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A brief history of proteoglycans

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Discovery of glycosaminoglycans

The study of proteoglycans dates back to the late 19th century when chondroitin sulfate was isolated from cartilage tissues[4]. In keeping pace with the development of carbohydrate chemistry, the early half of the 20th century was devoted to the discovery of other glycosaminoglycans and the refinement of their carbohydrate analyses. Among the pioneers of this period, Karl Meyer and his colleagues stood out for their monumental accomplishments, which include discovery of hyaluronan[20], dermatan sulfate[22], keratan sulfate I and II[23] and hyaluronidase[21], and the differentiation of chondroitin sulfate A and C[24], among others. By the 1950s, when the chemical bases of glycosaminoglycan (then termed as mucopolysaccharide) structures were established, their physicochemical nature, e.g., molecular weight and viscosity, were also studied[18]. The main biological function of the glycosaminoglycans was thought to be as ground substance which fills the extracellular space of connective tissues.

From glycosaminoglycans to proteoglycans

During the 1950s, it gradually became apparent that the purified glycosaminoglycans were associated with protein components. The nature of their association, whether it involves covalent bonds or not, was initially ambiguous. The isolation of the glycosaminoglycan-protein complex by Shatton and Schubert[31] provided the first compelling evidence that they are indeed covalently associated. In 1958, Muir first demonstrated that serine remained associated with glycosaminoglycans after extensive protease treatment, presenting strong evidence that this amino acid was involved in the covalent linkage of glycosaminoglycans to the protein[25]. The structure of the reducing end trisaccharide, Gal-Gal-Xyl, of glycosaminoglycans, which links repeating disaccharide units of glycosaminoglycans to the protein, was established by Rodén and Smith in 1966[27]. The framework of proteoglycan substructures was materialized when a new isolation procedure of intact proteoglycans from cartilage tissue was devised by Sajdera and Hascall[30], combining the extraction of the molecules with dissociative solvent and isopycnic density gradient centrifugation for purification. This development in purification procedures made the study of the biological functions of intact proteoglycans possible. Shortly after, Hardingham and Muir[7] demonstrated a specific interaction between the cartilage proteoglycan core protein and hyaluronan. The result of this work predicted that proteoglycans can form supramolecular complexes with hyaluronan in cartilage tissues (often termed as cartilage proteoglycan aggregates), one of the most prominent characteristics of the cartilage proteoglycans. Indeed, the cartilage proteoglycan aggregates were made visible by electron microscopy[29] several years later.

Heparin/heparan sulfate proteoglycans

Unlike other glycosaminoglycans, heparin was discovered because of its prominent biological activity, anticoagulation[19]. Subsequent structural determination verified the glycosaminoglycan nature of the molecule[11]. Because of its complex carbohydrate modifications, elucidation of the fine structure of heparin took until the 1970s when NMR analyses became widely available. From early on, heparin has been used in clinical medicine as an anticoagulant, e.g. to prevent post-surgical thrombosis and blood clotting in artificial kidney and heart-lung machines. The anticoagulant activity of heparin is mainly explained by its specific binding to antithrombin III. The elucidation in the last decade of the specific antithrombin-binding pentasaccharide structure in heparin[15] provided critical information for the safer and more effective use of heparin in medical applications.

A closely related glycosaminoglycan, heparan sulfate, was discovered by Jorpes and Gardell in 1948[12]. In contrast to heparin, which is only found in mast cells, heparan sulfate proteoglycans are widely distributed throughout animal cells in two major locations[34], at the cell surface as integral plasma membrane proteins[1] and in basement membranes. The biological functions of these proteoglycans were not noticed until the last decade. The involvement of heparan sulfate proteoglycans in many fundamental cell functions has now been recognized[1,14,34], and active research efforts in this field will unfold diverse functions of these proteoglycans.

Biosynthesis of glycosaminoglycans

Biosynthesis of glycosaminoglycans (except for hyaluronan[15]) occurs in the Golgi apparatus on core proteins which possess the specific amino acid sequence, Ser-Gly dipeptide, by the sequential addition of sugar

precursors by specific glycosyltransferases and sulfotransferases. Biosynthesis of heparin/heparan sulfate glycosaminoglycans is especially complex, requiring more than 10 different carbohydrate-modifying enzyme activities[17]. Pioneering work by Silbert[32] and later extensive work by Lindahl and coworkers have elucidated biosynthetic steps of heparin[17]. The isolation and cloning of these glycosaminoglycan-synthesizing enzymes, a key factor in the regulation of the biological functions of proteoglycans, are on the horizon.

Mucopolysaccharidoses

Mucopolysaccharidoses represent a relatively rare group of hereditary disorders affecting lysosomal hydrolases responsible for degradation of glycosaminoglycans[26]. Since the deficiency of glycosaminoglycan-degrading hydrolases was discovered in the early 1970s, rigorous efforts have been made to isolate and determine the structures of these glycosaminoglycan-degrading enzymes. Primary amino acid sequences of several of these enzymes have been elucidated by cDNA cloning techniques[10], and now mucopolysaccharidoses are excellent candidates for gene therapy.

The past decade

Research in biology during the past decade has been decisively shaped by the development and application of molecular biology. The proteoglycan field is no exception. The primary amino acid sequences of more than 10 distinct families of proteoglycans have now been elucidated by cDNA cloning techniques. Advances in biochemical techniques have refined the isolation procedures of proteoglycans, and enabled the detailed characterization of the molecules. In addition to the more predominant forms of proteoglycans, the list of minor forms (minor in their quantity but significant in their functions) has been ever growing. The days when the function of proteoglycans was considered as an inert ground substance are long gone. Now, the precise functions of individual proteoglycans are questioned, and remarkably diverse structures and functions of proteoglycans have been emerging. Forms and functions of proteoglycans, unimaginable even a few years ago, may surprise us in the near future.

This article has scanned the enormous surface of the history of proteoglycan research in a brief form. To those who are interested in more thorough historical accounts on proteoglycans, three excellent reviews are recommended as entry points; Brimacombe's review describing the discovery and early work on the structure of proteoglycans until the early 1960s[2], the extensive collection of literature on proteoglycans until the late 1970s by Kennedy[13], and Rodén's article on the history of heparin[28]. Other articles in this issue and the recent reviews on proteoglycans from many aspects[1, 3, 5, 6, 8, 9, 14, 15, 33, 34] further elaborate on the exciting developments in recent and current proteoglycan research.

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