The propeptide domain of secreted matrix metalloproteinases (MMPs) is responsible for maintaining the latency of these proteinases. Recently, the propeptide domain of the prototype membrane type matrix metalloproteinase (MT1-MMP) was demonstrated to act as an intramolecular chaperone (Cao, J., Hymowitz, M., Conner, C., Bahou, W. F., and Zucker, S. (2000) J. Biol. Chem. 275, 10917–10922). In the current study, the role of an unique four-amino acid sequence in the propeptide domain of MT1-MMP was examined. The sequence 42YGYL45 is conserved in the propeptide domain of all six members of the MT-MMP subfamily, but not in secreted MMPs. Mutant MT1-MMP cDNAs coding for alanine substitutions (single and double amino acid sequences) in this conserved propeptide region were transfected into COS-1 cells deficient in endogenous MT1-MMP. As demonstrated by immunofluorescence, mutant MT1-MMP protein was synthesized and displayed on the plasma membrane of transfected cells. Alanine substitutions within the 42YGYL45 sequence proved to be detrimental for enzyme function in terms of activation of proMMP-2 and binding TIMP-2 to the cell surface (MT1-MMP serves as a cell surface receptor for TIMP-2). In contrast to wild-type MT1-MMP-transfected cells, mutant MT1-MMP-transfected cells were incapable of degrading and migrating on a fibronectin substrate. These data indicate that the conserved 42YGYL45 sequence within the propeptide domain of MT-MMPs is required for intramolecular chaperone function of these intrinsic membrane proteinases.

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Matrix metalloproteinases (MMPs)† comprise a family of zinc-dependent enzymes that share a common domain structure and are involved in the degradation of various extracellular matrix and non-matrix components (1–3). The MMP family includes proteinases secreted from cells aszymogens, which become activated in the extracellular space, as well as membrane type MMPs (MT-MMPs), which have a hydrophobic transmembrane domain and a short cytoplasmic domain. The structure of MT-MMP genes differs from other MMP genes in exon organization, suggesting a relatively earlier divergence in evolution (4, 5). Activation of the MT-MMPs has been reported to occur in the trans-Golgi network by furin-mediated cleavage within the RRKR sequence of the propeptide domain. Indirect evidence suggests critical regulation of trafficking of the active proteinase to the plasma membrane (6–14).

Numerous functions for MT1-MMP, the most abundant and active MT-MMP, have been described in various biological settings; however, key structural elements that influence the activity of the enzyme still remain to be elucidated. In particular, the role of the MT1-MMP propeptide domain has not been established. The primary role of the propeptide region of secreted MMPs involves maintaining the latency of the proenzymes (1, 2). In the case of MT1-MMP, the propeptide region appears to mediate additional functions that are critical for enzymatic activity (6, 15). Recent studies indicate that the propeptide of MT1-MMP acts as an intramolecular chaperone for the enzyme (16). Genetically engineered MT1-MMP lacking the propeptide domain, ΔproMT1-MMP, is not functionally competent when expressed on the surface of transfected cells, despite the fact that this recombinant protein contains the essential sequence information of the mature form of MT1-MMP. Co-transfection of incompetent cells with propeptide cDNA of MT1-MMP in trans along with ΔproMT1-MMP restores proteolytic activity. Chaperone function has not been attributed to the propeptides of secreted MMPs. Specifically, it has been demonstrated that the propeptide of MMP-3 does not serve as a chaperone for this secreted enzyme (17).

The promembrane-bound bacterial proteinases, such as subtilisin and α-lytic protease (18–20), as well as that of human cysteine proteinases, cathepsin C and cathepsin S (21, 22), confer chaperone function to the respective enzymes. Propeptide-deleted forms or mutants in critical amino acids required for the chaperone function of these enzymes are misfolded and inactive. Denatured mature proteinases lacking the propeptide domain can be refolded properly in the presence of their respective propeptide domains and restore the enzymatic function (18).

Although membrane type MMPs share structure homology with secreted MMPs, they also contain unique sequences that may confer different features to the enzymes of this subgroup.
In this study we have addressed the role in folding and trafficking of MT1-MMP of a unique sequence in the propeptide of MT1-MMP, which is remarkably conserved in all six members of the membrane type MMPs, but not in secreted MMPs (Fig. 1A). To assess the contribution of this four-amino acid sequence, G42YGYL, to the chaperone activity of the propeptide, we targeted the sequence for mutational analysis. Constructs containing Ala substitutions for a single or a pair of adjacent residues in this sequence were expressed in COS-1 cells and tested in this function in terms of binding TIMP-2 and activation of proMMP-2. The function of the MT1-MMP constructs was also evaluated in the terms of the ability of respective transfected cells to migrate in situ over a fibronectin substrate (23). The study of cell migration, an important MT1-MMP-requiring event (24–29), provides the opportunity to better determine whether conserved sequences within the propeptide domain constitute key structural elements required for MT1-MMP function.

In the studies described here, mutations in this conserved G42YGYL region were demonstrated to be detrimental for MT1-MMP function. These results indicate that this four-amino acid sequence within the propeptide domain of MT1-MMP contains important conformational information for the folding of MT1-MMP and constitutes an important component of the intramolecular chaperone moiety of the propeptide.

MATERIALS AND METHODS

Reagents—Chemical reagents were purchased from Sigma except for fluorescein isothiocyanate (FITC) hydrochloride, which was purchased from Research Organics (Cleveland, OH). Human fibroblasts was from Invitrogen (Carlsbad, CA). Restriction enzymes were from Roche Molecular Biochemicals. The pSG5 expression vector was from Stratagene (La Jolla, CA).

Monoclonal antibody against the catalytic domain of MT1-MMP (Ab-114) was purchased from Calbiochem (San Diego, CA), polyclonal antibodies against the hinge region of MT1-MMP (Ab815) were from Chemicon International (Temecula, CA). Polyclonal antibodies against TIMP-2 were from Oncogene Research (Cambridge, MA), and monoclonal antibody against the HA epitope was from Roche Molecular Biochemicals. Horseradish peroxidase-conjugated anti-mouse or anti-rabbit antibodies were both from Amersham Biosciences, Inc. Anti-fibronectin antibody was from Genzyme Corporation. Anti-rabbit antibody was from Rockland (Gilbertsville, PA). Human proMMP-2 was purified from transfected COS-1 cells propagated in 24-well dishes was performed in duplicate as described previously (6). For equilibrium binding experiments, dilutions of 125I-labeled TIMP-2 (0.3–6 nm) in bovine serum albumin/PBS/Ca2+ were added to the cells in the presence or absence of excess unlabeled recombinant TIMP-2. After 3 h incubation at 4 °C, supernatant fluid and washed cells were counted in a gamma counter. Cell monolayers were then lysed in 0.1% SDS in 0.1 n NaOH and collected as the bound fraction. Bound and unbound 125I-TIMP-2 was measured by y counting.

For the detection of plasma membrane-bound MT1-MMP by immunofluorescence, fixed cells were subsequently treated as follows. After washing with PBS and blocking with 0.1% bovine serum albumin in PBS, cells were incubated with polyclonal antibody against the hinge region of MT1-MMP for 2 h at room temperature, washed four times with PBS, incubated with Texas Red isothiocyanate-conjugated goat anti-rabbit secondary antibody for 1 h at room temperature, and washed four times with PBS.

When detection of TIMP-2 (endogenous TIMP-2) bound on the cell surface or HA epitope was required, respective monoclonal antibodies against TIMP-2 or HA epitope were included in the solution of primary antibody against MT1-MMP, followed by incubation with a mixture of FITC-labeled anti-mouse antibodies together with the TRITC-labeled anti-rabbit antibody. Coverslips were then washed with PBS and finally mounted on microscope slides over a drop of Vecta-Shield anti-fading mounting solution. Staining was visualized using an Olympus IX70 fluorescence microscope.

Fibronectin Degradation/Cell Migration Assay—FITC-labeled fibronectin was prepared and used for coating glass coverslips as described by Nakahara et al. (23). Briefly, FITC-labeled fibronectin (50 μg/ml) was layered over 12-mm glass coverslips prequently with a thin film of cross-linked gelatin and allowed to bind at room temperature for 1 h. Coverslips were then washed with PBS, sterilized with 70% ethyl alcohol.
Fig. 1. A, similarity plots and multiple sequence alignment for the propeptide domain of MMP members. Following multiple sequence alignment, the similarity plots between MT1-MMP and representative members of secreted MMPs (MT1-MMP and secreted MMPs), as well as the similarity plot for all MT-MMP subfamily members (MT-MMPs) were created. The following MMPs were used as representative members of secreted MMPs: MMP-1 for collagenases, MMP-2 for gelatinases, and MMP-3 for stromelysins. For the similarity plot of all members of MT-MMPs the following members were used: MT1-MMP, MT2-MMP, MT3-MMP, MT4-MMP, MT5-MMP, and MT6-MMP. The dotted line shows the average similarity.
MT1-MMP Expression in COS-1 Cells Induces Cell Migration over a Fibronectin Substrate—COS-1 cells, transfected with the plasmid encoding wild-type MT1-MMP as described under “Materials and Methods,” were seeded on coverslips precoated with FITC-labeled fibronectin. Following 17 h of incubation, cells on coverslips were fixed and immunostained for cell-surface MT1-MMP. As shown in Fig. 2 (row B, middle panel), dark areas on the FITC-fibronectin film depict fibronectin degradation occurring contiguous with wild-type MT1-MMP-transfected cells. Examination of films indicate that transfected cells migrated along the degradation path (Fig. 2, row B, right panel, arrow).

MT1-MMP cDNA-transfected cells, positively stained for cell surface expression of the enzyme (Fig. 2, row B, right panel), and degraded fibronectin, whereas mock-transfected cells did not degrade the substrate (Fig. 2, row A, middle panel). These results indicate that the degradation of fibronectin and cell migration are associated with cell surface expression of MT1-MMP.

Incubation of MT1-MMP cDNA-transfected cells in the presence of TIMP-2 inhibited both fibronectin degradation and cell migration (Fig. 2, row D), thus both functions are MMP-dependent. On the other hand, the presence of TIMP-1, which has minimal effect on other MT1-MMP functions (6), had no effect on either fibronectin degradation or cell migration (Fig. 2, row C). These results suggest that degradation of fibronectin and cell migration are MT1-MMP-dependent and are not mediated by the function of a secreted MMP, which would have been inhibited by TIMP-1.

Cell Surface-bound Propeptide-deleted MT1-MMP Is Deficient in Both TIMP-2 Binding and Induction of a Migratory Phenotype in Transfected COS-1 Cells—COS-1 cells were transfected with wild-type MT1-MMP cDNA, a propeptide deletion mutant of MT1-MMP cDNA referred to as ΔproMT1-MMP, or with vector cDNA alone. Specific rabbit anti-MT1-

MT1-MMP antibodies were employed to demonstrate the localization of both wild-type MT1-MMP and Δpro MT1-MMP at the cell surface. To examine cell-surface binding properties, TIMP-2 binding (endogenous TIMP-2) was monitored by immunofluorescence. Incubation with an anti-TIMP-2 mouse monoclonal antibody was followed by incubation with an FITC-labeled anti-mouse IgG antibody. As shown in Fig. 3A, wild-type MT1-MMP cDNA-transfected cells (MT1-MMP) efficiently bound TIMP-2, but ΔproMT1-MMP cDNA-transfected cells (Δpro) did not. TIMP-2 binding was restored for ΔproMT1-

MT1-MMP-expressing cells when they were co-transfected in trans with a plasmid encoding the propeptide domain of MT1-MMP, MTPro (Fig. 3A, MTPRO + ΔproMT1-MMP), although the percentage of positively stained cells was less than that of wild-type MT1-

MT1-MMP-transfected cells.

across the entire propeptide sequence alignment. The position numbers correspond to MT1-MMP sequence. Three regions of above average similarity for the members of MT-MMPs are shown (I, II, III). The same regions are boxed in the sequence alignment. To simplify the diagram, the furin consensus sequence (RRKR, RRRR) common to all MT-MMPs has been excluded from the analysis. Comparison of the similarity plot of MT1-MMP and secreted MMPs and the similarity plot of all MT-MMPs, shows that, although regions II and III are very similar, region I is not similar for secreted MMPs and it shows remarkable similarity for all MT-MMP members. The multiple sequence alignment and similarity plots of the propeptide domain were performed utilizing the Genetics Computer Group (www.gcg.com) program PlotSimilarity SeqWeb version 2 of the Wisconsin Package, version 9.1. B, schematic diagram of MT1-MMP and mutants. MT1-MMP domains are represented by boxes. Propept., propeptide domain of MT1-MMP; TM, transmembrane domain; Cytop., cytoplasmic tail. Domains missing from the deletion mutants are depicted. HA box represents the inserted epitope in the position shown. Alanine substitutions in the full-length mutants contain Ala substitutions in the propeptide domain in amino acid positions shown.
Transfected cells were tested in the fibronectin degradation/migration assay. Although ΔproMT1-MMP cDNA-transfected cells (Fig. 3B, MTΔPro) or MTPro cDNA-transfected cells (data not shown) did not degrade the fluorescein substrate and subsequently migrate, COS-1 cells co-transfected with both ΔproMT1-MMP and MTPro in trans, exhibited both fibronectin degradation and migration similar to that observed with wild-type MT1-MMP cDNA-transfected cells (Fig. 3B). The percent-
age of transfected cells that displayed both degradation and migration was less than the respective wt MT1-MMP-transfected cells; this observation may be attributed to the lower efficiency of co-transfected cells expressing both ΔproMT1-MMP and MTPro cDNA (16). These results indicate that both functions, TIMP-2 binding and induction of the migratory phenotype of MT1-MMP-expressing cells, require cell synthesis of the propeptide domain of MT1-MMP for proper function of MT1-MMP at the cell surface (16).

The Propeptide Domain of MT1-MMP Is Displayed on the Cell Surface of Transfected COS-1 Cells—Previous studies from this laboratory showed that wild-type MT1-MMP is detected as a 63-kDa immunoreactive band in cell lysates of transfected COS-1 cells (6, 15, 16). This molecular size corresponds to the proenzyme form of MT1-MMP. However, these data cannot be used as evidence that MT1-MMP on the plasma membrane exists in the proenzyme form. Reports employing other types of cells have described furin-induced cleavage of MT1-MMP at the RRKR recognition motif in the trans-Golgi network and delivery of the mature (activated) enzyme to the cell surface (Refs. 9 and 32; data not shown from this laboratory). To investigate whether MT1-MMP is displayed on the cell surface in the form of the active enzyme or in the nonproc-...
propeptide region I of proMT1-MMP were prepared and tested using the fibronectin degradation/migration assay.

Three full-length mutants MT1-MMPs, carrying double alanine substitutions in amino acid positions 42–43, 43–44, and 44–45 of the propeptide region (Fig. 1B), were constructed and inserted in pSG5 plasmids. COS-1 cells transiently transfected with the respective mutant cDNAs were subsequently grown on coverslips precoated with FITC-labeled fibronectin as described. Fig. 4 shows that Y42A/G43A-transfected cells were deficient in fibronectin degradation/migration. A similar deficiency was demonstrated with MT1-MMP mutants G43A/Y44A and Y44A/L45A (data not shown). As confirmed by immunofluorescence (Fig. 4, Y42A/G43A, right panel), cells efficiently expressed and displayed the mutant protein on the cell surface of transfected cells; this was also true for G43A/Y44A and Y44A/L45A (data not shown). These data indicate that cell surface-expressed mutant MT1-MMP proteins were defective in degradation/migration on fibronectin.

Membrane-bound mutant enzymes were tested for their ability to activate exogenously added proMMP-2. As confirmed by zymogram of the conditioned media (Fig. 5B, left zymogram), Y42A/G43A, G43A/Y44A, and Y44A/L45A mutant-MT expressing cells show no activation of proMMP-2, whereas the proMMP-2 was effectively activated by wt MT1-MMP-expressing cells. This absence of enzymatic activity could not be attributed to diminished expression of the respective mutants because Western blots of cell lysates, comparing wt MT1-MMP cDNA-transfected cells and respective mutants, did not show significant differences in the amount of nonprocessed 63-kDa MT1-MMP expressed (Fig. 5A, left Western blot).

Mutant MT1-MMP-expressing cells were also tested for their ability to bind 125I-TIMP-2 (Fig. 6, B–D). TIMP-2 bound efficiently to cells expressing wt MT1-MMP, but no significant specific binding was identified with cells expressing Y42A/G43A, G43A/Y44A, or Y44A/L45A mutations of MT1-MMP.

To test the possibility that random double Ala substitution in the propeptide region might have an adverse effect on the function of the expressed enzyme, other pairs of adjacent amino acids upstream or downstream of the conserved peptide region were selected for Ala substitutions. Mutants for amino acid positions 34–35 and 48–49, respectively, were prepared and tested for function. As shown in Fig. 4, S34A/P35A or G48A/D49A were active proteolytically against the fluorescein substrate and subsequently provided respective transfected cells with the ability to migrate along the degradation pathway, although a reduction in the percentage of migrating cells was noted compared with respective wild-type MT1-MMP-transfected cells. The S34A/P35A and G48A/D49A mutants were functional in terms of activation of proMMP-2 (Fig. 5B, right zymogram), but somewhat diminished as compared with wt MT1-MMP. The $K_d$ for 125I-TIMP-2 binding was equivalent, but the number of cell surface TIMP-2 binding sites was diminished for S34A/P35A and G48A/D49A mutants as compared with wt MT1-MMP (Fig. 6, E and F). In addition, the...
108ARAA111 mutant-MT1-MMP (Fig. 1B), which contains three alanine substitutions, showed similar function to the one observed by wild-type MT1-MMP (Figs. 4 and 5B, middle zymogram).

Constructs containing single amino acid alanine substitutions in the same region of MT1-MMP, Y42A, G43A, and Y44A mutants (Fig. 1B) were transfected in COS-1 cells and tested functionally. As shown in Fig. 4, Y42A and G43A-transfected cells were functional in the fibronectin degradation/migration assay (Fig. 4), and in the activation of proMMP-2 (Fig. 5B, middle zymogram), although the percentage of mutant transfected cells performing migration was somewhat reduced compared with wt MT1-MMP-transfected cells; Y44A was nonfunctional in all assays (Figs. 4 and 5B, middle zymogram). These data suggest that the sequence spanning amino acids 42–45 of the propeptide domain is important for MT1-MMP function.

DISCUSSION

Transition of the phenotype of a cell from quiescent to migratory type, has been the focus of intense study aimed at elucidation of components necessary for this transition. MMPs participate in the migratory mechanism by virtue of their role
in extracellular matrix processing that affect cell detachment, cell-cell, and cell-matrix interactions (33, 34). MT1-MMP has been associated with cell migration and invasion (27–29, 35–37). When tumor cells, grown on a fibronectin or gelatin film, are induced to overexpress MT1-MMP, focal proteolysis of substrate occurs, accompanied by cell migration (23, 38). In addition, recent reports relate cell migration with MT1-MMP mediated cleavage of surface receptors involved in cell adhesion to extracellular components (24–26). It needs to be pointed out that the effect of MT1-MMP on cell migration may involve critical intermediate factors not yet characterized. For example, the degradation of extracellular matrix proteins, i.e. fibronectin, may expose cryptic sites within the protein that then promote cell migration; other proteases may be involved in this process (39, 40).

In this study we used a fibronectin degradation/migration assay to examine MT1-MMP function because we considered induction of a migratory phenotype to be an important effect of this MMP on cell behavior. Other studies have depicted different functional features of cell surface-bound MT1-MMP as compared with recombinant soluble transmembrane-deleted MT1-MMP (6, 23, 32, 41). Our study of native membrane-bound MT1-MMP in intact cells permits us to draw conclusions concerning MT1-MMP activity that more closely simulates in vivo function.

We transfected wild-type MT1-MMP cDNA into COS-1 cells and tested the behavior of these cells in the fibronectin degradation/migration assay. Wild-type MT1-MMP induced local degradation of the fibronectin film and migration of the transfected cells along the degradation path. Wild-type MT1-MMP on the cell surface of transfected cells was detected with an intact propeptide, thus indicating a nonprocessed form of the enzyme. This is in contrast with the general concept that the propeptide domain of MMPs needs to be cleaved in order for the enzyme to function. Alternative mechanisms of MT1-MMP activation in different types of cells independent of the well recognized furin-mediated activation pathway have been reported recently (10, 32, 42). In the case of COS-1 cells, we demonstrated that the presence of propeptide domain does not interfere with the function of the enzyme on the cell surface, because MT1-MMP in the form of a proenzyme caused fibronectin proteolysis. Therefore, the requirement of cleavage of the propeptide domain does not apply to COS-1 cells where membrane-mediated conformational change of proMT1-MMP presumably results in displacement of the propeptide domain from the catalytic site, leading to active enzyme function. This concept evolves from the original experiments leading to the cysteine switch hypothesis of MMP activation in which chemical reagents conferred enzymatic activity of latent MMPs independent of cleavage of the propeptide domain (43–44). The frequency of this MT-MMP phenotype in other cell types remains to be determined.

Cells expressing prodomain-deleted MT1-MMP were not functional in the fibronectin degradation/migration assay and in binding of TIMP-2. As confirmed by immunofluorescence, this deletion mutant was displayed on the cell surface. Both, function of cells expressing ΔproMT1-MMP in the fibronectin degradation/migration assay and TIMP-2 binding were restored when ΔproMT1-MMP cDNA was co-transfected in trans with the N-terminal MT1-MMP propeptide-encoding cDNA. These findings support previous results, which suggested that synthesis of the propeptide domain is required for MT1-MMP to be functional on the cell surface (6, 15, 16). Because MT1-MMP in other types of cells is active following propeptide cleavage in the trans-Golgi network, we propose that the major function of the propeptide occurs intracellularly and is related to proper folding prior to trafficking to the Golgi apparatus.

For most secreted serine and cysteine proteases where the propeptide domain has a chaperone function, the propeptide domain has also been reported to have an inhibitory effect on the native mature enzyme (18, 20, 22); this seems not to be the case for MT1-MMP. We demonstrated that intact proMT1-MMP on the cell surface is active while it still contains the propeptide domain. Furthermore, we found that in the case of ΔproMT1-MMP/propeptide in trans co-transfected cells, although the propeptide is bound to ΔproMT1-MMP on the cell surface, it does not inhibit its function. In addition, the presence of the propeptide on the surface of full-length, wild-type MT1-MMP-transfected cells, does not interfere with TIMP-2 binding. These results lead us to consider that the propeptide/mature MT1-MMP interaction site may be distinct from the TIMP-2 catalytic domain (45) interaction site in MT1-MMP. Crystal structure studies, similar to that described for the complex formed between the catalytic domain of MT1-MMP and TIMP-2 (45) will be required to characterize this interaction. Recently, Anders et al. (46) reported that the propeptide of another member of the metzincin family, ADAM-10, was reported to have an intramolecular chaperone function similar to our observation with MT1-MMP. In contrast to MT1-MMP, the prodomain of ADAM-10 has an inhibitory effect on the mature enzyme.

The propeptide domain of MT1-MMP contains a four-amino acid sequence, 42YGYL45, which is conserved in all six MT-MMP subfamily members. The fifth amino acid in this peptide sequence, proline 46, is also conserved in MT-MMPs, but, because of technical limitations, was not further analyzed in this report. In the cases of subtilisin and cathepsin C, critical amino acids or domains within the propeptide region required for the intramolecular function of the propeptide domain have been revealed by mutation analyses (18, 22). In a comparable fashion, we employed mutation analysis to examine the function of the four-amino acid sequence 42YGYL45, in the chaperone activity of the propeptide of MT1-MMP.

Ala substitution mutants for the conserved propeptide region 42YGYL45 were prepared and tested for function when expressed on the surface of transfected COS-1 cells. Ala was chosen as the substitution residue for the mutants because it eliminates the side chain beyond the β-carbon, yet does not alter the main chain conformation or contribute significantly to electrostatic or steric effects (47). Function of the mutants was determined with the use of the fibronectin degradation/migration assay. It was shown that double Ala substitutions Y42A/G43A, G43A/Y44A, and Y44A/L45A in the conserved propeptide domain 42YGYL45 abrogate proteolytic activity/migration over fibronectin. Furthermore, these mutant proteases were inactive in proMMP-2 activation and did not bind TIMP-2 on the cell surface. Single amino acid mutations for positions 42 and 43 had minimal effect on MMP function, whereas Ala substitution in position 44 abrogates the enzyme activity. Based on these data, it is concluded that conservation of the four-amino acid sequence 42YGYL45 is essential for MT1-MMP activity. Because these mutations affect the propeptide domain sequence, which is removed from MT1-MMP upon its activation in other types of cells and are distinct from the furin-activation site, such a dramatic effect on catalytic activity would not be expected.

Double Ala substitutions proximal or distal to the conserved 42YGYL45 sequence, but still within the MT1-MMP propeptide, in positions 34–35 or 48–49 resulted in functional enzymes with variably reduced activity as determined by their ability to activate proMMP-2, by their affinity for TIMP-2, and by their function in cell migration/degradation. Nevertheless, these mu-
tions did not have the catastrophic effect on the enzyme’s activity as demonstrated for mutations in the 42GYGL45 region. Reduction in the functional activity of S34A/P35A and G48A/D49A may be attributed to small changes in the conformation of the entire MT1-MMP propeptide, which appears to affect the function of the enzyme. Studies examining the role in enzyme latency of amino acid residues located proximal to the conserved cysteine-switch residue in the propeptide domain of secreted MMPs support this observation (48–50).

We conclude that the 42GYGL45 region within the propeptide of MT1-MMP is a major determinant of the enzyme’s correct folding and function. Other areas in the propeptide domain contribute to a lesser degree to the proper folding of the enzyme.

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A Conserved Sequence within the Propeptide Domain of Membrane Type 1 Matrix Metalloproteinase Is Critical for Function as an Intramolecular Chaperone
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