THE BINDING OF GLYCOSAMINOGLYCANS TO PEPTIDES

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ABSTRACT

The overall aim this study was to examine the possibility of using immobilised polypeptide chains to fractionate/separate Glycosaminoglycans (GAG's) from mixtures.

Initially individual samples of three GAG classes (chondroitin sulphate, dermatan sulphate and heparin) were characterised to establish purity and provide basic information. Once these samples had been characterised the samples were treated as standards.

Three short poly-l-lysine (PLL) chains with defined length and orientation were synthesized. As a control a PLL chain with 633 residues was immobilised. The interaction of the GAG standards with these resins did not replicate published solution binding behaviour of longer PLL chains. This suggested a different mode of binding. The interaction of two lengths of PLL (126 and 633 residues) and the K_8G peptide with the GAG standards in solution was investigated. These studies demonstrated that the mode of binding of GAG's to short PLL chains was radically different to the earlier reported solution binding studies. ß-Strand dominates with the short PLL chains instead of α-helix established in the published solution binding studies.

The interaction of two peptides PCI (264-283) and thrombospondin peptide with the GAG standards was studied using circular dichroism spectroscopy. In the case of the PCI peptide, each GAG induced different secondary structures. Chondroitin sulphate and heparin induced an α-helix, whereas dermatan sulphate gave ß-strands. Heparin and dermatan sulphate induced double the amount of secondary structure compared to chondroitin sulphate. The strength of the interaction of GAG's with the peptide was also measured by the concentration of salt required to dissociate 50% of the complex. The figures for dermatan sulphate and heparin were found to be 0.1 and 0.3 M salt respectively. The binding of the GAG standards to the thrombospondin

peptide did not elicit any detectable change in conformation of the peptide.

Critical examination of published material on the interaction of GAG's (principally heparin) with short peptides, prompted the writer to propose two **new** complementary models. The first model examines binding in terms of the conformation of the peptide induced by binding to the GAG. It is composed of three components, the periodicity of polar and nonpolar residues within the peptide sequence, the spacing of pairs of basic residues and the spacing of pairs of acidic and basic residues. This model is successfully able to rationalise the binding behaviour of a number of GAG/peptide interactions in terms of the dominant secondary structure and the biological activity. The model is able to make a number of specific predictions. The second model examines the strength of the interaction between heparin and peptides containing the proposed consensus sequences for GAG binding sites. A significant correlation between the binding strength and an attribute derived from the sequence of the peptide was found using only one assumption. The assumption was that the peptides in the correlation bound to heparin with significant levels of ß-strand.

For the **first time** it is possible to rationalise the behaviour of GAG/peptide interactions in a coherent manner. The design of peptides that are capable of binding to specific GAG's now seems possible.

This thesis is dedicated to the most unappreciated group of chemicals in the science of biochemistry: glycosaminoglycans. Thanks to my long suffering laboratory companions: Simon Burton, Lou Wen and Estela Campanella (a.k.a dancing in the streets). I can honestly say that it has certainly been an experience. Thanks to my supervisor David Harding for his support and intellectual contribution, at several critical points in the research project and the drafting of the thesis. Thanks to Darren Englebretsen (a.k.a. Dr Cellulose) and Jenny Cross of the former SSU for helpful advice on peptide synthesis and the synthesis of three peptides. Thanks to Marcia Baker of the Separation Science Programme (formerly the Separation Science Unit) for looking after the finances of the project. It is with regret that I must acknowledge the demise of the SSU, that supplied the intellectual climate in which enabled this project to be fostered. I hope that the unique climate will be allowed to continue to exist by the powers that be.

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Abbreviations

α	Alpha helix
ß	Beta sheet
C^{13}	Carbon thirteen
CS	Chondroitin sulphate
C4S	Chondroitin-4-sulphate
C6S	Chondroitin-6-sulphate
CD	Circular dichroism
DS	Dermatan sulphate
GAG	Glycosaminoglycan
Gal	Galactose
GalN	Galactosamine
GalNAc	N-acetyl-galactosamine
GlcN	Glucosamine
GlcNAc	Nacetylglucosamine
GlcNS	Glucosamine-Nsulphate
GlcNS(5S)	Glucosamine-Nsulphate-5Osulphate
GlcN($6SO_3$)	Glucosamine-Nsulphate-6Osulphate
GluA	Glucuronic acid
Hep	Heparin
HexA	Hexuronic acid
HS	Heparan sulphate
Ido2S	Iduronic acid-2Osulphate
IdoA	Iduronic acid
N_{eff}	Number of theoretical plates
PLA	Poly-L-arginine
PLL	Poly-L-lysine
R	Random coil