HEPARIN (AND RELATED POLYSACCHARIDES)

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Structural and Functional Properties

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To My Family

Series Editor's Preface

"*If a book is worth reading, it is worth buying.*" (John Ruskin)

Two trends, which are not especially favourable to a series such as this have become noticeable in recent years, *vis*—first, the reluctance of the polymer industry to invest in the development of entirely new homopolymers and, secondly, the increasing costs of scientific book production. Enquiry and feedback revealed, perhaps surprisingly, but nonetheless emphatically, a genuine need for authoritative up-to-date treatments on several existing polymers. Consequently, each volume is devoted to one specified polymer. The interests of an industry or an institution usually dictate that research and/or development be conducted over an extended period of time on the particular polymer of relevance. It is hoped that individuals will be able to select their specific volume from Polymer Monographs and thus be freed from the inconvenient and unfair obligation to pick sections out of the commoner, large treatises devoted to classes of polymer.

With regard to the topics themselves, proven useful application is considered a *sine qua non*, and hence macromolecules of purely academic interest are excluded from this series. An innovation is the inclusion of biopolymers and their synthetic prototypes. Since polymer science has now become truly interdisciplinary in scope, the monographs are addressed to a broad spectrum of potential readers.

These volumes are short, the aim being to present the maximum of current information in the minimum of space. Fortunately the authors, who are all pre-eminent in their respective fields, have not only complied with this difficult and stringent condition, but have succeeded in doing so without sacrificing readability. The pub-

lishers have endeavoured to make these books available at a price which is reasonable by present standards. They and I trust these volumes will prove useful and welcome comments and suggestions for future topics.

MALCOLM B. HUGLIN

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her effort in life. I am indebted to Rowly Dolman for all his help. I am most grateful to my parents-in-law for their tremendous help in our accommodation during periods of writing. And to my wife, who has suffered an author's emotional traumas, isolationism and other typical author's human responses when performing this exercise, and who has had two magnificent sons, Albert and Lee, one born at the commencement of writing this book, and the other on its completion (perhaps two of an author's typical human responses), I can only say her effort has been supreme.

Preface

Why write a book on heparin? It has intrigued me that, whereas our knowledge of the chemistry and structure of connective tissue polysaccharides (including heparin) is considerable, our knowledge of the biological role of these polysaccharides is far from clear. It is primarily for this reason that writing a book on heparin may satisfy my curiosity concerning this problem. Given the existence of a missing link between structure and function of heparin is always good material for those philosophically bent, it is clear that other problem-solving strategies should be adopted. A comprehensive treatment of the properties and interactions of heparin in a unified manner may help define the way.

The high quality work in areas of heparin anticoagulant activity, structural analysis, metabolism and interaction with cell surfaces has involved an explosive output of work in the last five years. These results and new concepts are still settling. In this arena of transience a book on heparin becomes more a newspaper article. The anticipated headlines will outdate the book quickly. However, in describing the events of the last decade I have tried to place, in parallel, recent results on heparin with its chequered past and sometimes hackneyed concepts. This may place the many diverse aspects of heparin in proper context, clear up apparent contradictions, reduce confusion and improve the chance of giving new insights into heparin.

I have placed major emphasis on the structural and functional aspects of endogenous heparin and related polysaccharides. Many important aspects of their clinical and pharmacological usage have not been included, except where it is considered appropriate that they focus on the question of the properties of endogenous material. I have frequently drawn upon many excellent review articles and the reader of both will find similarities. On the other hand, I have attempted to draw together and document much of the fragmentary and isolated information associated with the physicochemical properties of these polysaccharides.

WAYNE D. COMPER

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General Introduction

CHAPTER 1

Introduction

1.1 BRIEF HISTORY

The property of heparin, which has attracted most attention and resulted in its universal clinical use, is its anticoagulant activity. As a result, heparin is usually defined as an anticoagulant (as in most general reference works).

Attention was drawn to this property at the turn of the century where a number of workers (Pavlov, 1887; Schmidt, 1892; Morawitz, 1905; Doyon, 1912 cited in Best (1959) and Jaques (1978a)) had isolated preparations with anticoagulant properties. The significance of these findings was enhanced by the work of Jay MacLean [1891–1957] who, while working in Howell's laboratory as a medical student, set out to purify thromboplastic active materials previously reported by Howell (1912) to be due to cephalin-protein (MacLean, 1916). It was during this investigation that MacLean became interested in the deterioration of these preparations from various tissues as evidenced by their loss of thromboplastic activity. It was found that several preparations had not only lost their thromboplastic action, but actually achieved anticoagulant status. In 1918, Howell and Holt extended this study and called the material from liver, heparin derived from the Greek word *hepar* meaning liver. Isolation consisted of ethereal extraction and purification by repeated reprecipitations of the ether solution with acetone and then absolute ethanol. The antagonism between cephalin (thromboplastic active) and heparin on the clotting system was described in the Howell and Holt paper. However, there is some controversy as to whether preparations by MacLean, Howell and Holt did actually represent heparin (Jaques, 1978a) although MacLean is generally accredited with the dis-

covery of heparin. Serendipity did not apparently play a role in MacLean's 'discovery' as he states later in correspondence (Best, 1959) "Naturally I regard the statements in the literature that I discovered this (*sic* anticoagulant) 'accidentally' as not correct. It was discovered 'incidentally' in the course of the problem but not 'accidentally.'"

In 1922 Howell described under the same name 'heparin' an anticoagulant material prepared from dog liver by aqueous extraction and acetone precipitation, and in the period between 1922 and 1928 demonstrated its carbohydrate nature and the fact that it contained sulphur.

Heparin was first isolated from beef lung in sufficient purity for clinical use and for chemical study by Scott and Charles (1933). They demonstrated that it was an acidic carbohydrate capable of forming salts with metals and that it was reasonably free of other substances by crystallizing it as a barium salt (Charles and Scott, 1936). The identification of uronic acid (Schmitz and Fischer, 1933), sulphate (Jorpes, 1935) and glucosamine (Jorpes and Bergström, 1936) as the main components demonstrated that heparin was a highly sulphated anionic polysaccharide.

The realization that heparin preparations exhibited complex chemical heterogeneity was made as early as 1948, when Jorpes and Gardell isolated a by-product from a commercial preparation of heparin, which showed a positive optical rotation, but differed from heparin by the presence of acetyl groups and had low anticoagulant activity. They concluded that the substance, which they called heparin monosulphuric acid, contained one equivalent disaccharide of hexosamine, uronic acid, sulphate and acetyl moieties and thereby constituted material different from heparin. Similar material was isolated by Meyer *et al.* (Meyer *et al.*, 1956; Linker *et al.*, 1958) from the liver of a patient with amyloidosis and from other tissues and was called heparitin monosulphate. These substances were later to be more commonly known as heparan sulphates (Table 1.1).

1.2 HEPARIN AND HEPARAN SULPHATE

Heparin is the highest negatively charged molecule in tissues. It is widely distributed in mammalian tissues and fluids, the largest

TABLE 1.1
Nomenclature of the Glycosaminoglycans and Proteoglycans

Contemporary version	Other names	Abbreviation
glycosaminoglycan	glycosaminoglycuronan acid mucopolysaccharide acidic mucopolysaccharide sulphated mucopolysaccharide amino polysaccharide mucopolysaccharide	GAG
chondroitin 4-sulphate	chondroitin sulphate A chondroitin A	C4-S
chondroitin 6-sulphate	chondroitin sulphate C chondroitin C	C6-S
dermatan sulphate	chondroitin sulphate B chondroitin B B-heparin	DS
hyaluronate	hyaluronic acid	HA
keratan sulphate (I)	corneal keratan sulphate	KS (I)
keratan sulphate (II)	skeletal keratan sulphate keratosulphate keratan	KS (II)
heparin-like polysaccharides		
heparin	α-heparin or porcine heparin ω-heparin or whale heparin	Hep
heparan sulphate	heparitin sulphate heparitin sulphate (A, B, C, D) heparin monosulphate heparin sulphate N-acetylheparin sulphate	HS
proteoglycan	mucopolysaccharide-protein complex mucoprotein polysaccharide-protein protein polysaccharide	PG
multi-chain heparin	macromolecular heparin high-molecular weight heparin heparin proteoglycan	

amounts being in lung, spleen, liver and muscle. It is the only major representative of the glycosaminoglycan group in mammals which exists normally in an intracellular environment, apparently contributing to the frame-work of the granular cytoplasmic inclusions of

mast cells. The mast cells are located in connective tissues alongside the capillaries and in the wall of blood vessels and in other loose connective tissues. On the other hand, the other vertebrate glycosaminoglycans, including heparan sulphate, have a largely extracellular distribution in the amorphous extracellular matrix of connective tissues or, as with heparan sulphate, are attached to the cell surface. The non heparin-like glycosaminoglycans generally occur in much higher amounts in tissues as compared to the 'trace' nature of heparin and heparan sulphate.

The classical differences between heparin and heparan sulphate were registered on the basis of sulphate content and anticoagulant activity (heparin having relatively higher sulphate and anticoagulant activity). In broad terms, low sulphated, D-glucuronic acid-rich polysaccharides are classified as heparan sulphate, whereas high sulphated, L-iduronic acid-rich species are designated heparin. In more recent times, Lindahl (1976) has suggested that there are no sufficient criteria to clearly indicate differences between these molecules on a structural basis particularly for intermediate-type polymers and that both molecules represent the extremes for a spectrum of molecules. In view of this it seems more appropriate to consider both polymers as members of the same family, i.e. 'heparin-like polysaccharides' or heparin and related polysaccharides. However, the reader will find throughout the book references to heparin and heparan sulphate. This is not by choice (as it is confusing), but is used only to relay some continuity with published work. The clearest distinction between these two molecules is apparent in the amino acid composition of the protein cores of the proteoglycan or multi-chain forms of these polysaccharides (see Section 4.3). This distinction is hardly practical at the present time, as normal preparations are merely polysaccharide chain preparations in which the multi-chain forms have been degraded. It is not known whether the different protein cores of heparin and heparan sulphate do actually distinguish or determine the type of polysaccharide chain synthesized.

As the study of heparin is intimately related to that of the other glycosaminoglycans, it is pertinent to consider briefly some particular and general aspects of chemical structure in this group of compounds.