Cell surface binding and activation of gelatinase A induced by expression of membrane-type-1-matrix metalloproteinase (MT1-MMP)

Hiroshi Sato[a,*], Takahisa Takino[a], Takeshi Kinoshita[a], Kazushi Imai[b], Yasunori Okada[b], William G. Stetler Stevenson[c], Motoharu Seiki[a]

[a]*Department of Molecular Virology and Oncology, Cancer Research Institute, Kanazawa University, 13-1 Takaramachi, Kanazawa 920, Japan*
[b]*Department of Molecular Immunology, Cancer Research Institute, Kanazawa University, 13-1 Takaramachi, Kanazawa 920, Japan*
[c]*Laboratory of Pathology, National Cancer Institute, NIH, Bethesda, MD, USA*

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Abstract Gelatinase A is secreted as a proenzyme (progelatinase A) which is activated and bound on the surface of tumor and normal cells. We have reported that the expression of a membrane-type-1-matrix metalloproteinase (MT1-MMP) induces activation of progelatinase A. Here we demonstrate that the expression of MT1-MMP in COS-1 cells induces cell-surface binding of progelatinase A which is consequently processed to an intermediate form. Processing from the intermediate to the fully active form is dependent on the gelatinase A concentration. These results suggest that the cell-surface binding concentrates the gelatinase A intermediate form locally to allow autoproteolytic processing to the fully active form.

Key words: Metalloproteinase; Activation; Binding

1. Introduction

Gelatinase A/72-kDa type IV collagenase/ MMP-2 is implicated in the progression of tumors [1-3]. However, the abundance of inactive precursor of gelatinase A (progelatinase A) in normal tissues and fluids suggests that progelatinase A utilization is limited by its physiological activation rather than expression alone. Progelatinase A is unique among matrix metalloproteinases, in that it is not activated after digestion with exogenous proteinases such as plasmin, plasma kallikrein, neutrophil elastase or cathepsin G, which are putative physiological activators of other members of this family [4]. Progelatinase A is specifically activated on the surface of tumor and normal fibroblast cells exposed to 12-*O*-tetradecanoylphorbol acetate (TPA) or concanavalin A, as a result of proteolysis, and it is mediated by a fraction of the cell membrane that is sensitive to metalloproteinase inhibitors, including tissue inhibitor of matrix metalloproteinases-2 (TIMP-2) [5-8]. It has also been demonstrated that the activated form of gelatinase A is enriched in the plasma membranes of cancer cells particularly at the invadopodia which is the site of cellular invasion [9-13]. Although the specific binding of gelatinase A to the surface of tumor and normal fibroblast cells has been described, a membrane protein that functions as a gelatinase A receptor in the relevance to the activator has not been identified.

*Corresponding author. Fax: (81) (762) 60-7840;
E-mail: vhsato@icews1.ipc.kanazawa-u.ac.jp

Abbreviations: MT-MMP, membrane-type matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinases; TPA, 12-*O*-tetradecanoylphorbol acetate

We molecularly cloned a new matrix metalloproteinase (MMP) gene, of which the product was associated with the proteolytic activation of progelatinase A [14,15]. Since this MMP has a transmembrane domain and localizes on the cell surface, it was named membrane-type MMP (recently renamed membrane-type-1-MMP, MT1-MMP). MT1-MMP is overexpressed in malignant tumor tissues, including lung and stomach carcinomas that contain activated gelatinase A. This suggests that MT1-MMP is associated with the activation of progelatinase A in these tumor tissues [14,16,17]. MT1-MMP expression has been detected in HT-1080 cells treated with TPA and MDA-MB-231 cells stimulated with concanavalin A, which induce progelatinase A activation [18,19]. Although Strongin et al. isolated MT1-MMP-TIMP-2 complex using a carboxyl fragment of gelatinase A [18], no direct evidence was presented to show that the complex acts as the receptor or activator of progelatinase A.

In the present study we demonstrate that the expression of MT1-MMP induces not only processing but cell-surface binding of progelatinase A.

2. Materials and methods

2.1. Cell culture, transfection, immunoprecipitation and Western blotting

COS-1 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 5% fetal calf serum. A human gelatinase A and MT1-MMP cDNAs were isolated from human placenta cDNA library (Clontech) as described previously [14]. A full length MT1-MMP cDNA 3.4 kb fragment was inserted at the *Eco*RI site of the pSG5 plasmid (Stratagene) [20]. Efficient transfection of the expression plasmid into COS-1 cells was performed by modified calcium coprecipitation as described [21]. Western blotting using monoclonal antibodies was performed as described [14,22].

2.2. Cell surface binding assay

Progelatinase A was purified from the supernatant of COS-1 cells transiently transfected with gelatinase A expression plasmid according to a previously described method [23]. A sample of partially activated gelatinase A was spontaneously generated during a storage at −20°C. Purified progelatinase A was labeled with ^{125}I (Amersham). COS-1 cells (2×10^5) harvested at 24 h after transfection were incubated with ^{125}I-labeled proteins for 15 min in 10 μl of TNC buffer containing 10 mM Tris-HCl (pH 7.5), 150 mM NaCl and 20 mM $CaCl_2$. After washing with the same buffer three times by centrifugation, the cells were dissolved and separated by SDS-polyacrylamide gel electrophoresis. The ^{125}I-labeled proteins resolved on the gel were analyzed using a Bioimage Analyser, BAS1000 (Fuji Photo Film Co.).

3. Results and discussion

We examined the binding of ^{125}I-labeled progelatinase A to the cells expressing MT1-MMP. As shown in Fig. 1,

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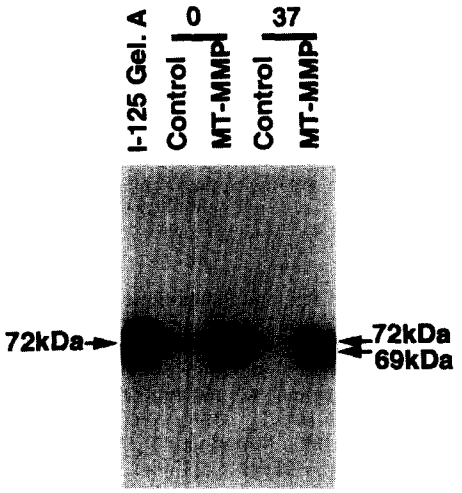

Fig. 1. Induction of progelatinase A binding and processing by expression of MT1-MMP. COS-1 cells transfected with MT1-MMP (MT-MMP) or control plasmid (Control) were harvested 24 h after transfection, suspended in TNC buffer (5×10^4 cells/10 μl) and incubated with 30 pg of progelatinase A labeled with ^{125}I for 15 min at 0 or 37°C (0 and 37, respectively). After washing with the same buffer, [^{125}I]gelatinase A bound to the cells was analyzed under reducing conditions on a 10% SDS-polyacrylamide gel. [^{125}I]Progelatinase A was run as a control (I-125 Gel. A).

[^{125}I]progelatinase A preparation was substantially pure and free from TIMP-2. COS-1 cells transfected with either the control or the MT1-MMP expression plasmid were incubated with 30 pg ^{125}I-labeled progelatinase A at 0 or 37°C for 15 min. After washing, ^{125}I-labeled progelatinase A bound to the cells was resolved by electrophoresis on a 10% polyacrylamide gel. Progelatinase A specifically bound to the cells expressing MT1-MMP but not to control cells, which remained in the latent form (72 kDa under reducing conditions) at 0°C (Fig. 1: '0', lanes denoted MT-MMP and Control, respectively) and was processed to the 69 kDa intermediate form at 37°C (Fig. 1: '37'). Incubation for a longer period at 37°C did not further convert the intermediate form. The progelatinase A-binding capacity of COS-1 cells expressing MT1-MMP was assessed by saturation studies and 1.4×10^5 binding sites per cell with a dissociation constant of 2.1×10^{-9} M were identified by Scatchard analysis (data not shown).

To analyze the mechanism of progelatinase A processing, COS-1 cells expressing MT1-MMP were incubated with various concentrations of [^{125}I]progelatinase A and then gelatinase A on the cell surface was analyzed (Fig. 2). Progelatinase A (72 kDa) incubated at lower concentration (30 pg/10 μl) with MT1-MMP expressing cells was processed only to the intermediate form (69 kDa) (Fig. 2, '30 pg') and was converted further to the fully active form (67 kDa) at higher concentrations (1 ng and 10 ng/10 μl) depending on the progelatinase A concentration (Fig. 2, lanes denoted '+1 ng' and '+10 ng', respectively).

These results raise the further question as to whether both latent and active forms bind to the cells in the same manner. A binding experiment with a sample of partially activated gelatinase A labeled with ^{125}I was carried out. As shown in Fig. 3, only a latent form of gelatinase A bound to MT1-MMP-expressing cells at 0°C which was then processed to an intermediate form at 37°C. However, the 67 kDa active form of gelatinase A failed to bind.

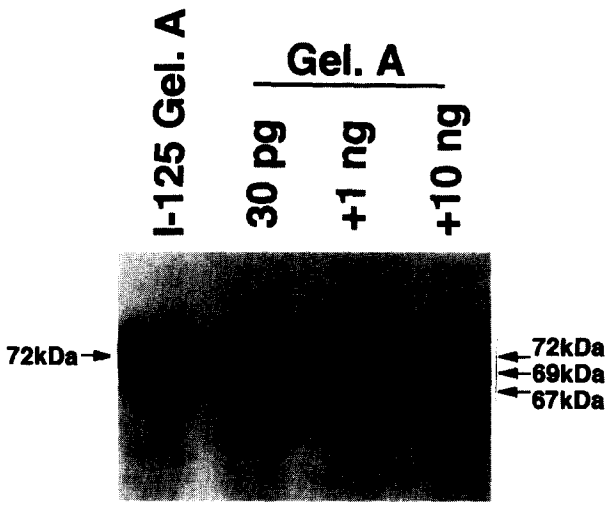

Fig. 2. Gelatinase A-concentration dependent processing to fully active form. COS-1 cells transfected with the MT1-MMP plasmid were incubated with 30 pg, 1 ng and 10 ng of ^{125}I-labeled progelatinase A (about 100 cpm/pg protein) (30 pg, 1 and 10 ng, respectively) for 15 min at 37°C, and then analyzed as described in the legend to Fig. 1.

Gelatinase A mutated such that the proposed active site at glutamic acid residue 375 was replaced by an alanine lost catalytic function [24]. To investigate whether the catalytic function of gelatinase A is required for its processing, gelatinase A or a mutant defective in catalytic function was co-expressed with MT1-MMP and their processing was compared (Fig. 4). Progelatinase A (72 kDa under reducing conditions) was converted to the 67 kDa fully active form due to MT1-MMP expression (Fig. 4, lanes denoted 'Gel. A' and 'Gel. A+MT', respectively). However, the progelatinase A mutant was converted only to the 69 kDa intermediate form (Fig. 4, lanes denoted 'E/A' and 'E/A+MT', respectively). This is consistent with the previous observation that a catalytically inactive mutant progelatinase A was converted to an intermediate form by incubation with CHO cells expressing MT1-MMP [25].

Here, we have demonstrated that the expression of MT1-MMP in COS-1 cells induces cell-surface binding of progelatinase A, which is consequently processed to the intermediate form. Strongin et al. isolated MT1-MMP-TIMP-2 complex

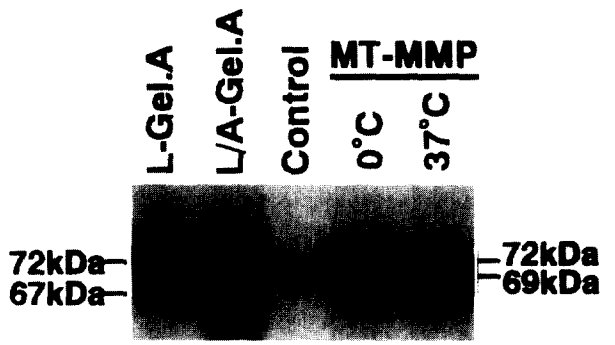

Fig. 3. Progelatinase A-specific binding. A sample of partially activated gelatinase A labeled with ^{125}I (L/A-Gel.A) was incubated with COS-1 cells transfected with control plasmid (Control) or MT1-MMP plasmid at 0 or 37°C (0°C and 37°C, respectively), and then analyzed as described in the legend to Fig. 1. [^{125}I]Progelatinase A was run as a control (L-Gel.A).

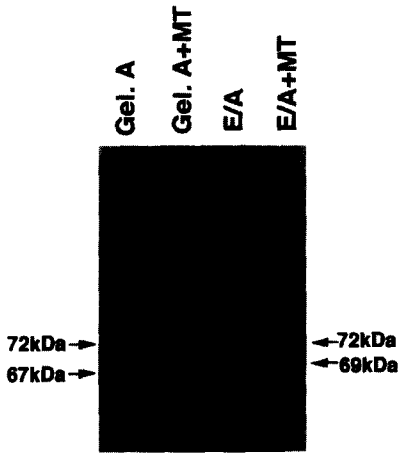

Fig. 4. Processing of the gelatinase A mutant by MT1-MMP expression. An expression plasmid for progelatinase A or a progelatinase A mutant (1 μg) was co-transfected with 3 μg of MT1-MMP or control plasmid into COS-1 cells (Gel. A+MT, E/A+MT, Gel. A and E/A, respectively). Culture supernatants were harvested 24 h after transfection and analyzed by Western blotting using a monoclonal antibody against gelatinase A.

using a carboxyl terminal fragment of gelatinase A and suggested that MT1-MMP-TIMP-2 complex is the receptor and activator of gelatinase A [18]. However, we observed that MT1-MMP expressed in COS-1 cells was substantially free of TIMP-2 as assessed by immunoprecipitation using a monoclonal antibody against TIMP-2 and exogenously added TIMP-2 bound to these cells which abolished binding of progelatinase A (data not shown). Thus, it remains to be clarified whether MT1-MMP by itself or MT1-MMP-TIMP-2 complex serves as a receptor and activator. TIMP-2 and TIMP-2-gelatinase A complex were reported to bind to the surface of MCF-7 cells [26], however, MCF-7 cells do not express a detectable level of MT1-MMP (unpublished data), suggesting an alternative mechanism for the cell-surface binding.

Gelatinase A processing from the intermediate to the fully active form was dependent on the gelatinase A concentration and the gelatinase A mutant defective in catalytic function was converted only to the intermediate form by MT1-MMP expression. These findings support the notion that the processing of progelatinase A to the intermediate form is catalyzed by the function of the activator and that the cell-surface binding concentrates the intermediate form locally to allow autoproteolytic processing to the fully active form. Furthermore, exogenously activated gelatinase A failed to bind to the cell surface. This may suggest that membrane activation is a sequential reaction which is initiated by the binding of progelatinase A to the plasma membrane followed by processing through the intermediate to the fully active form and thus, in vitro activation of progelatinase A results in conformational change which abolishes cell-surface association.

Young et al. have reported that the cell surface binding of progelatinase A not only promotes enzyme activation but also regulates enzyme activity by increasing the rate of substrate cleavage [27]. The cell surface localization of proteinases also has advantages over pericellular proteolysis. Probably MT1-MMP and its family member(s) play a central role in the cell surface localization and activation of progelatinase A and via this mechanism, tumor cells use exogenous progelatinase A to mediate the proteolysis associated with invasion and metastasis.

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