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Glycosaminoglycans as polyelectrolytes

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ABSTRACT

One of the barriers to understanding structure–property relations for glycosaminoglycans has been the lack of constructive interplay between the principles and methodologies of the life sciences (molecular biology, biochemistry and cell biology) and the physical sciences, particularly in the field of polyelectrolytes. To address this, we first review the similarities and differences between the physicochemical properties of GAGs and other statistical chain polyelectrolytes of both natural and abioitic origin. Since the biofunctionality and regulation of the structures of GAGs is intimately connected with interactions with their cognate proteins, we particularly compare and contrast aspects of protein binding, i.e. effects of both GAGs and other polyelectrolytes on protein stability, protein aggregation and phase behavior. The protein binding affinities and their dependences on pH and ionic strength for the two groups are discussed not only in terms of observable differences, but also with regard to contrasting descriptions of the bound state and the role of electrostatics. We conclude that early studies of the heparin–Antithromin system, proceeding to a large extent through the methods and models of protein chemistry and drug discovery, established not only many enabling precedents but also constraining paradigms. Current studies on heparan sulfate and chondroitin sulfate seem to reflect a more ecumenical view likely to be more compatible with concepts from physical and polymer chemistry.

Contents

Abbreviations: CS, Chondroitin sulfate; DS, Dermatan sulfate; FGF, Fibroblast growth factor; GAG, Glycosaminoglycan; GM-CSF, Granulocyte-macrophage colony stimulating factor; HA, Hyaluronic acid; Hp, Heparin; KS, Keratan sulfate; PE, Polyelectrolyte; SEC, Size exclusion chromatography; VEGF, Vascular endothelial growth factor.

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1. Introduction

Glycosaminoglycans (GAGs) are flexible linear bio-polysaccharides heterogeneously decorated with sulfate and carboxylate groups. They are ubiquitous on many cell surfaces and in connective tissues, and constitute an important component of the extracellular matrix. Many excellent reviews describe progress in GAG biochemistry [1–4], with notably recent findings on heparan sulfate[1]. As widely noted, the structural characteristics of GAGs involve multiple levels of heterogeneity (Fig. 1): the disaccharide building blocks (iduronic acid or glucoronic acid or galactose and glucosamine or galactosamine), sulfation type (4- or 6-, or exceptionally 3-O sulfation of the sugar rings), sulfation pattern (distribution of sulfates) and the overall chain length [5–7]. The diversity of the GAGs arising from such heterogeneity is a consequence of the non-template driven biosynthesis of these molecules which is nevertheless wonderfully regulated to allow for modifications of GAG structures in response to cell development, disease states and other variables only partly understood. Such diversity also influences physicochemical characteristics that strongly depend on environment such as chain flexibility, viscosity, and compressibility. One consequence of GAG heterogeneity is their ability to interact with numerous proteins. Through such interactions, GAGs, particularly the "heparinoids" heparan sulfate and heparin, regulate biological processes such as cell adhesion, cell growth and differentiation, cell signaling and anticoagulation [3,8]. The structure–function relationships governing these interactions are not well understood. A significant effort has been made to elucidate protein binding by the tools of molecular biology and by detailed structural characterization. In this sustained effort, the recognition of GAGs as polyelectrolytes has not had a high profile.

Polyelectrolytes (PEs) are linear or branched polymers that contain ionizable groups within their repeating units, resulting in charged chains with dissociable counterions in suitable polar solvents like water. Depending on the structural properties of their repeating units, PEs can display various levels of flexibility in solution. PEs do not have a secondary structure, hence they display randomized configurations in solution, denoted as "random coils". While proteins are sometimes described as PEs because some repeating units contain ionizable groups, their unique tertiary structures lead to solution behavior stongly divergent from those of random coil structure. Hence, classification of PEs exclude biomolecules with such defined tertiary structure, but can sometimes include other biomolecules, e.g. DNA and ionic polypeptides, with well-defined helical secondary structures and limited flexibility.

PEs readily interact with oppositely charged surfaces, and a substantial body of experiment and theory describes such polyelectrolyte adsorption and the resultant bound states [9]. The conformational flexibility of PEs allows for interactions with colloidal particles as well as flat surfaces. Particular significance has been attached to the interaction of polyelectrolytes with oppositely charged particles of many kinds including micelles, liposomes, dendrimers and inorganic colloids, with corresponding theoretical analyses [10,11]. PE binding to proteins must follow similar fundamental physics, but is distinctive in that binding occurs readily even when PE and protein have the same net charge. This is a consequence of protein charge anisotropy, allowing PEs to interact electrostatically with regions in which amino acids of opposite charge are clustered. The many aspects and applications of protein–PE interactions have been discussed in several reviews [12–14], while the interactions of GAGs with proteins are also described in detail in the reviews mentioned in the first paragraph, but often within notably different context. The extent to which protein–GAG interactions can be properly considered as a subset of protein–PE interactions is a central theme of this article.

While there is some debate about the propensity of GAGs to form non-transient local conformations, particularly in biofunctional complexes with proteins [15], it is clear that they behave in free solution as statistical semi-rigid (wormlike) chains [16,17]. Along with high linear charge density (the structural linear charge density of heparin exceeds that of any other biopolymer in non-helical state), these features should justify the inclusion of GAGs as polyelectrolytes. Since a rich and influential literature on the polyelectrolyte properties of DNA was already well-established 20 years ago, including the clear recognition of the role of electrostatics in DNA binding to proteins [18], one might ask why physicochemical and biochemical studies of GAGs have not yet followed a similar path towards convergence. Some possible reasons are (1) recognition of the immense importance of GAGs emerged nearly a half-century later than for nucleic acids; (2) the tremendous difficulty of characterizing the structure of GAGs has discouraged physical chemists (with some notable exceptions [17,19–22]) from physicochemical investigations of such ill-defined macromolecules; (3) the substitution of low MW GAGs, particularly low MW heparin–analogs, driven by the desires for both experimental convenience and new drug development, unparalleled in DNA research, has also provided a distraction from the polyelectrolyte viewpoint; and (4) the well-defined helicity of DNA is more consistent with conventional views of macromolecular structure in biology than the conformational irregularities of the native heparinoids.

The need to recognize the polyelectrolyte nature of GAGs was pointed out almost 20 year ago by Jaques et al. [23] who stated that this oversight could lead to erroneous conclusions from experimental data. Nevertheless, limited recognition of GAGs as polyelectrolytes over the following 15 years, presumably related to the obstacles noted above, can be seen from the number of publications that contain keywords "GAGs/Hp" and "protein interactions" and "electrostatic"(Fig. 2), chosen as an indicator of recognition of Hp/GAGs as PEs. Prior to 2007, less than 10% of papers on GAGs met this requirement. The significant increase in this fraction since 2007, indicates a shift in viewpoint. This might be correlated with the leveling off of papers on heparin–protein interactions with a shift to other GAGs, indicating that the non-electrostatic viewpoint was more characteristic of studies with heparin, for reasons that will be discussed below.

To address the roles of electrostatics in GAG biofunctionality, we first compare the physicochemical properties of GAGs with those of other statistical chain polyelectrolytes of both natural and abiotic origins, and then consider the protein binding of such polyelectrolytes vis-a-vis the interactions of GAGs with cognate proteins. This includes examination of the influences of GAG charge sequence heterogeneity and protein charge anisotropy on protein–GAG interactions. These comparisons bring up inconsistencies between the approaches arising from molecular biology and biochemistry vs. those deriving from physical and polymer chemistry. It may be useful to determine the extent to which these differences are semantic or arise from divergent paradigms.

2. The physicochemical behavior of GAGs is identical to those of polyelectrolytes

Should GAGs be considered as manifesting the behavior of polyelectrolytes, irrespective of their biological origin, in similar fashion to

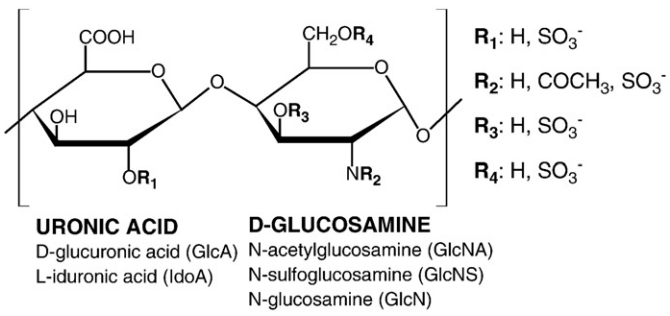

Fig. 1. The main repeating disaccharide unit of heparin indicating possible sulfation patterns at 2,4 and 6 positions.