The intermediates of aggrecanase-dependent cleavage of aggrecan in rat chondrosarcoma cells treated with interleukin-1

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We have examined the abundance and structure of intermediates in the chondrocyte-mediated degradation of aggrecan by aggrecanase(s). Degradation products were identified by Western-blot analysis with antibodies to cleavage-site neoepitopes and to peptides within the globular domains. Rat chondrosarcoma tumour contained full-length aggrecan and all of the individual peptides expected from single independent cleavages at each of the four aggrecanase sites in the chondroitin sulphate (CS) domain. Kinetic analysis of the products present in rat chondrosarcoma cell cultures treated with interleukin-1b showed that the first aggrecanase-mediated cleavages occurred at the four sites within the CS attachment region to generate two stable intermediates, Val^{1}–Glu^{1459} and Val^{1}–Glu^{1274}. These species were subsequently cleaved at the Glu^{373} site in the interglobular domain to form the terminal products, Val^{1}–Glu^{373}, Ala^{374}–Glu^{1274} and Ala^{374}–Glu^{1459}. It therefore appears that the aggrecanase-mediated processing of native aggrecan by chondrocytes *in situ* is initiated within the CS-attachment region and completed by cleavage within the interglobular domain. Since it has been shown that digestion of aggrecan monomer in solution with recombinant ADAMTS-4 [Tortorella, Pratta, Liu, Austin, Ross, Abbaszade, Burn and Arner (2000) Sites of aggrecan cleavage by recombinant human aggrecanase-1 (ADAMTS-4). J. Biol. Chem. **275**, 18566–18573] exhibits similar kinetics, it appears that preferential proteinase cleavage in the CS-rich region is determined by properties inherent in the aggrecan monomer itself, such as preferred peptide sequences for enzyme binding or enhanced accessibility to the core protein at these sites.

Key words: ADAMTS, cartilage, degradation, osteoarthritis, proteoglycan.

INTRODUCTION

Maintenance of the aggrecan composition and content of articular cartilage is critical to the function of the tissue throughout adult life. The process by which chondrocytes achieve this steady state must involve a delicate balance of biosynthetic and degradative processes [1]. It is now clear that accelerated degradation of aggrecan in cartilage, such as that which occurs in cytokine-stimulated cartilage explants [2–4] or in human osteoarthritis [5], is due largely to the activity of one or more aggrecanases. Aggrecanases-1 and -2 have been cloned and identified [6,7] as members of the ADAMTS gene family [a disintegrin-like and metalloprotease (reprolysin type) with a thrombospondin type-1 motif]. The ADAMTS proteins are composed of an N-terminal pro-sequence with a furin-processing site, a metzincin domain, a disintegrin-like domain and, uniquely, a variable number of thrombospondin-like repeats. The only members of the family with established substrates are ADAMTS-2 (procollagen-N-proteinase) and ADAMTS-4 and -5 (aggrecanases-1 and -2, respectively). The aggrecanases have been defined as a family of proteinases that cleave aggrecan at one or more of five specific Glu-Xaa sites in the aggrecan core protein [2–4]. In the rat, these sites are at Glu^{373} [within the interglobular domain (IGD) between aggrecan globular domains G1 and G2], and at Glu^{1274}, Glu^{1459}, Glu^{1564} and Glu^{1664} [all within the chondroitin sulphate (CS)-attachment region]. The significance of these multiple cleavage sites in the CS-attachment region of aggrecan is unknown. In bovine [8] and human [9] articular cartilages there is ample evidence for the presence in the tissue of aggrecan molecules that have been cleaved within the CS-attachment region and which have lost the C-terminal G3 domain. However, whether these species are products of aggrecanase and/or other proteinases has not been established fully.

In the present work, we have further defined the intermediates present in rat tumour *in vivo* and we have studied the process of aggrecanase-mediated degradation of aggrecan in a model system of interleukin-1b (IL-1b)-treated rat chondrosarcoma cells. Since this cell system produces only full-length aggrecan during matrix assembly it offers a unique opportunity to describe the time course of cleavage of the intact molecule at each site and the existence of natural intermediates in the pathway.

EXPERIMENTAL

Materials

Dulbecco's modified Eagle's medium (powder) and culture-tested distilled water were from Life Technologies, Grand Island, NY, U.S.A. Fetal bovine serum was from HyClone, Logan, UT, U.S.A. Mouse recombinant IL-1b was from Genzyme, Boston, MA, U.S.A. The ECL® detection system was from Amersham, Arlington Heights, IL, U.S.A., and was used according to the manufacturer's instructions. Goat anti-mouse IgG (peroxidase-conjugated) was from Sigma and goat anti-rabbit IgG and goat anti-mouse IgM (peroxidase-conjugated) were from Boehringer Mannheim. The production, characterization and use of the antiserum ATEGQV (anti-G1) has been described in [10]. In addition, an antiserum was raised to highly purified bovine aggrecan G1 domain (supplied by Dr Larry Rosenberg, Monte-

Abbreviations used: IL-1b, interleukin-1b; CS, chondroitin sulphate; IGD, interglobular domain; MMP, matrix metalloproteinase; ADAMTS, a disintegrin-like and metalloprotease (reprolysin type) with a thrombospondin type-1 motif.

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fiore Hospital, New York, NY, U.S.A.) and this was used to supplement the ATEGQV antiserum for detection of low-abundance products. Affinity-purified antisera NITEGE and VDIPEN were gifts from Dr John Mort (Shriners Hospital for Children, Montreal, Canada); Lec-7 was raised in rabbits against the keyhole-limpet haemocyanin-conjugated peptide DGHPM-QFENWRPNQPDN from the lectin domain of human aggrecan G3; 2-B-6 (which recognizes the unsaturated chondroitin 4-sulphate-bearing stub remaining on the core protein after chondroitinase digestion) and 3-B-3 (which recognizes the equivalent chondroitin 6-sulphate-bearing stub) were from ICN. Antisera TFKEEE, APTAQE and PTISQE were supplied by Merck, Rahway, NJ, U.S.A. The anti-TASELE antiserum was raised in rabbits against the ovalbumin-conjugated peptide CFTASELE by the methods described previously for anti-ATEGQV [10]. It should be noted that the TFKEEE and the PTIVSQE antisera were raised against the human peptide neoepitope sequences and were shown to detect the equivalent rat neoepitopes TFREEE and PTVSQE. BC-3 blots were done by Dr Liz Arner of DuPont Pharmaceuticals. Chondroitinase ABC (protease-free) was from Seikagaku (Rockville, MD, U.S.A.). The rat chondrosarcoma tumour aggrecan preparation was kindly supplied by Dr Jim Kimura (Henry Ford Hospital, Detroit, MI, U.S.A.).

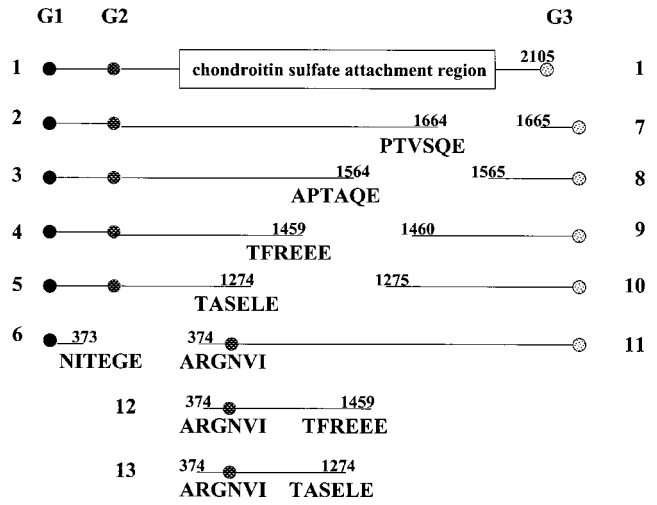

Figure 1 Schematic representation of aggrecanase-generated peptides

Depicted is the set of ten peptides (2–11) that would be generated by aggrecanase if all five possible sites were cleaved and any one cleavage prevented further cleavage. Also shown are peptides 12 and 13, which are generated by further cleavage at the Glu^{373}–Ala^{374} bond of peptides 4 and 5, respectively. The residue numbers and sequences are for rat aggrecan. (The residue numbering and sequences for human and bovine aggrecan at these sites are different and are summarized in [10].) G1, G2 and G3 are the aggrecan globular domains.

Methods

Rat chondrosarcoma cells (clone 11) were cultured and treated with IL-1b as described previously [11,12]. Western-blot analysis of rat chondrosarcoma tumour A1 (the aggrecan fraction of highest buoyant density isolated from a CsCl gradient run under associative conditions at a starting density of 1.5 g/ml) and clone 11 cultures was performed after digestion with chondroitinase ABC (protease-free) as described in [10,11]. Residue numbering in this article follows that previously described in [10,13,14], assuming a 19-residue signal sequence. Quantification and relative abundance of peptides by densitometric analysis of Western blots was done as described [11,12].

RESULTS

Characterization of antisera and analysis of rat chondrosarcoma tumour

We have previously described the use of anti-peptide antisera for characterization of aggrecan core species present in human cartilage and synovial fluid [10], rat chondrosarcoma tumour [10], rat chondrosarcoma cell cultures [11,12], bovine cartilage explants [11] and bovine chondrocyte culture [13]. This earlier work provided details of the properties of full-length aggrecan core and the terminal degradation products commonly described as G1-NITEGE (aggrecanase-generated) and G1-VDIPEN [matrix metalloproteinase (MMP)/cathepsin B-generated].

To further define the structure of aggrecan fragments generated *in vivo* we analysed the rat chondrosarcoma tumour A1 preparation (see the Experimental section for isolation procedures) with antisera to the G1 domain, the G3 domain, chondroitin 4-sulphate stubs (2-B-6), and the five possible aggrecanase-generated C-terminal neoepitopes (Figure 1). The neoepitope antisera, which were raised against human sequences, were PTVSQE (to detect peptide 2), APTAQE (to detect peptide 3), TFKEEE (to detect peptide 4), TASELE (to detect peptide 5), and NITEGE (to detect peptide 6). The Western-blot analysis (Figure 2) showed that the tumour A1 sample contains at least 10 discrete aggrecan-derived core species. On the basis of immunoreactivities and apparent size these appear to represent the full-length core (peptide 1, which reacts with the G1, G3, and 2-B-6 antibodies) and five other G1-bearing but G3-lacking species. These are peptide 2 (reacts with anti-G1 and PTVSQE), peptide 3 (reacts with anti-G1 and APTAQE), peptide 4 (reacts with anti-G1 and TFKEEE), peptide 5 (reacts with anti-G1 and TASELE) and peptide 6 (reacts with anti-G1 and NITEGE). Consistent with the suggested structures of G3-lacking fragments (Figure 1) all of these peptides reacted specifically with one neoepitope antiserum but not with the G3 antiserum (see Figure 2). Further, the G1-bearing fragments that would be expected to carry CS chains (peptides 2–5) apparently reacted with the 2-B-6 antiserum, whereas peptide 6, as expected, did not. The identity of the APTAQE-positive species that appears to co-migrate with peptides b and 10 (Figure 2, lane 5) is presently unknown.

Analysis of the tumour A1 with the G3 antiserum (Figure 2, lane 2) showed that peptides which react with the G3 but not the G1 antiserum are also abundant; these are therefore of a size and immunoreactivity consistent with the structures of peptides 7–10 (Figure 1). These structures are also supported by the 2-B-6 reactivity, which confirms the expected result that peptides 7–10 are all substituted with chondroitin 4-sulphate stubs after chondroitinase digestion. The relatively weak 2-B-6 reactivity of peptide 7 may be explained by the known relative paucity of CS-attachment sites in this extreme C-terminal region of the core protein. Interestingly, there was no evidence for the presence of the theoretically possible peptide 11 (Figure 1), which would be expected to be G3-positive and 2-B-6-positive and due to its size to migrate between peptides 1 and 2. The absence of peptide 11 was further supported by Western-blot analysis on a 4% gel (results not shown), where there was no evidence for more than a single high-molecular-mass G3-positive and 2-B-6-positive species (peptide 1) and no evidence for a BC-3-positive product larger than peptides 12 and 13. The G1-reactive peptide of about

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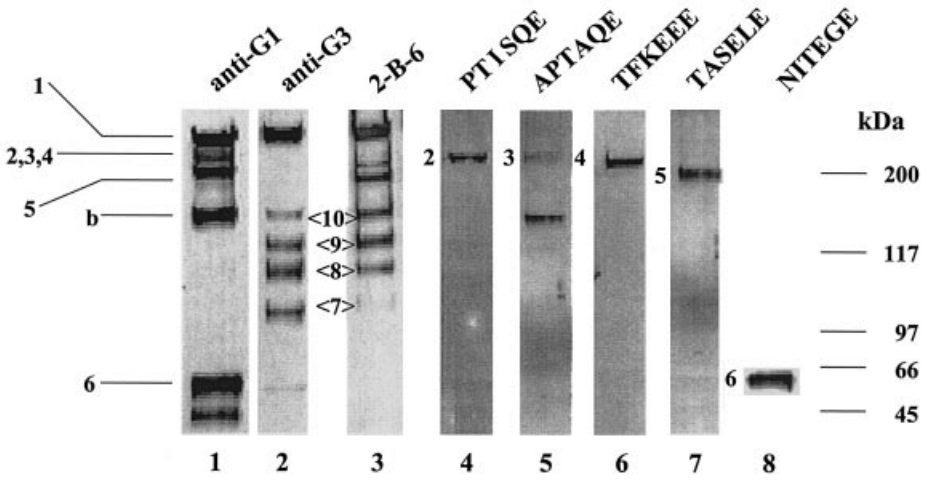

Figure 2 Characterization of antisera

Rat chondrosarcoma tumour aggrecan (A1) was chondroitinase ABC-digested and portions (about 1 μg of protein) were analysed by Western blotting on SDS/PAGE (4–12%) gels with the antisera shown across the top: anti-G1, anti-G3 (Lec-7), 2-B-6 (chondroitin 4-sulphate bearing CS stubs on chondroitinased aggrecan core protein), PTISQE (C-terminal of Glu^{1664}), APTAQE (C-terminal of Glu^{1564}), TFKEEE (C-terminal of Glu^{1459}), TASELE (C-terminal of Glu^{1274}) and NITEGE (C-terminal of Glu^{373}). Western blots were visualized using a Hewlett Packard Desk Scan II and Scion Image software. The data show that the tumour contains peptides with immunoreactivities and migration behaviours consistent with the structures of peptides 1–10 (labelled at the sides of lanes and structures shown in Figure 1). A major component (peptide b, lane 1) has not been identified fully (see text).

45 kDa migrating ahead of peptide 6 (Figure 2, lane 1) was identified as the MMP/cathepsin B product G1-VDIPEN since it alone reacted strongly with the anti-FVDIPEN antiserum (results not shown).

Since peptides 2–4 essentially co-migrated on the 4–12% gel (Figure 2), we next developed a 4% gel system to obtain complete separation of peptides 1–5. On this system, the migration behaviour could be predicted from the peptide size (residue number); thus the order of separation (1, 2, 3, 4 and 5) was also the size order, being 2105, 1614, 1564, 1459 and 1274 residues respectively (results not shown). In addition, probing of the 4% gel with the antiserum BC-3 to the aggrecanase-generated N-terminal ARGNVI gave no evidence for the presence of peptides 11–13 in the tumour, suggesting that they are degraded further or lost from the tissue.

Products of the aggrecanase response to IL-1b in rat chondrosarcoma cell cultures

In a previous paper [11] we established conditions for the optimum aggrecanase response of rat chondrosarcoma cells to treatment with IL-1b. Following 5 days of treatment the response was quantified by Western-blot analysis, on 4–12% gradient gels, of the presumed G1-TFKEEE (peptide 4) and G1-NITEGE (peptide 6) products. Given the recent availability of the TASELE and BC-3 antisera, and the improved separation on 4% gels of

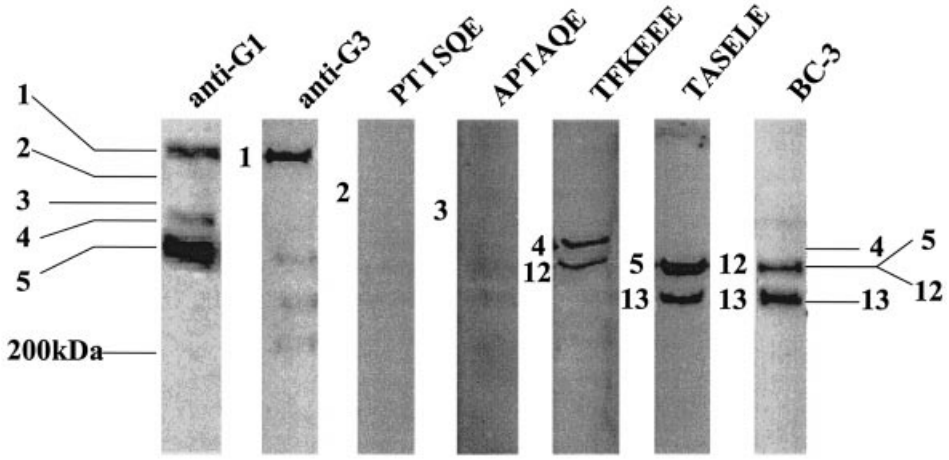

Figure 3 Characterization of terminal degradation products by Western-blot analysis with neoepitope and anti-peptide antisera

Degradation products present after 5 days of treatment with IL-1b were analysed by Western blotting on SDS/PAGE (4%) gels with antibodies ATEGQV (G1 domain), Lec-7 (G3 domain), PTISQE, APTAQE, TFKEEE, TASELE (see Figure 1 for details), and N-terminal of Ala^{374} (BC-3); Western blots were visualized using a Hewlett Packard Desk Scan II and Scion Image software. The positions of peptides 1, 4, 5, 12 and 13 are shown. Peptides 2 and 3 were not detected in the cell culture products but were identified in the tumour extract (see Figure 2).

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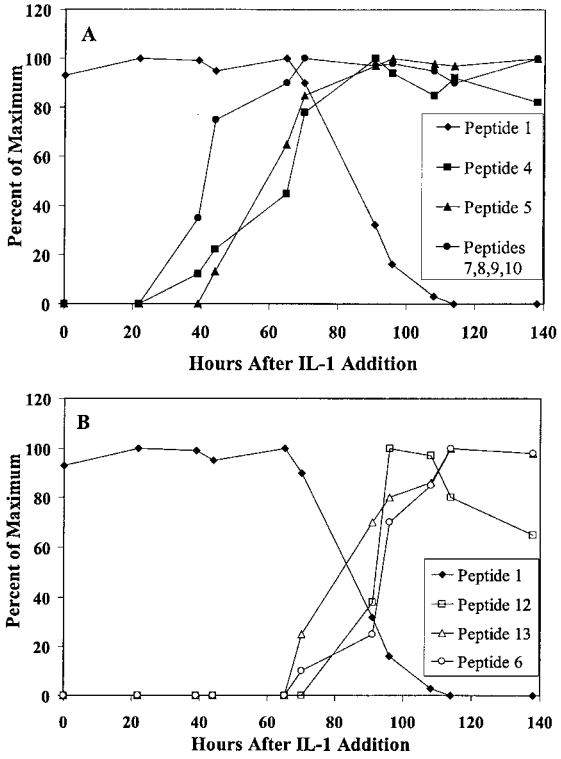

Figure 4 Description of the time-dependent change in abundance of peptides in chondrosarcoma cells treated with IL-1b

Time-course Western blots were scanned on NIH Image software and the integrated pixel-density values were used to calculate a percentage of maximum for each peptide shown. For any one antibody, all samples were run at the same time on two gels with the sample of the final time point loaded on each gel. Films were exposed to allow for integrated pixel-density capture in the linear detection range and signals from the two gels were corrected for minor differences seen in the data from the common sample. All data for any one peptide were then normalized to the maximum content observed and plotted relative to time. All species, except 7–10, were quantified on the basis of both N-terminal and C-terminal epitope reactivities and the data from both epitopes were compared to determine the validity of this approach. In all cases the comparison showed excellent agreement and therefore only the data on the C-terminal epitope reactivities is presented here. Also, since the time variation in abundance of the G3-reactive peptides 7–10 showed very close agreement, the data on these four peptides have been combined for presentation (**A**, ●).

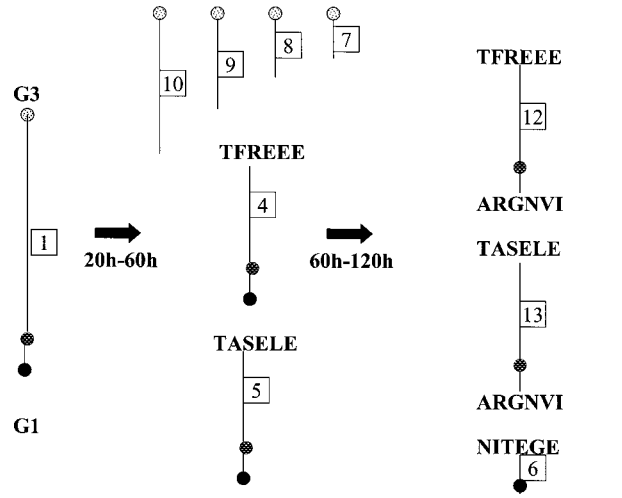

Figure 5 A model of the kinetics of the aggrecanase-mediated degradative pathway in rat chondrosarcoma cells

The model is based on the abundance analysis shown in Figure 4 and the peptide identifications shown in Figures 2 and 3. The peptide numbers and neoepitope sequences are for rat and refer to those shown in Figure 1.

peptides in the 200–400-kDa range we have now re-examined the composition of the 5-day medium from these cultures. Analysis on both 4–12 % (results not shown) and 4 % (Figure 3) gels both confirmed and extended the previous data.

First, peptide 6 (Figure 1) was confirmed as a major catabolic product with both anti-G1 and NITEGE antisera. Second, despite their presence in the tumour extract (Figure 2, lanes 4 and 5) there was no evidence for the presence of peptides 2 and 3 in the cell culture even though the corresponding G3-bearing peptides 7 and 8 were generated (see Figure 4). This suggests that in the cell system peptides 2 and 3, once formed, are processed rapidly, presumably by aggrecanase action, to the smaller peptides 4 and 5. Third, the previously described 220-kDa band [11], which had been ascribed to G1-KEEE (peptide 4) alone, was here resolved into two bands that were identified as peptide 4 and G1-SELE (peptide 5). Moreover, the relative reactivity with anti-G1 (Figure 3, lane 1, reading from the left) suggests that peptide 5 was much more abundant than peptide 4. Fourth, the combined use of the TASELE, TFKEEE and BC-3 antisera has also now clearly identified the expected peptides 12 and 13 in this material. Not surprisingly, peptide 5 (1274 residues; G1- and TASELE-reactive) and peptide 12 (1085 residues; BC-3- and TFKEEE-reactive) were not resolved, whereas peptide 4 (1459 residues; G1- and TFKEEE-reactive) and peptide 13 (900 residues; BC-3-and TASELE-reactive) were completely separated from each other and from the peptide 5/peptide 12 mixture (Figure 3). Finally, the G3-reactive peptides, 7–10, were all generated in the IL-1b-treated cell cultures and were all identified on the basis of their reactivity with the 2-B-6 and G3 antibodies, much as shown in the tumour extract (Figure 2, lane 3). In summary, the analyses suggested that the peptides 5, 6, 12 and 13, along with 7–10, were the major terminal products in the cell-culture system. Further, as was found for the tumour, there was no evidence for the presence of peptide 11 in the cell system, suggesting again that IGD cleavage does not occur without previous cleavage(s) in the CS-attachment region.

Kinetics of the aggrecanase-mediated process in cell culture

The antisera described above were next used on both 4–12 % and 4 % gels to examine the abundance of the 10 different peptides at various times after IL-1b addition. First, the only species present at 0 h was peptide 1, showing that there was no appreciable aggrecanase activity in this culture system before IL-1b addition (Figure 4). Second, there was a lag period after IL-1b addition of about 20 h before any catabolism occurred, and the first products, which appeared between 20 and 45 h were peptides 4, 5 and 7–10 (Figure 4A). These products (see Figure 5 for a schematic representation) are those expected from cleavage within the CS-attachment region but without IGD cleavage. A second phase of catabolism began at about 60 h (Figure 4B) with the appearance of peptides 12, 13 and 6. These are the terminal products expected from IGD cleavage of peptides 4 and 5 at the Glu^{373}–Ala^{374} site (Figure 5). The finding that the abundance of peptides 4 and 5 was apparently maintained up to 138 h suggests that their conversion to peptides 12 and 13 was accompanied by their replacement from the pool of peptide 1. Thus the abundance of peptide 1 remained essentially constant until about 60 h due to

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the continued synthesis of aggrecan over this period; consistent with this was the finding that the total CS content of the cultures typically increased from about 20 μg to about 45 μg between 0 and 60 h. A summary of the species identified in the cultures and the kinetics of conversion suggested by the data in Figure 4 is given in Figure 5.

DISCUSSION

Whereas it has been known for at least two decades that aggrecan exists in articular cartilage as the full-length molecule along with a range of C-terminally truncated species [8,9,14], the precise structure of these truncated forms and the proteinases responsible for their generation have never been established. In the present study we have analysed C-terminal truncation of aggrecan in the rat chondrosarcoma tumour tissue *in vivo* and also in tumour cells stimulated in culture by IL-1b. In both cases the large majority of the aggrecanolysis, as determined by fragment analysis, results from the action of the glutamyl-endopeptidases (aggrecanases), which belong to the ADAMTS family of proteinases. The only exceptions were found in the tumour extract and these were the MMP-generated aggrecan fragment, G1-VDIPEN341 (Figure 2, lane 1), and a relatively abundant G1-positive species labelled as band b. The migration behaviour of band b (similar to species 10, which is 830 residues) suggests that it is a G1–G2 'double-globe' species generated by proteolysis between the G2 domain (which terminates at Cys^{663}) and the CS-attachment region (which begins at Ser^{779}). While the exact C-terminus is unknown, this region of the core protein contains no strong sequence similarities to the five ADAMTS-sensitive sites, although it does contain a potential MMP-sensitive site (Ser^{668}--Val^{669}) that is characterized by the X-(clip)-hydrophobic-hydrophobic sequence present at the Asn^{341}–Phe^{342} bond, which is cleaved to generate G1-VDIPEN. The only other unidentified product is the fast-migrating APTAQE-positive band in Figure 2, lane 5, which one can speculate might represent the product of MMP cleavage at Ser^{668} and ADAMTS cleavage at Glu^{1564}.

The present study strongly indicates that proteolysis of aggrecan at the Glu^{373}–Ala^{374} bond by aggrecanase *in situ* is much less preferred than cleavage at the four sites within the CS domain. The time course of appearance of products (Figure 4) shows clearly that cleavage within the CS domain to generate the G3-bearing fragments (peptides 7–10) is essentially complete after about 65 h of IL-1b treatment, whereas the appearance of peptides 6, 12 and 13 (due to Glu^{373}–Ala^{374} cleavage) is barely initiated at this time. Whether IGD cleavage is dependent on prior CS cleavage, as suggested previously [15], cannot be concluded from the present work, although the absence of peptide 11 (which would be generated by IGD cleavage alone) both in the tumour and in the cell system, is consistent with this idea.

Particularly interesting is the observation that these results on the kinetics of chondrocyte-mediated aggrecanase action in cell culture and those described in explant culture [15] appear to be very similar to those reported recently for this process with recombinant ADAMTS-4 in solution studies [16]. In each case the conclusion has been made that the primary cleavages occur in the CS-rich region and that the IGD cleavage is slow and secondary. Since this order of cleavage is observed with recombinant enzyme on aggrecan monomers in dilute solution (about 0.5 mg/ml) and also with 'natural' enzyme on link-stabilized aggregates at high concentration (greater than 50 mg/ml in tissue), it appears that the structural features which render the CS-rich region sensitive and the IGD relatively insensitive are contained within the monomer structure alone.

An obvious difference between the two regions is the peptide sequences themselves. Alignment of all five sequences in rat aggrecan from P14 to P5′ reveals a consensus motif of XLXe-XXXXXXXeXE↓XXgXX (where the arrow indicates the position of the scissile bond; upper-case residues conserved $> 80\%$ and lower case conserved $> 50\%$). For human aggrecan this motif is XXveXXXXXXXeXE↓XXgXX. It therefore appears that in addition to the P1 glutamate there is an important recognition sequence at P13, P12 and P11 (P14, P13 and P12 at the SELE cleavage site in rat and human). Thus at all cleavage sites within the CS-attachment region this triplet is present as hydrophobic-hydrophobic-glutamate (generally LVE but also LIE or FVE). On the other hand, this triplet is absent at this site in the IGD of both rat and human aggrecan. The identity of residues at P14–P11 may therefore be critical in determining active site or exosite binding. Another clear difference is the presence of an N-linked oligosaccharide at P6 and a probable O-linked oligosaccharide at P3 in the IGD sequence only; such carbohydrate substitutions may interfere with enzyme–substrate binding.

While peptide sequence or oligosaccharide substitution may be major determinants of activity it is also interesting to note that the order of cleavage obtained with the ADAMTS activities (CS domain followed by IGD) has also been observed routinely with a range of other proteinases such as MMPs [17], trypsin [18] and clostripain [19]. In addition, we have recently found (J. D. Sandy and V. Thompson, unpublished work) that time-course digestion of human aggrecan monomer with recombinant MMP3 is initiated in the CS-rich region and only after prolonged digestion is the IGD cleaved extensively. It would therefore appear that this order of cleavage may be a consequence of more general structural features of the aggrecan, such as the high degree of CS/keratan sulphate substitution in the CS-rich region and their absence or paucity in the IGD. Thus sulphated glycosaminoglycan substitution and associated hydration and charge repulsion may maintain the associated core protein in an extended conformation, which might improve accessibility for proteinases in general. On the other hand the relatively hydrophobic IGD may adopt a particular tertiary structure in solution which protects it from rapid proteolytic attack.

The higher sensitivity of the CS-rich region to proteolysis provides a simple explanation for the presence of a range of G1--G2-bearing aggrecan species in mature cartilages from many species. In this regard it is interesting that the available evidence from human [20] and bovine cartilages [15,21] suggests that species with C-terminals other than those generated by ADAMTS cleavages are the major products of this kind in mature tissue. Thus in human articular cartilage the equivalent of peptide 4 (Val^{1}–Glu^{1714}) is present at low abundance throughout life and it represents only a minor proportion of the truncated tissue aggrecan which, from electrophoretic behaviour, appears as two discrete species that appear (results not shown) to be similar in structure to peptides 5 and b (Figure 2). The precise C-termini for these major tissue forms are under investigation.

On the other hand, analysis of human synovial fluids for aggrecan fragments has led to the conclusion [5] that the major species present are represented by peptides 12 and 13 (Figure 1), and recent Western-blot analysis of normal and osteoarthritic human synovial fluids with an anti-ARGSV antiserum and both anti-KEEE and anti-SELE (J. D. Sandy, C. Verscharen, V. Thompson and L. S. Lohmander, unpublished work) fully supports this idea.

Taken together the available data suggest that the MMP and ADAMTS groups of proteinases fulfil very different functions in aggrecan processing. It appears that steady-state proteolysis of

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aggrecan, to form or maintain the mature intercellular aggrecan matrix with a long half-life, is a function of non-ADAMTS proteinases such as MMPs. On the other hand, steady-state turnover of the short-half-life pool in the normal pericellular matrix, or accelerated degradation following cytokine exposure or in joint disease, appear to be primarily a function of the ADAMTS group.

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