Degradation of vitronectin by matrix metalloproteinases-1, -2, -3, -7 and -9

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Received 8 June 1995

Abstract The susceptibility of vitronectin (Vn) purified from human plasma to digestion by matrix metalloproteinases (MMPs) was examined. MMP-2, -3, -7 and -9 except for MMP-1 degraded Vn into multiple fragments. MMP-7 showed the highest activity to the substrate among these MMPs, digesting 8-, 30- and 44-fold more preferentially than MMP-2, -3 and -9, respectively. These data suggest that MMP-2, -3, -7 and -9 may be responsible for the pathological degradation and/or normal turnover of Vn.

Key words: Vitronectin; Matrix metalloproteinase; Degradation; Kinetics

1. Introduction

Vitronectin (Vn), an extracellular matrix component in various tissues, was originally identified as serum protein secreted from the liver [1]. However, recent studies demonstrated the expression of Vn mRNA in the extrahepatic tissues [2]. Vn can bind to plasminogen activator inhibitor type 1, plasminogen, complement complex, perforin and antithrombin III in the circulation, and to collagen and elastin microfibrils in extracellular milieu, suggesting that Vn may play important biological roles in the tissues [1]. Actually, Vn is a substrate for cellular adhesion and stimulates motility of the keratinocytes and smooth muscle cells, retinal neurite outgrowth, and migration of the embryonal neural crest cells [3]. In pathological conditions, Vn may be implicated in the tumor cell invasion and metastasis, since expression of the integrin type receptor for Vn is well correlated with the malignant phenotype of tumor cells such as melanoma, glioblastoma and lung carcinoma cells [4,5]. However, no information is so far available for the proteinases involved in turnover and pathological degradation of Vn. In the present study, we have examined susceptibility of Vn to five different matrix metalloproteinases (MMPs) and found that it can be digested by MMP-2 (gelatinase A; EC 3.4.24.24), MMP-3 (stromelysin 1; EC 3.4.24.17), MMP-7 (matrilysin; EC 3.4.24.23) and MMP-9 (gelatinase B; EC 3.4.24.35), but not by MMP-1 (tissue collagenase; EC 3.4.24.7).

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Abbreviations: APMA, 4-aminophenylmercuric acetate; EDTA, ethylenediaminetetraacetic acid; MMP, matrix metalloproteinase; PAGE, polyacrylamide gel electrophoresis; Vn, vitronectin.

2. Materials and methods

2.1. Materials

Materials were obtained as follows: Brij 35 and diisopropyl fluorophosphate from Sigma Chemical Co.; acrylamide, ethylendiaminetetraacetic acid (EDTA) and SDS from Wako Chem., Japan; 4-aminophenylmercuric acetate (APMA) from Aldrich Chemical Co.; heparin-Sepharose CL-6B from Pharmacia Biotech. The zymogens of MMP-1, MMP-2, MMP-3, MMP-7 and MMP-9 were purified and activated by incubation with APMA as described previously [6–9].

2.2. Purification of vitronectin

Purification protocol of Vn from human plasma was essentially the same as reported by Yatohgo et al. [10]. Human plasma was applied to heparin-Sepharose column chromatography equilibrated with 50 mM Tris–HCl, pH 7.5, and 0.15 M NaCl. The flow-through fractions were treated with 8 M urea and applied to the column equilibrated with 8 M urea in the buffer. Vn bound to the column was eluted with 50 mM Tris–HCl, pH 7.5, 0.5 M NaCl, 5 mM EDTA and 8 M urea. The purified Vn showed a doublet of 75,000- and 65,000-M_r bands as reported [10]. It was then dialyzed against 50 mM Tris–HCl, pH 7.5, 0.15 M NaCl, 0.05% Brij 35 and 0.02% NaN_3. After addition of 2 mM diisopropyl fluorophosphate, Vn was stored at −20°C until used.

2.3. Degradation of Vn by MMPs

Digestion of Vn was first carried out by incubation of the substrate at 37°C for up to 24 h with each MMP in an enzyme-to-substrate ratio of 1:30 in 50 mM Tris–HCl, pH 7.5, containing 0.15 M NaCl, 10 mM $CaCl_2$, 0.05% Brij 35 and 0.02% NaN_3. To show the digestion fragments, MMP-1, -2, -3, -7 and -9 were incubated for 4 h at 37°C with the substrate in enzyme-to-substrate ratios of 1:10, 1:20, 1:10, 1:700 and 1:10, respectively. The reactions were terminated with 20 mM EDTA and the products were analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) (10% acrylamide) under reducing conditions.

To determine catalytic efficiency of MMP-2, -3, -7 or -9, Vn (10 μg) was incubated for 2 h at 37°C with each APMA-activated MMP in a final reaction mixture of 70 μl (50 mM Tris–HCl, pH 7.5, 0.15 M NaCl, 10 mM $CaCl_2$, 0.05% Brij 35 and 0.02% NaN_3). Seven different concentrations of each MMP, i.e. enzyme-to-substrate ratios of 1:25–1:150 for MMP-2, -3 and -9 and 1:400–1:1000 for MMP-7, were used for the study. After SDS-PAGE, the protein bands in the gels were stained with 1% Coomassie Brilliant Blue and densitometrically scanned using computer assisted image analysis. The reaction velocity was quantitated by measuring the disappearance of 75,000- and 65,000-M_r bands of intact Vn, since the single cleavage product of Vn was not obtained. The disappearance patterns were linear with time until approximately 20–25% of the substrate was degraded. Based on the molecular masses of 51,929, 70,952, 52,220, 27,938 and 78,426 for MMP-1, -2, -3, -7 and -9, respectively, the extinction coefficients were calculated [8]. $E^{0.1\%}$ = 1.3, 1.9, 1.1, 1.6 and 1.3 ml/mg for MMP-1, -2, -3, -7 and -9, respectively, were used for the studies [8].

2.4. Determination of K_m and V_{max}

K_m and V_{max} of Vn degradation of MMPs were determined using the methods described by Welgus et al. [11]. Briefly, increasing amounts of Vn (8, 10, 12, 14 and 16 μg) were incubated with 1.6 μg of MMP-9, 400 ng of MMP-2 and MMP-3, or 16 ng of MMP-7 for 2 h at 37°C. The reaction was performed in 50 mM Tris–HCl, pH 7.5, 0.15 M NaCl, 10 mM $CaCl_2$, 0.05% Brij 35 and 0.02% NaN_3 at a final volume of 75 μl. The reaction mixtures were subjected to SDS-PAGE under reduction

SSDI 0014-5793(95)00752-0

and the initial velocity of disappearance of the 75,000- or 65,000-M_r bands in the gels was measured densitometrically as described above.

3. Results

Proteolytic fragments of human plasma Vn by MMP-1, -2, -3, -7 and -9 were analyzed on SDS-PAGE under reduction. Among these MMPs, MMP-2, -3, -7 and -9 but not MMP-1 digested Vn (Fig. 1). MMP-2, -3 and -9 digested it into similar major fragments ranging from 62,500- to 31,500-M_r, although some minor bands were different from each other. These products were resistant to further degradation even after longer incubation up to 24 h in a high enzyme-to-substrate ratio of 1:10 (data not shown). On the other hand, MMP-7 showed different digestion products ranging from 61,500- to 22,500-M_r (Fig. 1). Longer incubation of the reaction mixture degraded these fragments into smaller peptides and only the digestion product of 22,500-M_r species remained after 24 h incubation (data not shown). These results suggest that MMP-7 has the strong proteolytic activity to Vn and cleaves it at different sites of the substrate from those by MMP-2, -3 and -9.

The efficiency of the Vn-degrading MMPs was examined by incubation of Vn with different concentrations of the MMPs. Table 1 shows the relative catalytic efficiency of MMP-2, -3, -7 and -9 to cause 50% cleavage of the substrate. Vn was composed of 75,000- and 65,000-M_r species in a ratio of 3.5:6.5 by densitometrical analysis. Both species were equally susceptible to digestion by all the MMPs used in this study. MMP-7 was the most effective enzyme; the efficiency was about 8-, 30- and 44-fold higher than MMP-2, -3 and -9, respectively.

The kinetic parameters, K_m and K_{cat}, were determined for MMP-2, -3, -7 and -9. Since a single initial proteolysis could not be obtained by lowering enzyme concentrations or temperature, the stained gels were scanned densitometrically for the disappearance of intact Vn molecule. In these experiments, degradation of Vn was always kept to <25% to measure initial velocity, even at the lowest concentration of Vn employed and graphed by the method of Lineweaver and Burk. K_m of each

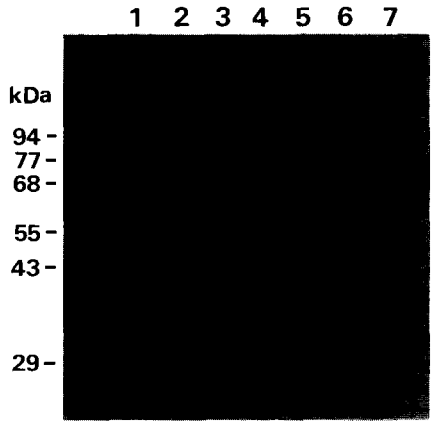

Fig. 1. SDS-PAGE of the reaction products of Vn by incubation with MMP-1, -2, -3, -7 and -9. The substrate (10 μg) was incubated with MMP-1 (1 μg, lane 2), MMP-2 (0.5 μg, lane 3), MMP-3 (1 μg, lane 4), MMP-7 (14 ng, lane 5), or MMP-9 (1 μg, lane 6) for 4 h at 37°C. After termination of the reaction with 20 mM EDTA, the digestion products were subjected to SDS-PAGE (10% acrylamide) under reduction and stained with 1% Coomassie Brilliant Blue. Lanes 1 and 7 are the control samples incubated with buffer alone for 0 and 4 h, respectively.

Table 1
Catalytic efficiency of vitronectin by matrix metalloproteinases

Metalloproteinase	Mol of enzyme producing 50% degradation of vitronectin	
	65,000-M_r form of vitronectin	75,000-M_r form of vitronectin
MMP-2	4.6×10^{-12}	3.7×10^{-12}
MMP-3	1.5×10^{-11}	1.5×10^{-11}
MMP-7	5.2×10^{-13}	4.7×10^{-13}
MMP-9	2.3×10^{-11}	2.1×10^{-11}

Each metalloproteinase was incubated with vitronectin (10 μg) at 37°C for 2 h using different enzyme concentrations as described in section 2. Degradation was extrapolated linearly to estimate the number of metalloproteinase required to produce 50% substrate cleavage.

MMP for both 65,000- and 75,000-M_r species of Vn was approximately equal (Table 2). Values for K_{cat} were observed within the range of 0.03–1.2 molecules of 65,000-M_r Vn/molecule of MMP/min and 0.02–1.1 molecules of 75,000-M_r Vn/molecule of MMP/min. K_{cat}/K_m, an index of specific activity, ranged from 2.0×10^4 M^{-1} min^{-1} to 0.9×10^6 M^{-1} min^{-1} for the 65,000-M_r species and for 2.0×10^4 M^{-1} min^{-1} to 1.1×10^6 M^{-1} min^{-1} for the 75,000-M_r species. The value of K_{cat}/K_m of MMP-7 was approximately 11-, 30- and 50-fold higher than that of MMP-2, -3 and -9, respectively.

4. Discussion

The present study is the first to demonstrate the proteolytic degradation of Vn. Among five MMPs used, i.e. MMP-1, -2, -3, -7 and -9, all but MMP-1 cleaved Vn, suggesting that Vn is a susceptible substrate of the MMP family members. Although MMP-3 and -7 share the substrates, degradation products of the extracellular matrix macromolecules such as fibronectin and laminin by the enzymes are dissimilar [8,12] and the digestion patterns of Vn are also different. Recent studies have indicated that MMP-7 has the highest activity against cartilage link protein, aggrecan, entactin, insoluble elastin, and tenascin [8,13–17]. The present study further demonstrates that MMP-7 is also the most effective enzyme for Vn digestion with 8-, 30- and 44-fold higher activities than MMP-2, -3 and -9, respectively. The data showing that the values of K_m of MMP-2, -3, -7 and -9 against 65,000- or 75,000-M_r species of Vn ($1.0 \sim 1.5 \times 10^{-6}$ M) are almost identical indicate similar binding activities of these MMPs to Vn. It is definite that the high specific activity of MMP-7 to Vn is ascribed to its catalytic activity. However, the precise molecular mechanism is unknown at the present time. It may be possible to speculate that this is because MMP-7 which lacks the COOH-terminal Vn-like domain can readily access the substrate. MMP-2 and -9 are structurally related and share the substrates such as gelatins and type IV and V collagens. However, our previous studies showed that MMP-2 degrades glycoproteins including laminin and fibronectin while MMP-9 cleaves type III collagen and α2 chain of type I collagen [6,9]. For the degradation of Vn MMP-2 was more effective enzyme than MMP-9, but both showed similar digestion products, suggesting the cleavage at the similar sites. MMP-1 was considered to exclusively hydrolyze fibrillar collagens, but recent studies have shown that it also digests other proteins including aggrecan, entactin, cartilage link pro-

Table 2
Kinetic parameters of MMPs on Vn

Metalloproteinase	K_m (M)		K_{cat} (mol of vitronectin digested/mol of enzyme/min)		K_{cat}/K_m (M^{-1}/min^{-1})	
	65,000-M_r form of vitronectin	75,000-M_r form of vitronectin	65,000-M_r form of vitronectin	75,000-M_r form of vitronectin	65,000-M_r form of vitronectin	75,000-M_r form of vitronectin
MMP-2	1.2×10^{-6}	1.0×10^{-6}	0.1	0.1	8.0×10^{4}	1.0×10^{5}
MMP-3	1.5×10^{-6}	1.4×10^{-6}	0.05	0.05	3.4×10^{4}	3.5×10^{4}
MMP-7	1.4×10^{-6}	1.0×10^{-6}	1.2	1.1	0.9×10^{6}	1.1×10^{6}
MMP-9	1.5×10^{-6}	1.3×10^{-6}	0.03	0.02	2.0×10^{4}	2.0×10^{4}

The K_m and K_{cat} of metalloproteinases on both 65,000- and 75,000-M_r species of vitronectin were determined as described in section 2. The ratio K_{cat}/K_m is used as an index of specific activity.

tein, and tenascin [13–15,17]. However, Vn is not a substrate for the enzyme.

Vn is known to be localized in the loose connective tissue, dermis, and atherosclerotic arterial wall [1]. Because of the wide distribution in elastin-containing tissues and the properties to interact with other extracellular matrix proteins, it is considered to serve as a molecular link between elastic fibers and the surrounding collagen/proteoglycan scaffold [1]. Vn is also a ligand for cell adhesion through integrins, urokinase receptor and/or cell surface-associated proteoglycan [1]. The expression of the intergrins for Vn in tumor cells is closely related with their invasive and metastatic phenotype [4]. In the current study, we have shown that MMP-2, -3, -7 and -9 degrade Vn. Since these MMPs are expressed in the tissues of artherosclerotic region, wound healing and cancer [19], they would degrade Vn under such pathological conditions. Actually, it has been reported that Vn extracted from the human adult dermis is composed of the degraded fragments with M_r 58,000, 50,000, 42,000, 35,000 and 27,000 [20], which appear to be similar to those obtained by digestion with MMPs. In addition, Seftor et al. [21] reported that treatment of melanoma cells with Vn or antibodies to the Vn receptor induces MMP-2 expression, enhancing cellular invasion through reconstituted basement membrane. Thus, it seems likely that MMPs may participate in the degradation and/or turnover of Vn in the tissues under the pathophysiological conditions and also modulate tumor cell invasion through degradation of Vn.

Acknowledgements: We are grateful to Dr. Björn Dahlbäck of the Department of Clinical Chemistry, the University of Lund, Sweden, for providing us with vitronectin in the earlier experiments and helpful suggestion.

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