FEBS Letters 352 (1994) 216–218

FEBS 14573

Susceptibility of tenascin to degradation by matrix metalloproteinases and serine proteinases

Kazushi Imai[a], Moriaki Kusakabe[b], Teruyo Sakakura[c], Isao Nakanishi[a], Yasunori Okada[a,*]

[a]*Department of Pathology, School of Medicine, Kanazawa University, 13-1 Takara-machi, Kanazawa, Ishikawa 920, Japan*
[b]*Laboratory of Cell Biology, Riken, Tsukuba Life Science Center, The Institution of Physical and Chemical Research, 3-1-1 Koyadai, Tsukuba, Ibaraki 305, Japan*
[c]*Department of Pathology, School of Medicine, Mie University, 2-174 Edobashi, Tsu, Mie 514, Japan*

Received 16 August 1994

Abstract The degradation of tenascin purified from human melanoma cells was examined by treatment with matrix metalloproteinases (MMPs) and serine proteinases. Among eight different types of proteinases examined, MMP-1, -3, and -7, cathepsin G and leukocyte elastase could digest tenascin, but MMP-2, MMP-9 and thrombin did not. This suggests that tenascin may be readily catabolized by extracellular matrix-degrading proteinases found in the pathophysiological conditions.

Key words: Tenascin; Matrix metalloproteinase; Serine proteinase

1. Introduction

Tenascin is a hexameric protein of more than 1,000 kDa, each subunit of which is in the range of 200–300 kDa depending on the species. It is identified in a wide range of species including chickens, rats, mice, cows, pigs and human, and variously referred to as tenascin, hexabrachion, cytotactin, myotendinous antigen, J1, glial-mesenchymal extracellular matrix protein, or neurotactin [1,2]. Tenascin is known to participate in cell adhesion and repulsion, guidance along cell migration-pathways, the shedding of epithelial cells, the demarcation of tissue boundaries, the stimulation of cell growth, and hemagglutination [2,3]. Localization studies have shown that tenascin is transiently observed in connective tissues during embryogenesis and in some adult tissues under the pathophysiological conditions such as malignant tumors [1]. During fetal lung development tenascin accumulated at the epithelial-mesenchymal interface is quickly degraded after decrease in the mRNA expression [4]. However, no information is so far available for the proteinases which can degrade tenascin.

We report here that tenascin is susceptible to proteolysis by matrix metalloproteinases (MMPs) including MMP-1 (tissue collagenase; EC 3.4.24.7), MMP-3 (stromelysin 1; EC 3.4.24.17) and MMP-7 (matrilysin; EC 3.4.24.23), cathepsin G (EC 3.4.21.20) and leukocyte elastase (EC 3.4.21.37).

2. Materials and methods

2.1. Materials

Materials were obtained as follows: Brij 35, diisopropyl fluorophosphate (DIFP), leukocyte elastase and thrombin (bovine) (EC 3.4.21.5) from Sigma Chemical Co.; acrylamide, ethylenediaminetetraacetic acid (EDTA) and sodium dodecyl sulfate from Wako Chem., Japan; 4-aminophenylmercuric acetate (APMA) from Aldrich Chemical Co.; Sephadex G-10 from Pharmacia Fine Chemicals; cathepsin G from ICN Biochemicals; $Na^{125}I$ (549 MBq/μg) from Amersham. The zymogens of MMP-1, -2 (gelatinase A; EC 3.4.24.24) and -3 were purified from the culture medium of rheumatoid synovial fibroblasts as previously described [5–7]. The zymogens of MMP-7 and -9 (gelatinase B; EC 3.4.24.35) were also purified from the culture media of CaR-1 rectal carcinoma cells (Imai et al., manuscript submitted) and HT-1080 fibrosarcoma cells [8], respectively. These proenzymes were fully activated by incubation with APMA as described previously [6–9].

2.2. Purification and radioiodination of tenascin

Human tenascin was purified from melanoma cell conditioned medium as previously reported [10]. Purified tenascin was iodinated according to the method of Fraker and Speck [11] and free ^{125}I was removed from the sample by spin column of Sephadex G-10 equilibrated with 50 mM Tris-HCl, pH 7.5, 0.15 M NaCl, 0.05% Brij 35 and 0.02% NaN_3 as previously described [7].

2.3. Digestion of tenascin by endopeptidases

Digestion of a mixture of ^{125}I-labelled and non-labelled tenascin was carried out by incubation with the proteinases in an enzyme-to-substrate ratio of 1:25 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 at 37°C. For the digestion with MMP-2 and MMP-9 the enzyme-to-substrate ratio was increased up to 1:1. The reactions were terminated with 20 mM EDTA and 2 mM DIFP for activities of MMPs and serine proteinases, respectively. The digestion products were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (5% acrylamide) under the non-reducing or reducing conditions, and the gels were autoradiographed.

3. Results

When tenascin purified from the human melanoma cells was analyzed by SDS-PAGE without reduction, two aggregated protein bands with very high molecular weight were identified in the upper gel and at the top of the lower gel, which may correspond to haxamer and trimer of the subunit, as previously noted [12] (Fig. 1A). Under the reducing conditions, they were migrated as a major protein band of ~250 kDa (Fig. 1B). Incubation of the substrate with MMP-1, -2, -3, -7 or -9 and subsequent analysis of the products by SDS-PAGE showed that MMP-1, -3 and -7 degrade tenascin (Figs. 1 and 2). MMP-1 and MMP-3 had similar actions on tenascin and digested it into major fragments of 210, 120 and 100 kDa under reduction (Fig. 1B). Under the non-reducing conditions, the huge aggregated

*Corresponding author. Fax: (81) (762) 34-4228.

Abbreviations: MMP, matrix metalloproteinase; DIFP, diisopropyl fluorophosphate; EDTA, ethylenediaminetetraacetic acid; APMA, 4-aminophenylmercuric acetate; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

SSDI 0014-5793(94)00960-0

form observed in the upper gel appeared to be processed to two fragments (Fig. 1A). Time course study of the digestion by MMP-1 and MMP-3 showed that the conversion of tenascin to the fragment of 210 kDa is initiated at 4 h incubation and completed after 12 h incubation without further degradation of the product by longer incubation up to 24 h (data not shown). On the other hand, MMP-7 degraded tenascin into several fragments with less than 190 kDa under reduction and the aggregated form found under non-reduction disappeared (Fig. 2A and B). In contrast to the actions of these MMPs, tenascin was resistant to MMP-2 or MMP-9 (Fig. 1A and B). This was the case even when the substrate was incubated with each MMP in a 1:1 enzyme-to-substrate ratio (data not shown).

Tenascin was extremely susceptible to cathepsin G and leukocyte elastase (Fig. 2A and B), whereas no degradation was observed with thrombin under the similar conditions (data not shown). These two serine proteinases digested the protein into multiple small fragments under both non-reducing and reducing conditions (Fig. 2A and B).

4. Discussion

The present study demonstrates for the first time that tenascin is degraded by MMP-1, MMP-3, MMP-7, cathepsin G and leukocyte elastase. The degradation by MMP-1 and MMP-3 seemed to be limited digestion, producing similar large fragments under reduction. On the other hand, tenascin was most susceptible to digestion by MMP-7 among MMP-1, -2, -3, -7 and -9. Obviously, MMP-7 degrades tenascin at multiple sites because of the many fragments generated by the incubation with MMP-7. Although MMP-1 is an enzyme active basically on the fibrillar collagens such as types I, II and III [13], the present result adds tenascin to the list of the substrates. The substrate specificity of MMP-3 and MMP-7 is reported to be similar [13]. This is the case with tenascin although the specific activity of MMP-7 seems to be greater than that of MMP-3. The present data that tenascin is completely resistant to the action of MMP-2 and MMP-9 suggest that these gelatinases play limitted or no role in the turnover in vivo. Cathepsin G and leukocyte elastase most effectively digested tenascin into small peptides. Because both serine proteinases are derived from polymorphonuclear leukocytes [14], their role in the tenascin degradation may be important in the tissues with suppurative inflammation.

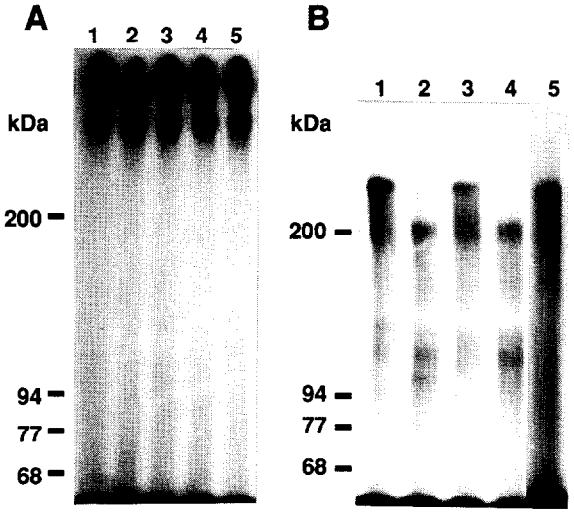

Fig. 1. SDS-PAGE of the reaction products of ^{125}I-labelled tenascin generated by incubation with MMP-1, -2, -3 and -9. The substrate (1 μg) was incubated with MMP-1 (lane 2), MMP-2 (lane 3), MMP-3 (lane 4) or MMP-9 (lane 5) (40 ng each) for 24 h at 37°C. The digestion products were subjected to SDS-PAGE (5% acrylamide) under non-reduction (A) or reduction (B) and the gels were autoradiographed. Lane 1 is the control sample incubated with buffer alone for 24 h at 37°C.

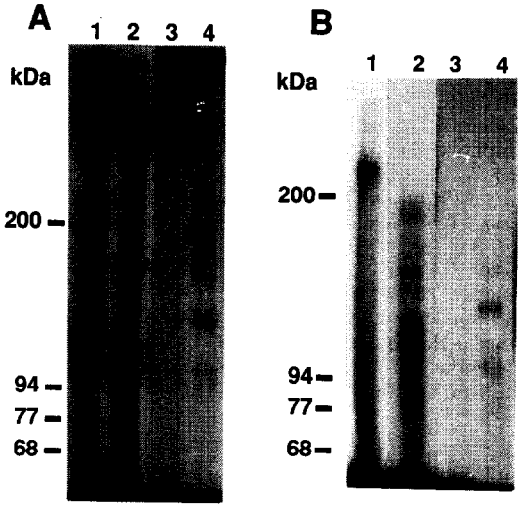

Fig. 2. SDS-PAGE of the degradation products of tenascin with MMP-7, cathepsin G and leukocyte elastase. The substrate was incubated with MMP-7 (lane 2), cathepsin G (lane 3) or leukocyte elastase (lane 4) and the reaction products were analyzed by SDS-PAGE under non-reduction (A) or reduction (B) as described in Fig. 1. Lane 1 is the control sample incubated with buffer alone.

Tenascin is a unique protein, which is transiently observed in the tissues during the embryogenesis and in some adult tissues such as the endometrium [15], the healing wounds [16] and the tumor stroma [1]. Although the tissue- and time-specific localization may be ascribed to the transient expression of the tenascin gene, it can also be explained by enhanced degradation of tenascin by proteinases in the tissues. In fact, tenascin-degrading MMPs are known to be expressed in these tissues; MMP-1, -3 and -7 in the endometrium [17,18], MMP-1 and -3 in the healing wounds [19,20], and many types of MMPs including MMP-1, -3 and -7 in the tumors [21]. Since the tissues are frequently infiltrated by leukocytes, both groups of the MMPs and serine proteinases may be implicated in the normal turnover and/or pathological degradation of tenascin in vivo.

References

[1] Chiquet-Ehrismann, R. (1993) Semin. Cancer Biol. 4, 301–310.
[2] Sakakura, T. and Kusano, I. (1991) Acta Pathol. Jpn. 41, 247–258.
[3] Erickson, H.P. and Bourdon, M.A. (1989) Annu. Rev. Cell Biol. 5, 71–92.
[4] Young, S.L., Chang, L.Y. and Erickson, H. (1994) Dev. Biol. 161, 615–625.
[5] Zhang, J., Fujimoto, N., Iwata, K., Sakai, T., Okada, Y. and Hayakawa, T. (1993) Clin. Chim. Acta 219, 1–14.
[6] Okada, Y., Morodomi, T., Enghild, J.J., Suzuki, K., Yasui, A., Nakanishi, I., Salvesen, G. and Nagase, H. (1990) Eur. J. Biochem. 194, 721–730.

[7] Okada, Y., Harris Jr., E.D. and Nagase, H. (1988) Biochem. J. 254, 731–741.
[8] Okada, Y., Gonoji, Y., Naka, K., Tomita, K., Nakanishi, I., Iwata, K., Yamashita, K. and Hayakawa, T. (1992) J. Biol. Chem. 267, 21712–21719.
[9] Crabbe, T., Willenbrock, F., Eaton, D., Hynds, P., Carne, A.F., Murphy, G. and Docherty, A.J.P. (1992) Biochemistry 31, 8500–8507.
[10] Oike, Y., Hiraiwa, H., Kawakatsu, H., Nishikai, M., Okinaka, T., Suzuki, T., Okada, A., Yutani, R. and Sakakura, T. (1990) Int. J. Dev. Biol. 34, 309–317.
[11] Fraker, P.J. and Speck Jr., J.C. (1987) Biochem. Biophys. Res. Commun. 80, 849–857.
[12] Erickson, H.P. and Lightner, V.A. (1988) Adv. Cell Biol. 2, 55–90.
[13] Birkedal-Hansen, H., Moore, W.G.I., Bodden, M.K., Windsor, L.J., Birkedal-Hansen, B., DeCarlo, A. and Engler, J.A. (1993) Crit. Rev. Oral Biol. Med. 4, 197–250.
[14] Bretz, U. and Baggiolini, M. (1974) J. Cell Biol. 63, 251–269.
[15] Vollmer, G., Siegal, G.P., Chiquet-Ehrismann, R., Lightner, V.A., Arnholdt, H. and Knuppen, R. (1990) Lab. Invest. 62, 725–730.
[16] Mackie, E.J., Halfter, W. and Liverani, D. (1988) J. Cell Biol. 107, 2757–2767.
[17] Salamonsen, L., Nagase, H. and Woolley, D.E. (1991) J. Cell Sci. 100, 381–385.
[18] Rodgers, W.H., Osteen, K.G., Matrisian, L.M., Navre, M., Giudice, L.C. and Gorstein, F. (1993) Am. J. Obstet. Gynecol. 168, 253–260.
[19] Saarialho-Kere, U.K., Kovacs, S.O., Pentland, A.P., Olerud, J.E., Welgus, H.G. and Parks, W.C. (1993) J. Clin. Invest. 92, 2858–2866.
[20] Quanglino Jr., D., Nanney, L.B., Kennedy, R. and Davidson, J.M. (1990) Lab. Invest. 63, 307–319.
[21] Stetler-Stevenson, W.G., Aznavoorian, S. and Liotta, L.A. (1993) Annu. Rev. Cell Biol. 9, 541–573.