arg-$NH_2$ (Mca substrate) [20] is an internally quenched fluorescent peptide cleaved by various matrix metalloproteinases at the Gly-Leu bond. Fluorimetric analysis was carried out using a Perkin-Elmer LS50 B spectrofluorimeter at 25°C. Fluorescence was determined at an excitation wavelength of 328 nm and emission at 393 nm. For the $K_m$ determination the assay mixture contained 330 ng cdMT1-MMP and different concentrations (0.17–0.43 μmol/l) of substrate (100 μg/ml in 15% DMSO) in 2 ml of assay buffer (20 mM Tris-HCl pH 7.3, 100 mM NaCl, 5 mM $CaCl_2$, 100 μM $ZnCl_2$, 0.02% $NaN_3$). The initial rate of cleavage of Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-$NH_2$ measured over 100 s was proportional to the substrate concentration. The assay of the inhibition of cdMT1-MMP by bovine TIMP-2 was carried out by preincubating the cdMT1-MMP (14 nmol) and TIMP-2 (0–9 nM) in assay buffer at 37°C for 15 min. Activity was measured for three different Mca peptide substrate concentrations at 25°C by following release of fluorescence with time.

### 2.5. Amino acid sequence determination

Amino-terminal sequence determinations were performed as published [21] using a microsequencer (Modell 810, Knauer, Berlin, Germany) and a modification of the method of Hunkapiller [22].

## 3. Results

### 3.1. Purification and activation of ($Ala^{21}$–$Ile^{318}$)proMT1-MMP

The coding region of ($Ala^{21}$–$Ile^{318}$)proMT1-MMP was amplified by PCR and expressed in *E. coli* utilizing the inducible T7 polymerase pET system. The recombinant protein was routinely accumulated in inclusion bodies to a level of 20% of the total wet weight of the *E. coli* mass. The inclusion body fraction was recovered from lysed bacteria, solubilized in 6 M urea and purified to approximately 95% homogeneity by Sephadex S-200 gel permeation chromatography as determined by reduced SDS-PAGE. The proenzyme migrated at the expected $M_r$ of 33.000 under reducing conditions. The N-terminal amino acid sequence of ($Ala^{21}$–$Ile^{318}$)proMT1-MMP (45 cycles) was found to be identical to the predicted propeptide sequence for the wild-type enzyme defined by Takino et al. [23]. In a typical purification 0.18 g of recombinant ($Ala^{21}$–$Ile^{318}$)proMT1-MMP was purified from 1 l of culture medium (Fig. 1A). Folding of the purified protein to the active enzyme was achieved by repeated dialysis against PBS buffer contain-

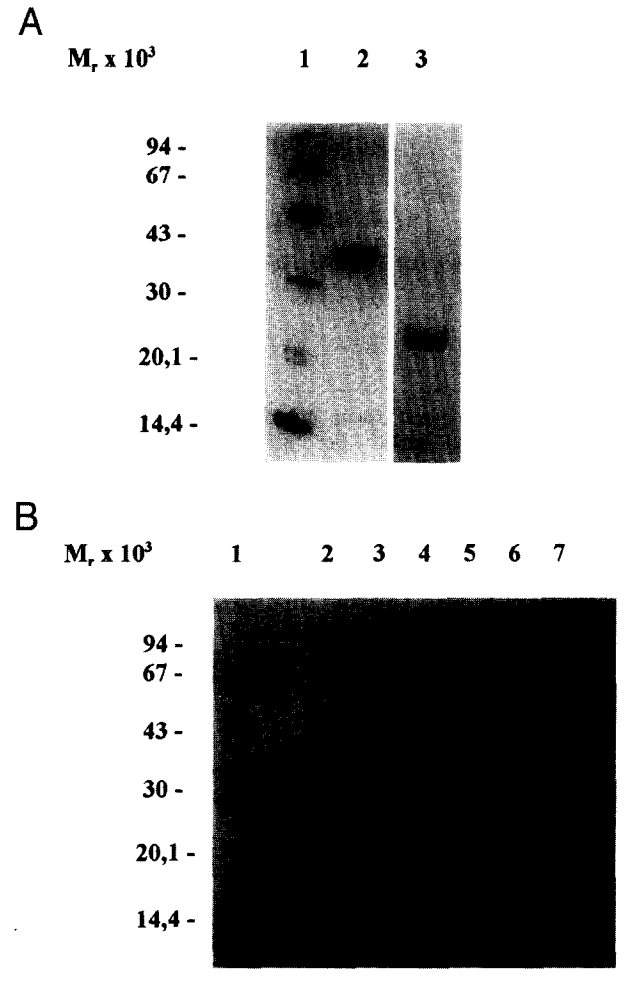


Fig. 1. A: SDS-PAGE analysis of the purification of recombinant ($Ala^{21}$–$Ile^{318}$)proMT1-MMP following Sephadex S-200 gel chromatography. The homogeneity is documented after silver staining. Lane 1: protein $M_r$ markers (low range standards); lane 2: purified ($Ala^{21}$–$Ile^{318}$)proMT1-MMP under denaturating conditions; lane 3: totally activated cdMT1-MMP followed by incubation at room temperature overnight. B: SDS-PAGE of the autoproteolytic activation of ($Ala^{21}$–$Ile^{318}$)proMT1-MMP under non-denaturating conditions. Aliquots of the refolded purified enzyme were incubated at room temperature over a period of 4 h and the reactions were terminated by addition of sample buffer (50 mM Tris-HCl pH 7.5, 30 mM NaCl, 5 M urea, 1% (v/v) 2-mercaptoethanol, 1% (w/v) SDS, 0.05% (w/v) BPB). Lane 1: protein markers (low range standards); lane 2: sample after concentration by ultrafiltration, maintained at 4°C; lanes 3–7: samples after incubation at room temperature for 15, 30, 60, 90 and 120 min, respectively.

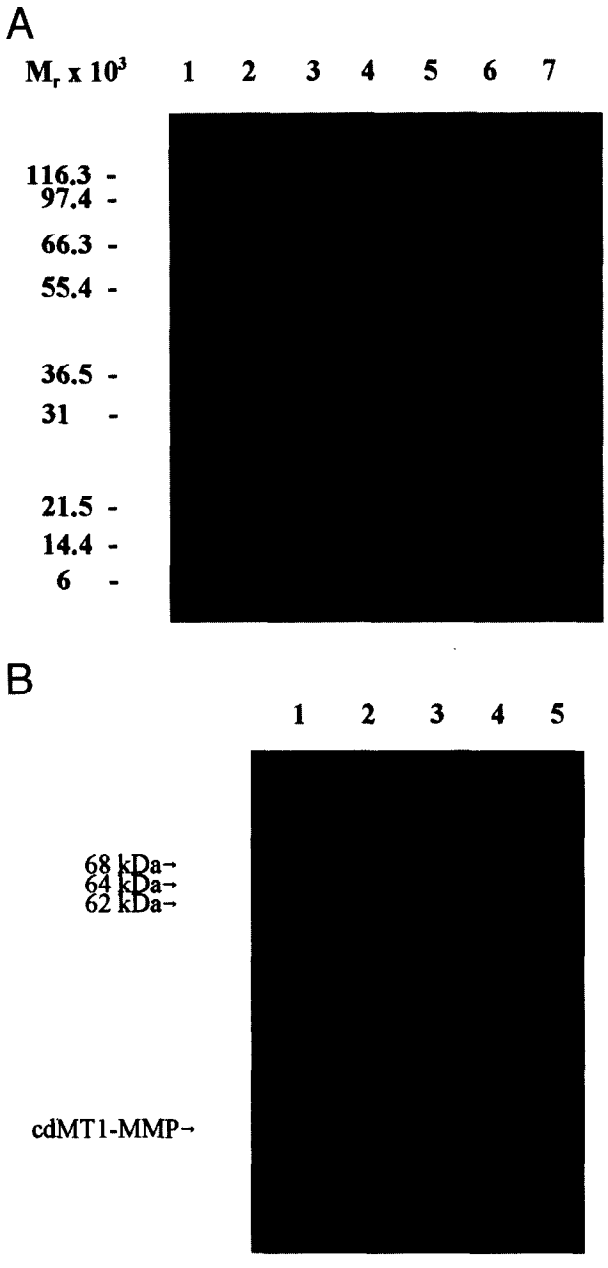


Fig. 2. Activation of progelatinase A by cdMT1-MMP. A: Aliquots of progelatinase A (15.6 pmol) were incubated with cdMT1-MMP (4.2 pmol) at various times and then subjected to SDS-PAGE (10% acrylamide). Lane 1: protein $M_r$ markers (wide range standards); lane 2: progelatinase A (control); lane 3: cdMT1-MMP; lanes 4–7: reaction mixture after exposure to cdMT1-MMP at 37°C for 15, 30, 60 and 90 min. B: Gelatin zymogram of progelatinase A activated by cdMT1-MMP. 150 ng progelatinase A was incubated with 100 ng of cdMT1-MMP for 30 min at 37°C. Lane 1: control: progelatinase A in the absence of cdMT1-MMP. Lanes 2–5: progelatinase A after incubation with cdMT1-MMP for 5, 15, 30 and 60 min.

ing 100 μM $ZnCl_2$. During the refolding procedure and the following protein reconcentration the truncated form of proMT1-MMP immediately autoactivated via successive cleavages of the propeptide to yield the stable active form of the catalytic domain. Maximal activated cdMT1-MMP was stable without loss of activity even after storage at 23°C for several days, although the proenzyme showed spontaneous autoproteolytic activation when stored at 4°C. During activation (Ala$^{21}$–Ile$^{318}$)proMT1-MMP of $M_r$ 33000 was processed initially to a major molecule of about $M_r$ 24000 and was then converted to the minor 22500 species (Fig. 1B). Both species showed proteolytic activity in gelatin (or casein) substrate gels. N-terminal amino acid sequencing of the products revealed that in both samples the Ala$^{113}$–Ile$^{114}$ peptide bond, two amino acids C-terminal of the RRKR sequence – a potential processing site for subtilisin-like enzymes – was cleaved. The generation of the minor form originates from an additional cleavage at the C-terminal site of the recombinant variant of MT1-MMP. Interestingly, the identified N-terminus is not identical with that reported for the processed full-length enzyme at Tyr$^{112}$ [15,24].

### 3.2. *Activation of progelatinase A and progelatinase A complexed with TIMP-2*

We demonstrated that C-terminal truncated cdMT1-MMP initiates activation of progelatinase A and progelatinase A complexed with TIMP-2 in vitro. Processing was monitored by SDS gels and gelatin zymography (Figs. 2 and 3). In gelatin substrate gels the 72 kDa progelatinase migrates as two faint bands, one at 68 kDa representing progelatinase A activated during the development of the zymogram by SDS [25], and a second one at 62 kDa originating possibly from autoactivation during the preparation (see Fig. 3B). Purified cdMT1-MMP itself also demonstrated gelatinolytic activity in the zymogram. However, for an equal proteolytic degradation of gelatin an enzyme level higher than that of gelatinase A was required. As shown in Fig. 3B lane 2 cdMT1-MMP dependent activation of progelatinase A followed a two step mechanism. The catalytic domain of MT1-MMP initially cleaved progelatinase A into a 64 kDa fragment, which was subsequently processed to a 62 kDa product. N-terminal sequence analysis of both gelatinase A fragments (5 cycles) revealed Leu$^{38}$ for the 64 kDa intermediate and Tyr$^{81}$ for the 62 kDa species. The minor fragment is known to be the final autocatalytic cleavage product when exposed to APMA [10]. Densitometric analysis of gelatinase A activation showed that 5 min after incubation with cdMT1-MMP at 37°C the percentage of proMMP-2 decreases to 23%. 1 h after incubation at 37°C the proform totally disappeared; the active form represents 48% of the reaction mixture. A distinct difference in the rate and extent of activation of the 72 kDa progelatinase was observed by treatment of the TIMP-2 free and TIMP-2 complexed proenzyme with cdMT1-MMP for varying time periods (Figs. 2 and 3). TIMP-2 free progelatinase A was more rapidly converted to lower molecular weight species than the TIMP-2 complexed form of the 72 kDa progelatinase. Furthermore, an excess amount of cdMT1-MMP was needed to activate the progelatinase A/TIMP-2 complex because the complex is a strong inhibitor for active MMPs [19,26].

### 3.3. *Kinetic studies*

In kinetic experiments we found that cdMT1-MMP cleaved the synthetic fluorogenic substrate Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-$NH_2$ at the Gly-Leu bond. The initial linear rates of peptide hydrolysis by a constant amount of cdMT1-MMP were determined in the presence of six different concentrations of the substrate. The resulting data were used in a Lineweaver-Burk plot (Fig. 4), from which the $K_m$ value of $5.65 \pm 0.26$ μM ($k_{cat}/K_m = 8.27 \times 10^8$ $s^{-1}$ $M^{-1}$) was calculated. In the case of MMP-2 the $K_m$ for the hydrolysis of Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-$NH_2$ was reported to be 70 μM

($k_{cat}/K_m = 6.29 \times 10^6$ $s^{-1}$ $M^{-1}$) [20] and $3.86 \pm 0.6$ μM ($k_{cat}/K_m = 1.69 \times 10^8$ $s^{-1}$ $M^{-1}$) for the catalytic domain of MMP-8 (M. Pieper, personal communication). This kinetic study revealed that the C-terminal truncated membrane-type metalloproteinase generally behaves like other catalytic domains of the MMP family because their $K_m$ values and their values of the specificity constants $k_{cat}/K_m$ were comparable. Comparison of the values of $k_{cat}/K_m$ for the catalytic domain of MT1-MMP and full-length MMP-2, however, showed that efficiency of substrate catalysis by cdMT1-MMP is increased 100-fold. These kinetic parameters must reflect a more favorable binding of the Mca substrate. The equilibrium inhibition constant for bovine TIMP-2 and cd1MT-MMP was determined in a similar manner. The addition of increasing amounts of TIMP-2 to a constant amount of cdMT1-MMP resulted in a decrease in enzyme activity. The $K_i$ value of TIMP-2 (104 pM) was determined from Dixon plots (Fig. 5). Our study indicated that TIMP-2 has a high affinity for cdMT1-MMP. It is evident that the C-terminal domain of MT1-MMP is not essential for inhibitor binding.

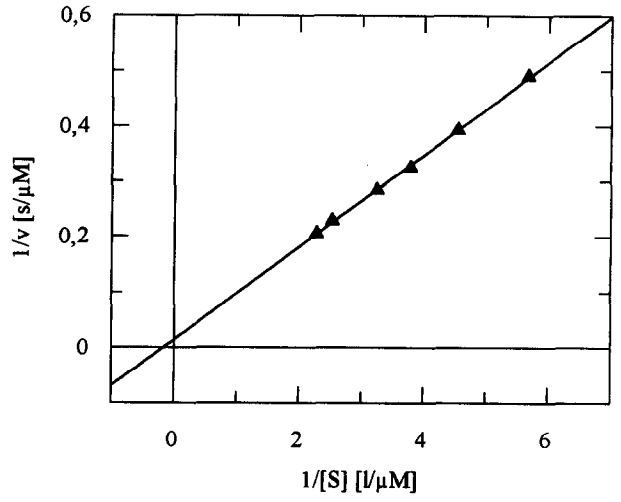


Fig. 4. Lineweaver-Burk plot for the digestion of the quenched fluorescent substrate Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-$NH_2$ by the catalytic domain of MT1-MMP. Assays were conducted under the conditions described in Section 2. The digestion of the Mca peptide was measured at six different substrate concentrations. Values are the means of three determinations.

## 4. Discussion

### 4.1. Activation of MT1-MMP

Our in vitro studies revealed that the zymogen form of MT1-MMP can be converted to the catalytic active enzyme (independent of other proteases or organomercury compounds) in an autocatalytic process. We found the amino-terminal residue of the mature protein to be $Ile^{114}$. ($Ala^{21}$–$Ile^{318}$)proMT1-MMP showed high autoproteolytic activity to such a degree that we failed to isolate the proform under non-denaturing conditions. The fact that the generated catalytic domain remains intact and keeps its activity even after storage at room temperature for several days could perhaps be explained by an optimal stabilization of the enzyme conformation. The N-terminal residue of most fully processed MMPs is often a phenylalanine or tyrosine, with the exception of mouse metalloelastase (leucine) [27]. From the crystal structure of the 'superactive' catalytic domain of neutrophil collagenase (MMP-8) it is known [28] that the N-terminal $Phe^{79}$ interacts with $Asp^{232}$ through a surface-located salt bridge formed between the N-terminal ammonium group and the carboxylate of $Asp^{232}$ which is strictly conserved in collagenases, resulting in an optimal conformation for catalytic activity. In the catalytic domain of MT1-MMP a tandem of three aspartates N-terminal of the putative 'Met-turn' occurs. Thus, it can perhaps be assumed that in a topologically equivalent manner the ammonium group of $Ile^{94}$ forms a salt bridge with the carboxylate moiety of one of these conserved aspartates. So far this phenomenon of 'superactivation' has only been observed in species having $Phe^{79}$ as N-terminal residue of the active form [29–31]. It remains to be determined whether MT-MMPs are processed by similar autocatalytic mechanisms in vivo. A number of studies have pointed out that MT1-MMP, like stromelysin-3, displays a four residue consensus sequence (RRKR or RQKR in stromelysin-3) which constitutes a prospective cleavage site for the subtilisin-like serine protease furin. Pei and Weiss determined that activation of MT1-MMP occurred by a proprotein convertase dependent pathway and found that the final cleavage site is the $Arg^{111}$-$Tyr^{112}$ peptide bond [16].

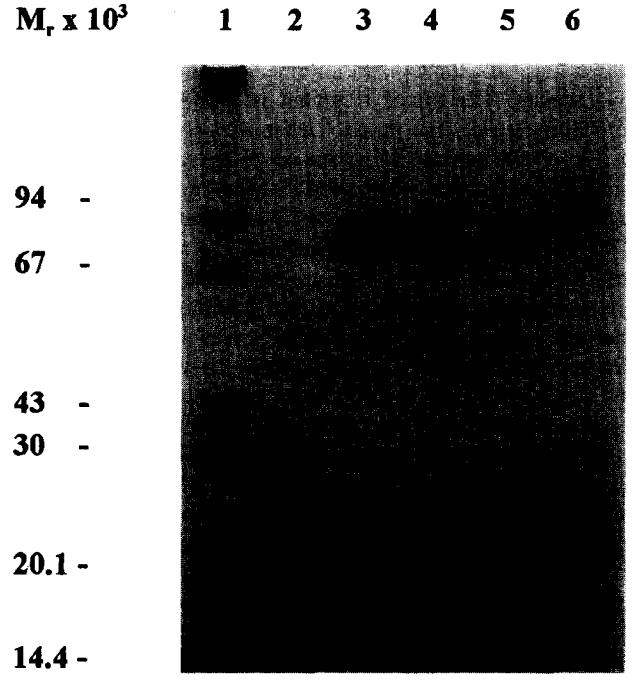


Fig. 3. Activation of progelatinase A complexed with TIMP-2 by cdMT1-MMP.The 72 kDa progelatinase A/TIMP-2 complex (210 ng samples each) was incubated with an excess of cdMT1-MMP for varying times at 37°C. It was then analyzed by SDS-PAGE (10% acrylamide). Lane 1: protein $M_r$ markers (low range standards); lane 2: cdMT1-MMP; lanes 3–5: proMMP-2/TIMP-2 complex in the presence of cdMT1-MMP incubated at 37°C for 15, 30 and 60 min respectively; lane 6: control: proMMP-2/TIMP-2 complex, incubation at 37°C for 60 min without cdMT1-MMP.

### 4.2. Activation of progelatinase A and progelatinase A complexed with TIMP-2

Observations that progelatinase A could be activated by components of fibroblast cell membranes led to the proposal that the in vivo activation might be a specific cell surface event [11,32]. During cell membrane-induced processing a progela-

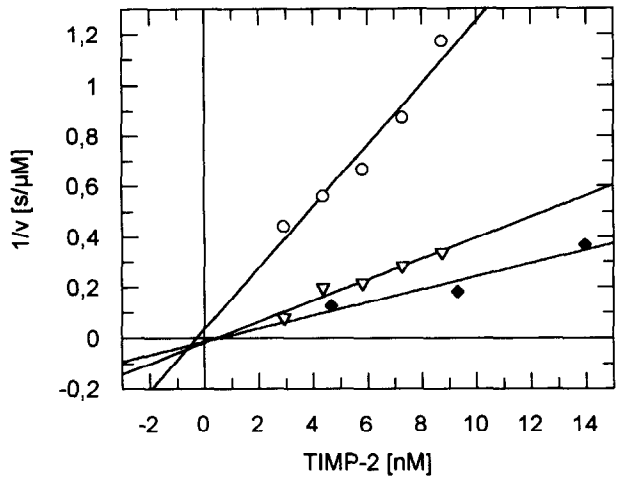


Fig. 5. Dixon plot for the inhibition of cdMT1-MMP by TIMP-2. A constant amount of truncated MT1-MMP (14 nM) was incubated with increasing amounts of TIMP-2 (0–9 nM) for 15 min at 37°C and assayed for its ability to cleave the fluorescent Mca peptide substrate. Inhibition experiments were carried out at three different substrate concentrations. Values are the means of four experiments.

tinase A intermediate cleaved at $Asn^{37}$-$Leu^{38}$ had been identified, which is further cleaved at $Asn^{80}$-$Tyr^{81}$. The possibility of a plasma membrane based activation process for the 72 kDa progelatinase has recently been substantiated by the important discovery of membrane-bound MMPs [13]. Strongin and co-workers [24] demonstrated that the formation of a ternary complex consisting of progelatinase A, TIMP-2 and activated MT1-MMP is necessary for proMMP-2 activation. Moreover, Cao et al. [14] stated that the C-terminal transmembrane domain is required as an essential structural element for the proteolytic activation of progelatinase A. In contrast to the above mentioned investigation the results of our in vitro studies demonstrate that processing of proMMP-2 to its mature form by MT1-MMP is independent of cell surface localization of the enzyme. cdMT1-MMP can initiate proMMP-2 activation via a two step mechanism: the cleavage of the $Asn^{37}$-$Leu^{38}$ peptide bond is followed by a cleavage of the $Asn^{80}$-$Tyr^{81}$ bond generating fully active gelatinase A. The precise mechanism of proMMP-2 activation in vivo is complicated by the presence of TIMP-2. Strongin et al. [24] recently reported that the MT1-MMP/TIMP-2 complex acts as the surface receptor for the gelatinase zymogen. The data presented here suggest that TIMP-2 and MMP-2/TIMP-2 complex are physiological inhibitors of MT1-MMP. It is likely that activation of proMMP-2 is a very complex process. Knowledge of the TIMP-2 binding affinities of both enzymes would help to clarify these still unsolved interactions.

### 4.3. Inhibition of cdMT1-MMP by TIMP-2

Among the well known MMP antagonists including chelating agents, substrate analogues and $\alpha_2$-macroglobulin the TIMPs probably have the most physiological relevance. Strongin et al. [24] reported that latent progelatinase A may bind to cell surface receptors (MT1-MMP) by interaction with TIMP-2. They suggested further that activation of proMMP-2 would require an assembly of a trimolecular stoichiometric complex involving the C-terminus of MMP-2, TIMP-2 and MT1-MMP. Small amounts of TIMP-2 should increase the affinity of MT1-MMP for progelatinase A. An excess of TIMP-2 completely inhibited proMMP-2 binding. Stronging and coworkers concluded that the cell surface receptor (MT1-MMP) saturated with TIMP-2 would not be able to bind the proMMP-2/TIMP-2 complex. Our present results show that the catalytic domain is able to activate proMMP-2 in the absence of TIMP-2. It can therefore be concluded that activation of proMMP-2 may be independent of a plasma membrane associated trimolecular non-covalent complex of MT1-MMP/TIMP-2 and progelatinase A and could proceed easily by shedded MT1-MMP. Furthermore, we found that TIMP-2 exerts a strong inhibitory effect on cdMT1-MMP with a $k_i$ value in the picomolar range. These results support the contention that the regulation of progelatinase A activation probably cannot be explained by a simple model of a trimolecular complex. Our observations point to the involvement of multiple factors in the regulation of progelatinase A activation. We suggest that to maintain proenzyme converting activity, the balance of proteases (MT1-MMP, progelatinase A and probably additional, unidentified ones) and inhibitors (TIMPs) is essential. It is conceivable that cell-cell interactions might influence this balance between MMPs and TIMPs.

*Note added in proof:* A paper on activation of progelatinase A by a soluble form of MT1-MMP comfirming these results appeared by Will, H., Atkinson, S.J., Butler, G.S., Smith, B. and Murphy, G. (1996) J. Biol. Chem. 271, 17119–17123.

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## References

[1] Birkedal-Hansen, H., Moore, W.G., Bodden, M.K., Windsor, L.J., Birkedal-Hansen, B., DeCarlo, A. and Engler, J.A. (1993) Crit. Rev. Oral Med. 4, 197–250.
[2] Stetler-Stevenson, W.G., Aznavoorian, S. and Liotta, L.A. (1993) Annu. Rev. Cell. Biol. 199, 541–573.
[3] Birkedal-Hansen, H. (1995) Curr. Opin. Cell. Biol 7, 728–735.
[4] Mignatti, P., Robbins, E. and Rifkin, D.B. (1986) Cell 47, 487–498.
[5] Alexander, C.M. and Werb, Z. (1992) Cell Biology of the Extracellular Matrix (Hay, E.D., Ed.) pp. 255–302. Plenum, New York.
[6] Matrisian, L.M. (1992) BioEssays 14, 455–463.
[7] Timpl, R., Martin, G.R., Bruckner, P., Wick, G. and Wiedemann, H. (1986) Eur. J. Biochem. 84, 43–52.
[8] Crouch, E., Sage, H. and Bornstein, P.(1980) Proc. Natl. Acad. Sci. USA 77, 745–749.
[9] Nagase, H., Ogato, Y., Suzuki, K., Enghild, I.J. and Salvesen, G.(1991) Biochem. Soc. Trans. 19, 715–718.
[10] Okada, Y., Morodomi, T., Enghild, I.J., Suzuki, K., Yasui, A., Nakanishi, I., Salvesen, G. and Nagase, H. (1990) Eur. J. Biochem. 194, 721–730.
[11] Strongin, A.Y., Marmer, B.L., Grant, G.A. and Goldberg, G.I. (1993) J. Biol. Chem. 268, 14033–14039.
[12] Lees, M., Taylor, D.J. and Woolley, D.E.(1994) Eur. J. Biochem. 223, 171–177.
[13] Sato, H., Takino, T., Okada, Y., Cao, J., Shinagawa, A., Yamamoto, E. and Seiki, M. (1994) Nature 370, 61–65.
[14] Cao, J., Sato, H., Takino, T. and Seiki, M. (1995) J. Biol. Chem. 270, No. 2, 801–805.
[15] Pei, D. and Weiss, S.J. (1996) J. Biol. Chem. 271, 9135–9140.
[16] Studier, F.W., Rosenberg, A.H., Dunn, J.J. and Dubendorff, I.W. (1990) Methods Enzymol. 185, 60–89.
[17] Birkedal-Hansen, H. and Taylor, R.E. (1982) Biochem. Biophys. Res. Commun. 107, 1173–1178.

[18] Hanemaaijer, R., Koolwijk, P., Leclerq, L., Devrel, W.J.A. and van Hinsbergh, V.W.M. (1993) Biochem. J. 296, 803–809.
[19] Kolkenbrock, H., Orgel, D., Hecker-Kia, A., Noack, W. and Ulbrich, N. (1991) Eur. J. Biochem. 198, 775–781.
[20] Knight, C.G., Willenbrock, F. and Murphy, G. (1992) FEBS Lett. 296, 263–266.
[21] Knäuper, V., Krämer, S., Reinke, H. and Tschesche, H. (1990) Eur. J. Biochem. 189, 295–300.
[22] Hunkapiller, M.W. (1985) Applied Biosystems User Bulletins 14, Protein Sequencer.
[23] Takino, T., Sato, H., Shinagawa, A. and Seiki, M. (1995) J. Biol. Chem. 270, 39, 23013–23020.
[24] Strongin, A.Y., Collier, I., Bannikov, G., Marmer, B.L., Grants, G.A. and Goldberg, G.I. (1995) J. Biol. Chem. 270, 5331–5338.
[25] Springman, E.B., Angleton, E.L., Birkedal-Hansen, H. and van Ward, H.E. (1990) Proc. Natl. Acad. Sci. USA 87, 364–368.
[26] Kolkenbrock, H., Hecker-Kia, A., Orgel, D., Ruppitsch, W. and Ulbrich, N. (1994) Biol. Chem. Hoppe-Seyler 375, 589–595.
[27] Shapiro, S.D., Griffin, G.L., Gilbert, D.J., Jenkins, N.A., Copeland, N.G., Welgus, H.G., Senior, R.M. and Ley, T.J. (1992) J. Biol. Chem. 267, 4664–4671.
[28] Reinemer, P., Grams, F., Huber, R., Kleine, T., Schnierer, S., Pieper, M., Tschesche, H. and Bode, W. (1994) FEBS Lett. 338, 227–233.
[29] Suzuki, K., Enghild, I. Morodomi, T., Salvsen, G. and Nagase, H. (1990) Biochemistry 29, 10261–10270.
[30] Knäuper, V., Wilhelm, S.M., Sepernack, P.K., DeClerk, Y.A., Langley, K.E., Osthues, A. and Tschesche, H. (1993) Biochem. J. 295, 581–586.
[31] He, C., Wilhelm, S.M., Pentland, A.P., Marmer, B.L., Grant, G.A., Eisen, A.Z. and Goldberg, G.I. (1989) Proc. Natl. Acad. Sci. USA 86, 2632–2636.
[32] Murphy, G., Willenbrock, F., Ward, R.V., Cockett, M.I., Eaton, D. and Docherty, A.J.P. (1992) Biochem. J. 283, 637–641.