

A NOVEL TAGGING PROCEDURE FOR THE ANALYSIS OF THE STRUCTURE AND SEQUENCE OF GLYCANS BY ESI-MS

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Heparin (*Figure 1*), heparan sulfates, and their structurally related glycosaminoglycans (GAGs) mediate many important biological activities in animal biochemistry and potent anti-inflammatory, antitumour and antimetastatic drugs have been developed by synthesising sulfated oligosaccharides that mimic heparin fragment analogues¹. Further insight into the sequence specificity of GAGs, in relation to their biological interactions and activities, should lead to the development of more powerful anticancer, antithrombotic and osteoarthritis drugs.

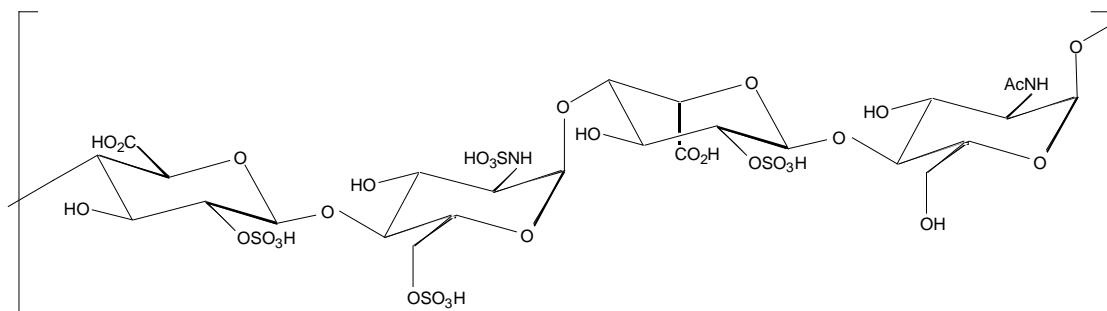


Figure 1, A tetrasaccharide portion of heparin

We have developed novel methodologies to “tag” heparin and heparan sulfate fragments derived from chemical and enzymatic degradation of the native compound. The methodology enables tagging of the fragments with both *O*- and *N*-substituted hydroxylamine derivatives. Both acidic and neutral oligosaccharides can be tagged in this way and the reaction is quantitative and utilises mild reaction conditions. This lessens unwanted degradation (eg desulfation) and improves chromatographic separations, as well as enhancing the sensitivity of these derivatives for mass spectrometric analysis.

GAGs, such as bovine lung and porcine mucosal heparin, were subjected to partial depolymerisation by treatment with limiting amounts of nitrous acid, or by *endo*-glycosidase treatments. The heparin fragments formed were then tagged and efficiently separated using ion-pairing HPLC. Positive and negative ion ESI-MS and ESI-MS/MS analysis, carried out on the collected fractions, allowed structural information to be obtained.

Although tagging of the heparin fragments enhances the MS sensitivity, extensive desulfation of the tagged fragment is still observed, both in ESI-MS analysis and in ESI-MS/MS analysis of the molecular species. Desulfation in the gas-phase therefore reduces the overall sensitivity and also decreases the amount of structural information available by MS/MS analysis. Investigations have therefore been initiated into methods for reducing the degree of desulfation which is observed under standard electrospray conditions.

1. Parish, C.R., Hindmarsh, E.J., Bartlett, M.R., Staykova, M.A., Cowden, W.B., and Willenborg, D.O., *Immunol. Cell Biol.*, 1998, 76, 104