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The cleavage of biglycan by aggrecanases

L. I. Melching Ph.D.†, W. D. Fisher M.D.‡, E. R. Lee Ph.D.†‡, J. S. Mort Ph.D.†‡ and P. J. Roughley Ph.D.†‡*

† *Shriners Hospital for Children, McGill University, Montreal, Quebec, Canada*

‡ *Department of Surgery, McGill University, Montreal, Quebec, Canada*

Summary

Objective: Aggrecanase-1 [a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)-4] and aggrecanase-2 (ADAMTS-5) have been named for their ability to degrade the proteoglycan aggrecan. While this may be the preferred substrate for these enzymes, they are also able to degrade other proteins. The aim of this work was to determine whether the aggrecanases could degrade biglycan and decorin.

Methods: Biglycan, decorin and aggrecan were purified from human and bovine cartilage and subjected to degradation by recombinant aggrecanase-1 or aggrecanase-2. *In vitro* degradation was assessed by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS/PAGE) and immunoblotting, and the cleavage site in biglycan was determined by N-terminal amino acid sequencing. SDS/PAGE and immunoblotting were also used to assess *in situ* degradation in both normal and arthritic human articular cartilage.

Results: Both aggrecanase-1 and aggrecanase-2 are able to cleave bovine and human biglycan at a site within their central leucine-rich repeat regions. Cleavage occurs at an asparagine–cysteine bond within the fifth leucine-rich repeat. In contrast, the closely related proteoglycan decorin is not a substrate for the aggrecanases. Analysis of human articular cartilage from osteoarthritic (OA) and rheumatoid arthritic (RA) joints showed that a biglycan degradation product of equivalent size is present in the extracellular matrix. No equivalent degradation product was, however, detectable in normal adult human articular cartilage.

Conclusion: Biglycan, which is structurally unrelated to aggrecan, can act as a substrate for aggrecanase-1 and aggrecanase-2, and these proteinases may account for at least part of the biglycan degradation that is present in arthritic cartilage.

Key words: Metalloprotease, Cartilage, Arthritis, Proteoglycan, Leucine-rich repeat, Decorin.

Abbreviations: ADAM a disintegrin and metalloproteinase, ADAMTS a disintegrin and metalloproteinase with thrombospondin motifs, α2M α_2-macroglobulin, MMP matrix metalloproteinase, OA osteoarthritis, RA rheumatoid arthritis, SLRP small leucine-rich repeat proteoglycan.

Introduction

Biglycan and decorin belong to the family of small leucine-rich repeat proteoglycans (SLRPs). The SLRPs are characterized by the presence of 6–10 adjacent leucine-rich repeats flanked by cysteine-rich domains[1,2]. Usually they are substituted with chondroitin sulfate, dermatan sulfate or keratan sulfate chains, which distinguish them from the larger family of leucine-rich repeat proteins which share the same leucine-rich motif[3]. The presence of the contiguous leucine-rich repeats causes the proteins to adopt a curved or horseshoe-like conformation[4,5], which is thought to be responsible for their functional ability to interact with other proteins within the extracellular matrix of tissues.

Biglycan and decorin are structurally similar, with both possessing 10 leucine-rich repeats and chondroitin sulfate or dermatan sulfate in the amino terminal regions of their core proteins[6]. In the case of biglycan there are two attachment sites for chondroitin sulfate or dermatan sulfate, whereas decorin has only one such site[7,8]. The presence of chondroitin sulfate or dermatan sulfate varies between tissues, though the functional significance of this change is unclear. Both proteoglycans can interact with a variety of other proteins, including growth factors[9–12] and collagens[13,14], and in so doing they can influence both the integrity of the extracellular matrix and its metabolism. The function of the two proteoglycans is not, however, equivalent, as their ability to interact with other proteins does vary. While decorin interacts with the surface of the fibrils formed by types I and II collagen[15–17], biglycan shows a preferential interaction for type VI collagen and aids in the formation of the type VI collagen network[14,18].

The structure of biglycan and decorin may vary with age in both their glycosaminoglycan chains and their core proteins. The chondroitin sulfate–dermatan sulfate chains may vary in length, position of sulfation and degree of uronic acid epimerization[19], whereas the core proteins may undergo proteolytic modification[20]. Such proteolytic modification is most apparent in biglycan, which undergoes cleavage within its amino terminal domain resulting in the separation of the terminal peptide bearing the glycosaminoglycan chains from the remainder of the core protein. The "no glycan" forms of biglycan appear to accumulate in the extracellular matrix with age[20]. Similar proteolytic processing also occurs in decorin, although to a lesser extent[21]. More extensive proteolytic modification of the core proteins

*Address correspondence and reprint requests to: Dr P. J. Roughley, Genetics Unit, Shriners Hospital for Children, 1529 Cedar Avenue, Montreal, Quebec H3G 1A6, Canada. Tel: 1-514-282-7156; Fax: 1-514-842-5581; E-mail: proughley@shriners.mcgill.ca

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occurs in disease states, such as in the articular cartilage of arthritic joints[22,23].

At present it is not clear whether the proteolytic modification of biglycan and decorin alters their function, though one might expect it to if both the glycosaminoglycan chains and the core protein contribute to the normal functions of the proteoglycans. It is also not clear which proteinases are responsible for the proteolytic modification, though one might predict the involvement of proteinases that are active in the extracellular matrix and that are secreted by the constituent cells. Metalloproteinases belonging to the matrix metalloproteinase (MMP) and a disintegrin and metalloproteinase (ADAM) families form ideal candidates for this role[24–26]. Of particular interest are the aggrecanases, members of the ADAM with thrombospondin motifs (ADAMTS) family[27–29]. Aggrecanase-1 (ADAMTS-4)[30] and aggrecanase-2 (ADAMTS-5)[31] are normally associated with their ability to degrade aggrecan[32,33], the major proteoglycan in the extracellular matrix of hyaline cartilages. However, they have also been described to degrade other substrates (Table I), and would be in the correct milieu to degrade the SLRPs.

The purpose of this study was to determine whether aggrecanase-1 or aggrecanase-2 is able to degrade biglycan or decorin, and assess whether the products of such degradation are present within the extracellular matrix of articular cartilage.

Methods

SOURCE OF CARTILAGE

Normal human articular cartilage was obtained from the femoral condyles of the knee at the time of autopsy from individuals, ranging in age from 7 months to 61 years, who had no evidence of a joint disorder. Cartilage was taken with the permission of the chief pathologist from individuals where the next of kin had given consent for a complete autopsy. Human articular cartilage was also obtained from the knees of individuals aged 50–75 years, who had undergone total knee replacement for osteoarthritis (OA) or rheumatoid arthritis (RA). Normal bovine nasal cartilage from animals aged 18 months to 2 years was obtained from the abattoir at the time of slaughter.

PREPARATION OF PROTEOGLYCANS

Biglycan and decorin were purified from juvenile human cartilage as previously described[7]. Briefly, the cartilage was extracted with 4 M guanidinium chloride in the presence of proteinase inhibitors and the extract was subjected to cesium chloride density gradient centrifugation under dissociative conditions at a starting density of 1.4 g/ml. Aggrecan was recovered from the bottom of the density gradient, while a preparation enriched in the SLRPs was recovered from the center. The SLRPs were further purified by anion exchange through DEAE-Sephacel and subsequent gel filtration chromatography through Sepharose CL-4B[34]. Bovine biglycan and decorin were a kind gift from Dr L. Rosenberg (Montefiore Hospital, New York, NY).

SOURCE OF AGGRECANASE

Recombinant human aggrecanase-1 and aggrecanase-2 were a kind gift from Dr J. Barnett (Roche Biosciences, Palo Alto, CA). They had been prepared by infecting insect cells with a recombinant baculovirus containing the full length aggrecanase cDNA[35]. The aggrecanases were purified from the culture medium by ion exchange chromatography through SP-Sepharose FF[35]. The molecular form of the recombinant aggrecanases was assessed by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS/PAGE) analysis and immunoblotting, as described below for analysis of proteoglycan degradation products. The primary antibodies used for analysis were raised in rabbits against synthetic peptides conjugated to ovalbumin[36]. The peptide sequences were derived from the published amino acid sequences of aggrecanase-1 (FASLS) and aggrecanase-2 (SISRA), and correspond to the amino terminal sequences of the mature proteinases following furin activation[37].

PROTEOGLYCAN DEGRADATION BY AGGRECANASE

Biglycan, decorin or aggrecan was incubated with aggrecanase-1 or aggrecanase-2 in 50 mM Tris/HCl, 250 mM NaCl, 5 mM $CaCl_2$ at either pH 7.5 or pH 8.5[35]. The proteoglycans were dissolved at 1 mg/ml in the digestion buffer, and incubation was carried out overnight at 37°C with sufficient aggrecanase to give limit digests. In some experiments incubations were carried out in the presence of either *o*-phenanthroline or ethylenediaminetetraacetic acid (EDTA) (1 mM or 10 mM final concentration, respectively).

CHONDROITINASE AND KERATANASE TREATMENT

Prior to subsequent analyses by SDS/PAGE, biglycan and decorin or their degradation products were treated with chondroitinase ABC (0.05 mU/μg proteoglycan) in 100 mM Tris/HCl, 100 mM sodium acetate, pH 7.3, at

Table I
Sites of aggrecanase cleavage in proteins

Molecule	Species	Sequence	Aggrecanase	Reference
Aggrecan*	Human	ITEGE–ARGSV	1, 2	33
	Human	ASELE–GRGTI	1, 2	33
	Human	FKEEE–GLGSV	1, 2	33
	Human	PTAQE–AGEGP	1, 2	33
	Human	TISQE–LGQRP	1, 2	33
Versican	Human	PEAAE–ARRGQ	1	52
Brevican	Rat	AVESE–SRGAI	1	53
Aggrecanase-1	Human	GSFRK–FRYGY	1	54
		GSALT–FREEQ	1	54
Aggrecanase-2	Human	VRIPE–GATHI	2	55
α_2M		ESDVM–GRGHA	1, 2	56
Biglycan	Bovine	LRNMN–CIEMG	1, 2	This work

*Equivalent cleavage sites have also been reported in bovine aggrecan[61,62].

37°C overnight. In the case of aggrecan and its degradation products, chondroitinase treatment was preceded by treatment with keratanase II (0.005 mU/μg proteoglycan) in 10 mM sodium acetate, pH 6.0, at 37°C overnight.

ANALYSIS OF PROTEOGLYCAN DEGRADATION

Proteoglycans or their aggrecanase-derived degradation products (1 μg) were analyzed by SDS/PAGE using 4–20% (w/v) gradient gels (Novex NuPage Bis–Tris gels; Invitrogen), according to the manufacturer's instructions. Fractionated proteins were then transferred to nitrocellulose membranes (Bio-Rad) by electroblotting[38] and identified by immunoblotting. Following blocking of the membranes in 5% skim milk (Carnation Instant Milk Powder), they were exposed to primary antibodies recognizing either the carboxy terminus of biglycan or decorin[20] or a mixture of epitopes in the G3 domain of aggrecan[39]. Immunoreactive proteins were identified using a secondary biotinylated anti-rabbit IgG, a streptavidin-biotinylated horseradish peroxidase complex, and a chemiluminescence substrate, followed by exposure to Hyperfilm (Amersham). In addition, extracts of normal and arthritic human articular cartilage were analyzed in an analogous manner for degradation products of biglycan.

N-TERMINAL AMINO ACID SEQUENCING

Degradation products resulting from aggrecanase treatment of biglycan were resolved by SDS/PAGE following chondroitinase ABC treatment, as described above. Fractionated proteins were then transferred to polyvinylidene difluoride membranes (Immobilon P, Bio-Rad) by electroblotting. The membranes were then stained briefly with 0.1% Coomassie Blue R-250 until bands were visible, then briefly destained in 50% methanol, 10% acetic acid, and subsequently washed well with water. Bands corresponding to biglycan degradation products were excised and analyzed directly by sequential Edman degradation to determine the amino terminal amino acid sequence[40]. Sequencing was carried out by the Sheldon Biotechnology Centre, McGill University.

Results

When degradation products of bovine decorin were analyzed by SDS/PAGE and immunoblotting, there was no evidence for aggrecanase-1-mediated degradation of the decorin core protein. The intact decorin migrated with a molecular size of about 70 kDa prior to chondroitinase ABC treatment [Fig. 1(A), lane 1] and about 48 kDa following chondroitinase ABC treatment [Fig. 1(A), lane 2]. Following aggrecanase treatment, the decorin core protein possessed a size analogous to that of the core protein prior to treatment [Fig. 1(A), lane 3]. The treated decorin core protein migrated slightly faster than that present without treatment due to the presence of an endoglycosidase activity produced by the insect cells used to generate the recombinant proteinase[41].

In contrast, aggrecanase-1 treatment did result in proteolytic cleavage of the bovine biglycan core protein. While intact biglycan is not visualized with the antibody used [Fig. 1(B), lane 1], the chondroitinase-treated core protein migrates with a molecular size of about 45 kDa [Fig. 1(B), lane 3]. Following aggrecanase treatment a degradation product of about 27 kDa was visualized by

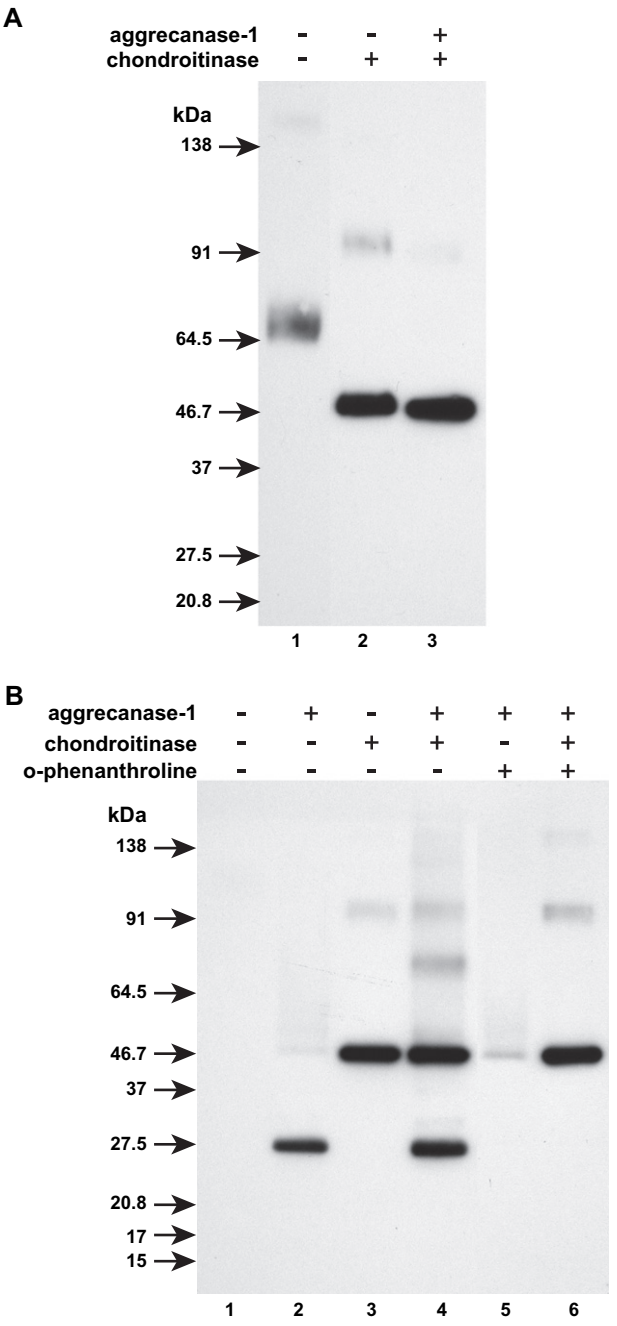

Fig. 1. Effect of aggrecanase-1 on bovine decorin and biglycan. The action of aggrecanase-1 on bovine decorin and biglycan was studied by SDS/PAGE and immunoblotting. (A) Lane 1, intact decorin; lane 2, intact decorin following chondroitinase treatment; lane 3, aggrecanase-treated decorin following chondroitinase treatment. (B) Lane 1, intact biglycan; lane 2, aggrecanase-treated biglycan; lane 3, intact biglycan following chondroitinase treatment; lane 4, aggrecanase-treated biglycan following chondroitinase treatment; lane 5, biglycan incubated with aggrecanase in the presence of *o*-phenanthroline; lane 6, biglycan incubated with aggrecanase in the presence of *o*-phenanthroline and subsequent chondroitinase treatment.

immunoblotting [Fig. 1(B), lane 2]. However, even under conditions of limiting digestion degradation did not appear to be complete, as chondroitinase treatment still revealed the presence of the intact core protein [Fig. 1(B), lane 4].

The size of the aggrecanase-derived degradation product was the same both before and after chondroitinase treatment, as expected for a fragment derived from the carboxyl terminus of the molecule. The cleavage of biglycan by aggrecanase was completely prevented by the presence of *o*-phenanthroline, a zinc-chelating inhibitor of metalloproteinases. Under these conditions only the intact biglycan core protein was evident [Fig. 1(B), lane 6]. A similar result was obtained when incubation was carried out in the presence of EDTA, another metal-chelating inhibitor of metalloproteinases.

Aggrecanase-1 treatment of human biglycan gave similar results to its bovine counterpart, with the carboxy terminal degradation product of about 27 kDa being detected (Fig. 2, lane 1). This and the previous studies on bovine biglycan had been carried out at pH 8.5, the pH optimum of the aggrecanases *in vitro*[42]. It was therefore important to establish whether biglycan cleavage could also occur at a more physiologically relevant pH. Incubation with aggrecanase-1 at pH 7.5 (Fig. 2, lane 2) gave identical results to those obtained at pH 8.5. It was, however, apparent that human biglycan cleavage was more extensive than that of bovine biglycan, with all the biglycan now undergoing degradation.

Aggrecanase-2 was also able to cleave the human biglycan with equal efficiency at both pH 8.5 and 7.5 (Fig. 2, lanes 3 and 4). The degradation product was of identical size to that obtained with aggrecanase-1. However, in contrast to aggrecanase-1, aggrecanase-2 gave incomplete degradation of biglycan, even under conditions of limit digestion. The diminished ability of aggrecanase-2 to cleave biglycan appears to be substrate specific, as when identical enzyme to substrate ratios were used with bovine aggrecan, both aggrecanases resulted in extensive cleavage of the core protein (Fig. 3). Moreover, when products containing the aggrecan G3 domain were visualized by immunoblotting, the extent of degradation appeared to be greater for aggrecanase-2 than aggrecanase-1. As with aggrecanase-1, there was no evidence that aggrecanase-2 could cleave decorin (data not shown).

As the substrate specificity of aggrecanases can depend upon their C-terminal processing, the molecular form of aggrecanases used in this study was established by SDS/PAGE and immunoblotting. Aggrecanase-1 was shown to be present in the recombinant enzyme preparation as a major component of about 70 kDa [Fig. 4(A)], whereas aggrecanase-2 was present as two components of about 56 and 62 kDa [Fig. 4(B)].

To investigate whether the aggrecanase-mediated biglycan degradation product was present in normal human articular cartilage, tissue ranging from the young juvenile

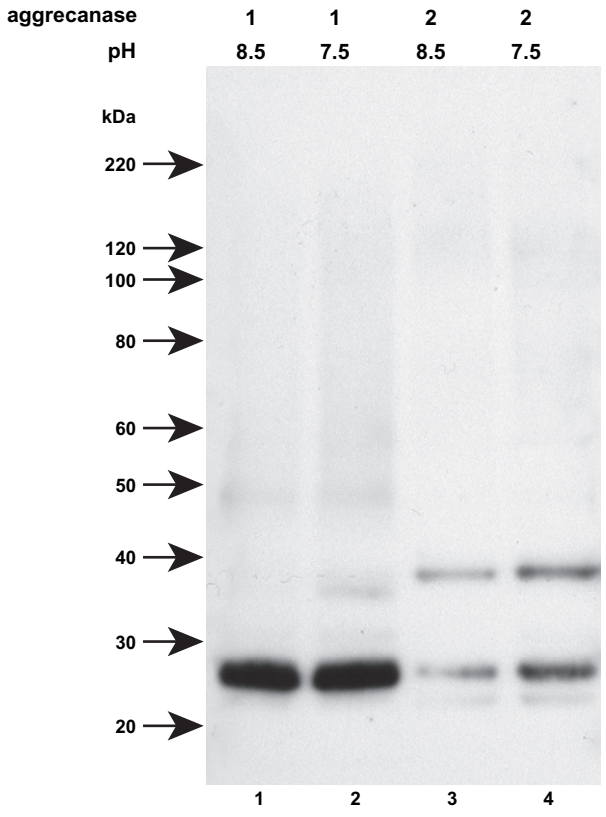

Fig. 2. Effect of aggrecanases on human biglycan. Aggrecanase-1 or aggrecanase-2 was incubated with human biglycan at either pH 8.5 or pH 7.5, and the products analyzed by SDS/PAGE and immunoblotting. All digestion mixtures were treated with chondroitinase prior to analysis. Lane 1, biglycan treated with aggrecanase-1 at pH 8.5; lane 2, biglycan treated with aggrecanase-1 at pH 7.5; lane 3, biglycan treated with aggrecanase-2 at pH 8.5; lane 4, biglycan treated with aggrecanase-2 at pH 7.5.

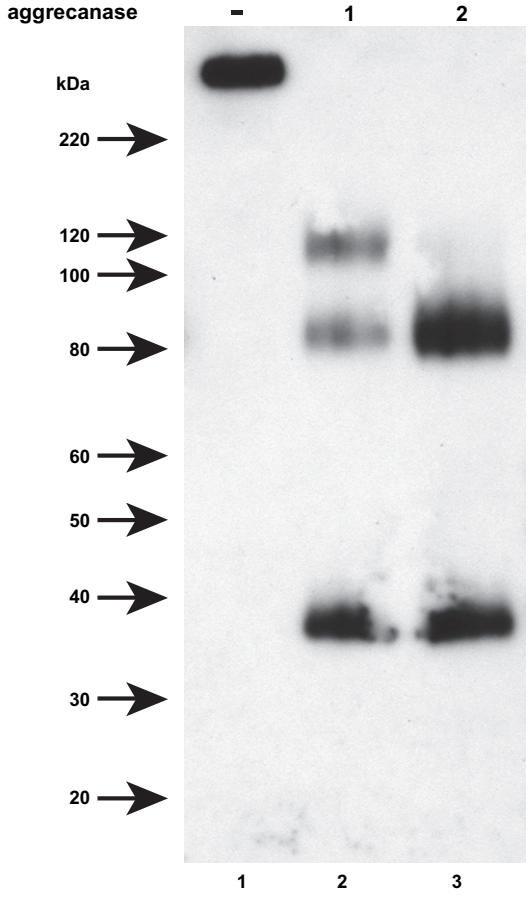

Fig. 3. Effect of aggrecanases on aggrecan. Aggrecanase-1 or aggrecanase-2 was incubated with bovine aggrecan at pH 7.5, and the products analyzed by SDS/PAGE and immunoblotting using an antibody to the aggrecan G3 domain. The digestion mixtures were treated with keratanase and chondroitinase prior to analysis. Lane 1, intact aggrecan; lane 2, aggrecan treated with aggrecanase-1; lane 3, aggrecan treated with aggrecanase-2.

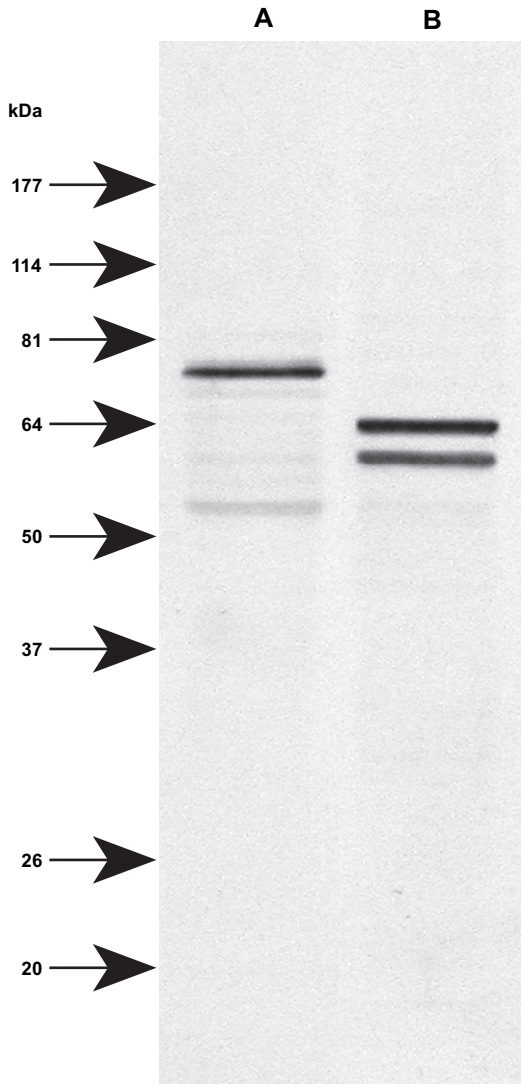

Fig. 4. Analysis of aggrecanase composition. The aggrecanase preparations were analyzed by SDS/PAGE and immunoblotting, using antibodies specific for each proteinase. (A) Aggrecanase-1; (B) aggrecanase-2.

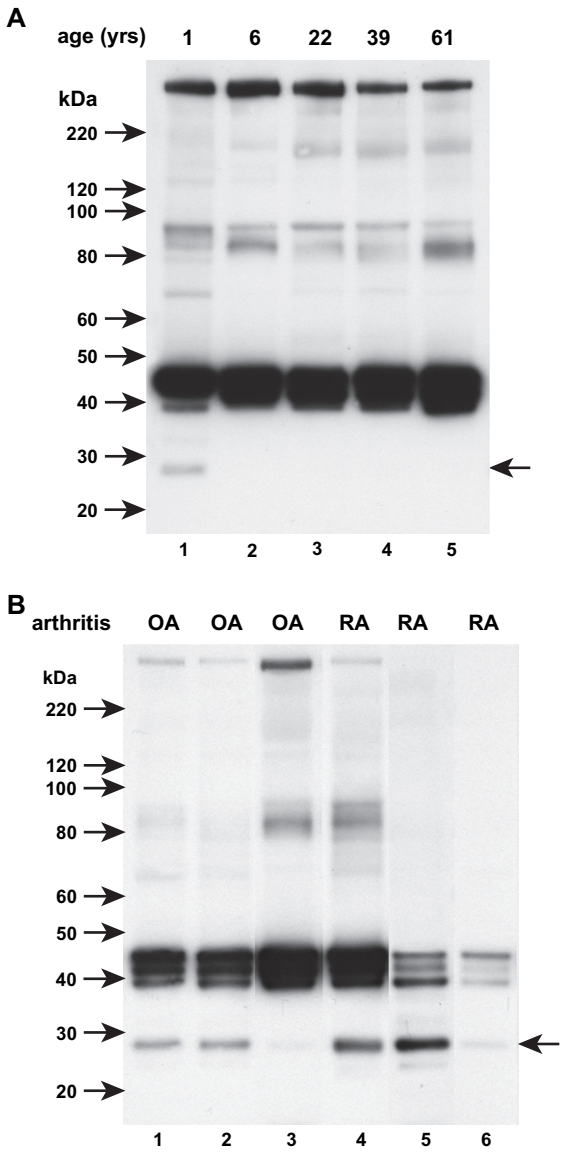

Fig. 5. Analysis of biglycan in normal and arthritic human cartilage. Extracts of human articular cartilage were analyzed by SDS/PAGE and immunoblotting using an antibody to biglycan. All extracts were treated with chondroitinase prior to analysis. (A) Samples were from normal individuals aged: (1) 7 months, (2) 6 years, (3) 22 years, (4) 39 years, and (5) 61 years. (B) Samples were from individuals with arthritic joints aged: (1) 73 years, (2) 75 years, (3) 74 years, (4) 50 years, (5) 77 years and (6) 62 years. Samples 1–3 were from OA cartilage, and samples 4–6 were from RA cartilage. Arrow indicates the position of the biglycan cleavage product.

to the mature adult was extracted and the resulting proteins analyzed by SDS/PAGE and immunoblotting. The biglycan degradation product was not detected in any normal adult cartilage examined, nor was it present in a 7-year-old juvenile cartilage [Fig. 5(A)]. However, a biglycan degradation product of equivalent size was present as a minor component in cartilage from a 7-month-old. In contrast, the biglycan degradation product was detected in extracts of adult human articular cartilage derived from individuals with either OA or RA [Fig. 5(B)]. In the mature cartilages, biglycan exists predominantly in three forms of 40–50 kDa. These have previously been shown to represent intact biglycan and two N-terminally processed forms of the molecule[20]. Such processing is not aggrecanase-mediated[43].

To establish the cleavage site at which the aggrecanases are acting on biglycan, the 27 kDa degradation product resulting from aggrecanase-1 action on bovine biglycan was isolated by SDS/PAGE and subjected to N-terminal amino acid sequencing. A single sequence was obtained for 10 cycles, XIEMGGNPLE. Comparison with the published amino acid sequence of bovine biglycan showed that the unidentified initial residue corresponds to cysteine, with cleavage having occurred between Asn149 and Cys150.

Discussion

Both aggrecanase-1 and aggrecanase-2 cleave bovine and human biglycan near the beginning of their fifth leucine-rich repeat domain. The amino acid sequence surrounding the cleavage site is conserved in all species described to date – mouse, rat, human, bovine[6,44–46], dog

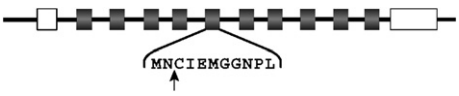

Fig. 6. Schematic representation of biglycan and its aggrecanase cleavage site. Biglycan is depicted as a line representing its mature core protein, with the location of the 10 leucine-rich repeats (filled boxes) and the flanking disulfide-bonded domains (open boxes) being indicated. The location of the aggrecanase cleavage site and its surrounding amino acid sequence are also indicated.

(AAB51244), sheep (AAB87988) and horse (AAB88305) – and occurs between asparagine and cysteine residues (Fig. 6). Cleavage generates a carboxy terminal fragment of about 27 kDa, commencing with the cysteine residue and ending at the carboxy terminus of the mature biglycan core protein. It is not clear if this is the only cleavage site within biglycan, as amino terminal fragments cannot be detected by the carboxy terminal antibody used for immunoblotting. However, no other sequence predicted to be compatible with an aggrecanase cleavage site occurs elsewhere in the biglycan core protein. The unique leucine-rich sequence cleaved by the aggrecanases in biglycan is not conserved in decorin[47–49] nor are any consensus aggrecanase cleavage sites present. Hence, it is not surprising that the aggrecanases should fail to cleave decorin. However, there has been one report that a C-terminally truncated recombinant form of aggrecanase-1 will cleave decorin[50], albeit very inefficiently, and it is possible that C-terminal truncation could result in altered substrate specificity. The aggrecanase-1 used in the present work was not C-terminally truncated.

The data presented in the present work indicate more substantial cleavage of human than bovine biglycan. One contributing factor is probably the decreased substrate to enzyme ratio used in the human work. Although all digestions were carried out using a constant proteoglycan to aggrecanase ratio, the bovine biglycan was pure whereas the human biglycan was present together with decorin, so lowering its effective concentration. It is unlikely that the data reflect a true species difference, as the amino acid sequence surrounding the cleavage site is identical. However, it is possible that cysteine modification could occur *in situ* at the aggrecanase cleavage site, and that differences in such modification could occur between the two biglycan preparations so influencing their susceptibility to cleavage. Also, one cannot exclude the possibility that biglycan forms dimers in a similar manner to decorin[5,51], but that there are differences in dimer conformation between the two biglycan preparations that influence access of the aggrecanases to their cleavage site.

Initially, aggrecanases were characterized by their ability to cleave aggrecan. Both aggrecanase-1 and aggrecanase-2 cleave aggrecan at the same five sites[33], each following a glutamic acid residue (Table I). Aggrecanase-1 is also able to cleave other members of the hyalectin family of proteoglycans to which aggrecan belongs, with single cleavage sites having been identified in both versican[52] and brevican[53]. These cleavage sites also occur following a glutamic acid residue. Recently it has been shown that the aggrecanases can cleave protein substrates other than proteoglycans. The first such sites were identified in aggrecanase-1 and aggrecanase-2 themselves following autocatalytic processing[54,55]. Perhaps unexpectedly, the cleavages in aggrecanase-1 did not occur following a glutamic acid residue. A similar situation has also been shown to occur in α_2-macroglobulin (α2M), where cleavage follows a methionine residue, with both aggrecanase-1 and aggrecanase-2 being active at this site[56]. Thus, biglycan represents another molecule in which the aggrecanases can cleave following a residue other than glutamic acid. At present there is no obvious relationship between the different cleavage sites, and it is not clear how the different sites may vary in their avidity toward cleavage.

Where studied, aggrecanase-1 and aggrecanase-2 appear to have identical substrate specificity, though the present work indicates that they do not necessarily cleave all sites with equal efficiency. A similar conclusion had been previously reported for cleavage of aggrecan[35]. In the case of aggrecan, aggrecanase-2 performs more extensive degradation, whereas with biglycan aggrecanase-1 is more active. It is possible that this difference may not be an innate property of the catalytic domain of the proteinases, but rather a reflection of their molecular state. Aggrecanase-1 has been shown to undergo initial proteolytic activation by cleavage within its amino terminal region by a furin-like proteinase[57]. Subsequently, autocatalytic cleavage can result in carboxy terminal truncation of the aggrecanase[58], and with such processing the affinity of the enzyme for cleavage at different sites does change[50]. In the present work, the size of the aggrecanase-1 is compatible with only amino terminal activation. The aggrecanase-2 used in the present work was present as two components, both of which were smaller than the aggrecanase-1. Such sizes are compatible with carboxy terminal truncation, and this could contribute to the altered substrate reactivity. Thus one cannot categorically state that equivalent forms of aggrecanase-1 and aggrecanase-2, whether intact or C-terminally truncated, will also show altered substrate reactivity.

Biglycan degradation products of a size compatible with aggrecanase cleavage appear to be a feature of adult human cartilage from arthritic joints but not that from normal joints. This is not too surprising as increased expression of aggrecanase via cytokine stimulation is associated with the arthritic joints[59]. A similar biglycan degradation product was also detected in the epiphyseal cartilage of a young juvenile human. This is a period of maximal growth rate, when aggrecanases may contribute toward rapid tissue remodeling. In the case of aggrecan, there is also evidence for proteolytic processing by aggrecanases in the juvenile[60]. At present it is unclear whether the biglycan degradation product is stably retained within the cartilage matrix or whether it is lost relatively rapidly. As such, it is possible that the immunoblotting analysis performed in this work may not give a complete reflection of the extent of biglycan processing. One must also accept that size equivalence of the *in vitro* and *in vivo* biglycan degradation products does not categorically prove that they have been generated by cleavage at identical sites, and conclusive proof awaits confirmation of the *in vivo* cleavage site by neoepitope analysis, amino acid sequencing or mass spectrometry. Anti-neoepitope antibodies have proven to be useful for detecting proteolytic cleavage products by immunoblotting. However, the presence of an amino terminal cysteine residue at the aggrecanase cleavage site of biglycan poses unique problems for this technique due to the possibility of oxidation or other derivatization occurring *in situ* or during antigen preparation for antibody production.

It is clear that aggrecanase-mediated cleavage of biglycan can occur under physiological conditions, and this raises the question of whether it is of any functional consequence. While detailed analyses of the structure/function

relationships of biglycan are lacking, it is thought that the leucine-rich repeat region is responsible for conferring the ability to interact with other proteins[3]. As such, cleavage within the central part of this region might be expected to impair biglycan function. Furthermore, cleavage would result in separation of the amino terminal portion of the biglycan core protein bearing the glycosaminoglycan chains from the remainder of the molecule. Such truncated biglycan has not been identified in the cartilage matrix and is likely lost. Again, this is unlikely to be beneficial to biglycan function. Thus it is likely that aggrecanase processing of biglycan may contribute to cartilage turnover under both normal and pathological conditions.

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