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Review

Structure, function, aging and turnover of aggrecan in the intervertebral disc

Sarit Sara Sivan [a,*,1], Ellen Wachtel [b,1], Peter Roughley [c]

[a] Department of Biotechnology Engineering, ORT Braude College, Karmiel 21982 Israel
[b] Faculty of Chemistry, Weizmann Institute of Science, Rehovot 76100, Israel
[c] Shriners Hospital for Children, Genetics Unit, 1529 Cedar Avenue, Montreal, Quebec H3G 1A6, Canada

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ABSTRACT

Background: Aggrecan is the major non-collagenous component of the intervertebral disc. It is a large proteoglycan possessing numerous glycosaminoglycan chains and the ability to form aggregates in association with hyaluronan. Its abundance and unique molecular features provide the disc with its osmotic properties and ability to withstand compressive loads. Degradation and loss of aggrecan result in impairment of disc function and the onset of degeneration.

Scope of review: This review summarizes current knowledge concerning the structure and function of aggrecan in the normal intervertebral disc and how and why these change in aging and degenerative disc disease. It also outlines how supplementation with aggrecan or a biomimetic may be of therapeutic value in treating the degenerate disc.

Major conclusions: Aggrecan abundance reaches a plateau in the early twenties, declining thereafter due to proteolysis, mainly by matrix metalloproteinases and aggrecanases, though degradation of hyaluronan and non-enzymic glycation may also participate. Aggrecan loss is an early event in disc degeneration, although it is a lengthy process as degradation products may accumulate in the disc for decades. The low turnover rate of the remaining aggrecan is an additional contributing factor, preventing protein renewal. It may be possible to retard the degenerative process by restoring the aggrecan content of the disc, or by supplementing with a bioimimetic possessing similar osmotic properties.

General significance: This review provides a basis for scientists and clinicians to understand and appreciate the central role of aggrecan in the function, degeneration and repair of the intervertebral disc.

1. Structure of aggrecan

Aggrecan belongs to the family of proteoglycans that are characterized by the presence of glycosaminoglycan (GAG) chains attached covalently to a core protein. In the case of aggrecan there may be over 100 GAG chains bound to its large core protein, making it the most glycosylated of the proteoglycans. The proteoglycans can be subdivided according to the type of GAG chain present and the function of the core protein. In this respect aggrecan can be described as a large chondroitin sulfate (CS)/keratan sulfate (KS) aggregating proteoglycan; the term aggregating refers to the ability to interact with hyaluronan (hyaluronic acid, HA). The structure of aggrecan has now been elucidated in many species, and while all show common features there are species-specific variations. All aggrecan molecules are composed of three globular regions termed G1, G2 and G3, with a short interglobular domain (IGD) separating G1 from G2, and a long GAG-attachment region separating G2 from G3 (Fig. 1) [1]. Most species variations are present in the GAG attachment region and influence the number of attachment sites on the core protein for KS and CS [2–6]. In addition, as there is no template for GAG synthesis, KS and CS chain length and sulfation pattern may also vary between species [7,8]. At present it is unclear whether such structural variations in aggrecan affect disc function and whether they contribute to the differences in the susceptibility to disc degeneration between species.

1.1. The G1 region

The G1 region is formed from the amino terminal portion of the aggrecan core protein, and is responsible for the interaction with HA.

Abbreviations: GAG, glycosaminoglycan; KS, keratan sulfate; HA, hyaluronic acid; IGD, interglobular domain; MMPs, metalloproteinases; CS, chondroitin sulfate; IVD, intervertebral disc; EGF, epidermal growth factor; CRP, complement regulatory protein; ECM, extracellular matrix; NP, nucleus pulposus; AF, annulus fibrosus; IL1, interleukin 1; TNFα, tumor necrosis factor α; HYALs, hyaluronidases; OSM, oncostatin M; BMP7, bone morphogenetic protein 7; TGFβ, transforming growth factor β; GDF5, growth and differentiation factor 5; AGEs, glycation end products; ADAMTS, A disintegrin and metalloproteinase with thrombospondin motifs; CML, carboxymethyllysine; CEL, carboxyethyllysine; Asp, aspartic acid

* Corresponding author. Tel.: +972 4 9901924; fax: +972 4 9901970.
E-mail address: ssivan@braude.ac.il (S.S. Sivan).

[1] Equal contribution.

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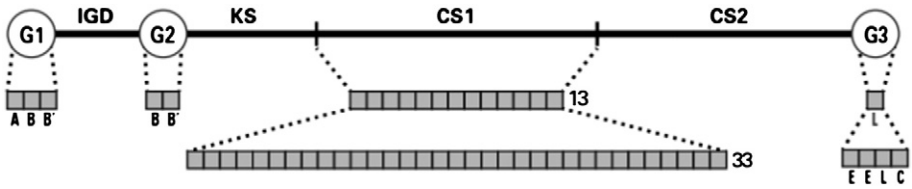

Fig. 1. The domain structure of aggrecan. The figure depicts the aggrecan core protein with 3 globular regions (G1, G2 and G3) and 4 extended domains (IGD, KS, CS1 and CS2). The G1 region is divided into three domains (A, B and B′) and the G2 region is divided into 2 domains (B and B′). The G3 region can possess 4 domains (E, E, L and C; though the 2 E domains and the C domain may be absent due to alternative splicing). The CS1 domain is composed of between 13 and 33 repeat sequences. IGD, interglobular domain; KS, keratan sulfate-rich domain; CS1, chondroitin sulfate-rich domain 1; CS2, chondroitin sulfate-rich domain 2; E, epidermal growth factor-like domain; L, lectin-like domain; C, complement regulatory protein-like domain.

It is composed of three disulfide-bonded domains termed A, B and B' [9]. The B and B' domains are responsible for the interaction with HA, but while both domains have the potential to interact, it is not clear if they are both functional in the same G1 region. The A domain is responsible for stabilizing the interaction with HA via its interaction with a link protein, which is structurally similar to the G1 region [10]. The G1 region can be glycosylated by N-linked oligosaccharides or KS [11], though this may vary with species, site and age. It is not clear whether glycosylation is of functional consequence.

1.2. The IGD

The IGD domain acts as a spacer separating the G1 and G2 regions. Unlike the G1 region which is relatively resistant to proteolysis, it is susceptible to cleavage by most proteinases [12], including the matrix metalloproteinases (MMPs) and aggrecanases commonly associated with aggrecan degradation in vivo. It may also be glycosylated and substituted with both N-linked and O-linked oligosaccharides or KS [11], which again may vary with species, site and age. While the functional role of glycosylation is unclear, it could influence the rate of proteolysis if it occurs adjacent to a cleavage site, as occurs for the aggrecanases.

1.3. The G2 region

The G2 region is composed of disulfide-bonded B and B' domains that are structurally similar to those of the G1 region. Surprisingly however, the G2 region does not have the ability to interact with HA [13]. As with the G1 region it may also be glycosylated. At present it is not clear if the G2 region serves any specific functional role.

1.4. The KS-rich domain

The KS-rich domain follows the G2 region and forms the first part of the GAG-attachment region. It is composed of multiple 6-residue amino acid repeats containing proline–serine [3]. The serine residues may be substituted with O-linked oligosaccharides or KS chains. The type of substitution varies with age, as the oligosaccharides are progressively replaced by KS [14]. The structure of the KS chains also varies with development and aging, but it is not clear if these changes have any functional significance. The number of repeats in the KS-rich region varies between species, being lowest in rats which are reported to be devoid of KS substitution in this region [15]. There is no evidence for CS substitution in this region [16].

1.5. The CS-rich domains

Aggrecan possesses two adjacent CS-rich domains, termed CS1 and CS2, which differ in their amino acid composition. The CS1 domain follows the KS-rich domain and is composed of repeats of 19 amino acids, with each repeat containing two glycine–serine sequences [3]. These serine residues are potential sites for CS attachment. The number of repeats varies between species, and it is unclear whether all potential attachment sites are always occupied or whether heterogeneous CS substitution occurs. The human CS1 domain is so far unique in showing a variation in repeat number between individuals; the number of repeats ranging from 13 to 33 [17], but with most individuals possessing 26–28 repeats. There is an evidence that a low number of repeats can adversely affect aggrecan function and be a predisposing factor in early disc degeneration [18]. The adjacent CS2 domain has a more variable amino acid structure and therefore a more random distribution of CS chains, which are again attached to serine residues. This variation in amino acid sequence gives the two domains a different susceptibility to proteolysis, with the CS2 domain being cleaved by aggrecanases but not the CS1 domain. The structure of the CS chains can vary between the two domains and also varies with age [19]. There is no evidence for KS substitution in either the CS1 or CS2 domains in the human [16].

1.6. The G3 region

The G3 region is formed from the carboxy terminal portion of the aggrecan core protein, and appears to be essential for normal trafficking of the molecule through the cell and subsequent secretion [20]. It is composed of several disulfide-bonded domains with homology to epidermal growth factor (EGF), C-type lectin and complement regulatory protein (CRP). The human G3 region is so far unique in showing splicing variation of the exons that encode its two EGF domains and one CRP domain [21]. As a result, aggrecan for all individuals possesses a G3 region containing the lectin domain, but may or may not contain the other domains. It is not clear if these splicing variations are of any functional consequence. The G3 region, via its lectin domain, is able to interact with a variety of extracellular matrix proteins in vitro [22], but it is not clear whether such interactions persist in vivo. Due to proteolysis, the G3 region is often absent from the mature aggrecan molecules present in the extracellular matrix (ECM) [23].

2. Aggrecan function in the disc

Aggrecan function depends on both its core protein and GAG constituents. The core protein endows aggrecan with the ability to aggregate, giving the molecules a vast size, limiting their diffusion and maintaining their location within the tissue. It is interesting to note that upon secretion by the cells, individual aggrecan molecules do not interact well with HA, and only attain this ability within the ECM [24]. This may allow the molecule time to diffuse away from the HA-rich coat present at the cell surface into the more remote matrix. The GAG chains provide the aggrecan with its osmotic properties, including the ability to swell and resist compressive loads [25]. These osmotic properties are driven by the highly sulfated nature of the CS and KS chains. The ability to resist compression is a hallmark of tissues that are rich in aggrecan, such as the disc and articular cartilage.

When aggrecan finds itself in an aqueous environment, it swells as the sulfated GAG chains become hydrated. Within a tissue, swelling is limited by the collagenous framework and, if sufficient aggrecan is present, a positive swelling potential is maintained, which is balanced by the tensile forces imposed on the collagen fibrils. The higher the aggrecan content of the tissue, the higher this equilibrium swelling potential

will be. In the healthy disc, swelling is mainly associated with the nucleus pulposus (NP) where aggrecan content is highest, and is mainly resisted by the collagen fibrils of the surrounding annulus fibrosus (AF). Upon applying compression to the tissue, water is displaced and the concentration of aggrecan at the site of compression is increased, thereby increasing its swelling potential (Fig. 2). This increased swelling potential is dissipated upon the removal of the compressive load as the aggrecan swells, drawing water back until the initial equilibrium is restored. This process explains the diurnal variation in disc height experienced in humans, as loading of the discs by gravity during the day is removed when lying flat in bed. It is thought that this process may also help maintain adequate nutrition of the disc, as it facilitates the removal of waste with the displacement of water and the inflow of nutrients upon its return. The swelling properties of aggrecan also contribute to the pathology associated with disc herniation, where a tear in the AF occurs. This results in a weakness in the AF and a focal decrease in the ability to resist the NP swelling, which may result in protrusion of the disc at the site of the tear or even extrusion of disc material if the tear is severe.

For ideal tissue function, it is essential that the aggrecan concentration, charge and size remain as large as possible. Thus, the optimal aggrecan molecules would have maximal substitution by GAG chains, the longest and most sulfated GAG chains, and form the largest aggregates in association with HA. Unfortunately, such molecules do not persist throughout life and can be deficient in some skeletal dysplasias, due to either truncation or impaired interaction of the aggrecan core protein [26–29] or undersulfation of the GAG chains [30]. Loss of these properties is an initiating factor in disc degeneration [31]. The link between disc degeneration and CS1 polymorphism may relate to the lower number of CS chains that are present on aggrecan molecules with the lowest number of repeats within the CS1 domain [18].

In addition to providing a resistance to compression, the aggrecan content of the disc is also a contributor to its avascular and aneural nature in all but the peripheral regions of the AF. High concentrations of aggrecan are an impediment to blood vessel [32] and nerve [33] ingrowth into the tissue, and hence they cannot penetrate into the more central regions of the disc where aggrecan content is highest. In disc degeneration, which is associated with aggrecan loss, such ingrowth can occur. The high aggrecan content may also help prevent calcification of the disc by using calcium ions as counterions for the sulfate, thereby limiting the amount of free calcium ions available to promote mineral deposition. However, aggrecan loss associated with disc degeneration may result in more free calcium ions being available and thereby contribute to concomitant calcification [34].

3. Age-related changes in aggrecan structure

The aggrecan molecules present in the disc do not maintain a constant structure or abundance throughout life. These age-related changes may be due to intracellular events affecting GAG synthesis, as well as extracellular events affecting core protein and HA degradation or modification (Fig. 3).

3.1. GAG synthesis

During development and growth, the structure of the GAG chains on aggrecan changes. The major changes are a decrease in the length of CS and a change in its sulfation from the 4-position of N-acetyl galactosamine to the 6-position, and an increase in the length of KS. It is not clear whether the variation in sulfation position on CS has any functional significance, but the decrease in CS chain length can be viewed as detrimental. However, in terms of maintaining charge, this could be at least partially compensated by the increase in KS length. It has been suggested that the greater abundance of KS in the adult disc is related to its increased avascular nature and resulting deficiency in vascular-derived oxygen. Unlike CS, the synthesis of KS does not require an oxidation step to convert hexose to uronic acid. It is not clear whether the changes in GAG chain length are accompanied by changes in the degree of GAG substitution along the core protein, though this could occur if the expression of the glycosyl transferases involved in the initiation of GAG synthesis vary with age.

3.2. Core protein degradation

Aggrecan has little inherent resistance to proteolytic degradation, and most proteinases having access to aggrecan will cleave it in one or more of its domains, particularly the IGD and the domains of the GAG attachment region. Within the GAG-attachment region, the CS1 domain is least sensitive to proteolysis as its highly repetitive amino acid sequence limits the number of proteinases having the required substrate specificity. While most proteinases are capable of degrading aggrecan in vitro,

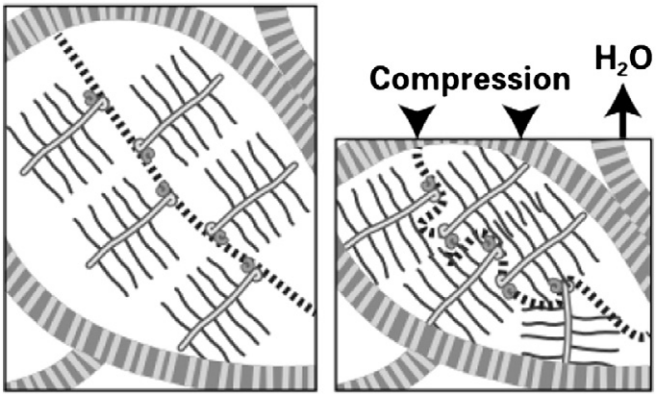

Fig. 2. The function of aggrecan. The figure depicts a proteoglycan aggregate, possessing a central filament of hyaluronan with five attached aggrecan molecules, entrapped by collagen fibrils within the nucleus pulposus of the disc. The left hand panel and the right hand panel show the tissue under relaxed and compressed conditions, respectively. Under compression, water is displaced and the aggrecan molecules are brought into closer proximity. On removing the compression, the aggrecan expands, drawing water back into the compressed tissue to restore the relaxed state.

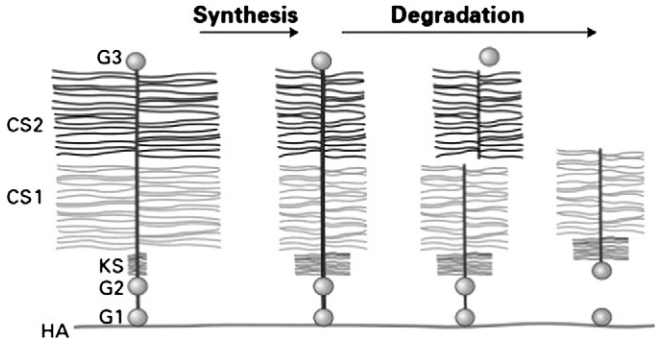

Fig. 3. Age-related changes in aggrecan structure. The figure depicts structural variations associated with synthesis or degradation processes occurring within the disc. Changes due to synthesis occur mainly during growth and result in a decrease in CS chain length and an increase in KS chain length. Throughout life, changes due to proteolytic degradation result in loss of the G3 region and truncation of the aggrecan core protein, ultimately leaving only the G1 region bound to HA. The fragments of aggrecan that are unable to interact with HA remain in the disc for many years until slowly being lost from the tissue by diffusion. G1, G2 and G3, globular regions of the aggrecan core protein; KS, keratan sulfate-rich domain; CS1, chondroitin sulfate-rich domain 1; CS2, chondroitin sulfate-rich domain 2; HA, hyaluronan.

only a limited number play a major role in vivo. Foremost among these are the MMPs and aggrecanases [35], which can be produced by the disc cells themselves in response to adverse mechanical loading [36] or the presence of inflammatory cytokines such as interleukin 1(IL1) and tumor necrosis factor α (TNFα) [37]. All MMPs appear to cleave aggrecan, but do so at vastly different rates in vitro [38]. The collagenases (MMP1, 8 and 13) and gelatinases (MMP2 and 9) are least active, while MMP3, 7 and 12 are most active. In the case of aggrecanases, cleavage is exclusively within the IGD and CS2 domain [39]. The aggrecanases belong to the ADAMTS (A Disintegrin And Metalloproteinase with Thrombospondin Motifs) family, with the most active being ADAMTS4 and 5 (aggrecanase 1 and 2, respectively). In vitro, ADAMTS5 is more active than ADAMTS4 [40].

Irrespective of which proteinase cleaves aggrecan, the consequence is similar as all cleavages generate a C-terminal fragment that is no longer bound to HA as part of the aggregate. This fragment is able to diffuse more freely within the tissue. In the case of articular cartilage such fragments are rapidly lost from the tissue into the synovial fluid. However in the case of the disc, loss from the NP is restricted by the surrounding AF and adjacent vertebra, and accumulation of these non-aggregating fragments occurs. Proteolysis results in loss of the G3 domain, truncation of the core protein, and ultimately only the G1 domain remains bound to HA. All of these events can be viewed as being detrimental to disc function and predisposing to disc degeneration.

3.3. HA degradation

While there is little evidence for extracellular degradation of CS or KS, extracellular degradation of HA does occur. Such degradation of HA is not a benign event, as it reduces the size of the aggrecan aggregates and so can promote their diffusion. HA degradation may proceed by enzymatic or non-enzymatic mechanisms. Enzymatic degradation can occur via the action of hyaluronidases (HYALs), with HYAL2 being the most likely candidate responsible for HA depolymerization within the ECM [41]. While such degradation can be viewed as a means of mobilizing the aggregate to allow normal turnover, excessive degradation would be detrimental. Increased hyaluronidase action can be induced by cytokines such as oncostatin M (OSM) [38]. HA is also susceptible to depolymerization by free radicals [42], particularly the hydroxyl radical that may be generated from hydrogen peroxide and superoxide. However, it is unclear to what extent this contributes to HA degradation in vivo. Within the disc, the HA within the aggregates is protected to a certain extent from depolymerization due to either hyaluronidases or free radicals by the incorporation of link proteins [43]. The link protein not only stabilizes the interaction of each aggrecan molecule with HA, but can also block access by the hyaluronidases and can act as a scavenger for the free radicals.

3.4. Core protein modification

It has been known for some time that non-enzymatic glycation of aggrecan mediated by reducing sugars such as ribose or glucose can occur, resulting in initial modification of lysine residues and ultimately advanced glycation end-products (AGEs) such as pentosidine [44]. It is also known that lysine residues within the aggrecan G1 region play a major role in the ability to interact with HA. At least in vitro, ribose is able to modify aggrecan such that it no longer is able to interact with HA, and more importantly, can result in the disassembly of link protein-stabilized aggregates. If such a scenario also occurs in vivo, it would help explain why many of the non-aggregating fragments of aggrecan that accumulate in the disc possess a G1 domain. Such a process would be expected to be detrimental to disc function and may at least in part explain why diabetes is associated with disc degeneration.

4. Turnover of aggrecan in IVD

4.1. Racemization and glycation

A reliable method for assessing protein age and turnover is the measurement of the accumulation of the D-isomer of amino acids. Due to stereochemical constraints, amino acids are synthesized in nature as the L-isomers. However, spontaneous, non-enzymatic racemization slowly converts L-form amino acids into a racemic mixture of L- and D-forms. The characteristic racemization rate for each amino acid depends on protein conformation as well as on the temperature, pH and ionic strength of the environment [45]. Since aspartic acid (Asp) has one of the highest racemization rates, it is possible to measure its D-isomer accumulation in human subjects (an average increase of ~0.1% per year) in proteins that are not renewed or that turnover slowly [45,46]. Published measurements of age-dependent racemization in human and animal tissues containing metabolically-stable, long-lived proteins include studies of enamel and dentin [46,47], white matter of the brain [48], eye lens [49], aorta [50], cartilage, [51,52], skin [53], bone [54,55], tendon [56], arteries [57] and IVD [58–61]. Since the relationship between the age of a protein and its D-Asp content depends on both racemization and turnover rates, and since racemization correlates with the age of long-lived proteins, it can be used as a molecular clock of protein aging and turnover.

Under normal physiological conditions, protein longevity is observed to vary widely. Three distinct patterns of D-Asp accumulation have been observed: (i) proteins with high turnover, such as hemoglobin [62], with in vivo lifetime of 120 days, are exchanged before a measurable accumulation of D-Asp with age can occur. (ii) Proteins with longer half-life can exhibit measurable accumulation of D-Asp. However, due to protein turnover, the lifetime relationship between D-Asp accumulation and age may not be linear; age-related equilibrium between accumulated D-Asp residues and L-Asp of newly synthesized proteins can result in zero net accumulation of the D-isomer. (iii) Proteins with very slow (if any) turnover exhibit an approximately linear relationship between accumulation of D-Asp and age. This is the basis for the identification of long-lived proteins that age in parallel with the human organism.

There are other post-translational modifications associated with aging and degeneration which correlate with racemization of aspartyl residues [44,63–69]. As noted above, the spontaneous reaction between sugars and lysine residues in proteins results in the formation of AGEs; pentosidine is a crosslink formed between lysine and arginine, whereas carboxymethyllysine (CML), and carboxyethyllysine (CEL) are lysine adducts. AGEs have also been cited as relevant factors in a number of diseases [64,67,68]. Pentosidine, one of the most extensively characterized of the AGEs, accumulates with age in tissues containing proteins which turnover very slowly, such as articular cartilage [69,70]. Previous studies showed that accumulation of AGEs in cartilage collagen [52] resulted in increased stiffness and brittleness of the tissue, making it more prone to mechanical damage [65,66,69]. Such damage may eventually lead to the development of osteoarthritis.

4.2. Aspartic acid racemization in human IVD aggrecan

In human IVD aggrecan, the racemization pattern of L- to D-Asp is similar to that of the type ii proteins described above. The amount of D-Asp increases with donor age until ~60 years (Fig. 4). The average accumulation rates during this time, defined as the rate of change of the ratio of the D-isomer to total Asp content are $4.74 \pm 0.44 \times 10^{-4}$ and $2.78 \pm 0.43 \times 10^{-4}$ year^{-1} for aggrecan from normal and degenerate IVD, respectively [59]. Beyond 60 years, accumulation appears to level off. From this, we infer that in aggrecan, equilibrium between accumulated D-Asp and newly synthesized L-Asp and/or preferential loss of D-Asp is established. By contrast, for longer-lived proteins in IVD, such as collagen and elastin, a steady accumulation of D-Asp with age

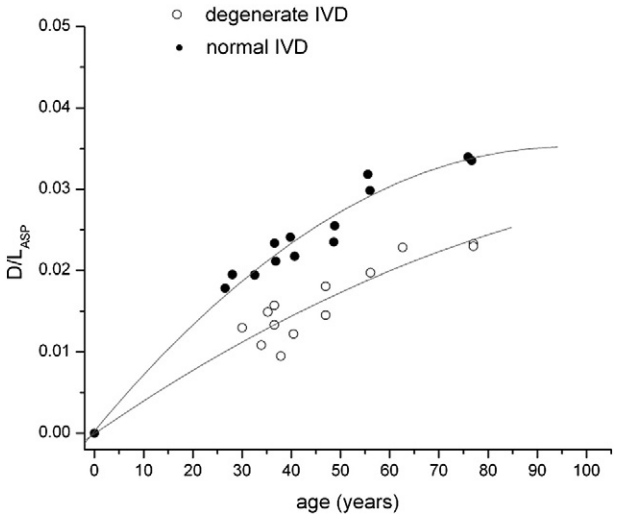

Fig. 4. Accumulation of the D-isomer of aspartic acid in aggrecan obtained from normal (•) and degenerate (o) human IVD as a function of donor age. A quadratic curve was found to give the best fit ($n = 8$, $r = 0.96$ and $n = 8$, $r = 0.97$ for normal NP and AF, respectively; and $n = 9$, $r = 0.87$ and $n = 6$, $r = 0.73$ for degenerate NP and AF, respectively) [59]. No significant difference in the rate of accumulation of the D-isomer with age in NP as compared to AF in normal or degenerate discs ($p > 0.05$, t-test) was found.

(similar to type iii proteins described above) throughout the human life span is observed. The accumulation rates for collagen from normal and degenerate IVD are 6.74 ± 0.44 and $5.18 \pm 0.44 \times 10^{-4}$ year^{-1}, respectively [61] and for elastin from normal and degenerate IVD (until the mid 50s) are 16.2 ± 3.1 and $11.7 \pm 3.1 \times 10^{-4}$ year^{-1}, respectively [60]. It should be noted that in none of the human IVD samples studied [59,61] did the accumulation of D-Asp in the nucleus and the annulus differ significantly. However, in the case of whole, healthy human IVD tissue, Ritz [58] did find a higher D-Asp accumulation rate in the aggrecan-rich nucleus (23×10^{-4} year^{-1}) that decreased towards the peripheral collagen-rich annulus (10.8×10^{-4} year^{-1}).

From D-Asp accumulation rates and an independent evaluation of the characteristic rate of Asp racemization, the rate at which human IVD aggrecan turns over can be determined. A rapid turnover implies a short residence time and frequent population renewal, whereas a slow turnover implies a long residence time, suggesting that it is more difficult for cells to repair defects, which therefore may accumulate over time during the human life span [47,71]. Aggrecan turnover is not constant during a lifetime. The calculated change in turnover rate with age is shown for aggrecan from both normal and degenerate IVD in Fig. 5A. The variation of aggrecan half-life (defined as ln(2)/turnover rate) for different age groups is shown in Fig. 5B. Turnover is a decreasing function of age and is consistently higher for degenerate IVD as compared with normal. The mean turnover rate for normal aggrecan is 0.126 per year and the mean half-life, 5.7 years.

IVD aggrecan can be separated into fractions A1D1–A1D6. These fractions display progressively decreasing mass due to extracellular proteolysis of the core protein [72,73] and to differences in the extent to which the core protein is substituted with CS and KS chains [72,74]. The carbohydrate content decreases towards A1D6, which is primarily composed of free G1 domain and link protein. An inverse relationship between mean turnover rate and carbohydrate content was found [59]. D-Asp content is approximately stable with age in A1D1, while the maximum accumulation is observed for A1D6. The measured mean turnover rate for A1D6 is 0.097 year^{-1}, with corresponding mean half-life of 7.4 years. The much shorter mean half-life of normal aggrecan as compared to that of collagen (~150 years) [61], is due in part to the fact that aggrecan is more susceptible to proteolysis. As described above, small degraded fragments are produced that are able to slowly diffuse out of the disc and are subsequently replaced by newly-

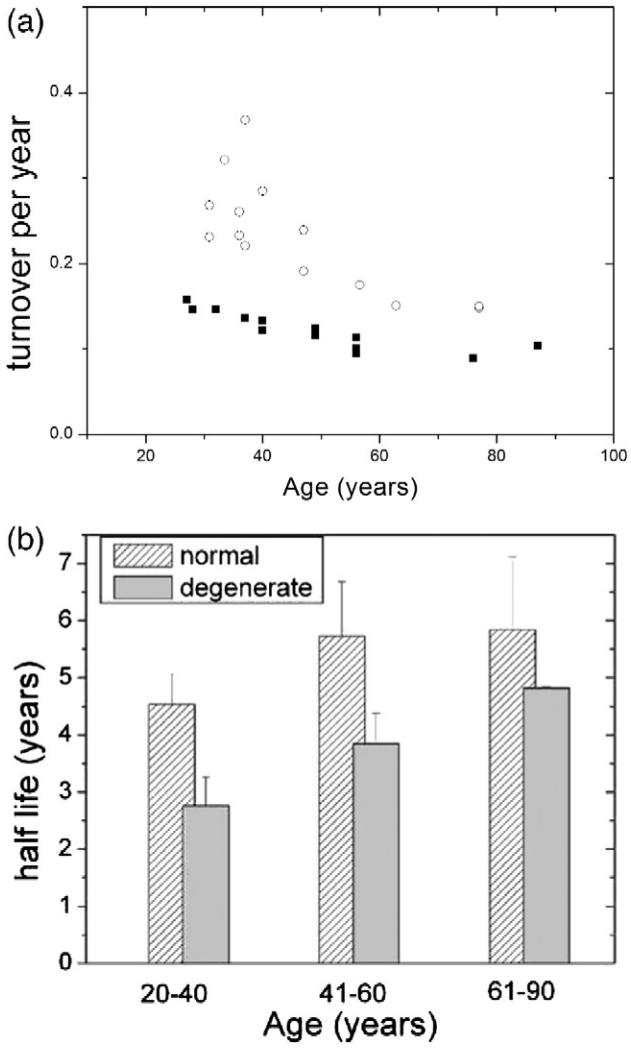

Fig. 5. (A) Age dependent change in the rate of turnover of human IVD aggrecan as determined by the accumulation of the D-isomer of aspartic acid. (o) Degenerate; (■) normal. (B) Half-life of the A1 preparation of aggrecan as a function of age.

synthesized, intact molecules. The fact that half-life is an increasing function of age reflects changes in the rate of protein synthesis. That half-life is consistently lower for degenerate IVD tissue when compared with normal tissue, suggests that there is more rapid protein synthesis in degenerate tissue.

4.3. Pentosidine formation on IVD aggrecan-correlation with aspartic acid racemization

In addition to racemization, the most frequently investigated post-translational modifications of proteins are oxidation, glycation and deamidation [67,75–78]. These processes may occur in parallel. It was shown that both aspartyl residue racemization and pentosidine formation in IVD proteins correlate well with disc age; the amount of pentosidine increases with age in both normal and degenerate tissue [44,63,79]. The linear correlation obtained between the levels of D-Asp and pentosidine in IVD proteins supports the previously noted relevance of both these markers to the biology of aging [79]. The correlation of turnover rate (obtained from D-Asp racemization) and pentosidine content is shown for aggrecan from both normal and degenerate IVD in Fig. 6. The fact that there is no statistical significance ($p < 0.05$) to the difference in slopes between normal and degenerate tissue, suggests that these two markers change at a relatively similar pace during aging and/or degeneration. This correlation may exclude the possibility of

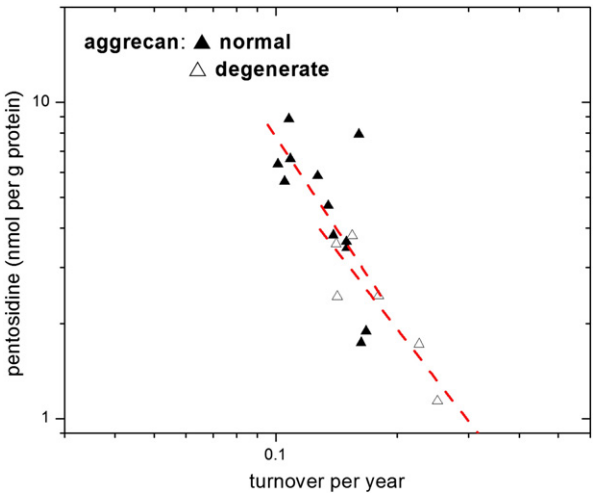

Fig. 6. Correlation between the accumulation of pentosidine and the rate of turnover of aggrecan obtained from human IVD. The R-factor for data obtained from aggrecan in degenerate IVD is 0.89, with P = 0.017. For aggrecan obtained from normal IVD, the corresponding numbers are 0.69 and 0.013.

considering Asp racemization as a simple molecular event independent of other macromolecular processes such as glycation. The origin of the correlation is not clear; however, it has been suggested that racemization of aspartyl residues may induce posttranslational stereochemical modifications, altering structure–function relationship of proteins [71,80], which in turn could result in reactions such as crosslinking [81], higher susceptibility to degradation [66,82,83], as well as to conformational instability [84]. In the case of structural proteins undergoing slow turnover, protein aggregation could also occur [84–87].

5. Consequence of age-related changes in aggrecan

Age-related changes in aggrecan include altered tissue content, impaired ability to interact with HA and fragmentation, all of which can affect tissue function. A decrease in aggrecan content and interaction and an increase in fragmentation can all be viewed as being detrimental. In contrast, it is not clear whether the change in GAG structure that accompanies growth has detrimental or beneficial consequences.

In the fetal and neonatal human disc, the majority of aggrecan resides in the gelatinous NP, and its abundance at this site increases throughout juvenile life to reach a maximum in the young adult [31]. However, even at this age much of the aggrecan exists in a fragmented form and is unable to interact with HA. With further increase in age the concentration of aggrecan in the NP decreases, though this is somewhat compensated by an increase in the aggrecan content of the inner AF [31]. In the mature adult the aggrecan content of the AF may exceed that of the NP, and the inner AF may represent the major compression resisting tissue. The aggrecan content of the AF also shows increasing levels of fragmentation with age. The decrease in aggrecan abundance may in part be due to a diminished capacity for synthesis, but is mainly associated with degradation in the ECM. However, loss of aggrecan degradation products from the center of a large human disc is a slow process, as it is impeded axially by the endplates of adjacent vertebrae and laterally by the dense collagenous lamellae of the AF. It is likely that as long as the degraded fragments remain in the disc, they may serve a functional role in resisting compression, as they maintain the fixed charge density of the tissue. It appears that extensive decrease in size due to proteolysis is needed for loss to occur. As proteolysis in the healthy disc originates from the disc cells themselves and these are present in low abundance, fragmentation is a slow process and it may take decades for functionally significant loss to occur.

It is easy to envisage that a decrease in the aggrecan content of the disc, with its concomitant decrease in charge density, will result in impaired ability of the disc to resist compression, and such a scenario has been linked to the early stages of disc degeneration. It is also apparent that those individuals in whom this loss occurs most rapidly will experience the greatest functional deficit and an earlier onset of disc degeneration. The factors driving premature disc degeneration may be genetic, mechanical or environmental in nature [88–90], and in the latter cases may be able to be minimized by lifestyle changes. Aggrecan depletion may also result in increased innervation of the disc, resulting in the discogenic back pain that is commonly associated with disc degeneration [91]. The degenerate disc may also result in back pain by other mechanisms, as it can exhibit lateral protrusion causing nerve root impingement and a decrease in disc height causing increased loading of the facet joints leading to their degeneration [92]. It is however still unclear why not all degenerate discs become symptomatic.

One intriguing question is whether aggrecan fragmentation itself is detrimental to disc function even in the absence of fragment loss. If the fragments can be retained there would be no decrease in charge density, but it is likely that the fragments would be more motile within the disc, and this can in itself be viewed as detrimental particularly under the asymmetric compression associated with bending. In such a scenario it is ideal if the aggrecan can be maintained in its original site so as to best counteract compressive forces.

6. Implications for the repair of disc degeneration

At present there is no medical treatment for the repair of the degenerate disc, and ultimately surgical intervention is required for symptomatic relief. This can involve excision of a disc protrusion or, if degeneration is more extensive, removal of the whole disc followed by spinal fusion or insertion of a disc prosthesis. These procedures are not benign and do not restore normal disc function. In the case of fusion, the mechanics of the spine are altered and degeneration of the discs at levels adjacent to the fusion may result. Thus, there is a need for a therapy that can retard or even halt the early degenerative process. As aggrecan loss is a major driving force of early disc degeneration, it seems reasonable that such a therapy should ideally promote aggrecan synthesis and at the same time prevent its degradation.

Growth factors such as bone morphogenetic protein 7 (BMP7), transforming growth factor β (TGFβ) and growth and differentiation factor 5 (GDF5) can fulfill these functions, and have shown therapeutic potential in animal models of disc degeneration [93]. However, they are costly to use and risk adverse side effects should they escape from the environs of the disc. These deficits can be overcome by the use of a synthetic peptide (LinkN) derived from the N-terminus of the link protein that stabilizes the interaction of aggrecan with HA. This 16 amino acid residue peptide (DHLSDNYTLDHDRAIH) was initially recognized as being able to stimulate proteoglycan synthesis by chondrocytes, and this property was later shown to be exhibited by disc cells [94]. LinkN not only stimulates both NP and AF cells to produce aggrecan, but also down-regulates the transcription of many of the metalloproteinases associated with disc degeneration and can function even if inflammatory cytokines are present [95]. It appears to function by binding to the BMP receptor and signaling via Smad 1 and 5 [96]. However, unlike the BMPs it does not stimulate osteogenesis, so avoiding one undesirable side effect [97].

LinkN has been shown to partially restore disc height in a rabbit in vivo model of disc degeneration [98], to replenish the disc aggrecan content in a bovine organ culture model of early disc degeneration, and to stimulate aggrecan production in intact human discs in organ culture. Hence, its interest as a potential therapeutic agent. However, at present its effect on the whole disc has only been shown following intradiscal injection, and this would probably not be a favorable option in the human if repeated injections were necessary to maintain its functional benefit [99]. It would be more desirable to have a systemic means

of delivery, but a means of targeting LinkN to the disc awaits development. There is also the question of when to administer LinkN. It is unlikely that LinkN will prove to be effective in the later stages of disc degeneration once extensive disruption of the collagen network of the AF has occurred, and its value may be as a prophylactic agent in the early stages of disc degeneration. Here it may retard degeneration, inhibit painful nerve ingrowth, and delay the need for surgery. Even in patients with advanced degeneration, it may be useful as an adjunct following surgery to prevent subsequent degeneration in adjacent spinal segments.

Thus while the properties of aggrecan can be viewed as the cause of disc degeneration, in the future they may also be its savior.

7. Aggrecan biomimetics and disc repair

While restoration of the aggrecan content of the disc may be the ideal means of restoring disc function in early stage degeneration, it may not be effective unless ongoing proteolysis can be prevented. Otherwise the newly synthesized aggrecan will suffer the same fate as its predecessors. To overcome this problem, recent research has focused on the development of biomimetics that replicate the functional properties of aggrecan but are not susceptible to proteolytic degradation. One such biomimetic is a GAG analog formed by polymerizing sulfonated monomers of acrylamido 2-methylpropane sulfonic acid (AMPS) and sulfopropyl acrylate (SPA) in the presence of a crosslinking agent (Fig. 7) [100]. The sulfonate groups on the GAG analog mimic the sulfate groups on the CS chains of aggrecan, and provide the molecule with osmotic properties similar to those of aggrecan. The GAG analog can be formed in situ following the injection of the monomers into the disc, and the polymerization process does not appear to be detrimental to cell viability.

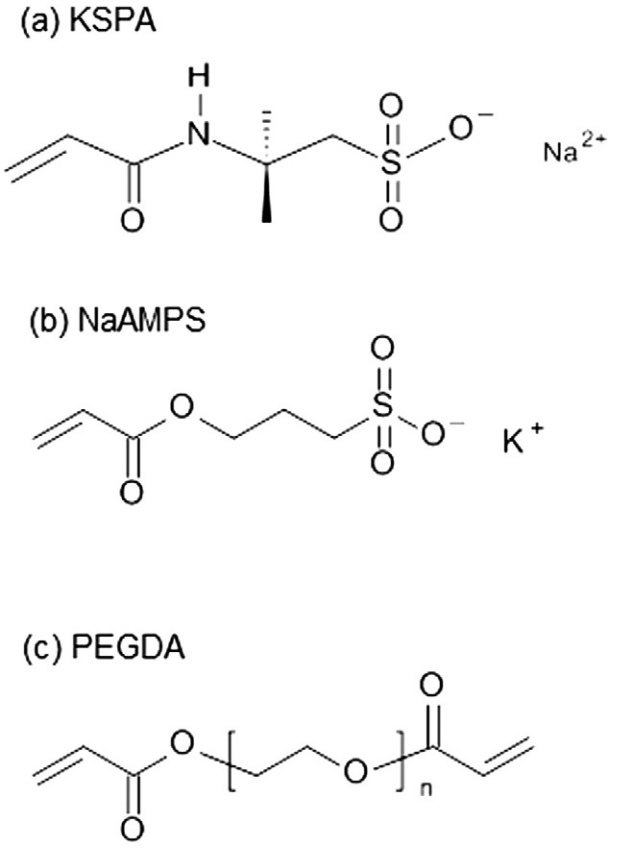

Fig. 7. The chemical structure of the monomers used for the preparation of the GAG analogs: (a) sulfopropyl acrylate (SPA), (b) acrylamido 2-methylpropane sulfonic acid (AMPS) and (c) poly(ethyleneglycol) diacrylate (PEGDA), used as the crosslinker.

An alternative aggrecan biomimetic has been developed for use in articular cartilage repair. In this case the mimetic consists of CS chains functionalized by periodate oxidation, to which specific HA-binding peptides have been added [101]. This enables the mimetic to possess similar osmotic properties to the CS chains of aggrecan and the ability to be localized within the ECM of a tissue by interaction with HA. When used in model systems, the mimetic has been shown to be resistant to proteolysis and to both up-regulate aggrecan production and down-regulate metalloproteinase production [102,103]. These latter features add to its value as a repair agent, as it may not only provide functional restoration but also help eliminate an ongoing degenerative process. However, while this biomimetic molecule has considerable potential, it remains to be established if it would be of value in disc repair.

One problem with using aggrecan biomimetics in vivo for disc repair, is how to administer them without causing damage to the disc, as it is likely that direct intradiscal injection is not a benign procedure [99]. If this problem can be solved, then the application of biomimetics may be the future of disc repair.

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