

GRAPHITISED CARBON LC-MS ANALYSES OF OLIGOSACCHARIDES FROM PROTEOGLYCANS

Ruby P. Estrella¹, John M. Whitelock¹, Nicolle H. Packer² and Niclas G. Karlsson^{2,3}

¹*Graduate School of Biomedical Engineering, University of New South Wales, Sydney Australia*

²*Proteome Systems Ltd, Sydney, Australia*

³*Centre for BioAnalytical Sciences, Chemistry Department, NUI Galway Ireland*

Proteoglycans are distinguished by their possession of long unbranched saccharide polymers called glycosaminoglycans (GAGs) and they are essential to maintaining joint cartilage tissue integrity and function. In disease states such as osteoarthritis, damage to joint cartilage leads to modifications in GAG composition. Therefore, it is imperative to elucidate GAG structural differences from biological samples in order to detect disease initiation and progression. To confront this challenge, we have developed a novel approach using standard glycoproteomic separation techniques and enzymatic digests of proteoglycans prior to analysing their GAG structures with mass spectrometry.

Aggrecan from bovine articular cartilage which was electrophoresed in 1D AgPAGE gel, electroblotted on to PVDF membrane or straight in solution was digested with Chondroitinase ABC to produce Chondroitin Sulfate (CS) repeat region disaccharides, Δ di-COS, Δ di-C6S and Δ di-C4S. Digest products were separated by microfiltration and reduced by NaBH₄ while the remaining hexassacharide linkage regions still attached to the protein core were cleaved by reductive β -elimination. The resultant oligosaccharides were introduced to an electrospray LC-MS ion trap mass spectrometer with an on-line graphitised carbon column. Western Blot analysis using the CS stub antibodies 1B5, 3B3 and 2B6 on both the Chondroitinase ABC digested and undigested aggrecan samples were also performed to correlate linkage region identity.

Our data demonstrates how graphitised carbon LC-MS provides unique resolution and highly sensitive identification of the CS repeat region disaccharides and the predominant linkage region oligosaccharides conforming to the format Δ UA-GalNAc-GlcA-Gal-Gal-Xylitol, where GalNAc can be unsulfated, 6-sulfated or 4 sulfated. Furthermore, we verified the presence of each linkage region by showing reactivity with the respective CS stub antibodies on the digested versions of aggrecan. Our glycomic strategy will be applied to examine glycosylation differences amongst other proteoglycans such as lubricin from different sources to provide a better understanding of their structure and potential role in osteoarthritis.