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Purification to homogeneity of pig leucocyte catabolin, a protein that causes cartilage resorption *in vitro*

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Catabolin, a protein that causes proteoglycan resorption in explants of living cartilage, was purified to homogeneity from culture medium conditioned by culturing buffy-coat leucocytes from 60 litres of pig blood in the presence of concanavalin A. The purification steps were (1) gel filtration of concentrated medium, (2) chromatofocusing, (3) hydroxyapatite chromatography, (4) anion-exchange chromatography (Mono Q), (5) reversed-phase high-pressure liquid chromatography (h.p.l.c.) (Zorbax ODS). These achieved approx. 9000-fold purification from the starting material. The purified protein when reduced ran as a single band on sodium dodecyl sulphate (SDS)/polyacrylamide-gel electrophoresis with M_r 21000. On isoelectric focusing its pI was 4.8–5.0, and there was evidence of micro-heterogeneity. The protein co-migrated with active material on h.p.l.c., isoelectric focusing and SDS gels (15 and 12.5% acrylamide) under both reducing and non-reducing conditions. The pure protein caused proteoglycan release from cultured bovine nasal cartilage at 20pM. Its possible identity with interleukin 1 is discussed.

Resorption of connective tissues occurs in a variety of physiological and pathological situations. Elucidation of the biochemical mechanisms by which connective-tissue cells are stimulated to remove their matrix is potentially of importance to our understanding of such diverse processes as chronic inflammatory diseases, wound healing, fracture repair, tissue remodelling during growth and tumour invasion. Cartilage is a simple and convenient tissue in which to study resorption. When explanted into organ culture, it will normally maintain its matrix of proteoglycan and collagen, but the chondrocytes will resorb the matrix if they are appropriately stimulated by vitamin A (Fell & Mellanby, 1952) or products of synovium (Fell & Jubb, 1977).

Previous work from our laboratory has shown that cultured explants of soft connective tissues such as synovium, as well as mononuclear leucocytes stimulated with lectins, produce materials that induce resorption of cartilage proteoglycan *in vitro*. Originally, the active agent from pig synovium was identified as a small (M_r 20000) acidic (pI 4.5–5.0) protein and called 'catabolin' (Saklatvala & Dingle, 1980; Saklatvala, 1981). Subsequently, a physicochemically indistinguishable protein was shown to be made by pig mononuclear leucocytes if they were stimulated by concanavalin A (Saklatvala & Sarsfield, 1982). More recently, both human synovium and mononuclear leucocytes have been shown to produce a protein that induces cartilage resorption (Saklatvala *et al.*, 1983; Jasin & Dingle, 1981). This human 'catabolin' was of similar size and charge (pI 5.2) to the pig catabolin.

In the present paper we report that we have now succeeded in purifying to homogeneity the catabolin from pig leucocytes.

Abbreviations used: h.p.l.c., high-pressure liquid chromatography; SDS, sodium dodecyl sulphate.

Experimental

Materials

Dulbecco's modified Eagle's medium was from Gibco. Concanavalin A, cortisol, whale chondroitin sulphate (Grade III), fluorescamine and protein standards for gel chromatography were from Sigma. 1,9-Dimethyl Methylene Blue chloride was from Serva Feinbiochemica. Gelatin and acetonitrile for h.p.l.c. were from Fisons. Ultrogel AcA-54 and Ampholines were from LKB, hydroxyapatite suspension was from BDH, and Polybuffer exchanger PBE94 and Polybuffer 74 were from Pharmacia.

Ultrafiltration apparatus (Diaflo and PM10 membranes) were from Amicon. Zorbax ODS columns were from du Pont de Nemours and Co.

Cartilage resorption bioassay for catabolin

The principle of the assay is to measure the release of proteoglycan from cartilage stimulated to resorb *in vitro*. Discs (2mm diameter × 1mm thick) of bovine nasal cartilage (obtained immediately after slaughter), were cultured for 3 days in microtitre plates in Dulbecco's modified Eagle's medium containing 5% (v/v) heat-inactivated normal sheep serum and cortisol (0.1μg/ml) at 37°C in CO_2/air (1:19). Each well contained a single disc and 0.15ml of culture medium. Samples, such as column fractions to be tested for activity, were included in the medium for the whole culture period and were assayed on four separate discs. After culture, the medium was recovered and its content of chondroitin sulphate was measured by use of the metachromatic dye dimethyl Methylene Blue. Samples (10μl) were added to 5ml of 0.0016% dimethyl Methylene Blue dissolved in formate buffer (30mM-sodium formate/29mM-formic acid/0.5% ethanol, pH3.5) and the change in A_{535} was measured immediately. The assay was calibrated with chondroitin sulphate over the range 5–50μg. Each unstimulated disc of cartilage released about 50μg of chondroitin sulphate over the culture period, but discs that had been stimulated to resorb released up to about 400μg. The discs used generally contained a total of around 500μg of chondroitin sulphate. The amount of chondroitin sulphate released in these bioassays increases approximately linearly with the amounts of catabolin added, up to about 70% release (see the dose–response curves in Saklatvala, 1981). Results were plotted as the mean release (±S.E.M.) of chondroitin sulphate.

To estimate recoveries and purification factors, an arbitrary unit of activity was used. This was the amount required to increase 3-fold the release of chondroitin sulphate during the culture (from a control release of around 50μg, or about 10%, to a value of around 150μg, or about 30%). This unit was used to calculate the data in Table 1, and all the measurements were made on the same batch of cartilage. Fractions were stored at −20°C before assay, with no loss of activity. Full discussion of the use of the cartilage bioassay procedure and the use of dimethyl Methylene Blue to assay chondroitin sulphate in culture medium can be bound elsewhere (Saklatvala, 1981; Farndale *et al.*, 1982).

Preparation and culture of pig leucocytes

Blood was collected from pigs as they were slaughtered at a local abattoir; 10ml of a sterile solution of 50% (w/v) trisodium citrate/litre was added as an anticoagulant. Then 332ml of a sterile (autoclaved) solution of 3% (w/v) gelatin dissolved in phosphate-buffered saline (1mM-NaH_2PO_4/18mM-Na_2HPO_4/145mM-NaCl, pH7.4) containing 1mM-EDTA was added to each litre of blood, mixed well, and the erythrocytes were allowed to sediment for 30min at 37°C. The leucocyte-rich supernatant plasma was decanted and centrifuged at 400 *g* for 15min at room temperature. The supernatant was discarded and the cells were resuspended in 0.83% (w/v) NH_4Cl (about 40ml for cells from 1 litre of blood). This procedure haemolysed most of the contaminating erythrocytes, and after 10min the suspension was diluted 10-fold with phosphate-buffered saline containing 1mM-EDTA and was centrifuged as above. The cells were then washed three times by resuspending and centrifuging them in the same buffer. Finally, they were resuspended at 50×10^6cells/ml in Dulbecco's modified Eagle's medium containing concanavalin A (0.05mg/ml), and cultured at 37°C in CO_2/air (1:19) in 14cm-diameter Petri dishes (70ml/dish) for 48h.

After culture, the supernatant was carefully decanted from the carpet of cells and was clarified by centrifugation (5000 *g* for 15min). This conditioned leucocyte medium was stored at −20°C.

Purification of catabolin

Ultrafiltration. Conditioned leucocyte medium (15 litres) was passed through a Millipore filter (0.45μm pore size) and concentrated 400-fold over a PM 10 membrane in a 2.0-litre stirred ultrafiltration cell (Amicon 2000A). The concentrated material was clarified by centrifuging at 20000 *g* for 30min.

Gel chromatography (*Fig. 1*). The concentrated leucocyte material (45ml) was applied to a column (4.4cm × 100cm) of Ultrogel AcA-54. The column was eluted with phosphate-buffered saline at a flow rate of 1ml/min at 4°C. Fractions (18ml) were collected and assayed for activity on cartilage.

Chromatofocusing (*Fig. 2*). Active fractions from gel chromatography were pooled (indicated by the horizontal bar in Fig. 1), concentrated to about 30ml over a PM10 membrane, and the buffer was changed to 25mM-histidine adjusted to pH6.2 with HCl. This sample was applied to a column (1.6cm × 40cm) of Polybuffer PBE 94 exchanger, equilibrated with the histidine buffer. The column was eluted at 4°C with Polybuffer 74, diluted 1:8 and adjusted to pH4.0 with HCl. Fractions (10ml) were collected and assayed for activity.

Chromatography on hydroxyapatite (*Fig. 3*). The active fractions between pH4.45 and 4.6, indicated by the bar in Fig. 2, were pooled, adjusted to pH7.0 with NaOH, and pumped on to a small column (1.3cm × 0.5cm) of hydroxyapatite equilibrated wtih distilled water. The column was washed well with water until the A_{280} of the eluate returned to zero.

Active protein was then eluted from the column with 150mM-sodium phosphate buffer, pH 7.0.

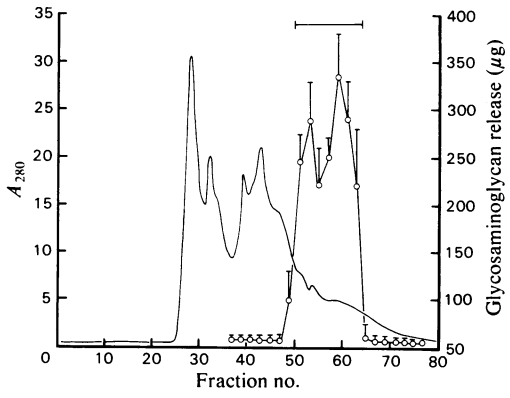

Fig. 1. *Gel chromatography of concentrated leucocyte culture medium*

Leucocyte culture medium (15 litres) was chromatographed on a column (4.4cm × 100cm) of Ultrogel AcA-54. Full details are given in the Experimental section. ——, A_{280}; O, glycosaminoglycan release from cartilage discs caused by fractions assayed at 1μl/ml. Vertical bars indicate the S.E.M. The horizontal bar indicates fractions pooled for chromatofocusing.

Anion-exchange chromatography (*Fig. 4*). This was done on a Mono Q HR 5/5 column in a Pharmacia FPLC (fast protein liquid chromatography) system fitted with two P500 pumps and a gradient controller. The column was equilibrated with 20mM-Tris/HCl buffer, pH 7.9. The active fractions from hydroxyapatite chromatography were diluted 1:5 in the Tris buffer and applied to the Mono Q column, which was then eluted at a flow rate of 1ml/min with a gradient of NaCl (17.5mM/ml) up to a limit of 250mM. Fractions (1ml) were assayed for activity on cartilage.

Reversed-phase h.p.l.c. (*Fig. 5*)

A Zorbax ODS column (4.6mm i.d. × 25cm) was used with a du Pont 970 pump module and a series-8800 gradient controller. The column was equilibrated with 0.1M-NaH_2PO_4, adjusted to pH 2.1 with H_3PO_4, and proteins were eluted with a gradient of acetonitrile (O'Hare & Nice, 1979). The active fractions from anion-exchange chromatography were pooled as indicated by the bar in Fig. 4, and were injected in 0.5ml batches on to the column, which was maintained at 35°C. The column was then eluted at a flow rate of 1ml/min [pressure of about 9MPa (90 bar)], for 5min with the primary solvent, and then with a two-segment binary gradient with acetonitrile as the secondary solvent: 0–3min, 0–25%; 3–33min, 25–55%; 33–36min, 55% aceto-

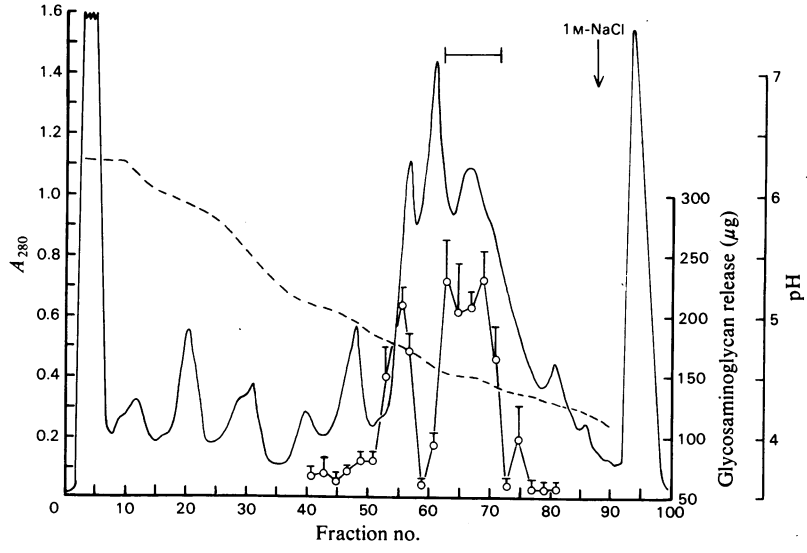

Fig. 2. *Chromatofocusing of leucocyte proteins from gel filtration*

The active material from gel filtration was chromatographed on a column (1.6cm × 40cm) of Polybuffer exchanger, PBE 94, as described in the Experimental section. ——, A_{280}; ----, pH of fractions; O, glycosaminoglycan released (±S.E.M.) from cartilage cultured in the presence of the fractions at 4μl/ml. At the position marked by the vertical arrow, the column was washed with 1M-NaCl; no active material was found in the peak of protein that eluted, nor was there any active material in fractions 1–40 (assays not shown). The horizontal bar shows fractions pooled for further purification.

nitrile. Eluted proteins were detected by u.v. absorption at 225nm, and 1ml fractions were collected, adjusted to pH7.0 (with NaOH), and assayed for activity.

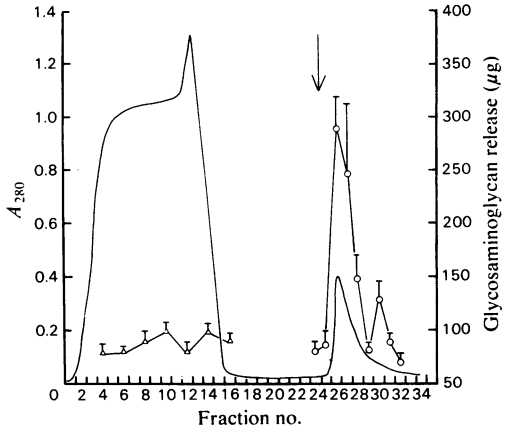

Fig. 3. *Chromatography on hydroxyapatite of the main active component from chromatofocusing*
The fractions containing the main active component from chromatofocusing were passed through a 1ml column of hydroxyapatite as described in the Experimental section. The column was eluted with 150mM-sodium phosphate buffer, pH7.0, at the position of the vertical arrow. Fractions (4.5ml) were assayed for their ability to cause glycosaminoglycan release from cultured cartilage at 20μl/ml (△) and 1μl/ml (○). Bars represent S.E.M.; ——, A_{280}.

Other methods

SDS/polyacrylamide-gel electrophoresis. This was carried out in vertical slab gels of either 12.5 or 15% total acrylamide concentration with the glycine/2-amino-2-methylpropane-1,3-diol (Ammediol) HCl buffer system described by Wyckoff *et al.* (1977). Samples were boiled for 5min in sample buffer containing 1% SDS, upper gel buffer (84mM-Ammediol/62mM-HCl, 40% (v/v) glycerol and 0.5% 2-mercaptoethanol (the last was omitted if samples were to be run unreduced). For some experiments the samples were not boiled before electrophoresis. Gels were stained for protein with Coomassie Brilliant Blue G250 [0.1% (w/v) in methanol/acetic acid/water (5:2:3, by vol.)] and destained in methanol/formic acid/water (30:1:69, by vol.). Standard proteins run as M_r markers were bovine serum albumin (68000), carbonic anhydrase (29000), soya-bean trypsin inhibitor (21500), myoglobin (17000) and cytochrome *c* (12500). Some gels were not stained, but were sliced and eluted to detect an active component. Slices (2mm) were each placed in a dialysis bag, crushed and suspended in 1ml of 20% (v/v) propanol in phosphate-buffered saline. The bags were dialysed against two changes of the propanol-containing buffer, then against the buffer alone and finally against two changes of Dulbecco's modified Eagle's medium. The contents of each bag were then retrieved and assayed on cartilage.

Isoelectric focusing. This was done in 7.5%-polyacrylamide gel cylinders (0.4cm × 6cm) containing 1% Ampholine of the desired pH range. The anode solution was 0.2% H_3PO_4; the cathode

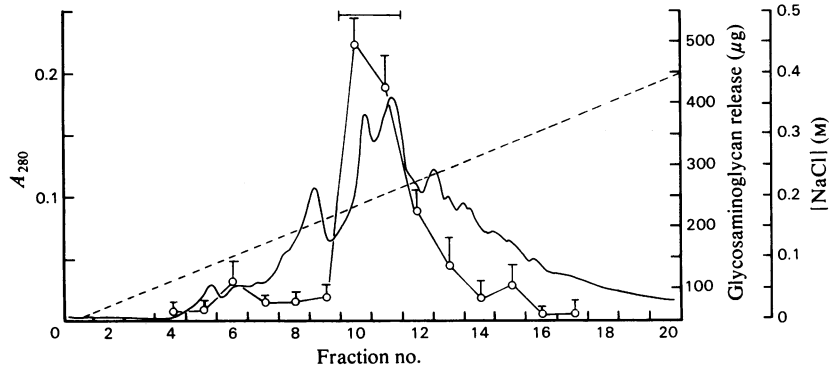

Fig. 4. *Mono Q anion-exchange chromatography of active protein from hydroxyapatite chromatography*
The active protein eluted from the hydroxyapatite column was diluted 1:5 with 20mM-Tris/HCl buffer, pH7.9, and chromatographed on a Mono Q HR 5/5 column as described in the Experimental section. ○, Glycosaminoglycan released (±S.E.M.) from cultured cartilage in the presence of 0.2μl of fractions/ml. ——, A_{280}; – – – –, gradient of NaCl in the mixing chamber before the column as computed by the gradient controller; this is not the salt concentration in the fractions. The horizontal bar indicates fractions pooled for h.p.l.c.

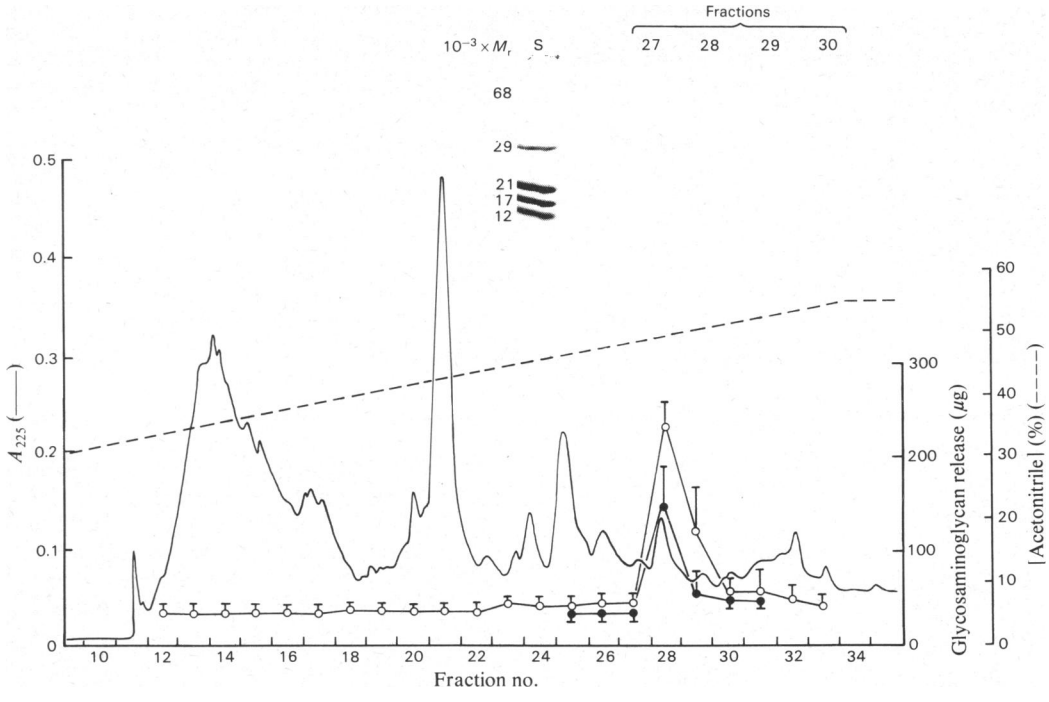

Fig. 5. *Reversed-phase h.p.l.c. on Zorbax ODS of active material from anion-exchange chromatography*
Active protein from the Mono Q column was injected directly on to a Zorbax ODS column [4.6mm (internal diameter) × 25cm] and chromatographed as described in the Experimental section. Fractions were assayed for their ability to cause glycosaminoglycan release from cartilage at 1μl/ml (○) and 0.2μl/ml (●). Bars indicate the S.E.M. ——, A_{225}; – – – –, gradient of acetonitrile as computed at the mixing valves (this is not the acetonitrile concentration of the fractions). The inset photograph shows a polyacrylamide 15%/SDS-electrophoresis gel run and stained for protein as described in the Experimental section. The samples were boiled and reduced and loaded into the tracks as follows: S, standard proteins, 1μg each of bovine serum albumin (M_r 68000), carbonic anhydrase (29000), soya-bean trypsin inhibitor (21500), myoglobin (17000) and cytochrome *c* (12500); 27–30, 40μl samples of the respective fractions.

solution was 0.1% NaOH. Focusing was carried out for 1.5h to a limit voltage of 300V. Gels were stained for protein with Coomassie Brilliant Blue G250 (method A of Vesterberg *et al.*, 1977). The pH gradient was measured by slicing one gel, eluting each slice in 1ml of water and measuring the pH of the eluate. Some gels were sliced and protein was eluted to test for activity on cartilage as described for SDS/polyacrylamide gels, except that the propanol was omitted.

Protein determination. This was done with fluorescamine (Weigele *et al.*, 1972), and bovine serum albumin was used as a standard. Appropriate controls were done for samples containing histidine, Tris and Polybuffer.

Dialysis tubing. Visking tubing (18/32in) was acetylated before use by immersion overnight in 25% (v/v) acetic anhydride in pyridine, followed by thorough washing in distilled water.

Results and discussion

Purification of catabolin

The purification procedure is summarized in Table 1. The starting material consisted of 7.8g of protein that had been prepared from leucocytes obtained from about 60 litres of blood. The unit of catabolin activity is quite arbitrary and obviously imprecise, but it serves to illustrate the progress of the purification. An active peak was obtained from the final h.p.l.c. step (Fig. 5), which was estimated to contain about 4μg of protein. SDS/polyacrylamide-gel electrophoresis of reduced samples of the fractions (inset, Fig. 5) showed a protein of M_r 21000 corresponding to this small peak. The specific activity of this protein indicated a purification factor of about 9000-fold over the starting material. The overall recovery of activity was low (0.45%) and the worst loss occurred during h.p.l.c.: the recovery at

Table 1. *Purification of catabolin from leucocyte culture medium*

The unit of catabolin activity is arbitrarily defined as the quantity required to cause a 3-fold increase in the amount of glycosaminoglycan released from the stimulated cartilage discs compared with unstimulated controls during the culture period (3 days). The cartilage was from one animal, and the rationale for the procedure is discussed in the Experimental section.

Purification step	Total protein (mg)	Total activity (arbitrary units)	Specific activity (units/mg)	Purification factor	Recovery of activity (%)
Concentrated culture medium	7832	1100000	140	1	100
Ultrogel AcA-54	1080	300000	278	2	27
Chromatofocusing (pH 4.5–4.9)	45	110000	2444	17	10
Hydroxyapatite	6.0	80000	13300	95	7.3
Mono Q	1.3	72000	55400	395	6.5
Zorbax ODS (h.p.l.c.)	0.004*	5000	1250000	8900	0.45

* This concentration is estimated from the u.v. absorption of the peak in Fig. 5.

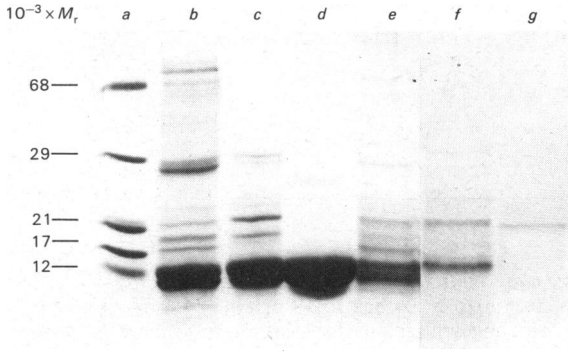

Fig. 6. *SDS/polyacrylamide-gel electrophoresis of samples from purification steps for catabolin*

Boiled and reduced samples were run on a 12.5%-acrylamide gel and stained for protein as described in the Experimental section. Samples were: *a*, standard proteins as for Fig. 5 inset; *b*, concentrated leucocyte culture medium; *c*, active material from gel filtration; *d*, active material from chromatofocusing marked by the horizontal bar in Fig. 2; *e*, active material from hydroxyapatite chromatography (Fig. 3); *f*, active material from anion-exchange chromatography (Fig. 4); *g*, active peak from h.p.l.c. (fraction 28, Fig. 5).

this step was only about 7%, although it achieved over 20-fold purification. The protein may well have been damaged by both the low pH and the high concentration of acetonitrile. The other major loss of activity occurred during the first step, when only 27% was recovered from gel filtration. It may be that some of the loss of activity during purification was due to separation of the protein from substances that enhanced its activity, as well as to loss and denaturation of protein itself. Alternatively, there may be another cartilage-resorbing protein that is lost because it is labile.

Chromatofocusing (Fig. 2) revealed two main peaks of activity, namely a major one at pH 4.4–4.6 and a minor one at pH 4.8. This was consistent with the previous results of isoelectric focusing (Saklatvala, 1981). The smaller peak may be a differently charged species of the protein of the main peak, since it had the same retention time (28 min) on Zorbax ODS when run with the gradient shown in Fig. 5. The progress of the purification is illustrated in Fig. 6, which shows SDS/polyacrylamide-gel-electrophoresis patterns at successive stages of purification. The bands that can be seen in lanes *e* and *f* are very faint in lane *d* and are not visible in the photograph.

Electrophoresis and focusing experiments

These were done to obtain evidence that the protein purified by h.p.l.c. was the biologically active component. A sample was run without reduction or boiling in two lanes of a SDS/15%-polyacrylamide electrophoresis gel. One lane was stained, and two protein bands were revealed, one of M_r 42000 and the other of M_r 21000 (Fig. 7). The parallel lane was then sliced to include the M_r-21000 band in slice 12 and the other band in slice 6. The eluates of these slices were assayed on cartilage discs, and Fig. 7 shows that by 70h the material from slice 12 had caused release of proteoglycan. By 92h, there was even greater release from the slice-12 eluate and a slight increase in release from slice 6. Since the protein ran as a single band when boiled and reduced (Figs. 5 and 6), the M_r-42000 band is a dimer and is relatively inactive, since the bands appear to contain similar amounts of protein. Fig. 8 shows the result of another experiment in which the protein was boiled and reduced and run on a SDS/12.5%-polyacrylamide gel. Under these conditions, a single band was seen, and active material was again associated with it. Since the band was too faint to show photographically, its position is indicated diagrammatically.

Isoelectric focusing of 1 μg of purified protein in a pH 4–6 gradient in polyacrylamide gels revealed one major and two minor stained bands. They were all

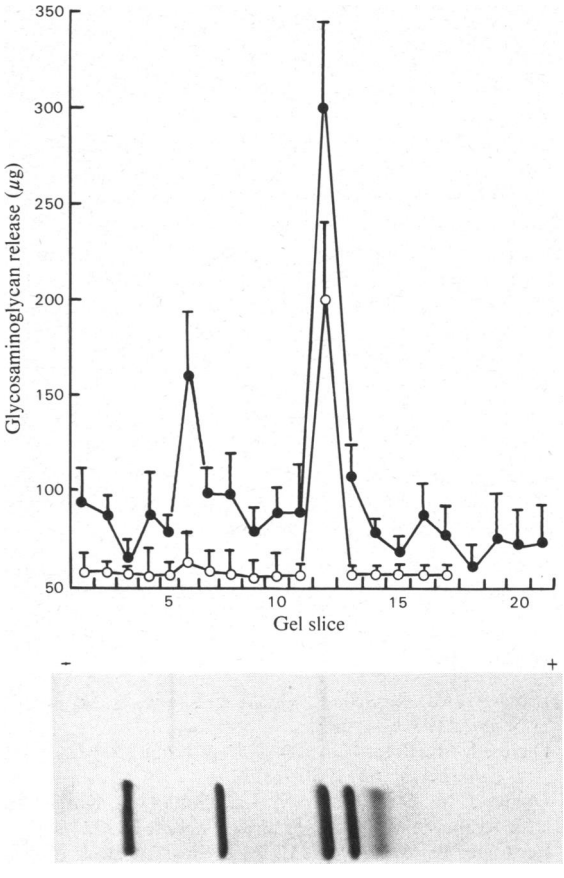

Fig. 7. *Location of active protein after running purified catabolin in SDS/polyacrylamide-gel electrophoresis*

The protein purified by h.p.l.c. was electrophoresed with SDS in a 15%-polyacrylamide gel. Samples were not reduced or boiled. One lane was loaded with 50ng of pure protein and after electrophoresis was sliced (2mm-thick slices) and eluted as described in the Experimental section. A parallel lane contained 200ng and was stained for protein. A photo of this lane, together with standard proteins (as in Fig. 5 inset), is shown below the activity profile. The material eluted from gel slices was assayed on bovine nasal cartilage and the release of glycosaminoglycan was measured after 72h (○) and 96h (●). Bars indicate the S.E.M.

rather faint, owing to the small amount of protein available, and are shown diagrammatically (Fig. 9). Assay of the eluates of slices of a parallel gel showed active material in the position corresponding to the stained bands. It was concluded that the protein showed heterogeneity with respect to charge and that its isoelectric point was in the range pH4.8–5.0. This was within that range previously reported for experiments with impure material (Saklatvala, 1981; Saklatvala & Sarsfield, 1982).

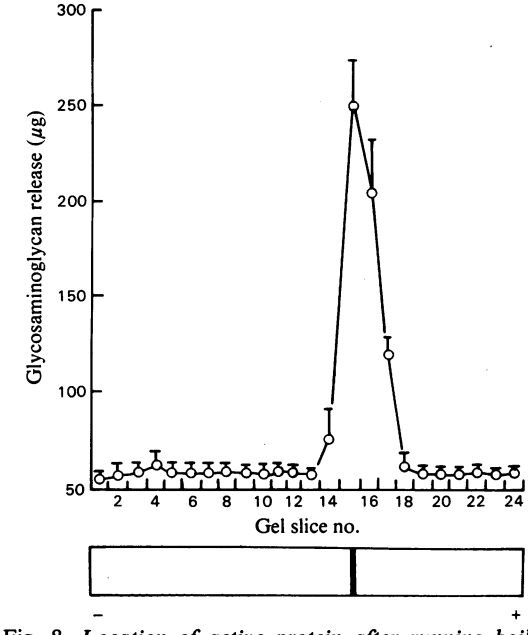

Fig. 8. *Location of active protein after running boiled and reduced catabolin in SDS/polyacrylamide-gel electrophoresis*

The purified protein was run on a 12.5%-acrylamide gel and the samples were boiled and reduced as described in the Experimental section, otherwise the procedure was exactly as that for the experiment shown in Fig. 7. A 25ng sample of pure protein was loaded into the lane that was sliced and 50ng into the lane that was stained for protein. Since the protein band was faint, its position is shown in the diagram at the base of the Figure.

Since the protein and the active material coincided on reversed-phase h.p.l.c., on SDS/polyacrylamide-gel electrophoresis and on analytical isoelectric focusing, it was reasonable to conclude that the protein was pure catabolin.

Fig. 10 shows a dose–response curve for catabolin assayed on bovine nasal cartilage. The protein was active at 20pM in this system (assuming M_r 21000), and the shape of the curve was the same as that obtained for the crude leucocyte culture medium from which the catabolin had been purified. The resistance of the activity of the protein to SDS, reduction and boiling, together with its being active at such a low concentration, suggests that catabolin is unlikely to be a catalytic molecule and that it may bind to cells very tightly.

Conclusions

The purification reported here has enabled us to identify the leucocyte protein that causes the release of proteoglycan from explants of cartilage, but an improved procedure with a higher recovery will be needed to obtain it in quantities sufficient for

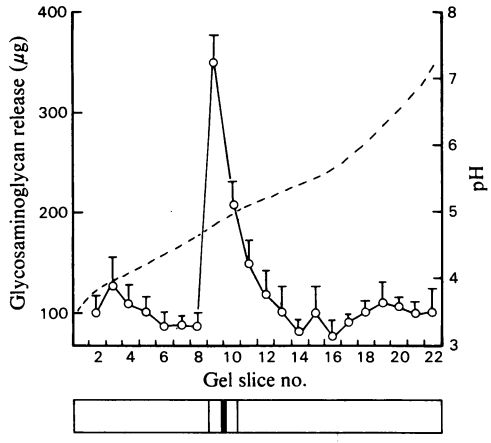

Fig. 9. *Isoelectric focusing of purified catabolin*
Three polyacrylamide gels were run with pH 4–6 Ampholine as described in the Experimental section. One gel contained 1 μg of purified catabolin and was stained for protein; the bands seen are shown diagrammatically at the base of the Figure. One gel contained 0.2 μg of the purified protein and was sliced and eluted for activity. The third gel was used for measuring the pH gradient. ○, Glycosaminoglycan released from cultured cartilage; ----, pH gradient.

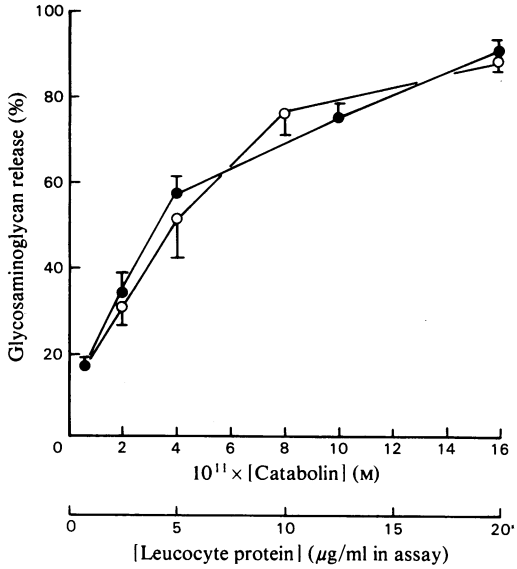

Fig. 10. *Dose–response curves of purified catabolin and the leucocyte culture medium on bovine nasal cartilage*
The assay was an 8-day cartilage culture and the glycosaminoglycan contents of the medium and cartilage (papain-digested) were measured at the end of the culture. The results are expressed as the percentage of total glycosaminoglycan that was released and are means ± S.E.M. for quintuplicate assays. ●, Purified catabolin; ○, leucocyte culture medium.

structural studies. The purification of small amounts of a homogeneous protein will make it possible to define its effects on connective-tissue cells, and to establish whether it is the pig homologue of interleukin 1.

Interleukin 1 is a protein made by macrophages (and monocytes) that, by definition, enhances the proliferation of mouse thymocytes. It is now thought to be identical with the 'mononuclear-cell factor' which stimulates synovial fibroblasts to make collagenase and prostaglandin E_2 (Dayer *et al.*, 1977, 1979, 1981), because it has not been possible to separate the activities chromatographically or electrophoretically (Mizel *et al.*, 1981; Postlethwaite *et al.*, 1983). Interleukin 1 may also stimulate proliferation of fibroblasts (Schmidt *et al.*, 1982). An appealing hypothesis is that the cartilage-resorbing action of catabolin is due to the cells being stimulated to release proteinases, and this possibility requires investigation.

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References

Dayer, J.-M., Russell, R. G. G. & Krane, S. M. (1977) *Science* **195**, 181–183
Dayer, J.-M., Breard, J., Chess, L. & Krane, S. M. (1979) *J. Clin. Invest.* **64**, 1386–1392
Dayer, J.-M., Stephenson, M. L., Schmidt, E., Karge, W. & Krane, S. M. (1981) *FEBS Lett.* **124**, 253–256
Farndale, R. W., Sayers, C. A. & Barrett, A. J. (1982) *Connect. Tissue Res.* **9**, 247–248
Fell, H. B. & Jubb, R. W. (1977) *Arthritis Rheum.* **20**, 1359–1371
Fell, H. B. & Mellanby, E. (1952) *J. Physiol. (London)* **116**, 320–349
Jasin, H. E. & Dingle, J. T. (1981) *J. Clin. Invest.* **68**, 571–581
Mizel, S. B., Dayer, J.-M., Krane, S. M. & Mergenhagen, S. E. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 2475–2477
O'Hare, M. J. & Nice, E. C. (1979) *J. Chromatogr.* **171**, 209–226
Postlethwaite, A. E., Lachman, L. B., Mainardi, C. L. & Kang, A. H. (1983) *J. Exp. Med.* **157**, 801–806
Saklatvala, J. (1981) *Biochem. J.* **199**, 705–714
Saklatvala, J. & Dingle, J. T. (1980) *Biochem. Biophys. Res. Commun.* **96**, 1225–1231
Saklatvala, J. & Sarsfield, S. J. (1982) *Biochem. J.* **202**, 705–708
Saklatvala, J., Sarsfield, S. J. & Pilsworth, L. M. C. (1983) *Biochem. J.* **209**, 337–344
Schmidt, J. A., Mizel, S. B., Cohen, D. & Green, I. (1982) *J. Immunol.* **128**, 2177–2182
Vesterberg, O., Hanson, L. & Sjosten, A. (1977) *Biochim. Biophys. Acta* **491**, 160–166
Weigele, M., Debernardo, S. L., Tangi, J. P. & Leimgruber, W. (1972) *J. Am. Chem. Soc.* **94**, 5927–5928
Wyckoff, M., Rodbard, D. & Chrambach, A. (1977) *Anal. Biochem.* **78**, 459–482