02 *ABSTRACT*

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The Role of Keratan Sulphate in the Modulation of Aggrecan Catabolism

profile

Amanda J. Fosang

Amanda Fosang is a Senior Research Fellow of the National Health and Medical Research Council (NHMRC) of Australia. After completing her postgraduate training in proteoglycan biochemistry at Monash University, Melbourne, Australia, she undertook four years post-doctoral studies at the Kennedy Institute of Rheumatology, London, UK, investigating aggrecan structure and function. She returned to the University of Melbourne Department of Medicine, Australia in 1990 and commenced her independent research career focussing principally on aggrecan catabolism. In 1994 Dr. Fosang was awarded an NHMCR RD Wright Fellowship and moved to the University of Melbourne Department of Paediatrics at the Royal Children's Hospital. In 1997 she was awarded an NHMRC Research Fellowship and in 2001, Senior Research Fellowship. Her present appointment is in the University of Melbourne Department of Paediatrics and she is also a research affiliate of the Murdoch Childrens Research Institute. She participates regularly at Matrix Metalloproteinase and Proteoglycan Gordon Research Conferences and sits on committees for several National Funding bodies. Dr. Fosang has published extensively on the role of matrix metalloproteinases in aggrecanolysis and more recently on the involvement of the ADAMTS family of proteinases in aggrecan breakdown. She holds a patent for an aggrecan monoclonal neoepitope antibody that has potential as a clinical diagnostic reagent. Her recent interest in keratan sulphate biochemistry, and its potential role in regulating aggrecan turnover, has been generously supported by the Mizutani Foundation for Glycoscience.

Key words Aggrecan, keratan sulphate, matrix metalloproteinase, aggrecanase, vaccinia virus

The destruction of articular cartilage in arthritis is accompanied by proteolysis and loss of the large proteoglycan, aggrecan. We have investigated the enzymes involved in aggrecan degradation, and more recently, focussed our attention on the possibility that keratan sulphate (KS) glycosaminoglycans substituted on the aggrecan core protein may have a role in regulating aggrecan catabolism.

The matrix metalloproteinase (MMP) family and the aggrecanase family (comprising ADAMTS-4, ADAMTS-5 and ADAMTS-1) are the principal families involved in aggrecan catabolism *in vivo*. Our studies have focussed on MMP and aggrecanase cleavage in the aggrecan interglobular domain (IGD). The IGD is located between the G1 globular domain (which binds to hyaluronan to immobilise aggrecan in the tissue) and the G2 domain (Figure. a). The IGD is substituted with KS chains but not CS (chondroitin sulphate) and is particularly sensitive to proteinases. *We are interested in the possibility that the structure of aggrecan KS may not be uniform, and that KS chains in the IGD may have a microstructure that is unique and of biological significance.* Although amino acid sequence primarily determines sites of proteolytic cleavage, there is evidence to suggest that enzyme specificity may also be influenced by glycosaminoglycans [1-3], either directly, by interactions between glycosaminoglycans and enzyme, or indirectly.

KS is distributed along the aggrecan core protein in the CS-rich region, the IGD, and a short KS-rich region, located C-terminal to the G2 domain. KS in the IGD is variably substituted on amino acids Thr_{352}, Thr_{357}, Asn_{368} and Thr_{370} [4], and we have confirmed that the 32-amino acid fragment Phe_{342}-Glu_{373} released from aggrecan following dual cleavage at both the MMP and aggrecanase sites, contains KS chains [5](Figure. b). We are using Fluorophore-Assisted-Carbohydrate-Electrophoresis (FACE) analysis to assess whether KS on the IGD 32mer fragment is distinct from total KS and KS in the KS-rich region; these analyses are being done with aggrecan fragments purified from pig laryngeal and pig articular cartilage, for comparison. The KS-rich region is purified by published methods and we are developing methods for purifying the 32mer fragment that contains the majority of the KS in the IGD.

The generation of KS disaccharides for FACE analysis relies on the specificity of the hydrolases used to digest the KS chains. Plaas et al [6] have reported that keratanase II (*Bacillus sp*) contains a contaminating exo-glycosidase activity that is not inhibited by β-galactosidase inhibitors. Thus the quantitation of keratanase II-derived monosulphated disaccharides is often complicated by their conversion to monosaccharides. During our analyses of gel-purified 32mer fragment we observed that low concentrations of SDS could selectively inhibit contaminating keratanase II exo-glycosidase activity, without diminishing the yield of the expected mono- and disulphated disaccharides. We are characterising this potentially useful feature of SDS and its applicability for improving the quantitative yield of monosulphated disaccharides from keratanase II digests for FACE.

To further investigate the influence of KS on aggrecanolysis in the IGD we are expressing a human recombinant G1-G2 fragment, using a vaccinia virus expression system, with the aim of generating a range of rG1-G2 glycoforms that vary in the type and degree of KS substitution. The vaccinia expression system was chosen because the virus can infect almost any mammalian or avian cell. In addition, our collaborators have used vaccinia virus to express decorin and biglycan in cells capable of CS biosynthesis [7,8] and found that CS substitution was heterogenous, yielding a spectrum of recombinant proteins ranging from unsubstituted to fully substituted. At present, there are no reports in the literature of KS substitution on recombinant proteins and indeed KS production is markedly reduced when cells are grown in culture. We have now successfully produced rG1-G2 containing KS in primary bovine and chicken keratocytes. rG1-G2 containing approximately 5kDa KS was purified by hyaluronan-affinity chromatography and the authentic monosulphated KS disaccharides, Galβ1,4GlcNAc6S and GlcNAc6Sβ1,3Gal, were identified by FACE following keratanase II and endo-β-galactosidase digestion, respectively. The KS-containing rG1-G2 fragment was susceptible to cleavage by MMPs and aggrecanases. We are continuing to investigate KS expression on rG1-G2 using agents that may enhance the level of KS substitution [9]. The recombinant KS-containing G1-G2 glycoforms will be useful substrates in our studies to elucidate the involvement of KS in the modulation of aggrecanolysis.

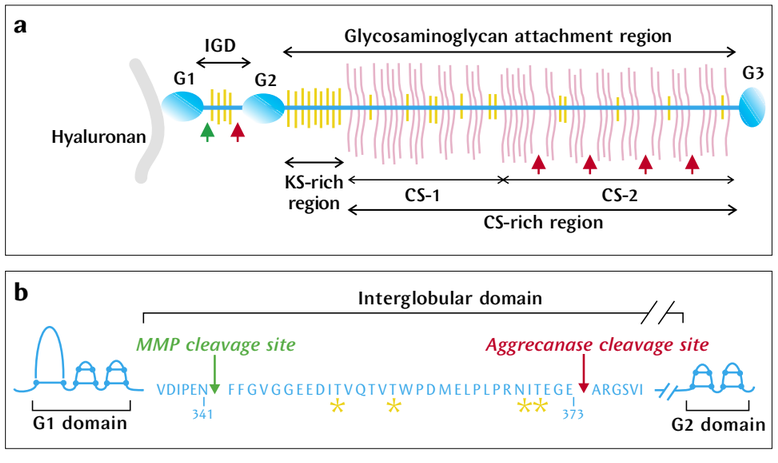

Figure.a) Schematic of aggrecan showing the core protein (blue) with G1, G2 and G3 globular domains, CS chains (pink, wavy) substituted in the CS-rich region and KS chains (yellow, straight) substituted along the length of the protein core but mostly in the KS-rich region. The major MMP cleavage site in the IGD is marked with a green arrow. The known aggrecanase cleavage sites in the IGD and CS-2 region are marked with red arrows. The interglobular domain (IGD) between G1 and G2 is expanded in **b)** to show the amino acid sequence in the proteinase sensitive region and the MMP cleavage site at N_{341}-F and the aggrecanase cleavage at E_{373}-A. Cleavage at both these sites releases a KS-containing 32 amino acid fragment, F_{342}-E_{373}.

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