

## The Structure, Degradation and Lifespan of Aggrecan in the Human Intervertebral Disc

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**INTRODUCTION:** Intervertebral discs consist of an outer annulus fibrosus (AF) and a central nucleus pulposus (NP). The ability of the discs to resist compression is aided by their high proteoglycan content, particularly in the NP. Intervertebral disc degeneration involves loss of proteoglycan in the NP, which to some extent is compensated by an increase in the inner AF. Disc proteoglycans exist in two populations – those that are aggregated with hyaluronan and those that are non-aggregated. The aggregated proteoglycans are derived from aggrecan, as in cartilage, though their structure has not been well characterized. At present it is not clear if the non-aggregated proteoglycans are also derived by proteolysis of aggrecan or whether they are distinct.

**METHODS:** AF and NP from lumbar discs was extracted with 4 M guanidinium chloride containing proteinase inhibitors. Proteoglycans were recovered by associative CsCl density gradient centrifugation, and fractionation through Sepharose CL-2B separated aggregated from non-aggregated proteoglycans. The aggregated proteoglycans were further subdivided by dissociative CsCl density gradient centrifugation. Proteoglycan structure was analyzed by gel electrophoresis in 1.2% agarose, following trypsin, chondroitinase ABC or keratanase II digestion. Products were identified directly by toluidine blue staining or immunologically following transfer to CPC-coated nitrocellulose. Antibodies to the CS1 core protein or to KS chains were used for immune detection. Proteoglycans were also analyzed for aspartic acid racemization to assess their residence time within the extracellular matrix.

**RESULTS:** Trypsin digestion cleaves the human aggrecan core protein in all structural regions, with the exception of the CS1 region, which remains intact and can be separated from other fragments by virtue of its larger size and slower electrophoretic mobility. Anti-KS analysis showed that CS1 fragment was devoid of KS, and treatment with keratanase prior to

analysis did not alter its mobility. Treatment with chondroitinase did alter the mobility of the CS1 region, as expected, but did not alter that of the KS fragments, indicating that the majority of the CS2-derived fragments are devoid of KS chains. Analysis of the non-aggregated proteoglycans after trypsin treatment revealed that the CS1 region was present in the molecules of larger size but not in those of smaller size. KS-containing fragments of identical size to those present in the aggregated proteoglycans were present in both large and small non-aggregated proteoglycans. The proportion of D-aspartic acid in the proteoglycan increased with age, indicating long residence times within the extracellular matrix, with the proteoglycans having a mean half-life of 10-15 years.

**DISCUSSION & CONCLUSIONS:** The aggrecan core protein possesses three adjacent regions to which glycosaminoglycan (KS or CS) chains are attached - the KS-rich region, the CS1 region, and the CS2 region. Current models of aggrecan structure suggest that KS may be present in all regions. However, the present work indicates that the CS1 region is devoid of KS, and that if KS occurs in the CS2 region then it is only in areas devoid of CS. The present work also indicates that the majority of disc non-aggregated proteoglycans are derived from aggrecan by proteolysis, as their trypsin-generated fragments were identical to those derived from the CS1 region and the KS-rich region of aggrecan. The relatively long half-life of the disc proteoglycans accounts for the high proportion of non-aggregated molecules present in the disc matrix, as degradation products can accumulate. The reason for the retention of the non-aggregated proteoglycans in the disc is unclear, but it is probably related to the large size and avascular nature of the tissue. Finally, it was apparent that proteoglycan structure and turnover in the AF is analogous to that in the NP.

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