Review

Heparan sulfate-protein interactions: therapeutic potential through structure-function insights

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Abstract. Heparin and the related glycosaminoglycan, heparan sulfate, bind a myriad of proteins. The structural diversity of heparin and heparan sulfates is enormous, but differences in the conformational flexibility of the monosaccharide constituents add extra complexity and may influence protein binding. Silencing genes for heparin/heparan sulfate biosynthetic enzymes profoundly affects mammalian development. Thus, altering the structure of heparan sulfate chains can alter protein binding and embryo development. Different heparan sulfate structures are located in particular tissue sites, and these structures are recognised by different sets of proteins. Regulation of certain heparan sulfate-protein interactions by pH or cations is described. Heparin/heparan sulfate structures are viewed as potential therapeutics for a variety of diseases. An understanding at the molecular and functional levels of the specificity and affinity of heparan sulfate-protein interactions is crucial for designing heparin-inspired drugs. How the development of synthesis techniques is facilitating structure-function analyses and drug development is discussed.

Key words. Heparan sulfate; heparin; heparin-like therapeutics; heparan sulfate structure; biosynthetic enzymes; binding; protein interactions.

Introduction

Although heparin has been in clinical use for decades, the extent of the importance of heparin and the related glycosaminoglycans (GAGs), heparan sulfates, in biology and medicine has not been recognised until recently. Heparan sulfate and heparin-like structures appeared very early in metazoan evolution and have been preserved in modern organisms. Virtually all cells secrete, or have associated with their cell surface, a type of glycosaminoglycan. This means that all proteins outside of the cell, regardless of function, have evolved in the presence of sulfated polysaccharides. It is thus not surprising that there are large numbers of heparin/heparan sulfate binding proteins and that protein-GAG interactions have profound effects on vertebrate and invertebrate physiology. Heparin or heparan sulfate family members have been detected in a wide range of marine invertebrates [1]. Perhaps the best illustration that heparan sulfate-protein interactions were fundamental to metazoan development comes from the finding that a protein binding a sulfated polysaccharide mediates cell-cell adhesion in the simplest of all metazoans, a marine sponge [2]. Data from a partial characterisation of the sulfated polysaccharide revealed the presence of glucuronic acid, N-sulfated glucosamine and O-sulfates. As the polysaccharide was cleaved by nitrous acid, collectively these data point to it being a heparan sulfate family member [2]. Although

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many invertebrates have also been reported to have chondroitin sulfates, it has been proposed that the wide, virtually ubiquitous distribution of heparan sulfate-type structures indicates that this was the original GAG in the metazoan lineage [3]. It is now accepted that the heparin/heparan sulfate class of GAGs binds to a wide range of proteins of diverse function. Heparin was initially discovered because of its profound effect on coagulation, and it was in that capacity that in 1935 it was used in clinical trials and subsequently in the clinic [4]. Heparin was later found to bind to antithrombin III, causing a conformational change within that protein, which enhanced the neutralization of thrombin leading to anticoagulant effects [5]. However, only one-third of the chains in commercial heparins have this capacity, which indicated that heparin chains with high affinity for antithrombin III must contain particular oligosaccharide sequences. Subsequent structural analysis revealed that a unique pentasaccharide (fig. 1) was required for high-affinity binding and that the relatively rare modification of a 3-O-sulfate on the glucosamine, the third monosaccharide in the sequence, was essential [6]. The possibility that other proteins may bind heparin or heparan sulfate with similar exquisite specificity is a continuing source of interest and controversy.

In recent years, technological advances in the structural analyses of heparin and heparan-sulfate oligosaccharides [7, 8] and in the modeling of GAG-protein binding events [9, 10] have assisted our understanding of structural aspects of these interactions. However, studies to unravel the functional relevance of GAG-protein interactions must be performed alongside structural analyses before the biological implications can be ascertained. In some cases, the interaction with GAGs serves to regulate protein stability and activity. In others, the interaction of proteins with GAGs acts to sequester proteins or infectious agents to particular locations. Growth factors, particularly those of the fibroblast growth factor (FGF) family, are a well-studied example of the types of proteins that bind GAGs.

It was the finding in 1991 that heparan sulfate is required for the binding of basic FGF (or FGF-2) to its high-affinity receptor and for receptor activation [11, 12] that spearheaded heparan sulfate into a centre-stage position in cell biology. The idea that particular heparan sulfate structures of a certain length may be required to bind various FGF family members followed shortly thereafter [13]. In 1993 a paper was published entitled ‘Minimal sequence in heparin/heparan sulfate required for binding of basic fibroblast growth factor’ [14]. Indeed, different heparin/heparan sulfate structures were required to bind and activate different FGFs [15–17]. The possibility that heparin/heparan sulfate mediated dimerisation of FGF molecules triggered or facilitated receptor dimerisation, and hence activation, was proposed to explain the role of these oligosaccharides in receptor activation [13, 18–20]. A further key finding was that heparan sulfate also bound FGF receptors (FGFR). This was first demonstrated with FGFR-1 [21]. Crystal structures of FGF FGFR-heparin complexes confirmed that heparin makes contact with both the growth factor and the receptor [22, 23]. It is now known that the heparan sulfate structure and length required for activating FGFs is dictated by the particular FGF-FGFR pair [24]. The FGF-FGFR-heparin story is a complex one, and its pre-eminence in heparan sulfate biology has shaped, rightly or wrongly, much of current thinking in relation to heparin/heparan sulfate growth factor interactions. However, it is probable that not all growth factor interactions with these GAGs will be reminiscent of that of the FGF family. As we move into an era where heparin/heparan sulfate-like structures are being examined for their therapeutic potential, it is important not to have our thinking unduly biased by the FGF-FGFR-heparin story.

Nevertheless, as a result of the huge body of work on the FGF-FGFR-heparin/heparan sulfate interaction, GAGs and particularly heparin/heparan sulfate-like structures are now attracting considerable interest as a source of new therapeutics for the treatment of infectious diseases, inflammation and allergic diseases, and cancers. Crucial issues to be understood if the potential of GAGs as therapeutics is to be realised include how GAG-protein interactions are regulated in the tissues, whether particular GAG epitopes are localised within tissues, whether biological activity requires high-affinity GAG-protein binding, and how specific GAG-protein interactions are in vivo. Some of these issues will be examined in the course of this review.

**Structure of heparin and heparan sulfates**

**Saccharide composition and arrangement**

Heparin and heparan sulfates are mixtures of linear chains that display extraordinary structural diversity, the different chains of these molecules having different patterns of sulfation. Yet, the underlying structural regularities of heparin-like-GAGs (HL-GAGs) allows the deduction of structural details from molecular weight data. Heparin and