Vol. 96, No. 3, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 16, 1980 Pages 1225-1231

IDENTIFICATION OF CATABOLIN, A PROTEIN FROM SYNOVIUM WHICH INDUCES DEGRADATION OF CARTILAGE IN ORGAN CULTURE

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Received July 28,1980

SUMMARY

Explants of porcine synovium produce a factor which causes degradation of the matrix of live cartilage in organ culture. Cartilage degradation was measured as release of glycosaminoglycan from explants of bovine nasal septum. Fractionation of synovial culture medium showed the factor to be a protein of 20,000 mol.wt. and iso-electric point pH = 4.6. The factor has been named catabolin.

INTRODUCTION

The soft tissue that lines synovial joints (the synovium) induces degradation of the proteoglycan and collagen matrix of articular cartilage in organ culture. The original experiments, which were co-cultures of porcine synovium and articular cartilage, showed that when the tissues were not in contact the presence of synovium caused loss of matrix from live, but not from dead, cartilage (1). Subsequently it was shown that medium conditioned by synovium contained a factor which would induce degradation of both porcine articular cartilage and cartilage from bovine nasal septum (2). The factor was precipitated from the synovial medium by ammonium sulphate. It was retained by dialysis membrane, was thermolabile and was destroyed by digestion with either chymotrypsin or pancreatic elastase. Like whole synovial medium it caused degradation of cartilage in organ culture if the chondrocytes were alive, but not if they were dead (2). These results suggested that the factor produced by synovium might be a protein. Further experiments now show that this protein, referred to as 'catabolin', has a molecular weight of 20,000 on gel chromatography and an iso-electric point of pH 4.6.

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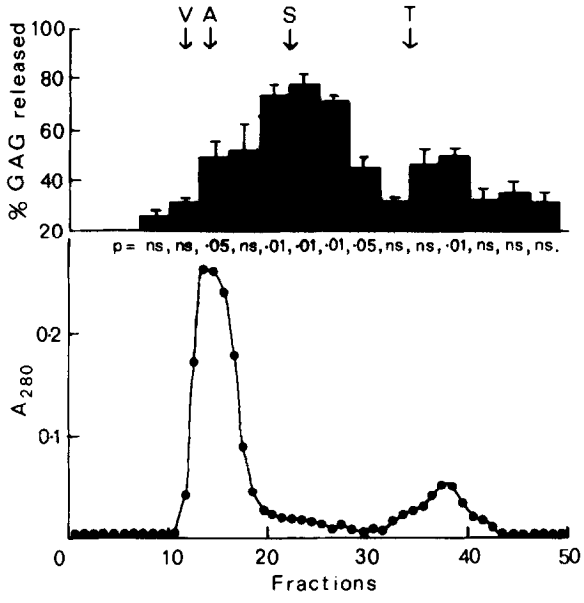

Fig.1. Gel filtration chromatography of the 60% - 95% $(NH_4)_2SO_4$ fraction of porcine synovial medium

2ml of $(NH_4)_2SO_4$ fraction (4mg of protein) were applied to a 1.5 x 90 cm column of acrylamide/agarose gel AcA 54, equilibrated with phosphate buffered saline. 3.7ml fractions were collected and the A_{280}(●) was measured. The column was calibrated with the following: dextran blue (V); bovine serum albumin, mol.wt. 68,000 (A); soya bean trypsin inhibitor, mol.wt. 21,500 (S) and Trasylol, mol. wt. 6,200 (T); their elution positions are shown by the arrows. Fractions were pooled in threes and dialysed against Dulbecco's MEM. 9ml of each pool was added to 9ml of Dulbecco's MEM containing 10% normal heat-inactivated sheep serum. These solutions were assayed on bovine nasal cartilage. Glycosaminoglycan (GAG) released is shown by the blocks; the values are means of 5 discs and the bars are SEM. The control discs released 31 ± 3% and the p values are for Student's t test between this group and those containing samples of column fractions. n.s. = not significant.

MATERIALS AND METHODS

Ultrogel AcA 54 and Ampholines were from LKB, DEAE-cellulose was DE-52 from Whatman and 1,9-dimethyl methylene blue was from Serva Feinbiochemica.

Culture of synovium

Synovium dissected from the metacarpophalangeal joints of freshly slaughtered young pigs was minced finely with scissors and cultured in Dulbecco's MEM (2g/100ml) at 37°C in CO_2/air (1:19). Medium was changed on alternate days up to day 10, it was pooled, and an active fraction was prepared by ammonium sulphate precipitation at 4°C (2). Material precipitating between 60% and 95% saturation with ammonium sulphate was redissolved in phosphate buffered saline (137mMNaCl, 25mMKCl, 1.1mMKH_2PO_4, 8.1mMNa_2HPO_4, pH 7.4) to 1% of the original volume.

Assay of cartilage degradation

Cartilage degradation was measured as release of glycosaminoglycan by explants

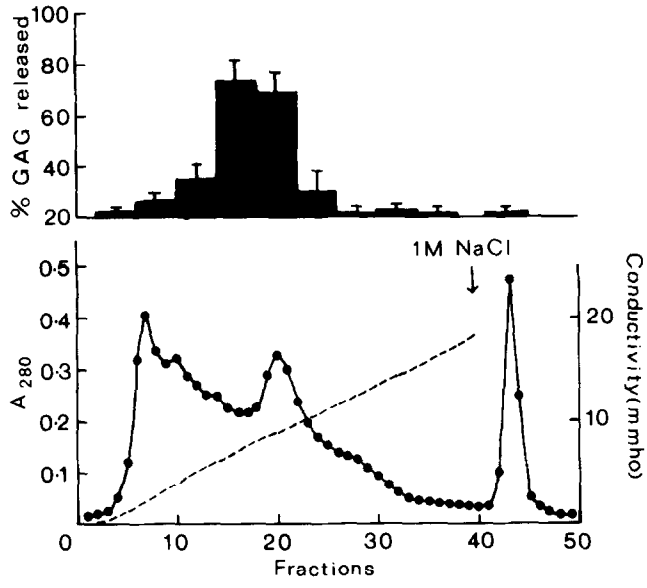

Fig.2. DEAE-cellulose chromatography of active porcine synovial material from gel filtration chromatography

100ml of 60% - 95% ammonium sulphate precipitate was concentrated to 15ml and chromatographed on a column (4.5 x 100cm) of AcA 54. Fractions that were active on cartilage (corresponding to fractions 20-28 in Fig.1) were pooled and dialysed against 20mM glycine/NaOH buffer pH 8.6. This sample (about 25mg protein) was applied to a column (1.5 x 4.5cm) of DEAE-cellulose equilibrated with the same buffer. The column was developed with a gradient formed by 100ml of buffer and 100ml of buffer containing 0.35M NaCl (---). After the gradient, the column was eluted with 1M NaCl in the buffer (at arrow). 5ml fractions were collected and the A_{280} was recorded (●). Fractions were pooled in fours, dialysed against 1% glycine, and assayed on bovine nasal cartilage discs at 0.04ml/ml of culture medium. % GAG released is shown by the blocks (means of 5 discs), the bars are SEM. The release from controls was 20.5 ± 2.1%. Only the activity in fractions 15-22 was significant ($p = 0.01$).

of cartilage from bovine nasal septum (2). Discs (0.5cm diameter x 0.1cm thickness) of cartilage were cut from freshly slaughtered animals, and were maintained at 37°C in CO_2/air (1:19) for 8 days in Dulbecco's MEM (1.5ml/disc) containing 5% heat-inactivated normal sheep serum (56°C for 30 min). Medium was changed after 4 days. Samples under test were incorporated in the medium and each was assayed on 5 separate discs. After culture the discs were digested with papain, and glycosaminoglycan was measured in the digests and culture medium by use of the dye dimethyl methylene blue (2,3). Results were expressed as the percentage of total glycosaminoglycan (medium + tissue) released into the medium during the 8 days of culture. Control discs generally released 20% - 30% of their glycosaminoglycan over the culture period. The release induced by synovial material was approximately linearly related to the amount added (2).

RESULTS

Fig.1 shows the results of gel filtration chromatography of the ammonium sulphate fraction. The most active fractions eluted just after the main

protein peak. It was necessary to pool the fractions in threes in order to accommodate the whole chromatograph in one set of assays using cartilage from a single animal. Highest activity was found in fractions 23-26, whose elution volume corresponded to a molecular weight of about 20,000. The smaller peak of activity that emerged later (after the Trasylol marker) was significant, but it has not been found consistently in subsequent experiments. The recovery of activity in the main peak was judged to be better than 60%.

The active peak from gel filtration was then subjected to chromatography on DEAE-cellulose (Fig.2). About 10% of the active material failed to adsorb to the cellulose (not shown on the figure) but the rest eluted in fractions 15-22, just before the second protein peak. No further activity was recovered from the column when it was washed with 1M NaCl. The overall recovery of activity was estimated to be about 60%. The active fractions from ion-exchange chromatography (15-22) were next subjected to iso-electric focusing in polyacrylamide gels. Sliced gels were eluted and assayed for activity on bovine nasal cartilage. The results of electrofocusing in wide (pH 3-10) and narrow (pH 4-6) gradients are shown in Figs.3a & b. Active material was eluted at pH 4.5 on the pH 3-10 gradient and pH 4.6 on the pH 4-6 gradient. In both gradients the activity was sharply localised in one slice suggesting that there was only a single active component present. Gels run in parallel, which were stained for protein, showed 2 or 3 visible bands in the region of the pH 4-6 gradient from which active material was eluted (not shown). Recovery of activity from the focusing procedure was estimated to be about 30%.

DISCUSSION

Cartilage degradation induced by synovial explants is a complex chain of events. Initially there is loss of proteoglycan, followed by loss of collagen and proliferation of chondrocytes which assume a fibroblastic appearance (1,2). Because the first visible change is loss of proteoglycan, and since it can be easily measured as loss of glycosaminoglycan, we have used it as an assay for substances which may trigger cartilage degradation. The factor from synovial

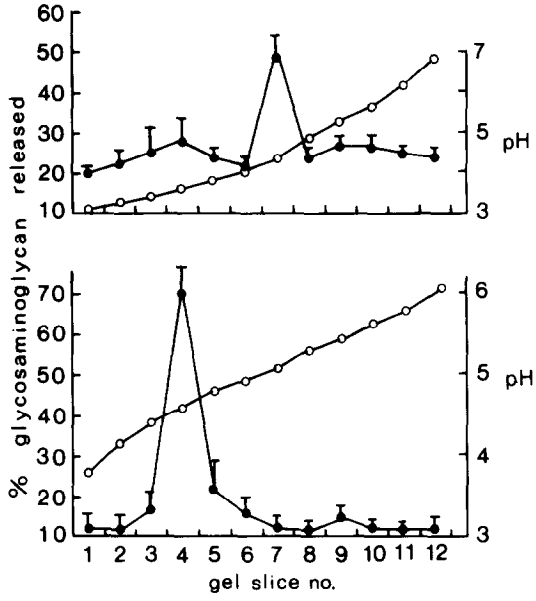

Fig.3. Iso-electric focusing of active porcine synovial material from DEAE-cellulose chromatography

(a) Active material from DEAE-cellulose chromatography (fractions 15-22 in Fig. 2 was dialysed against 1% glycine and 0.8ml samples were focused in 7.5% polyacrylamide gels (1.2ml) containing 1% Ampholine pH 3-10 (4). After focusing, the gels were sliced. Slices from 1 gel were eluted in distilled water (1ml/slice) for pH measurement (o). Slices from 2 gels were homogenized and eluted for 48hrs in 3ml of PBS. These eluates were dialysed, first against PBS and then against culture medium. The samples were finally assayed on discs of bovine nasal cartilage at 0.3ml/ml of culture medium. Samples from blank gels did not affect the release of glycosaminoglycan from discs cultured in the presence or absence of the active ammonium sulphate fraction. Values of % glycosaminoglycan released (●) are means of 5 determinations per gel slice ± SEM. The control release was 22.0 ± 2.6% and only slice 7 contained significant activity ($p = 0.01$).

(b) Exactly as for (a) except that pH 4-6 Ampholine was used. The % glycosaminoglycan released from controls was 16.3 ± 2.7%. Only slice 4 showed significant activity ($p \simeq 0.01$).

explants which initiates cartilage degradation is apparently a protein of 20,000 mol.wt. and iso-electric point pH 4.6; we have named it catabolin. Whether or not it accounts for all of the degradation caused by whole synovial medium is not yet known. The recovery of activity from the medium by ammonium sulphate fractionation was low (20% - 30%), but this may have been because precipitation was inefficient due to the very low concentration of protein (only 20-30 μg/ml total protein). Alternatively, there may have been other active components which were lost during the procedure.

The cells of the synovial tissue must be alive to produce catabolin since no activity was obtained from freeze-thawed synovial explants. It is not stored in the tissue in detectable amounts since no activity was found in homogenates; it is apparently produced as a result of the tissue being put into culture. Whether it is synthesised by the cells in culture or is generated by some other means, for instance by limited proteolysis of a precursor, is not known. Other soft tissues (e.g. sub-dermal connective tissue and tendon) produce catabolin-like activity in culture, so it is unlikely to be a specific product of synovium (JS and JTD unpublished observations).

The effect of catabolin on cartilage could be due either to an increase in degradation, or to an inhibition of synthesis of matrix macromolecules. Cartilage explants do synthesise glycosaminoglycans (5,6) but the turnover rate is not known although evidence from in vivo and in vitro experiments suggests that it may be very slow (7). Since catabolin can cause release of 90% of the glycosaminoglycan from explants in four days, an increase in degradation seems very likely. Its action is superficially similar to that of vitamin A, which has long been known to induce cartilage degradation as well as metaplasia of embryonic skin in organ culture (8,9). There is no evidence that catabolin is a proteinase which directly attacks the matrix since it causes no degradation of dead cartilage. Its action is to stimulate the chondrocytes to degrade the matrix, perhaps by causing release of proteinases.

Cartilage degradation in rheumatoid arthritis has often been attributed to the release of proteinases from the synovium (10-12). The experiments described here suggest another possibility. If catabolin, or cognate substances, were formed during inflammation, they might cause chondrocytes to deplete the matrix. Such a process could be concurrent with degradation by enzymes from synovium.

ACKNOWLEDGEMENTS

We thank Mrs.Valerie Curry and Miss Helen Williams for skilled technical assistance, and the Arthritis and Rheumatism Council for financial support.

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