Biology of Extracellular Matrix

Mauro S.G. Pavão
Editor

Glycans in Diseases and Therapeutics

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Foreword

I am delighted to provide a foreword to “Glycans in Diseases and Therapeutics” edited by Dr. Mauro Pavao, at his invitation. Although I do not have as much knowledge of all the glycans highlighted in this book as is necessary to introduce the recent advances in individual glycan fields, I found the book intriguing as both a glycobiologist and a structural biochemist, and useful not only for researchers but also for graduate students to learn about the rapidly growing molecular fields, which were ignored for decades, but are now recognized as essential to understand the numerous critical biological phenomena in development or life processes.

I believe that the structure of polysaccharides from marine invertebrates analogous to that of mammalian heparinoids turned Dr. Pavao’s scientific interests to the structure–activity relationship and the possibility of therapeutic applications, almost 20 years ago. I have always thoroughly enjoyed his work, which is often impressive since he provides solid structural data and describes interesting activities of complicated molecules. I think, I first met him at one of the Gordon Conferences on Proteoglycans in 2000. I have enjoyed the discussions with him almost every year since then at the same and other conferences on proteoglycans.

Over the years, his interests have been turned to various pharmacological activities of glycans such as antimetastasis, antithrombosis, and anti-inflammation. This book is unique that it was designed to encompass the most recent advances in biological and pharmacological research on various glycans including not only heparan sulfate and chondroitin/dermatan sulfate glycosaminoglycans (GAGs), but also *N*- and *O*-glycans of glycoproteins. Dr. Pavao invited young independent frontier researchers, who are at the cutting-edge in each research field, to be the authors.

When I entered this field, almost 35 years ago, it was small and relatively inactive. However, it is now clear, based on recent accumulating and direct evidence, that glycans are essential components for life. It is now timely to compile review articles of representative sections of the most interesting studies. I express my respect for Dr. Pavao for his contribution to the field as an editor, and to all the authors for their efforts.

Hokkaido University, Hokkaido, Japan Kazuyki Sugahara

Preface

Carbohydrates are a major component of the extracellular matrix (ECM) where they associate with proteins to form glycoproteins or proteoglycans, or exist as long-chain disaccharides (e.g., hyaluronic acid). All ECM proteins except elastin have associated sugar, and, in some cases, ECM proteins require proper glycosylation to achieve full biological activity. It is also now clear that many ECM proteins have carbohydrate-binding domains that specifically recognize and interact with glycoconjugates on other matrix components and on the cell surface. Carbohydrates have been implicated in a wide variety of processes, ranging from cell adhesion and migration to matrix assembly, growth factor sequestration and regulation, involvement in many aspects of immune function, binding of plasma proteins, and control of thrombogenesis. Proteoglycans and other glycoconjugates were reviewed in earlier volumes in this series (*Cell Surface and Extracellular Glycoconjugates: Structure and Function*, David D. Roberts and Robert P. Mecham, editors; and *Biology of Proteoglycans*, Thomas N. Wight and Robert P. Mecham, editors). This volume reviews the most recent findings on the role of glycans in the development of diseases and the possible therapeutic use of this class of molecules. It shows how the interaction of glycans with growth factors, growth factor-binding proteins, extracellular proteases, protease inhibitors, chemokines, morphogens, and adhesive proteins regulates inflammation, infection, cancer, atherosclerosis, thrombosis, and embryonic stem cell biology. Further, an extensive survey of the structure and pharmacological effects of unique marine GAGs, and also the possibility to use these glycans as therapeutic agents are discussed.

Heparan sulfate is a linear polysaccharide composed of glucosamine and uronic acid (glucuronic acid or iduronic acid) disaccharide repeats with various types of sulfation modifications. In tissues, heparan sulfate covalently attaches to core proteins to form heparan sulfate proteoglycans (HSPG), and are abundant at the cell surface and in the ECM. In Chap. 1, Wang reviews our current understanding of the cellular and molecular mechanisms of heparan sulfate in the regulation of inflammation and angiogenesis. This chapter focuses on the regulatory roles of heparan sulfate on key inflammatory molecules and on the vascular endothelial

growth factor (VEGF), the master proangiogenic factor of angiogenesis. For most intracellular pathogens, cell surface HSPGs serve as a scaffold that facilitates the interaction of microbes with secondary receptors that mediate host cell entry. Consistent with this mechanism, addition of heparan sulfate (HS) or its pharmaceutical functional mimic, heparin, inhibits microbial attachment and entry into cultured host cells, and HS-binding pathogens can no longer attach or enter cultured host cells whose HS expression has been reduced by enzymatic treatment or chemical mutagenesis. In Chap. 2, Bartlett and Park provide a mechanistic overview of our current understanding of how certain microbial pathogens subvert HSPGs to promote their infection, using specific HSPG-pathogen interactions as representative examples.

Alteration in cellular glycosylation is a common phenotypic change associated with malignant transformation and cancer progression. Cell surface oligosaccharides carried on glycoproteins and glycolipids mediate communication among cells and facilitate cell adhesion, processes that are central during cancer progression. Accumulating evidence indicates that glycans contribute to tumor invasion, metastasis, and angiogenesis. Borsig (Chap. 3) provides an overview on cancer-specific changes of glycosylation on *O*- and *N*-glycans, with the focus on the function of these oligosaccharides in cancer progression.

Chapter 4 by Vicente, Godoy and Werneck focuses on the roles of different GAGs located in the vessel wall in the pathogenesis of atherosclerosis and thrombosis. GAGs in atherosclerosis can help to regulate atherogenesis through their ability to retain lipoproteins in the vessel wall. Prolonged retention of lipoproteins may render them susceptible to chemical modifications, leading to their aggregation, cellular uptake, and lipid accumulation. GAGs can also act as anticoagulant molecules because of their ability to interact with anticoagulant proteins like antithrombin and heparin cofactor II, promoting their activation and increasing their ability to inhibit thrombin.

In Chap. 5, we return to heparan sulfate where Pickford, Holley, Meade, and Merry discuss the role of this important glycoconjugate in early development. Mouse embryonic stem (ES) cells produce a poorly sulfated HS that may protect from prodifferentiation cues (e.g., fibroblast growth factors), and HS epitopes can identify cells with hemangioblast potential. Therefore, HS-sequences can identify functionally unique populations of cells and so have potential applications in the development of cell-replacement therapies for degenerative conditions. How HS chains influence differentiation events is unclear, but possible mechanisms include interactions of heparan with cytokines and chemokines. Indeed, a well-known function of HS is its ability to serve as a coreceptor to modulate cell growth, survival, and movement. These functions become relevant to heparan's role in disease as well as development. In Chap. 6, Gassar, Ibrahim, and Götte discuss the role of HS in tumor progression and cancer therapy. As matrix receptors, HSPGs act in concert with integrins to regulate tumor cell motility. As binding partners for matrix metalloproteinases and protease inhibitors, they regulate the proteolytic microenvironment of tumors, thus modulating metastatic spread. The development of glycan-based drugs targeting these biological functions has become

an area of intense research in cancer biology. This chapter discusses some of the promising results that have been obtained both in animal models and in clinical trials.

The final chapter by Kozlowski, Gomes, Silva, Pereira, Silva, and Pavão focuses on the interesting sulfated GAGs present on marine organisms. These glycoconjugates possess pharmacological properties ranging from anticoagulant and antithrombotic to antimetastatic and anti-inflammatory. In this chapter, the authors review the phylogenetic distribution, the structure, and the biological effects of the marine GAGs, as well as the molecular mechanisms involved in some of their biological activities. The possibility to use these glycans as therapeutic agents is also discussed.

It is hoped that the information in this volume will provide useful information to the research community about the broad range of functions associated with glycoconjugates in the context of development and disease. This field is especially exciting to follow because it impacts basic, applied, and clinical aspects of biomedical research. It is clear that additional practical applications will be forthcoming with advances in our basic knowledge of glycans in disease.

Rio de Janeiro, Brazil Mauro S.G. Pavão

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Chapter 1
Heparan Sulfate Proteoglycan in Inflammation and Angiogenesis

Lianchun Wang

Abstract Heparan sulfate is a linear polysaccharide composed of glucosamine and uronic acid (glucuronic acid or iduronic acid) disaccharide repeats with various types of sulfation modifications. More than 26 enzymes participate in the biosynthesis of heparan sulfate, which includes two major steps of chain polymerization and chain modification. The chain modification steps proceed sequentially but are incomplete, resulting in enormous structural diversity in mature heparan sulfate. In tissues, heparan sulfate covalently attaches to core proteins to form heparan sulfate proteoglycans, and are abundant at the cell surface and in the extracellular matrix. Studies have demonstrated that heparan sulfate interacts with growth factors, growth factor binding proteins, extracellular proteases, protease inhibitors, chemokines, morphogens, and adhesive proteins to critically regulate cell functions under both physiological and pathological conditions. In this chapter, I will review our current understanding of the cellular and molecular mechanisms, and the structure–function relationship of heparan sulfate in the regulation of inflammation and angiogenesis, with particular focus on the regulatory roles of heparan sulfate on the key inflammatory molecules, selectin and chemokine, and on the vascular endothelial growth factor, the master proangiogenic factor of angiogenesis.

1.1 Introduction

Heparan sulfate and heparin, a highly sulfated form of heparan sulfate, are linear polysaccharides composed of glucosamine and uronic acid (glucuronic acid or iduronic acid) disaccharide repeats with various types of sulfation modifications (Bernfield et al. 1999; Esko and Selleck 2002). Heparan sulfate covalently attaches to core proteins in tissues to form heparan sulfate proteoglycans (HSPGs) and are

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abundant at the cell surface and in the extracellular matrix (Bernfield et al. 1999; Esko and Selleck 2002). Heparin is restricted in mast cells of connective tissue and is enriched in liver, lung, intestine, and skin (Forsberg et al. 1999; Bishop et al. 2007). Although heparin has been used clinically as a potent anticoagulant for more than 70 years, the physiological function of endogenous heparin appears to be unrelated to blood coagulation. Instead, heparin is essentially required for protease storage in the secretory granules in mast cells (Forsberg et al. 1999). The biological functions of heparan sulfate have also gradually been recognized and been shown to critically regulate various aspects of cell functioning under both physiological and pathological conditions by interacting with growth factors, growth factor binding proteins, extracellular proteases, protease inhibitors, chemokines, morphogens, and adhesive proteins (Bernfield et al. 1999; Bishop et al. 2007). Intriguingly, studies have further emerged showing that the regulatory roles of heparan sulfate occur in a spatio-temporal manner and are implicated in distinct arrays of molecular pathways during different biological processes (Allen et al. 2001; Ledin et al. 2004). For better understanding of the structure, biosynthesis and general biological functions of heparan sulfate and HSPGs, readers are referred to other chapters in this book as well as excellent reviews in the literature (Bernfield et al. 1999; Esko and Selleck 2002; Bishop et al. 2007; Hacker et al. 2005; Beauvais and Rapraeger 2004; Gotte 2003; Sasisekharan et al. 2002; Iozzo and San Antonio 2001; Gallagher 2001; Forsberg and Kjellen 2001; Esko and Lindahl 2001; Park et al. 2000b; Varki and Varki 2002; Parish 2006). In this chapter, the recent progress in understanding the cellular and molecular mechanisms and the structure–function relationship of heparan sulfate and heparin in regulating inflammation and angiogenesis will be reviewed, with a particular focus on their interactions with the key inflammatory molecules, selectin and chemokine, and with the vascular endothelial growth factor (VEGF), the master proangiogenic factor of angiogenesis (Wang et al. 2002, 2005; Fuster et al. 2007).

1.2 The Anti-inflammatory Function of Heparin

1.2.1 Leukocyte Trafficking and Inflammation

An essential feature of the inflammatory response is the rapid recruitment of leukocytes from the blood to the site of inflammation, usually through postcapillary venules (Parish 2006; Springer 1994). This recruitment occurs as a multistep process, including the initial attachment and rolling of leukocytes on the inflamed endothelium, activation of leukocytes by endothelial cell-bound chemokines, stable adherence of the activated leukocytes to the endothelium, degradation of the subendothelial basement membrane, and migration of leukocytes along chemokine gradients into the target tissue. The recruited leukocytes at the target tissues facilitate host responses to tissue injury and pathogen invasion (Springer 1994).

The complex leukocyte recruitment process is mediated and coordinated by diverse and multiple molecules at different stages. Initial tethering and rolling of leukocytes on the inflamed endothelium are mediated by the selectin family members, including lymphocyte (L)-, platelet (P)- and endothelial-cell (E)-selectin, and their counter ligands (Springer 1994; Norgard-Sumnicht et al. 1993). L-selectin is constitutively expressed by leukocytes, and P- and E-selectin are expressed on endothelial cells activated by proinflammatory cytokines. P-selectin is rapidly mobilized to the surface of endothelial cells exposed to thrombin or histamine, whereas the expression of E-selection by endothelial cells appears several hours after receiving the inflammatory stimuli, such as IL-1, TNF-α and endotoxin. Therefore, P-selectin and L-selectin appear to mediate the initial events in leukocyte infiltration due to their rapid appearance on activated endothelium and constitutive expression on leukocytes, respectively (Springer 1994; Parish 2006). Blockage of the L- and P-selectin-mediated leukocyte tethering and rolling in blood vessels represents a reasonable approach to inhibit unwanted inflammatory responses (Parish 2006; Springer 1994; Ebnet et al. 1996; Koenig et al. 1998; Wang et al. 2002).

1.2.2 Heparin Inhibits Inflammation Mainly Through Blockage of L- and P-Selectin-Mediated Leukocyte Recruitment

All three selectins contain an amino terminal, a calcium-dependent carbohydrate recognition domain that binds to sialylated, fucosylated carbohydrate antigens related to sialyl Lewis^x [SLe^x, Neu5Acα2,3Galβ1,4(Fucα1,3)GlcNAcβ-] (Varki 1994). The SLe^x determinants present on specific selectin ligands, such as P-selectin glycoprotein ligand-1, E-selectin ligand-1 and glycosylation-dependent cell adhesion molecule-1 (Varki 1994; Leppanen et al. 2000). The binding of selectin to these ligands appears to involve sulfate residues either on the carbohydrate moiety or on the protein as sulfated tyrosine residues in proximity to the carbohydrate chain (Hemmerich et al. 2001; Leppanen et al. 2000). Heparin, a highly sulfated form of heparan sulfate and a classic anticoagulant, was found to bind L- and P-selectin but not E-selectin, and to inhibit the binding of L- and P-selectin to SLe^x-related compounds and to SLe^x determinants on HL-60 cells (Norgard-Sumnicht et al. 1993; Nelson et al. 1993; Norgard-Sumnicht and Varki 1995; Giuffre et al. 1997; Koenig et al. 1998). Furthermore, injected heparin inhibits inflammatory responses in vivo (Tyrrell et al. 1999; Nelson et al. 1993). In our studies, we observed that heparin inhibits the binding of L- and P-selectin to immobilized SLe^x and the adhesions of SLe^x-bearing U937 cells to immobilized P- and L-selectin and to P-selectin-expressing endothelial cells (Table 1.1) (Wang et al. 2002). These observations validated and also extended previous findings of others, and suggested that the anti-inflammatory effects of heparin might be due to a blockage of P- and L-selectin-mediated leukocyte recruitment. This idea was tested by examining the

Table 1.1 Anticoagulant activity of heparin and its chemically modified derivatives

Heparinoids	Anti-Xa activity (units/mg)	Inhibition of selectin–PAA–sLe^x binding (IC_{50}, μg/ml)		Inhibition of cell-selectin adhesion (IC_{50}, μg/ml)		Inhibition of cell–cell adhesion (IC_{50}, μg/ml)
		L-selectin	P-selectin	L-selectin	P-selectin	
Heparin	149	0.1	2.5	0.4	0.3	0.5
Oversulfated heparin (OS)	12.5	0.01	1.8	0.1	0.02	0.08
2-O, 3-O desulfated heparin (2/3DS)	1.7	0.4	20	3	4	2
N-desulfated/*N*-acetylated heparin (NDS)	0.1	1	10	0.7	0.9	25
N-,2-,3-*O*-desulfated heparin (N/2/3DS)	0.1	4	100	5	8	50
6-*O*-desulfated heparin (6DS)	0.1	85	450	25	30	350
Carboxyl reduced heparin (CR)	0.1	2	10	3	8	50

The anticoagulant activity of the heparinoids was analyzed by aminolytic antifactor Xa assay. All the raw data were converted into percentages for comparative purposes using the formula: % of maximum = [(average of duplicates) − (negative control)]/[(positive control) − (negative control)] × 100. IC_{50} values represent heparinoid concentrations that achieve 50% inhibition in the corresponding assays

anti-inflammatory effect of heparin in L- and P-selectin single knockout mice as well as in P/L-selectin double knockout mice in vivo, including the inflammatory models of thioglycollate-induced acute peritonitis and allergic (delayed-type hypersensitivity) contact dermatitis (Wang et al. 2002). It was observed that, compared to wildtype mice, the L- and P-selectin single knockout mice both showed impaired inflammation in the two models (Fig. 1.1b), indicating that the inflammatory responses in the two models are mediated by both L- and P-selectin. The two models are appropriate to use in testing whether the anti-inflammatory effect of heparin in vivo is due to the blockage of L- and P-selectin-mediated leukocyte recruitment. Compared to wildtype mice, the inflammatory responses in the L- and P-selectin single knockout mice were attenuated, and heparin treatment further extended this attenuation (Fig. 1.1b). However, the extended attenuation was not seen in P/L-selectin double-deficient mice (Fig. 1.1b). The enhanced attenuated inflammatory responses and lack of an additional attenuation by heparin treatment in the P/L-selectin double-deficient mice further confirmed that the inflammatory responses in both models were both P- and L-selectin-dependent, and also indicated that the anti-inflammatory effect of heparin was achieved primarily by blocking both P- and L-selectin functions (Wang et al. 2002).

Heparin has been known to interact with multiple components of the inflammation cascade, including integrins, cytokines, neutrophil-derived elastases, complement activation, and platelet-activating factors (Tyrrell et al. 1999). However, in our studies we demonstrated that the in vivo anti-inflammatory effect of heparin was mainly achieved by blocking L- and P-selectin functions. This might be explained by the fact that P- and L-selectin-mediated interactions are the necessary first step in the

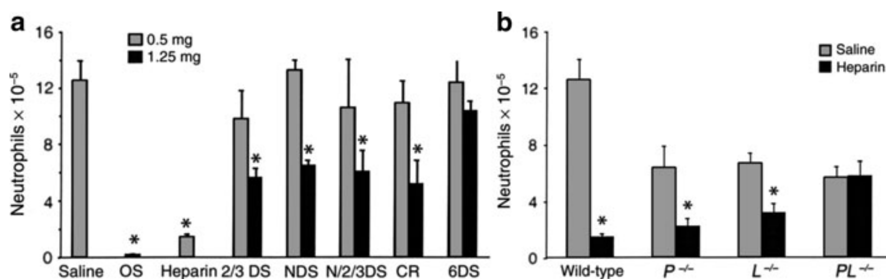

Fig. 1.1 Heparin inhibits thioglycollate-induced acute peritonitis. (**a**) Heparin and heparinoids at 0.5 (*gray bar*) or 1.25 mg (*black bar*) per wildtype mouse were given intravenously 5 min after thioglycollate intraperitoneal injection. The number of neutrophils recruited in the peritoneal cavity was quantified. *Significant statistical difference when comparison was made with heparin treatment. (**b**) Thioglycollate-induced acute peritonitis was elicited in wildtype C57BL/6 and selectin-deficient mice, and the mice received heparin treatment at 0.5 mg/mouse as described in (**a**). *Significant statistical difference when comparison was made between heparin and saline treatment. $P^{-/-}$, P-selectin knockout mice; $L^{-/-}$, L-selectin knockout mice; $PL^{-/-}$, P- and L-selectin double knockout mice

inflammatory cascade, occurring prior to the participation of integrins, cytokines, proteases, and to chemokine presentation and gradient-directed leukocyte migration. Thus, the potential inhibitory effects of heparin on these later steps of inflammatory response might occur, but are not reflected in our inflammatory models.

1.2.3 The Anti-inflammatory Effect of Heparin Does Not Correlate with Its Anticoagulant Activity and Critically Depends on the Presence of 6-O-Sulfate of Glucosamine Residues

Heparin consists of repeating disaccharide units containing D-glucuronic acid (GlcA) or L-iduronic acid (IdoA), and a glucosamine residue that is *N*-sulfated (GlcNS), *N*-acetylated (GlcNAc), or occasionally unsubstituted (GlcNH2). The disaccharides may be further sulfated at C6 or C3 in the glucosamine residues and at C2 in the uronic acid residues. The potent anticoagulant activity of heparin depends on a specific arrangement of sulfated sugar units and uronic acid epimers encompassing $GlcA_{NS6S}$–IdoA–$GlcA_{NS3S6S}$–$IdoA_{2S}$–$GlcA_{NS6S}$, which form a binding site for antithrombin (Esko and Selleck 2002). Much less information is available about the specific oligosaccharide structures in heparin that interact with P- and L-selectin (Nelson et al. 1993; Norgard-Sumnicht et al. 1993; Norgard-Sumnicht and Varki 1995; Koenig et al. 1998; Giuffre et al. 1997; Borsig et al. 2001). Thus, we sought to understand the relationship of heparin fine structure in its interaction with L- and P-selectin. Using heparin as the parent compound and various chemical approaches, several heparinoids were generated, including oversulfated

heparin (OS-heparin), *N*-desulfated/*N*-acetylated heparin (NDS-heparin), 2-*O*, 3-*O*-desulfated heparin (2/3DS-heparin), 6-*O*-desulfated heparin (6DS-heparin), and carboxyl-reduced heparin (CR-heparin) (Fig. 1.2). As expected, the desulfation as well as the reduction in heparin carboxyl groups resulted in the heparinoids losing anticoagulant activity, which was determined by the aminolytic antifactor Xa assay (Table 1.1) (Wang et al. 2002). To determine the structure– function relationship of heparin and its anti-inflammatory effect, the heparinoids were initially tested for their inhibition on the interaction of L- and P-selectin with SLe^x among various experimental systems, including the binding of L- and P-selectin to SLe^x, and SLe^x-bearing U937 cell adhesions to immobilized P- and L-selectins and to P-selectin expressing endothelial cells. It was observed that, compared to heparin, the OS-heparin showed an enhanced blocking effect, whereas all the desulfated-heparins and the CR-heparin exhibited reduced inhibitory roles (Table 1.1). Intriguingly, among the desulfated-heparins and the CR-heparin, 6DS-heparin showed the least blocking effect, which was about 4–45 times lower than others. In heparin, *N*-sulfate is the most abundant sulfation modification. Therefore, these observations indicate that the dramatic loss in the capacity of 6DS-heparin to bind to L- and P-selectin is not due to a reduction in the negative charge of the molecule, but is, instead, mainly due to the removal of the

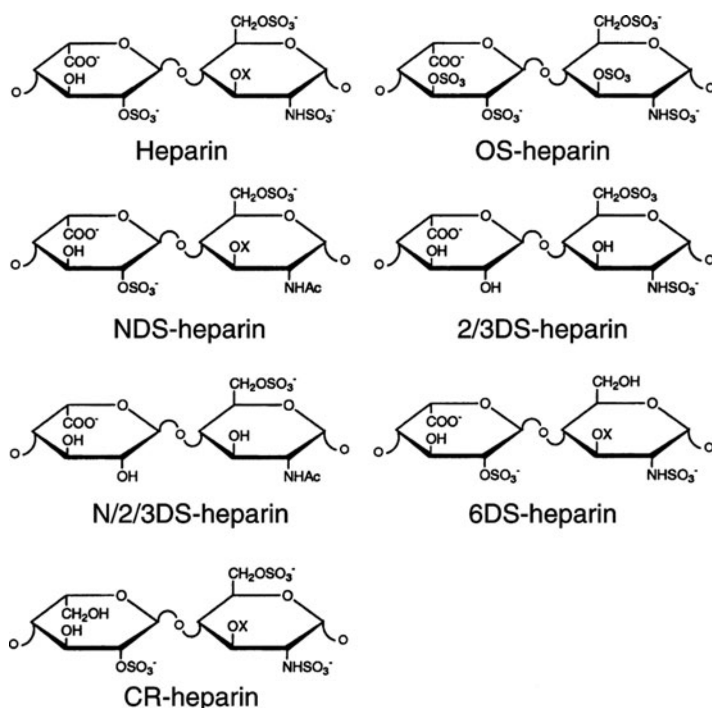

Fig. 1.2 Representative disaccharide units of heparin and chemically modified heparinoids. Each disaccharide illustrates a characteristic unit in the indicated preparation and does not represent the overall structure of the chains. X: H or SO_3^-

6-*O*-sulfate in GlcN residues, thus revealing that the 6-*O*-sulfate of heparin plays a dominant role in determining heparin's interaction with L- and P-selectin. This observation also suggests that the 6-*O*-sulfates of GlcN residues may be critically required for heparin's anti-inflammatory effect in vivo. This was further tested in the mouse inflammatory models of thioglycollate-induced acute peritonitis and allergic contact dermatitis. The in vivo studies recapitulated the in vitro results (Fig. 1.1a), and demonstrated that the anti-inflammatory effect of heparin critically requires the presence of the 6-*O*-sulfates in GlcN residues. Furthermore, with the exception of 6DS-heparin, all other heparinoids retained potent anti-inflammatory effects, even though they lacked anticoagulant activities (Table 1.1; Fig. 1.1a). Therefore, these observations also revealed that the anticoagulant activity of heparin is not required for its anti-inflammatory effect. Together, these structure–function relationship studies laid out a structural basis for the development of non-anticoagulant heparin as potent anti-inflammatory drugs, which can be administrated at high concentrations without fear of the unwanted bleeding side effects that occur with the clinically used heparin.

In summary, these studies demonstrate for the first time that the anti-inflammatory effect of heparin is achieved mainly by blocking the L- and P-selectin-mediated leukocyte recruitment. The structure–function studies further revealed that the 6-*O*-sulfate groups of GlcN residues are critically required for heparin's anti-inflammatory effect and some non-anticoagulant heparin may be developed as potent, novel anti-inflammatory drugs.

1.3 The Role of Heparan Sulfate in Leukocyte Recruitment in Inflammation

Studies have shown that heparin efficiently blocks inflammation in vivo, but heparin is rarely found in blood vessels where L- and P-selectin-mediated leukocyte recruitment occurs during inflammation. Instead, heparan sulfate is abundantly present on the vascular cell surface and in the perivascular matrix, particularly in the basement membrane, suggesting that heparan sulfate may mimic heparin's function to participate in inflammatory response in vivo. Indeed, biochemical and cell-based in vitro studies established that endothelial heparan sulfate is a potential ligand for P- and L-selectin (Norgard-Sumnicht et al. 1993; Norgard-Sumnicht and Varki 1995; Koenig et al. 1998; Giuffre et al. 1997), and can bind proinflammatory chemokines (Witt and Lander 1994; Luster et al. 1995; Hoogewerf et al. 1997), transport chemokine across endothelial cells and present chemokine to leukocytes (Middleton et al. 1997). However, the precise role of heparan sulfate during the inflammatory response in vivo was unclear. In our recent studies we generated heparan sulfate-deficient mice and specifically examined the role of endothelial and leukocyte heparan sulfate in inflammation in vivo (Wang et al. 2005). These studies revealed that endothelial heparan sulfate essentially modulates multiple stages of the inflammatory response (Wang et al. 2005).