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N-Terminal sequence of proteoglycan fragments isolated from medium of interleukin-1-treated articular-cartilage cultures

Putative site(s) of enzymic cleavage

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Bovine articular cartilage was cultured both in the presence and in the absence of human recombinant interleukin-1α (IL-1) (100 units/ml). Addition of this cytokine stimulated matrix degradation approx. 3-fold. This increased degradation permitted characterization of the large chondroitin sulphate proteoglycan (aggrecan) fragments accumulating in the media. When compared with controls, the proteoglycans isolated from the medium of cultures treated with IL-1 exhibited a decrease in the $K_{av.}$ (control 0.25; IL-1-treated 0.37), determined by Sepharose CL-2B chromatography. This decrease in proteoglycan size was accompanied by a decreased ability of these monomers to associate with hyaluronic acid. Thus only 20% of the proteoglycans isolated from the medium of IL-1-treated cultures, compared with 39% for control cultures, had the capacity to form high-M_r aggregates with hyaluronic acid. SDS/PAGE analysis of the proteoglycans from the media of IL-1-treated cultures demonstrated several large proteoglycan protein-core bands (M_r 144000–380000). The protein-core bands with M_r 144000–266000 exhibited a significantly decreased reactivity with monoclonal antibody 1-C-6 (specific for domains G1 and G2). The *N*-terminal amino acid sequence of four of these protein-core bands (M_r 144000, 173000, 214000 and 266000) yielded sequences LGQRPPV–Y–PQLF(E), AGEGP(S)GILEL–GAP(S)–AP(D)M, GLG–VEL—LPGE and (A)RGSVIL–AKPDFEV–P–A. A comparison of these *N*-terminal amino acid sequences with the published proteoglycan sequence for bovine nasal cartilage [Oldberg, Antonsson & Heinegård (1987) Biochem. J. **243**, 255–259], rat chondrosarcoma [Doege, Sasaki, Horigan, Hassell & Yamada (1987) J. Biol. Chem. **262**, 17757–17769] and human articular cartilage [Doege, Sasaki, Kimura & Yamada (1991) J. Biol. Chem. **266**, 894–902] permitted assignment of their relative positions on the core protein. Furthermore, on the basis of this similarity to published sequence, putative sites of enzymic cleavage were constructed. These theoretical cleavage sites revealed a glutamic acid residue in the P1 position and an uncharged polar or non-polar residue in the P1′ position.

INTRODUCTION

The large chondroitin sulphate-containing proteoglycan and collagen comprise the major matrix components of cartilage. When they are in the appropriate configuration, these macromolecules provide the compressive and tensile strength that is characteristic of cartilage (Muir, 1979; Kempson, 1980; Maroudas *et al.*, 1985). Under homoeostatic conditions, the resident chondrocytes of articular cartilage maintain the appropriate balance between deposition and resorption of these matrix molecules. However, in the case of a pathological process such as osteoarthritis, or following the addition of cytokines, this balance may be disrupted. Thus interleukin-1 (IL-1) has been shown to induce proteoglycan degradation *in vitro* (Saklatvala *et al.*, 1984) and *in vivo* (Pettipher *et al.*, 1986), and to inhibit proteoglycan synthesis (Benton & Tyler, 1988). These increases in proteoglycan degradation have been shown to be associated with elevated concentrations of neutral metalloproteinases (Pelletier *et al.*, 1983; Martel-Pelletier *et al.*, 1984; Nojima *et al.*, 1986; Martel-Pelletier & Pelletier, 1987). A potential consequence of this increased proteolytic activity is the separation of the hyaluronic acid (HA)-binding region from the chondroitin sulphate-containing portion of the proteoglycan monomers (Roughley *et al.*, 1984, 1985; Nguyen *et al.*, 1989). The aim of the present study was to utilize the IL-1-induced stimulation of proteoglycan degradation to characterize further cartilage proteoglycan turnover by defining the *N*-terminal amino acid sequence of the major fragments accumulating in the culture medium.

EXPERIMENTAL

Materials

Sepharose CL-2B was obtained from Pharmacia (Uppsala, Sweden). Dulbecco's modified Eagle's medium with high glucose concentration was purchased from Hazelton (Lenexa, KS, U.S.A.); CsCl was obtained from BRL (Gaithersburg, MD, U.S.A.); guanidinium chloride was from Mallinckrodt (Paris, KY, U.S.A.). Chondroitin ABC lyase and keratanase were purchased from ICN (Lisle, IL, U.S.A.). HA was a gift from Dr. T. Wollen, Mobay (Kansas City, MI, U.S.A.). Human recombinant IL-1α (IL-1) and insulin–transferrin–selenium were obtained from Collaborative Research (Bedford, MA, U.S.A.). Six-well flat-bottom (35 mm) dishes were from Corning Glass Works (Corning, New York, U.S.A.). Triton X-100, penicillin/streptomycin, L-glutamine and Hepes were from Gibco (New York, NY, U.S.A.). Acrylamide was obtained from Enprotech (Hyde Park, MA, U.S.A.). Immobilon-P transfer membrane was from Millipore (Bedford, MA, U.S.A.). Problott membranes were purchased from Applied Biosystems (Foster City, CA, U.S.A.). Monoclonal antibody 1-C-6, specific for domains G1 and G2 of the proteoglycan monomer (Stevens *et al.*, 1984), was obtained from Developmental Studies Hybridoma Bank (Baltimore, MD, U.S.A.). High-M_r markers were from Bio-Rad Laboratories

Abbreviations used: IL-1, interleukin-1; HA, hyaluronic acid.
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(Richmond, CA, U.S.A.). The enhanced-chemiluminescence Western-blotting detection system and the peroxidase-linked anti-(mouse Ig) peroxidase-linked antibody were from Amersham (Arlington Heights, IL, U.S.A.). Dimethyl Methylene Blue was from Polysciences (Worrington, PA, U.S.A.) EDTA, SDS, benzamidine, 6-aminohexanoic acid, phenylmethanesulphonyl fluoride, Alcian Blue and all other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Methods

Cartilage cultures. Bovine articular-cartilage slices were prepared from metacarpal–phalangeal joints of calves (1–3 months old) (Morales *et al.*, 1984) obtained immediately after they were killed from Fortes Meat Market (Branford, CT, U.S.A.). Approx. 0.5 g of articular-cartilage explant was incubated in 5.0 ml of serum-free Dulbecco's modified Eagle's medium supplemented with 20 mM-Hepes, 50 units of penicillin/ml, 50 μg of streptomycin/ml, 2 mM-L-glutamine, insulin–transferrin–selenium (insulin, 5 μg/ml; transferrin, 5 μg/ml; selenious acid, 5 ng/ml) and 60 μg of BSA/ml for 2 h before the addition of the appropriate concentration of IL-1. The cultures were maintained at 37 °C in a humidified atmosphere of 5% CO_2 in air. The medium was exchanged at 48 h and the cultures were terminated after 96 h.

Extraction of proteoglycans. The matrix and the 48 h and 96 h pooled media were extracted with 4 M-guanidinium chloride/50 mM-sodium acetate buffer, pH 5.8, containing 1% Triton X-100 at 4 °C for 48 h (Hascall & Kimura, 1982). The following proteinase inhibitors were present throughout the extraction and purification: 10 mM-EDTA, 5 mM-benzamidine, 10 mM-phenylmethanesulphonyl fluoride, 0.1 M-6-aminohexanoic acid and 10 mM-*N*-ethylmaleimide.

Dissociative CsCl gradients. The extracted proteoglycans from the medium and cartilage were subjected to CsCl-density-gradient centrifugation (Roughley & White, 1980). Briefly, CsCl was added at an initial density of 1.5 g/ml and the gradient was formed in a Beckman VTi50 rotor at 45000 rev./min. for 48 h at 20 °C. The high-buoyant-density proteoglycans recovered from the D1 fraction (buoyant density $\geqslant$ 1.7 g/ml) were dialysed at 4 °C against water in the presence of proteinase inhibitors (as discussed above) and freeze-dried. Sulphated proteoglycans were assayed by the dimethylMethylene Blue assay (Farndale *et al.*, 1982).

Sepharose CL-2B chromatography. To determine quantitatively the presence of functional HA-binding region proteoglycans from each fraction were resuspended in an associative buffer (0.1 M-Tris/HCl/0.1 M-NaCl buffer, pH 7.0) and incubated overnight at 4 °C with or without 2% (w/v) HA. These samples were then chromatographed at a flow rate of 0.25 ml/min on a Sepharose CL-2B column [void volume (V_0) 19 ml, total volume (V_t) 53 ml] utilizing the associative buffer detailed above as the mobile phase. Proteoglycan elution was monitored at 214 nm and by the dimethylMethylene Blue assay. The relative amount of proteoglycans capable of aggregating with HA was determined by calculating the percentage of total proteoglycans eluted in the V_0.

SDS/PAGE and immunoblot analysis of core protein. Proteoglycans were deglycosylated enzymically (Thonar *et al.*, 1986). Briefly, a 0.1 mg sample of proteoglycan was incubated with 0.1 unit of chondroitin ABC lyase and 0.1 unit of keratanase for 2 h in the presence of proteinase inhibitors, then dissolved in an equal volume of a buffer containing 10% (w/v) SDS, 10% (v/v) glycerol, 0.01% Bromophenol Blue, 10% (v/v) 2-mercaptoethanol and 62.5 mM-Tris/HCl buffer, pH 6.8. The sample was incubated at 60 °C for 15 min, boiled for 15 min and then subjected to SDS/PAGE (Laemmli, 1970) in a 2–12% gradient gel. Electrophoresis was at 30 mA per 12.5 cm of gel until the tracking dye was about 2 mm from the bottom of the gel (approx. 3.5 h). The gels were then fixed and sequentially stained with Alcian Blue and ammoniacal $AgNO_3$ (Krueger & Schwartz, 1987). The relative mobilities of the core protein samples were determined with high-M_r protein standards including laminin.

Medium and matrix proteoglycans were electrophoretically transferred from unstained gels on to Immobilon-P transfer membranes for 2 h by a previously described method (Towbin *et al.*, 1979) except that methanol was excluded from the transfer buffer. Non-specific binding sites on Immobilon-P membranes were blocked with 3% BSA/10 mM-Tris/HCl/150 mM-NaCl buffer, pH 8.0, containing 3% (w/v) BSA and 0.05% Tween 20. Antibody dilutions for 1-C-6 and the peroxidase-linked anti-(mouse Ig) antibody conjugate were 1:1000 and 1:5000 respectively. The peroxidase reaction was developed by using the ECL system (Amersham).

Proteoglycans from the medium fraction of IL-1-treated cultures were subjected to SDS/PAGE, electroblotted on to Problott membranes and stained with Coomassie Blue. The *N*-terminal amino acid sequence of the appropriate band was determined by using an ABI 477A gas-phase sequencer with an ABI 120A on-line h.p.l.c. and Nelson Analytical model 2600 chromatography software. Sequence comparisons were performed by using the Protein Identification Resource program RELATE (Dayhoff, 1978) and Genetics Computer Group programs Find and BestFit (Devereux *et al.*, 1984).

RESULTS

IL-1-induced cartilage matrix resorption as a model for proteoglycan fragmentation

Cartilage was cultured in the presence or in the absence of IL-1 in defined medium for 96 h (Fig. 1). Proteoglycans from both the control and IL-1-treated cultures were continually released from the cartilage explants into the medium. During the incubation period of 96 h, control cartilage cultures released approx. 6% of the total proteoglycans into the medium. In the presence of 10 and 100 units of IL-1/ml the proteoglycan release into the media was increased to 10% and 16% of total respectively.

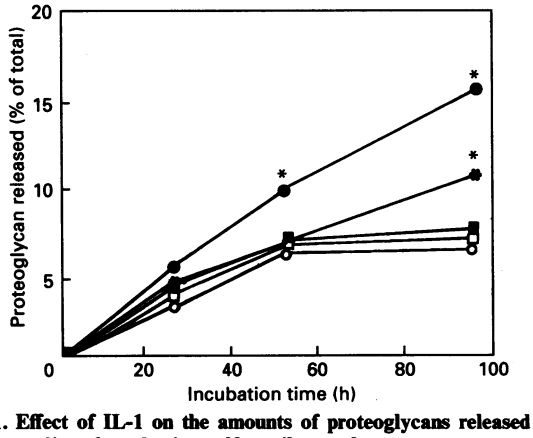

Fig. 1. Effect of IL-1 on the amounts of proteoglycans released into the medium from bovine calf cartilage cultures

Cartilage tissue was incubated, as described in the Experimental section, in medium supplemented with 0 (□), 0.1 (○), 1.0 (■), 10.0 (✱) or 100 (●) units of human recombinant IL-1 α/ml. Sulphated proteoglycans were assayed with dimethylMethylene Blue. Each point represents the mean for eight samples with less then 10% variation between samples. *Significantly different from control, $P \leqslant 0.01$, *t* test.

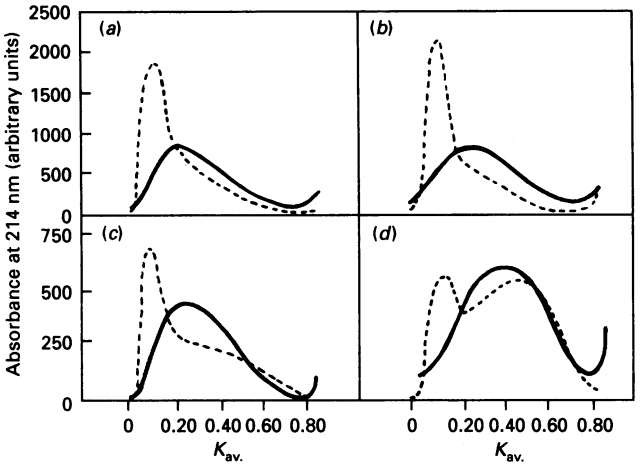

Fig. 2. Re-association of matrix- (*a* and *b*) and medium- (*c* and *d*) derived proteoglycans with HA

Proteoglycans were prepared from cartilage matrix and pooled medium extracts by density-gradient centrifugation. Dialysed D1 fractions (approx. 200 μg of glycosaminoglycan) were chromatographed directly (——) or after preincubation with HA (-----) on a Sepharose CL-2B column (V_0 19 ml, V_t 53 ml), as described in the Experimental section. Profiles are shown for proteoglycans extracted from matrix of (*a*) control or (*b*) IL-1-treated cultures and for proteoglycans isolated from the medium of (*c*) control and (*d*) IL-1-treated cultures. Absorbance units are arbitrary units derived from the Gilson h.p.l.c. 116 u.v. recorder.

In order to test the competency of the HA-binding region, proteoglycans isolated from the D1 fraction of a dissociative CsCl gradient were incubated with 2% HA as described in the Experimental section. Under these associative conditions, monomers with a functional HA-binding region form complexes with HA and are eluted in the V_0 of a Sepharose CL-2B column (Campbell *et al.*, 1989). As can be seen in Fig. 2, much of the proteoglycan monomer isolated from the matrix of control and IL-1-treated culture formed aggregates with HA. Thus 54% of the matrix proteoglycans from control cultures and 50% of the matrix proteoglycans isolated from IL-1-treated cultures were eluted in the column V_0. In contrast, proteoglycan monomers isolated from the media demonstrated a greatly diminished capacity for HA binding (39% and 20% for control and IL-1-treated cultures respectively) (see Fig. 2). Therefore many of the monomers released into the medium during the 96 h incubation period had non-functional or missing HA-binding region. Sepharose CL-2B chromatography of proteoglycans in the absence of HA demonstrated that this diminished HA-binding capacity was accompanied by a decrease in the hydrodynamic size of these proteoglycans (see Fig. 2). Compared with control cultures, proteoglycans from IL-1-treated cultures demonstrated a decrease in size from control ($K_{av.}$ 0.20) to IL-1-treated ($K_{av.}$ 0.25) for matrix fractions and from control ($K_{av.}$ 0.25) to IL-1-treated ($K_{av.}$ 0.37) for medium fractions. Furthermore, the proteoglycans with the smallest hydrodynamic size were the non-aggregating proteoglycans from the medium of IL-1-treated cultures (panel *d*). These proteoglycans exhibited a $K_{av.}$ of approx. 0.47.

Analysis of cartilage matrix and medium proteoglycans by SDS/PAGE and immunoblotting

In order to examine the nature of the proteoglycan fragmentation pattern, de-glycosylated proteoglycan core proteins from control and IL-1-treated cartilage cultures were subjected to SDS/PAGE. As can be seen in Fig. 3(*a*), several discrete high-

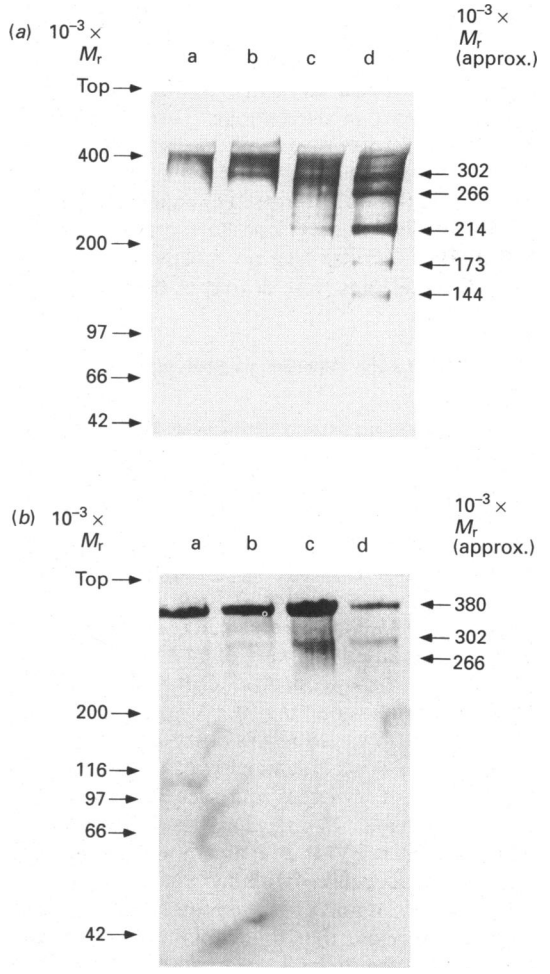

Fig. 3. Silver stain (*a*) and Western blot (*b*) of a 2–12% gradient slab gel of proteoglycans core proteins derived from cartilage matrix and medium fractions

Proteoglycans from pooled 48 h and 96 h medium (approx. 6 μg of protein) and matrix (approx. 3 μg of protein) fractions were digested with chondroitin ABC lyase and keratanase. Lane designation is as follows: matrix proteoglycans from control and IL-1-treated cultures, lanes a and b respectively; medium fractions from control and IL-1-treated cultures, lanes c and d respectively. Electro-transferred proteoglycans were treated with monoclonal antibody 1-C-6 as described in the Experimental section.

M_r protein-core bands were observed. A major high-M_r proteoglycan species with an M_r of 380000 was observed in all fractions from control and IL-1-treated cultures (lanes a, b, c and d). In addition, a protein-core band with an M_r of 302000, visible in all fractions, appeared enhanced in the medium and matrix of IL-1-treated cultures. Additional bands with apparent M_r of 144000–266000 were also observed in both medium fractions (lanes c and d) but were most prominent in the medium from IL-1-treated cultures. A densitometric scan (LKB Ultrascan X) of silver-stained lane d revealed that the protein bands with M_r values of 302000, 266000, 214000, 173000 and 144000 represented approx. 14%, 13%, 29%, 6% and 13% respectively of the total proteoglycan added (scan not shown).

In order to characterize further the cartilage proteoglycan fragments from the medium and matrix fractions, proteoglycans were subjected to SDS/PAGE, electro-transferred and immunoblotted with the monoclonal antibody 1-C-6. High-M_r proteoglycan protein-core bands from the cartilage fraction of IL-1-treated and control cultures and the medium fraction of

control cultures (Fig. 3*b*, lanes a, b and c) reacted with monoclonal antibody 1-C-6, demonstrating the presence of domains G1 and/or G2. In contrast, medium proteoglycan protein-core bands from IL-1-treated cultures (lane d) demonstrated a decrease in the presence of this epitope. This lack of domains G1 and/or G2 was progressive with decreasing M_r. Thus the prominent proteoglycan protein-core band with an M_r of 266000 (Fig. 3*a*, lane d) exhibited decreased G1 and/or G2 epitope (Fig. 3*b*, lane d). Smaller proteoglycan core protein bands with M_r values of 144000–214000 had no reactivity with monoclonal antibody 1-C-6 and thus were devoid of both domains G1 and G2.

N-Terminal amino acid sequence of proteoglycan fragments in the medium

In order to verify and extend the SDS/PAGE and immunoblot data sited above, the *N*-terminal amino acid sequences of four high-M_r protein bands from the medium of IL-1-treated cultures (A, M_r 144000; B, M_r 173000; C, M_r 214000; D, M_r 266000) (see Fig. 3*a*, lane d) were determined subsequent to electrotransfer on to Problott membranes. Tentative sequence assignments are shown in parentheses.

Protein band A (M_r 144000) yielded the sequence LGQRPPV–Y–PQLF(E) at an initial coupling of 14.8 pmol. Protein band B (M_r 173000) yielded the sequence AGEGP(S)GILEL–GAP(S)–AP(D)M at an initial coupling of 63.9 pmol. Protein band C (M_r 214000) yielded two sequences observed at 7.4 and 3.7 pmol respectively. The first was identical with the sequence obtained for protein band B, whereas the second was GLG–VEL––LP–GE. Protein band D (M_r 266000) yielded the sequence (A)RGSVIL–AKPDFEV–P–A at an initial coupling of 5.5 pmol. In addition to the sequences described above, BSA was detected in protein bands A, B and C as a minor *N*-terminal amino acid sequence. We speculate that this contaminant was introduced during the chondroitin lyase/keratanase de-glycosylation step.

When the four bovine proteoglycan sequences were compared with the proteoglycan sequences reported for rat chondrosarcoma (Doege *et al.*, 1987) and human articular cartilage (Doege *et al.*, 1991), the following analogous matches were found: fragments A and B belong to the second chondroitin sulphate domain (beginning at amino acid positions 1684 and 1584 for the rat proteoglycan and positions 1939 and 1839 for human proteoglycan). Fragment C was aligned with amino acid positions 1479 and 1734 for rat and human proteoglycans respectively, and fragment D matched a sequence in the interglobular domain (beginning at amino acid position 393 for either species) (see Table 1). Direct analysis of the entire CsCl D1 fraction yielded an *N*-terminal amino acid sequence identical with that observed for protein band C (results not shown).

DISCUSSION

The ability of cartilage to deform reversibly from repetitive mechanical loading is due in large measure to the HA–proteoglycan complex trapped within the collagen network (Kempson *et al.*, 1976). The normal homoeostasis of matrix proteoglycan metabolism within cartilage can be disrupted by cytokines, most notably IL-1 (Saklatvala *et al.*, 1984; Pettipher *et al.*, 1986; Benton & Tyler, 1988; Hubbard *et al.*, 1988; Campbell *et al.*, 1988; Smith *et al.*, 1989). Accordingly, our experiments demonstrate that IL-1 (100 units/ml) addition to cartilage, under culture conditions approaching 'basal levels' (Hascall *et al.*, 1983), increased auto-resorption of proteoglycans almost 3-fold. This increased proteoglycan degradation and subsequent fragment accumulation in the medium has been exploited to characterize proteoglycan degradation further.

The majority of the proteoglycans that were released from cartilage into the medium in the presence of IL-1 did not have a functional HA-binding region. This conclusion was deduced from the inability of medium proteoglycans to aggregate with HA as judged by Sepharose CL-2B chromatography and is consistent with previous reports (Campbell *et al.*, 1984, 1989; Ratcliffe *et al.*, 1986; Campbell & Handley, 1987). Furthermore, immunoblot analysis with the antibody 1-C-6 detected significantly less epitope (G1 and/or G2) in the protein-core bands isolated from the medium of IL-1-treated cultures. Thus, from the perspectives of both function and epitope detection, the IL-1-induced release of proteoglycans from the cartilage resulted from or was concomitant with the loss of the HA-binding region. The data also agree with a report demonstrating destruction of G2 *in vitro* by purified stromelysin (Hughes *et al.*, 1990).

Although the temporal relationship of proteoglycan degradation has not been addressed in this study, sedimentation field-flow fractionation analysis of cartilage proteoglycan monomers and aggregates (Arner & Kirkland, 1989) has suggested that proteoglycans are first released from the aggregate before being further degraded. Clearly, however, as shown by our SDS/PAGE analysis and the work of others (Tyler, 1985), degradation of proteoglycan may occur at a variety of sites along the protein core. The end result of this proteolytic degradation is an assortment of large proteoglycan fragments that possess M_r values of 144000–380000. This proteoglycan fragmentation is consistent with that reported by others (Campbell *et al.*, 1986; Pelletier *et al.*, 1988).

In order to characterize the IL-1-induced degradation of cartilage proteoglycan fragments, the proteoglycans released into the medium were subjected to *N*-terminal amino acid analysis. Four sequences were detected. These sequences were similar to four regions of the rat chondrosarcoma (Doege *et al.*, 1987) and human proteoglycan (Doege *et al.*, 1991). Three of the obtained sequences (A, B and C) matched the published bovine sequence (Oldberg *et al.*, 1987). These sequence alignments were consistent with both their M_r values (Fig. 3*a*) and their reactivity

Table 1. Proposed sites of cleavage for the proteoglycans released from cartilage organ culture

N-Terminal amino acid sequence was determined for four medium proteoglycan fragments from IL-1-treated cultures (see the Results section). Each sequence is shown aligned with corresponding region of the rat chondrosarcoma (Doege *et al.*, 1987), bovine (Oldberg *et al.*, 1987) and human proteoglycans (Doege *et al.*, 1991). Tentative sequence assignments are shown in parentheses. * denotes the residue number assignments for the published sequences at the P1′ position.

	P 321	P′ 123
A (M_r 144000)		LGQRPPVT
Bovine	VEGVTEPTVSQE	LGQRPPVT
Rat *1684	TVSQE	LGQRPPVT
Human *1939	TISQE	LGQRPPVT
B (M_r 173000)		AGEGPSGI
Bovine	VESVTQAPTAQE	AGEGPSGI
Rat *1584	PTAQE	AGEGPSSI
Human *1839	PTAQE	AGEGPSGI
C (M_r 214000)		GLGSVELS
Bovine	VEVTPTTFKEEE	GLGSVELS
Rat *1479	FREEE	GLGSVELS
Human *1734	FKEEE	GLGSVELS
D (M_r 266000)		(A)RGNVILT
Rat *393	ITEGE	ARGNVILT
Human *393	ITEGE	ARGSVILT

with monoclonal antibody 1-C-6 (Fig. 3*b*). Thus fragments A and B were assigned to the second chondroitin sulphate domain and fragment C was assigned to the first chondroitin sulphate domain. The largest fragment analysed, fragment D, showed sequence similarity to the interglobular domain of both the rat and human proteoglycan molecules. The diminution of antibody 1-C-6 reactivity of this fragment (M_r 266000) would be consistent with the loss of only the G1 domain. Although not directly evaluated in this study, it is tempting to speculate that this fragment (M_r 266000) represents a preliminary site of proteolytic attack for the release of the proteoglycans from the aggregates. The *N*-terminal amino acid sequence of this latter fragment has also been recently observed (Sandy *et al.* 1991).

When the partial *N*-terminal amino acid sequences of the proteoglycan fragments were aligned with the published sequences (see Table 1), hypothetical sites of enzymic degradation could be constructed. These putative degradation sites exhibited several common features. The P1 (where P represents substrate features complementary to the enzyme specificity subsites) position of all the putative degradation sites was a glutamic acid residue, and the P'1 position in all cases comprised a non-polar or uncharged polar residue, alanine, leucine or glycine. Furthermore, glycine residues accounted for almost 50% of the amino acid residues in the P'1–P'3 positions of the four fragments. In the three fragments aligned with the published bovine sequence, Val-Glu, Val-Thr and Pro-Thr peptide pairs were evident in the residues P4–12. In addition, it is noteworthy that, as has been previously suggested (Campbell *et al.*, 1989), all of the putative chondroitin sulphate domain cleavage sites (A, B, C and D) are in areas relatively devoid of glycosaminoglycan consensus sequence sites.

We conclude, then, that in the presence of IL-1 proteoglycan turnover proceeds in a non-random fashion as a single or multiple degradative event(s).

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