Review

Aggrecanases and cartilage matrix degradation

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Abstract

The loss of extracellular matrix macromolecules from the cartilage results in serious impairment of joint function. Metalloproteinases called 'aggrecanases' that cleave the Glu^{373}–Ala^{374} bond of the aggrecan core protein play a key role in the early stages of cartilage destruction in rheumatoid arthritis and in osteoarthritis. Three members of the ADAMTS family of proteinases, ADAMTS-1, ADAMTS-4 and ADAMTS-5, have been identified as aggrecanases. Matrix metalloproteinases, which are also found in arthritic joints, cleave aggrecans, but at a distinct site from the aggrecanases (i.e. Asn^{341}–Phe^{342}). The present review discuss the enzymatic properties of the three known aggrecanases, the regulation of their activities, and their role in cartilage matrix breakdown during the development of arthritis in relation to the action of matrix metalloproteinases.

Keywords: ADAMTS, chondrocytes, matrix metalloproteinases, osteoarthritis

Introduction

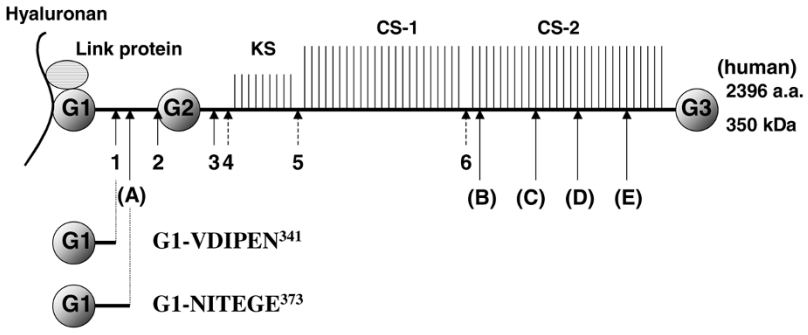
Cartilage consists of a relatively small number of chondrocytes and abundant extracellular matrix (ECM) components. While numerous macromolecules have been identified in cartilage, the major constituents are collagen fibrils and aggrecan, a large aggregating proteoglycan [1]. Collagen fibrils consisting mainly of type II collagen and, to a lesser extent, of collagen type IX and type XI form an oriented meshwork that provides the cartilage with tensile strength. Aggrecans fill the interstices of the collagen meshwork by forming large aggregated complexes interacting with hyaluronan and link proteins. Aggrecan monomers are approximately 2.5 million Da and consist of a 250-kDa core protein to which chondroitin sulfate and keratan sulfate glycosaminoglycan (GAG) chains are covalently attached. Aggrecans are highly hydrated because of their negatively charged long polysaccharide chains, and thus provide the cartilage with its ability to resist compressive loads.

Chondrocytes synthesize and catabolize ECM macromolecules, while the matrix in turn functions to maintain the homeostasis of the cellular environment and the structure of cartilage. In diseases such as osteoarthritis (OA) and rheumatoid arthritis (RA), degradation of the ECM exceeds its synthesis, resulting in a net decrease in the amount of cartilage matrix or even in the complete erosion of the cartilage overlying the bone at the joint surface. Although many possible causes of cartilage destruction have been suggested, such as hypoxic conditions and oxygen-derived free radicals [2,3], the primary cause of this process is thought to be an elevation in the activities of proteolytic enzymes. The loss of aggrecan is considered a critical early event of arthritis, occurring initially at the joint surface and progressing to the deeper zones. This is followed by degradation of collagen fibrils and mechanical failure of the tissue.

The matrix metalloproteinases (MMPs) have been considered the main enzymes responsible for degradation of aggrecan and collagens in cartilage [4]. The expression of several MMPs is elevated in cartilage and synovial tissues of patients with RA and OA [4,5]. Those overexpressed in cartilage (e.g. MMP-3, MMP-13 and MMP-14) are considered to be key enzymes in the development of OA, as

ADAM = protein with a disintegrin and metalloproteinase domain; ADAMTS = a disintegrin and metalloproteinase domain with thrombospondin motif; ECM = extracellular matrix; G1 = N-terminal globular domain; GAG = glycosaminoglycan; IC_{50} = inhibitor concentration that gives 50% enzyme inhibition; IGD = interglobular domain; IL = interleukin; K_i = inhibition constant; MMP = matrix metalloproteinase; OA = osteoarthritis; RA = rheumatoid arthritis; TNF-α = tumour necrosis factor alpha.

Figure 1



Sites cleaved by aggrecanases	
(A)	…RNITEGE^{373} ^{374}ARGSVIL…
(B)	…STASELE^{1545} ^{1546}GRGTIGI...
(C)	...TTFKEEE^{1714} ^{1715}GLGSVEL...
(D)	...QAPTAQE^{1819} ^{1820}AGEGPSG...
(E)	...EPTISQE^{1919} ^{1920}LGQRPPV...

Sites cleaved by MMPs	
1	…FVDIPEN^{341} ^{342}FFGVGGE…
2	…TAFTSED^{441} ^{442}LVVQVTA…
3	…HAFCFRG^{666} ^{667}ISAVPSP…
4	not determined
5	not determined
6	not determined

Aggrecan cleaved by aggrecanases and matrix metalloproteinases (MMPs). Aggrecan core protein has three globular domains (G1, G2 and G3). The N-terminal G1 domain interacts with hyaluronan with the help of a link protein. G1-VDIPEN^{341} and G1-NITEGE^{373} are G1-bearing N-terminal products generated by MMPs and aggrecanases, respectively. Sites cleaved by aggrecanases are shown as (A)–(E), and sites cleaved by MMPs are shown as 1–6. The dotted arrows are sites predicted based on SDS-PAGE analysis of Little *et al.* [90] and of Sandy and Verscharen [96]. KS, keratansulfate rich region; CS, chondroitinsulfate rich region. Residues and numbering in parentheses indicate bovine sequences.

characteristic lesions develop in the centre of the articular cartilage surface, well away from the synovial membrane, with no infiltration of inflammatory cells [6]. A recently discovered group of metalloproteinases called 'aggrecanases', however, are now thought to also play an important role in aggrecan breakdown. This topic has been covered by several recent reviews [7–11]. In the present article, we describe recent progress in the field and discuss the role of aggrecanases in cartilage matrix degradation in relation to the actions of MMPs.

Discovery of aggrecanases

One well-characterized site that MMPs cleave in the aggrecan core protein is the Asn^{341}–Phe^{342} bond in the interglobular domain (IGD) between the N-terminal globular domain (G1) and the second globular domain (G2) [12–14] (see Fig. 1). In 1991, however, Sandy *et al.* [15] reported that when bovine articular cartilage was treated with IL-1, an inflammatory cytokine that evokes cartilage breakdown, aggrecan cleavage occurred at the Glu^{373}–Ala^{374} bond in the IGD, but not at the Asn^{341}–Phe^{342} bond. The enzyme responsible for this new proteolytic activity was referred to as 'aggrecanase'.

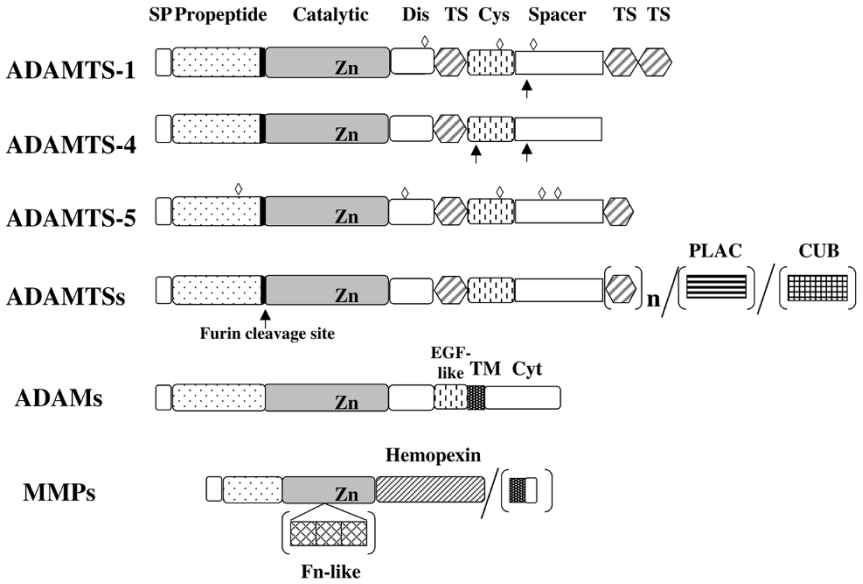
Additional hydrolysis found at TAQE^{1819} ~ AGEG and VSQE^{1919} ~ LGQR (~ denoting the scissile bond) was also thought to be aggrecanase mediated [16,17]. Aggrecan fragments resulting from the cleavage of the Glu^{373}–Ala^{374} bond accumulate in the synovial fluids of patients with OA and inflammatory arthritis [18,19], emphasizing the potential importance of aggrecanases *in vivo*.

The first aggrecanase, called 'aggrecanase 1', was reported by a research group at DuPont in 1999 [20], who subsequently reported a second enzyme, 'aggrecanase 2' [21]. Aggrecanase 1 and aggrecanase 2 are now designated as ADAMTS-4 and ADAMTS-5, respectively. They are zinc metalloproteinases whose structure and domain arrangements are homologous to ADAMTS (a disintegrin and a metalloproteinase domain with thrombospondin motifs) proteins (see [22,23]). More recent studies have shown that ADAMTS-1 also has aggrecanase activity [24,25]. ADAMTS-1 transcripts are found in cartilage [26].

Aggrecanase structure and function

The ADAMTSs and the proteins with a disintegrin and metalloproteinase (ADAMs) belong to the metallopeptidase family M12 [27]. The metalloproteinase domains of ADAMs are related to snake venom metalloproteinases or reprolysins. There are currently 30 ADAM genes [28] and 18 ADAMTS genes known in humans [29].

Figure 2



Domain arrangements of ADAMTS, ADAMs and MMPs. N-linked glycosylation sites (◇) and post-translational processing sites of ADAMTS-1 and ADAMTS-4 (↑) are indicated. Some ADAMTSs have PLAC and CUB domain at the C terminus. ADAMs are type I membrane proteins but ADAMTSs lack a transmembrane domain. MMP-2 and MMP-9 have three repeats of a fibronectin type II-like domain and membrane-type MMPs have a transmembrane domain and a cytoplasmic tail. SP, signal peptide; Dis, disintegrin-like domain; TS, thrombospondin type I motif; Cys, cysteine-rich domain; PLAC, proteinase and lacunin domain; CUB, complement C1r/C1s–urchin epidermal growth factor–bone morphogenetic protein-1 domain; TM, transmembrane domain; Fn, fibronectin.

ADAMs are type I transmembrane proteins with extracellularly located N-termini. Their genes encode an N-terminal signal peptide, a relatively large prodomain (about 170 amino acids), a metalloproteinase domain (about 230 amino acids), a disintegrin domain, a cysteine-rich region usually containing an epidermal growth factor-like domain, and a transmembrane domain followed by a cytoplasmic tail at the C-terminus (Fig. 2). The metalloproteinase domains are well conserved, but only 19 out of 30 have the zinc binding catalytic site consensus sequence HEXXHXXGXXH. Other ADAMs lacking this motif are likely to be proteolytically inactive.

Biological functions of many ADAMs are not clearly understood. Among those whose function is known are: ADAM-1 and ADAM-2 (fertilin α and fertilin β), which play a role in sperm–egg fusion during fertilization [30]; ADAM-12 (metrin α), which participates in myoblast fusion [31] and which releases heparin-binding epidermal growth factor from the plasma membrane [32]; ADAM-10 (Kuzbanian in *Drosophila*), which processes Notch and Notch ligand Delta during neural development [33,34]; and ADAM-17 (tumour necrosis factor alpha [TNF-α] converting enzyme), which releases TNF-α, TNF-α receptors and other cell surface molecules [35,36].

ADAMTS proteins are related to ADAMs, but they are not membrane-anchored proteins as they lack a transmembrane domain (Fig. 2). The common domain modules of ADAMTSs are a signal peptide, a prodomain, a metalloproteinase domain, a disintegrin domain, a thrombospondin type I motif, a spacer domain, and a second thrombospondin module of a variable number of repeats at the C-terminal region. Some ADAMTSs have a PLAC (protease and lacunin) domain [37] and a CUB (complement C1r/C1s–urchin epidermal growth factor–bone morphogenetic protein-1) domain [38] at the C-terminus (see Fig. 2).

ADAMTSs have highly selective proteolytic activities (Table 1). ADAMTS-2, ADAMTS-3 and ADAMTS-14 have N-procollagen processing activity [39–41]. ADAMTS-13 cleaves von Willebrand factor, and a decrease in ADAMTS-13 activity results in congenital and acquired thrombotic thrombocytopaenic purpura [42–44]. ADAMTS-1 (METH-1) has been identified as an IL-1-inducible gene in mice, and ADAMTS-1 and ADAMTS-8 (METH-2) have anti-angiogenic activity [45]. The proteolytic activity of ADAMTS-8 has not been investigated. The functions of other ADAMTSs remain unknown.

Catalytic activity of aggrecanases

Besides the Glu^{373}–Ala^{374} bond in the IGD, ADAMTS-4 and ADAMTS-5 cleave at least four other sites in the chondroitin sulfate-rich CS-2 region of bovine aggrecan: $GELE^{1480}$ ~ GRGD, $KEEE^{1667}$ ~ GLGS, $TAQE^{1771}$ ~ AGEG, and $VSQE^{1871}$ ~ LGQR [46–48]. These sites are

Table 1

Biological activities of ADAMTSs

Enzyme	Substrate	Activity	Reference
ADAMTS-1	Aggrecan, versican, α_2-macroglobulin	Cleavage of proteoglycan core proteins, anti-angiogenic	[25,45,50,51]
ADAMTS-2	Procollagen I, procollagen II	Processing of N-propeptide of procollagen	[39]
ADAMTS-3	Procollagen II	Processing of N-propeptide of procollagen	[40]
ADAMTS-4	Aggrecan, versican, brevican	Cleavage of proteoglycan core proteins	[20,52,50]
ADAMTS-5	Aggrecan	Cleavage of aggrecan core protein	[21]
ADAMTS-8		Anti-angiogenic	[45]
ADAMTS-13	von Willebrand factor	Reduced activity results in thrombotic Thrombocytopaenic purpura	[43]
ADAMTS-14	Procollagen I	Procollagen N-proteinase	[41]

ADAMTS, a disintegrin and metalloproteinase domain with thrombospondin motif.

much more readily cleaved than the Glu^{373}–Ala^{374} bond [46,47] (Fig. 1). The structural requirements for different rates of hydrolysis are not known, but they may be influenced by the location of polysaccharide chains as well as of amino acid sequences around the cleavage site in the core protein. ADAMTS-1 cleaves the Glu^{1480}–Gly^{1481} and Glu^{1871}–Leu^{1872} bonds of bovine aggrecan [25]. In addition, ADAMTS-1 [25] and ADAMTS-4 [49] hydrolyse the Asn^{341}–Phe^{342} bond at a high enzyme to substrate ratio, suggesting that these two ADAMTSs may also cleave at the so-called 'MMP-cleavage site'. Other substrates include versican and α_2-macroglobulin for ADAMTS-1 [50,51], and brevican and versican for ADAMTS-4 [50,52]. When the 'bait region' of α_2-macroglobulin is hydrolyzed by a proteinase, α_2-macroglobulin entraps the enzyme and sterically hinders it from accessing large protein substrates [53]. It is therefore likely that α_2-macroglobulin is an endogenous inhibitor of ADAMTS-1.

ECM components such as collagens, fibronectin and thrombospondin, and general proteinase substrates such as casein and gelatin are not cleaved by ADAMTS-1, ADAMTS-4 or ADAMTS-5 [47]. The highly selective specificity of these enzymes can be attributed to the non-catalytic domains. Tortorella *et al.* [54], who reported that the ADAMTS-4 proteinase domain alone does not cleave aggrecan core protein, suggest that the thrombospondin type I domain is critical for aggrecan recognition and cleavage. The cleavage of the Glu^{373}–Ala^{374} bond in the IGD by aggrecanases is enhanced by the presence of keratan sulfate chains in this domain [55]. Little activity is detected when the full-length ADAMTS-4 is incubated with the deglycosylated aggrecan [54], indicating that interaction of polysaccharide chains and the enzyme is important for the aggrecanase activity. A study using a recombinant IGD and its deletion mutants has indicated that at least 32 residues at the N-terminal side of the cleavage site (P residues of substrate) and 13 residues at the C-terminal side (P′ residues) are required for aggrecanases to cleave the Glu^{373}–Ala^{374} bond [56]. MMP-cleaved IGD is no longer susceptible to aggrecanase, whereas aggrecanase-cleaved IGD is hydrolyzed by MMPs at the Asn^{341}–Phe^{342} bond [57]. Not only the primary sequence, but also the secondary structure of the IGD thus appears to be critical for substrate recognition by aggrecanases.

Post-translational processing of ADAMTSs

ADAMTSs are synthesized as pre-proproteins and are targeted to the secretory pathway. All members possess a furin cleavage site just before the proteinase domain, and therefore they are most probably activated intracellularly by a proprotein convertase and secreted as active enzymes. ADAMTS-1 may undergo further processing extracellularly, with a C-terminal part of the spacer domain and the two thrombospondin type I domains being removed [58] (see Fig. 2). This processing reduces both the affinity of the enzyme for heparin and the ability of the enzyme to suppress endothelial cell proliferation [58]. The mature full-length ADAMTS-4 (75 kDa) is also further processed extracellularly to 60-kDa and 50-kDa forms by MMPs [59]. These additional processing events greatly increase the aggrecanase activity of the enzyme [59], indicating that post-translational processing may be an important regulatory mechanism for this enzyme *in vivo*.

TIMP-3 as an endogenous inhibitor of aggrecanases

The aggrecanase activity from bovine cartilage is inhibited by TIMP-1 with an IC_{50} (inhibitor concentration that gives 50% enzyme inhibition) of 210 nM [60]. ADAMTS-1 is inhibited by TIMP-2 and TIMP-3, but only a very high concentration (500 nM) was tested [25]. TIMP-3 is a potent inhibitor of ADAMTS-4 and ADAMTS-5 [61,62]. The recombinant N-terminal inhibitory domain of human

TIMP-3 inhibits ADAMTS-4 and ADAMTS-5 with K_i values in the subnanomolar range, considerably lower than those for MMPs [61], indicating that TIMP-3 is a potent endogenous inhibitor of aggrecanase 1 and aggrecanase 2.

TIMPs are generally considered specific inhibitors of MMPs, but TIMP-3 is exceptional in that it also inhibits some other metalloproteinases, such as TNF-α converting enzyme (ADAM-17) [63], ADAM-10 [64] and ADAM-12 [65]. Another unique feature of TIMP-3 is its ability to tightly bind to negatively charged polysaccharides [66]. TIMP-3 is expressed in skeletal tissues during development of mouse embryos [67] and in normal bovine and human chondrocytes and synoviocytes, and the levels of expression are elevated in human OA synovium [68]. TIMP-3 expression in cultured chondrocytes, and synovial fibroblasts is upregulated by transforming growth factor beta [69] or oncostatin M [70]. Treatment of human rheumatoid synovial fibroblasts with the anti-arthritic agent calcium pentosan polysulfate increases TIMP-3 protein levels, without altering its mRNA levels [71]. This increase of TIMP-3 is due to an enhanced transition of the mRNA without affecting the stability and secretion of newly synthesized TIMP-3 [71]. The increase of TIMP-3 production is further augmented by cotreatment of the cells with IL-1 [71]. Calcium pentosan polysulfate inhibits the IL-1-stimulated and retinoic acid-stimulated aggrecan breakdown in bovine articular cartilage [72]. This effect is probably due to an elevated production of TIMP-3 in the cartilage [71] and to direct inhibition of aggrecanase activity [73]. Increased levels of TIMP-3 may therefore be beneficial for protecting cartilage from degradation.

Synthetic aggrecanase inhibitors

Synthetic inhibitors designed for MMPs often inhibit aggrecanase activity [74], but some selective inhibitors for aggrecanase have been reported recently. Succinate-based hydroxamic acid compounds containing 3-hydroxyphenyl and *cis*-(*1S*)-(*2R*)-amino-2-indanol moieties have good selectivity for aggrecanases [75]. The best compound has an IC_{50} value of 12 nM against aggrecanase, with the K_i values for MMP-1, MMP-2 and MMP-9 in a micromolar range (4–33 μM), and it is orally available [75].

Compounds with a biphenylmethyl group in the P_1' position show improved potency for aggrecanase with IC_{50} values in the low nanomolar range [76]. These compounds have excellent selectivity over MMP-1 and MMP-9, but only moderate selectivity over MMP-2. Information about other MMPs and specific ADAMTSs is not available as the aggrecanase enzyme used was not defined in these studies, but once the inhibitory activities of these compounds against each aggrecanase (ADAMTS-1, ADAMTS-4 and ADAMTS-5) and other MMPs are known, they may be useful agents to test the role of aggrecanases and MMPs in various models of cartilage degradation.

Regulation of aggrecanase activity and the expression of ADAMTS-1, ADAMTS-4 and ADAMTS-5

Aggrecanase activity was first described in bovine articular cartilage treated with IL-1 [15], but it is also enhanced in cartilage treated with TNF-α, retinoic acid [7], IL-17 [77], ceramide [78] or the 45 kDa fibronectin fragment containing collagen/gelatin binding motifs [79]. It is therefore reasonable to consider that some ADAMTS genes are transcriptionally regulated. However, reports describing mRNA levels of aggrecanases in response to inductive stimuli are not consistent at present.

For example, the treatment of normal human cartilage in culture with IL-1, TNF-α or retinoic acid increases aggrecanase activity, but it has no effect on mRNA levels for ADAMTS-1, ADAMTS-4 and ADAMTS-5 [26]. This suggests that enhanced aggrecanase activity may be regulated post-transcriptionally or that the increased activity is due to unidentified aggrecanases. On the other hand, human chondrocytes [80], bovine chondrocytes [81], bovine articular cartilage [81,82] and porcine articular cartilage [83] treated with IL-1 increase ADAMTS-4 mRNA levels. In the case of immortalized human chondrocytes, however, the levels of ADAMTS-4 mRNA increase only if treated with IL-1 and oncostatin M, but not with either cytokine alone [84]. Several studies indicate that IL-1 has little or no effect on ADAMTS-5 mRNA levels [80–82]. Two studies report that IL-1 treatment increases ADAMTS-5 mRNA levels in porcine articular cartilage [83] and in immortalized human chondrocytes [84]. The variability and inconsistency among these reports may indicate that the regulatory mechanisms of ADAMTS-4 and ADAMTS-5 transcription and translation depend on the species and age of the tissue and culture conditions of isolated cells. The stability and half-life of the mRNA may also affect results.

Synovial tissues in culture also produce and release soluble aggrecanase activity [48]. However, the treatment of bovine synovium with IL-1 or retinoic acid does not alter mRNA levels of ADAMTS-4 and ADAMTS-5 [48]. Similar results have been obtained for human synoviocytes treated with IL-1 or TNF-α [85], even though these cytokines are potent inducers of MMP production in synoviocytes. Nevertheless, Yamanishi *et al*. [85] found that transforming growth factor beta significantly increases ADAMTS-4 mRNA in human synoviocytes along with increasing the production of a 90-kDa protein thought to be the precursor form of the enzyme. ADAMTS-5 mRNA is constitutively produced in both RA and OA synoviocytes, and the 70-kDa protein is detected in cell lysates, but neither mRNA nor protein levels are regulated by transforming growth factor beta [85]. These observations again emphasize that the regulation of ADAMTS-4 and ADAMTS-5 genes in response to cytokines and growth factors depends on the cell type.

Other important findings regarding the regulation of aggrecanase activity have been made by Caterson and colleagues, who reported that cyclosporin A and n-3 fatty acids downregulate ADAMTS-4 and/or ADAMTS-5 mRNAs [81,83,86]. Treatment of porcine articlar cartilage with cyclosporin A abrogates the IL-1-enhanced ADAMTS-4 and ADAMTS-5 mRNAs [83]. Supplementation of bovine chondrocytes with n-3 fatty acid reduces the IL-1-inducible mRNAs for ADAMTS-4 and cyclooxygenase 2, but not those for ADAMTS-5 [81]. A similar suppressive effect on ADAMTS-4 mRNA is seen in human OA cartilage treated with n-3 fatty acid along with reduction of aggrecanase activity in the cartilage [86]. Supplementation with n-3 fatty acid also reduced mRNA levels of MMP-3, MMP-13, cyclooxygenase 2, 5-lipoxygenase, 5-lipoxygenase activating protein, TNF-α, IL-1α and IL-1β [86]. The mechanisms by which these genes are regulated by n-3 fatty acid and cyclosporin A are not known, but elucidation of such mechanisms could suggest useful ways to manipulate expression of the genes associated with inflammation and joint destruction.

Aggrecanases versus MMPs in cartilage degradation

Because several MMPs are elevated in arthritic joints [4,5], and because the MMP-generated G1-VDIPEN^{341} fragment and the aggrecanase-generated G1-NITEGE^{373} fragment are found in cartilage [87] and synovial fluids [18,19,88] from patients with RA and OA, there is a debate regarding which group of enzymes plays the major role in aggrecan degradation under biological and pathological conditions. In short-term *in vitro* models of cartilage explants stimulated with IL-1, TNF-α or retinoic acid, aggrecanases appear to be the primary enzymes that degrade aggrecan, at least in the first week [89,90]. Little contribution is made by MMPs although the mRNA levels of MMP-3 and MMP-13 are elevated [89]. After about 3 weeks of incubation, however, MMP-dependent cleavage of aggrecan core protein can be detected, at which time collagen breakdown also starts to occur [90].

Fosang *et al*. [91] reported that porcine cartilage treated with IL-1 or retinoic acid for 5 days increased the MMP-generated aggrecan fragments in cartilage, but a later report indicated that this is an experimental artefact [92]. Thus, in the *in vitro* cartilage explant systems, the initial enzymes responsible for degrading aggrecan are aggrecanases, followed by MMPs at a later stage [90]. It is notable, however, that the responses to catabolic stimuli differ in various tissues [93]. Bovine nasal cartilage stimulated with IL-1 or retinoic acid releases GAG primarily due to aggrecanase. In human cartilage, little GAG release is seen with IL-1, but aggrecanase-dependent GAG release is seen with retinoic acid [93]. By contrast, treatment of foetal bovine epiphyseal cartilage with retinoic acid, but not with IL-1, releases GAG without degrading the core protein [93]. This novel mechanism of GAG release is yet to be investigated.

Both G1-VDIPEN^{341} and G1-NITEGE^{373} fragments remain in the cartilage by interacting with hyaluronan, and they can be detected by antibodies detecting the C-terminal neoepitope of each fragment (Fig. 1). Using this approach, both MMPs and aggrecanases are shown to contribute to the lysis of aggrecan at distinct sites during the development of the secondary ossification centre in the cartilaginous epiphysis of rat long bone [94]. In normal human cartilage, both neoepitopes are also found and increase with age, but they remain at a steady state after the age of 20–30 years [87]. This probably reflects the much slower turnover rate of the G1 domain (0.027/year with a half-life of 25 years) compared with that of the large aggrecan monomer (0.206/year with a half-life of 3.4 years) [95]. The concentration of the MMP-generated VDIPEN neoepitope in adult joint cartilage represents 15–20% of the resident aggrecan molecules within the matrix, and the proportion of G1-VDIPEN in OA and RA cartilage is about the same as in adult joint cartilage, although high levels of staining are seen in areas of cartilage damage [87]. The distribution of the aggrecanase-generated NITEGE neoepitope is similar to the VDIPEN neoepitope in most cases, but in some cases the NITEGE neoepitope is detected in regions where the VDIPEN neoepitope is not found [87], indicating that two groups of enzymes may function at different sites in cartilage.

More recent studies by Sandy and Verscharen [96] have indicated that normal human adult cartilage contains at least seven main G1-bearing species, which include the full-length, G1-NITEGE^{373} and G1-VDIPEN^{341} fragments, and four other fragments (90 kDa, 110 kDa, 160 kDa and 250 kDa after deglycosylation). The latter four fragments (see Fig. 2 for the potential cleavage sites) represent at least 50% of the total core protein, and they are most probably generated by MMPs *in vivo*. Interestingly, the core protein composition in the cartilage does not change in OA cartilage. Synovial fluids, on the other hand, contain primarily the fragments generated by aggrecanases, and fluids from patients with late-stage OA contain more excessively cleaved fragments. In acutely injured joints there is a marked increase in the ratio of G1-NITEGE to G1-VDIPEN both in the cartilage and synovial fluids. Based on these observations, these investigators propose that excessive aggrecanase activity is destructive to cartilage matrix, whereas MMP activity is nondestructive since it trims mostly the C-terminal region of the aggrecan molecule and much of the GAG-bearing product is retained in the tissue [96] (see Fig. 1).

Some *in vivo* models of arthritis indicate that MMPs may participate in cartilage destruction. In antigen-induced arthritis and collagen-induced arthritis mouse models,

NITEGE neoepitopes are present, but VDIPEN neoepitopes are not, during the early phase of aggrecan depletion [97]. VDIPEN neoepitopes are detected in the antigen-induced arthritis model when aggrecan degradation has progressed, and this coincides with collagenase cleavage of type II collagen [98]. However, cartilage from MMP-3$^{(-/-)}$ mice exhibits neither VDIPEN neoepitopes nor collagenase-cleaved neoepitopes during antigen-induced arthritis, but proteoglycan depletion occurs to a similar extent in MMP-3$^{(-/-)}$ and wild-type mice [98]. The probable mediators of aggrecan degradation are aggrecanases. Nevertheless, cartilage destruction was not observed in MMP-3$^{(-/-)}$ mice even 2 weeks after arthritis induction, suggesting that MMP-3 may play a key role in later progression of cartilage erosion in the antigen-induced arthritis model. By contrast, in the more severe collagen-induced arthritis model, MMP-3$^{(-/-)}$ mice develop arthritis to a similar extent as the wild-type mice, and there is no obvious decrease of VDIPEN epitope [99]. This activity is most probably due to the induction of other MMPs. It is also possible that ADAMTS-1 and ADAMTS-4 [25,49] or cathepsin B [100] released from chondrocytes, in part, participate in this process.

STR/ort mice spontaneously develop OA in the medial tibial cartilage of the knee joint. The lesions are not accompanied by inflammation and they closely resemble those in the knee of human OA [101]. In nonarthritic joints, MMP and aggrecanase neoepitopes map to different locations in cartilage, suggesting that two groups of enzymes function at different sites in normal turnover of aggrecan [102]. When the disease progresses, distributions of VDIPEN and NITEGE neoepitopes become similar, suggesting that both MMPs and aggrecanases play a role in cartilage destruction in STR/ort mice [102].

Concluding remarks

Three ADAMTSs have been identified as aggrecanases. Aggrecan products generated by these metalloproteinases are found in normal, OA and RA cartilage, and in synovial fluids, supporting the notion that these enzymes participate in aggrecan catabolism in the tissue. Since the ADAMTSs have been only recently discovered, however, limited information is available regarding the biological and pathological significance of these enzymes. It is yet to be investigated which and to what extent these ADAMTSs are responsible for cartilage degradation *in vivo*. It is also not known whether other ADAMTSs can degrade the aggrecan core protein. The ADAMTSs have highly selective substrate specificities, seemingly associated with the noncatalytic domains of these enzymes, as exemplified by ADAMTS-4. An understanding of the molecular interactions mediating such specificities will shed light on the mechanism of action of ADAMTSs on aggrecan and may suggest novel ways of inhibiting aggrecan breakdown.

The regulation of various ADAMTS genes in articular cartilage needs further investigation since data on the expression patterns of these enzymes in response to stimulatory factors are variable. Aggrecanases are also expressed in other tissues [21]. The expression of ADAMTS-1 mRNA increases in the injured motor neurons [103], and aggrecanase-mediated degradation of nerve tissue proteoglycans is seen in mouse brain and peripheral nerves [104], in developing and adult rat spinal cord, and after injury [105]. Levels of ADAMTS-4 mRNA increase in astrocytes treated with β-amyloid [106]. These observations indicate that aggrecanases also play an important role in the catabolism of aggrecan and other aggrecan-like molecules in normal nerves and in neuronal tissue remodelling. Little is known about the promoter regions of ADAMTSs or about the enhancer elements that increase expression. Further studies on this topic may help explain tissue- and age-dependent aggrecanase expression.

Several lines of evidence have been provided that MMPs also function as aggrecan-degrading enzymes *in vivo*. However, it is yet to be investigated whether MMPs function primarily in the normal turnover of aggrecan or whether they are actively involved in cartilage degradation during disease progression. Elevated levels of MMPs including MMP-3 and MMP-13 are found in OA cartilage, and levels of a number of other MMPs are increased in the rheumatoid synovium, but they are produced as inactive zymogens. Once activated, they may also participate in aggressive aggrecan degradation. As the disease progresses, the local pH of the cartilage may fall [107], and cathepsin B, cathepsin L [108] and cathepsin K [107] from chondrocytes may participate in further cartilage destruction. Several proteinases are therefore likely to be involved in cartilage destruction in the advanced stages of arthritis. To further advance our understanding of the precise *in vivo* functions of these proteinases in cartilage degradation during the progression of OA and RA, selective inhibitors of each enzyme and the deletion of specific proteinase genes may be necessary. The information obtained by such experiments may also provide useful insights for developing therapeutic agents to prevent progressive destruction of the cartilage matrix.

Competing interests

None declared.

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