FEBS 22966 FEBS Letters 462 (1999) 261–266

Expression, purification and charaterization of recombinant mouse MT5-MMP protein products

Xing Wang, Jun Yi, Jianxun Lei, Duanqing Pei*

Department of Pharmacology, 6-120 Jackson Hall, 321 Church St. S.E., University of Minnesota, Minneapolis, MN 55455, USA

Received 29 September 1999

Edited by Thomas L. James

Abstract We have recently identified the fifth member of the membrane-type matrix metalloproteinase subfamily, MT5-MMP/MMP24, which is expressed in a brain specific manner (Duanqing Pei (1999) J. Biol. Chem. 274, 8925–8932). To further characterize its enzymic properties, an expression construct was engineered to produce MT5-MMP as a soluble and active form by truncating its transmembrane domain. Stable expression cell lines were subsequently established from MDCK cells transfected with this construct. Unfortunately, purification of MT5-MMP from the culture media in large quantity proves to be difficult initially due to its rapid turnover via a mechanism which can be inhibited by a broad spectrum metalloproteinase inhibitor, BB94. Thus, BB94 was included in the cell culture medium and throughout the purification process except the final step of chromatography to protect MT5-MMP from destruction. Purified to homogeneity and free of the synthetic inhibitor, MT5-MMP can activate progelatinase A efficiently in a TIMP2 sensitive fashion. A preliminary screen for its potential substrates among extracellular matrix components identified the proteoglycans as the preferred substrates for MT5-MMP. Furthermore, it is determined that the stability of purified MT5-MMP is temperature dependent with rapid destruction at 37°C, but being relatively stable at temperatures 4°C or lower. These observations establish MT5-MMP as a proteoglycanase with a short half-life at body temperature, which may be critical for tightly controlled turnover of ECM components such as those in the brain.

Key words: MMP; Proteolysis; ECM degradation; Brain

1. Introduction

The extracellular matrix (ECM) undergoes extensive remodeling under both physiological as well as pathological conditions [1–3]. Among the proteinases discovered so far, members of the matrix metalloproteinase (MMP) family have been singled out as the main mediators of ECM destruction by virtue of their abilities to degrade all ECM components under physiological conditions [2,3]. Indeed, the expression of one or multiple MMPs has been documented extensively in tissues or organ sites under active ECM remodeling [2,4,5]. However, the relative contribution of individual MMPs toward tissue destruction remains poorly defined.

Among MMPs discovered so far, two main categories can be recognized based on their cellular localizations: soluble vs. membrane-bound. The soluble MMPs are divided into the collagenases (MMP1, MMP8 and MMP13). gelatinases (MMP2 and MMP9), stromelysins (MMP3, MMP12) and those yet to be classified such as MMP7, MMP11, MMP18/19, etc. [5,6]. The membrane-bound subfamily currently has five members: MT1, 2, 3, 4, and 5-MMPs with a characteristic transmembrane segment at their C-termini [7–11]. Although localized differently, these two classes of MMPs are expected to cooperate in ECM degradations. In fact, a well-defined property of the MT-MMPs is their ability to interact with and activate the proforms of MMP2 and MMP13, thus forming a proteolytic complex on cell surface [7,8,11–13]. With the expansion of the MT-MMP subfamily, it becomes apparent that they play a far greater role in ECM remodeling events than zymogen activation. For example, MT1, 2, 3-MMPs have been demonstrated to have powerful intrinsic activities toward ECM components such as fibronectin, laminin, type I and III collagens, nidogen, tenascin, aggrecan and perlecan, thus, demonstrating a degree of versatility for these important enzymes under many physiological as well as pathological states [14–19]. Thus, there have been intense interests in the characterization of these membrane-bound MMPs as central regulators of ECM degradation [20,21].

MT5-MMP, the latest addition to this subfamily with a serial designation as MMP24, is a brain specific MT-MMP capable of activating progelatinase A [11]. It is significant that brain is the main adult organ expressing high level of MT5-MMP message RNA because other MT-MMPs have much broader tissue distributions [7–11]. Furthermore, MT5-MMP exhibits an unusual tendency to be shed from cell surface [11]. Despite these interesting properties, the role of MT5-MMP in tissue remodeling events remains undefined. To begin to understand its enzymic potentials, we report here the expression, purification and characterization of MT5-MMP active enzyme. With a short half-life as an active proteinase, MT5-MMP appears to be an efficient proteinase against proteoglycan components of the extracellular matrix.

2. Materials and methods

2.1. Cell lines and reagents

MDCK cells were obtained and maintained as described previously [11]. General chemical reagents were from Fisher Scientific (Pittsburgh, PA, USA). Reagents for DNA restriction and modification were from Promega (Madison, WI, USA). Tissue culture media and reagents were from Gibco-BRL (Rockville, MD, USA). Oligonucleotide primers were made by the University of Minnesota microchemical core facility. ECM components were purchased from Collaborative Research (Boston, MA, USA). FLAG peptides, anti-FLAG M2 antibody and M2 affinity agarose gel were purchased from Sigma (St. Louis, MO, USA). Rabbit polyclonal anti-MT5-MMP antisera were prepared as described previously [22].

*Corresponding author. Fax: (1)-612-625-8408.
E-mail: peixx003@tc.umn.edu

0014-5793/99/$20.00
PII: S0014-5793(99)01534-3

2.2. Construction of expression vectors and generation of stable cell lines

The expression vector for full-length MT5-MMP as well as its stable transfectant MT591 have been described [11]. The sequence encoding MT5-MMP$_{1-570}$ was isolated by high-fidelity PCR with the pfu polymerase employing two primers: T3 primer (on the pBluescript vector) and the 3′ MT5-MMP specific primer GTC ACT TGT CAT CGA CGT CCT TGT AGT CGT TCA CAG AGC CTG GCA C. The fragment was subsequently cloned into pCR3.1uni expression vector and characterized as described previously [11]. Since the 3′ end primer also contains the FLAG sequence, the resulting protein is tagged with the FLAG epitope to facilitate detection and purification. The resulting expression vector was transfected into MDCK cells and stable clones were established by neomycin selection as described [23]. One of the clones, MT5Δ44, was selected for further studies based on a relatively high level of expression for MT5-MMP$_{1-570}$.

2.3. Purification and characterization of MT5-MMP products

MT5Δ44 cells were grown to confluence and washed with phosphate-buffered saline three times before fresh medium was added. Due to stability concerns, we have purified MT5-MMP with or without BB94 (5 μM). For purification without BB94, all steps from conditioned medium collection to affinity chromatography were carried out free of this inhibitor. For the ones with BB94, it was included throughout the purification procedure except the final immuno-affinity step to protect MT5-MMP from auto-degradation. Briefly, the conditioned medium was first cleared of debris by centrifugation at 20 000 rpm, loaded onto a Q-sepharose column (2×20 cm) and eluted with a gradient of 0.15 to 1 M NaCl as described [14]. Positive fractions were identified by Western blotting, pooled together and then stored at −80°C. The final purification step was accomplished with an immuno-affinity column made of anti-FLAG M2 monoclonal antibody (Sigma, St. Louis, MO, USA). The Q-sepharose fractions were loaded onto a pre-washed M2 column twice to maximize the binding. Unbound materials as well as residual BB94 were removed by extensive washing with Tris-buffered saline (TBS, 150 mM NaCl, 50 mM Tris-HCl, 5 mM $CaCl_2$, 0.1% Brij35). The bound materials were eluted with two column equivalents of FLAG peptides in TBS. The purified materials were analyzed by SDS-PAGE, Western blotting and zymography, as described [14].

Substrate degradation experiments were carried out essentially as described [14]. Briefly, ECM substrates (2–5 μg) were incubated with various amounts of purified MT5-MMP (10–160 ng) in the activity buffer (150 mM NaCl, 50 mM Tris-HCl, 5 mM $CaCl_2$, 1 mM $ZnCl_2$, 0.1% Brij 35) at 37°C for 2 to 24 h. Degradation of native type I collagen was carried out in the presence of 100 mM arginine at 25°C with the same activity buffer as above. The reaction mixtures were analyzed by SDS-PAGE and visualized by Coomassie staining as described [14].

The stability of MT5-MMP is determined by incubating the same amount of purified MT5-MMP at 4°C, 20°C and 37°C in the activity buffer or the same buffer without Brij35 for 0 min, 30 min, 1 h, 3 h and 24 h. At each time point, reactions were stopped with SDS-PAGE sample buffer before being stored at −80°C. The reaction mixtures were analyzed by Western blotting using anti-MT5-MMP antisera [11].

3. Results

3.1. Expression of MT5-MMP as a soluble and active enzyme

As reported previously, MT5-MMP was identified as a membrane-anchored proteinase and soluble ones shed from cell surface [11]. To further characterize the enzymic properties of MT5-MMP, an expression vector was engineered to express a fully secretory form of MT5-MMP by deleting the transmembrane domain, i.e. the full-length ecto-enzyme (Fig. 1A). A FLAG tag was fused at the C-terminus to facilitate detection and purification as described previously (Fig. 1A, [23]). Based on previous experience with MT1-MMP, the ecto-enzyme should be synthesized as a proenzyme and processed in the *trans*-Golgi network into active species prior to secretion by furin or related proprotein convertases recognizing the RXKR motif sandwiched between its pro- and catalytic domains [14]. Indeed, transient transfection of the vector carrying MT5-MMP$_{1-570}$ into COS cells yielded MT5-MMP as a secreted form (data not shown). Stable cell lines were subsequently established to express MT5-MMP$_{1-570}$ in MDCK cells as described [23]. One of them, MT5Δ44, was chosen for further characterization for its relatively high yield of the secreted MT5-MMP proteinases. To compare between secretion of the ecto-enzyme and shedding of the full-length molecule, serum-free conditioned media were collected in the absence or presence of BB94 (50 μM) from MT5Δ44 cells as well as MT591, a cell line expressing the full-length construct, and subsequently analyzed by zymography and Western blotting. As reported previously, MT591 sheds into the conditioned medium a predominantly 28 kDa gelatinolytic species which is reduced in the presence of BB94 with concomitant increase of higher molecular weight forms (Fig. 1B, lanes 3 vs. 4, [11]). As expected, MT5Δ44 cells secrete a combination of gelatinolytic species ranging from 28 to 58 kDa (Fig. 1B, lanes 5 and 6). Curiously, a 28 kDa species, apparently identical to the one shed from MT591, is also present in conditioned medium from MT5Δ44 cells (Fig. 1B, lower arrowhead). In addition, a major species around 58 kDa and minor ones at 40 and 49 kDa were also detected from MT5Δ44 cells (Fig. 1B, lanes 5 and 6, upper arrowhead and short/long arrows). The multiple banding pattern on zymography suggests that the 58 kDa active MT5-MMP species may autocatalytically process itself into smaller species (Fig. 1B, lane 5). To test this possibility, BB94, a synthetic MMP inhibitor, was added to the culture medium to prevent the autocatalytic processing. As shown in Fig. 1B, BB94 in general inhibited the levels of the smaller molecular weight forms of MT5-MMP and protected the larger species (lanes 4 and 6). In fact, the 58 kDa species was stabilized enough for further purification. For reason unclear, BB94 also altered the apparent migration rate of progelatinase B in all three cell lines analyzed (Fig. 1B). To confirm the identity of putative MT5-MMP gelatinolytic activity identified on zymography, BB94-containing conditioned media from control, MT591 and MT5Δ44 cells were analyzed by Western blotting using MT5-MMP antisera. As shown in the right panel of Fig. 1B, a major 58 kDa species, as well as minor smaller species were identified only in the MT5Δ44 cells, not in the control or MT591 cells (lane 9), consistent with the zymography data described above.

3.2. Purification of MT5-MMP protein products

Based on data from Fig. 1, it is apparent that MT5-MMP undergoes extensive processing after secretion into the conditioned medium, and consequently, fails to accumulate significantly as a full-length ecto-enzyme in the culture medium without supplementing BB94. Thus, BB94 becomes a critical supplement in the purification procedure to prevent the fragmentation of the full-length MT5-MMP ecto-enzyme similar to that described for the purification of stromelysin-3 [22]. As described in Section 2, conditioned media were collected from MT5Δ44 cells without or with BB94 (5 μM) and subjected to chromatography, first on a Q-sepharose column, then, an anti-FLAG M2 immuno-affinity column. At the final elution step during affinity chromatography, the columns were washed extensively with at least 15× of the column volume, and the bound materials were eluted competitively with

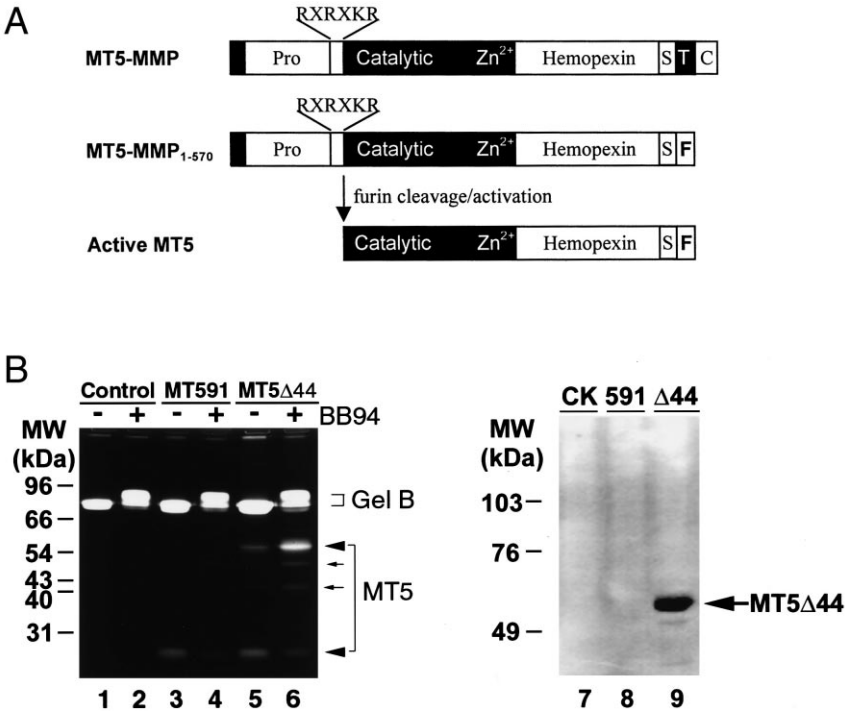

Fig. 1. Expression of mouse MT5-MMP as a soluble, processed ecto-enzyme in mammalian cells. A: Domain structures of wild-type (upper), ecto- (middle) and fully processed ecto- (lower) MT5-MMPs are presented schematically (see Section 2). The ecto-portion of MT5-MMP ($MT5\text{-}MMP_{1-570}$) is tagged with a FLAG epitope for detection and purification. Pro: prodomain; S: stem; T: transmembrane; C: cytosolic tail; F: FLAG. B: Stable expression of MT5-MMP as a soluble enzyme. MDCK cells were transfected stably with control (lanes 1, 2 and 7), full-length (lanes 3, 4 and 8) and ecto- (lanes 5, 6 and 9) MT5-MMP expression constructs, to generate the corresponding cell lines: control, MT591 and MT5Δ44 as described in Section 2. Confluent cultures were washed three times in PBS and allowed to be conditioned under serum-free condition in the absence (lanes 1, 3 and 5) or presence (lanes 2, 4, 6–9) of BB94 (50 μM) for 48 h. The harvested supernatants were analyzed by zymography (lanes 1–6) or Western blotting (lanes 7–9) as described [11]. CK: control, Gel B: gelatinase B; MT5: MT5-MMP.

FLAG peptide at 2:1 molar ratio (peptide:antibody). For the purification of MT5-MMP in the presence of BB94, the final wash step was carried out without the inhibitor to remove the residual inhibitor from the bound materials. To evaluate the purification procedures, the starting medium, flow-through and eluted materials were analyzed by SDS-PAGE followed by Coomassie staining (Fig. 2, lanes 2–4 and 8–10). The eluted products were further analyzed by immunoblotting with both anti-MT5 and anti-FLAG antibody, or zymography (Fig. 2, lanes 5–7 and 11–13). As shown in Fig. 2, the final purified products (lanes 4 and 10) are significantly different between procedures with or without BB94. In the absence of BB94, MT5-MMP was purified as a mixture of fragments ranging from 45–58 kDa, whereas only the 58 kDa species was obtained in its presence with a significantly higher yield (lanes 4 vs. 10 of Fig. 2). Since the proteins were purified with the anti-FLAG M2 affinity column, all eluted products should contain an intact FLAG epitope at the C-termini. Thus, only the largest species at 58 kDa represents the full-length ecto-enzyme whereas the smaller species have N-terminal truncation in the catalytic domain. Indeed, all eluted proteins visualized by SDS-PAGE/Coomassie staining were also detected by anti-FLAG antibody, suggesting the presence of the FLAG epitope (Fig. 2, lanes 5 and 11). On the other hand, the 28 kDa gelatinolytic species present in the conditioned medium of MT5Δ44 cells were not recovered during this purification procedure due to their lack of intact C-termini to which the FLAG tag was linked, thus, failed to bind to the M2 affinity column. Nevertheless, all purified products appear to be MT5-MMP derived protein products since they can all be detected by anti-MT5-MMP antisera (lanes 6 and 12).

Smaller fragments of MT5-MMP are only present in the eluted products purified without BB94, but absent from those purified in the presence of BB94 (Fig. 2, lanes 6 and 12). Since these smaller fragments were also reactive to M2 antibody, they are truncated at the N-termini, i.e. the catalytic domain, potentially rendering the remaining molecule inactive (Fig. 2, lanes 5 and 11). Indeed, when the purified products were analyzed by zymography, only the full-length ecto-enzyme exhibited gelatinolytic activity, suggesting that the smaller fragments were indeed truncated in the catalytic domain of MT5-MMP (Fig. 2, lanes 7 and 13). Thus, the synthetic inhibitor, BB94, is critical in the purification of full-length MT5-MMP ecto-enzyme, providing at least five fold more full-length ecto-MT5-MMP enzyme.

3.3. Enzymic function of MT5-MMP

Based on zymography, it is clear that MT5-MMP degrades gelatin efficiently (Fig. 1B). To begin to analyze the enzymic properties of MT5-MMP, we examined its activity against gelatin in a dose dependent manner. Significant degradation of gelatin was observed with as little as 20 ng of enzyme in a 2 h incubation period (Fig. 3A, lane 3). All gelatin substrates were completely destroyed by 160 ng of enzyme under identical conditions (Fig. 3A, lane 6). At the mean time, known MMP inhibitors such as EDTA, BB94 and TIMP2 completely blocked the activity of MT5-MMP (Fig. 3A, lanes 7–9). It is interesting that the inhibitors blocked not only substrate deg-

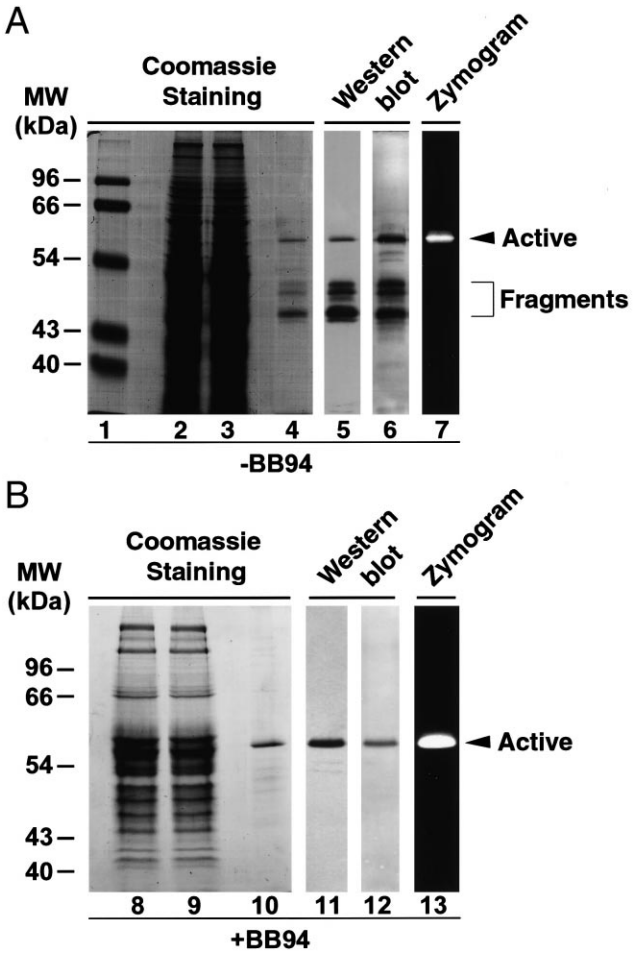

Fig. 2. Purification and analysis of MT5-MMP ecto-enzyme. Serum-free media conditioned from the stable cell line MT5Δ44 in the absence (A) or presence (B) of MMP inhibitor BB94 (5 μM) were fractionated on Q-sepharose ion exchange columns with or without BB94 (5 μM) as indicated. MT5-MMP positive fractions were pooled (lanes 2 and 8) and loaded onto M2-agarose column twice. Most of the proteins flow through without binding to the column (lanes 3 and 9). After washing to remove unbound materials and BB94, the columns were eluted with 2 column equivalents of FLAG peptide to give rise to purified MT5-MMP materials (lanes 4–7, 10–13). Materials from each step (20 μl per lane) were analyzed by SDS-PAGE stained with Coomassie (lanes 1–4, 8–10). The purified materials (50 ng each) were analyzed by Western blotting using anti-FLAG monoclonal antibody (lanes 5 and 11) or rabbit polyclonal antisera raised against human GST-MT5-MMP fusion protein (lanes 6 and 12), or gelatin zymography (lanes 7 and 13) as described [11]. The arrowheads indicate MT5-MMP ecto-enzymes. The bracket marks MT5-MMP fragments generated in the absence of BB94.

radation, but also the fragmentation of enzyme itself (Fig. 3A, lanes 6 vs. 7–9).

To confirm that the purified enzyme has similar properties as the native enzyme, we tested its ability to activate gelatinase A, the putative physiological substrate for the MT5-MMP. As shown in Fig. 3B, MT5-MMP activated progelatinase A to completion (lane 2), in a manner sensitive to MMP inhibitor BB94 as expected (Fig. 3B, lane 3).

3.4. Substrate profile of MT5-MMP

To further analyze the proteolytic properties of MT5-MMP, the purified enzyme was incubated with various extracellular matrix components including fibronectin, laminin, chondroitin sulfate proteoglycans, type I collagen and dermatan sulfate proteoglycans as described previously [14]. The resulting reaction mixtures were analyzed as described in Section 2 to monitor any potential degradation. As shown in Fig. 4, the proteoglycans were degraded completely by MT5-MMP (lanes 12 and 18) in a BB94 sensitive fashion (lanes 13 and 19). In addition, MT5-MMP degraded fibronectin partially under similar conditions (Fig. 4, lane 6). Type I collagen and laminin are apparently resistant to MT5-MMP (lanes 6 and 9).

3.5. Stability of MT5-MMP

While characterizing the enzymic properties of the purified MT5-MMP products, we noticed a steady decrease in the amount of enzyme in the reaction mixtures without MMP inhibitors (Fig. 3A). Since no other proteolytic enzymes are present in the reaction buffer, it is highly likely that the decay is autocatalytic, consistent with a similar observation made

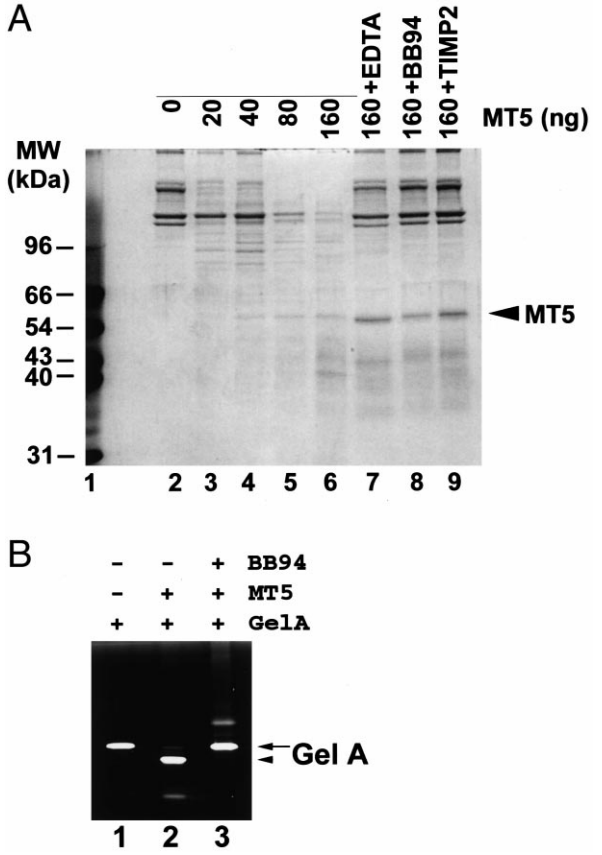

Fig. 3. Proteolytic activity of MT5-MMP ecto-enzyme. A: Degradation of gelatin by MT5-MMP and inhibition by EDTA, BB94 and TIMP2. Gelatin (5 μg) was incubated either alone (lane 2), or purified MT5-MMP at a dose of 20 ng (lane 3), 40 ng (lane 4), 80 ng (lane 5) and 160 ng (lanes 6–9) for 2 h at 37°C. Metalloproteinase inhibitors, EDTA (10 mM, lane 7), BB94 (2.5 μM, lane 8) or TIMP 2 (300 ng lane 9), were added individually into the selected reactions as indicated. At the end of the experiment, portions of each reaction (~1.5 μg of gelatin) were analyzed by SDS-PAGE which was stained with Coomassie blue. B: Activation of progelatinase A by purified MT5-MMP. Progelatinase A from COS conditioned media (20 ml unconcentrated) were either incubated alone (lane 1) or with purified MT5-MMP (40 ng) without (lane 2) or with (lane 3) 2.5 mM of BB94 for 24 h at 37°C. The reaction mixtures were analyzed by gelatin zymography.

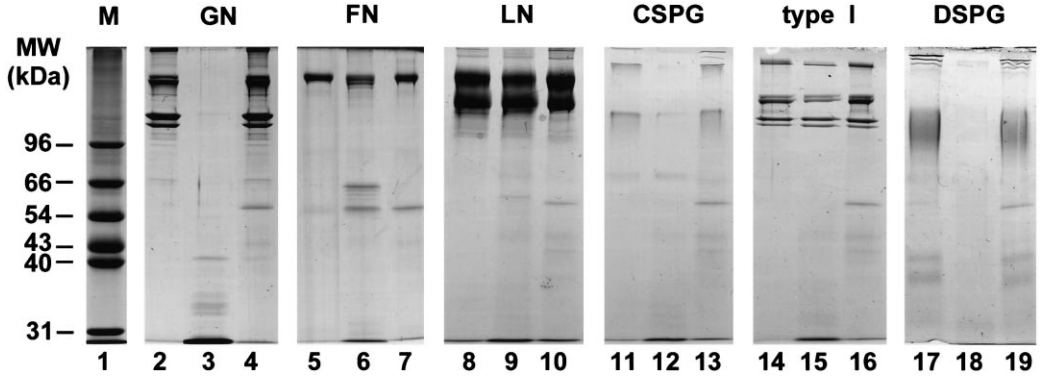

Fig. 4. ECM degradation by MT5-MMP. ECM components (2 μg each) including gelatin (GN, lanes 2–4), fibronectin (FN, lanes 5–7), laminin (LN, lanes 8–10), chondroitin sulfate proteoglycan (CSPG, lanes 11–13), native type I collagen (type I, lanes 14–16) and dematin sulfate proteoglycan (DSPG, lanes 17–19) were either incubated alone (lanes 2, 5, 8, 11, 14 and 17) or with purified MT5-MMP (50 ng, lanes 3, 4, 6, 7, 9, 10, 12, 13, 15, 16, 18 and 19) in absence (lanes 3, 6, 9, 12, 15 and 18) or presence (lanes 4, 7, 10, 13, 16 and 19) of BB94 (5 μM) for 24 h at 37°C (lanes 2–13, 17–19) or 25°C (lanes 14–16). The reaction mixtures were then analyzed by SDS-PAGE and stained as described [14].

during the purification procedure (Fig. 2A). Since the stability of an enzyme affects the kinetics of its substrate degradation, it becomes critical to determine the rate by which MT5-MMP decays autocatalytically. To this end, the purified MT5-MMP was incubated at various temperatures under the identical buffer system used for substrate characterization. As shown in Fig. 5, MT5-MMP fragments itself rapidly at 37°C. More than 50% of the enzyme is converted into smaller fragments within 30 min of incubation (Fig. 5, lane 7). The enzyme is even less stable in the absence of Brij 35 (Fig. 5, lane 8). Within 24 h, MT5-MMP lost all its activity at 37°C, and almost 95% at 20°C (Fig. 5, lanes 18–20). At lower temperature, however, MT5-MMP was relatively stable, able to maintain almost 45% of the enzyme in its original form after 24 h (Fig. 5, lane 17). Based on these observations, it is concluded that MT5-MMP has a relatively short half-life.

4. Discussion

Currently, five distinct members of the MT-MMP subfamily have been identified and characterized [7–11]. One of the hallmark functions for MT-MMPs is their ability to activate progelatinase A on cell surface [7,13]. The exception is perhaps MT4-MMP whose ability to activate progelatinase A has yet to be demonstrated. Based on sequence similarity analysis, MT4-MMP appears to be quiet distant from the rest of the MT-MMP members [6,11], thus, may have different enzymic properties. In addition to progelatinase A, MT1-MMP has been shown to be able to activate procollagenase 3 [12]. Thus, it appears that the primary function for MT-MMPs is to activate a selective group of proMMPs. However, with the expansion of the MT-MMP subfamily, it is unlikely that these five membrane-bound MMPs have been evolved solely to serve as activators for progelatinase A or procollagenase 3.

In this report, we demonstrated that MT5-MMP degrades ECM components directly against at least two proteoglycans-dermatan sulfate proteoglycans and chondroitin sulfate proteoglycans (Fig. 4). By expressing MT5-MMP as a truncated form lacking its transmembrane domain, we were able to purify the ecto-enzyme of MT5-MMP into homogeneity. The availability of this enzyme preparation made it possible to characterize the properties of MT5-MMP in further details. First of all, purified MT5-MMP activates progelatinase A as predicted from previous co-transfection experiments [11]. More significantly, we also demonstrated that MT5-MMP has intrinsic matrix degrading activity. This is in general agreement with the view that MT-MMPs are powerful matrix degrading enzymes themselves, a notion first demonstrated for MT1-MMP by engineering and purifying its ecto-enzyme [14]. It was subsequently confirmed, expanded and extended to MT2, 3-MMPs [15,16,18,24,25]. As a group, MT-MMPs have been shown to be able to degrade fibronectin, laminin, type I, II and III collagen, nidogen, tenascin, aggrecan and perlecan [15,16,18,24,25]. With the availability of purified enzymes, it will be interesting to compare and contrast directly their activities towards not only the traditional substrates such

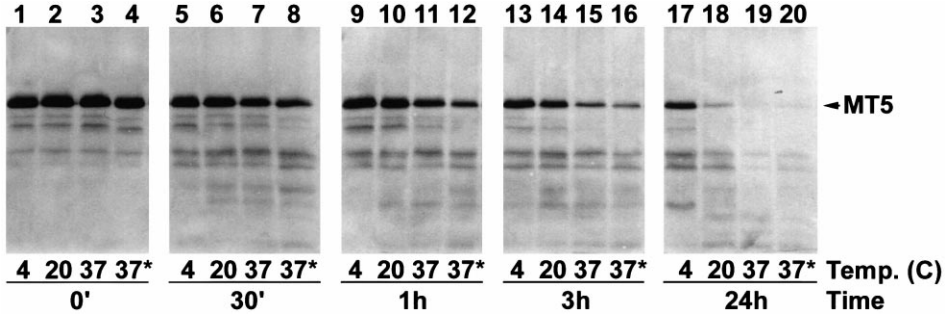

Fig. 5. Stability of MT5-MMP. Purified MT5-MMP (40 ng per reaction) was incubated at 4°C, 20°C, or 37°C for 0 min, 30 min, 1 h, 3 h, and 24 h as indicated. Reactions were terminated by boiling in SDS-PAGE sample buffer and analyzed by Western blotting using rabbit polyclonal anti-MT5-MMP antisera as described previously [11]. * indicates the incubation buffer without 0.1% Brij.

as progelatinase A or procollagenase 3, but also many ECM components.

By design, this study focused on the full-length ecto-enzyme of MT5-MMP. It is interesting to point out that the active proteinase for MT5-MMP may also include the 28 kDa species under some circumstances such as the shed one present in culture supernatants of MT591 expressing the intact molecule. Although BB94 can shift the dynamics from the 28 kDa to the 58 kDa ones when included in the culture medium (Fig. 1), it is not clear whether the 58 kDa can be autocatalytically converted into the 28 kDa species. If so, some of the substrate degrading activities detected in our studies may represent the activity of both the 58 kDa and 28 kDa species, similar to that of stromelysin-3 [22,26]. Further investigation will be needed to clarify the relative contribution of the 28 and 58 kDa species as well as the membrane-bound vs. soluble forms of MT5-MMP in pathobiological processes.

MT5-MMP has a short half-life as an active proteinase. Based on data presented in this paper, it is clear that MT5-MMP has a short half-life at physiological temperature. Almost 50% of the full-length ecto-enzyme undergoes autocatalytic destruction into smaller fragments. Although the half-life for intact MT5-MMP on cell membrane has not been determined, this property along with its tendency to be shed from cell surface serves to distinguish MT5-MMP from the other MT-MMPs. It is interesting to note that its closest relative, MT3-MMP, is degraded rapidly after maturation, although detail stability studies have yet to be reported [19]. On the other hand, MT1-MMP, with less sequence homology to MT5-MMP, appears to be fairly stable [14]. Thus, the stability of each MT-MMP may be a function of its primary amino acid sequence and there may be specific sequence determinants for stability. Furthermore, the half-life of active enzymes, along with latency and TIMPs, may be a critical mechanism for MT-MMPs to achieve precise proteolytic activity at ECM remodeling sites [11,13,14].

Acknowledgements: The author wishes to thank Dr. R. Fridman (Wayne State University) for providing TIMP1 and 2; Helen Mills of British Biotech for providing BB94; Drs. Stephen J. Weiss and Hideaki Nagase for encouragement. This study was supported in part by Grant CA76308 from the National Cancer Institute, American Heart Association Grant-in-Aid 9750197N, Elsa Pardee Foundation, University of Minnesota grant-in-aid, Minnesota Medical Foundation.

References

[1] Chambers, A.F. and Matrisian, L.M. (1997) J. Natl. Cancer. Inst. 89, 1260–1270.
[2] 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 Biol. Med. 4, 197–250.
[3] Werb, Z. (1997) Cell 91, 439–442.
[4] Lochter, A., Sternlicht, M.D., Werb, Z. and Bissell, M.J. (1998) Ann. N.Y. Acad. Sci. 857, 180–193.
[5] Matrisian, L.M. (1992) BioEssays 14, 455–463.
[6] Massova, I., Kotra, L.P., Fridman, R. and Mobashery, S. (1998) FASEB. J. 12, 1075–1095.
[7] Sato, H., Takino, T., Okada, Y., Cao, J., Shinagawa, A., Yamamoto, E. and Seiki, M. (1994) Nature 370, 61–65.
[8] Takino, T., Sato, H., Shinagawa, A. and Seiki, M. (1995) J. Biol. Chem. 270, 23013–23020.
[9] Will, H. and Hinzmann, B. (1995) Eur. J. Biochem. 231, 602–608.
[10] Puente, X.S., Pendas, A.M., Llano, E., Velasco, G. and Lopez-Otin, C. (1996) Cancer Res. 56, 944–949.
[11] Pei, D. (1999) J. Biol. Chem. 274, 8925–8932.
[12] Knauper, V., Will, H., Lopez-Otin, C., Smith, B., Atkinson, S.J., Stanton, H., Hembry, R.M. and Murphy, G. (1996) J. Biol. Chem. 271, 17124–17131.
[13] Nagase, H. (1998) Cell. Res. 8, 179–186.
[14] Pei, D. and Weiss, S.J. (1996) J. Biol. Chem. 271, 9135–9140.
[15] d'Ortho, M.P., Will, H., Atkinson, S., Butler, G., Messent, A., Gavrilovic, J., Smith, B., Timpl, R., Zardi, L. and Murphy, G. (1997) Eur. J. Biochem. 250, 751–757.
[16] Fosang, A.J., Last, K., Fujii, Y., Seiki, M. and Okada, Y. (1998) FEBS Lett. 430, 186–190.
[17] Kolkenbrock, H., Hecker-Kia, A., Orgel, D., Ulbrich, N. and Will, H. (1997) Biol. Chem. 378, 71–76.
[18] Matsumoto, S., Katoh, M., Saito, S., Watanabe, T. and Masuho, Y. (1997) Biochim. Biophys. Acta 1354, 159–170.
[19] Shofuda, K., Yasumitsu, H., Nishihashi, A., Miki, K. and Miyazaki, K. (1997) J. Biol. Chem. 272, 9749–9754.
[20] Polette, M. and Birembaut, P. (1998) Int. J. Biochem. Cell. Biol. 30, 1195–1202.
[21] Sato, H., Okada, Y. and Seiki, M. (1997) Thromb. Haemost. 78, 497–500.
[22] Pei, D., Majmudar, G. and Weiss, S.J. (1994) J. Biol. Chem. 269, 25849–25855.
[23] Pei, D. and Yi, J. (1998) Protein Expr. Purif. 13, 277–281.
[24] d'Ortho, M.P., Stanton, H., Butler, M., Atkinson, S.J., Murphy, G. and Hembry, R.M. (1998) FEBS Lett. 421, 159–164.
[25] Ohuchi, E., Imai, K., Fujii, Y., Sato, H., Seiki, M. and Okada, Y. (1997) J. Biol. Chem. 272, 2446–2451.
[26] Murphy, G., Segain, J.P., O'Shea, M., Cockett, M., Ioannou, C., Lefebvre, O., Chambon, P. and Basset, P. (1993) J. Biol. Chem. 268, 15435–15441.