

Structure and functions of Glycosaminoglycans: a short review (with some personal scientific contributions)

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Abstract

This present article wishes to underline the strong contribution to both structure and functions of living bodies of a group of acidic polysaccharides, the Glycosaminoglycans (GAGs), usually covalently linked to proteins to give Proteoglycans (PGs): GAGs and PGs are structural components of connective tissues and of tissue extracellular matrix, also present on cell membrane surface and in secretory granules. Their basic structure is very simple: a linear repeat of disaccharide units, each composed of uronic acid and hexosamine moieties. However, except unsulphated high molecular weight Hyaluronan, they show very high structural heterogeneity, due to the chemical nature of both uronic acid and aminosugar components, the degree of monosaccharide sulphation, the distribution of O- and N-sulphate groups, the length of the chain and its copolymeric degree, the nature of interacting protein in PGs. So they are able to interact with a large variety of molecular structures, including matrix proteins, growth factors, cytokines and chemokines, and enzymes. Many functions in connective tissues are known GAG-depending. Many others in the body are expected. In this short review, to each one of the selected topics, GAGs synthesis, distribution, catabolism and related inborn errors, PGs functions, some personal scientific contributions will be recalled.

Key Words: Glycosaminoglycans, Proteoglycans, Extracellular Matrix, Mucopolysaccharidoses, human plasma

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Introduction

Glycosaminoglycans (GAGs) are long unbranched chains of repeating disaccharide units, which are sulphated in chondroitins (CS), dermatans (DS), heparans (HS) and keratans (KS), while hyaluronan (HA) is not. Sulphated GAGs are usually covalently linked to proteins to give proteoglycans (PGs), which are main components of connective tissues and of polymer matrices outside the cells, the extracellular matrices (ECMs). HS PGs are particularly associated with cells and basement membranes. My personal first approach to the field is dated more than fifty years ago, when GAGs were still named mucopolysaccharides and both their distribution and their binding to protein were still under study (1,2).

GAGs structure (3)

The GAGs basic structure is composed of a linear sequence of disaccharide units, each made up by a hexosamine moiety, linked to an uronic acid moiety. Hexosamine is N-acetyl-D-glucosamine in HA, unsulphated GAG, and in HS and KS (where it is O-sulphated and, in HS, N-sulphated). N-acetyl-D-galactosamine is present in CS and DS, usually O-sulphated. Uronic acid is usually D-

glucuronic acid, which is replaced by L-iduronic acid in HS, and by galactose in KS. These basic structures, made up by the simple repeating of the same disaccharide unit, the one typical of each GAG, are otherwise largely varied by a number of modification of the original scheme, especially by the extent and the position of the sulphate groups in both uronic acid and hexosamine moieties, by the length of the chain, by the copolymerisation with disaccharide unit typical of another GAG. So that a very high heterogeneity is present in these only apparently simple repeating of disaccharide units. In addition, the heterogeneity of protein to which the polysaccharide chain (or a variable number of chains) is covalently linked greatly increases the reactive properties of the resulting proteoglycans (PGs), and then of their metabolic functions. The covalent link with protein is a glycosidic link to serine of the pentose xylose, which through galactose, galactose and glucuronic acid residues is connected to the first hexosamine of the GAG chain. The exception is KS. For one type of KS, N-acetylated glucosamine is connected to protein through N-asparaginyl bond. In my doctoral thesis at the University of Pavia I showed the origin in cartilage of xylose from glucuronic acid (1) and also I showed for the first time the presence of N-asparaginyl bond in KS: however, this last result was not enough supported by multiple experimental data, and was underestimated (as friendly noted by John E. Scott, who mentioned the result in the book *Keratan sulphate: chemistry, Biology, Chemical Pathology*, Editors Greiling, H., Scott, J.E, published in 1989 by The Biochemical Society).

GAGs biosynthesis (4)

The biosynthesis of GAGs (but HA) takes place at the Golgi apparatus, on the core protein previously synthesized in the rough endoplasmic reticulum and moved to Golgi. The biosynthesis of GAGs CS/DS and HS/heparin initiates by linkage to a serine residue of core proteins of xylose residue, using xylosyl transferase and UDP-xylose as donor. To xylose, two galactose residues and a glucuronyl residue are sequentially added, from their UDP derivatives, by galactosyl and glucuronosyl transferase enzymes. To this common linkage tetrasaccharide N-acetylgalactosamine and glucuronic acid moieties are sequentially added by specific transferases in CS/DS polymerization, with simultaneous modification reactions, including sulphation (by PAPS, 3'-phosphoadenyl-5'-phosphosulphate) and epimerization of glucuronic to iduronic acid. Different glycosyltransferases, epimerases and sulphotransferases are used to synthesize quite different sulphation motif oligosaccharide domains, so that numerous different ligand binding domains are produced to allow a number of different specific association with molecules, as growth factors and cytokines, regulating cellular differentiation and proliferation, or molecules for tissue structure (5). Heparin and HS assemble on linkage tetrasaccharide as copolymers of N-acetyl-glucosamine and glucuronic acid residues, by specific transferases: a series of modification reactions including

sulphation and epimerization of glucuronic to iduronic acid and N-deacetylation and N-sulphation of glucosamine occurs simultaneously. The HS chains consist of different domains that vary in the extent of modification, with N-sulphated domains and N-acetylated domains. KS, N-linked to asparagine residue or O-linked to threonine or serine of the core protein by means of N-acetyl galactosamine, is synthesized by alternate addition of galactose and N-acetyl glucosamine. Sulphation of the chain may be on position 6 of the aminosugar and of galactose.

The unsulphated GAG, HA, is synthesized at the inner surface of the cell membrane by three isoenzymes, named HA synthase 1,2 and 3, catalyzing the transfer of glucuronic acid and N-acetyl glucosamine from their UDP derivatives (6).

GAGs catabolism and related inborn errors (Mucopolysaccharidoses)

Proteoglycans of the extracellular matrix or membrane associated structure are proteolytically cleaved, and GAG chains enter the lysosomes for intracellular digestion. Each GAG type shows a different pathway of lysosomal degradation. Ten different enzymes, four glycosidases, five sulphatases, and one nonhydrolytic transferase are involved. Deficiencies of each one of these enzymes have been reported and result in seven storage diseases, called Mucopolysaccharidoses (MPSoses), with typical and highly compromising symptoms, usually explained by abnormal accumulation of undegraded GAGs within the lysosomes (7-9), although this concept has been recently challenged (10). Traditionally, MPSoses are recognized through quantitation of urinary GAGs (11), and urinary GAG analysis may be useful to differentiate the MPSoses types (12). We studied also the GAG-derived oligosaccharides in urine of MPSoses (13). To these analytical methods, useful as screening for MPSosis occurrence, although unable to differentiate the different types, significant contribution was given by enzymatic assay of the defective enzyme (8), and actually the MPSoses are subjected to therapy by enzyme replacement and gene therapy (14)

GAGs occurrence in human and animal plasma

All mammalian cells produce PGs and secrete them into the extracellular matrix: so GAGs are widely distributed. At Baylor University in Houston, Texas, in Di Ferrante's laboratory on connective tissue, we investigated GAGs in human term placenta (15) and in human plasma (2). The contribution on plasma GAGs was well accepted in the international GAG scientific family, and for many years it was the basis of any other further characterization in the field. At the Universities of Pavia and then of Messina, the topic was also further investigated and the study extended to animal species (16-19). Especially important was the GAG measurement, at the University of Messina, in plasma of patients with chronic lymphocytic leukaemia and essential thrombocythaemia (20): we showed that the proteoglycan Decorin (see below), that is not synthesized in normal lymphocytes, neither in lymphocytes of patients with chronic lymphocytic leukaemia of standard evolution, is present in

lymphocytes of patients with chronic lymphocytic leukaemia of very low evolution, undergoing no therapy, so suggesting a role as natural antioncogenic factor (21) for decorin, already exhibiting potent oncosuppressive action in tumourigenesis (22). Method of plasma GAG measurement was also improved (23).

Proteoglycans (PGs): types and functions

As pointed out, PGs are produced by the different types of mammalian cells: they vary in structure for a number of factors, especially the heterogeneity of the protein core linked to GAG chain and the type (and its heterogeneity due to the structural variations, i.e. number and location of sulphate groups, of their repeating disaccharide units) and number of GAG chain linked to protein core.

PGs are usually classified according to their location, or in extracellular matrix, or at membrane level, or in secretory granules. The biological functions of PGs depend on the interaction of the GAG chains with different protein ligands.

Among PGs secreted in ECM the aggrecan family, showing the protein moiety able to bind Hyaluronic acid (HA). Aggrecan contains in humans 100 CS chains and also KS chains, and many PGs structures are linked to the same HA chain, so that a huge macromolecular complex is generated, able to bind tremendous quantity of water and form hydrated matrices capable of absorbing high compressive loads by water desorption and resorption. Among PGs of ECM, the small leucine-rich PGs (SLRPs), as decorin, contain CS, DS and KS chains, and organize collagen fibers, by determining their ordered spacing in all connective tissue (24-25). In few words and schematic way, the protein core of decorin is on the axis of collagen fiber, while the polysaccharide chains are at 90 degree in the space around collagen fiber (they “decorate” the fiber): the length of the polysaccharide chain and the position on it of the polysaccharide to polysaccharide binding sequences on overlapping chains from two different collagen fibers define the distance between and the parallel deposition of collagen fibers, different in the different connective tissues. In corneal stroma, the highly ordered system of collagen fibers defined by PGs ensures transparency. ECMs are maintaining animal shapes: the module of collagen fibers held together by PGs strings are elastic: external pressure forces water out, that returns post compression (26)

As for secretory granules PGs, serglycin (27-28) is the major PG present in cytoplasmic secretory granules in hematopoietic cells, in tumor cells, and in several non-hematopoietic cells as chondrocytes, endothelial cells, smooth muscle cells, fibroblasts. In human serglycin, the 158 aminoacid core protein contains eight serine/glycine repeats, each potential GAG (CS/HS) attachment site. Serglycin interacts with several inflammatory mediators, such as chemokines,

cytokines, growth factors and proteases. The membrane PGs are mainly Syndecans and Glypicans: the syndecan family (29) components are membrane spanning PGs containing CS and HS chains (syndecans 1 and 3), or only HS chains (syndecans 2 and 4). Syndecans consist of an N-terminal extracellular domain, where GAG chains attach distally from the plasma membrane, followed by the transmembrane domain and a short cytoplasmic domain. Syndecans are able to regulate cell adhesion and migration, by associating with actin cytoskeleton; and regulate calcium metabolism; they are considered key molecules during cancer initiation and progression, by interacting with extracellular matrix molecules and with intracellular kinases; syndecans interact with cell surface growth factor receptors and integrins, activating signaling pathways, critical for cellular behavior (30). Syndecans play a major role in inflammation (31). Glypicans are at the surface of the membrane, linked by a GPI (glycosylphosphatidylinositol) anchor, and carry only HS chains. They can bind many factors essential for development and morphogenesis. The important member of the family is Glypican-3, a regulator of growth (32). Among other membrane PGs, CD44, a transmembrane cell-surface receptor, able to bind HA, highly expressed in many cancers that regulates metastasis (33).

Antioxidant effect of GAGs

In the last years of my activity at the University of Messina, the interest of the research group was addressed, with the very important input of Prof. Giuseppe M. Campo, to evaluate the protective effect of GAGs, especially chondroitin sulphate, in animal (rat) models of human pathologies (rheumatoid arthritis, liver injury, pancreatitis) and in cell cultures. The protective effect was considered to depend on the antioxidant activity of the polysaccharides. Commercially available GAG molecules and GAGs obtained from human plasma were utilized. The effect in cell culture and in vivo of the GAGs addition has been studied in terms of expression and regulation of metalloproteases and of nuclear factor NF- κ B and caspases (34).

Since cytokines stimulate cellular synthesis of hyaluronic acid (HA), the cytokine effect on HA synthesis and the effect of HA at different molecular weight has been studied on NF- κ B expression and caspase activity, and also for receptor Toll-like. Hyaluronic acid at high molecular weight is anti-inflammatory, while at small molecular weight it is pro-inflammation: receptors involved are CD44, the HA receptor, and toll-like receptor-4 (TLR-4). Modulation of these receptors has been studied in cell cultures and in animal (35). These research topics were further highly investigated by Prof. Campo research group in the following years (36-37).

Conflicts of interest: The authors declare no conflict of interest.

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