EFFECTS OF LYSOSOMAL ENZYMES ON THE TYPE OF COLLAGEN SYNTHESIZED BY BOVINE ARTICULAR CARTILAGE

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Received May 24, 1973

SUMMARY

Bovine articular cartilage normally synthesizes a collagen containing three identical α-chains. After pre-incubation with rat liver lysosomal enzymes, it begins to synthesize significant amounts of the more ubiquitous collagen of the $(\alpha_1)_2\ \alpha_2$ type. Since lysosomes are increased in osteoarthritis, it is possible that the abnormal biosynthetic patterns exhibited by cells in areas of degeneration are caused by such enzymes.

Articular cartilage is an avascular tissue with very low cell density, composed primarily of extracellular substances such as collagen, proteoglycans, and glycoproteins. The structural integrity of this tissue depends on the relative proportion, nature, and structural organization of these components. Until recently, the destruction of cartilage seen in osteoarthritis was considered to result from a "wear and tear" process. This concept is not substantiated by recent ultrastructural and biochemical findings. Cellular activity in the involved areas leads to enlarged clones of chondrocytes containing increased numbers of intracellular organelles reflecting synthetic and secretory activity (1).

There is an inverse correlation between the severity of the degenerative changes and the glycosaminoglycan content of the tissue (2-7). On the other hand, radioactive sulfate incorporation increases in osteoarthritis, an indication of the attempts made by the cells involved to repair the lesion (2). The nature of the proteoglycans synthesized under these conditions (less keratan sulfate and more chondroitin-4-sulfate) reflect the behaviour of immature chondroblasts (3-8). Lysosomal proteases have been associated with the degradation of the matrix (9-12). Cathepsin-D and a neutral protease which degrade proteoglycans at pH 5.0 and 7.0 respectively are considerably increased in early osteoarthritic lesions (13-15). Although the collagen content of cartilage does not change in osteoarthritis, qualitative differences may exist. Recently, we have shown that whereas normal human cartilage synthesizes only cartilage type collagen or $(\alpha_1\text{-Type II})_3$, osteoarthritic cartilage synthesizes in addition significant amounts of $(\alpha_1)_2\ \alpha_2$ collagen (skin type) (16). Articular cartilage collagen is quite different from other ubiquitous forms of mammalian collagens. In addition to containing three identical α-chains, it has four to five times more hydroxylysine and glycosidically associated carbohydrate than collagen from other tissues (17). It is quite possible that the abnormal collagen deposited by the cells at the site of degeneration may give rise to a mechanically weaker structure and lead to a loss of cartilage. While attempting to elucidate the mechanism underlying this abnormal metabolic pattern, it became apparent that lysosomal enzymes can alter the function of normal cartilage cells causing them to synthesize non-specific collagen molecules.

MATERIALS AND METHODS

Fresh bovine articular cartilage was obtained from local slaughter houses, cleaned, and chopped into small pieces. Lysosomes were separated from the livers of young rats weighing 70-80 grams (18). One gram aliquots of bovine cartilage slices was suspended in 0.1 M Na acetate buffer, pH 5 or in 0.1 M Tris HCl buffer, pH 7.0.

The lysosomal preparation was divided into two parts and suspended in 2 ml of buffer, either pH 5 or 7. The suspensions were frozen and thawed two or three times (or mixed with 0.1% triton x-100) to break the membrane. Lysosomal suspensions (0.5 ml) were added to the cartilage and the mixtures incubated at 37°C for two hours with gentle shaking. Cartilage incubated similarly at pH 5 or 7 without the addition of lysosomes served as a control. Lysosomal preparation alone exhibited no synthetic activity. At the end of the incubation period, the mixtures were centrifuged, the cartilage pieces washed three times with saline, and incubated with 10 ml of Dulbecco's phosphate buffered solution (GIBCO) containing 50 μCi of 2,3 H^3-L-proline (Sp.Act. 29.8 Ci/mMole) at 37°C for six hours with gentle shaking.

The cartilage slices were removed, washed with saline, homogenized with a Virtis-45 in 0.45 M NaCl buffered to pH 7.0 with 0.02 M Na phosphate and kept shaking overnight at 4°C. The mixture was centrifuged and the residue incubated with papain at 4°C for 24 hours (17). The enzyme was inactivated with iodoacetic acid, the cartilage washed thoroughly with saline and again extracted with 0.45 M NaCl, pH 7 at 4°C for 24 hours. The pre-papain and post-papain neutral salt extracts were dialyzed against 0.05 M Tris HCl buffer, pH 7.5, containing 0.2 M NaCl.

The dialyzed extracts were chromatographed on a DEAE cellulose column (0.6 x 30 cms) and eluted with 0.05 M Tris HCl buffer containing 0.2 M NaCl (19). After collecting 50-60 ml of effluent volume, the NaCl concentration was raised to 1 M and the elution continued. The flow rate was kept at 15 ml/hr and 2 ml fractions were collected.

The radioactive peak containing collagen was lyophilized, dialyzed against 0.06 M Na acetate buffer, pH 4.8, and mixed with rat skin acid soluble collagen which served as a carrier. The mixture was chromatographed on a CM-cellulose column (0.8 x 5 cm) equilibrated with 0.06 M Na acetate buffer, pH 4.8, and eluted with a linear gradient between 0 and 0.1 M NaCl (19). Fractions were read at 230 mμ and the radioactivity counted with a Beckman liquid scintillation counter.

RESULTS AND DISCUSSION

The distribution of radioactivity among the collagen subunits synthesized by normal untreated cartilage is shown in Figure 1. Radioactivity is restricted to the α_1 peak indicating that the cartilage cells are synthesizing only $[(\alpha_1)II]_3$ collagen. Figure 2 shows the distribution of radioactivity in the extracts of bovine cartilage which had been pretreated with rat liver lysosomes. In this case, more than one kind of collagen is synthesized since radioactivity is also associated with α_2. The ratio of α_1 to α_2 subunits varies from 3 to 3.5, this ratio being slightly higher when cartilage was incubated with lysosomes at pH 7 than at pH 5.0. It is also worthwhile to note that the extent of incorporation of the radioactivity into the pre-papain NaCl extract is greater than that in the post-papain NaCl extract, probably a reflection of the relatively short incubation periods.

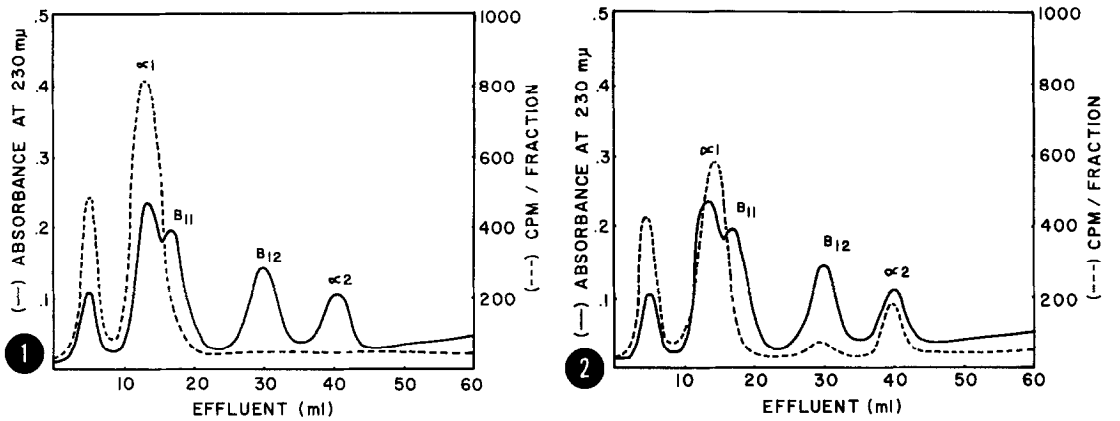

Figure 1: CM-cellulose chromatogram of biosynthetically labeled cartilage collagen. Normal bovine articular cartilage was incubated with ^{3}H-proline, extracted with 0.45 M NaCl and collagen separated from proteoglycans on DEAE-cellulose. The collagen fraction was mixed with rat skin acid soluble collagen and eluted from CM-cellulose using an 0.06 M Na acetate buffer (pH 4.8) with a linear gradient between 0 and 0.1 M NaCl.

Figure 2: Bovine articular cartilage was incubated with rat liver lysosomes at pH 5.0, then incubated with ^{3}H-proline and processed as described in figure 1 and in the text.

Nevertheless, the relative proportions of α_1 and α_2 chains in both extracts is similar. The effect of lysosomes on the cartilage was studied at pH 5 and pH 7 to cover the optimum pH range for the lysosomal enzymes and neutral proteases. Pre-incubation of normal cartilage in the absence of lysosomes at both these pH's showed that this treatment did not affect the synthesis of collagen by normal chondrocytes.

The increase in lysosomal enzyme activity associated with osteoarthritis has been well documented (11-15, 20). Until

now, it was felt that these enzymes acted only on the proteoglycan matrix and therefore responsible for the loss of metachromasia. The present experimental findings suggest that these enzymes may also modify cell behaviour. Since bovine articular cartilage contains only one type of collagen synthesizing cell, the synthesis of a new type of collagen containing α_2 chains could result either from the stimulation of an inactive cell type or from a change in the phenotypic expression of the chondrocyte. The suggestion that cell surfaces play a key role in the control of morphogenesis and differentiation is widely viewed (21-25). It is therefore quite likely that lysosomal enzymes by modifying the environment around the chondrocyte or by directly reacting with cell surface components may be altering drastically the cell behaviour. The phenotypic expression encompassed by the synthesis of $(\alpha_1)_2$ α_2 collagen could result from dedifferentiation of a chondrocyte to a more primitive fibroblastic form. This pattern of behaviour is consistent with the finding showing that osteoarthritic cartilage synthesizes glycosaminoglycans which reflect the presence of immature cells (3). One can envision how this combination of abnormal proteoglycans and collagen fibers could generate a mechanically inadequate matrix which could lead to cartilage destruction and exposure of the bony surface.

ACKNOWLEDGEMENTS

We thank Mrs. Sylvia Padilla for her excellent technical assistance. This research was supported by a special fellowship (AM-53414) and grants (AM-10358 and DE-02848) from the National Institutes of Health; The Arthritis Foundation; and The Robert E. and May R. Wright Foundation.

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