STUDY OF THE SUBSTRATE SPECIFICITY AND CATALYTIC MECHANISM OF GLYCOSAMINOGLYCAN LYASES AS POTENTIAL ANALYTICAL TOOLS

by

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ABSTRACT

Glycosaminoglycans (GAGs) are a large class of polysaccharides common to all mammalian cells. GAGs are found on most cell surfaces and extracellular matrix in the form of proteoglycans (PGs), exerting a wide range of biological functions through interaction with protein ligands involved in cellular processes such as anticoagulation, angiogenesis, inflammation, viral and microbial pathogenesis, and multiple developmental processes. The fine structure of the engaged polysaccharide plays pivotal roles in controlling the activities of the GAG-binding proteins and their effect on the corresponding cellular functions. The focus of this research falls upon the structure-function relationship study of the GAG-interacting proteins from two aspects, the GAG degradation lyases and heparan sulfate (HS)-binding angiogenic growth factors.

The highly diversified biological roles of GAGs can majorly be attributed to their structural complexity with respect to various stereochemistries, patterns and levels of sulfation and acetylation. To better characterize the influence of these distinct structures on protein functions, an oligosaccharide library produced from either chemical or enzymatic depolymerization of GAGs provides excellent materials for such purposes. The first part of this study present a generic approach for the preparation of a heparin-derived oligosaccharide library with sizes ranging from disaccharide to octadecasaccharide. Analysis of these oligosaccharides adds to the current understanding of the heparin structure as well as the substrate specificity of the heparin lyases being applied. Oligosaccharides with heparin or HS representative structures have afforded potential reagents for HS-binding protein activity studies.

The catalytic mechanism and substrate specificity of heparin lyase II was first investigated using the prepared heparin and HS oligosaccharides. Heparin lyase II is distinguished from the other types of heparin lyases due to its broad substrate selectivity on both heparin and heparan sulfate-like regions of GAGs, regardless of their sulfation patterns. The underlying mechanism for its wide specificity was exploited by mutagenesis, enzymatic characterization, crystallography, and molecular simulation. Critical active site amino acids involved in catalysis were hypothesized based on the three-dimensional structure of the enzyme. The solved crystal structure also allowed tailoring on the substrate specificity of heparin lyase II by site-directed mutagenesis.

These efforts might lead to useful enzyme-based analytic tools to benefit future studies in sequencing and structural determination.

The action patterns of chondroitin lyases from various bacterial sources were next investigated. Previously studies have employed UV spectrometry, PAGE analysis, CE, TLC and HPLC as detection methods. In this study, liquid chromatography-mass spectrometry (LC-MS) was examined as new approach to determine the digestion profiles and action patterns of these lyases. Minor sequence heterogeneities having a profound importance in chondroitin sulfate biology were also identified using LC-MS. The new methodology offers an excellent technique for the future study of other polysaccharide lyases.

A further application of the heparin oligosaccharides involves characterizing the heparin-specific binding of vascular endothelial growth factor (VEGF), a much-recognized angiogenic growth factor that regulates multiple aspects of vascular development, functioning through interactions with its receptors and cellular HSPGs. With emerging evidence that VEGF promotes tumor angiogenesis, GAG-derived, or mimicking oligosaccharide inhibitors represent an exciting new direction for antitumor drug development. Nevertheless, it still remains unclear how the complex structures of GAGs influence the targeting of angiogenic molecules. This study examines the minimum binding size of heparin for $VEGF_{165}$ in an effort to compete with that binding. Knowledge of this binding should provide effective guidelines in antiangiogenesis drug design involving heparin and heparan sulfate oligosaccharides.

This thesis improves our understanding and knowledge of GAGs and the GAG-interacting proteins. Probing and characterizing these interactions through use of an array of GAG-derived oligosaccharides represents a potent approach. GAGs and GAG-related proteins are widely involved in a great many of physiological and pathological events in organisms. Knowing their underlying biology should enable their exploitation in biomedical research.