The Propeptide Domain of Membrane Type 1-Matrix Metalloproteinase Acts as an Intramolecular Chaperone when Expressed in trans with the Mature Sequence in COS-1 Cells*

Jian Cao†, Michelle Hymowitz§, Cathleen Conner§, Wadie F. Bahou‡, and Stanley Zucker†‡§¶§

From the †Department of Medicine, State University of New York at Stony Brook, Stony Brook, New York 11794 and §Department of Veterans Affairs Medical Center, Northport, New York 11768

It has been assumed that cleavage of the N-terminal propeptide domain of membrane type-1 matrix metalloproteinase (MT1-MMP) is required for enzyme function. We recently demonstrated that the propeptide domain of MT1-MMP is not cleaved and actually is required for function of the membrane-bound enzyme in transfected COS-1 cells (Cao, J., Drews, M., Lee, H. M., Conner, C., Bahou, W. F., and Zucker, S. (1998) J. Biol. Chem. 273, 34745-34752). In this report, we have inserted the cDNA encoding the signal and propeptide sequences of MT1-MMP (MT1pro) and the cDNA encoding propeptide-deleted mature MT1-MMP (MT1pro) in expression vectors that were then transfected into matrix metalloproteinase-deficient COS-1 cells. Co-expression of both the mature sequence and the prosequence of MT1-MMP as independent polypeptides (in trans) in COS-1 cells resulted in reconstitution of MT1-MMP function in terms of facilitating 125I-labeled tissue inhibitor of metalloproteinase 2 binding to transfected cells and subsequent activation of progelatinase A. Transfection of cells with either cDNA alone resulted in non-functional cells. These results are consistent with the propeptide sequence of MT1-MMP functioning as an intramolecular chaperone involved in protein folding and trafficking to the cell surface.

Membrane-type matrix metalloproteinases (MT-MMPs) are a newly described family of MMPs (1) that are distinguished by their localization to the plasma membranes of cells by a stretch of hydrophobic amino acids (transmembrane domain) (2). MT-MMPs have been the subject of intense interest because of their role in activating a secreted MMP, progelatinase A, at the cell surface (1) and in directly cleaving collagen and other extracellular matrix proteins (9). Because activation of all soluble latent MMPs described to date is accompanied by cleavage of the N-terminal propeptide, thereby exposing the active site zymogen to dissociation from the conserved cysteine in the prodomain, the assumption has been made that an analogous mechanism is responsible for the activation of MT1-MMP on the cell surface.

Utilizing recombinant wild-type (wt) MT1-MMP cDNA and mutant MT1-MMP CDNAs transfected into cells lacking endogenous MT1-MMP, we have recently examined the function of the N-terminal propeptide domain of membrane-bound MT1-MMP. Contrary to expectation, we demonstrated that the N-terminal prodomain of MT1-MMP is required for function of the intact membrane-bound enzyme in COS-1 cells. Transfected COS-1 cells containing a deletion of the N-terminal propeptide domain of MT1-MMP or a chimeric construction in which the prodomain of MT1-MMP is substituted by the N-terminal propeptide domain of collagenase-3 were functionally inactive in terms of binding 125I-labeled TIMP-2 to the cell surface and in initiating the activation of progelatinase A (4). These data led us to hypothesize that in its native plasma membrane-inserted form, the prodomain of MT1-MMP serves to facilitate the function of MT1-MMP as a receptor for TIMP-2 that subsequently immobilizes progelatinase A, thereby forming a trimolecular complex on the cell surface. A second free MT1-MMP molecule is then required to cleave the prodomain leading to activation of membrane-bound progelatinase A. The presence of excess TIMP-2 saturates all available MT1-MMP molecules, thereby interfering with progelatinase A activation. We proposed that conformational effects induced by the plasma membrane provide functional activity to latent cell surface-bound MT1-MMP without cleavage of the molecule (4, 5). It needs to be emphasized that our understanding of the importance of the propeptide domain of MT1-MMP was possible only because COS-1 cells are defective in proteolytic processing of certain newly synthesized proteins (6). Even following co-transfection of furin cDNA and MT1-MMP cDNA, COS-1 cells do not process latent MT1-MMP (60 kDa) to mature MT1-MMP (58 kDa) (7). In contrast, both the latent and mature forms of MT1-MMP are prominently displayed in several types of non-transfected cells capable of inducing progelatinase A activation (5, 8, 9); hence it was not previously possible to distinguish between the function of native versus mature enzyme.

The goal of this report is to further clarify the role of the propeptide domain of MT1-MMP in maintaining the function of the plasma membrane-inserted enzyme. To this end, we have independently transfected or co-transfected COS-1 cells (in trans form) with the N-terminal prodomain cDNA for MT1-MMP and MT1-MMP cDNA lacking the propeptide domain (mature sequence). The results demonstrate that synthesis of the prosequence is required to obtain functional MT1-MMP...
and that a covalent link of the prosencep with the mature sequence of MT1-MMP in COS-1 cells is not required.

MATERIALS AND METHODS

Reagents—Chemical reagents were purchased from Sigma. Restriction enzymes were purchased from Stratagene (La Jolla, CA). The pcDNA3 expression vector was purchased from Invitrogen (San Diego, CA). The monoclonal antibody to hemagglutinin (HA) tag was purchased from Roche Molecular Biochemicals (clone 12 CA5). Rabbit polyclonal antibodies to a synthetic peptide (CDGNIOTWAMLGEM) within the catalytic domain (10), to the hinge region of MT1-MMP (Chemicon International, Temecula, CA), and to the prodomain of MT1-MMP (Chemicon) were employed. Recombinant progelatinase A, produced by COS-1 cells transfected with gelatinase A cDNA, was purified as described previously (2, 7). Interstitial collagenase-1 cDNA was generously provided by Dr. Chitra Biswas (Tufts University School of Medicine) (11).

Cell Culture and Transfection—COS-1 cells were maintained in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) containing 10% fetal bovine serum (Life Technologies, Inc.) and 2 mM glutamine under 5% CO2 atmosphere. Plasmids were transfected into cells using calcium phosphate as described previously (2).

Construction of Plasmids—MT1-MMP cDNA encoding an open reading frame from amino acid residues Met1–Val1582 and mutant MT1-MMP were cloned in a pcDNA3 expression vector employing a cytomegalovirus (CMV) promoter (4). MT1pro lacking the entire N-terminal propeptide domain was utilized in this study as we have previously described (4).

A chimera between collagenase-3 and MT1-MMP (Col-3/MT) was constructed by a two-step polymerase chain reaction using the introduced Sacl and BamHI sites. The electrophoresed proteins in the gel were transferred onto 0.5% Polyvinylidene fluoride membrane and immunoblotted with polyclonal antibodies to a synthetic peptide (CDGNIOTWAMLGEM) within the catalytic domain of MT1-MMP and mutants. The domain structure of MT1-MMP displays the typical do-

RESULTS

Synthesis of Mutant Propeptide Domains of MT1-MMP by Transfected COS-1 Cells—The expression plasmid encoding the cDNA for deletion mutants of MT1-MMP lacking all but the
signal peptide and the entire propeptide domain was constructed and transfected into COS-1 cells as depicted in Fig. 1. Western blotting of conditioned media from the transfected COS-1 mutant MT1–109 using an antibody to the propeptide domain of MT1-MMP, resulted in staining of a single protein band migrating predictably at ~9 kDa, based on its truncated mass markers listed on the left. Co-transfection with cDNA for the prodomain of MT1-MMP (MT1–109) was transiently transfected into COS-1 cells. A plasmid encoding the N-terminal prodomain of MT1-MMP (MT1–109), MT1–109, and MT1pro, or MT1–109 using various combinations of plasmids. Serum-free conditioned medium was collected after an 18-h incubation of cells at 37 °C, and gelatin zymography was performed. Co-expression of MT1pro and the prodomain of MT1-MMP (MT1–109) in trans resulted in activation of progelatinase A indicating that the independently synthesized prodomain of MT1-MMP has associated (non-covalent) with prodomain deleted MT1-MMP and resulted in proteolytic activity.

FIG. 2. A, expression of mutant MT1-MMP (N-terminal propeptide domain) in COS-1 cells. A plasmid encoding the N-terminal prodomain of MT1-MMP (MT1–109) was transiently transfected into COS-1 cells. The secreted protein in serum-free conditioned medium was precipitated with 10% trichloroacetic acid and analyzed by immunoblotting with an antibody to the prodomain of MT1-MMP. The molecular mass of wild type MT1-MMP prodomain was detected at ~9 kDa. Molecular mass markers are listed on the left. B, reconstitution of function (activation of progelatinase A) of N-terminal prodomain-deleted MT1-MMP by the prodomain of MT1-MMP (MT1–109) is dose-dependent, as demonstrated by co-transfection of plasmids in COS-1 cells (cDNA for the N-terminal prodomain-deleted MT1-MMP (MT1pro) and the prodomain of MT1-MMP (MT1–109)). COS-1 cells were transiently transfected with cDNA for progelatinase A, as well as cDNA, for vector (pCDNA3), MT1-MMP, MT1pro, or MT1–109, using various combinations of plasmids. Serum-free conditioned medium was collected after an 18-h incubation of cells at 37 °C, and gelatin zymography was performed. Co-expression of MT1pro and the prodomain of MT1-MMP (MT1–109) in trans resulted in activation of progelatinase A indicating that the independently synthesized prodomain of MT1-MMP has associated (non-covalent) with prodomain deleted MT1-MMP and resulted in proteolytic activity.

To examine the specificity of these observations, we determined whether the prodomain of MT1-MMP is able to provide function to an inactive chimeric mutation of MT1-MMP in which the propeptide domain of MT1-MMP is replaced by the propeptide domain of collagenase-3 (Col-3/MT). COS-1 cells, transiently transfected with both MT1–109 cDNA and Col-3/MT cDNA (Fig. 3, lane 5), did not activate progelatinase A. In this construct, the isolated prodomain of MT1-MMP presumably is unable to displace the prodomain of the inactive chimeric molecule; hence, activation of progelatinase A does not occur.

We further determined whether the prodomain of a secreted MMP (MMP-1) was able to reconstitute the function of MT1pro (Fig. 4). To this end, MMP-1–99 cDNA was co-transfected into
COS-1 cells in trans along with MTΔpro cDNA; 24 h later recombinant progelatinase A was added, and cells were incubated as described above. Gelatin substrate zymography demonstrated that co-transfection with MMP-1Δpro did not alter the lack of function of MTΔpro. Synthesis of MMP-1Δpro cDNA by transfected COS-1 cells was documented by the introduction of an influenza HA epitope in the C terminus of MMP-1Δpro. Western blotting of conditioned medium using an anti-HA antibody demonstrated a peptide of approximately 11 kDa, consistent with the anticipated molecular mass of MMP-1Δpro. To determine whether the HA epitope might alter function of an MMP prodomain, the HA epitope was added to the cDNA for MT1-MMP. The HA tag did not impair the reconstitution of MT1-MMP with MTΔpro as shown by functional assay in terms of progelatinase A activation. This result is consistent with the specific requirement for the prodomain of MT1-MMP for function of the molecule in cells.

We next examined the effect of adding the recombinant N-terminal propeptide domain of MT1-MMP to COS-1 cells transfected with MTΔpro cDNA. Recombinant MT1Δ109 protein isolated from conditioned medium of COS-1 cells transfected with MT1Δ109 cDNA did not reconstitute the activation of progelatinase A by COS-1 cells transfected with MTΔpro (Fig. 5). This result suggests that the association between MTΔpro and MT1-109 protein takes place intracellularly during synthesis, folding, and transport of these proteins to the plasma membrane and cannot be replicated after insertion of MTΔpro into the plasma membrane (4).

Binding of 125I-labeled TIMP-2 to the Reconstituted MT1-MMP Molecule in COS-1 Cells—In previous experiments we demonstrated that 125I-labeled TIMP-2 did not bind to COS-1 cells transfected with N-terminal propeptide-domain-deleted MT1-MMP (MTΔpro) cDNA but readily bound to the surface of cells transfected with wtMT1-MMP cDNA. Furthermore, we demonstrated that TIMP-2 binds to the catalytic domain of intact membrane-inserted MT1-MMP (5).

To further examine the role of the N-terminal propeptide of MT1-MMP in TIMP-2 binding, we have examined COS-1 cells transfected with N-terminal propeptide-deleted MT1-MMP (MTΔpro) cDNA and N-terminal propeptide (MT1-109) cDNA in trans. Radiolabeled recombinant TIMP-2 (varying doses) was incubated with COS-1 cells transfected with MT1-109 cDNA alone versus dual transfection with MTΔpro and MT1-109 cDNA. Whereas 125I-labeled TIMP-2 did not bind to cells individually transfected with either MTΔpro or MT1-109 cDNA alone (data not shown; nonspecific binding exceeded specific binding. See FIG. 6.

![Graph A](http://www.jbc.org/)

**Fig. 6.** 125I-labeled TIMP-2 binds to COS-1 cells dual-transfected with N-terminal propeptide-deleted MT1-MMP (MTΔpro) cDNA and N-terminal propeptide (MT1-109) cDNA. Radiolabeled recombinant TIMP-2 (varying doses) was incubated with COS-1 cells transfected with MTΔpro cDNA (panel A) and both prodomain-deleted MT1-MMP (MTΔpro) cDNA and N-terminal propeptide (MT1-109) cDNA in trans (panel B) in the presence (nonspecific) or absence (total) of 20-fold excess non-radiolabeled recombinant TIMP-2. Specific binding was calculated as the difference between total and nonspecific binding. The curves for specific activity were fitted to the binding data using the following rectangular hyperbola: Bound = Boundmax × TIMP-2 concentration/Kd + TIMP-2 concentration). The total and nonspecific curves were drawn by best-fit. The Scatchard plot describes 125I-labeled TIMP-2 bound specifically to cells transfected with MT1-MMP and MTΔpro plus MT1-109 cDNA; the number of receptors per cell was 48,000 and 7,000, respectively. Nonspecific binding exceeded specific binding of 125I-labeled TIMP-2 bound to MT1-109 cDNA alone-transfected cells (data not shown) or bound to MTΔpro cDNA-transfected cells (see Ref. 5).
MMP functions as a chaperone involved in protein folding and these data suggest that the N-terminal propeptide of MT1-MMP acts as a chaperone involved in protein folding and stability. In contrast, substitution of the furin consensus sequence of MT1-MMP (MT1–109) in trans with MT1-MMP cDNA lacking the propeptide domain (MTαpro). This co-transfection, leading to independent synthesis of MT1–109 and MTαpro proteins, resulted in reconstitution of MT1-MMP function in terms of binding TIMP-2 and activating gelatinase A at the cell surface. These results are consistent with the concept that the isolated propeptide domain of MT1-MMP is able to associate (presumably in the endoplasmic reticulum) with the remainder of the MT1-MMP molecule (catalytic/hinge/haemopexin domain) prior to trafficking to the plasma membrane and provide function to the reconstituted protein. However, the number of functioning MT-MMP units on the cell surface is diminished, presumably because of decreased efficiency of the binding process in the endoplasmic reticulum. For unexplained reasons, the dissociation constant (Kd) for TIMP-2 binding to non-covalent linked, reconstituted MT1-MMP was reproducibly lower than that of the wild-type enzyme.

To further examine the function of the prodomain of membrane-bound MT1-MMP, we herein have studied the effect of transfecting COS-1 cells with cDNA encoding the propeptide sequence of MT1-MMP (MT1–109) in trans with MT1-MMP cDNA lacking the propeptide domain (MTαpro). This co-transfection, leading to independent synthesis of MT1–109 and MTαpro proteins, resulted in reconstitution of MT1-MMP function in terms of binding TIMP-2 and activating gelatinase A at the cell surface. These results are consistent with the concept that the isolated propeptide domain of MT1-MMP is able to associate (presumably in the endoplasmic reticulum) with the remainder of the MT1-MMP molecule (catalytic/hinge/haemopexin domain) prior to trafficking to the plasma membrane and provide function to the reconstituted protein. However, the number of functioning MT-MMP units on the cell surface is diminished, presumably because of decreased efficiency of the binding process in the endoplasmic reticulum. For unexplained reasons, the dissociation constant (Kd) for TIMP-2 binding to non-covalent linked, reconstituted MT1-MMP was reproducibly lower than that of the wild-type enzyme.

Co-transfection of COS-1 cells with the chimeric Col-3/MTαpro cDNA and MT1–109 cDNA or MTαpro cDNA and MMP-1 1–99 cDNA did not reconstitute gelatinase A activation function. These experiments strengthen our proposition that the isolated prodomain of MT1-MMP is capable of non-covalent bonding with MTαpro, presumably in the endoplasmic reticulum/Golgi apparatus prior to trafficking to the plasma membrane.

Additional studies will be required to determine which amino acids in the prodomain are essential for enzyme function. As depicted in Fig. 1B, the sequence YGYLP is highly conserved in the propeptide of all MT-MMP subfamily members described to date, which leads us to suspect an important function for this sequence. Our previous study of membrane-inserted mutants of MT1-MMP suggested an important role of the Ser34–Arg1 position in the prodomain (4), but it should be pointed out that a large deletion of this type may exert a deleterious effect on protein structure and stability. In contrast, substitution of the furin consensus sequence of MT1-MMP (Arg108–Arg109–Lys110–Arg111 with Ala108–Arg109–

**DISCUSSION**

The unique feature of MT-MMPs is their insertion into the plasma membrane of cells by a stretch of hydrophobic amino acids followed by a short cytoplasmic sequence. Because purified MT-MMPs cannot readily be evaluated in their membrane-bound state, most experiments designed to study the function of MT-MMPs have employed secreted (soluble) mutant MT1-MMP recombinants lacking the transmembrane domain (15–18). Based on these studies, it has been concluded that cleavage of the N-terminal domain of membrane-bound MT-MMPs by furin, presumably in the trans-Golgi network, is required for function of the enzyme in activating gelatinase A and degrading other substrates (15, 16, 18, 19).

Recent reports by Cao et al. (4) and Butler et al. (20) have demonstrated that membrane-bound MT1-MMP reacts differently than soluble MT1-MMP (extracted from cell membranes by detergents or membrane domain-deleted MT1-MMP). Stoichiometric concentrations of TIMP-2, which forms an essential bridge between plasma membrane MT1-MMP and gelatinase A at the cell surface, are not required for activation of gelatinase A by soluble MT1-MMP (20). As with naturally secreted MMPs, TIMP-2 function is limited to binding and inhibition of enzymatic activity of soluble prodomain-deleted MT1-MMP (16, 18, 20). We further demonstrated that cleavage of the N-terminal prodomain of MT1-MMP on the surface of transfected COS-1 cells was not required for function of the enzyme in terms of binding TIMP-2 and activating gelatinase A. In other cell types (i.e. endothelial cells, HT-1080 fibrosarcoma (9)), however, furin-induced cleavage of the N terminus is required for function of MT-MMP.

To further examine the function of the prodomain of membrane-bound MT1-MMP, we herein have studied the effect of transfecting COS-1 cells with cDNA encoding the propeptide sequence of MT1-MMP (MT1–109) in trans with MT1-MMP cDNA lacking the propeptide domain (MTαpro). This co-transfection, leading to independent synthesis of MT1–109 and MTαpro proteins, resulted in reconstitution of MT1-MMP function in terms of binding TIMP-2 and activating gelatinase A at the cell surface. These results are consistent with the concept that the isolated propeptide domain of MT1-MMP is able to associate (presumably in the endoplasmic reticulum) with the remainder of the MT1-MMP molecule (catalytic/hinge/haemopexin domain) prior to trafficking to the plasma membrane and provide function to the reconstituted protein. However, the number of functioning MT-MMP units on the cell surface is diminished, presumably because of decreased efficiency of the binding process in the endoplasmic reticulum. For unexplained reasons, the dissociation constant (Kd) for TIMP-2 binding to non-covalent linked, reconstituted MT1-MMP was reproducibly lower than that of the wild-type enzyme.

Co-transfection of COS-1 cells with the chimeric Col-3/MTαpro cDNA and MT1–109 cDNA or MTαpro cDNA and MMP-1 1–99 cDNA did not reconstitute gelatinase A activation function. These experiments strengthen our proposition that the isolated prodomain of MT1-MMP is capable of non-covalent bonding with MTαpro, presumably in the endoplasmic reticulum/Golgi apparatus prior to trafficking to the plasma membrane.

Additional studies will be required to determine which amino acids in the prodomain are essential for enzyme function. As depicted in Fig. 1B, the sequence YGYLP is highly conserved in the propeptide of all MT-MMP subfamily members described to date, which leads us to suspect an important function for this sequence. Our previous study of membrane-inserted mutants of MT1-MMP suggested an important role of the Ser34–Arg11 region of the prodomain (4), but it should be pointed out that a large deletion of this type may exert a deleterious effect on protein structure and stability. In contrast, substitution of the furin consensus sequence of MT1-MMP (Arg108–Arg109–Lys110–Arg111 with Ala108–Arg109–

**FIG. 7.** Cell surface localization of MTαpro. COS-1 cells transfected with MTαpro (F and G), MT1-MMP (C and D), or pcDNA3 alone (A and B) were fixed in 3.7% formaldehyde, permeabilized with 0.1% Nonidet P-40, and incubated first with MT1-MMP hinge antibody and later with fluorescein isothiocyanate-coupled goat anti-rabbit secondary antibody. The cells were analyzed by fluorescent confocal microscopy. A, C, and E show transmission images with a white light, and B, D, and F show fluorescent images with a UV light. A and B were in the same field, as well as C and D and E and F. The arrow identifies cell surface immunofluorescence.
Ala\(^{110}\)–Ala\(^{111}\) in COS-1 cells resulted in no loss of function of this membrane-inserted mutant MT1-MMP (7) indicating that this conserved sequence is not required for progelatinase A activation at the COS-1 cell surface.

The N terminus of all secreted MMPs maintains the latency of the proteinase, while permitting controlled activation by cleavage of the propeptide (21). In contrast, the propeptide regions of other types of enzymes serve different functions. An intact prosequence of lactase-phlorizin hydrolase, an intestinal brush border enzyme, is necessary for enzyme trafficking to the plasma membrane (22). Some proregions act as potent inhibitors of the mature enzyme following cleavage (23, 24), whereas longer proregions may act as intramolecular chaperones (25). Relevant to our reconstitution experiments with MT\(_{\text{pro}}\) cDNA and MT\(_{1-109}\) cDNA dual-transfected cells (see above), covalent linkage of the prosequence to the propeptide domain of a protein is not essential for proper folding of subtilisin (25, 26), α-lytic protease (27), and thermolysin (28). In these instances, the prosequence can mediate the folding of the protease when added as a separate polypeptide chain (25–27) or when the protein is synthesized in trans with the inactive mature enzyme in bacteria (28, 29). Relevant to this discussion, a mutation lacking a single domain (designated MATH) of meprin (30) has been reported to be folded sufficiently for secretion of the protein but not sufficiently to generate an active protease from the proform of the enzyme (30).

MT1-MMP has a proregion with 77 amino acids at the N terminus, which is quite similar to other MT-MMP members. Our data suggest that proregion-deleted MT1-MMP progressed through the trafficking pathway in COS-1 cells more slowly than wild-type MT1-MMP and accumulated in the perinuclear region presumably as a result of delayed trafficking between the endoplasmic reticulum, the Golgi apparatus, and the plasma membrane. Although MT\(_{\text{pro}}\) is expressed on the cell surface eventually, it has lost the striking features of MT-MMP function (binding TIMP-2 and activation of progelatinase A). Thus, we conclude that the proregion of MT1-MMP differs from the proregion of secretory MMPs by functioning as an intramolecular chaperone in trafficking the molecule to the cell surface and in maintaining the molecule in proper conformation for full enzymatic activity.

Acknowledgments—We thank Drs. Y. DeClerck and K. Langley for providing recombinant TIMP-2 antigen, Dr. Carlos Lopez-Otin for providing collagenase-3 cDNA, and Dr. Jolyn Jesty for advice in preparation of this manuscript.
The Propeptide Domain of Membrane Type 1-Matrix Metalloproteinase Acts as an Intramolecular Chaperone when Expressed in trans with the Mature Sequence in COS-1 Cells

Jian Cao, Michelle Hymowitz, Cathleen Conner, Wadie F. Bahou and Stanley Zucker

doi: 10.1074/jbc.M001920200 originally published online July 10, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M001920200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 28 references, 16 of which can be accessed free at http://www.jbc.org/content/275/38/29648.full.html#ref-list-1