THE BINDING OF GLYCOSAMINOGLYCANS TO PEPTIDES

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GRANT JOHN TAYLOR
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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. B-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 β-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
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 β-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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Chapter 1: Introduction

1.1 REVIEW OF GLYCOSAMINOGLYCANS STRUCTURE AND FUNCTION

1.1.1 Classification of Glycosaminoglycans (GAG’S)

1.1.1.1 Common GAG types

1.1.1.2 Heterogeneity of disaccharide repeat sequences

1.1.2. Biological roles of GAG’s

1.1.3 GAG properties

1.1.3.1 pH, of ionizable groups

1.1.3.2 Flexibility of uronic acid sugar residues

1.1.3.3 Hydrophobic patches

1.1.3.4 Secondary Structure

1.1.3.5 Stereochemistry of charged groups

1.1.3.6 Binding to polycations

1.1.3.4 Summary of important GAG properties

1.2 SEPARATION OF GAG’s

1.2.1. Ion exchange resins

1.2.2. Fractional solvent precipitation

1.2.3. Polymersine resins

1.2.4 Hydrophobic interactive chromatography

1.2.5 Bioaffinity chromatography

1.2.6 Summary of GAG purification procedures

1.3 BINDING OF GAG’S TO PROTEINS

1.3.1 Sequence specific binding

1.3.2 Protein consensus sequences for GAG binding

1.3.3 Conformational change of protein

1.3.4 Examples of GAG binding peptides

1.4 Scope of this study

CHAPTER TWO: GAG STANDARDS

2.1 INTRODUCTION

2.2 MATERIAL AND METHODS

2.2.1 Equipment

2.2.2 Chemicals

2.2.3 Preparative GAG fractionation procedures
2.2.3.1 Calcium salt ethanol fractionation ........................................... 36
2.2.3.2 Calcium salt to sodium salt conversion ..................................... 37
2.2.3.3 Selective precipitation of heparin ........................................... 37
2.2.3.4 Alkaline copper precipitation ................................................ 38
2.2.4 Characterisation of GAG samples .............................................. 38
2.2.4.1 Cellulose acetate electrophoresis ........................................... 38
2.2.4.2 Optical rotation measurements .............................................. 40
2.2.4.3 Chondroitinase ABC and AC lyase digestion .............................. 40
2.2.4.4 C\textsuperscript{13} NMR spectroscopy ........................................ 41
2.2.4.5 Elemental analysis ............................................................ 41
2.2.4.6 Uronic acid assay ............................................................. 42
2.2.4.7 Dimethylmethene blue assay for GAG’s ................................... 42
2.2.4.8 Intrinsic viscosity determination ......................................... 43
2.2.4.9 Iduronic-20 sulphate assay ................................................ 43
2.3 RESULTS AND DISCUSSION ....................................................... 44
2.3.1 Alkaline copper precipitation ................................................... 44
2.3.2 Ethanol precipitation of calcium salts ........................................ 45
2.3.3 2M KOAc, pH 5.7 precipitation ............................................... 47
2.3.4 Characterisation of GAG samples .............................................. 49
2.3.4.1 Enzymatic digestion ............................................................. 49
2.3.4.2 C\textsuperscript{13} NMR Spectroscopy ........................................... 53
2.3.4.3 Charge density of GAG standards ......................................... 60
2.3.4.4 Iduronic 20S levels ............................................................. 62
2.3.4.5 Molecular weight and molecular weight distribution .................. 63
2.4 SUMMARY OF CHAPTER ....................................................... 63

CHAPTER 3: PEPTIDE SYNTHESIS .................................................. 66
3.1 INTRODUCTION ........................................................................... 66
3.2: MATERIALS AND METHODS .................................................. 69
3.2.1 Equipment ............................................................................... 69
3.2.2 Chemicals .............................................................................. 69
3.2.3 Quantitative ninhydrin procedure .............................................. 70
3.2.4 Picrate titration of resin bound amine groups .............................. 70
3.2.5 Synthesis of K\textsubscript{4}G and K\textsubscript{8}G peptide resins ............... 71
3.2.5.1 Reaction of Perloza with FmocGLYOBt .................................. 71
3.2.5.2 Coupling of the aminoacids to the resin ................................ 71
3.2.5.3 Theoretical calculation of substitution levels ........................... 72
3.2.5.4 Amino acid analysis ............................................................. 72
3.2.5.5 Removal of protecting groups ............................................. 73
3.2.6 Automated synthesis of K\textsubscript{4}G and K\textsubscript{8}G peptide resins .......... 73
3.2.7 Synthesis of free peptides ..................................................... 73
3.2.7.1 Purification of peptides ......................................................... 73
3.2.8 Characterisation of purified peptides ........................................ 74
3.2.8.1 Reverse phase HPLC ............................................................ 74
3.2.8.2 Capillary electrophoresis (C.E.) ............................................. 74
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2.8.3 Fast atom bombardment (FAB)-mass spectrometry</td>
<td>75</td>
</tr>
<tr>
<td>3.3 RESULTS AND DISCUSSION</td>
<td>75</td>
</tr>
<tr>
<td>3.3.1 KxG Resins</td>
<td>75</td>
</tr>
<tr>
<td>3.3.2 Synthesis of free peptides</td>
<td>77</td>
</tr>
<tr>
<td>3.3.2.1 Protease C inhibitor peptide (residues 264-283)</td>
<td>77</td>
</tr>
<tr>
<td>3.3.2.2 Thrombospondin peptide</td>
<td>78</td>
</tr>
<tr>
<td>3.3.2.3 KxG peptide</td>
<td>80</td>
</tr>
<tr>
<td>3.3.3 Capillary electrophoresis of free peptides</td>
<td>81</td>
</tr>
<tr>
<td>3.4 SUMMARY</td>
<td>83</td>
</tr>
<tr>
<td>Chapter 4: BINDING OF GAG's TO IMMOBILISED PEPTIDE RESINS</td>
<td>84</td>
</tr>
<tr>
<td>4.1 INTRODUCTION</td>
<td>84</td>
</tr>
<tr>
<td>4.2 MATERIALS AND METHODS</td>
<td>87</td>
</tr>
<tr>
<td>4.2.1 Chemicals</td>
<td>87</td>
</tr>
<tr>
<td>4.2.2 Equipment</td>
<td>87</td>
</tr>
<tr>
<td>4.2.3 Preparation of immobilised polylysine resin</td>
<td>87</td>
</tr>
<tr>
<td>4.2.4 Binding of GAG standards to polylysine resins</td>
<td>88</td>
</tr>
<tr>
<td>4.2.4.1 Binding and elution experiments</td>
<td>90</td>
</tr>
<tr>
<td>4.2.5 GAG binding capacities of the peptide resins</td>
<td>90</td>
</tr>
<tr>
<td>4.2.6 Ligand displacement chromatography on PLL resin</td>
<td>91</td>
</tr>
<tr>
<td>4.3 RESULTS AND DISCUSSION</td>
<td>91</td>
</tr>
<tr>
<td>4.3.1 Immobilisation of polylysine (PLL)</td>
<td>91</td>
</tr>
<tr>
<td>4.3.2 Validation of relative binding strength</td>
<td>92</td>
</tr>
<tr>
<td>4.3.3 Binding of GAG standards to PLL-resin</td>
<td>93</td>
</tr>
<tr>
<td>4.3.4 Binding and elution of GAG standard from KxG peptide resins</td>
<td>93</td>
</tr>
<tr>
<td>4.3.5 Comparison of the binding behaviour of the PLL-resin and KxG resins</td>
<td>96</td>
</tr>
<tr>
<td>4.3.6 PLL-resin: Ligand displacement chromatography</td>
<td>100</td>
</tr>
<tr>
<td>4.3.7 CHAPTER SUMMARY</td>
<td>101</td>
</tr>
<tr>
<td>Chapter 5: SOLUTION BINDING OF GAG'S TO PEPTIDES</td>
<td>103</td>
</tr>
<tr>
<td>5.1 INTRODUCTION</td>
<td>103</td>
</tr>
<tr>
<td>5.2 MATERIALS AND METHODS</td>
<td>106</td>
</tr>
<tr>
<td>5.2.1 Chemicals</td>
<td>106</td>
</tr>
<tr>
<td>5.2.2 Equipment</td>
<td>106</td>
</tr>
<tr>
<td>5.2.3 Stoichiometry of GAG/peptide interaction</td>
<td>107</td>
</tr>
<tr>
<td>5.2.3.1 Purification of methylene blue</td>
<td>107</td>
</tr>
<tr>
<td>5.2.3.2 Methylene blue titration</td>
<td>107</td>
</tr>
<tr>
<td>5.2.3.3 Data Analysis</td>
<td>108</td>
</tr>
<tr>
<td>5.2.4 Secondary structure determination</td>
<td>109</td>
</tr>
<tr>
<td>5.2.5 Secondary structure prediction</td>
<td>111</td>
</tr>
<tr>
<td>5.3 RESULTS AND DISCUSSION</td>
<td>113</td>
</tr>
<tr>
<td>5.3.1 Hydrophobic profiles</td>
<td>113</td>
</tr>
<tr>
<td>5.3.2 CD conformational study</td>
<td>115</td>
</tr>
</tbody>
</table>
5.3.2.1 Calibration of the technique ............................................. 115  
5.3.2.2 Polylysine/ GAG interaction ............................................. 118  
5.3.2.3 Thrombospondin peptide ................................................. 123  
5.3.2.4.1: PCI peptide .......................................................... 124  
5.3.2.4.2 Fine structure of heparin .............................................. 127  
5.3.3.1 Stoichiometry of GAG- peptide binding ................................. 129  
5.3.3.2 Stoichiometry of GAG/lysine interaction ............................... 130  
5.3.3.3 A comparison of binding stoichiometry: solution versus solid phase ............................................................... 132  
5.4 CHAPTER SUMMARY ............................................................ 132  
5.4.1 PLL/GAG interaction .......................................................... 132  
5.4.2 GAG/peptide interaction ..................................................... 133  

Chapter 6: A REEXAMINATION OF PUBLISHED WORK ............................................. 134  
6.0 INTRODUCTION ................................................................. 134  
6.1 GAG binding sites on proteins ................................................ 135  
6.1.1 Chi squared test ............................................................ 135  
6.1.2 Cardin and Weintraub’s consensus sequences ............................ 136  
6.1.3 Sobel’s consensus sequence ................................................ 139  
6.1.4 Margalit et al’s GAG binding consensus sequence ........................ 141  
6.1.5 Hyaluronan binding site ..................................................... 142  
6.1.6 Ligands for sulphate binding ............................................... 142  
6.1.7 Summary of consensus sequences ........................................ 143  
6.2 Conformational change of peptide on binding GAG’s ....................... 144  
6.2.1 Importance of the conformational change ................................ 144  
6.2.2 PLL/GAG interaction .......................................................... 145  
6.2.3 Peptide/GAG interactions .................................................... 146  
6.2.3.1 A stereochemical model for GAG binding ............................. 149  
6.2.3.2 Biological binding behaviour of peptides .............................. 150  
6.2.3.3 Discussion of the model ................................................... 154  
6.3 Binding strength of GAG/peptide interaction ................................ 155  
6.4.1 Specificity of peptide/GAG interactions ................................ 159  
6.4.2 ATIII/heparin interaction .................................................... 160  
6.4.3 Peptide affinity columns .................................................... 164  
6.4.4 Energetics of peptide/heparin interaction ................................ 164  
6.4.5 Summary ................................................................. 165  
6.5 Sequence specific binding ................................................... 165  
6.6 CHAPTER SUMMARY ............................................................ 167  

CHAPTER 7: CONCLUSION ............................................................. 169  
7.1 INTRODUCTION ................................................................. 169  
7.2 GAG STANDARDS .............................................................. 170  
7.3 GAG BINDING BEHAVIOUR OF PLL RESINS ............................... 171  
7.4 GAG BINDING PETIDES ........................................................ 172
7.5 MODELS OF THE GAG/PEPTIDE INTERACTION ........................................ 174
  7.5.1 Stereochemical model ............................................................... 174
  7.5.2 Consensus sequence model ....................................................... 176
  7.5.3 Implications of the models ....................................................... 176

7.6 OUTLINE OF FUTURE WORK ............................................................. 177

Appendix 1: Supplementary material to Chapter 1. ........................................ 179
  A1.1: GAG properties ................................................................. 179
  A1.2 Heparan sulphate proteoglycans (HSPG) and amyloidoses ................. 180
    A1.2.1 Alzheimer’s Disease ....................................................... 182
    A1.2.2 AA Amyloidosis ........................................................... 183
    A1.2.3 Prion disorders ........................................................... 183

Appendix 2: Supplementary material to Chapters 2 and 3 ............................... 185
  A2.1 Worked examples of viscosity molecular weight determination .......... 185
  A2.2 Worked examples of calculation of substitution menu ...................... 185

Appendix 3: Supplementary material to Chapter 4 ....................................... 187
  A3.1 Data analysis of GAG binding to ion exchange resins ....................... 189
  A3.2 GAG binding to peptide resins ............................................... 189
  A3.3 Sample calculation of stoichiometry of GAG binding to polylysine resin ............................................... 192

Appendix 4: Supplementary material to Chapter 5 ..................................... 194
  A4.1 Calculation of hydrophobic moments ......................................... 194
  A4.2 CD spectral data for peptide/GAG complexes ................................ 194
  A4.3 Sample calculation for stoichiometry of solution binding ................ 198

Appendix 5: Supplementary information to Chapter 6 ................................... 200
  A5.1 Distribution of acidic, aromatic and basic residues within consensus sequences ............................................... 200
    A5.1.1 Cardin and Weintraub type I ............................................ 200
    A5.1.2 Cardin and Weintraub’s type II consensus sequence .................. 201
    A5.1.3 Sobel’s et al consensus sequence .................................... 202
  A5.2 Preferred ligand for sulphate binding ....................................... 202
  A5.3 Worked examples for stereochemical model .................................. 205
    A5.3.1 Peptides with no acidic residues ..................................... 205
    A5.3.2 Peptides containing acidic residues ................................... 207
    A5.3.3: Peptide data for stereochemical model ................................ 208
  A5.4 Peptide data for consensus sequence binding model ........................ 210
  A5.5: Specificity of ATIII peptide heparin interaction .......................... 210
List of Figures

Figure 1.1: Structures of monosaccharide units in GAG’s 3

Figure 2.1: Electrophoretic analysis of Calcium ppt Technique 46

Figure 2.2: Electrophoretic analysis of GAG fractionation procedures 48

Figure 2.3a: Chondroitinase ABC lyase digestion 51

Figure 2.3b: Chondroitinase AC lyase digestion 51

Figure 2.4: Electrophoretic analysis of enzymatically degraded GAG standards 52

Figure 2.5: Chondroitin and Dermatan sulphate C\textsuperscript{13} NMR spectra 55

Figure 2.6: C\textsuperscript{13} NMR spectra of slow and fast heparin 58

Figure 2.7: Intrinsic viscosity determination 64

Figure 3.1: HPLC profiles of PCI peptide 78

Figure 3.2: CE profiles of purified PCI peptide 79

Figure 3.3: HPLC profiles of Thrombospondin peptide 79

Figure 3.4: CE profiles of purified Thrombospondin peptide 80

Figure 3.5: HPLC profiles of $K_8G$ peptide 81

Figure 3.6: CE profiles of purified $K_8G$ peptide 82

Figure 4.1: Shape of gradients used in resin binding experiments 89

Figure 4.2: Elution of GAG standards from PLL-resin 95

Figure 4.3: Typical profile for PLL-resin with ligand displacement chromatography 101

Figure 5.1: Hydrophobic moment profiles for the peptides 114

Figure 5.2: CD spectra of GAG standards 117

Figure 5.3: CD spectra of long PLL/GAG complexes 119

Figure 5.4: CD spectra of short PLL/GAG complexes 120

Figure 5.5: CD spectra for $K_8G$/GAG complexes 122

Figure 5.6: CD spectra of Thrombospondin/GAG complexes 123

Figure 5.7: CD spectra of PCI peptide/GAG complexes 125

Figure 5.8: Salt dissociation curves for PCI peptide/GAG complexes 127

Figure 5.9: Methylene Blue titration curve 129

Figure 6.1: Proposed origin of Sobel’s consensus sequence 140

Figure 6.2: Salt strength binding correlations 157
Figure A3.1: Cumulative Elution of GAG’s from Dowex 1X2 resin 187
Figure A3.2: Cumulative Elution of GAG’s from ECTEOLA-cellulose 188
List of Tables

Table 1.1: Classification of glycosaminoglycans 2
Table 1.2: Summary of Gelmans solution binding experiments 15
Table 1.3: Sequences specific binding sites on GAG's 23
Table 1.4: Proposed consensus sequences for Heparin binding 25
Table 1.5: Influence of Polysaccharide binding on peptide conformation 29
Table 1.6: Free peptides synthesized as part of this thesis 31
Table 2.1: Constants for Mark-Houwink Equation 35
Table 2.2: Typical Optical rotations of GAG classes 40
Table 2.3: Preparative precipitation of calcium salts 46
Table 2.4: Heparin fractionation 48
Table 2.5: Assignment of C\textsuperscript{13} Peaks for dermatan and chondroitin sulphate 56
Table 2.6: Assignment of C\textsuperscript{13} Peaks for Fast and Slow heparin 61
Table 2.7: Charge density and percentage sulphate and nitrogen of GAG's 62
Table 2.8: Epoxidation reaction results 62
Table 2.9: Summary of viscosity results for GAG standards 64
Table 2.10: Summary of the properties of the GAG standards 65
Table 3.1: Biolynx continuous flow peptide synthesizer synthesis cycle 72
Table 3.2: Run parameters for Capillary electrophoresis 75
Table 3.3: Analytical data on K\textsubscript{4}G resins 76
Table 3.4: Deprotection of K\textsubscript{4}G Resins 77
Table 3.5: Analytical data for cleavable K\textsubscript{4}G peptide 80
Table 3.6: Comparison of the CE buffers performance 82
Table 4.1: Binding behaviour of GAG's on ion exchange resin. 85
Table 4.2: Details of gradients used in resin binding experiments 90
Table 4.3: Substitution level of PLL resin 92
Table 4.4: Validation of relative binding strength for K\textsubscript{4}G resin 93
Table 4.5: Elution of GAG standards from PLL-resin 94
Table 4.6: Summary of elution experiment on peptide resins 94
| Table 4.7: Comparison of GAG binding | 97 |
| Table 4.8: Stoichiometry of GAG binding to polylsine resins | 99 |
| Table 5.1: Set up parameters for CD analysis | 110 |
| Table 5.2: Amphipathic indexes of GAG binding peptides | 114 |
| Table 5.3: Optical rotation of Dermatan sulphate and Heparin at 222 nm | 116 |
| Table 5.4: Secondary structure elements for long PLL/GAG complexes | 119 |
| Table 5.5: Secondary structure elements for short PLL/GAG complexes | 120 |
| Table 5.6: Comparison of induced helix for PLL GAG binding | 121 |
| Table 5.7: Secondary structure elements for K₈G/GAG complexes | 122 |
| Table 5.8: Secondary structure elements of Thrombospondin/GAG complexes | 124 |
| Table 5.9: Secondary structure elements of PCI peptide/GAG complexes | 124 |
| Table 5.10: Relative rate increase of GAG for PCI inhibition | 126 |
| Table 5.11: Time course of heparin binding | 130 |
| Table 5.12: A comparison of the stoichiometry of the GAG/polylsine interaction. | 131 |
| Table 5.13: PLL chain binding behaviour solution and resin bound | 132 |
| Table 6.1: Cardin's Type I consensus sequence | 137 |
| Table 6.2: Cardin's type II consensus sequence | 138 |
| Table 6.3: Distribution of basic residues in Sobel's consensus sequence | 139 |
| Table 6.4: Comparison of basic amino acid tendencies | 140 |
| Table 6.5: A comparison of induced helix and disaccharide repeat distance | 146 |
| Table 6.6: Evaluation of the model | 151 |
| Table 6.7: Summary of Camejo et al’s results | 152 |
| Table 6.8: FN-C/HII peptide results | 153 |
| Table 6.9: Summary of peptides for used in the salt strength correlation | 158 |
| Table 6.10: Summary of peptide modelling of ATIII/heparin interaction | 162 |
| Table 6.11: Peptide modelling of the specificity of the ATIII/heparin interaction | 164 |
| Table 6.12: A comparison of the two GAG binding models | 168 |
| Table A1.1: Properties of the GAG’s Used by Gelman | 179 |
| Table A1.2: Elution behaviour of GAG’s on Suzuki’s PLL resin | 179 |
| Table A1.3: Ionizing groups per disaccharide units of GAG’s | 180 |
Table A2.1: Data processing for viscosity determination 185
Table A2.2: Substitution menu for K₄G 186
Table A3.1: Binding of GAG's to two ion exchange resins 188
Table A3.2: K₄G resin ECONO system runs 189
Table A3.3: K₄G resin FPLC runs 189
Table A3.4: K₄G resin FPLC runs 190
Table A3.5: K₁₂G resin FPLC runs 191
Table A3.6: PLL resin FPLC runs 192
Table A4.1: Sample calculation of hydrophobic moment at 80° degree's for PCI peptide 195
Table A4.2: PLL (long)/GAG interaction 196
Table A4.3: PLL (short)/GAG interaction 196
Table A4.4: K₄G/GAG interaction 197
Table A4.5: PCI peptide/GAG interactions 197
Table A4.6: Thrombospondin peptide/GAG interaction 197
Table A5.1: Distribution of residues within type I consensus sequences, 200
Table A5.2: Distribution of the outcomes at position 2,3 and 4 201
Table A5.3: Distribution of the residues within the type II consensus sequence 202
Table A5.4: distribution of the residues within Sobel's Consensus sequence 203
Table A5.5: Preferred ligands for sulphate binding 204
Table A5.6: Preference Among Basic Ligands 205
Table A5.7: Multiples of Disaccharide repeat distances for GAG's 206
Table A5.8: Distances between basic residues in an alpha helical conformation 206
Table A5.9: Distances between basic residues in a beta strand conformation 206
Table A5.10: Distances between acidic and basic residues 207
Table A5.11: Distances between basic residues in an alpha helical conformation 207
Table A5.12: Distances between basic residues in a beta strand conformation 208
Table A5.13: Summary of the peptides used in the stereochemical model 209
Table A5.14: Summary of peptides used in the consensus sequence model 210
Table A5.15: Data treatment for Bae et al's peptides 211
**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>α</td>
<td>Alpha helix</td>
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</tr>
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<td>N-acetyl-galactosamine</td>
</tr>
<tr>
<td>GlcN</td>
<td>Glucosamine</td>
</tr>
<tr>
<td>GlcNAc</td>
<td>N-acetylglucosamine</td>
</tr>
<tr>
<td>GlcNS</td>
<td>Glucosamine-Nsulphate</td>
</tr>
<tr>
<td>GlcNS(5S)</td>
<td>Glucosamine-Nsulphate-50sulphate</td>
</tr>
<tr>
<td>GlcN(6SO$_3$)</td>
<td>Glucosamine-Nsulphate-60sulphate</td>
</tr>
<tr>
<td>GluA</td>
<td>Glucuronic acid</td>
</tr>
<tr>
<td>Hep</td>
<td>Heparin</td>
</tr>
<tr>
<td>HexA</td>
<td>Hexuronic acid</td>
</tr>
<tr>
<td>HS</td>
<td>Heparan sulphate</td>
</tr>
<tr>
<td>Ido2S</td>
<td>Iduronic acid-20sulphate</td>
</tr>
<tr>
<td>IdoA</td>
<td>Iduronic acid</td>
</tr>
<tr>
<td>N$_{err}$</td>
<td>Number of theoretical plates</td>
</tr>
<tr>
<td>PLA</td>
<td>Poly-L-arginine</td>
</tr>
<tr>
<td>PLL</td>
<td>Poly-L-lysine</td>
</tr>
<tr>
<td>R</td>
<td>Random coil</td>
</tr>
</tbody>
</table>