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ALL THREE CHAINS OF 1α2α3α COLLAGEN FROM HYALINE CARTILAGE RESIST HUMAN COLLAGENASE

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A previous report that the 3α collagen chain of hyaline cartilage was cleaved by human collagenase could not be confirmed when the 1α2α3α collagen fraction was freed of all contaminating type II collagen. All three minor collagen chains, 1α, 2α and 3α, were totally resistant to highly purified collagenases from both rheumatoid synovial and gastric mucosal tissues. This finding and CNBr-peptide patterns suggest that, despite the close homology with α1(II), the 3α chain is a unique collagen component, possibly combined with 1α and 2α in heterotrimeric molecules. In contrast, a 3α-like component from fibrocartilage was cleaved by collagenase and gave a CNBr-peptide pattern more typical of α1(II) than of the collagenase-resistant 3α of hyaline cartilage.

Hyaline cartilage contains, in addition to type II collagen, several minor species of collagen not seen in other tissues. In adult cartilage the most prominent minor collagen fraction consists of three distinct chains called 1α, 2α, and 3α (1). These chains have been found in the various types of growing and adult human, bovine, porcine and chicken hyaline cartilages (1-7). In contrast, the comparable fraction from a fibrocartilage contained type V collagen and a 3α-like component instead of the 1α, 2α, and 3α chains (7). Based on amino acid compositions and CNBr-peptide patterns the 1α and 2α chains are distinct gene species, whereas the 3α chain appears closely homologous if not genetically identical to the α1(II) chain (1,3,4,8). The chain compositions and number of different native collagen molecules that give rise to the 1α, 2α and 3α chains are unknown. Attempts to fractionate potential parent molecules such as $[3\alpha]_3$ and $[1\alpha]_2[2\alpha]$, have failed (1,4). Nevertheless, the possibility that 3α represents a post-translational variant of type II collagen (1,8), was

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supported by a finding that partially purified human collagenase cleaved 3α but not 1α and 2α chains in a collagen preparation from chick cartilage (9).

The susceptibility of the minor collagens of cartilage to human collagenase was re-examined using highly purified and well characterized synovial and gastric mucosal enzymes and collagens prepared from bovine articular and meniscus cartilages.

MATERIALS AND METHODS

Collagen isolation: Articular cartilage was sliced from the femoral condyles of 2 year-old adult steers. The slices were extracted in 4M guanidine HCl, 0.05M Tris, pH 7.5 for 24 hours to remove proteoglycans and then equilibrated with water at 4°C and freeze-dried. Collagen was solubilized by digestion with pepsin (1 to 50 by dry weight) in 3% acetic acid for 24 hours. After centrifugation the supernatant was sequentially brought to 0.7M and 1.8M NaCl, collecting the precipitated collagen at each step (2). Collagen recovered in the 1.8M fraction was redissolved in 0.1M acetic acid and further fractionated by adjusting the NaCl concentration to 0.9M then 1.2M, collecting the precipitates by centrifugation. Each precipitate was dialyzed against 0.1M acetic acid and freeze-dried. Similar fractions were prepared from the fibrocartilaginous menisci of adult steer knees, discarding vascular and peripheral regions as previously described (7).

Collagenase: The human enzyme was isolated from explant cultures of rheumatoid synovia and purified as described previously (10). Non-specific proteinase activity was absent as judged using hemoglobin, fibrinogen and albumin as non-collagenase substrates. Human gastric mucosal collagenase was prepared and purified similarly (11).

Enzyme digests: Collagen substrates were dissolved at 3 mg/ml in 0.1M acetic acid and dialyzed against 0.4M NaCl, 0.05M Tris, 50 mM arginine, 10 mM $CaCl_2$, pH 8.0. Aliquots of substrate were mixed with the enzyme dissolved in 0.17M NaCl, 0.05M Tris, 10 mM $CaCl_2$, pH 8.0, and the mixture was incubated at 25°C or 35°C for 22-24 hours. The reaction was stopped by adding 5% by volume of 100 mM 1,10-phenanthroline, 250 mM Na_2 EDTA, pH 8.0.

Electrophoresis: Aliquots of the enzyme digests and of substrates incubated with buffer alone were mixed with two volumes of electrophoresis buffer (1% SDS, 10% glycerol, 0.05M Tris, pH 6.8), heat denatured at 100°C for 2 min. and run in SDS-7.5% polyacrylamide slabs by the method of Laemmli (12). Individual α-chains and collagenase-derived fragments were excised, digested with CNBr and the digests were run on SDS-15% polyacrylamide essentially as described (7).

RESULTS AND DISCUSSION

About 1% of the pepsin-solubilized collagen from bovine articular cartilage was recovered in the initial 1α, 2α, 3α fraction precipitating at 1.8M NaCl. On reprecipitation of this material, about 60% went to the 0.9M fraction and 40% to the 1.2M fraction. Figure 1 shows the effects of synovial collagenase on these fractions. In neither was their any sign of cleavage of 1α and 2α. In the 0.9M fraction some of the material migrating with the 3α-

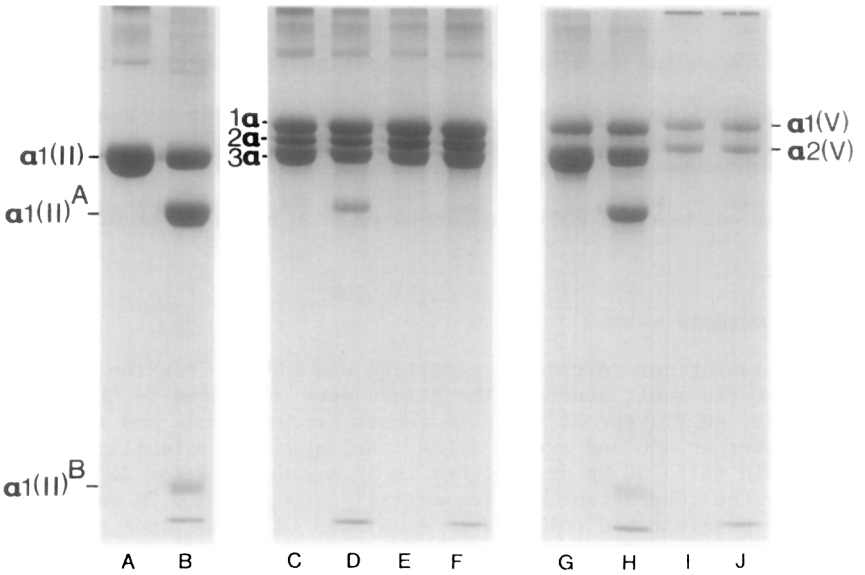

Figure 1. Electrophoresis of cartilage collagen fractions before and after incubation with human synovial collagenase for 24 hours at 25°C. Samples (25 μg) were run in slabs (1.5 mm) of SDS-7.5% polyacrylamide and stained with Coomassie blue R250. Samples in lanes A-F were prepared from articular cartilage and in lanes G-J from meniscus fibrocartilage. A. Type II - 0.7M NaCl fraction; C. 1α2α3α - 0.9M NaCl fraction; E. 1α2α3α - 1.2M NaCl fraction; G. Type V + 3α - 0.9M NaCl fraction; I. Type V - 1.2M NaCl fraction. The enzyme digests of each substrate are run in adjacent lanes B, D, F, H and J.

component was degraded to three-quarter and one-quarter products (Fig. 1, lanes C and D), whereas essentially none of the 3α component of the 1.2M fraction was degraded (lanes E and F). Furthermore, exposure of the 1.2M fraction to much higher levels of enzyme activity, together with incubations at 35°C, failed to degrade any of the 1α, 2α and 3α chains. Such findings were confirmed using the purified human gastric mucosal collagenase (not shown).

The component in the 0.9M NaCl fraction which migrates with 3α and is cleaved by collagenase is probably contaminating type II collagen. Such contamination probably explains a previous observation that 3α from chick cartilage was cleaved by collagenase (9). This is consistent with the apparent failure to cleave 3α quantitatively in that study (9). Others have noted a high ratio of 3α to 1α and 2α in preparations from chick cartilage, again consistent with contamination by type II collagen (4).

The 3α-like component from meniscus was almost fully cleaved by synovial collagenase in 24 hours (Fig. 1, lanes G and H). Neither α1(V) nor α2(V) were degraded (lanes I and J), as previously reported for type V collagen prepared from other tissue sources (13-15), and observed when using the present synovial enzyme with type V from fetal calf skin (not shown). This result suggests that all the 3α-like component in fibrocartilage is present as a homotrimeric molecule which represents a post-translational variant of type II collagen (7). It may be comparable to the small fraction of type II collagen that initially co-precipitates with 1α2α3α collagen from hyaline cartilage. Its greater solubility and the slower mobility of its α1(II) chains and derived CNBr-peptides compared with the bulk of type II collagen may be due to a higher content of hydroxylysine glycosides.

The results indicate that in hyaline cartilage there is a distinct 3α component present in roughly equal amounts with 1α and 2α which is not cleaved by collagenase. Its CNBr-peptide electrophoretic pattern (Fig. 2, lane B) was basically similar to that of the α1(II) chain (lane C). However, in addition to the originally noted slower mobility of all the major CNBr-peptides (1) it was distinguished from α1(II) by having a much larger peptide in place of α1(II)CB9,7 (the band midway between the position of CB9,7 and CB8 in lane B, Fig. 2). The 3α-like chain from fibrocartilage (Fig. 2, lane A) resembled the α1(II) chain (lane C) in this region of the peptide pattern, supporting its identity as a post-translational variant of type II collagen.

The composition of the parent molecule of the collagenase-resistant 3α chain of hyaline cartilage is still undefined. If it is $[3\alpha]_3$, it may resist collagenase because of structural differences from $[\alpha1(II)]_3$, probably in primary sequence as the CNBr-peptide profile suggests (Fig. 2) rather than in post-translational chemistry. Alternatively, 3α may be combined with 1α and 2α in heterotrimeric molecules. If so, the resistance to collagenase might be due to steric hindrance by the other chain types.

It is notable that 1α, 2α and 3α are present in roughly equal amounts in the 1.2M preparation, and in the 0.9M preparation after collagenase digestion

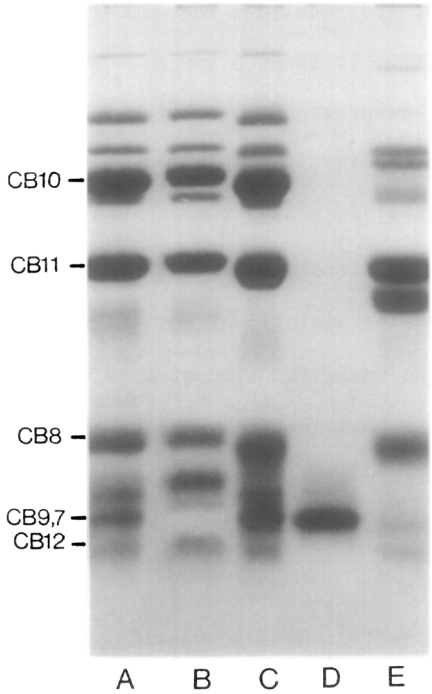

Figure 2. Electrophoresis of CNBr-digests of the various cartilage collagen α-chains and collagenase fragments. The polypeptides were isolated by electrophoresis (as in Fig. 1), digested with CNBr and re-run in SDS-15%-polyacrylamide.
A. 3α-like chain from meniscus. B. 3α from the 1.2M fraction of articular cartilage. C. α1(II) (0.7M fraction). D. $\alpha 1(II)^{B}$ fragment. E. $\alpha 1(II)^{A}$ fragment.

(Fig. 1), consistent with a parent molecule of composition $[1\alpha,2\alpha,3\alpha]_3$. This possibility is supported by the presence of multiple β-components on electrophoresis of the 1.2M fraction in 5% polyacrylamide (not shown). At least four β chains are resolved, and their mobilities suggest various heterodimers between 1α, 2α and 3α. No homodimers of 3α are evident. However, since it is not known whether these dimers derive from inter- or intra-molecular cross-linking, no firm conclusions are possible on the number and composition of native parent molecules. Nevertheless, the findings endorse the original concept that the 1α, 2α and 3α chains are intimate components of a distinct species of cartilage collagen (1).

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