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Minireview

Structural view of glycosaminoglycan–protein interactions

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Dedicated to the memory of Professor Nikolay K. Kochetkov

Abstract—The essential role of protein–glycosaminoglycan interactions in the regulation of various physiological processes has been recognized for several decades but it is only recently that the molecular basis underlying such interactions has emerged. The different methodologies to elucidate the three-dimensional features of glycosaminoglycans along with the interactions with proteins cover high resolution NMR spectroscopy, X-ray crystallography, molecular modeling, and hydrodynamic measurements. The structural results that have accumulated have been organized in databases that allow rapid searching with entries related either to the type of glycosaminoglycan or the type of protein. Finally, three selected examples enlightening the complexity of the nature of the interactions occurring between proteins and glycosaminoglycans are given. The example of interactions between heparin and antithrombin III illustrates how such a complex mechanism as the regulation of blood coagulation by a specific pentasaccharide can be dissected through the combined use of dedicated carbohydrate chemistry and structural glycobiology. The second example deals with the study of complexes between chemokines and heparin, and shows how multimolecular complexes of proteins can be organized in space throughout the action of glycosaminoglycans. Again, the synthesis of chemical mimetics offers an unexpected route to the development of novel glycotherapeutics. Finally, the area of enzymes/glycosaminoglycans complexes is briefly covered to realize the limited knowledge that we have for such an important class of biomacromolecular complexes.

Keywords: Glycosaminoglycans; Interactions; Molecular modeling; Crystallography

Contents

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1. Introduction

Glycosaminoglycans (GAGs) are linear polysaccharides present on all animal cell surfaces and in the extracellular matrix. They are usually found attached covalently to core proteins forming the proteoglycan family. Each tissue produces specific repertoires of glycosaminoglycans (GAGs), some of which are known to bind and regulate a number of distinct proteins, including chemokines, cytokines, growth factors, morphogens, enzymes, extracellular matrix, or adhesion molecules. GAGs can be classified into four groups: the hyaluronic acid type, the chondroitin/dermatan sulfate type, the heparan sulfate/heparin type, and the keratan type (Fig. 1). These polysaccharides consist of a repeating disaccharide unit, composed of an *N*-acetyl-hexosamine and a hexose or hexuronic acid, either or both of which may be sulfated on different positions. They have molecular masses ranging from few kDa to 2×10^4 kDa. Hyaluronic acid (HA) consists of β-D-glucuronic acid (1→3)-linked to 2-acetamido-2-deoxy-β-D-glucopyranose (linkages between disaccharide units are 1→3). It is the only GAG that does not contain sulfate groups. Keratan sulfate (KS) disaccharide consists of β-D-galactose (1→4)-linked to 2-acetamido-2-deoxy-β-D-glucopyranose. In chondroitine sulfate (CS), the disaccharide unit is a β-D-glucuronic acid (1→3)-linked to a 2-acetamido-2-deoxy-β-D-galactopyranose (linkages between disaccharide units are 1→4). Galactosamine can be sulfated on the C-4 or C-6 (or both) positions. β-D-Glucuronic acid in CS is converted to α-L-iduronic acid in dermatan sulfate (DS) by C-5 epimerization. Repeating disaccharide units in heparin and heparan sulfate (HS) are either α-L-iduronic acid (1→4)-linked to glucosamine or β-D-glucuronic acid (1→4)-linked to glucosamine. The glucosamine is either N-sulfated or N-acetylated, and can be ester O-sulfated at the C-3 or C-6 position while the uronic acid can be sulfated on C-2 position. Depending on the nature, the extent and the position of these sulfate groups along the chain, these molecules can display a very large variety of structures. In heparan sulfate, these modifications occur in restricted domains (called S-domain, usually of about 5–10 disaccharides) that are hyper variable, and interspersed within poorly sulfated regions (called A-domain). Heparin is similar to HS but with a degree of sulfation, which is much higher and homogeneously distributed along the chain. Except for the hyaluronic acid, epimerization at the C-5 position of uronic acids, and N- and O-sulfation provide numerous sources of micro-heterogeneity. GAGs assume extended structures in aqueous solutions because of their strong hydrophilic nature based on their extensive sulfation patterns, which is further enhanced 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

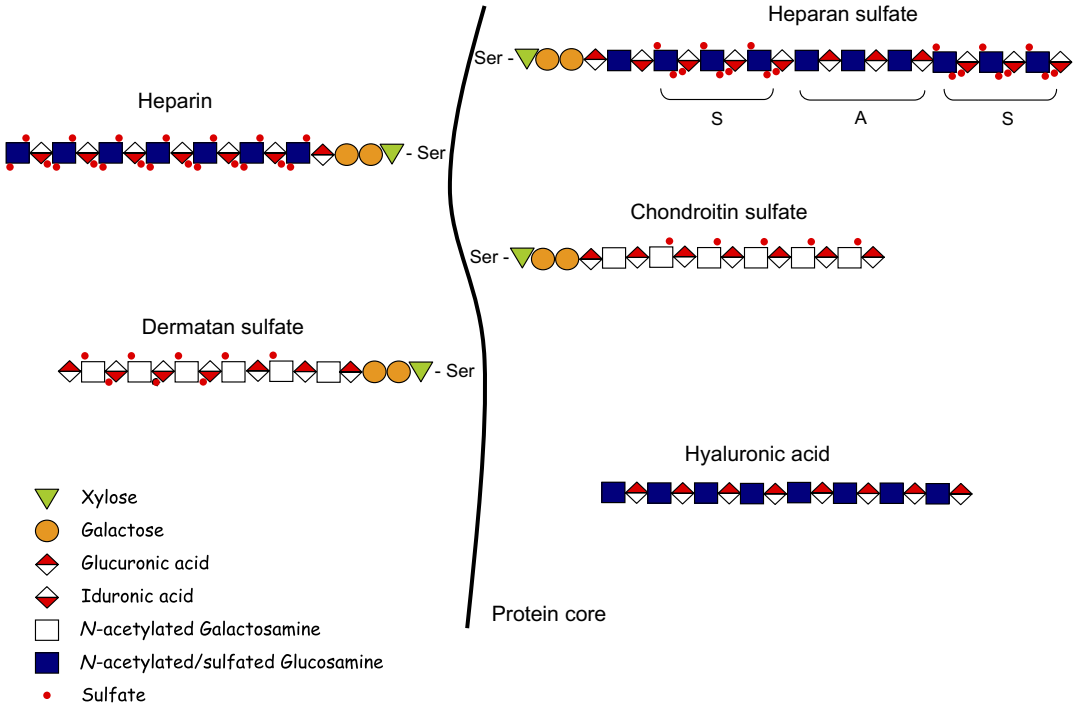

Figure 1. Schematic representation of the different glycosaminoglycans.

factors and functional proteins. These interactions are often crucial to the biological functions of these proteins. As such, glycosaminoglycan domains play a role in the regulation of many processes, such as hemostasis, growth factor control, anticoagulation and cell adhesion, inflammation, pathogens attachment.[1] Given the importance of protein–glycosaminoglycan interactions, oligosaccharide fragments are important targets for drug design, an area where major successes have been obtained. Considerable effort has been invested in establishing the structure of glycosaminoglycan fragments with biological activity, either in solution or in their bioactive conformation, that is in interaction with a protein.

Recognition of glycosaminoglycan oligosaccharides by proteins depends on their structural arrangements and such knowledge is required for the rational design of carbohydrate-derived drugs. Several approaches have been used to characterize the three-dimensional structure of protein/glycosaminoglycan complexes. The complexes involving heparan sulfate, ubiquitously present on animal cell surfaces and in extracellular matrix (ECM) and heparin, the highly sulfated analogs, have been most studied.[2] In solution, the flexibility of certain glycosidic linkages produces multiple conformations, which co-exist in equilibrium and NMR alone is often not sufficient for defining the population of conformers.[3] Protein crystallography had met little success for a long time, but in the recent years, the structures of several proteins of biological interest have been solved in complexes with GAG ligands. Molecular modeling has also been demonstrated to be a tool of major importance for establishing the structural features of GAG/protein complexes.[4] The present article is devoted to the second point. It starts with a brief presentation of the methodological tools that are available for characterizing the three-dimensional features of the complexes; it proceeds with selected examples, that illustrate the variety of the binding modes that GAG exhibit toward proteins.

2. Different methodological approaches

2.1. NMR spectroscopy

NMR has been used to characterize the conformational changes that can be observed on either the oligosaccharide ligand or the protein. In the case of heparin fragments, nuclear Overhauser effects (NOEs) and coupling constants have been successfully used to characterize the conformation of the flexible iduronate ring that exists as an equilibrium between $^{1}C_{4}$, $^{4}C_{1}$, and $^{2}S_{O}$ conformers in the free state.[5] When looking at the interaction between antithrombin III and a synthetic analog of the natural pentasaccharide ligand, the skew $^{2}S_{O}$ form appears to be favored upon binding and is accompanied by the conformational change of one of the glycosidic linkages.[6] Oppositely, histamine binds to heparin trisaccharides with the iduronate ring in the $^{1}C_{4}$ conformation.[7] The fibroblast growth factor FGF-1 binds to heparin-like synthetic hexasaccharide with no locking of the iduronate ring.[8]

High field NMR can be used to analyze the conformational change of protein or peptide upon GAGs binding. Many of the GAGs binding proteins are small enough so that their structure can be determined by NMR. Comparison of the free and hyaluronan-bound conformation of the link module of human TSG-6 revealed the opening of a groove upon binding.[9] In addition, NMR experiments at a range of pH values identified protein groups that titrate due to their proximity to a free carboxylate of hyaluronate ligand.[10] For growth factor FGF-1, comparison of the relaxation data for the free and the complex with heparin hexasaccharide demonstrated that some portion of protein backbone presents reduced motion in its bound state.[11] Very recently, the solution structure of a human FGF-1 monomer activated by a hexasaccharide heparin analogue confirmed that the dimerization of the protein is not an absolute requirement for biological activity.[12] It is also possible to use NMR for studying the interaction of peptides with GAGs such as the C-terminal peptide of interferon-gamma (IFNγ) with heparin oligosaccharides.[13] Marked changes have been observed for the chemical shifts of both peptide and oligosaccharide compared with the free state, providing evidence for strong electrostatic interactions between the charged side chains of the protein and the sulfate groups of heparin.

2.2. X-ray crystallography

The crystallization of protein/GAG complexes appears to be specially challenging due (1) to the difficulty to isolate or synthesize homogeneous GAG fragments and (2) to the nature of the ionic interaction of GAGs with protein that may allow several binding modes, generating heterogeneity. Significant and rapid progresses arising from the use of synchrotron radiations are providing access to highly resolved three-dimensional structures. As a consequence, the first complex, obtained in 1997 and involving antithrombin III and a synthetic analog of the pentasaccharide fragment of heparin unraveled the occurrence of a very specific sulfation pattern, and therefore a very unique binding mode to its receptor.[14]

Recent crystallographic elucidations have provided structural information about the binding of some low molecular weight oligosaccharides from GAGs in interaction with proteins. These are chemokines, complement proteins, extracellular matrix proteins, enzymes, growth factors, and viruses. Most of these GAG binding proteins establish salt bonds between basic groups of amino acid

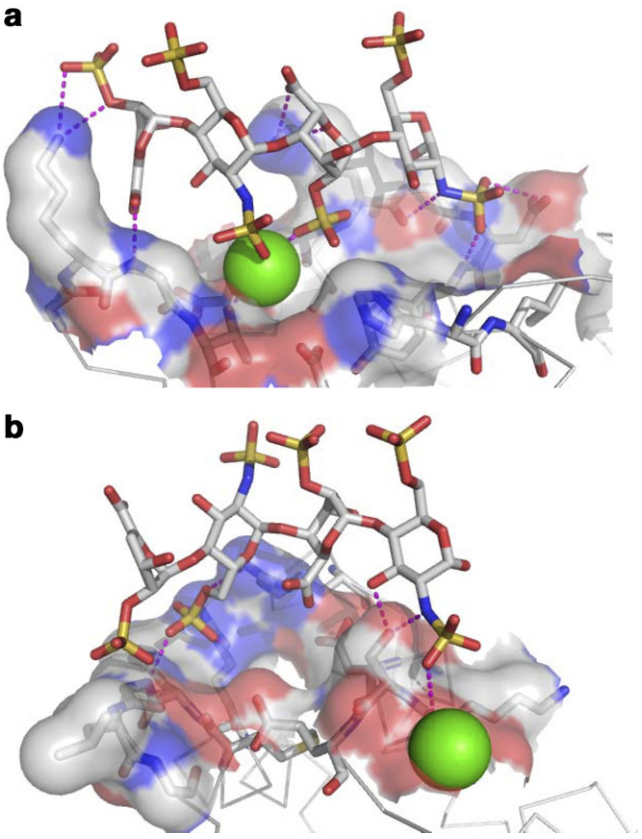

Figure 2. Graphical representation of the heparin binding site of annexin A2,[15] and annexin V.[16] In both structures, the accessible surface of the protein is displayed. Hydrogen bonds are represented by violet dotted lines and calcium ions by green spheres. Plots are made using Pymol (Delano Scientific LLC).

side chains and sulfated or acidic groups of the ligand. The involvement of bridging calcium ions that is observed in several lectins is very rarely occurring in heparin binding. Only annexins, a family of calcium-dependent membrane-binding eukaryotic, require calcium for binding to heparan sulfate. In the crystal structure of annexin A2/heparin oligosaccharide complex,[15] two sulfate groups of the carbohydrate ligand participate in the coordination of one calcium ion that bridges to two oxygens of the protein main chain (Fig. 2a). In the other available crystalline complex, that is, annexin V/heparin oligosaccharide,[16] the calcium ion does not interact directly with the ligand but induces the conformation of protein loops necessary for binding (Fig. 2b).

2.3. Molecular modeling

The docking of glycosaminoglycan oligosaccharides onto protein binding sites presents two major difficulties: (i) the shape of the binding site does not generally adopt a pocket or crevasse shape that will allow for easy identification; (ii) both the ligand and the protein present high flexibility of side chains. Indeed, taking into account all possible conformations of oligosaccharide sulfate and hydroxyl groups, all rotamers of lysine and arginine side chains together with different conformations and orientations of the oligosaccharide ligand represents a computational problem of too high complexity to be solved at the present time.

The location of the glycosaminoglycans binding site at the protein surface can be performed by looking for the most positively charged patches of amino acids. The MOLCAD program[17] associated with the GRID program has been proven to be useful for mapping the most favorable position of sulfate groups at the surface of the proteins.[4] When putative binding regions are identified, the AUTODOCK program[18] may be used for predicting the orientation of the oligosaccharide on the protein surface. Such approach has been successively used for predicting the binding geometry of heparin to endostatin,[19] and to a variety of chemokines (see below).

2.4. Other methods

The study of GAGs and their complexes is made difficult by their heterogeneous structure, the redundancy of the sulfate groups leading to multiple possibilities of interaction, and the possible flexibility of the partners. Most of the 3D structures of protein/GAG complex solved up to now involve relatively small oligosaccharides (di- to hexasaccharides). This is particularly true for heparin–heparan sulfate, for which heterogeneity (and thus purification difficulty) rapidly increases with length. However, a number of proteins interact with rather large GAG fragments. This has been first evidenced with the study of interferon-gamma (IFNγ), which in contrast to many other heparin binding proteins, does not significantly interact with isolated S-domains (i.e., heparin-like fully sulfated oligosaccharides). The IFNγ binding HS fragment encompasses as many as 24 disaccharide units and consists of two terminal sulfated domains, each binding to one IFNγ monomer, separated by a nonsulfated G1cA-rich sequence.[20] Such a finding points toward an important general concept: properly spaced sequences of the appropriate structure along a GAG chain may form functional domains that act in a concerted manner. This binding motif, called SAS for sulfated–acetylated–sulfated structure has been subsequently found to be recognized by a number of other proteins (Fig. 3), including platelet factor 4 (PF4),[21] interleukin-8 (Il-8),[22] MIP-1α,[23] RANTES,[24] endostatin,[25] or vascular endothelial growth factor (VEGF).[26] Most of these proteins are either multimeric in solution or multimerised upon binding to HS. The requirement for two distant S-domains reflects the fact that at least two basic domains of different subunits have to interact with the HS polymer to reach high binding constants.

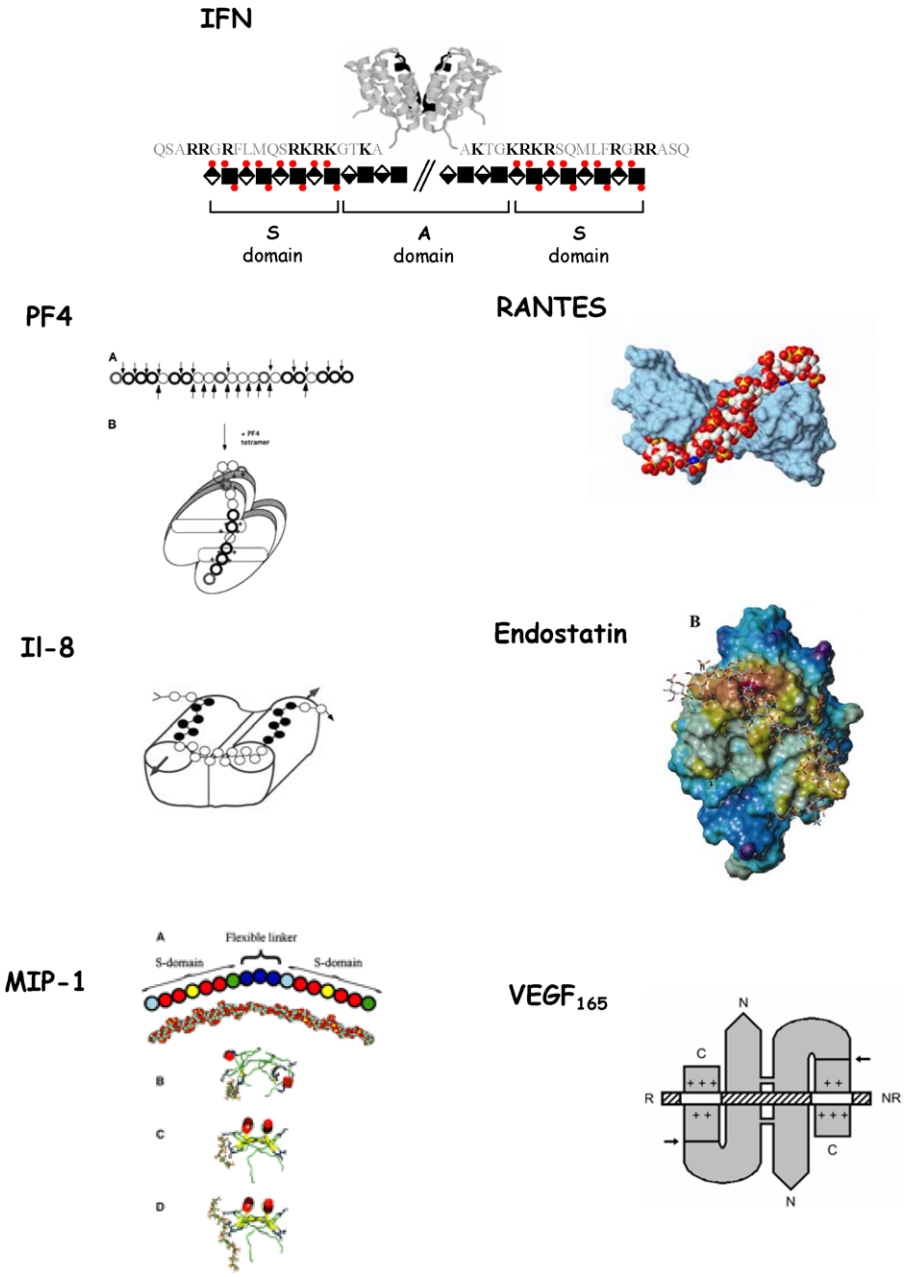

Figure 3. Graphical representation for sulfated–acetylated–sulfated (SAS) heparan sulfate fragment interacting with protein receptors including IFNγ,[20] platelet factor 4 (PF4),[21] RANTES,[24] interleukin-8 (Il-8),[22] endostatin,[25,19] MIP-1α,[23] and endothelial growth factor (VEGF).[26] (Adapted with permission from the American Chemical Society, the American Society for Hematology and the American Society for Biochemistry and Molecular Biology).

Due to the structural heterogeneity of HS, such complex binding motif is obviously impossible to obtain in pure form and in large quantity from natural sources thus precluding any high resolution structural studies. The synthesis of mimics of such structures in which S-domains were artificially spaced by different linkers has been reported and may be helpful for structural approaches.[27]

Hydrodynamic methods can be appropriate to understand the structural organization of such complexes, and have been used to study the IFNγ-heparin association.[28] In that study, different stoichiometries and hydrodynamic radii for IFNγ-heparin complexes were deduced from complementary sedimentation velocity and size exclusion chromatography experiments. In combination with hydrodynamic modeling, where properties of rigid beads assemblies representing the complexes were calculated and compared to experimental data, these approaches permit to evaluate the compactness of the complex, the eventual conformational changes of the partners and the possible location of the protein along the GAG chain.

Because HS activity might help organizing multimolecular complex of proteins thanks to their SAS design, such method should be useful in a number of other systems. This includes, for example, fibroblast growth factors (FGFs) and their various receptors, which form ternary complexes with HS, and where signaling also depends on HS domains appropriately spaced on the molecule.[29]

3. Databases and selected examples

3.1. Databases

About 60 crystal structures of complexes between protein and glycosaminoglycans are available in the Protein Data Bank.[30] These structures, together with structural and bibliographic information have been gathered in a database of three-dimensional structures set at CERMAV (http://www.cermav.cnrs/glyco3d). The different proteins have been co-crystallized with heparin oligosaccharides as listed in Table 1. Most of them are of animal origin with the exception of one bacterial enzyme and two viral proteins. Enzymes are limited to only two cases: one heparinase and one sulfotransferases illustrating that a large number of protein interacting with heparin sulfate are receptors.

Tables 2 and 3 list all crystal structures of complexes between proteins and KS/DS and HA, respectively. In these cases, the complexes have been mostly obtained with enzymes.

3.2. Heparin–antithrombin III interactions

Heparin is recognized by a variety of proteins[31] such as growth factors, chemokines and protease inhibitors of the blood coagulation cascade, etc. Whereas some proteins recognize the most regular regions of heparin, others have affinity only for unique irregularities in this structure. Of particular interest is the dissection of the mechanism of regulation of blood coagulation by a specific pentasaccharide from heparan sulfate present on blood vessels (see Ref. 32 for historical review).

The quest for understanding the molecular basis underlying the anticoagulant activity of heparin was undertaken using all the tools of structural biology. The study of the interaction between the heparin pentasaccharide and its receptor antithrombin III is not a

Table 1. Crystal structures of complexes between protein and heparan sulfate or heparin fragments

PDB	Protein	Origin	Nature	Length of oligosaccharide[a]	Ref.
2BRS	Eosinophil-granule major basic protein (EMBP)	Human	Lectin	disaccharide	49
1G5N	Annexin V	Rat	Extracell. prot.	Δtetrasaccharide[b]	16
2HYU	Annexin A2	Human	Extracell. prot.	Δtetrasaccharide	15
2HYV	Annexin A2	Human	Extracell. prot.	Δhexasaccharide	15
1XT3	Cardiotoxin A3	Cobra	Toxin	hexasaccharide	50
1ZA4	Thrombospondin 1 (N-term domain)	Human	Extracell. prot.	pentasaccharide (Arixtra)	51
1QQP	Foot-and-mouth disease virus capsid	Viral	Capsid	pentasaccharide	52
1RID	Vaccinia complement protein	Viral	Complement	hexasaccharide	53
1U4L	RANTES	Human	Chemokine	Δdisaccharide	45
1U4M	RANTES	Human	Chemokine	Δdisaccharide	45
1BFB	Basic fibroblast growth factor (bFGF)	Human	Growth factor	Δtetrasaccharide	54
1BFC	Basic fibroblast growth factor (bFGF)	Human	Growth factor	Δhexasaccharide	54
1AXM	Acidic fibroblast growth factor (aFGF)	Human	Growth factor	Δhexasaccharide	55
2AXM	Acidic fibroblast growth factor (aFGF)	Human	Growth factor	Δhexasaccharide	55
1E0O	FGF1/ectodomain of receptor FGFR2	Human	Growth factor/receptor	decamer	56
1FQ9	FGF2/ectodomain of receptor FGFR1	Human	Growth factor/receptor	decamer	57
1GMN	N-term domain of hepatocyte growth factor (NK1)	Human	Growth factor	tetrasaccharide	58
1GMO	N-term domain of hepatocyte growth factor (NK1)	Human	Growth factor	tetrasaccharide	58
2FUT	Heparinase II	*Pedobacter heparinus*	Enzyme	Δdisaccharide	59
1T8U	3-*O*-Sulfotransferase 3	Human	Enzyme	Δtetrasaccharide	60
1AZX	Antithrombin	Human	Serpin	synthetic penta	14
1E03	Antithrombin	Human	Serpin	synthetic penta	14
1NQ9	Antithrombin	Human	Serpin	pentasaccharide	61
1XMN	Thrombin	Human	Protease	hexamer	62
1TB6	Antithrombin/thrombin	Human	Serpin/protease	heparin mimetic	38
2B5T	Antithrombin/thrombin	Human	Serpin/protease	heparin mimetic (SR123781)	37
1SR5	Antithrombin/anhydrothrombin	Human	Serpin/protease	heparin mimetic	36
2GD4	Antithrombin/factor Xa	Human	Serpin/protease	pentasaccharide (Fondaparinux)	39

[a] The reported length corresponds to the fragment observed in the crystal structure.
[b] Δ Indicates the presence of ΔUA at the nonreducing end (1,4-dideoxy-5-dehydro glucuronic acid).

Table 2. Crystal structures of complexes between protein and hyaluronan fragments

PDB	Protein	Origin	Length of oligosaccharide[a]	Ref.
1FCV	Hyaluronidase	*Apis mellifera* (Honeybee)	tetrasaccharide	47
1I8Q	Hyaluronate lyase	*Streptococcus agalactiae*	two Δdisaccharides[b]	63
1LXM	Hyaluronate lyase	*Streptococcus agalactiae*	hexasaccharide	64
1C82	Hyaluronate lyase	*Streptococcus pneumoniae*	two Δdisaccharides	65
1LXK	Hyaluronate lyase	*Streptococcus pneumoniae*	tetrasaccharide	66
1LOH	Hyaluronate lyase	*Streptococcus pneumoniae*	hexasaccharide	66
1N7Q	Hyaluronate lyase W291A/W292A	*Streptococcus pneumoniae*	hexasaccharide	67
1N7R	Hyaluronate lyase W291A/W292A/F343V	*Streptococcus pneumoniae*	hexasaccharide	67
1HM3	Chondroitinase AC	*Pedobacter heparinus*	Δdisaccharide	68

[a] The reported length corresponds to the fragment observed in the crystal structure.
[b] Δ Indicates the presence of ΔUA at the nonreducing end (1,4-dideoxy-5-dehydro glucuronic acid).

Table 3. Crystal structures of complexes between protein and chondroitin/dermatan (sulfate) fragments

PDB	Protein	Origin	Length of oligosaccharide[a]	Ref.
1DBO	Chondroitinase B	*Pedobacter heparinus*	Δdisaccharide dermatan sulfate[b]	69
1HM2	Chondroitinase AC	*Pedobacter heparinus*	tetrasaccharide dermatan sulfate	68
1HMU	Chondroitinase AC	*Pedobacter heparinus*	Δdisaccharide dermatan sulfate	68
1OFL	Chondroitinase B	*Pedobacter heparinus*	four Δdisaccharides dermatan sulfate	70
1RWF, 1RWG, 1RWH	Chondroitinase AC	*Arthrobacter aurescens*	two Δdisaccharides dermatan sulfate	71
1HMW	Chondroitinase AC	*Pedobacter heparinus*	tetrasaccharide chondroitin sulfate	68
1OFM	Chondroitinase B	*Pedobacter heparinus*	Δtetrasaccharide chondroitin sulfate	70
1RWF	Chondroitinase AC	*Arthrobacter aurescens*	Δdisaccharide chondroitin sulfate	71
1OJM	Hyaluronate lyase	*Streptococcus pneumoniae*	Δdisaccharide chondroitin	72
1OJN	Hyaluronate lyase Y408F	*Streptococcus pneumoniae*	Δdisaccharide chondroitin sulfate	72
1OJO	Hyaluronate lyase Y408F	*Streptococcus pneumoniae*	Δdisaccharide chondroitin sulfate	72
1OJP	Hyaluronate lyase	*Streptococcus pneumoniae*	Δdisaccharide chondroitin sulfate	72

[a] The reported length corresponds to the fragment observed in the crystal structure.
[b] Δ Indicates the presence of ΔUA at the nonreducing end (1,4-dideoxy-5-dehydro glucuronic acid).

simple task, due to the fact that a conformational change occurs in the protein upon binding.[33] The first model obtained using homology modeling for the protein and hand-docking of the pentasaccharide, allowed the determination of the basic amino acids involved in the recognition of the sulfate and carboxylate groups.[34] A more recent study,[35] making use of several recently developed docking programs, arrived at the same prediction for the binding site. The crystal structure of the complex between antithrombin and the pentasaccharide was a pioneering work in structural glycobiology[14] and it unraveled the contact between the ligand and side chains of arginine and lysine residues on three different helices of the protein. However, one single structure could not describe the very large conformational changes that occur in antithrombin upon activation. Recently the crystal structures of ternary complexes between antithrombin/thrombin/heparin[36–38] and antithrombin/factor Xa/heparin[39] have been solved using inactive forms of the proteases (Fig. 4). The use of synthetic heparin fragments of heparin mimetics has been crucial for such studies since it allowed for using stereochemically pure oligosaccharides that cannot be obtained by fragmentation and purification.

3.3. Complexes between chemokines and heparin

Chemokines are small proteins sharing only one type of fold organized around a triple-stranded antiparallel β-sheet overlaid by a C-terminal α-helix. From this monomer template, several oligomerization modes are observed (for review, see Refs. 40 and 41). All chemokines interact with heparan sulfate and this binding affects their localization and therefore their many roles in inflammation. Chemokines may exist in solution as monomers of dimers (sometimes tetramers) but in most cases they appear to bind GAGs in the dimeric or tetrameric state. Depending on the dimerization mode and positions of basic amino acids in the peptide sequences, chemokines will present positively charged clusters on their accessible surfaces that define several possibilities for binding heparan sulfate.[40,41] Docking simulations predicted that IL8/CXCL8 binding site is orthogonal to the two α-helices,[35,40] while SDF-1α/CXCL12 one runs parallel to the β-sheet.[42] Different heparan sulfate binding modes have been proposed for RANTES/CCL5,[24] MIP-1α/CCL3,[23] and CDF/CX3CL1 illustrating the varieties of the type of interaction that may exist (Fig. 3). Two chemokines, PF4 and MCP-1/CCL2 are

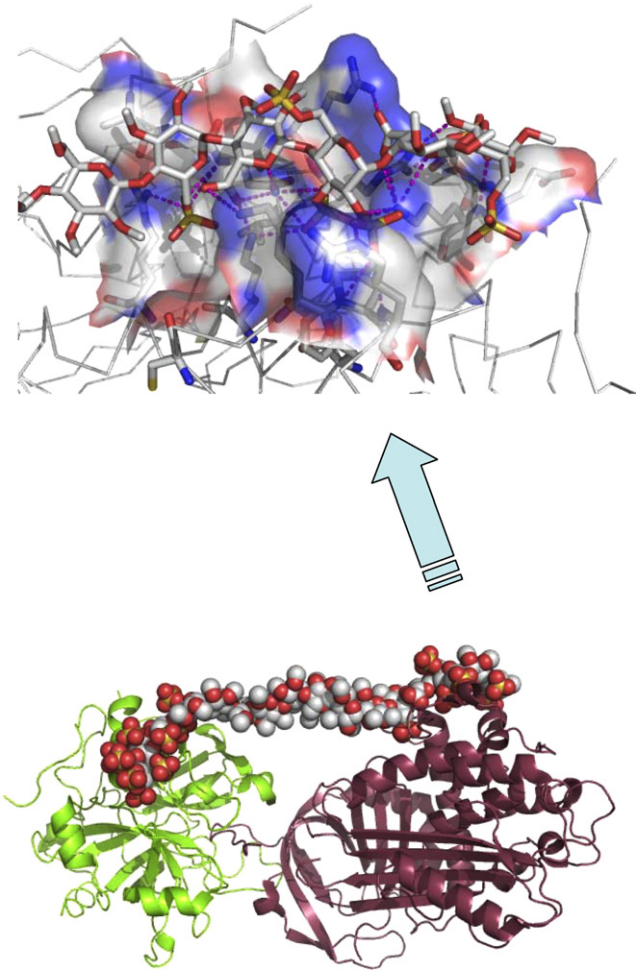

Figure 4. General view (bottom) of the crystal structure of ternary complexes between antithrombin (violet ribbon), thrombin (green ribbon), and heparin analog.[36,38] Top: blow up of the binding site of antithrombin interacting with the specific heparin fragment.

predicted to bind heparan sulfate in the tetrameric state.[43,44] Complexes of chemokines with heparin fragments have been very difficult to obtain. Only the crystallographic structures of the chemokine RANTES in the presence of heparin-derived disaccharide analogs have been determined by X-ray crystallography; it provides a description of the molecular interaction, including the occurrence of unexpected associations in the 30s loop and at the amino terminus.[45]

3.4. Enzymes/GAGs complexes

While heparin fragments have been mostly crystallized with a variety of different proteins, the other GAGs have been co-crystallized with two enzymes, chondroitinases and hyaluronidases. Disaccharide and tetrasaccharide fragments of HA, DS, and CS are present in crystal structures of bee hyaluronidase, a glycosyl hydrolase, and several bacterial chondroitine lyases and hyaluronane lyases (Tables 2 and 3). These latter enzymes are β-helical polysaccharide lyases and cleave the β-(1→4) linkage yielding 4,5-unsaturated disaccharides as the product (see review[46]). Hyaluronan lyases are classes of enzymes that degrade hyaluronan, although they have the limited ability to degrade chondroitin and chondroitin sulfate. Mammalian hyaluronases have sequence similarity with the bee enzymes. By degrading hyaluronan, the human hyaluronases participate in the precise regulation of the polysaccharide catabolism, which is involved in the preservation of its biophysical properties. The three-dimensional structures of the bee venom along with the enzyme–tetrasaccharide complex suggest a possible mechanism of action.[47] A large and positive charge and the hydrophobic character of the surface of the cleft allow for binding negatively charged and also hydrophobic substrates.

4. Conclusions

Whereas the essential role of protein–glycosaminoglycans interactions in the regulation of various physiological processes has been recognized for several decades, it is only recently that the molecular basis underlying such interactions has emerged. The combination of powerful methods of structural elucidation, along with availability of synthetic oligosaccharides and their mimetics have played a dramatic role in the understanding of the interactions. A fairly complex image is emerging from the dissection of the mechanism of regulation of blood coagulation by a specific pentasaccharide from heparin present on blood vessels. Considering this example as a prototypical one, it may be anticipated that cases of similar or higher complexity (i.e., induced conformational changes and fits generating dramatic structural rearrangements) may be found in other interactions involving other members of the glycosaminoglycan family. Understanding such relationships between structural and biological functions is of course opening the route to rational design of highly specific therapeutics agents, and the interplay between structural glycobiology and carbohydrate chemistry is obvious. One should not only restrict its attention to the bioactive sequences of GAG oligosaccharides, but also consider the major influence that an ionic polysaccharide may have in terms of spatial occupancy in the matrix. The well documented case of the interaction between interferon-gamma and high oligomers of heparan sulfate indicates how GAG may help in organizing multimolecular complex of proteins. In such case, the functional role is related to the 'polymeric' nature of the substrate. Again, the synthesis of chemical mimetics of such spatial templates offers an unexpected route to develop glycotherapeutics.[48] Our recent comprehension is that GAG may display multiple functions: fine recognition processes are carried on

chains that can induce the formation of functional domains of proteins. This is of course complementary to their functional role occurring from their physico-chemical and rheological properties.

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