GlycosaminoGlycans (GAGs) Discover

Description

GlycosaminoGlycans (GAGs)
Glycosaminoglycan (GAGs) are linear polysaccharides present on all animal cell surfaces and in the extracellular matrix, where they are usually found to be attached covalently to core proteins to form the proteoglycan family. Each tissue produces specific repertoires of glycosaminoglycans (GAGs), some of which are known to bind and regulate chemokine activity. GAGs can be classified into four groups : the hyaluronic acid type, the chondroitin / dermatan sulfate type, the Heparan/Heparin type, and the Keratan type. They are acidic polysaccharides made of disaccharide repeating units (ranging from 40 to 100) which consists of uronic acid and hexosamines. Except for the hyaluronic acid, epimerization at the C-5 position of uronic acids, and N- and O- sulfation provide numerous sources of microheterogeneities. GAGs assume extended structures in aqueous solutions because of their strong hydrophilic nature based on their extensive sulfation, which is further exaggerated when they are covalently linked to core proteins. They hold a large number of water molecules in their molecular domain and occupy enormous hydrodynamic space in solution. A complementary remarkable property of the glycosaminoglycans, which is particularly significant in heparan sulfate and heparin, is their capability to specifically interact with a number of important growth factors and functional proteins. Such interactions are often crucial to the biological functions of these proteins.

Hyaluronic acid ?4(–?-D-GlcA-1?3-?-D-GlcNAc-1?)n

Chrondroitin / Dermatan ?4(–?-D-GlcA-1?3-?-D-GalNAc-1?)n
Sulfate type ?4(–?-L-IdoA -1?3-?-D-GalNAc-1?)n
Sulfation at position 2 of IdoA and 4 and 6 of GalNAc

Heparan sulfate / Heparin ?4(–?-D-GlcA-1?4-?-D-GlcN-R-1?)n
?4(–?-L-IdoA -1?4-?-D-GlcN-R-1?)n
with R = -COCH3 or SO3-
Sulfation at position 2 of GlcA and 3 and 6 of GlcNR

Keratan sulfate ?3(–?-D-Gal-1?3-?-D-GlcNAc-1?)n
Sulfation at position 6 of GlcA and 6 of GlcNR

Fig. 1 A proteoglycanglycan : a large molecular complex formed by the linking of many GAGs (composed of oligosaccharide chains attached to the core protein) through a long hyaluronate strand.

Fig. 2 A schematic representation of a glycosaminoglycan (Mucopolysaccharide) its location and density

Hyaluronic acid : Hyaluronan

Hyaluronan, alos know as hyaluronic acid, is the only unsulfated member of the glycosaminoglycan

family. It is found in mammalian connective tissues, where it forms the central core of the proteoglycan aggregate. Apart from its role in connective tissues, other known biological functions of hyaluronan occur in cartilage, umbilical cord, cornea…. reference-in-status-bar. There has been an extensive number of X-ray diffraction investigations on well-oriented fibers incorporated different couner-ions (sodium, potassium, calcium) as well as some molecular modeling studies aiming at characterizing the conformational behaviour of the polysaccharide in different environments. All these investigations explored the polymorphic behaviour of hyaluronan.

ig. 3 A 2D representation of the Hyaluronan structure

From X-ray investigation, seven polymorphs have been characterized which can be classified under three major categories that range form 4-fold to 3-fold left-handed helices. These are only some of the possible theoretical helical conformations that hyaluronan can adopt.

Fig. 4 A schematic drawing of hyaluronic acid segment. Each disaccharide repeat has the potential to participate in 4 hydrogen bonds (2 across the 1?4 linkage and 2 across the 1?3 linkage)

The 3-fold conformation is limited to a fully stretched value of 9.5 Å for the disaccharide repeat. The 4-fold conformation is more tolerant to stretching and upon external conditions such the nature of the cation, and/or the relative humidity, can adopt conformations such that the length of the disaccharide repeat may vary from full extension of 9.5 Å to 8.5 Å. Under the influence of potassium ions, a double helical structure may occur.

Sodium Hyaluronate I anhydrous. The fiber diffraction diagram for sodium hyaluronate in its anhydrous form can be interpreted based on a 4-fold (43) helix symmetry reference-in-status-bar. The molecular model best compatible with the X-ray data is a left-handed helix having a repeat of 33.94 Å. with the noticable occurrence of two interesidues hydrogen bonds. The orientation of the hydroxymethyl group in the GlcNAc residue is gauche-trans; whereas the carboxylated orientation in the glucuronate residue is c = -91°.

Fig. 5 A 3D representation of (a.) hyaluronate I sodium (b.) hyaluronate II sodium (c.) hyaluronate III sodium

The space group symmetry has been identified as P43212, and two helices passes through the unit cell. They are oriented in an anti-parallel fashion, into a tight packing anhydrous arrangement. Sodium being the only guest molecule interacts with the carboxylate group. It dispays an octahedral coordination. Anti-parallel and parallel helices interacts via hydrogen bonds, respectively O3H….O62 (2.59 Å) and O2H…O6 (2.37 Å) and O6H…O7 (2.79 Å). (Note the O2H…O6 distance of 2.37 Å is notably too short thereby indicated a minor flaw in the elaboration of the model).

Sodium Hyaluronate I hydrated. Upon control relative humidity at 75%, one observes an alteration of the unit cell and space group symmetry of the anhydrous form reference-in-status-bar. This is accompanied by a change in helix symmetry from 43 to 21 in a way such that the new repeat consists of the two disaccharides. This hydrated structure corresponds to an amount of 4 water molecules per tetra saccharide repeat, with still one sodium ion near every carboxylate group. The presence of water does not bring significant change in the intra-chain hydrogen–bonding, and brings some perturbation in the interchain hydrogen bonds.

Fig. 6 A 3D representation of the structure reported by Guss et al. reference-in-status-bar.

Potassium Hyaluronate I. Upon replacement of sodium per potassium, and while maintaing a relative humidity at 85% an X-ray fiber diffractogram that can be indexed in a tetragonal space, P43212 group occurs reference-in-status-bar. Whereas some changes are observed at the level of intrahelical hydrogen bonds, the rest of the structural features remains fairly close to the previous sodium salt. The packing arrangement with one potassium ion and two water molecules is analoguous to that described for the sodium salt.

Fig. 7 A 3D representation of the structure of Potassium Hyaluronate I

Potassium Hyaluronate II. Another fiber diffraction pattern can be found for potassium hyaluronate reference-in-status-bar which crystallizes in an orthorhombic unit cell. With respect to the previous structure, the helix symmetry is slightly perturbed and the extension of the disaccharide repeat is 8.85 Å, i.e. about 0.35 Å longer than in the other polymorph. Such a change does not preclude the occurrence of two intrachain hydrogen bonds, but only one of the two acetamido groups is able to make an intrachain hydrogen bond. Each tetrasaccharide is associated with two potassium ions which lie in the vicinity of the carboxylate groups, and two water molecules. The potassium ion is seven fold coordinated , throughout intarction with a carboxylate oxygen atom, a water molecules, and five remainig ligands from surrounding helices. Two hyaluronate helices cross the unit cell; they are arranged in an anti-parallel fashion, but with rotational arrangement being 90° relative to that in the orthorhombic form. This illustrates some of the low energy interchain arrangements that hyaluronate chains can take.

These structural models have been confirmed throughout independant investigations reference-in-status-bar.

Sodium Hyaluronate III. This is another hydrated polymorph that is obtained when some of the fibers are prepared at 40°C with 90% relative humidity. Under these conditions, diffraction patterns are obtained, that correspond to the occurrence of 3-fold helix symmetry reference-in-status-bar. A trigonal space group corresponds best to the indexaton of the pattern. A three-fold left handed helix having full extension of 9.5 Å for the disaccharide repeat characterises helical chain conformation. Whereas the acetamido group does not participate in any intrachain hydrogen bonding, the low energy helix conformation is stabilized by hydrogen bonds.Fig. 8 A 3D representation of the structure of Sodium Hyaluronate III

The unit cell of the P3121 space group encompasses two helices, one sodium ion per disaccharide and about 3.5 water molecules. The sodium ions have a total of 6 ligands and an octahedral

coordination. The helices are aligned in an anti-parallel arrangement, and are associated via carboxylate…sodium…O2 (glucuronate) / O7 (carbonyl interactions), with a further stabilisation by an interchain hydrogen bond (O6-H… O3 (2.72 Å).

Calcium Hyaluronate. X-ray fiber diffractogram of calcium hyaluronate obtained at 75% relative humidity (and higher) can be interpreted on the basis of a 3-fold helix symmetry, occurring in a trigonal spce group, P3112, with a unit-cell having large dimensions. reference-in-status-bar. Such a left-handed 3-fold helical symmetry implies that 3 disaccharides constitute one turn of the helix. This can be obatined while relaxing some of the conformation about the glycosidic angles, but still maintaining some of the intra-chain hydrogen bonds. As a result, the hydroxymethyl groups undergo some orientational changes.

Fig. 9 A 3D representation of the structure of Calcium Hyaluronate

Potassium Hyaluronate III. When prepared at low pH (3.0 – 4.0) well oriented fibers of potassium hyaluronate correspond to a hemi or fully protonated state of the polysaccharide. The interpretation of the corresponding X-ray diffractogram reference-in-status-bar has been debated and as a result, a careful re-examination has produced an excellent double helix in which the inter-twinned chains are closey interacting, not only by direct hydrogen bonds, but also water bridges. Each chain is extensively hydrogen bonded across the glycosidic linkages.

Fig. 10 A 3D representation of the structure of Potassium Hyaluronate III

The cation are regularly placed between the duplexes, and have been considered to be redundant for the stability and the survival of the double-helical structure. Inter double-helix hydrogen bonds are mediated between hydroxymethyl and N-acetly groups.

Rationalising the conformational versatility of hyaluronan chains
The conformational polymorphism displayed by hyaluronan chain is the solid state has been rationalised via computational methods reference-in-status-bar that could predicit the occurrence of three different helical conformations 32, 43, 21, and explained them by weak energetic costs (<0.7 kcal/mol.dimer) associated to small variations in ?, ? glycosidic torsion angles leading to the different types of helices. Other helical conformations which are likely to occur were also predicted. Fig. 11 Helices of hyaluronic acid predicted by molecular modelling Finally the feasibility of a double helix with anti-parallel strands for hyaluronan chains was evaluated and compared to the two models derived from X-ray experiments. The calculations demonstrated that the formation of a duplex by chain folding is possible; though marginally observed, the anti-parallel arrangement of the chains in the double helix is more favourable than the parallel one for hyaluronic acid. The energetic evaluation of the X-ray models, in comparison to the established theoretical model, indicated that the first model originally proposed by Sheehan and coworkers is not viable, and that the second one proposed by Arnott et al. is much more reasonable, with may be an over-evaluated hydrogen bond network. Chondroitin sulfate The repeating unit of chondroitin is a disaccharide similar to that in hyaluronan, except that GalNAc replaces GlcNAc. Fig. 12 Chemical repeat unit of Chondroitin sulphate In the native state, the galactose residues are O-sulfated (either at position 4 or at position 6). Molecular modelling investigations reference-in-status-bar reference-in-status-bar have indicated the the occurrence of chondroitin-4-sulfate chains as left handed in which the extension of the disaccharide repeat vries from 9.3 to 9.8 Å. In the case of chondroitin-6-sulfate similar trends have been postulated to occur. Sodium Chrondroitin 4-sulfate and Potassium Chondroitin 4-sulfate. For both salts X-ray analysis from fiber

diffractogram has established the occurrence of a left-handed 3 fold helix with the sulfate groups pointing towards the periphery where they interact with one surrounding cation and water molecules reference-in-status-bar reference-in-status-bar. In both cases the helix is stabilised by a O3H…O5 hydrogne bond (O…O distance = 2.74 Å) across every 1?4 linkage. Within the P3221 space group, the chains are packed in the unit cell in an anti-parallel fashion. The potassium ions have an octahedral coordination. Fig. 13 A 3D representation of the structure of Sodium Chondroitin 4-sulphate Fig. 14 A 3D representation of the structure of Potassium Chondroitin 4-sulphate Sodium Chrondroitin 4-sulfate. The replacement of monovalent ions with divalent ions such as calcium induces a conformational transition of the chain from 3 fold helix to 2-fold helix symmetry reference-in-status-bar. This is accompanied by an extension of the pitch. As a result, the length of the disaccharide repeat is 9.82 Ang. which corresponds to a maximum extension and it is accompanied by the occurence of two intrachain hydrogen bonds O3H….O5 (2.64 Å) across the 1-4 linkages and O2H…O7 (2.99 Å) across the 1?3 linkages. In the unit cell, the chains are arranged in anti-parallel fashion. The calcium ion are playing an important role assuming the coordination via bridges involving the sulfate groups. Discover Polysaccharides Glycosaminoglycans (GAGs) Introduction Hyaluronic acid : Hyaluronan Chondroitin sulfate Dermatan sulfate Keratan 6-sulfate Heparin …….. Introduction Glycosaminoglycan (GAGs) are linear polysaccharides present on all animal cell surfaces and in the extracellular matrix, where they are usually found to be attached covalently to core proteins to form the proteoglycan family. Each tissue produces specific repertoires of glycosaminoglycans (GAGs), some of which are known to bind and regulate chemokine activity. GAGs can be classified into four groups : the hyaluronic acid type, the chondroitin / dermatan sulfate type, the Heparan/Heparin type, and the Keratan type. They are acidic polysaccharides made of disaccharide repeating units (ranging from 40 to 100) which consists of uronic acid and hexosamines. Except for the hyaluronic acid, epimerization at the C-5 position of uronic acids, and N- and O- sulfation provide numerous sources of microheterogeneities. GAGs assume extended structures in aqueous solutions because of their strong hydrophilic nature based on their extensive sulfation, which is further exaggerated when they are covalently linked to core proteins. They hold a large number of water molecules in their molecular domain and occupy enormous hydrodynamic space in solution. A complementary remarkable property of the glycosaminoglycans, which is particularly significant in heparan sulfate and heparin, is their capability to specifically interact with a number of important growth factors and functional proteins. Such interactions are often crucial to the biological functions of these proteins. Hyaluronic acid ?4(–?-D-GlcA-1?3-?-D-GlcNAc-1?)n Chrondroitin / Dermatan ?4(–?-D-GlcA-1?3-?-D-GalNAc-1?)n Sulfate type ?4(–?-L-IdoA -1?3-?-D-GalNAc-1?)n Sulfation at position 2 of IdoA and 4 and 6 of GalNAc Heparan sulfate / Heparin ?4(–?-D-GlcA-1?4-?-D-GlcN-R-1?)n ?4(–?-L-IdoA -1?4-?-D-GlcN-R-1?)n with R = -COCH3 or SO3- Sulfation at position 2 of GlcA and 3 and 6 of GlcNR Keratan sulfate ?3(–?-D-Gal-1?3-?-D-GlcNAc-1?)n Sulfation at position 6 of GlcA and 6 of GlcNR GAG-Fig-1 Fig. 1 A proteoglycanglycan : a large molecular complex formed by the linking of many GAGs (composed of oligosaccharide chains attached to the core protein) through a long hyaluronate strand GAG-Fig-2 Fig. 2 A schematic representation of a glycosaminoglycan (Mucopolysaccharide) its location and density Hyaluronic acid : Hyaluronan Hyaluronan, alos know as hyaluronic acid, is the only unsulfated member of the glycosaminoglycan family. It is found in mammalian connective tissues, where it forms the central core of the proteoglycan aggregate. Apart from its role in connective tissues, other known biological functions of hyaluronan occur in cartilage, umbilical cord, cornea…. reference-in-status-bar. There has been an extensive number of X-ray diffraction investigations on well-oriented fibers incorporated different couner-ions (sodium, potassium, calcium) as well as some molecular modeling studies aiming

at characterizing the conformational behaviour of the polysaccharide in different environments. All these investigations explored the polymorphic behaviour of hyaluronan. GAG-Fig-3 Fig. 3 A 2D representation of the Hyaluronan structure From X-ray investigation, seven polymorphs have been characterized which can be classified under three major categories that range form 4-fold to 3-fold left-handed helices. These are only some of the possible theoretical helical conformations that hyaluronan can adopt. GAG-Fig-4 Fig. 4 A schematic drawing of hyaluronic acid segment. Each disaccharide repeat has the potential to participate in 4 hydrogen bonds (2 across the 1?4 linkage and 2 across the 1?3 linkage) The 3-fold conformation is limited to a fully stretched value of 9.5 Å for the disaccharide repeat. The 4-fold conformation is more tolerant to stretching and upon external conditions such the nature of the cation, and/or the relative humidity, can adopt conformations such that the length of the disaccharide repeat may vary from full extension of 9.5 Å to 8.5 Å. Under the influence of potassium ions, a double helical structure may occur. Sodium Hyaluronate I anhydrous. The fiber diffraction diagram for sodium hyaluronate in its anhydrous form can be interpreted based on a 4-fold (43) helix symmetry reference-in-status-bar. The molecular model best compatible with the X-ray data is a left-handed helix having a repeat of 33.94 Å. with the noticable occurrence of two interesidues hydrogen bonds. The orientation of the hydroxymethyl group in the GlcNAc residue is gauche-trans; whereas the carboxylated orientation in the glucuronate residue is c = -91°. a. GAG-Fig-5a-hyaluronate-I-Na b. GAG-Fig-5b-hyaluronate-I-Na c. GAG-Fig-5c-hyaluronate-III-Na Fig. 5 A 3D representation of (a.) hyaluronate I sodium (b.) hyaluronate II sodium (c.) hyaluronate III sodium The space group symmetry has been identified as P43212, and two helices passes through the unit cell. They are oriented in an anti-parallel fashion, into a tight packing anhydrous arrangement. Sodium being the only guest molecule interacts with the carboxylate group. It dispays an octahedral coordination. Anti-parallel and parallel helices interacts via hydrogen bonds, respectively O3H….O62 (2.59 Å) and O2H…O6 (2.37 Å) and O6H…O7 (2.79 Å). (Note the O2H…O6 distance of 2.37 Å is notably too short thereby indicated a minor flaw in the elaboration of the model). Sodium Hyaluronate I hydrated. Upon control relative humidity at 75%, one observes an alteration of the unit cell and space group symmetry of the anhydrous form reference-in-status-bar. This is accompanied by a change in helix symmetry from 43 to 21 in a way such that the new repeat consists of the two disaccharides. This hydrated structure corresponds to an amount of 4 water molecules per tetra saccharide repeat, with still one sodium ion near every carboxylate group. The presence of water does not bring significant change in the intra-chain hydrogen–bonding, and brings some perturbation in the interchain hydrogen bonds. GAG-Fig-6 Fig. 6 A 3D representation of the structure reported by Guss et al. reference-in-status-bar. Potassium Hyaluronate I. Upon replacement of sodium per potassium, and while maintaing a relative humidity at 85% an X-ray fiber diffractogram that can be indexed in a tetragonal space, P43212 group occurs reference-in-status-bar. Whereas some changes are observed at the level of intrahelical hydrogen bonds, the rest of the structural features remains fairly close to the previous sodium salt. The packing arrangement with one potassium ion and two water molecules is analoguous to that described for the sodium salt. GAG-Fig-7 Fig. 7 A 3D representation of the structure of Potassium Hyaluronate I Potassium Hyaluronate II. Another fiber diffraction pattern can be found for potassium hyaluronate reference-in-status-bar which crystallizes in an orthorhombic unit cell. With respect to the previous structure, the helix symmetry is slightly perturbed and the extension of the disaccharide repeat is 8.85 Å, i.e. about 0.35 Å longer than in the other polymorph. Such a change does not preclude the occurrence of two intrachain hydrogen bonds, but only one of the two acetamido groups is able to make an intrachain hydrogen bond. Each tetrasaccharide is associated with two potassium ions which lie in the vicinity of the carboxylate groups, and two water molecules. The potassium ion is seven fold coordinated , throughout intarction with a carboxylate oxygen atom, a water molecules, and five

remainig ligands from surrounding helices. Two hyaluronate helices cross the unit cell; they are arranged in an anti-parallel fashion, but with rotational arrangement being 90° relative to that in the orthorhombic form. This illustrates some of the low energy interchain arrangements that hyaluronate chains can take. These structural models have been confirmed throughout independant investigations reference-in-status-bar. Sodium Hyaluronate III. This is another hydrated polymorph that is obtained when some of the fibers are prepared at 40°C with 90% relative humidity. Under these conditions, diffraction patterns are obtained, that correspond to the occurrence of 3-fold helix symmetry reference-in-status-bar. A trigonal space group corresponds best to the indexaton of the pattern. A three-fold left handed helix having full extension of 9.5 Å for the disaccharide repeat characterises helical chain conformation. Whereas the acetamido group does not participate in any intrachain hydrogen bonding, the low energy helix conformation is stabilized by hydrogen bonds. GAG-Fig-8 Fig. 8 A 3D representation of the structure of Sodium Hyaluronate III The unit cell of the P3121 space group encompasses two helices, one sodium ion per disaccharide and about 3.5 water molecules. The sodium ions have a total of 6 ligands and an octahedral coordination. The helices are aligned in an anti-parallel arrangement, and are associated via carboxylate…sodium…O2 (glucuronate) / O7 (carbonyl interactions), with a further stabilisation by an interchain hydrogen bond (O6-H… O3 (2.72 Å). Calcium Hyaluronate. X-ray fiber diffractogram of calcium hyaluronate obtained at 75% relative humidity (and higher) can be interpreted on the basis of a 3-fold helix symmetry, occurring in a trigonal spce group, P3112, with a unit-cell having large dimensions. reference-in-status-bar. Such a left-handed 3-fold helical symmetry implies that 3 disaccharides constitute one turn of the helix. This can be obatined while relaxing some of the conformation about the glycosidic angles, but still maintaining some of the intra-chain hydrogen bonds. As a result, the hydroxymethyl groups undergo some orientational changes. GAG-Fig-9 Fig. 9 A 3D representation of the structure of Calcium Hyaluronate Potassium Hyaluronate III. When prepared at low pH (3.0 – 4.0) well oriented fibers of potassium hyaluronate correspond to a hemi or fully protonated state of the polysaccharide. The interpretation of the corresponding X-ray diffractogram reference-in-status-bar has been debated and as a result, a careful re-examination has produced an excellent double helix in which the inter-twinned chains are closey interacting, not only by direct hydrogen bonds, but also water bridges. Each chain is extensively hydrogen bonded across the glycosidic linkages. GAG-Fig-10 Fig. 10 A 3D representation of the structure of Potassium Hyaluronate III The cation are regularly placed between the duplexes, and have been considered to be redundant for the stability and the survival of the double-helical structure. Inter double-helix hydrogen bonds are mediated between hydroxymethyl and N-acetly groups. Rationalising the conformational versatility of hyaluronan chains The conformational polymorphism displayed by hyaluronan chain is the solid state has been rationalised via computational methods reference-in-status-bar that could predicit the occurrence of three different helical conformations 32, 43, 21, and explained them by weak energetic costs (<0.7 kcal/mol.dimer) associated to small variations in ?, ? glycosidic torsion angles leading to the different types of helices. Other helical conformations which are likely to occur were also predicted. GAG-Fig-11 Fig. 11 Helices of hyaluronic acid predicted by molecular modelling Finally the feasibility of a double helix with anti-parallel strands for hyaluronan chains was evaluated and compared to the two models derived from X-ray experiments. The calculations demonstrated that the formation of a duplex by chain folding is possible; though marginally observed, the anti-parallel arrangement of the chains in the double helix is more favourable than the parallel one for hyaluronic acid. The energetic evaluation of the X-ray models, in comparison to the established theoretical model, indicated that the first model originally proposed by Sheehan and coworkers is not viable, and that the second one proposed by Arnott et al. is much more reasonable, with may be an over-evaluated hydrogen bond network. Chondroitin sulfate The repeating unit of

chondroitin is a disaccharide similar to that in hyaluronan, except that GalNAc replaces GlcNAc. GAG-Fig-12 Fig. 12 Chemical repeat unit of Chondroitin sulphate In the native state, the galactose residues are O-sulfated (either at position 4 or at position 6). Molecular modelling investigations reference-in-status-bar reference-in-status-bar have indicated the the occurrence of chondroitin-4-sulfate chains as left handed in which the extension of the disaccharide repeat vries from 9.3 to 9.8 Å. In the case of chondroitin-6-sulfate similar trends have been postulated to occur. Sodium Chrondroitin 4-sulfate and Potassium Chondroitin 4-sulfate. For both salts X-ray analysis from fiber diffractogram has established the occurrence of a left-handed 3 fold helix with the sulfate groups pointing towards the periphery where they interact with one surrounding cation and water molecules reference-in-status-bar reference-in-status-bar. In both cases the helix is stabilised by a O3H…O5 hydrogne bond (O…O distance = 2.74 Å) across every 1?4 linkage. Within the P3221 space group, the chains are packed in the unit cell in an anti-parallel fashion. The potassium ions have an octahedral coordination. GAG-Fig-13 Fig. 13 A 3D representation of the structure of Sodium Chondroitin 4-sulphate GAG-Fig-14 Fig. 14 A 3D representation of the structure of Potassium Chondroitin 4-sulphate Sodium Chrondroitin 4-sulfate. The replacement of monovalent ions with divalent ions such as calcium induces a conformational transition of the chain from 3 fold helix to 2-fold helix symmetry reference-in-status-bar. This is accompanied by an extension of the pitch. As a result, the length of the disaccharide repeat is 9.82 Ang. which corresponds to a maximum extension and it is accompanied by the occurence of two intrachain hydrogen bonds O3H….O5 (2.64 Å) across the 1-4 linkages and O2H…O7 (2.99 Å) across the 1?3 linkages. In the unit cell, the chains are arranged in anti-parallel fashion. The calcium ion are playing an important role assuming the coordination via bridges involving the sulfate groups. Dermatan sulfate Dermatan 4 sulfate differs from chondroitin by having ?-L-iduronate instead of ?-D-glucuronate residues. As with the other members of the family of Glycosaminoglycan, dermatan sulfate displays polymorphism. Three distinct forms of molecular structure and packing arrangements have been identified from X-ray diffraction patterns. A first polymorph reference-in-status-bar has been described as a three-fold left handed helix with a repeat of 28.2 Å crystallizing in a trigonal packing arrangement analoguous to that of potassium chondroitin 4-sulfate. Fig. 15 A 3D representation of the structure of Dermatan sulphate – allomorph I A second polymorph displays a two-fold helix pitch of 18.8 Å and bears some similraity to the structure of calcium chloride chondroitin 4-sulfate. Fig. 16 A 3D representation of the structure of Dermatan sulphate – allomorph II A fairly novel form was found for a third polymorph which was identified as a right-handed helix with 83 symmetry (disaccharide extension of 9.2 Å) made up of a tetrasaccharide in which two slightly different disaccharides repeat. It this model, chains are packed in an anti-parallel fashion in the P43212 tetragonal space group. Fig. 17 A 3D representation of the structure of Dermatan sulphate – allomorph III Keratan 6-sulfate X-ray diffraction patterns from stretched films of keratan 6-sulfate show only continuous intensities from which one can identify meridional reflections on even layers indicating the occurrence of a two-fold helix with an extension of 9.45 Å per disaccharide reference-in-status-bar. Such a conformation may correspond to a sinuous helix that can incorporate the occurrence of an inter-residue hydrogen bond between O3H and O5 (distance : 2.8 Å). Not unexpectedly the sulfate gorups occur on the periphery of the helix, thereby providing points of interactions with the counter ions and further establishing associative interhelices interactions. Fig. 18 A 3D representation of the structure of Keratan 6-sulphate Heparin Heparin is the most therapeutically (and commercially) important member of the GAG family. For the first 30 years of its clinical use, not much was known about its structure except that iy was composed of glucosamine and uronic acid, with heavy sulfate substitution. Heparin has much higher specific anticoagulant activity than any other sulfated polysaccharides. The main structural components of heparin were established as containing N- and 6-O-sulfated ?-D-glucosamine, 2-O-

sulfated a-l iduronic acid along with N-acetyl D-glucosamine and ?-D-glucuronic acid reference-in-status-bar. Determination of the pentasaccharide structural motif in heparin with high affinity for antithrombin came after years of laborious works from groups in France, Sweden, Italy and the USA. Fig. 19 Chemical structure representations of heparin and heparin sulphate (a) Precursor polysaccharide for both heparin & heparan sulphate, ?-D-GlcA-1,4-?-D-GlcNAc, which is also a predominant structure in heparan sulphate. (b) Post-polymerization, enzymes remove (i) remove N-acetyl and replace it with N-sulphate (ii) epimerize ?-D-glucuronate residues at the resucing side of N-sulphate to ?-L-iduronate (iii) introduce 2-O-sulphate and (iv) introduce 6-O-sulphate. The final structure from all these changes results of these transformations leads to the formation of the main repeating unit of heparin ?-L-IdoA[2SO3-]-(1,4)-?-D-GlcNSO3-[6SO3-] (c) The pentasaccharide in heparin ?-D-GlcNAc[6SO3-]-(1?4)-?-D-GlcA-(1?4)-?-D-GlcNSO3-[3, 6-diSO3-]-(1?4)-?-L-IdoA[2SO3-]-(1?4)-?-D-GlcNSO3-[6SO3-] which is the minimal structure with high affinity for anti-thrombin, the serine protease in plasma which inhibits several coagulation enzymes. X-ray fiber diffraction pattern has been obtained for heparin sulfate reference-in-status-bar. The sodium form of heparan sulfate used in the investigation had few sulfate groups which were unevenly distributed along the polysaccharide chain. Since the presence of ?-L-iduronic acid was restricted to the sulfated portion of the polysaccharide, it was deduced that sections composed of alternating d-glucuronic acid and N-acetyl d-glucosamine units occupied a major portion of the macromolecule. From the X-ray pattern obtained from heparin sulfate (see Fig. 20) the layer-line spacing of 18.6 Ang. Is observed, along with a meridional reflection on even layer lines; giving a value for the axially projected disaccharide repeat (h) of 9.3 Ang. On the basis of the value of the repeating unit and primitive molecular modeling, a two-fold helical conformation incorporating intra-chain hydrogen bonds across both glycosidic linkages was established. Fig. 20 X-ray fibre-diffraction patterns obtained from heparan sulphate The ?-D-glucuronic and &alpha-D-N-acetyl glucosamine makes up most of the structure of heparan sulfate, interrupted by N-sulfated iduronate – containing sequences, which have been denotated as S-regions. It is through these heparin-like S-domains that heparan sulfate interacts with proteins. Conformational studies of heparin and molecular modeling have shown that the polysaccharide chain has a relatively well-defined shape, regardless of its patter, of sulfate substitutions. Nevertheless, sequence determines the three-dimensional structure of the chains, at more than one level. Detailed sequences of highly sulfated regions may influence affinity for specific interactions with proteins. In addition, the length and spacing of these highly sulfated sequences which are separated by unsulfated domains may also be crucial components. The sulfated S-domains offer space for internal dynamics of the conformationally flexible iduronate rings. New studies of the relative degrees of flexibility of sulfated and unsulfated domains lead to an overall model of heparin/heparan sulfate in which proteins-dinding, highly sulfated S-domains with well-defined conformations are separated by more flexible domains reference-in-status-bar.

Category

1. News