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PROTEASE SUSCEPTIBILITIES OF HMW, 1α, 2α, BUT NOT 3α CARTILAGE COLLAGENS ARE SIMILAR TO TYPE V COLLAGEN

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The protease susceptibilities of recently identified cartilage collagens HMW, 1α, 2α, and 3α were investigated. Mammalian skin collagenase cleaved the 3α chain under conditions where HMW, 1α and 2α were not degraded. A tumor cell derived type V collagenolytic metalloproteinase degraded HMW, 1α and 2α, but not 3α. Plasmin or leucocyte elastase failed to significantly degrade any of the cartilage collagens when digestion was performed at 25°C (15 hours, enzyme to substrate ratio 1:100). At 36°C but not 33°C α thrombin degraded HMW, 1α and 2α, with little or no degradation of 3α. This pattern of protease susceptibility for HMW, 1α and 2α is therefore similar to type V collagen. The cleavage of 3α by skin collagenase but not by elastase is similar to type II collagen. These results suggest that HMW, 1α and 2α are part of the type V collagen family.

INTRODUCTION

Normal hyaline cartilage contains several minor collagenous components which are chemically distinct from type II collagen. Burgeson and Hollister (1) identified three collagenous chains designated 1α, 2α and 3α. The 3α chain has substantial sequence homology to type II collagen and it may be an α1 (II) chain modified at multiple sites by hydroxylation and glycosylation (1, 2). Chains designated 1α and 2α exhibit CNBr peptide maps distinct from 3α and type V collagen, but show similarities to type V collagen in their amino acid composition, their migration in polyacrylamide gels, and with regard to solubility in high concentrations of NaCl (2). Reese and Mayne (2) identified two new collagenous molecules from pepsinized chicken cartilage designated HMW and LMW. Both of these molecules are similar to α_1 (V) and α_2 (V) in amino acid composition, but differ in that they contain disulfide bridges. Since it has been shown that type V collagen is considerably larger than that observed after pepsinization (3, 4), it is possible that HMW, LMW, 1α and 2α are part of the type V collagen family.

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We investigated whether the protease susceptibility pattern of the minor cartilage collagens was similar to any of the other collagen types. Collagen types I, II, III, IV and V all show marked difference in their susceptibility to both metalloproteinases and serine proteases (5-9). Interstitial collagens I, II and III but not collagens IV and V are susceptible to classic vertebrate collagenase. Using a type V collagenolytic metalloproteinase isolated from tumor cells, leucocyte elastase, plasmin and α thrombin, we found a clear difference between the protease susceptibility of 3α compared to all the other minor cartilage collagens.

MATERIALS AND METHODS

Collagen substrates. Minor collagens of chicken hyaline cartilage were solubilized by limited pepsinization (2). After precipitation of type II collagen at 0.9 M NaCl 0.5 M HAc, HMW, 1α, 2α and 3α were obtained by further dialysis and precipitation in 1.2 M NaCl 0.5 M HAc as described previously (1, 2). Additional collagenous molecules HMW and LMW were present in the 2.0 M NaCl 0.5 M HAc precipitate (2). Type V collagen [AB_1, or α_1 (V) and α_2 (V)] and interstitial collagens were purified as described previously (5, 6, 10).

Proteases. Type V collagenolytic metalloproteinase was partially purified from the media of cultured reticulum cell sarcoma cells, as described previously (6), using ammonium sulfate precipitation, molecular sieve chromatography and high pressure liquid chromatography. The enzyme appears as a doublet of 80000 Mr on polyacrylamide gel electrophoresis and has an isolectric point of pH 7.5. Leucocyte elastase was generously supplied by Dr. James Gadek (Pulmonary Branch, National Institutes of Health) (7) and verified to specifically cleave type III collagen (25°C, 15 hours enzyme to substrate ratio 1:100) and to produce multiple fragments from pepsinized type IV collagen. Plasmin and α thrombin were obtained and verified for purity as described previously (11). Human skin collagenase was partially purified from organ cultures of human skin using the method of Eisen et al. (15).

Digestion conditions. The collagen substrate was solubilized in 0.5 M HAc and neutralized in 0.5 M TRIS-HCl, 0.2 M NaCl, 5 mM $CaCl_2$ pH 7.4. Incubations were performed at various temperatures and times stated in the figure legends with a constant enzyme to substrate ratio of 1:100. The digestion mixture was precipitated with 95% ice cold absolute ethanol and immediately subjected to SDS-polyacrylamide gel electrophoresis as described previously (6).

RESULTS

Skin collagenase degraded 3α collagen under conditions where HMW, 1α, 2α, α_1 (V) and α_2 (V) were not affected (Fig. 1). The large cleavage product of 3α collagen comigrated with the $\alpha_1(I)^A$ cleavage product of

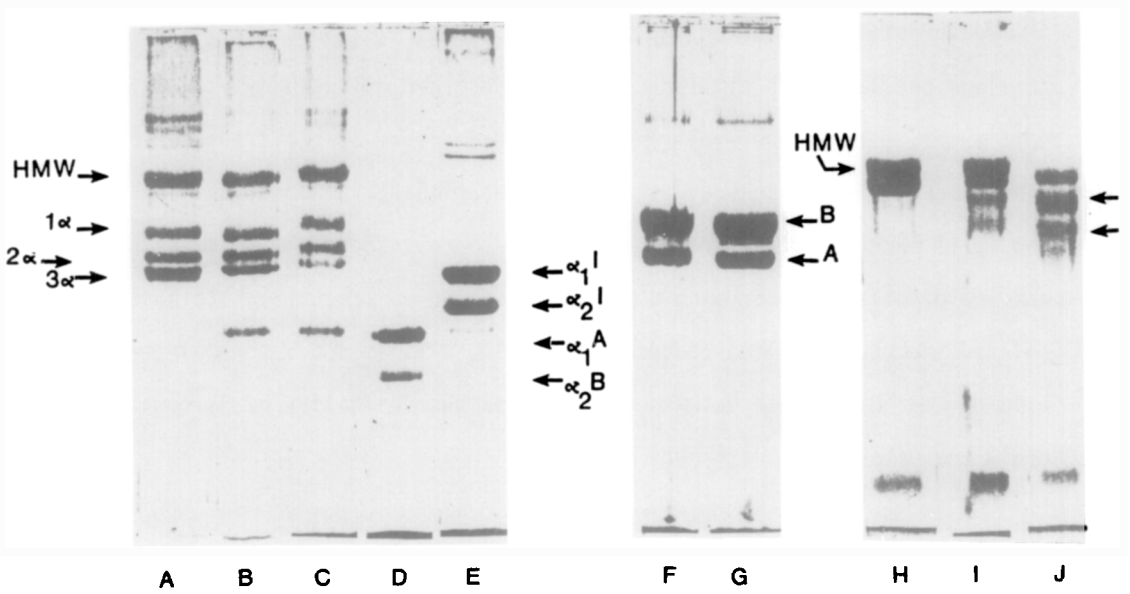

Figure 1. SDS-polyacrylamide gel (5%) electrophoresis of cartilage collagens HMW, 1α, 2α and 3α incubated with human skin collagenase, and HMW collagen incubated with a tumor type V collagenolytic metalloproteinase. Digestion conditions were 25°, enzyme to substrate ratio 1:100, pH 7.4. A. Cartilage collagens HMW, 1α, 2α, 3α alone (50 μg). B. Cartilage collagens (50 μg) plus skin collagenase 8 hours. C. Cartilage collagens (50 μg) plus skin collagenase 16 hours. The 3α chain is cleaved. D. Type I collagen (50 μg) plus skin collagenase 15 hours. E. Type I collagen (50 μg) alone. F. Type V collagen alone (50 μg). G. Type V collagen (50 μg) plus skin collagenase. H. HMW collagen (60 μg) alone. I. HMW collagen (60 μg) plus tumor metalloproteinase 8 hours. J. HMW collagen (60 μg) plus tumor metalloproteinase 16 hours. Specific cleavage products are denoted with arrows. All samples were unreduced.

skin collagenase (Fig. 1 B-D). Partially purified type V collagenolytic metalloproteinase produced specific cleavage products from type V collagen (AB_2) as described previously (6) (Fig. 2) and also degraded HMW, 1α and 2α producing specific limited products (Figs. 1 and 2). The large cleavage products of 1α and 2α and AB_2 migrated faster than the alpha chains of type I collagen but corresponding smaller products analogous to TC^B were not identified. HMW but not LMW was degraded to produce two large cleavage products which migrated slightly slower than 1α and 2α (Fig. 1 I and J).

Plasmin failed to degrade any of the minor collagen components when digestions were performed at 33°C (enzyme to substrate ratio 1:100). Leucocyte elastase produced a small change in the staining intensity of HMW but failed to degrade any of the other minor cartilage collagens (Fig. 3 A, B) under conditions where this enzyme degraded type III collagen (25°C, 15

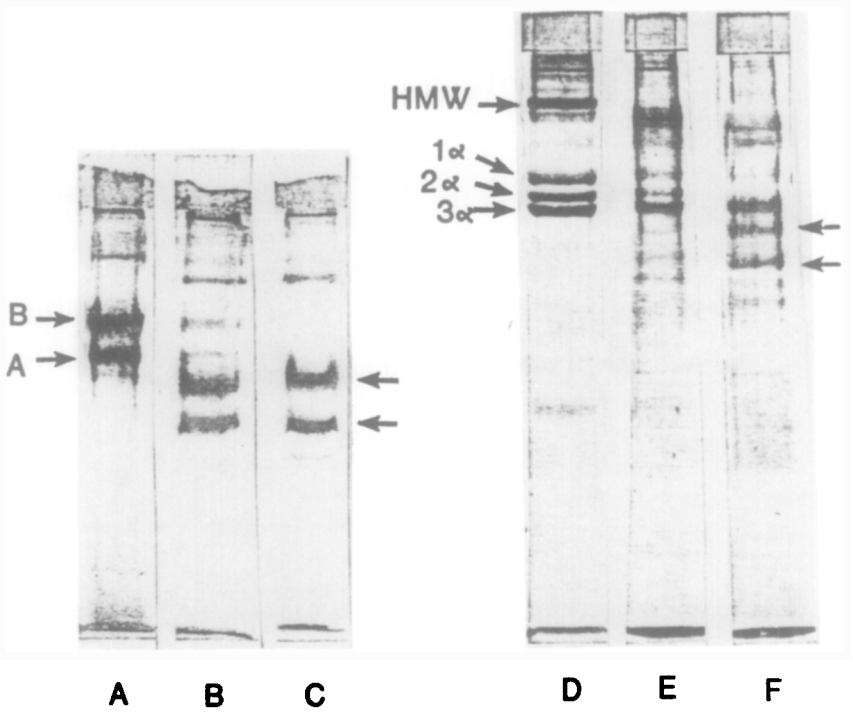

Figure 2. SDS-polyacrylamide gel electrophoresis of type V (AB_2) collagen and cartilage collagens incubated with a tumor metalloproteinase. A. Type V collagen (50 µg) alone. B. Type V collagen (50 µg) plus tumor metalloproteinase (0.5 µg) 25°, 15 h. C. Type V collagen (50 µg) plus tumor metalloproteinase (1 µg) 25°, 15 h. D. Cartilage collagens alone (50 µg). E. Cartilage collagens plus tumor metalloproteinase (0.5 µg) 30°, 4 h. F. Cartilage collagens plus tumor metalloproteinase (0.5 µg) 30°, 15 h. Cleavage products were produced from 1α, 2α and HMW (arrows). All samples were unreduced.

hours, enzyme to substrate ratio 1:100 (7, 8)). Purified α thrombin failed to degrade any of the minor cartilage collagens at 33°C. However, at 36°C this enzyme degraded HMW and removed the 1α and 2α chains producing a series of low molecular weight cleavage products (Fig. 3).

DISCUSSION

The ability of a tumor-derived type V collagenolytic metalloproteinase to degrade minor cartilage collagens was compared with human skin collagenase and other serine proteases. The type V collagenolytic metalloproteinase degraded both α1 (V) and α2 (V) chains at 25°C (6) (Fig. 2). HMW, 1α and 2α also served as substrates for this enzyme and specific large cleavage products were identified (Figs. 1 and 2). Although collagens IV and V are not

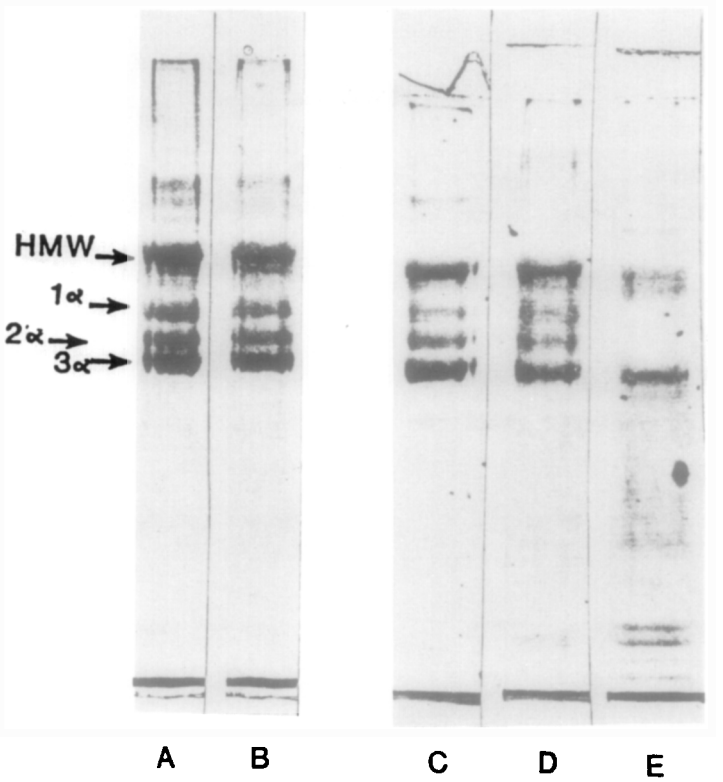

Figure 3. SDS-polyacrylamide gel electrophoresis of cartilage collagens incubated with leucocyte elastase or alpha thrombin. A. Cartilage collagens alone (50 µg). B. Cartilage collagens (50 µg) plus elastase (0.5 µg) 25°, 15 h. C. Cartilage collagens (50 µg) plus alpha thrombin (0.5 µg) 25°, 15 h. D. Cartilage collagens (50 µg) plus alpha thrombin (0.5 µg) 33°, 15 h. E. Cartilage collagens (50 µg) plus alpha thrombin (0.5 µg) 36°, 15 h. At the high temperature HMW, 1α and 2α but not 3α are digested, producing fragments which run near the front of the gel.

degraded by classic vertebrate collagenase, at least three groups have identified separate metalloproteinases which can cleave native collagens IV and V (5, 6, 9, 10 & personal communication, Dr. J. Reynolds). Leucocyte elastase failed to degrade 1α, 2α and 3α but did produce a small reduction in the staining intensity of the HMW band (Fig. 3). Human leucocyte elastase has been reported to degrade pepsinized type IV collagen and type III collagen but not collagens I, II or V (7, 8).

In the case of the minor cartilage collagens, α thrombin degraded 1α, 2α and HMW at 36°C but not at 33°C. However, no specific high molecular weight cleavage products could be identified. Alpha thrombin has been shown to exhibit a temperature-dependent degradation of type V collagen (12, 13).

At temperatures below 35°C no cleavage is observed. However, at higher temperatures when the type V collagen molecules may be partially denatured, they are degraded to produce cleavage products which are distinct from those produced by the type V collagenolytic metalloproteinase (12-14).

In the present study plasmin failed to degrade HMW or any other of the cartilage collagens (data not shown). Human plasmin can degrade disulfide bonded glycoproteins such as fibronectin and laminin (13, 14). This enzyme fails to degrade any of collagens I, II, III, IV or V at 25°C (13).

Taken together, the pattern of protease susceptibility for HMW, 1α and 2α is identical to type V collagen and distinct from interstitial collagens. On the other hand, 3α contains the vertebrate collagenase cleavage site (Fig. 1) and is resistant to elastase. This is in keeping with the hypothesis that 3α is closely related to type II collagen, and that HMW, 1α and 2α may be members of the type V collagen family.

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