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Molecular engineering of glycosaminoglycan chemistry for biomolecule delivery ☆

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ABSTRACT

Glycosaminoglycans (GAGs) are linear, negatively charged polysaccharides that interact with a variety of positively charged growth factors. In this review article the effects of engineering GAG chemistry for molecular delivery applications in regenerative medicine are presented. Three major areas of focus at the structure–function–property interface are discussed: (1) macromolecular properties of GAGs; (2) effects of chemical modifications on protein binding; (3) degradation mechanisms of GAGs. GAG–protein interactions can be based on: (1) GAG sulfation pattern; (2) GAG carbohydrate conformation; (3) GAG polyelectrolyte behavior. Chemical modifications of GAGs, which are commonly performed to engineer molecular delivery systems, affect protein binding and are highly dependent on the site of modification on the GAG molecules. The rate and mode of degradation can determine the release of molecules as well as the length of GAG fragments to which the cargo is electrostatically coupled and eventually released from the delivery system. Overall, GAG-based polymers are a versatile biomaterial platform offering novel means to engineer molecular delivery systems with a high degree of control in order to better treat a range of degenerated or injured tissues.

1. Introduction

Glycosaminoglycans (GAGs) are a class of linear polysaccharides that are ubiquitous in the human body and possess multiple biological functions essential for life [1]. Such functions consist of: (1) osmotically attracting water and thereby maintaining hydrostatic pressure to confer mechanical stability in connective tissues such as cartilage [2–6]; (2) covalent attachment to proteoglycans that regulate cell function [7]; (3) acting in conjunction with proteins on cell surfaces via receptors or co-receptors to modulate the local biological environment [8]. Based on their numerous biological functions, GAGs have been extensively explored as biomaterials for controlled protein delivery to improve the treatment of a variety of diseases [9–12].

Many of their biological functions are conferred by the unique chemical structure of GAGs, consisting of repeating disaccharide units that are specific for each GAG species. Sulfated GAG species such as chondroitin sulfate (CS), heparin, heparan sulfate (HS), dermatan sulfate (DS), and keratan sulfate (KS) bear negative charges that vary in density and position within the disaccharide units [13]. In addition to sulfated GAGs, hyaluronic acid (HA) is not sulfated and therefore is the GAG with the least net negative charge [14]. Based on this negative net charge, GAGs attract positively charged proteins, however, these binding processes are very challenging to investigate because they are governed by the complex inherent chemical properties of GAGs [15–17]. For protein delivery applications a number of GAG-based approaches have been developed that mimic the interactions that occur naturally between GAGs in the extracellular matrix (ECM) and growth factor binding partners. GAGs can possess specific carbohydrate sequence-specific electrostatic binding sites for some growth factors, or they can bind growth factors via a non-sequence-specific electrostatic mechanism [18].

Although protein-specific binding sites including conformational changes upon binding have been reviewed previously [19–21], this work focuses on reviewing the chemical properties and modifications of GAGs for protein binding and incorporation into complex biomolecule delivery systems. Besides considering the effects on protein binding, chemical modifications affect degradation processes [22,23], which, in turn, influence the molecular release characteristics; therefore, degradation mechanisms are also discussed in detail here. A thorough understanding of the chemical properties of GAGs, both native and modified, and how they relate to protein binding is a key factor for successful implementation of GAG-based biomaterial strategies in tissue engineering and drug

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delivery applications. As described throughout this review, a better understanding of GAG chemistry will lead to enhanced predictability of protein uptake and release from GAG-based biomaterials, and thus the ability to design more efficacious strategies for harnessing the unique innate properties of GAGs for a broad range of regenerative medicine applications.

2. GAG–protein binding: a function of sulfation pattern, three-dimensional conformation and polyelectrolyte properties

Generally it is believed that net negative charge is primarily responsible for mediating GAG interactions with oppositely charged proteins, but polyelectrolyte complexation does not fully explain protein affinity for GAGs. The primary structure of GAGs is determined by carbohydrate repeat units with their specific sulfation patterns, which influence complex three-dimensional (3-D) structures that contribute to the pharmacological activity of GAGs. Moreover, most GAG species do not exist in vivo in an isolated state, but, instead, are synthesized and secreted in the form of proteoglycans (PGs) or serve as co-receptors for GAG–growth factor complex formation on cell surfaces [24]. GAG attachment to PGs is not directly linked to the sulfation pattern, but to a specific carbohydrate end group sequence by which GAG chains are linked to the PG core protein. GAG–PG attachment has already been reviewed [7,25] and, thus, this section focuses on the importance of GAG 3-D structure combined with sulfation pattern on growth factor binding for each major representative of the GAG family.

2.1. Carbohydrate structures and nomenclature

For the reader's reference this section summarizes the most important monosaccharide structures, conformations and configurations of GAG subunits in order to better understand the specific epitopes presented in the following sections. Recommendations on sugar nomenclature rules and determination of conformation were published by the International Union of Pure and Applied Chemistry (IUPAC) [26,27]. Monosaccharide units of relevance for GAGs are uronic acid and amino sugars (Fig. 1). Such monosaccharides can acquire different solution conformations (Fig. 2A). Among the most well-known conformations are chair (*C*), boat (*B*), and envelope (*E*). The chair conformation and intermediate conformation between chair and boat, skew-boat (*S*), play an important role in antithrombin III (AT III) binding to heparin (Fig. 2A). In solution each carbohydrate is in equilibrium with its different conformations. The α/β- and D,L- nomenclature is also used to distinguish between different configurations (Fig. 2B).

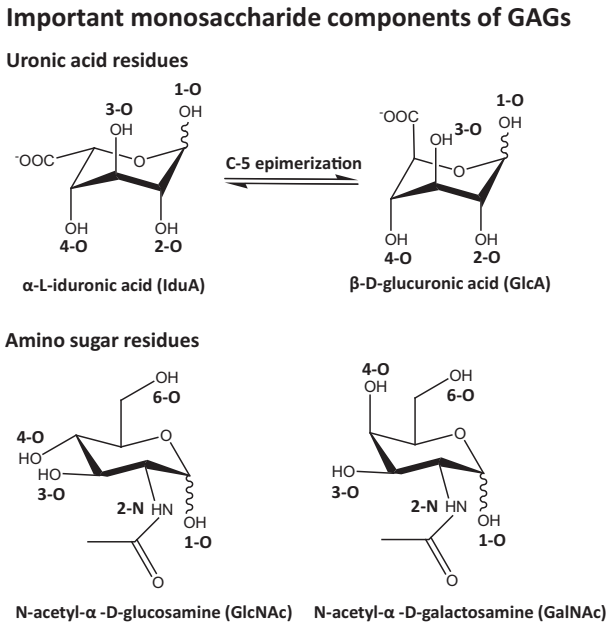

Fig. 1. Most prominent monosaccharides present in GAGs. Uronic acid sugars possess a carboxyl function connected to C5 of the ring atom, whereas amino sugars have an amino function at position C2. This amino moiety may exist as a free amine (rare) or be acetylated (shown above) or sulfated (see Fig. 3) within GAG polysaccharides.

2.2. Structure of heparin and heparan sulfate

The carbohydrate compositions of heparin and HS are similar but differ in monosaccharide ratio and sulfation pattern distribution. The most prominent disaccharide repeat unit in heparin consists of 2-O-sulfated L-iduronic acid (IduA2S, α-1,4) and a mixture of either N- and 6-O-sulfated (GlcNS6S) or N-acetylated D-glucosamine (GlcNAc, α-1,4). In HS, instead of IduA2S as in heparin, the majority of uronic acid residues are D-glucuronic acid (GlcA, β-1,4). These repeat units are connected in a complex pattern including other residues with additional O- and N-sulfated groups: GlcNAc can be additionally 6-O-sulfated (GlcNAc6S) or GlcNS less commonly 3-O-sulfated (GlcNS3.6S) [28]. Unfractionated heparin has a molar mass of between 3 and 30 kDa (15 kDa average) [29], whereas heparan sulfate, e.g. from human liver, has a molar mass of around 24 kDa [30]. HS is a key component of PGs secreted into the ECM, such as perlecan [31] and agrin [32], but HS transmembrane PGs can also serve as receptors or co-receptors (e.g. syndecans) [33]. Consequently, HS is present in many tissues and can serve multiple functions, while the presence of heparin in humans is limited to very few tissues. The best described occurrence of heparin is in mast cell granules, where its function and evolutionary role is still not fully understood [34,35].

Besides tissue distribution and function, there are general structural differences between HS and heparin. Specifically, these species differ in the overall charge distributions along the polymeric chain: heparan sulfate exhibits sulfate-rich ("S-rich") regions [36] separated by disaccharide units that contain mainly unsulfated, acetylated glucosamine and GlcA (NA-regions) [37]. Interestingly, the number of HS sulfate clusters changes during cell differentiation, leading to more sulfated regions with greater differentiation, whereas stem cells exhibit fewer sulfate clusters [38,39]. The combination of sulfated and non-sulfated regions in HS leads to a very flexible conformational structure because the alternating structure consisting of regions with high and low sulfation may cause HS to bind and "wrap" around a variety of proteins through non-carbohydrate sequence-specific interactions [40,41]. Although HS–protein interactions may not always be sequence specific, different cell types produce HS derivatives with various repeating monosaccharide patterns in the sulfated regions that potentially account for some protein specificity in certain tissues, but the exact physiological role of tissue-dependent HS compositions remains unknown [42].

In contrast to non-sequence-specific interactions, carbohydrate sequence-specific GAG–protein interactions have been elucidated for heparin/HS. The most well-investigated example is basic fibroblast growth factor (FGF-2) binding to heparin/HS, for which specificities and effects have been studied since the 1980s [43]. The FGF family consists of 22 distinct isoforms that are sub-divided into seven sub-families [44]. The transmembrane tyrosine kinase receptor for FGF is activated by heparin or HS as a co-factor, which induces FGF dimerization and enhances FGF signaling [45]. From a series of studies on this topic a minimal pentasaccharide sequence [46] from heparin was found to be responsible for FGF-2 pairing.