Biosynthesis of Extracellular Matrix Components, Glycosaminoglycans, Proteoglycans, Collagens, Elastin and Structural Glycoproteins

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Contents

Abstract

Most polysaccharides of higher vertebrates are in the extracellular matrix (connective tissues) either free or bound to proteins. Their composition and biological role will be described in decreasing order of their polysaccharide content.

Keywords

Extracellular matrix • Hyaluronan • Proteoglycans • Glycosylated collagens • Structural or matrix glycoproteins

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1 Introduction

Extracellular matrix (ECM) is the macromolecular component of connective tissues (CT), which comprise also the cellular elements, essential fibroblasts, and some other cell types which produce ECM rich in carbohydrates, unevenly distributed among its macromolecular components: glycosaminoglycans (GAGs), proteoglycans (PGs), structural or matrix glycoproteins (SGPs), collagens, and elastin. All four types of macromolecular components play essential roles in tissue structure and function to be described in the next sections, in decreasing order of the importance of their carbohydrate components. The biological role of ECM components depends on their chemical composition, their quality, quantity, and type of linkage of carbohydrates. Several of these macromolecules are used since immemorial times (as collagen for leather and for other industrial purposes). The emphasis is on vertebrate – mammalian tissues, first of all human connective tissues. We have to remind here that polysaccharides and carbohydrate-rich macromolecules appeared early during evolution, from prokaryotes, bacteria, algae, etc., both in the plant and animal kingdoms. The best known among the plant polysaccharides is cellulose, a polymer of glucose, of industrial use since antiquity. These polysaccharides were also intensely studied and will be mentioned in other chapters of this book.

2 Connective Tissue Polysaccharides

Hyaluronic acid, or better hyaluronan as called by scientists specialized in its study, is a high molecular weight polysaccharide composed of alternative units of a hexosamine (*N*-acetylglucosamine) and a uronic acid (D-glucuronic acid). Its very long chains can reach several millions of molecular weight (Balazs 1970). Its most interesting and unique properties are a high capacity to retain water and viscoelasticity. These properties are closely linked. It is the high water content which confers to this polysaccharide its remarkable viscoelasticity. This quality determines also its physiological roles in tissues. Young skin is rich in hyaluronan and confers to skin elasticity and hydration, component together with resistance to pressure. With age, the hyaluronan content of the skin decreases, with it decreases also the hydration of the skin. This contributes to the loose, wrinkly aspect the skin takes on old people. The long hyaluronan polysaccharide chains are susceptible to degradation by enzymes, called hyaluronidases, and also by free radicals (or better reactive oxygen species, abbreviated as ROSs, comprising also hydrogen peroxide (H_2O_2)). A simple experiment to demonstrate this physiologically important property of hyaluronan is to add to its solution a small amount of vitamin C (ascorbic acid) with some ferrous iron (Fe^{++}) to see the viscosity of the polysaccharide to decrease rapidly – measured with a viscosimeter – as a result of the rapid degradation of the polysaccharide by free radicals generated by this reagent. A testicular extract, rich in hyaluronidase, will exhibit the same effect. Both processes, degradation by ROSs and by hyaluronidases, are of great biological

importance. Because of its exceptional physicochemical properties, hyaluronan is industrially produced for medical purposes as, for instance, injection in arthritic articulations (as, for instance, the knee joint) for lubrication. A cross-linked hyaluronan preparation is used to fill wrinkles on the face. High molecular weight hyaluronan is used also in eye surgery. Injected before opening the eyeball, it will prevent the dislocation of intraocular structures during the surgical intervention. The inventor of this method, E.A. Balazs in the USA, called this type of surgery under the protection of hyaluronan "viscosurgery."

3 Polysaccharides of Proteoglycans

Proteoglycans (PGs) are also important macromolecular components of connective tissues. They are composed of a protein backbone carrying from one to hundreds of polysaccharide chains, called, according to their composition, chondroitin sulfates or dermatan sulfates. They are composed, as hyaluronan, of alternating units of a hexosamine and uronic acid, with one exception, keratan sulfate to be described later. There are several types of chondroitin sulfates: chondroitin A sulfate or better chondroitin 4-sulfate is composed of a succession of alternating hexosamines and uronic acids, different from those in hyaluronan. Chondroitin 4-sulfate is composed of an acetylgalactosamine-4-sulfate linked to 2-sulfated glucuronic acid. Chondroitin 6-sulfate is similar, but its *N*-acetylgalactosamine components are sulfated on position 6 as the only difference. Dermatan sulfate, another component of proteoglycans, is a polysaccharide chain composed of 4-sulfated *N*-acetylglucosamine linked to iduronic acid which can be also sulfated on position 2 of its ring, but not all iduronic acids in the polysaccharide chains are sulfated. Keratan sulfate, mentioned above, is a different polysaccharide, differing from the chondroitin and dermatan sulfates by the absence of uronic acid. In its chains, *N*-acetylglucosamine is 6-sulfated and linked to a unit of galactose (an isomer of glucose), being therefore different from chondroitin or dermatan sulfates, all containing uronic acids.

4 Heparin and Heparan Sulfates

These polysaccharides differ both in composition and localization from chondroitin or dermatan sulfates. Their hexosamine component is glucosamine which can be acetylated and/or *N*-sulfated, linked to an iduronic acid which also can be 2-sulfated. These polysaccharide chains are located on cell membranes where they play important roles in "helping" the fixation of growth factors enhancing cell proliferation. Heparin is well known for its anticoagulant capacity, used in medicine.

In the proteoglycans, these polysaccharide chains are mostly linked by an O-glycosidic linkage to the OH – group of the amino acids serine and threonine. Some polysaccharides are however linked to asparagine residues by an N-glycosidic linkage of the hexosamine component of the polysaccharide.

When bound to the central protein of proteoglycans, these polysaccharide chains are sticking out laterally and interact with collagen fibers, oriented parallel to the protein part of the proteoglycans. These interactions consolidate the orientation of collagen fibers as well as the construction of fiber bundles, important for the solidity of tissues.

5 Matrix or Structural Glycoproteins (SGPs)

Glycoproteins were first isolated from blood plasma or serum. Most plasma proteins are glycated, with the exception of serum albumin which is not, at least in bovine and human blood. The glycan content and composition are variable in quantity – from a few % to 20 % or more of the molecular weight – as well as in quality. For most, but not all, glycoproteins, the glycan chains are linked to the protein by an N-glycosidic linkage. The usual monosaccharide constituents are glucosamine, mannose, galactose, fucose, and sialic acid, at least in vertebrates and in human plasma. The detailed structure of these glycan chains was elucidated and described in great detail, among others by Jean Montreuil in Lille. The interest in blood glycoproteins increased considerably when it was demonstrated that some of them, as orosomucoid or better α_1-acid glycoprotein as well as haptoglobin, increase in blood during inflammatory processes. For this reason, determinations were described and widely used in hospital laboratories to follow the course of such diseases. One of the precursors of this medical research, M.F. Jayle at the Paris Medical School, who discovered haptoglobin – a glycoprotein forming a 1 to 1 complex with hemoglobin – proposed that connective tissues, actively participating in the inflammatory process, are the source of the glycans attached to inflammatory glycoproteins. Although this hypothesis could not be confirmed – most circulating glycoproteins are elaborated in the liver – we started to look for similar glycoproteins in connective tissues. Using an avascular tissue, cornea, we could show that it contains and synthetizes glycoproteins, similar in composition to circulating glycoproteins (Labat-Robert et al. 1986). Their glycan portion was of a comparable importance (of ~12 %) compared to circulating glycoproteins. The composition of the glycan chain was also similar; for the cornea preparation, it contained mannose, galactose, glucosamine, fucose, and sialic acid, and the only difference with plasma glycoproteins is that it was sulfated. To distinguish circulating glycoproteins from tissue glycoproteins, we called them structural glycoproteins (SGPs). We also demonstrated, using radioisotope-labeled precursors, that SGPs, present in all tissues investigated, were synthesized locally by connective tissue cells, fibroblasts, and others. A large number of such tissue-originated glycoproteins were described, forming a large part of the matrisome, the molecular component of ECM. The most studied component of these tissues – glycoproteins – is fibronectin (FN), which acts as a molecular linkage fastening cells to the surrounding ECM. Only adherent cells survive and detached cells die by apoptosis, a sort of cellular suicide. FN links ECM components to cells by interacting with cell receptors called integrins (Hynes and Yamada 2012). This adhesion process enables

cells to exchange "informations" with ECM components and is also "informed" of the behavior and fate of ECM components. This exchange of information, called "inside-out" and "outside-in" by R.O. Hynes, who discovered integrins, is of crucial importance for the homeostasis (normal physiology) of tissues.

6 Collagens

Collagens are quantitatively the most important components of ECM. They appeared with the sponges during the "Cambrian explosion"; the original, ancestral gene coding for the typical collagen sequences X–Y–glycine, where X and Y are often proline or hydroxyproline, was demonstrated in fungi (mushrooms) and even in some large viruses. Collagen molecules are composed of three peptide chains of the above composition, forming a triple helix. These peptide chains contain lysine and hydroxylysine residues. Some of the hydroxylysine residues are glycosylated, containing O-linked glucose or galactosyl-glucose residues. Therefore, collagen molecules are also part of the glycoprotein family. There are a number of different collagen types coded by different genes and differing also as far as their glycan content is concerned. Among the collagen types in vertebrates, only type VI is rich in glycans; an important part of this molecule has the typical composition of a glycoprotein, quite different from collagen composition. Therefore, it can be considered as a structural glycoprotein. Some tissues, as the cornea, are rich in type VI collagen (about 20 %), suggesting an important structural role for this molecule. As a matter of fact, the perfectly regular arrangement of collagen fibers is the condition of corneal transparency. This regular arrangement is created by the interaction of collagen type VI and proteoglycans of the cornea during its development in the embryo.

7 Elastin

As stated above, elastin is the only nonglycated macromolecule of the ECM. Its subunit, tropoelastin (TE), is not glycated and remains unglycated after its cross-linking by the formation of specific cross-link – amino acids, desmosine and isodesmosine, catalyzed by an enzyme called lysyl oxidase (LOX), as elucidated by Miles Partridge in GB. In order to form elastic fibers in tissues as the large, "elastic" blood vessels, elastin must interact with "microfibrils." These are composed of a number of SGPs, among them the fibrillins, which all are glycated. Such microfibrils (MFs) were shown to be present also in tissues, except elastin, and were present at earlier stages of phylogenesis, before the appearance of elastin with the vertebrates. Microfibrils, rich in fibrillins, replace elastin in invertebrate tissues. With aging, and even more during the development of the atherosclerotic process, the elastic fibers are degraded by elastolytic enzymes, elastases, liberating elastin peptides in the blood circulation reaching an average concentration in human blood serum of about 10 μg/ml. This is not a neutral process. The liberated elastin peptides

interact with an elastin receptor on cell membranes, triggering a series of reactions, some of which are harmful, as the release of more elastases and free radicals. This is a vicious circle, playing an important role in tissue aging.

Finally, let us say a few words on the biological importance of glycoconjugates, in particular of their glycan portion. As they exhibit specific carbohydrate sequences with their stereospecific conformations, they can specifically interact with complementary surfaces on other macromolecules and especially with lectins. These are proteins possessing stereospecific conformations enabling them to interact specifically with some carbohydrates. One example is the class of galectins specific for galactose end groups on glycan chains. A component of the elastin receptor acts as a galectin. Lectins are widespread in plants; also, the best known is concanavalin which found some practical applications in biochemistry. Glycan chains on most if not all glycoconjugates interact with stereo-compatible conformations on other molecules to fulfill their biological roles.

8 Conclusions

Oligo- and polysaccharides attached to proteins (glycoproteins) formed an important family of biological macromolecules. Most, but no all, proteins of the body are glycoproteins. The glycan chains, of specific composition, impart characteristic properties to glycoproteins and define their biological roles. Glycoproteins are present in tissues as well as in the blood circulation. Another family of polysaccharides, hyaluronan, and protein–polysaccharide complexes, proteoglycans, are important tissue components especially in connective tissues, designated as the Matrisome. They fulfill specific functions of vital importance for the body.

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