Human progelatinase A can be activated by matrilysin

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Abstract

The activation of human progelatinase A by other matrix metalloproteinases was studied by following both the loss of its N-terminal propeptide and the accompanying increase in the rate of hydrolysis of a synthetic substrate. Activated stromelysin 1 was unable to cause any activation of progelatinase A beyond that slowly occurring by autolysis, but an 8 h incubation with activated matrilysin was able to produce 64% of the activity generated by incubation with (4-aminophenylmercuric)acetate (APMA). Wild-type progelatinase A and a mutant proenzyme that cannot become active were both cleaved by matrilysin to a lower molecular weight species that had lost the propeptide. This shows that matrilysin activates progelatinase A by removing the propeptide in a process that does not require any autolytic cleavages.

Key words. Progelatinase A, Enzyme activation, Matrilysin

1. Introduction

The matrix metalloproteinase (MMP) family participate in the turnover of extracellular matrix components [1]. They are secreted by connective tissue cells as latent precursors that must lose an approximately 80 amino acid propeptide from the N-terminus in order to become activated. This can be achieved by stepwise autolysis induced by organomercurials such as (4-aminophenylmercuric) acetate (APMA). It is proposed that activation in vivo is the result of a two step mechanism involving the removal of the first thirty or so amino acids by a serine endoproteinase, followed by an autolytic cleavage to remove the remaining portion of the propeptide [2]. This mechanism has been demonstrated in vitro for all the MMPs except gelatinase A (EC 3.4.24.24; MMP2; 72kDa gelatinase), which has proved resistant to activation by any of the endoproteinases so far tested [3,4]. We have recently shown, however, that its entire propeptide can be removed by an intermolecular autolytic cleavage catalysed by previously activated gelatinase A [5]. The reaction only proceeds at an appreciable rate with greater than μM concentrations of proenzyme but the ability of progelatinase A to bind to cell surfaces [6] means that localised concentrations might approach this level in vivo. In order to initiate this mode of activation it is necessary that some active gelatinase A is already present. This report describes how it might be generated for we show that another MMP, matrilysin (EC 3.4.24.23, MMP7), is also able to remove the progelatinase A propeptide.

2. Materials and methods

2.1. Materials

Recombinant forms of human progelatinase A, human prostromelysin-1, human tissue inhibitor of metalloproteinases-1 (TIMP-1), human TIMP-2 and the active site mutant of human progelatinase A, proE375A, were all purified from medium conditioned by the relevant transfected mouse myeloma cell line as previously described [5,7-11].

2.2. Proenzyme activation

Maximum progelatinase A activity was generated by the incubation of a 1 μM solution at 23°C for 4 h in the presence of 1 mM APMA. Prostromelysin-1 was activated by incubation with 3 μg/ml TPCK-treated porcine trypsin for 45 min at 37°C. The trypsin was then inactivated by the addition of PMSF to 1 mM. Promatrilysin was activated by incubation at 53°C for 5 h as previously described [7]. All activations were performed in 25 mM Tris-HCl, 30 mM NaCl, 10 mM CaCl2, pH 7.5 (Buffer A) Activated gelatinase A and stromelysin-1 were prepared on the day of assay whilst activated matrilysin was stored prior to its use at -70°C.

2.3. Enzyme assay

Matrix metalloproteinase activity was assayed by following the increase in fluorescence that accompanied hydrolysis of the synthetic substrate, (7-methoxycoumarin-4-ylaceetyl-Pro-Leu-Gly-Leu-[3-(2,4-dinitrophenyl)-l-2,3-diaminopropony]-Ala-Arg-NH₂, which has proved resistant to activa-

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Abbreviations: MMP, matrix metalloproteinase; APMA, (4-amino-phenylmercuric)acetate; TIMP-1 and -2, tissue inhibitor of metallopro-
teinases-1 and 2; proE375A, active site mutant of progelatinase A; TPCK, N-tosyl-phenylalanine chloromethyl ketone; PMSF, phenylmethylsulphonylfluoride; McaPLGLDpaAR, (7-methoxycoumarin-4-ylacetyl-Pro-Leu-Gly-Leu-[3-(2,4-dinitrophenyl)-l-2,3-diaminopropony]-Ala-Arg-NH₂.
2.5. Protein concentration

Progelatinase A concentration was determined by absorbance at 280 nm using $e = 122800 \text{ M}^{-1} \text{ cm}^{-1}$. The concentrations of active stromelysin-1 and active matrilysin were determined by active site titration with known amounts of purified TIMP-2 [7,10].

2.6. N-terminal amino acid sequencing

Proteins were separated by SDS-PAGE run under reducing conditions using precast 4-20% polyacrylamide gels (Daichii, Tokyo) and transferred to a polyvinylidene difluoride membrane (Immobilon PSQ, Millipore). After staining with Ponceau S the relevant bands were excised and sequenced on a 470A Protein Sequencer (Applied Biosystems) with an on-line 120A HPLC.

3. Results

The activation of progelatinase A by either matrilysin or stromelysin 1 was followed by measuring the rate of hydrolysis of the synthetic substrate McaPLGLDpaAR (Fig. 1A). The results demonstrate that, whilst incubation with matrilysin caused a significant increase in activity, stromelysin 1 was unable to activate progelatinase A to a level beyond that observed with no additions. The slow rate of background activation is presumably due to intermolecular autolysis, catalysed initially by the small amount of active gelatinase A that copurifies with the proenzyme [5]. Increasing the matrilysin concentration elevated the maximum observed activity and the rate of its attainment but the values still fell short of those obtained with APMA. This was because gelatinase A is also subject to inactivation occurring a rate that is independent of proteolysis [5,11].

The increase in activity was mirrored by the cleavage of the proenzyme to two active, lower molecular weight species (Fig. 1B). The major product migrated alongside APMA-activated gelatinase A suggesting that it too was produced the removal of the propeptide (amino acids 1-80 [16]). At the later time points of the matrilysin incubation, the second lower molecular weight species became visible. This is likely to be a breakdown product of activated gelatinase A [13]. Both cleavages were caused by MMP activity because they did not occur if the 0.6 μM matrilysin incubation was performed in the presence of 4 μM 11MMP-1 (results not shown).

It is conceivable that the processing of progelatinase A was entirely due to autolytic cleavages that were somehow stimulated by the presence of matrilysin. In order to discount this possibility it was necessary to examine the effect of activated matrilysin on proE375A; a mutant of progelatinase A that cannot become active because a catalytically essential glutamic acid residue has been replaced by one that prevents peptide bond hydrolysis [11]. The results in Fig. 2 show that, in the presence of matrilysin, proE375A is cleaved at a rate comparable to that of the wild-type proenzyme. The major cleavage product was similar to APMA-activated gelatinase A in that it migrated at 67 kDa and had the N-terminal amino acid sequence YNFFPR [3]. This shows that matrilysin is able to hydrolyse the N80-Y81 peptide bond that is also cleaved in the autolytic activation of progelatinase A [3,5]. Of the minor cleavage products produced, the species migrating at 26 kDa is likely to be the catalytically inactive gelatinase A C-terminal domain [17]. In the 1 h time point sample there can also be seen a cleavage product that migrates to a position between that of proE375A and the 67 kDa species. Insufficient quantities were produced for N-terminal sequencing but, because there is a
must be pointed out that the rate of activation by matrix
a combination of autolytic and matrilysin activity. It
the remaining propeptide was more rapidly removed by
type progelatinase A but it is likely that in this instance
intermediate was not seen during the activation of wild-
mechanism is a hallmark of MMP activation [2]. The
formation of an intermediate because this sort of stepwise
little autolytic activation was occurring. It is of interest
that activation by matrilysin might proceed via the for-
demonstrating that, at the proenzyme concentration tested,
stromelysin 1, however, show that not all the members
of this enzyme family possess the specificity of action
obtained with either APMA-induced autolytic activation
or the activation of other MMPs by serine endopro-
tin mRNA levels [19]. Matrilysin might, for example, gener-
ate the small amount of gelatinase A activity required to
initiate an autolytic activation. Experiments designed to
test this hypothesis are currently being performed.

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4. Discussion

The results presented in this report demonstrate that,
unlike all other endoproteinases so far tested, matrilysin
can activate progelatinase A by cleaving away its
propeptide. As the propeptide can also be removed by
autolysis it was expected that other MMPs would also
be able to catalyse the reaction. The results obtained with
stromelysin 1, however, show that not all the members
of this enzyme family possess the specificity of action
necessary to cause significant activation.

Matrilysin processed wild-type progelatinase A and
the mutant at approximately equivalent rates demon-
strating that, at the proenzyme concentration tested,
little autolytic activation was occurring. It is of interest
that activation by matrilysin might proceed via the for-
formation of an intermediate because this sort of stepwise
mechanism is a hallmark of MMP activation [2]. The
intermediate was not seen during the activation of wild-
type progelatinase A but it is likely that in this instance
the remaining propeptide was more rapidly removed by
a combination of autolytic and matrilysin activity. It
must be pointed out that the rate of activation by matri-
disulphide bond linking the two ends of the gelatinase A
C-terminal domain [4,5], it must have been the result of a
clavage within the N-terminal propeptide. Activated
stromelysin 1 produced a significantly smaller amount of
the 67 kDa species confirming that it is much less able
to remove the propeptide from progelatinase A.

Fig. 2. Analysis by SDS-PAGE of the action of matrilysin on
proE375A. ProE375A at a concentration of 4.0 µM in Buffer A was
incubated at 37°C in the presence of either 0.6 µM matrilysin, 0.9 µM
stromelysin 1 or with no additions. At various time points, samples
from each incubation were removed and analysed by non-reduced SDS-
PAGE. Lanes 1-6, matrilysin incubation (lane 1, 0 h; lane 2, 1 h; lane
3, 2 h; lane 4, 4 h; lane 5, 8 h; lane 6, 24 h); lane 7, 24 h with no additions;
lane 8, 24 h with stromelysin 1. The positions of migration of matrilysin
(M) and stromelysin (S) are as indicated.