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Degradation of type IX collagen by matrix metalloproteinase 3 (stromelysin) from human rheumatoid synovial cells

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The degradation of type IX collagen, a minor collagen in cartilage, was examined by treatment with three different types of matrix metalloproteinases (MMPs) purified from the culture medium of rheumatoid synovial cells. Neither MMP-1 (collagenase) nor MMP-2 (so-called 'gelatinase') could digest type IX collagen, but MMP-3 (stromelysin) readily degraded it into smaller fragments. This suggests that MMP-3 may be responsible for the pathological degradation and/or normal turnover of type IX collagen.

Extracellular matrix; Degradation; Proteinase

1. INTRODUCTION

Type IX collagen is a recently characterized minor collagen in cartilage. Biochemical and molecular biological studies [1–4] have demonstrated that it is a heterotrimer of three disulfide-bonded polypeptide chains (α1, α2, α3) each containing three triple-helical domains (COL1–3), interspersed with noncollagenous domains (NC1–4). One of the unique properties of the molecule is the presence of chondroitin and dermatan sulfate-hybrid glycosaminoglycan chains covalently attached to the α2(IX) chain [5,6]. This collagen is co-distributed with type II collagen throughout the extracellular matrix of cartilage [7]. Electron-microscopic studies of cartilage fibrils by Vaughan et al. [8] showed that the individual type IX molecules align along the type II fibril projecting COL3 arm and the NH_2-terminal globular (NC4) domain out of the fibril with a d-periodic distribution. It is suggested that the NC4 domain with pI = 9.7, located away from the fibril, may facilitate interaction with the polyanionic proteoglycans in cartilage [9]. Moreover, biochemical studies demonstrated that type IX and II collagens are covalently attached through trivalent hydroxypyridinium cross-links [10] formed between one of the chains of type IX collagen at the central triple helical (COL2) domain and two of the α1(II) chains at either the NH_2-telopeptide [11,12] or the COOH-telopeptide [11] region. These data suggest that type IX collagen plays an important role in maintaining the structural integrity of cartilage. Recently, the ability of human neutrophil elastase to digest type IX collagen was reported by Gadher et al. [13], but at present no information is available regarding the connective tissue proteinases which may be responsible for the degrada-

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Note: collagenase (EC 3.4.24.7), 'gelatinase' and stromelysin are referred to here as MMP-1, MMP-2 and MMP-3, respectively, for systematic distinction of the three matrix metalloproteinases found in the same culture medium [14]

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tion and/or normal turnover of this collagen in vivo. We report here that type IX collagen is degraded by matrix metalloproteinase 3 (MMP-3) purified from the culture medium of rheumatoid synovial cells [14], but is resistant to MMP-1 and MMP-2.

2. MATERIALS AND METHODS

Type IX collagen was isolated from 13-day-old chick embryo vitreous body by some modification of the purification methods reported previously [15]. The purified collagen showed immunological cross-reactivity with and similar biochemical properties to the type IX collagen derived from sternal cartilage except for the longer glycosaminoglycan chains attached to the α2(IX) chain and lower M_r of the α1(IX) chain (Yada, T. and Kimata, K., in preparation). The substrate was dissolved in 50 mM Tris-HCl (pH 7.4)/0.15 M NaCl/10 mM $CaCl_2$/0.05% Brij 35/0.02% NaN_3 and reacted with MMP-1, -2 or -3.

An active form of MMP-3 (M_r 45000) was purified to homogeneity from the medium of rheumatoid synovial cells treated with rabbit macrophage-conditioned medium as in [14]. MMP-1 and MMP-2 were partially purified from the samples obtained during the MMP-3 purification steps by removal of contaminating metalloproteinases using Affi-Gel–gelatin and anti-(MMP-3) IgG-Sepharose columns for the purification of MMP-1, and anti-(MMP-1) and anti-(MMP-3) IgG-Sepharose columns for the purification of pro-MMP-2. One unit of MMP-1 or MMP-2 activity is defined as that digesting 1 μg collagen or 1 μg gelatin per min at 37°C as described by Cawston and Barrett [16] and Harris and Kraned [17], respectively. One unit of MMP-3 produces 1 μg reduced, carboxymethylated transferrin fragments soluble in 3.3% (w/v) trichloroacetic acid in 1 min at 37°C [14].

Type IX collagen (~15 μg) with or without glycosaminoglycan chains was incubated with MMP-3 (1 unit, 200 ng), MMP-2 (1 unit) or MMP-1 (1 unit) at 35°C for 6–26 h. After terminating the reactions with 20 mM EDTA the reaction products were analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) under nonreducing and reducing conditions and the gels were stained with Coomassie brilliant blue. Type IX collagen free of glycosaminoglycans was prepared by incubation with chondroitinase ABC (0.1 U/ml; Seikagaku Kogyo, Tokyo) for 12 h at 25°C.

3. RESULTS AND DISCUSSION

When chondroitinase ABC-treated type IX collagen was analyzed by SDS-PAGE (5% total acrylamide) without reduction, one major protein band of M_r 280000 was identified (fig.1A). Two minor protein bands of M_r 70000 were derived from the chondroitinase ABC preparation. The protein was susceptible to bacterial collagenase. Incubation of this material with MMPs and subsequent analysis of the products by SDS-PAGE showed that only MMP-3 degraded type IX collagen, initially into an intermediate fragment of M_r 260000 and then into a smaller fragment of M_r 230000 (fig.1A). The latter fragment of M_r 230000 was resistant to subsequent treatment with MMP-2, indicating that it largely retains the triple-helical structure (not shown). Lower-M_r fragments corresponding to M_r 170000, 49000 and 24000 were also identified when larger amounts of the substrate (~20 μg) were reacted with MMP-3 and analysed by SDS-PAGE with 6% acrylamide (fig.2). Type IX collagen was resistant to MMP-2 and MMP-1 (fig.1A). Similar specific degradation of type IX collagen by MMP-3 was also observed with biosynthetically radiolabeled type IX collagen from chick sternal cartilage (not shown). The slight decrease in density of the type IX collagen band after treatment with MMP-2 may be due to partial denaturation of the collagen molecule during preparation and/or incubation with chondroitinase ABC, since MMP-2 did not cause any further degradation of the collagen or emergence of its degradation fragments even after prolonged incubation (fig.1A). Without chondroitinase ABC treatment neither type IX collagen nor its degradation products by MMP-3 entered the stacking gel (3% total acrylamide). This suggests that the major proteolytic fragments, as well as the original type IX collagen, retain long glycosaminoglycan side chains.

Under reducing conditions α1(IX) and α3(IX) chains of intact type IX collagen were observed, but the α2(IX) chain was not visualized since it migrates diffusely at a position above the α1(IX) chain due to the heterogeneity in size of the glycosaminoglycan chains covalently attached to this chain [5] (fig.1B). When type IX collagen was treated with chondroitinase ABC and electrophoresed under reducing conditions, α chains wer not readily identified, since several protein bands derived from the chondroitinase ABC preparation migrated near the α chains. Therefore, analyses of the reaction products of glycosaminoglycan-free type IX collagen under reducing conditions were not carried out. As shown in fig.1B, it was clear that both the α1(IX) chain of M_r 61000 and the α3(IX) chain of M_r 68000 were digested by MMP-3 to a major fragment of M_r 47000. However, the α chain from which it was derived was not determined. No degradation of either

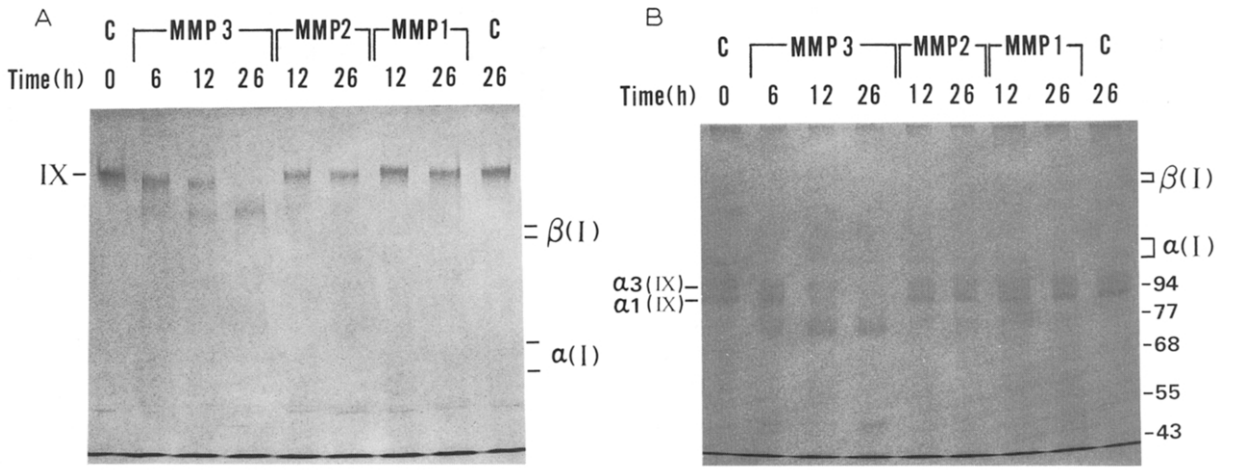

Fig.1. SDS-PAGE of the reaction products of type IX collagen generated by incubation with MMPs. (A) Type IX collagen free of glycosaminoglycan chains was incubated with MMP-1–3 at 35°C for 0–26 h and samples were analysed by SDS-PAGE (5% total acrylamide) without reduction. (B) Samples of type IX collagen with glycosaminoglycan chains reacted in the same way were run on a gel (7% total acrylamide) with reduction. α and β chains of type I collagen and M_r values ($\times 10^{-3}$) of globular standard proteins are indicated to the right. Approximate M_r values of type IX collagen and its degradation products were calculated by comparison with the mobility of collagen standards.

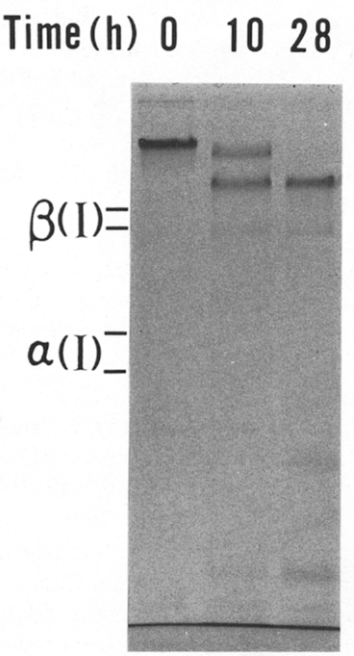

Fig.2. SDS-PAGE of the reaction products of type IX collagen free of glycosaminoglycans generated by incubation with MMP-3. Substrate (~20 μg) was incubated with MMP-3 at 35°C for 0–28 h and samples were subjected to SDS-PAGE (6% total acrylamide) without reduction. Positions where α and β chains of type I collagen migrate are indicated.

chain by MMP-1 and MMP-2 confirms the observation that type IX collagen is not susceptible to these enzymes as shown in fig.1A.

This study is the first to demonstrate the degradation of type IX collagen by a metalloproteinase secreted from connective tissue cells, i.e. by MMP-3. Although at present the sites of cleavage by MMP-3 of the collagen remain unelucidated, it seems likely that noncollagenous domains are susceptible to MMP-3 since MMP-3 can remove N-propeptides from type I procollagen, but does not digest the helical region of type I collagen [14]. The initial slight decrease in M_r is derived from the degradation at either the amino-terminal or carboxy-terminal non-helical region. The fragment of M_r 230000 may be generated by proteolytic cleavage at either the NC3 or NC2 domain. The removal of either the COL3 or COOH-terminal CO1 domain from type IX collagen results in a fragment of similar size of which three α chains are retained to be linked by disulfide bonds. Cysteinyl residues involved in interchain disulfide bonds are located in NC1 and NC3 domains [2]. Smaller fragments generated after longer incubation are probably due to the degradation of disulfide-bonded regions (fig.2).

MMP-3 is not only synthesized and secreted by

cultured rheumatoid synovial cells [14] but is also produced by the synovial lining cells of rheumatoid synovium (Okada, Y. et al., submitted). MMP-3 digests, in addition to the action on type IX collagen reported here, cartilage proteoglycans, fibronectin, type IV collagen, laminin and removes N-propeptide of type I procollagen at neutral pH [14]. It has also been reported that MMP-3 is essential for full activation of procollagenase [18,19]. More recently, the proteoglycan-degrading neutral metalloproteinase isolated from human articular cartilage has been identified as MMP-3 (stromelysin) [20]. Thus, the elevated proteoglycan-degrading activity found in osteoarthritic cartilage [21] may be largely due to the increased production of MMP-3. These observations suggest that MMP-3 may play an important role in the destruction of articular cartilage under various pathological conditions such as rheumatoid arthritis and osteoarthritis deformans by degrading both proteoglycans and type IX collagen.

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