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Chemistry, Biochemistry and Pharmaceutical potentials of Glycosaminoglycans and Related Saccharides

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15.1
Introduction

The first description of glycosaminoglycans (GAGs) was by J. Müller in 1836, who isolated "chondrin", a sugar-related substance from cartilage that was later shown to contain a sulfo group by Mörner (1889) and renamed "chondroitsäure" [1]. It was not until 1935 that Karl Meyer discovered hyaluronic acid, initiating the exploration of GAG biochemistry and the identification of different types of GAGs. The past half-century has resulted in substantial progress in the elucidation of GAG fine structure, biosynthesis, and biological functions.

GAGs are unbranched, polydisperse, acidic polysaccharides, often covalently linked to a protein core to form proteoglycans (PGs). GAGs extend from a protein core in a brush-like structure. The core protein size ranges from 10 kDa to >500 kDa, and the number of attached GAG chains varies from 1 to >100 [2]. Except for hyaluronic acid, all GAGs are biosynthesized as PGs. The linkage region is the same in all PGs (except for keratan sulfate) and consists of the tetrasaccharide – glucuronic acid (GlcAp), galactose (Galp), Galp, and xylose (Xylp) – linked to the hydroxyl group of serine in the polypeptide core (Fig. 15.1) [3]. PGs occur in the membranes of all animal tissues, intracellularly in certain cells (usually in secretory granules) or extracellularly in the matrix, where they are exported to perform a variety of biological functions.

GAG biosynthesis is initiated with the synthesis of the core protein, rich in serine-glycine repeats [4], to which the linkage region and GAG are attached. GAGs are characterized (with the exception of keratan sulfate) by a repeating core disaccharide structure comprised of uronic acid and hexosamine residues. The amino group of the hexosamine residue is either *N*-acetylated or *N*-sulfonated, the uronic acid being either D-glucuronic acid or L-iduronic acid. Moreover, the repeating disaccharide units are *O*-sulfonated to varying degrees at the 3-, 4-, or 6-positions of the hexosamine residue and at the 2-position of the uronic acid residues. The most common GAGs are heparin, heparan sulfate (HS), hyaluronic acid (HA), chondroitin sulfate (CS), dermatan sulfate (DS), and keratan sulfate (KS) (Tab. 15.1).

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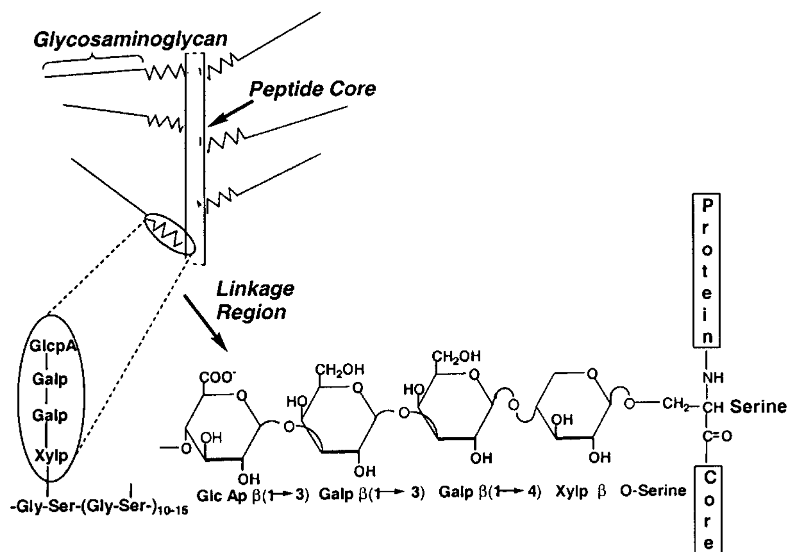


Fig. 15.1 Structure of the GAG linkage to protein in PGs.

Tab. 15.1 The structures of the sulfated GAGs.

GAG	*Disaccharide Unit*	*Modifications*
Heparan sulfate (HS)/ Heparin	[GlcAp/IdoApβ/α1-4GlcNpAcα1–4]	*N*-deacetylation, *N*-sulfation C5-epimerization of GlcAp C2-sulfonation on GlcAp/IdoAp C3,C6-sulfonation on GlcNpAc
Chondroitin sulfate (CS)/ Dermatan sulfate (DS)	[GlcAp/IdoApβ/α1–3GalNpAcα1–4]	C5-epimerization of GlcAp C2-sulfonation on GlcAp/IdoAp C4,C6-sulfonation on GlcNpAc
Keratan sulfate (KS)	[Galpβ1-4GlcNpAcβ1–3]	C6-sulfonation on Galp/GlcNpAc

15.1.1 Biological Activities

The PG family consists of 30 members, which display a wide variety of biological functions [5]. They play important roles in the extracellular matrix organization, influence cell growth and tissue maturation, and participate in the regulation of matrix turnover by binding, inactivating protease inhibitors.

The biological interactions and events mediated by PGs are believed to be due primarily to the presence of GAG chains. Since the PGs are often localized on cell surfaces and in the extracellular matrix, they function in the important role of

cell-cell interaction, binding a variety of proteins and localizing these at the cell surface [6, 7]. The heparin/HS GAGs are known to bind over 100 different proteins, including enzymes, protease inhibitors, lipoproteins, growth factors, chemokines, selectins, extracellular matrix proteins, receptor proteins, viral coat proteins, and nuclear proteins [8]. Some of the biological roles of GAGs have been exploited for the design and preparation of therapeutic drugs.

15.1.2 Heparin and Heparan Sulfate

15.1.2.1 Structure and Properties

Heparin, an anticoagulant isolated from animal tissue, is an important and chemically unique polysaccharide of considerable biological significance. It was discovered in 1916 by Jay McLean, working under the directions of William Howell at John Hopkins University [9]. To ascertain the origin of a substance causing blood coagulation, McLean isolated fractions from mammalian tissues. These, however, instead of clotting blood, prevented its coagulation [10]. Howell recognized the importance of his student's discovery, suggesting heparin's therapeutic use to treat coagulation disorders.

Heparin is a polydisperse, highly sulfated, linear polysaccharide made up of repeating $1 \rightarrow 4$ linked uronic acid and glucosamine residues (Fig. 15.2) [11, 12]. Although heparin has been used clinically as an anticoagulant for the past 70 years, its precise structure remains unknown. The failure to understand heparin's structure completely is not the result of a lack of effort, but rather is due to its extremely complex nature. The structural complexity of heparin can be considered

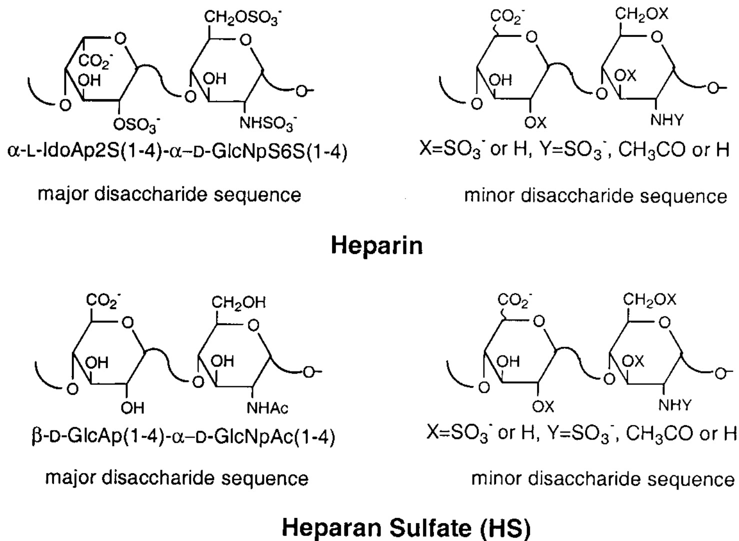


Fig. 15.2 Structures of heparin and heparan sulfate glycosaminoglycans.

at several levels. At the PG level, different numbers of polysaccharide chains (possibly with different saccharide sequences) can be attached to the various serine residues present in the protein core. Once freed from the protein core, through the action of tissue proteases, peptidoglycan heparin (a small peptide to which a single long polysaccharide chain (M_r 100,000) is attached) is formed [13]. This peptidoglycan is short-lived, as it is immediately processed by a β-endoglucuronidase to form a number of smaller polysaccharide chains (only one of which, corresponding to the original site of attachment to the core protein, should contain peptide) called GAG heparin. At the GAG level, some of heparin's structural complexity results from its polydispersity. GAG heparin has a molecular weight (MW) ranging from 5–40 kDa (degree of polymerization (dp) 10–80) with an MW (average) of 13 kDa. Even the heparin chain corresponding to the most prevalent dp represents a mere 5 mol% of a typical GAG heparin preparation [14]. GAG heparin has a second level of structural complexity associated with its primary structure or sequence [15]. The heparin/HS structure has been partially characterized by study both of its biosynthesis [16, 17] and of its chemical structure, by chemical, enzymatic, and spectroscopic techniques [10].

While GAG HS is structurally similar to GAG heparin (Fig. 15.2) [12], the core proteins of PG HS and heparin are different. HS is primarily found in the extracellular matrix and in cell membranes [18, 19], while heparin is only intracellular. Although structurally similar, HS and heparin have different ratios of *N*-acetyl to *O*-sulfo groups and can be often distinguished by differences in their sensitivity to heparin lyases [20].

15.1.2.2 **Biosynthesis and Biological Functions**

Heparin is synthesized in connective tissue-type mast cells, as part of the serglycin PG [2]. HS is produced by most animal cells and is bound to a variety of core proteins, corresponding to syndecan, glypican, perlecan, and agrin PGs [21]. After translation in the rough endoplasmic reticulum, the core proteins are transported to the Golgi apparatus, where the enzymes responsible for HS/heparin biosynthesis are located. Selected serine units are *O*-substituted with the GlcAp-Galp-Galp-Xylp-"linkage region" that connects the GAG chain to the core protein [22]. Next, the stepwise transfer of monosaccharide units from the appropriate UDP-sugars to the nonreducing termini of the nascent chains generates a precursor polysaccharide, $[\beta 1,4\text{-GlcAp-}\alpha 1,4\text{GlcNpAc-}]_n$. The linear polysaccharide chain is extended by approximately 300 sugars before its synthesis is terminated [17]. Conversion of the $(\text{GlcAp-GlcNpAc})_n$ precursor structure into the products recognized as heparin/HS occurs through a series of polymer-modification reactions initiated while the chain is still under elongation [23]. The first modification step is the *N*-deacetylation and *N*-sulfonation of GlcNAc residues. The resultant *N*-sulfo groups are prerequisite to all subsequent modifications, which include *C*-5 epimerization of GlcAp to L-iduronic acid (IdoAp) units, 2-*O*-sulfonation of GlcAp and IdoAp units, and 6-*O*- and 3-*O*-sulfonation of GlcNp residues. The process occurs in a stepwise fashion, with the products of a given reaction providing the substrate for

subsequent reactions [21]. However, most of these reactions do not go to completion, and a fraction of the potential substrate residues in each step escapes modification. While it is unclear what control (if any) is exerted on the extent of modification, such partial polymer modification is a fundamental feature of the biosynthetic process, and results in the diversified domain structure of HS.

PG heparin is primarily found in the granules of mast cells. When mast cells degranulate, heparin is released as GAG heparin, the result of processing by proteases and endo-β-glucuronidases [13, 24]. Although the GAG heparin released on mast cell degranulation demonstrates anticoagulant activity, the role of this activity is unclear. There is no evidence that endogenous mast cell heparin plays a role in maintaining blood flow through the vasculature, even though this is the primary application for exogenously administered GAG heparin [25, 26]. The true biological function of heparin still remains contested [8].

The biological activities of HS PGs result primarily from the specific interaction of proteins with their GAG chains. Some of the functions of syndecan, a HS PG, are: (1) organization of extracellular matrix, through binding to collagens, fibronectin, thrombospondin, tenascin, etc., (2) organization of the epithelia, (3) affording non-thrombogenic vascular endothelial surfaces, through binding to antithrombin III (ATIII), protein C, and protease nexins, (4) as a co-receptor for fibroblast growth factor (FGF), and (5) regulation of development in early embryogenesis and in cancer [8]. Syndecans are expressed in tissue-specific patterns; their expression is highly regulated and this regulation may be affected by their cell surface residence time. Syndecans are shed from the cell surface as the result of their cleavage at a protease-susceptible site near the plasma membrane. The extracellular matrix at the basal cell surface might slow syndecan release.

15.1.2.3 Applications of Heparin and Heparan Sulfate

Although the biological roles of endogenous PG heparin and PG HS are not completely understood, this has not precluded the use of GAGs derived from these natural products – as well as GAG fractions, oligosaccharides, and synthetic analogues – for a variety of medical applications. Heparin is the most commonly used clinical anticoagulant. Over 33 metric tons of heparin, representing over 500 million doses, are manufactured worldwide each year [10]. Since orally administered heparin is inactive and heparin has a low bioavailability when administered *subcutaneously* [27, 28], it is usually injected *intravenously*. The success of low molecular weight (LMW) heparins is primarily a result of their high *subcutaneous* bioavailability [29]. LMW heparins are prepared by the controlled chemical or enzymatic depolymerization of heparin. The clinical use of LMW heparin has recently surpassed the use of heparin in the US. In addition to heparin's anticoagulant activity, it has a wide variety of other activities (Tab. 15.2).

Heparin and its Antithrombotic Activity The antithrombotic action of heparin is due mainly to its ATIII-mediated anticoagulant activity. ATIII, a serine proteinase

Tab. 15.2 Potential therapeutic applications of heparin and heparin analogues.

Application	*Status*	*Reference*
Anticoagulant/antithrombotic	Currently in use	30
Antiatherosclerotics	Clinical trials	31
Complement inhibitors	Clinical trials	32
Anti-inflammatory	Animal studies	33
Antiangiogenic agents	Animal studies	34
Anticancer agents	Animal studies	35
Antiviral agents	Animal studies	36
Anti-Alzheimer agents	Animal studies	37

inhibitor (SERPIN) [38], is an anionic [39] glycoprotein of molecular weight 58,000 [40]. This SERPIN forms tight, irreversible, equimolar complexes with its target enzymes (thrombin, factor Xa, etc.) through the formation of an ester between an arginine residue of its active site and the serine residue of the active site of the enzyme. This slow, time-dependent inhibition process is accelerated 2000-fold by heparin [39]. The reversible interaction of heparin with ATIII induces a conformational change in ATIII, which enhances its anticoagulant activity [39].

Apart from its ATIII-mediated activity, heparin (and dermatan sulfate) stimulates the inactivation of thrombin by heparin cofactor II (HCII). HCII has a molecular weight and pI similar to those of ATIII [41, 42]. The physiological role of HCII might be as a reserve of thrombin inhibitor when the plasma concentration of ATIII becomes abnormally low [43]. Unlike ATIII, HCII specifically inhibits thrombin and no other coagulation proteases [44, 45].

The antithrombotic activity of heparin is not limited to its anticoagulant effect; heparin also influences other factors significant in thrombogenesis. Heparin increases the electronegative potential and consequently the antithrombogenic character of the vessel wall [46]. It may increase the production and release of anticoagulant-active HS from the endothelium [47]. Heparin affects platelet function and the release of substances important to homeostasis from the platelets [48]. For instance, heparin releases the tissue factor pathway inhibitor (TFPI), which enhances antithrombogenesis [49].

Antiatherosclerotic Activity Atherosclerosis develops from a disturbed homeostasis between the blood and the vessel wall. Blood-borne constituents cause repeated injuries to the vessel wall in various target organs, provoking a chronic, inflammatory fibroproliferative response [50], which ultimately results in arterial obstruction and insufficient blood supply to the organs. A chronically elevated plasma low-density lipoprotein (LDL) cholesterol level plays a key role in damaging the arterial wall [51]. Inflammatory heparin-binding constituents, such as complement, C-reactive protein, fibrinogen, tumor necrosis factor, viruses, and lipoprotein a, are implicated, together with homocysteine and mechanical shear forces, in promoting the progression of atherogenesis [52, 53]. Repeated injuries to the endotheli-

um weaken its resistance, resulting in transmigration of blood-borne constituents, such as LDLs, into the artery walls [52, 53].

When heparin is administered *intravenously*, lipoprotein lipase (LPL) is mobilized from the vascular endothelial surface into the blood. This may result in increased triglyceride lipolysis in the bloodstream, lowering the concentration of cholesterol-rich remnant particles in contact with the arterial wall [54–57]. The effect of heparin on the release and activation of LPL has been well studied [54, 55]. Heparin's application as an antiatherosclerotic agent is limited by its primary activity as an anticoagulant and its lack of oral bioavailability [58]. Heparin analogues might be useful in circumventing these problems.

The proliferation of smooth muscle cells (SMCs) after damage to endothelium is an important part of atherogenesis, resulting in further occlusion of vessels [59–61]. Both anticoagulant and non-anticoagulant heparin have demonstrated the ability to inhibit the proliferation of SMCs [62]. This activity results from heparin's interaction with growth factors including fibroblast growth factor (FGF) and endothelial cell growth factor (ECGF) [61].

Ability to Inhibit Complement Activation The complement system, important in the immune and inflammatory responses, involves proteins present in the bloodstream in an inactive form [32]. Activation of these complement proteins results in increased vascular permeability, activation of neutrophils, and alterations in cell membranes, ultimately causing cell lysis and death [63]. Activation of the complement system involves the sequential interaction of the serum complement proteins. Since complement activation can potentially produce profound effects, the system has inhibitors. C1 esterase inhibitor is a serum protein that inhibits the activation of the first component of complement. Heparin greatly potentiates this inhibitory activity [64]. Heparin is believed to bind simultaneously to C1 and to C1 esterase inhibitor, bringing the molecules into close proximity, kinetically favoring and stabilizing their interaction [65]. The C1 macromolecule may also be directly inactivated by heparin [66]. Purified C1q has two high-affinity binding sites for heparin, and heparin inhibits interaction of C1q with other C1 components to form hemolytically active C1 [67]. Complement activation ultimately involves amplification of the third component of complement (C3) through the formation of an amplification convertase. Heparin inhibits the generation of this amplification convertase [68] and this is independent of its antithrombin-binding activity [69].

Anti-inflammatory Activity Heparin may act as an anti-inflammatory agent through its interaction with selectins and chemokines [70]. Selectins are a family of transmembrane glycoproteins found on endothelium, platelets, and leukocytes [71]. When cells are activated, L-selectin on the cell surface increases, allowing their migration to the lymph nodes. In acute inflammation, neutrophils move through the vascular wall [8]. Initially, neutrophils anchor to the endothelium through a carbohydrate-protein interaction [71]. Although the putative ligand on the endothelium responsible for leukocyte interaction with selectins is sialyl Lewis X, HS has also been shown to play a role in this interaction [72].

Chemokines are soluble GAG-binding proteins involved in the recruitment and activation of leukocytes [73]. There are at least 15 known chemokines and in vitro they exhibit overlapping activities. Some selectivity is observed in vivo, and may correlate to differential localization of chemokines in tissues. The GAG chains of cell surface PGs have been postulated to play a role in this differential localization. The heparin/HS family of GAGs are most responsible for chemokine binding.

Role of Heparin/HS in Angiogenesis Angiogenesis (neovascularization) involves the growth of new capillary blood vessels. It plays an important role in normal development and in the physiology of reproduction [74]. Angiogenesis is also essential in wound repair, peptic ulcers, and myocardial infarction [75, 76]. In both physiologic and repair conditions, angiogenesis is regulated to switch on and off at predictable times. In a variety of disease processes, such as tumor growth and metastasis, however, angiogenesis is unregulated. After a tumor takes hold it grows slowly under oxygen limitations and remains quite small. When new capillaries come sufficiently close for oxygen to diffuse into the tumor, though, the vascularized tumor cells multiply rapidly. The process of angiogenesis requires the induction of proteases, degradation of the basement membrane, migration of the endothelial cells into the interstitial space, endothelial cell proliferation, lumen formation, generation of new basement membrane with the recruitment of pericytes, fusion of the newly formed vessels, and initiation of blood flow.

Heparin may play a variety of roles in angiogenesis. Immediately before capillary ingrowth, mast cells containing heparin accumulate at the site of the tumor [77]. The heparin from these mast cells can stimulate endothelial cell migration. Protamine and the chemokine platelet factor 4, both of which bind and inactivate heparin, can inhibit angiogenesis [78]. Heparin can localize, activate, stabilize, and stimulate angiogenic growth factors such as FGF and ECGF [79–81].

The FGFs are a multi-ligand, multi-receptor family in which one receptor can bind several ligands with high affinity. The assembly of 2FGF-2FGFR-2HS chains comprises a signal transduction complex that results in cell replication [82]. Interactions are coordinated through the HS PG, which can either promote or restrict growth factor binding to a particular receptor [83]. Heparin and heparin oligosaccharides (in the presence of angiostatic steroids) [84, 85] and even heparinase [86] can inhibit angiogenesis. In the presence of other factors such as steroids, the HS PG lining the endothelium restrains capillary growth [79]. This quiescent microvasculature can rapidly respond to heparin-modulated growth factors produced during ovulation, by wounds [87], or in inflammation (as occurs in stroke, where the damaged blood brain barrier requires repair [88]), and to the release of endogenous angiogenesis inhibitors (such as angiostatin and endostatin) [89].

Heparin and Cancer Heparin affects the progression of cancer in many ways. Because of its anticoagulant function, heparin can inhibit thrombin and fibrin formation induced by cancer cells. Heparins may therefore potentially inhibit intravascular arrest of cancer cells and thus promote metastasis. In addition to their

anticoagulant function, heparins bind to growth factors and extracellular matrix proteins and consequently can affect proliferation and migration of cancer and angiogenesis in tumors [90]. Heparin has been found to inhibit expression of oncogenes and to affect the immune system [91]. Heparin has both stimulatory and inhibitory effects on proteolytic enzymes essential for invasion of cancer cells through the extracellular matrix [92]. Heparin also reduces tumor cell-platelet adhesion, reducing metastasis [93]. This wide variety of activities makes the ultimate effect of heparin on cancer still uncertain [34].

Antiinfective Activity HS, ubiquitously found on the surfaces of animal cells, represents an ideal means for pathogens to localize on the membrane of the target cells that they infect [94, 95]. Moreover, heparin in mast cells [77] also binds pathogens and one of its roles might be to adhere to pathogens and to target them to dendritic or phagocytic cells.

Viruses gain entry into cells by using the HS PGs, which line the surface of most mammalian tissues, as receptors. Herpes simplex virus (HSV) anchors onto HS and localizes on the cell surface prior to its entry [96, 97]. Human immunodeficiency virus (HIV) binds HS PG on T-lymphocytes through an extended loop in its GP120 coat protein [96], and heparin can block this interaction [99]. Heparin has been clinically tested for the treatment of AIDS and is a potent and selective inhibitor of HIV-1 replication in cell culture [100]. Heparin, a highly sulfated heparin decasaccharide, and suramin, a small synthetic heparin analogue, can block dengue virus infection [36] by binding dengue envelope protein [101].

Malaria parasite circumsporozoites infect human liver by binding to the highly sulfated human liver HS PG [102]. Liver HSPG also appears to act as the receptor for the apolipoprotein E (apoE) [103].

The bacterial protein BGP from *Borrelia burgdorferi,* which causes Lyme disease, is a heparin-binding protein [104], suggesting that infection takes place through bacterial interaction with a HS acceptor.

Alzheimer's Disease Alzheimer's disease (AD) is characterized by the deposition of amyloid plaque and neurofibrillary tangles in the brain [105]. The amyloid peptide contains a specific sequence capable of binding heparin at the low pH values present within these plaques [106]. Heparin also inhibits a protease nexin found predominantly in the brain and possessing an activity associated with AD [107]. Basic FGF is found bound to HS PGs in plaques as well as in inclusions in Parkinson disease, suggesting a possible role of HS PG in the formation of intraneuronal inclusions [108]. ApoE4, encoded by a genetic marker closely associated with late onset AD, binds tightly to heparin [103], and this interaction is associated with the neurotoxicity of apoE4 [109]

Interactions of Heparin with Proteins The biological activity of heparin is usually attributed to its interaction with heparin-binding proteins [94, 110]. Heparin has been found to bind to a large number of proteins (Tab. 15.3). These proteins can be classified as: (1) enzymes, (2) protease inhibitors, (3) lipoproteins, (4) growth

Tab. 15.3 Selected heparin/HS binding enzymes and proteins.

1. **Enzymes**
   Lipolytic enzymes
   Kinase
   Phosphatases
   Enzymes acting on carbohydrates
   Proteases/esterases
   Nucleases, polymerases, and topoisomerases
   Other enzymes, oxidases, synthases
2. **Protease inhibitors (serpins)**
   Antithrombin III (AT III)
   C1 Inhibitor proteins
   Heparin cofactor II (HCII)
   Protease nexin
   Thrombomodulin
3. **Lipoproteins**
   Low and very low density lipoproteins
   Apolipoprotein B-100
   Apolipoprotein E
4. **Growth factors**
   Fibroblast growth factors
   Epidermal growth factors
   Hepatocyte growth factor
   Platelet-derived growth factor
   Smooth muscle cell growth factor
   Transforming growth factor
   Vascular endothelial growth factor
5. **Chemokines**
   Interleukin 8 (IL-8)
   Neutrophil activating peptide 2
   Platelet factor IV
6. **Selectins**
   L-selectin
   P-selectin
7. **Extracellular matrix proteins**
   Collagens I–VI
   Fibronectin
   Laminin
   Tenascin
   Vitronectin (S-protein)
8. **Receptor proteins**
   CD4 receptor
   FGF receptor (FGF1–4)
   Glycoprotein 330 (LDL receptor)
9. **Viral coat protein**
   gp120 of HIV-1
   gp140 and gp160 of HIV-2
   Herpes simplex virus-1 (HSV-1)
   Dengue envelope protein
10. **Nuclear proteins**
   Histones
   Transcription factors
11. **Other proteins**
   Fibrin
   Immunoglobulin G
   Protein C inhibitor
   Alzheimer β-amyloid precursor protein (APP)
   Platelet/endothelial cell adhesion molecule-1 (GMP-140)

factors, (5) chemokines, (6) selectins, (7) extracellular matrix proteins, (8) receptor proteins, (9) viral coat proteins, (10) nuclear proteins, and (11) others [111]. The specificities and strengths of these interactions are probably the result of ionic binding between the sulfo groups of heparin and basic amino acid residues of the interacting protein [112]. In some cases binding can occur through nonionic interactions such as hydrogen bonding [94].

15.2 Dermatan and Chondroitin Sulfates

15.2.1 Structure and Biological Role

Dermatan sulfate (DS) and chondroitin sulfate (CS) make up a second GAG family, called galactosaminoglycans. CS is the most abundant GAG in the body, and occurs in both skeletal and soft tissue. Much of our knowledge of GAGs is derived from studies of chondroitin 4-sulfate (CS-A), the first member of this class to be isolated in a pure state, from cartilage. CS consists of repeating units of GlcAp and GalNpAc. The two most common isomers contain *O*-sulfo groups at positions 4 (CS-A) or 6 (CS-C) of the galactosyl residue (Fig. 15.3, structure I). The size of the CS chain varies greatly, with an average of about 40 repeating disaccharide units for the cartilage proteoglycan, corresponding to a molecular weight of about 20,000 [113]. The number of sulfo groups also varies, with some galactosyl residues containing none, and some containing multiple sulfo groups at positions 2 and 4 of GalNpAc and at position 2 of the uronic acid residue. The average number of *O*-sulfo groups in CS is ~0.8 per disaccharide [113].

DS (chondroitin sulfate B), found mainly in mucosa and skin, is a polydisperse, microheterogeneous sulfated copolymer of D-GalpNAc and primarily L-IdoAp acid, with *O*-sulfo groups most commonly found on the 4-position of D-GalpNAc residues and occasionally on the 6-position of D-GalpNAc and the 2-position of L-IdoAp residues [114] (Fig. 15.3, structure II). Several DS core proteins have been identified [115, 116]. These include large PGs with up to 25–30 DS polysaccharide chains and small PGs such as decorin and biglycan, with one and two DS polysaccharide chains, respectively.

Decorin DS PG binds collagen types I and II and plays an important role in the organization of collagen fibrils. The specific blend of PG and collagen determines the elasticity and transparency properties of the tissue [115]. Biglycan DS PG is found in cell surface and pericellular environments and its function is not well understood. DS PGs, biglycan, and decorin may play roles in regulating the extravascular activities of thrombin. In solution, biglycan, decorin, and their GAG chains accelerate HCII inhibition of thrombin. Both biglycan and decorin exhibit the same activity when bound to type V collagen. This observation suggests that

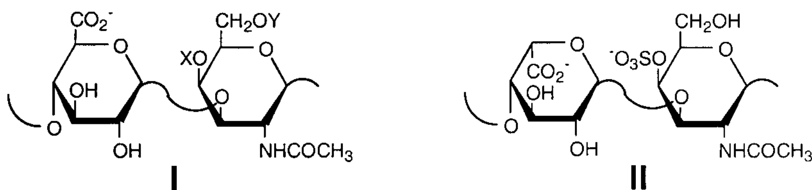


Fig. 15.3 The major disaccharide repeating units of chondroitin sulfates (structure I) and dermatan sulfate (structure II). In CS-A, $X = SO_3^-$ and $Y = H$, and in CS-C $X = H$ and $Y = SO_3^-$.

one function of these DS PGs is to provide a thromboresistant extravascular surface [117]. Thrombomodulin is a PG found on the luminal surface of the vascular endothelium and on underlying SMCs. It contains a single CS/DS GAG chain. Thrombomodulin binds thrombin, presumably through interaction both with its protein core and with its GAG chain [118]. Once bound, thrombin can act on protein C to form protein Ca, an activated serine protease, which inactivates factors Va and VIIIa, preventing the generation of factor Xa and thrombin, and inhibiting coagulation. Thrombin bound to thrombomodulin is also sensitive to inhibition by antithrombin III [114].

The GAG chains of decorin, biglycan, and thrombomodulin can act as anticoagulants by inhibiting thrombin, either directly through HCII or ATIII, or indirectly through protein C activation [117]. Since these PGs are found both on the luminal surface and on the subluminal surface, they provide a localized anticoagulant affect, affording thromboresistent surfaces at site in both intact and damaged vessels.

DS also acts as an antithrombotic agent by inhibiting the thrombin-induced aggregation of platelets and may activate the fibrinolytic pathway by causing the release of tissue plasminogen activator (tPA) [119]. The most thoroughly studied activity associated with DS is its acceleration of HC II-mediated inhibition of thrombin [120]. DS may also play a role in lipid metabolism by binding and releasing endothelial lipoprotein lipase into the circulation [119]. The compositions of the endothelial cell surface HS and DS PGs change during atherogenesis [121].

15.2.2
Therapeutic Applications

15.2.2.1 Dermatan Sulfate

Like HS, DS is a relatively weak anticoagulant in vitro (70 times less potent than heparin). Apart from its inhibition of thrombin-induced platelet aggregation [122], DS does not interact with platelets [48, 123]. Its anticoagulant effect is mainly based on enhancement of HCII activity [124]. To enhance HCII-mediated thrombin inhibition, DS requires at least seven to eight disaccharide units in addition to an IdoAp-GalNpAc4S disaccharide unit and IdoAp2S residues [125].

Recently, several DS preparations have been developed for prophylaxis of venous thromboembolism. In comparison with heparin, DS is a less active, but safer antithrombotic drug for *intravenous* administration, due to reduced hemorhagic complications [126]. The high molecular weight of DS inhibits its absorption when administered *subcutaneously*. Low molecular weight DSs, such as Desmin 370 (OP370) [124], are less potent in vitro, but show improved pharmacokinetic properties, including increased bioavailability and duration of action [127]. Oversulfated DS derivatives, with two to three sulfo groups per disaccharide unit, have also been examined [128, 129]. Antithrombotic activity increases with increasing *O*-sulfo group content [128], but these derivatives show a concomitant increase in hemorrhagic complications [130].

Other applications for DS include the preparation of medical devices and artificial tissues. Stone [131] has patented a prosthetic meniscus for use as a knee im-

plant, which acts as a scaffold for the regrowth of native meniscal tissue. The material is composed of collagen fibrils interspersed with DS. DS has also been useful in the development of artificial tissues [132]. Small arterial prostheses composed of a microporous polyurethane tube coated with a gel containing a mixture of type I collagen and DS have been designed. This gel promoted the adhesion and growth of endothelial cells, and reduced platelet adhesion in vitro. Grafts seeded with endothelial cells were highly antithrombotic when implanted into the carotid arteries of dogs.

15.2.2.2 Chondroitin Sulfates

Human plasma contains free CS at a concentration of $\sim$0.1 mg/100 ml [133]. As a component of thrombomodulin, CS is essentially involved in the inhibition of thrombin clotting activity [134]. The moderate antithrombotic action of chondroitin 4-sulfate in vivo is partly due to its anticoagulant effect, which is only partially mediated by ATIII [135]. To improve its antithrombotic activity, CS was chemically sulfonated, resulting in a so-called semisynthetic analogue (SSHA) with a mean MW of 7 kDa [136]. In several clinical studies comparing the prophylactic effect of SSHA with that of standard low dose heparin, SSHA was shown to be as effective as heparin, but without heparin's increased bleeding risk [136]. Thus, despite its weak anticoagulant activity, SSHA has significant antithrombotic potential.

CS has been widely used as a neutriceutical for the treatment of osteoarthritis [137]. Clinical trials have suggested that CS may have some efficacy in treating osteoarthritis symptoms [138]. While it is unclear how CS works, particularly with regard to its low oral bioavailability, it may act as a weak anti-inflammatory [139]. CS from human milk has also been found to inhibit HIV glycoprotein gp120 binding to its host cell CD4 receptor in vitro [140]. CS-C has been used as a component of artificial skin [141].

15.3 Hyaluronan

15.3.1 Structure and Properties

Hyaluronan (HA), first isolated from the vitreous body of the eye by Meyer and Palmer in 1934 [142], consists of repeating disaccharide units of [$\rightarrow$4)-β-D-GlcAp(1 $\rightarrow$ 3)-β-D-GlcNpAc(1$\rightarrow$]$_n$, where n can be up to 25,000 (Fig. 15.4). The contour length of an HA (M_r 4×10^6) is 10 μm [143]. HA is polymerized by plasma membrane-bound HA synthase and is not subjected to any type of covalent modification during its synthesis.

Nuclear magnetic resonance studies of the shape of HA performed by Scott [144] have shown the existence of internal hydrogen bonds that stabilize the chain in a stiffened helical conformation. HA chains have the capacity to self-aggregate

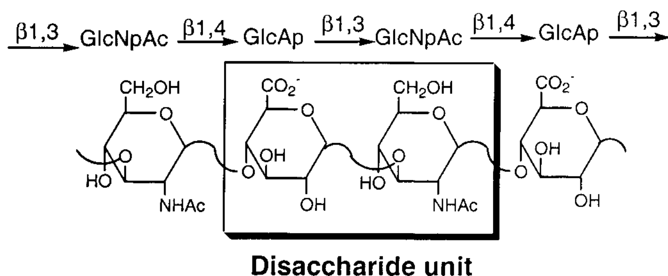


Fig. 15.4 The chemical structure of hyaluronan. The polymer is made up of alternating glucuronic acid (GlcAp) and *N*-acetylglucosamine (GlcNpAc).

Tab. 15.3 HA concentrations in various human organs and fluids [154].

Organ or fluid	***Concentration*** (μg g^{-1})
Aqueous humor	0.3–2.2
Brain	35–115
Dermis	200
Plasma (serum)	0.01–0.1
Synovial fluid	1400–3600
Thoracic lymph	8.5–18
Umbilical cord	4100
Urine	0.1–0.3
Vitreous body	140–340

in aqueous solutions and can be visualized by electron microscopy of rotary-shadowed [145]. The aggregation of two anti-parallel HA molecules is promoted through hydrogen bonds between the acetamido group on one chain and the carboxyl group on the other [146]. High molecular weight HA is extremely viscous.

15.3.2
Tissue Distribution and Biosynthesis

HA is synthesized by almost all animals, certain bacteria, and viruses [147, 148]. HA is found mainly in the extracellular space, where it accumulates, but the polymer can also be bound to the cell surface or be located intracellularly around the nucleus and in the lysosomes [149–151]. The largest storage of HA in humans is in the skin, constituting about 50% of the body's HA [152] (Tab. 15.4).

The first cell-free studies of HA biosynthesis used Group A streptococcal bacteria. Markovitz and co-workers showed that the streptococcal HA synthase, located in the cell membrane, required Mg^{++} ions and used the two sugar nucleotide substrates UDP-GlcAp and UDP-GlcNpAc to polymerize a HA chain [154]. HA synthase has two different glycosyltransferase activities and exhibits at least six different functions (Fig. 15.5).

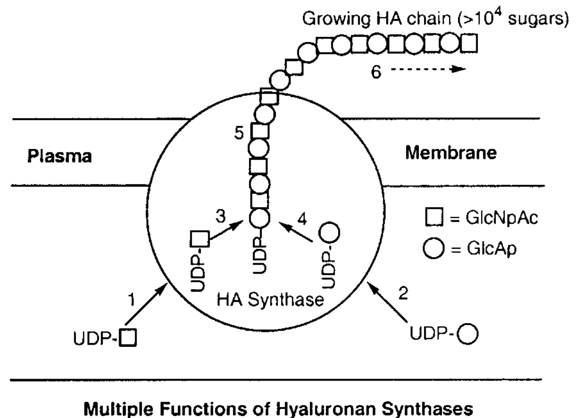


Fig. 15.5 Enzyme functions needed for hyaluronan biosynthesis. The diagram shows the membrane-bound hyaluronan synthase and the six independent activities required for the enzyme to make a disaccharide unit and extend the growing hyaluronan chain.

In 1983, Prehm [155] proposed a novel mechanism, distinctly different from that of other GAGs, for HA biosynthesis. He proposed that HA synthesis occurs at the reducing terminus of a growing HA chain by a two-site mechanism. In this mechanism, the reducing end sugar of the growing HA chain (either in the GlcNpAc or GlcAp site) would remain covalently bound to a terminal UDP, and the next sugar to be added from the second site would be transferred as the UDP-sugar onto the reducing end sugar with displacements of its terminal UDP [156]. The HA chain would then be in the second site. HA synthase assembles this high M_r HA, which is simultaneously extruded through the membrane into the extracellular space [156]. The HA chain has some hydrophobic character but is primarily hydrophilic, so it was a dilemma as to how this growing chain could be transferred across the hydrophobic lipid barrier of the plasma membrane. Weigel and co-workers proposed that cardiolipin, a common phospholipid, helps transfer HA by creating a pore-like passage within the enzyme through which the growing HA chain passes [157, 158].

15.3.3 Functions and Applications

Although the major biological function of HA is still unclear, many roles have been suggested. One important function is its ability to immobilize specific proteins (aggrecan, versican, neurocan, brevican, CD44) in desired locations within the body [159, 160]. The networks that HA forms are efficient insulators, since other macro-

molecules have trouble in finding room inside the HA network [145, 147]. With this property, HA (and other GAGs) can regulate (for example) the distribution and transport of major part of plasma proteins into the tissues. In the joints, HA probably has an important role as a lubricant between the joint surfaces [161].

HA is produced at high levels during cell proliferation, especially during mitosis. HA may help the cells to detach from the matrix, making it easier for them to divide, while some cell surface receptors (i.e., CD44 and RHAMM) bind HA, immobilizing them in the desired location [162].

HA has been reported to be involved in various events during morphogenesis and differentiation. Its concentrations increase in the areas where cell migration begins, suggesting that HA opens paths for cells to migrate through [163]. Cancer cells are often enriched with HA, and intense intracelluar staining for HA is a weak prognostic indicator for cancer therapy [164].

The production of HA is increased during inflammation, and generally, the viscous solutions seem to inhibit cell activities. HA increases phagocytosis in monocytes and granulocytes, but the importance of this phenomenon is unknown [165].

HA is often present in the pericellular space, probably protecting cells from lymphocytes and viruses [166]. The embryo is also covered by a thick HA coating during certain stages of development, which is probably important in differentiation [167]. How these coats are attached to the cell surface and what other molecules they contain is unknown.

Since the HA polymer does not itself exhibit any sequence diversity, its function is in part due to its chain length. The inductive role of HA in angiogenesis is attributed to oligosaccharides with four to 25 disaccharide residues [168], whereas high molecular weight HA exerted an inhibitory effect [169]. HA fragments have also been reported to evoke an inflammatory response in macrophages [170].

15.3.3.1 **Medical Applications**

The high water-binding capacity of HA and its high viscoelasticity give HA a unique profile among biological materials and make it suitable for various medical and pharmaceutical applications. One of the most successful medical applications of HA is the use of sodium hyaluronate (NaHA) for the treatment of osteoarthritis [144]. NaHA suppresses cartilage degeneration and release of proteoglycans from the extracellular matrix in cartilage tissues, protects the surface of articular cartilage [171], normalizes the properties of synovial fluids [172], and reduces pain perception [173, 174]. The mechanisms of these effects, however, have not yet been fully elucidated.

The application of HA in ophthalmology represents another medical application. In cataract surgery known as “viscosurgery,” viscoelastic materials such as NaHA are used to maintain operative space and to protect the endothelial layer of the cornea or other tissues from physical damage [175]. In cataract surgery, a new technique known as phacoemulsification and aspiration (PEA) uses ultrasound to emulsify the nucleus of the opaque lens, which is then removed. Highly viscoelastic NaHA is used to protect the endothelium of the cornea from injury during PEA [176].

15.3.3.2 Hyaluronic Acid Biomaterials

HA has been blended with other materials to produce novel biomaterials with desirable physicochemical, mechanical, and biocompatible properties. These include blends with poly(vinylalcohol) for ophthalmic use, and with carboxymethylcellulose (carbodiimide crosslinked) to produce a bioabsorbable film (Seprafilm®) for prevention of postsurgical adhesions, for wound-healing applications (with collagen) and for preparing immunologically 'unrecognizable' liposomes [177].

Conversion of the carboxylic groups of HA to *N*-acylhydrazides affords derivatives useful for controlled drug release [177]. HA esters have been prepared and fabricated into hydrophobic gauzes and microspheres, for use in transmucosal drug delivery. HA or sulfonated HA have been conjugated to the surface polymers used in medical devices, by use of either chemical or photochemical activation, to furnish coated materials with novel cell adhesive (or non-adhesive) properties.

Several different crosslinking strategies have been applied to the preparation of HA-derived hydrogels [178]. Bis-epoxides, formaldehyde, and divinylsulfone have been used under alkaline conditions to crosslink hydroxy groups in the preparation of hydrogels. Milder crosslinking conditions using polyhydrazides afford biocompatible HA hydrogels that allow covalent attachment of therapeutic molecules [178].

In vivo, HA is degraded by the specific enzyme hyaluronidase and by hydroxy radicals, resulting in degradation of the interstitial HA network at sites of inflammation [177]. Hyaluronidase has a high affinity for polyanionic substrates; it binds and processes chondroitin sulfate and heparin more slowly than HA. Chemical modification of the carboxylic acid and hydroxyl groups of HA can significantly reduce its susceptibility towards hyaluronidase. For example, while a bis-epoxide crosslinked HA hydrogel is degraded in response to inflammation, it is barely affected in healthy tissues. Similarly, surface-immobilized HA and fully esterified HA are not degraded by hyaluronidase.

15.4 Keratan Sulfate

15.4.1 Structure and Distribution

The term keratan sulfate (KS), originally applied to the major GAG of corneal tissue, is now generally used to describe oligo- or polysaccharides containing repeating sulfated Galp$\beta(1 \rightarrow 4)$GlcNpAc$\beta(1 \rightarrow 3)$ disaccharides (Fig. 15.6). Such KS sequences are found in several dissimilar PGs from cornea, cartilage, brain, and bone [179], as well as in carbohydrate moieties of less well characterized sulfated glycoproteins.

KS is typically heterogeneous in charge and size. It is usually of relatively low molecular weight, with nearly equal amounts of sulfo groups on the 6-positions of the D-Galp and the D-GlcNpAc [180]. It differs from other GAGs in the linkage region to the protein core, lacking the Xyl-serine linkage. In corneal tissue, the

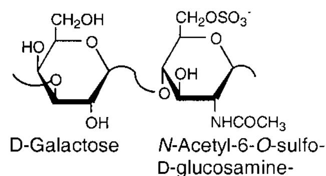


Fig. 15.6 The disaccharide repeating unit of keratan sulfate.

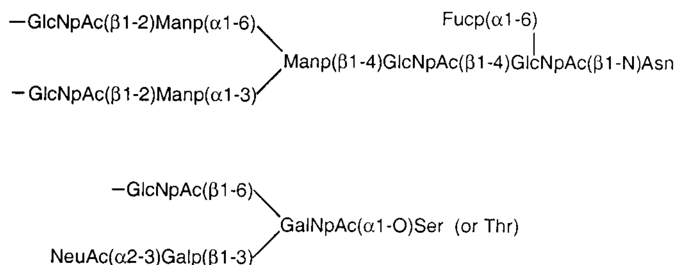


Fig. 15.7 Linkage region of corneal and skeletal keratan sulfate. The upper structure shows the linkage region in KS type I (corneal), the lower structure the linkage region in KS type II (skeletal).

linkage is *N*-glycosidic between GlcNpAc and an asparagine [181] (Fig. 15.7), while in skeletal tissue KS is linked to the hydroxy groups of serine or threonine residues by an *O*-glycosidic bond to GalNpAc [182].

15.4.2
Chemistry and Biosynthesis of Linkage Regions

15.4.2.1 Keratan Sulfate on Cartilage Proteoglycans

In addition to CS chains and *N*-linked oligosaccharides, the large PG from cartilage contains more than 100 serine and threonine residues substituted with *O*-linked KS or oligosaccharide chains [183]. The structures of *O*-linked oligosaccharides indicate that they provide the linkage region for initiating elongation of KS chains. Synthesis of the *O*-linked oligosaccharides and KS occurs entirely in the Golgi complex [179]. The *O*-linked oligosaccharides and KS chains enhance the ability of the PGs to resist compressive deformation and protect the core protein against proteolysis.

15.4.2.2 Keratan Sulfate on Corneal Proteoglycans

The small KS PG from corneal tissue contains 1–2 attachment sites, each with the possibility of carrying two chains, and 1–2 *N*-linked oligosaccharides bound to a 45 kDa core protein [184]. The linkage region for the KS chains is a *N*-linked oli-

gosaccharide of the complex type [185], synthesized in the endoplasmic reticulum, while the KS chains are elaborated in the Golgi. This KS PG appears to interact at specific sites along the collagen fibrils in the stroma and has a function in maintaining the optical transparency of the cornea [186].

15.4.3
Biological Roles of Keratan Sulfate

The cartilage matrix is rich in KS PGs that interact with HA and link protein to form aggregates. The exact function of the KS GAG chains is not well understood. While the highly negatively charged KS contributes to the physicochemical properties of the PG, it does not appear to be essential for the ability to bear load effectively [187]. Although KS is found mostly in cartilage, small amounts of KS, possibly resulting from cartilage breakdown [188], can be detected in blood, providing important diagnostic information [189]. Patients with polyarticular osteoarthritis have abnormally high levels of serum KS, suggesting that a high rate of PG catabolism during turnover may predispose the development of arthritis [190].

15.4.3.1 Role of KS in Macular Corneal Dystrophy

The corneal stroma contains a KS PG and a DS PG as major non-collagenous components of the extracellular matrix [191]. These PGs are absent from opaque corneal wounds [192]. Macular corneal dystrophy (MCD) is an inherited disorder, clinically characterized by the accumulation of opaque deposits in the corneal stroma [193, 194]. A post-translational error in KS PG biosynthesis affords an unsulfated glycoconjugate [195, 196].

15.5
Other Acidic Polysaccharides

15.5.1
Acharan Sulfate

Acharan sulfate is a GAG isolated from the giant African snail *Achatina fulica*. This polysaccharide has a repeating →4)-α-D-GlcNpAc (1→4)-α-L-IdoAp2S (1→) disaccharide structure (Fig. 15.8). The natural function of this molecule in the snail, while still unclear, may be as an anti-desiccant, a metal chelator, an anti-infective, or a locomotive (slime) agent. The unusual structure of acharan sulfate, similar but distinctive from both heparin and HS has resulted in studies of its biological activities. Chemically modified acharan sulfate, *N*-sulfoacharan sulfate, shows a heparin-like effect on basic FGF2 mitogenicity but at a greatly reduced level [197] and is a moderately active for the inhibitor of thrombin [198].

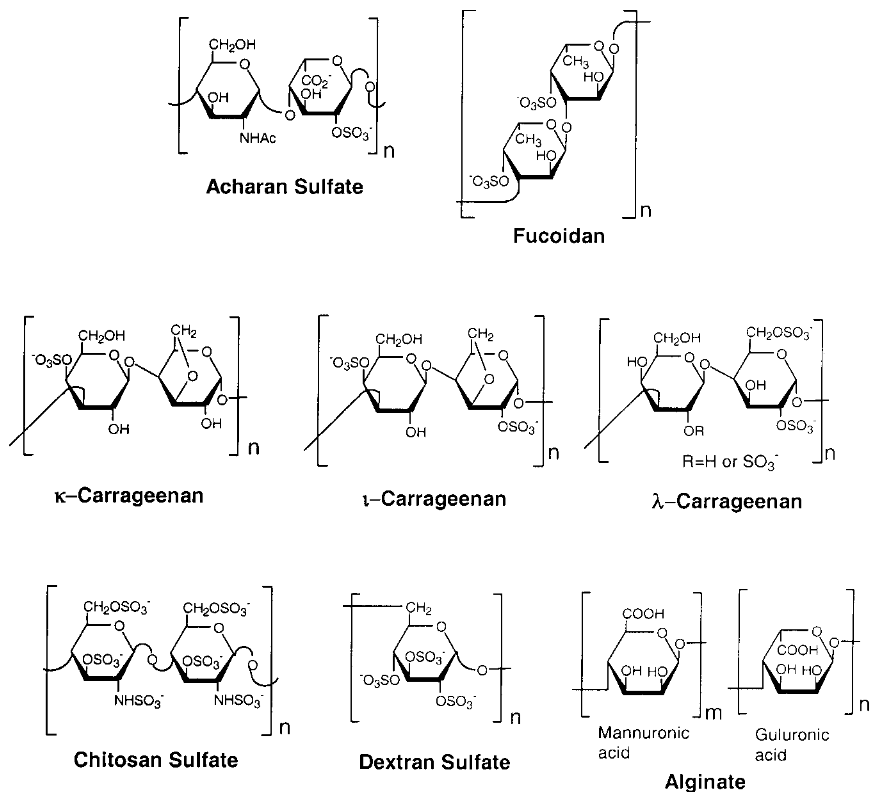


Fig. 15.8 Structures of other acidic polysaccharides.

15.5.2
Fucoidins

Fucoidin is a complex sulfated polysaccharide, derived from marine brown algae [199–201], the jelly coat from sea urchin eggs [202], and the sea cucumber body wall [203]. These glucuronofucoglycans and fucoglucuronans comprise a wide, continuous spectrum of low sulfate polymers. Fucoidins are primarily composed of α-(1 → 3)-linked units of 4-sulfo-L-fucose with branching or a second sulfo group at position 3 (Fig. 15.8) [204]. Most investigations into their biological activity have focused on fucoidans from brown algae, *Fucus vesiculosus*, which exhibit a variety of biological effects on mammalian cells. *Fucus* fucoidan has anticoagulant activity [205–207], and is a potent activator of both ATIII and HCII [207]. Fucoidan inhibits both the initial binding of sperm and subsequent recognition [208]. It also prevents infection of human cell lines by several enveloped viruses [209]. Fucoidan blocks cell-cell binding mediated by P- or L-selectin but not by E-selectin [210]. Furthermore, it demonstrates differential binding to interleukins [211] and the hepatocyte growth factor [212]. Since this polysaccharide causes no toxicity or

irritation, it may be useful as an anticoagulant, antiviral, anti-inflammatory, or contraceptive agent [213–215]. On oral administration fucoidans have been effective in healing and preventing gastric ulcers in animal models [201].

15.5.3 Carrageenans

Carrageenans are sulfated polysaccharides derived from various species of red algae. There are three main types: ι, κ, and λ. Both ι- and κ-carrageenan contain *O*-3-substituted *O*-4-sulfo-β-D-Galp units, while λ-carrageenan contains *O*-3-substituted *O*-2-sulfo-β-D-Galp units. In addition to the above moieties, ι-carrageenan also contains *O*-4-substituted *O*-2-sulfo-3,6-anhydro-α-D-Galpl units, κ-carrageenan also contains *O*-4-substituted 3,6-anhydro-α-D-Galp units, and λ-carrageenan also contains *O*-4-substituted *O*-2,6-disulfo-α-D-Galp units (Fig. 15.8) [216]. An otherwise perfect alternating sequence in each of these polymers is complicated by occasional modifications in the placement and number of sulfo groups or – for ι- and κ-carrageenans – by the absence of the 3,6-anhydro-linkage [217].

Carrageenans (Irish moss) have been used medicinally for centuries. The moss has been used as a cough medicine [218], and a degraded ι-carrageenan is marketed in Europe as an anti-ulcer preparation [217]. High molecular weight carrageenans have a wide variety of applications in the food industry, serving as thickeners, stabilizers, and emulsifiers. Carrageenan activates Hageman factor, one of the blood coagulation factors, which has cardiotonic activity [219]. On oral administration in animals, both κ- and ι-carrageenan show anti-tumor activity [220]. Some concerns have recently been raised about the toxicity of low molecular weight carrageenans [221].

15.5.4 Sulfated Chitins

Chitin, poly(2-acetamido-2-deoxy-D-glucopyranose), is the main structural element of the cuticles of crab, shrimp, and insects, and is also widespread in the cell walls of fungi [222]. Chitin can be de-*N*-acetylated to prepare chitosan, which on chemical sulfation and/or carboxymethylation results in a polymer with certain structural similarities to heparin (Figs. 15.2 and 15.8) [223]. These chitosan derivatives show anticoagulant activity related to their degree of sulfation. Carboxymethylated sulfochitosan inhibits thrombin activity through ATIII to almost the same degree as heparin.

15.5.5 Dextran Sulfate

Dextran, a $(1 \rightarrow 4)$-β-D-,$(1 \rightarrow 3)$-α-D-branched Glcp polymer [222], can be chemically sulfonated to prepare dextran sulfate (Fig. 15.8) [224]. Both dextran (a plasma extender) and dextran sulfate have been used in pharmaceuticals. Dextran sulfate has low anticoagulant activity with high LPL-releasing activity [225]. This has per-

mitted the exploitation of this agent as an anti-atherosclerotic in Japan [226]. Dextran sulfate has been used as a heparin replacement in anticoagulation and has been immobilized on plastic tubes to prepare non-thrombogenic surfaces [227]. Dextran sulfate is an inhibitor of HIV binding to T-lymphocytes, but its low oral bioavailability has precluded its use in the treatment of AIDS [228].

15.5.6 Alginates

Alginate is a commercially important component of brown seaweeds and is the most important mucilaginous polysaccharide, preventing desiccation of the seaweed when it is exposed to air [201]. It consists of D-mannuronic acid (ManAp) and L-guluronic acid (GulAp). The distribution of ManAp and GulAp in alginate chains give rise to three different block types, namely blocks of poly-ManAp, blocks of poly-GulAp and alternating blocks of the ManAp-GulAp (Fig. 15.8) [229]. Alginates are used as low-price viscosifiers or thickeners in a wide range of products. In the pharmaceutical field, alginates have been used for many years to treat wounds and gastric ulcers [201]. Alginate also depresses the plasma cholesterol level. Alginic acid strongly inhibits hyaluronidase and mast cell degranulation, involved in allergic reactions [230].

15.5.7 Fully Synthetic Sulfated Molecules

15.5.7.1 Polymers

Synthetic polymers such as poly(vinyl sulfate) and poly(anethole sulfonate) (Fig. 15.9) exhibit anticoagulant activity [231] and have been exploited in vitro to collect blood and plasma samples for assay. A biphenyl disulfonic acid urea copolymer shows potent anti-HIV activity [232]. These agents are highly toxic because of their long half-lives, the result of their failure to be cleared either through filtration or metabolism.

15.5.7.2 Small Sulfonated Molecules

Suramin (Fig. 15.9) was the first heparin analogue used clinically in a wide variety of applications [233]. These applications include its activity as an anthelmintic, an antiprotozoal, an antineoplastic, and an antiviral agent [36, 102]. Despite its potent activities, suramin has a very long half-life in the body and exhibits a wide range of toxic effects.

Naphthalene sulfonates (Fig. 15.9) show potent anti-HIV activity, but limited toxicological data are currently available [234]. A series of simple aliphatic disulfates and disulfonates have been tested for their ability to arrest amyloidosis in vivo as potential agents for the treatment of Alzheimer's disease [235]. Several alkyl malto-laminaro-α oligosaccharides, such as the highly sulfated dodecyl laminaropentaoside (Fig. 15.9), have also been investigated for anti-HIV activity [236].

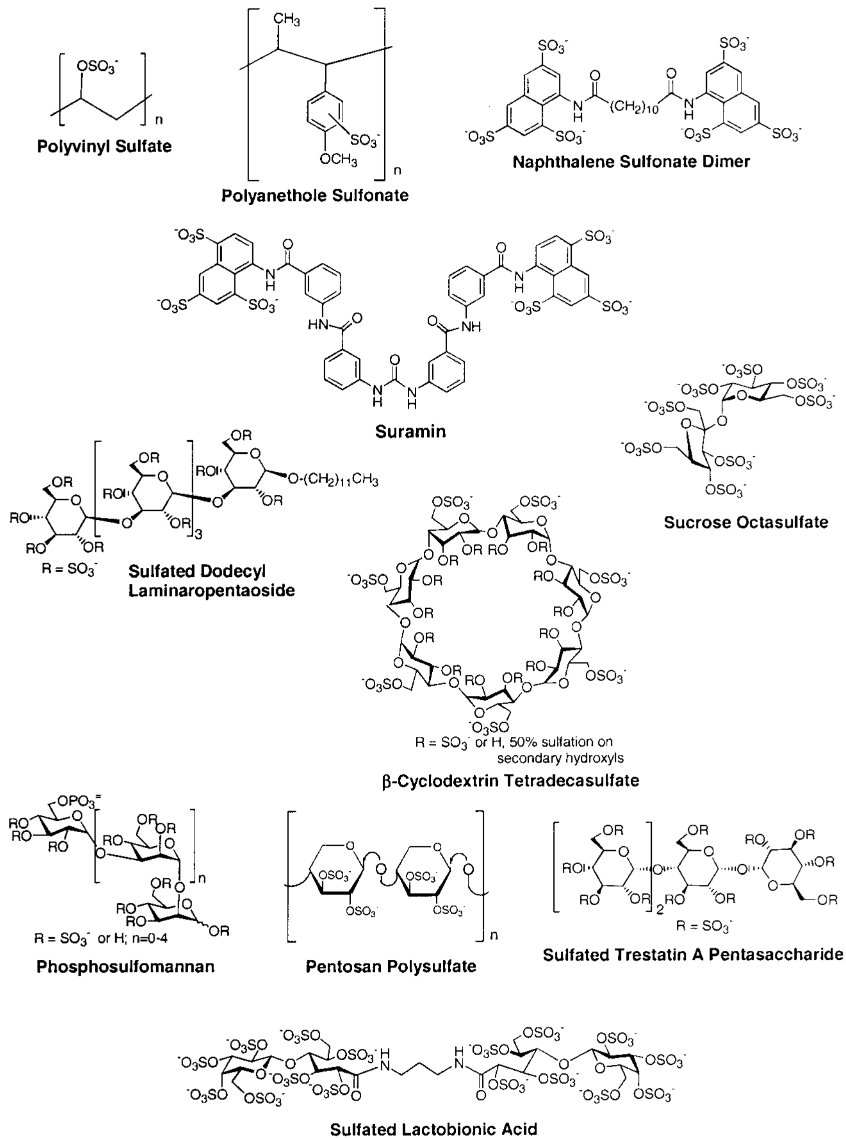


Fig. 15.9 Structures of several synthetic sulfonated molecules.

Sucralfate (Carafate®), insoluble aluminium sucrose octasulfate (SOS) (Fig. 15.9), is used in the treatment of ulcers [8]. The water-soluble sodium SOS binds in place of heparin/HS within the FGF-FGFR signal transduction complex. SOS derivatives lacking sulfo groups at specific positions prevent the assembly of active complex and are being investigated as potential anticancer agents [237].

Sulfated cyclodextrins such as β-cyclodextrin tetradecasulfate (Fig. 15.9) have demonstrated anticancer activity, presumably through an anti-angiogenic mechanism [238]. Sulfated cyclodextrins also inhibit complement activation [239].

Phosphosulfomannan, PI-88 (Fig. 15.9), is a chemically sulfonated phosphomannan oligosaccharide mixture derived from the yeast *Pichia holstii* [240]. PI-88 has recently been identified as a promising inhibitor of tumor cell growth and metastasis and is currently undergoing Phase I clinical trials. PI-88 is believed to block tumor growth by interfering in HS interaction with FGF and FGFR [241] and prevents metastasis by inhibiting heparanase [242], blocking the breakdown of extracellular matrix, preventing the spread of tumor cells. PI-88 also shows substantial antithrombotic activity through its catalysis of HCII-mediated inhibition of thrombin [243].

Pentosan, a linear Xylp polymer extracted from the bark of the birch tree *Fagus sylvantica*, when fully sulfonated and partially depolymerized (Fig. 15.9), is an anticoagulant with one tenth of heparin's activity on a weight basis [244]. Its primary anti-IIa activity has been postulated to be HCII-mediated [245]. Other xylans derived from corncobs, birchwood, and oatspelts provided heparinoids more active than pentosan polysulfate.

Trestatin A (Fig. 15.9), a pseudo-nonasaccharide obtained from strains of *Streptomyces dimorphogenes*, is a potent α-amylase inhibitor. A highly sulfated maltotriosyl trehalose pentasaccharide chemically modified from the Trestatin substructure has an antiproliferative activity comparable to that of heparin [246].

Sulfated lactobionic acid (Aprosulfate, LW10082) (Fig. 15.9), prepared through the chemical sulfonation of a lactose dimer, is an antithrombotic agent in animal models. It acts primarily through HCII but showed some toxicity precluding its clinical use [247, 48].

15.6
Pharmaceutical Potential and Challenges

This chapter describes a multiplicity of important biological functions that are associated with GAGs. Heparin and LMW heparin, for example, command a one billion dollar market worldwide, and are the preeminent clinical anticoagulants in use today. Furthermore, GAG-based (and potentially other carbohydrate-based) agents have unique properties – such as high specificity, delocalized binding sites, low antigenicity, and multivalency – difficult to replicate with other classes of molecules. The biological functions of GAGs might be enhanced by administration of exogenous GAGs, modified GAGs, GAG oligosaccharides, or GAG analogues. Such agonists might be useful, for example, as anticoagulants, as anti-infectives, in promoting cell growth, and for other important GAG-related activities. Alternatively, molecules might be designed as antagonists, to block normal GAG function, for use as procoagulants, or in inhibiting the assembly of signal transduction complexes and preventing cell replication in the treatment of cancers. Despite the great potential of new GAG-based drug therapies, the development of

LMW heparins represent the only significant success story in this class of molecules in the past 20 years of extensive research effort. Moreover, this success is limited to anticoagulant/antithrombotic agents, a role that was already being fulfilled by heparin itself. Thus, the agents could be viewed as merely "improved heparins" or a second-generation replacement for a relatively successful drug. The slow development of new GAG-based therapeutic agents is primarily associated with a number of difficult problems that still need to be addressed.

15.6.1
GAG-Based Agents Are Heterogenous

Heparin and LMW heparins are heterogeneous mixtures. This complicates their application as drugs in a number of ways. Firstly, the production of these agents must be controlled in order to obtain reproducible mixtures corresponding to those desired for the drug products. Secondly, analytical and quality control issues, while solvable, are more complicated than for homogenous products. Thirdly, biological evaluation of mixtures (half-life, pharmacokinetics, metabolism, and bio-activity) is more complicated than for a single entity. Fourthly, regulatory questions for drug mixtures can be formidable, making the drug approval process more difficult. Fifthly, a patent position is more difficult to establish for a mixture in which composition of matter is difficult to define.

15.6.2
GAG-Based Agents and Sulfonated Analogues Have Low Bioavailability

Heparin is only used by *intravenous* administration, while LMW heparins are effective when given either by *intravenous* or *subcutaneous* administration. This has resulted in a movement, particularly in the US, away from heparin and towards LMW heparins. Much of the biological potential of heparin, particularly its prophylactic uses (anti-atherosclerosis, anti-infective, etc.), cannot easily be exploited by a drug requiring injection. Heparin is not orally bioavailable because of its highly charged nature and its high molecular weight. Moreover, GAG-based analogues, while often having reduced molecular weights, are frequently still highly charged molecules. Three approaches might be used to solve these bioavailability problems. Firstly, excipients, drug carriers, or salt forms might be used to carry GAG-based drugs through membranes, facilitating their oral absorption. Approaches have ranged from ion-pairing agents (such as quaternary ammonium salts) [248], surfactants (such as saponins) [249], to small hydrogen-bonding peptoids (such as SNAC) [250]. Up to now, none of these approaches has afforded a clinically useful oral drug product. Secondly, the GAG-based drugs can be simplified by decreasing the number of required sulfo groups, reducing charge and molecular weight to make oral bioavailability possible. While this is a realistic and promising approach, no one has yet successfully designed an orally bioavailable GAG analogue for clinical use. Thirdly, it might be possible to mimic GAG binding without the use of charged sulfo groups and in the absence of a large extended linear template.

15.6.3 GAGs Have a Myriad of Biological Activities

The large number of biological activities of GAGs is a double-edged sword: while GAGs are interesting drug targets, it is difficult to obtain an agent with a single activity in the absence of complicating side effects. The multi-pharmacological profile of GAGs may be advantageous in treating complex disease processes in which all the properties align in the same direction. Furthermore, as in the case of the synthetic ATIII pentasaccharide, it may be possible to pair away other activities to obtain a homogeneous agent with a single prominent pharmacological activity.

15.6.4 Carbohydrate-Based Drugs Are Expensive and Difficult to Prepare

The synthetic heparin pentasaccharide, corresponding to the ATIII binding site, originally required over 30 synthetic steps [251]. This is over three times the number of synthetic steps required for prostaglandin synthesis, and the heparin pentasaccharide has to be administered in multi-milligram (not microgram) doses. Improvements in carbohydrate synthesis, including new enzymatic approaches [252, 253] and solid-phase synthesis [254], might one day make complex synthetic carbohydrate-based drugs economically viable. Indeed, the synthetic ATIII pentasaccharide is now clinically used as a highly specific anticoagulant agent in Europe.

The conformational flexibility of carbohydrates and their polyol structures give them many of their unique biological properties, low antigenicity, but limit the binding affinity to their protein targets. One approach is to design an alternative molecular scaffold, either flexible or inflexible, that can be used to recognize and occupy carbohydrate binding sites or protein-based receptors and act either as an agonist or as an antagonist. Our improved understanding of heparin-protein interactions [94] should aid in the design of such new agents.

15.7 Conclusion

In conclusion, while GAGs and their analogues have current therapeutic value, their further exploitation to treat a myriad of diseases depends on a number of new chemical, biological, and pharmaceutical advances. Future research on the fundamental physical, chemical, and biological properties of this important class of molecules should certainly provide the necessary knowledge to expand their therapeutic applications.

15.8
References

1 P.-D. Kittlick, *Glycosaminoglycans: Recent Biochemical Results in the Fields of Growth and Inflammation*, VEB Gustav Fischer, Germany, **1985**.

2 L. Kjellén, U. Lindahl, *Annu. Rev. Biochem.* **1991**, *60*, 443–475.

3 P. Kristian, T.D. Knut, *J. Cell Sci.* **2000**, *113*, 193–205.

4 J.H. Kimura, L.S. Lohmander, V.C. Hascall, *J. Cell Biochem.* **1984**, *26*, 261–278.

5 R.V. Iozzo, *Annu. Rev. Biochem.* **1998**, *67*, 609–652.

6 D.M. Templeton, *Crit. Rev. Clin. Lab. Sci.* **1992**, *29*, 141–184.

7 T.E. Hardingham, A.J. Fosang, *FASEB J.* **1992**, *6*, 443–475.

8 R.J. Linhardt, T.T. Toida, *Carbohydrates as Drugs*, Marcel Dekker, New York, **1996**.

9 A.B. Foster, A.J. Huggard, *Adv. Carbohydr. Chem. Biochem.* **1955**, *10*, 335–368.

10 R.J. Linhardt, *Chem. & Indust.* **1991**, *2*, 45–50.

11 B. Casu, *Adv. Carbohydr. Chem. Biochem.* **1985**, *43*, 51–134.

12 W.D. Comper, *Heparin (and related polysaccharides)* Vol. 7, Gordan and Breach Science Publ., Germany, **1981**.

13 A.A. Horner, E. Young, *J. Biol. Chem.* **1982**, *257*, 8749–8754.

14 R.J. Linhardt, Z.M. Merchant, K.G. Rice, Y.S. Kim, G.L. Fitzgerald, A.C. Grant, R. Langer, *Biochemistry*, **1985**, *24*, 7805–7810.

15 R.J. Linhardt, D.M. Cohen, K.G. Rice, *Biochemistr*, **1989**, *28*, 2888–2894.

16 L. Kjellén, I. Petterson, E. Unger, U. Lindahl, *Adv. Exp. Med. Biol.* **1992**, *313*, 107–111.

17 U. Lindahl, D.S. Feingold, L. Roden, *TIBS* **1986**, *11*, 221–225.

18 J.T. Gallagher, J.E. Turnbull, M. Lyon, *Adv. Exp. Med. & Biol.* **1992**, *313*, 49–57.

19 M. Bernfield, R. Kobeyesi, M. Kato, M. T. Hinkes, J. Spring, R.L. Gallo, E. J. Lose, *Ann. Rev. Cell Biol.* **1992**, *8*, 365–393.

20 R.J. Linhardt, *Polysaccharide Lyases for Glycosaminoglycan Analysis*, Wiley Interscience, V 2, 17.13.17–17.13.32, **1994**.

21 B. Casu, U. Lindahl, *Adv. Carbohydr. Chem. Biochem.* **2002**, *57*, 159–206.

22 R.N. Krishna, P.K. Agrawal, *Adv. Carbohydr. Chem. Biochem.* **2000**, *56*, 201–234.

23 K. Lidholt, L. Kjellén, U. Lindahl, *Biochem. J.* **1989**, *261*, 999–1007.

24 R.L. Stevens, C.C. Fox, L.M. Lichtenstein, K.F. Austen, *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 2284–2287.

25 R.J. Linhardt, D. Loganathan, *Heparin, Heparinoids and Heparin Oligosaccharides: Structure and Biological Activities*, Plenum Press, New York, **1990**.

26 J.A. Marcum, R.D. Rosenberg, *Semin. Thromb. Haemostasis* **1987**, *13*, 464–474.

27 R. Langer, R.J. Linhardt, C.L. Cooney, M. Klein, D. Trapper, S.M. Hoffberg, A. Larsen, *Science* **1982**, *217*, 261–263.

28 M.D. Freedman, *J. Clin. Pharmacol.* **1992**, *32*, 584–596.

29 N.S. Gunay, R.J. Linhardt, *Semin. Thromb. Hemostasis* **1999**, *25*, 5–16.

30 S.G. Frangos, A.H. Chen, B. Sumpio, *J. Am. Coll. Surg.* **2000**, *191*, 76–92.

31 B.R. Jaeger, *Ther. Apher.* **2001**, *5*, 207–211.

32 R.E. Edens, R.J. Linhardt, J.M. Weiler, *Compl. Prof.* **1993**, *1*, 96–120.

33 M.A. Fath, X. Wu, R.E. Hileman, R.J. Linhardt, M.A. Kashem, R.M. Nelson, C.D. Wright, W.M. Abraham, *J. Biol. Chem.* **1998**, *273*, 13563–13569.

34 S.M. Smorenburg, C.J. Van Noorden, *Pharmacol. Rev.* **2001**, *53*, 93–105.

35 R. J. Hettiarachchi, S.M. Smorenberg, J. Ginsberg, M. Levine, M.H. Prins, H.R. Buller, *Thromb. Haemost.* **1999**, *82*, 947–952.

36 Y. Chen, T. Maguire, R.E. Hileman, J.R. Fromm, J.D. Esko, R.J. Linhardt, R.M. Marks, *Nat. Med.* **1997**, *3*, 866–871.

37 B. Dudas, U. Cornelli, J.M. Lee, M.J. Hejna, M. Walzer, S.A. Lorens, R.F. Mervis, J. Fareed, I. Hanin, *Neurobiol. Aging* **2002**, *23*, 97–104.

38 R. Huber, R.W. Carrell, *Biochemistry* **1989**, *28*, 8951–8966.

39 Y.S. Kim, K.B. Lee, R.J. Linhardt, *Thromb. Res.* **1988**, *51*, 97–104.

40 K. Kurachi, G. Schmer, M.A. Hermodson, D.C. Teller, E.W. Davie, *Biochem. J.* **1976**, *15*, 368–372.
41 D.M. Tollefsen, M.K. Blank, *J. Biol. Chem.* **1982**, *257*, 2162–2169.
42 E. Ersdal-Badju, A. Lu, Y. Zuo, V. Picard, S.C. Bock, *J. Biol. Chem.* **1997**, *272*, 19393–19400.
43 T.H. Tran, B. Zbinden, B. Lammle, F. Duckert, *Semin. Thromb. Haemost.* **1985**, *11*, 342–346.
44 R.D. Rosenberg, P.S. Damus, *J. Biol. Chem.* **1973**, *248*, 6490–6505.
45 D.M. Tollefsen, *Nouv, Rev. Fr. Hematol.* **1984**, *26*, 233–237
46 L.B. Jaques, *Hämostaseologie* **1985**, *5*, 121–126.
47 H.B. Nader, L. Toma, M.S. Pinhal, V. Buonassisi, P. Colburn, C.P. Dietrich, *Semin. Thromb. Hemost.* **1991**, *17*, 47–56.
48 H.L. Messmore, B. Griffin, M. Koza, J. Seghatchian, J. Fareed, E. Coyne, *Semin. Thromb. Hemost.* **1991**, *17*, 57–64.
49 E. Gerlach, B.F. Becker, *Zeitschrift für Kardiologie* **1993**, *5*, 13–21.
50 R. Ross, *Nature* **1993**, *24*, 1447–1451.
51 B.R. Jaeger, *Therapeutic Apheresis* **2001**, *5*, 207–211.
52 V. Fuster, A. Lewis, *Circulation* **1994**, *90*, 2126–2145.
53 E.B. Smith, I.B. Massie, K.M. Alexander, *Atherosclerosis* **1976**, *25*, 71–84.
54 H. Engelberg, *Pharmacol. Rev.* **1984**, *36*, 91–110.
55 Z.M. Merchant, E.E. Erbe, W.P. Eddy, R.J. Patel, R.J. Linhardt, *Atherosclerosis* **1986**, *62*, 151–158.
56 S. Santamarina-Fiojo, H.B. Brewer Jr., *Int. J. Clin. Lab. Res.* **1994**, *24*, 143–147.
57 P. Sivaram, S.Y. Choi, L.K. Curtis, I.J. Goldberg, *J. Biol. Chem.* **1994**, *269*, 9409–9412.
58 T.K. Sue, L.B. Jaques, E. Yuen, *Can. J. Physiol. Pharmacol.* **1976**, *54*, 613–617.
59 J.J. Castellot Jr., D.L. Beeler, R.D. Rosenberg, M.J. Karnovsky, *J. Cell Physiol.* **1984**, *120*, 315–320.
60 J.J. Castellot Jr., J. Choay, J.-C. Lormeau, M. Petitou, E. Sache, M.J. Karnovsky, *J. Cell Biol.* **1986**, *102*, 1979–1984.
61 T.C. Wright, J.J. Castellot, J.R. Diamond, M.J. Karnovsky, *Chemical and Biological Properties Clinical Applications*, CRC Press, London, **1989**.
62 L.A. Pukac, G.M. Hirsch, J.-C. Lormeau, M. Petitou, J. Choay, M.J. Karnovsky, *Am. J. Pathol.* **1991**, *139*, 1501–1509.
63 S. Ruddy, I. Gigli, K.F. Austen, *New Engl. J. Med.* **1972**, 489–495.
64 H. Garg, B.T. Thompson, R.J. Linhardt, C.A. Hales, *Proteoglycans and Lung Disease*, in press, **2002**.
65 G.B. Caughman, R.J. Boackle, J. Vesely, *Mol. Immunol.* **1982**, *19*, 287–295.
66 C.R. Minick, *Immunity and Atherosclerosis*, Academic Press, New York, **1980**.
67 S. Alameda, R.D. Rosenberg, D.H. Bing, *J. Biol. Chem.* **1983**, *258*, 785–791.
68 H. Engelberg, *Adv. Lipid Res.* **1983**, *20*, 219–255.
69 M.D. Kazatchkine, D.T. Fearon, D.D. Metcalfe, R.D. Rosenberg, K.F. Austen, *J. Clin. Invest.* **1981**, *67*, 223–228.
70 M.P. Bevilacqua, R.M. Nelson, A. Venot, R.J. Linhardt, I. Stamenkovic, *Ann. Rev. Cell Biol.* **1995**.
71 R.P. McEver, *Curr. Opin. Immunol.* **1994**, *6*, 75–84.
72 R.M. Nelson, O. Cecconi, W. G. Roberts, A. Aruffo, R.J. Linhardt, M.P. Bevilacqua, *Blood* **1993**, *82*, 3253–3258.
73 D.P. Witt, A.D. Lander, *Curr. Biol.* **1994**, *4*, 394–400.
74 W.F. Long, F.B. Williamson, *Med. Hypoth.* **1984**, *13*, 385–394.
75 J. Folkman, Y. Shing, *Heparin and Related Polysaccharides*, Plenum Press, New York, **1992**.
76 T.K. Hunt, D.R. Knighton, K.K. Thakral, W.H. Goodson III, W.S. Andrews, *Surgery,,* **1984**, *96*, 48–54.
77 D. Ribatti, A. Vacca, B. Nico, E. Crivellato, L. Roncali, F. Dammacco, *Brit. J. Haematol.* **2001**, *115*, 514–521.
78 S. Taylor, J. Folkman, *Nature* **1982**, *97*, 307–312.
79 J. Folkman, D.E. Ingber, *Chemical and Biological Properties Clinical Applications*, CRC Press, London, **1989**.
80 J.J. Mason, *Cell* **1994**, *78*, 547–552.
81 K. Shiokawa, M. Asano, C. Shiozaki, *Jap. J. Clin. Med.* **1992**, *50*, 1893–1901.
82 J. Schlessinger, A.N. Plotnikov, O.A. Ibrahimi, A.V. Eliseenkova, B.K. Yeh,

A. Yayon, R.J. Linhardt, M. Mohammadi, *Molecular Cell* **2000**, *6*, 743–750.

83 R. Reich-Slotky, D. Bonneh-Barkay, E. Shaoul, B. Bluma, C.M. Srahn, D. Ron, *J. Biol. Chem.* **1994**, *269*, 32279–32285.

84 J. Folkman, R. Langer, R.J. Linhardt, C. Haudenschild, S. Taylor, *Science* **1983**, *221*, 719–725.

85 R. Crum, S. Szabo, J. Folkman, *Science* **1985**, *230*, 1375–1378.

86 R. Sasisekharan, M.A. Moses, M.A. Nugent, C.L. Cooney, R. Langer, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 1524–1528.

87 J. Denekamp, *Prog. Appl. Microcirc.*, Karger, Basel, **1984**.

88 D.W. Beck, J.J. Olson, R.J. Linhardt, *J. Neuropath. Exper. Neurol.* **1986**, *45*, 503–512.

89 T. Sasaki, H. Larsson, J. Kreuger, M. Salmivirta, L. Claesson-Welsh, U. Lindahl, E. Hohenester, R. Timpl, *The EMBO Journal* **1999**, *18*, 6240–6248.

90 T. Miralem, A. Wang, *J. Biol. Chem.* **1996**, *271*, 17100–17106.

91 D.J. Tyrrell, A.P. Horne, K.R. Holme, J.M. Preuss, C.P. Page, *Adv. Pharmacol.* **1999**, *46*, 151–208.

92 M. Nakajima, T. Irimura, G.L. Nicolson, *J. Cell Biochem.* **1988**, *36*, 157–167.

93 L. Borsig, R. Wong, J. Feramisco, D.R. Nadeau, N.M. Varki, A. Varki, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 3352–3357.

94 I. Capila, R.J. Linhardt, *Angewandte Chemie* **2002**, *41*, 1000–1022.

95 D. Sawitzky, *Microbio. Immunol.* **1996**, *184*, 155–161.

96 B.C. Herold, R.J. Visallai, N. Susmarski, C.R. Brandt, P.G. Spear, *J. Gen. Virol.* **1994**, *75*, 1211–1222.

97 B.C. Herold, S.I. Gerber, T. Polonsky, B.J. Belval, P.N. Shaklee, K. Home, *Virology* **1995**.

98 C.C. Rider, C.R. Coombe, H.A. Harrop, E.F. Hounsell, C. Bauer, J. Feeny, B. Mulloy, N. Mahmood, A. Hay, C.R. Parish, *Biochemistry* **1994**, *33*, 6974–6980.

99 C.C. Rider, *Glycoconjugate J.* **1997**, *14*, 639–642.

100 M. Witvrouw, E. De Clercq, *Gen. Pharmac.* **1997**, *29*, 497–511.

101 R.M. Marks, H. Lu, R. Sundaresan, T. Toida, A. Suzuki, T. Imanari, M.J. Hernaiz, R.J. Linhardt, *J. Med. Chem.* **2001**, *44*, 2178–2187.

102 D. Rathore, T.F. McCutchan, M.J. Hernaiz, L.A. LeBrun, S.C. Lang, R.J. Linhardt, *Biochemistry* **2001**, *40*, 11518–11524.

103 J. Dong, C.A. Peters-Libeu, K.H. Weisgraber, B.W. Segelke, B. Rupp, I. Capila, M.J. Hernaiz, L.A. LeBrun, R.J. Linhardt, *Biochemistry* **2001**, *40*, 2826–2834.

104 N. Parveen, J.M. Leong, *Molecul. Microbiol.* **2000**, *35*, 1220–1234.

105 L. Buee, W. Ding, A. Delacourte, H. Fillit, *Brain Res.* **1993**, *601*, 154–163.

106 K.R. Brunden, N.J. Richter-Cook, N. Chaturvedi, R.C. Frederickson, *J. Neurochem.* **1993**, *61*, 2147–2154.

107 R.W. Scott, B.L. Bergmann, A. Bajpai, R.T. Hersh, H. Rodriguez, B.N. Jones, C. Barreda, S. Watts, J.B. Baker, *J. Biol. Chem.* **1985**, *260*, 7029–7034.

108 G. Perry, P. Richey, S.L. Siedlak, P. Galloway, M. Kawai, P. Cras, *Brain Res.* **1992**, *579*, 350–352.

109 H.G. Bazin, M.A. Marques, A.P. Owens, R.J. Linhardt, K.A. Krutcher, *Biochemistry* **2002**.

110 B. Mulloy, R.J. Linhardt, *Curr. Opin. Struct. Biol.* **2001**, *11*, 623–628.

111 R.L. Jackson, S.J. Busch, A.D. Cardin, *Physiol. Rev.* **1991**, *71*, 481–537.

112 A.D. Cardin, H.J.R. Weintraub, *Arteriosclerosis* **1989**, *9*, 21–32.

113 R.V. Iozzo, *Lab. Invest.* **1985**, *53*, 373–396.

114 R.J. Linhardt, R.E. Hileman, *Gen. Pharmac.* **1995**, *26*, 443–451.

115 L, Cöster, *Biochem. Soc. Trans.* **1991**, *19*, 866–868.

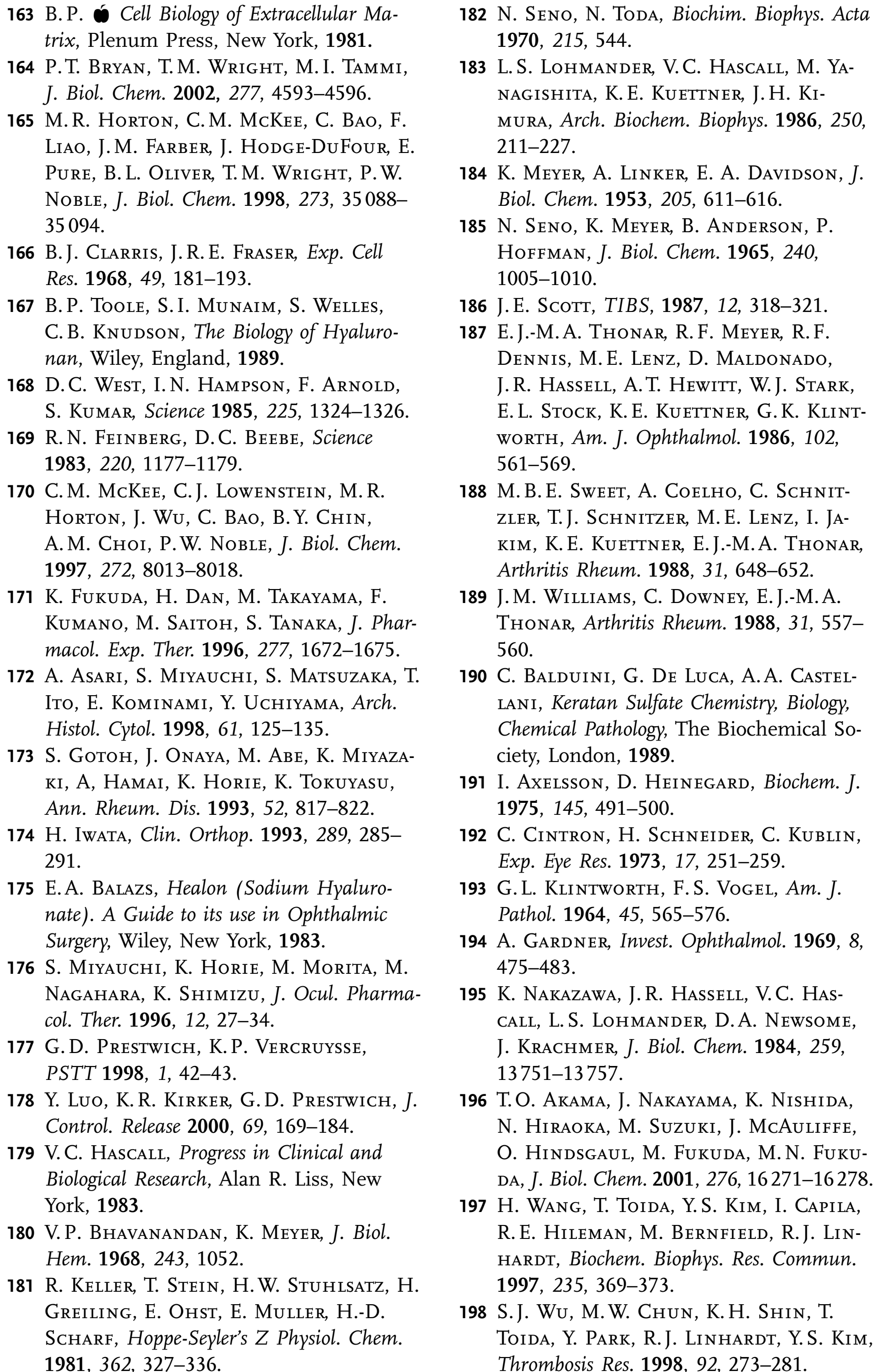
116 H. Kresse, H. Hausses, E. Schönherr, *Experimentia* **1993**, *49*, 403–416.

117 H.C. Whinna, H.U. Choi, L.C. Rosenberg, F.C. Church, *J. Biol. Chem.* **1993**, *268*, 3920–3924.

118 M.C. Bourin, U. Lindahl, *Biochem. J.* **1993**, *289*, 313–330.

119 J. Pangrazzi, F. Gianese, *Haematologica* **1987**, *72*, 459–464.

120 D.M. Tollefsen, *Adv. Exp. Med. Biol.* **1992**, *313*, 167–176.
121 I.J. Edwards, W.D. Wagner, R.T. Owens, *Am. J. Path.* **1990**, *136*, 609–621.
122 E. Cofrancesco, M. Colombi, F. Gianese, M. Cortellaro, *Thromb. Res.* **1990**, *57*, 405–414.
123 D. Hoppensteadt, J. Walenga, J. Fareed, *Semin. Thromb. Hemost.* **1991**, *17*, 60–64.
124 P. Sié, D. Dupouy, C. Caranobe, M. Petitou, B. Boneu, *Blood* **1993**, *81*, 1771–1777.
125 G. Mascellani, L. Liverani, P. Bianchini, B. Parma, G. Torri, G. Bisio, M. Guerrini, B. Casu, *Biochem. J.* **1993**, *296*, 639–648.
126 S. Alban, *Carbohydrates as Drugs* Marcel Dekker: New York, **1996**.
127 M. Barbanti, F. Calanni, M.R. Milani, E. Marchi, N. Semeraro, M. Colucci, *Thromb. Haemost.* **1993**, *69*, 147–151.
128 F. Dol, M. Petitou, J.C. Lormeau, J. Choay, C. Caranobe, P. Sié, S. Saivin, G. Houin, B. Boneu, *J. Lab. Clin. Med.* **1990**, *115*, 43–51.
129 S.J. Brister, M.R. Buchanan, C.C. Griffin, C.L. van Gorp, R.J. Linhardt, US Patent 5922690, **1999**.
130 J. Van Ryn-McKenna, F.A. Ofosu, J. Hirsh, M.R. Buchanan, *Br. J. Haemat.* **1989**, *71*, 265–269.
131 K.R. Stone, US Patent 4880429, **1989**.
132 H. Miwa, T. Matsuda, *J. Vasc. Surg.* **1994**, *19*, 658–667.
133 N. Volpi, M. Cusmano, T. Venturelli, *Biochim. Biophys. Acta* **1995**, *1243*, 49–58.
134 K. Nawa, K. Sakano, H. Fujiwara, Y. Sato, N. Sugiyama, T. Teruuchi, T. Iwamoto, Y. Marumoto, *Biochim. Biophys. Res. Commun.* **1990**, *171*, 729–737.
135 T.D. Bjornsson, P.V. Nash, R. Schaten, *Thromb. Res.* **1982**, *27*, 15–21.
136 D. Bergqvist, B. Lindblad, T. Mätzsch, *Heparin and related Polysaccharides*, Plenum Press, New York, **1992.**
137 H.J. Hanselmann, *Best Pract. Res. Clin. Rheumatol.* **2001**, *15*, 595–607.
138 J.Y. Reginster, V. Gillot, O. Bruyere, Y. Henrotin, *Curr. Rheumatol. Rep.* **2000**, *2*, 472–477.
139 F. Ronca, L. Palmieri, P. Panicucci, *Osteoarthritis & Cartilage* **1998**, *6*, 14–21.
140 M. Konlee, *Positive Health News* **1998**, *17*, 4–7.
141 C.S. Osborne, W.H. Reid, M.H. Grant, *Biomaterials* **1999**, *20*, 283–290.
142 K. Meyer, J.W. Palmer, *J. Biol. Chem.* **1934**, *107*, 629–634.
143 J.H. Fessler, L.I. Fessler, *Proc. Natl. Acad. Sci. USA* **1966**, *56*, 141–147.
144 J.E. Scott, *The Biology of Hyaluronan*, Wiley, England, **1989**.
145 J.E. Scott, C. Cummings, A. Brass, Y. Chen, *Biochem. J.* **1991**, *274*, 699–705.
146 J.E. Scott, F. Heatley, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 4850–4855.
147 T.C. Laurent, J.R. Fraser, *Faseb J.* **1992**, *6*, 2397–2404.
148 P.L. DeAngelis, *Cell. Mol. Life Sci.* **1999**, *56*, 670–682.
149 J.A. Ripellinio, M. Bailo, R.U. Margolis, R.K. Margolis, *J. Cell Biol.* **1988**, *106*, 845–855.
150 P.S. Eggli, W. Graber, *J. Histochem. Cytochem.* **1995**, *43*, 689–697.
151 T.C. Laurent, J.R.E. Fraser, *Degradation of Bioactive Substance: Physiology and Pathology*, CRC Press, Boca Raton, **1991**.
152 R.K. Reed, K. Lilja, T.C. Laurent, *Acta Physiol. Scand.* **1988**, *134*, 405–411.
153 J.R. Fraser, T.C. Laurent, U.B. Laurent, *J. Intern. Med.* **1997**, *242*, 27–33.
154 M. Markovitz, J.A. Cifonelli, A. Dorfman, *J. Biol. Chem.* **1959**, *234*, 2343–2350.
155 P. Prehm, *Biochem. J.* **1983**, *211*, 191–198.
156 P.H. Weigel, V.C. Hascall, M. Tammi, *J. Biol. Chem.* **1997**, *272*, 13 997–14 000.
157 V.L. Tlapak-Simmons, E.S. Kempner, B.A Baggenstoss, P.H. Weigel, *J. Biol. Chem.* **1998**, *273*, 26 100–26 109.
158 V.L. Tlapak-Simmons, B.A. Baggenstoss, T. Clyne, P. Weigel, *J. Biol. Chem.* **1999**, *274*, 4239–4245.
159 A.S. Day, J.K. Sheehan, *Curr. Opin. Struct. Biol.* **2001**, *11*, 617–622.
160 M.I. Tammi, A.J. Day, E.A. Turley, *J. Biol. Chem.* **2002**, *277*, 4581–4584.
161 M. Hlavacek, *J. Biomechanics* **1993**, *26*, 1145–1150.
162 P. Herrlich, J. Sleeman, D. Wainwright, H. Konig, L. Sharman, F. Hilberg, H. Ponta, *Cell Adhes. Commun.* **1998**, *6*, 141–147.



163 B.P. *Cell Biology of Extracellular Matrix*, Plenum Press, New York, **1981.**

164 P.T. Bryan, T.M. Wright, M.I. Tammi, *J. Biol. Chem.* **2002,** *277,* 4593–4596.

165 M.R. Horton, C.M. McKee, C. Bao, F. Liao, J.M. Farber, J. Hodge-DuFour, E. Pure, B.L. Oliver, T.M. Wright, P.W. Noble, *J. Biol. Chem.* **1998**, *273*, 35 088–35 094.

166 B.J. Clarris, J.R.E. Fraser, *Exp. Cell Res.* **1968**, *49*, 181–193.

167 B.P. Toole, S.I. Munaim, S. Welles, C.B. Knudson, *The Biology of Hyaluronan*, Wiley, England, **1989**.

168 D.C. West, I.N. Hampson, F. Arnold, S. Kumar, *Science* **1985**, *225*, 1324–1326.

169 R.N. Feinberg, D.C. Beebe, *Science* **1983**, *220*, 1177–1179.

170 C.M. McKee, C.J. Lowenstein, M.R. Horton, J. Wu, C. Bao, B.Y. Chin, A.M. Choi, P.W. Noble, *J. Biol. Chem.* **1997**, *272*, 8013–8018.

171 K. Fukuda, H. Dan, M. Takayama, F. Kumano, M. Saitoh, S. Tanaka, *J. Pharmacol. Exp. Ther.* **1996**, *277*, 1672–1675.

172 A. Asari, S. Miyauchi, S. Matsuzaka, T. Ito, E. Kominami, Y. Uchiyama, *Arch. Histol. Cytol.* **1998**, *61*, 125–135.

173 S. Gotoh, J. Onaya, M. Abe, K. Miyazaki, A, Hamai, K. Horie, K. Tokuyasu, *Ann. Rheum. Dis.* **1993**, *52*, 817–822.

174 H. Iwata, *Clin. Orthop.* **1993**, *289*, 285–291.

175 E.A. Balazs, *Healon (Sodium Hyaluronate). A Guide to its use in Ophthalmic Surgery*, Wiley, New York, **1983**.

176 S. Miyauchi, K. Horie, M. Morita, M. Nagahara, K. Shimizu, *J. Ocul. Pharmacol. Ther.* **1996**, *12*, 27–34.

177 G.D. Prestwich, K.P. Vercruysse, *PSTT* **1998**, *1*, 42–43.

178 Y. Luo, K.R. Kirker, G.D. Prestwich, *J. Control. Release* **2000**, *69*, 169–184.

179 V.C. Hascall, *Progress in Clinical and Biological Research*, Alan R. Liss, New York, **1983**.

180 V.P. Bhavanandan, K. Meyer, *J. Biol. Hem.* **1968**, *243*, 1052.

181 R. Keller, T. Stein, H.W. Stuhlsatz, H. Greiling, E. Ohst, E. Muller, H.-D. Scharf, *Hoppe-Seyler's Z Physiol. Chem.* **1981**, *362*, 327–336.

182 N. Seno, N. Toda, *Biochim. Biophys. Acta* **1970**, *215*, 544.

183 L.S. Lohmander, V.C. Hascall, M. Yanagishita, K.E. Kuettner, J.H. Kimura, *Arch. Biochem. Biophys.* **1986**, *250*, 211–227.

184 K. Meyer, A. Linker, E. A. Davidson, *J. Biol. Chem.* **1953**, *205*, 611–616.

185 N. Seno, K. Meyer, B. Anderson, P. Hoffman, *J. Biol. Chem.* **1965**, *240*, 1005–1010.

186 J.E. Scott, *TIBS*, **1987**, *12*, 318–321.

187 E.J.-M.A. Thonar, R.F. Meyer, R.F. Dennis, M.E. Lenz, D. Maldonado, J.R. Hassell, A.T. Hewitt, W.J. Stark, E.L. Stock, K.E. Kuettner, G.K. Klintworth, *Am. J. Ophthalmol.* **1986**, *102*, 561–569.

188 M.B.E. Sweet, A. Coelho, C. Schnitzler, T.J. Schnitzer, M.E. Lenz, I. Jakim, K.E. Kuettner, E.J.-M.A. Thonar, *Arthritis Rheum.* **1988**, *31*, 648–652.

189 J.M. Williams, C. Downey, E.J.-M.A. Thonar, *Arthritis Rheum.* **1988**, *31*, 557–560.

190 C. Balduini, G. De Luca, A.A. Castellani, *Keratan Sulfate Chemistry, Biology, Chemical Pathology*, The Biochemical Society, London, **1989**.

191 I. Axelsson, D. Heinegard, *Biochem. J.* **1975**, *145*, 491–500.

192 C. Cintron, H. Schneider, C. Kublin, *Exp. Eye Res.* **1973**, *17*, 251–259.

193 G.L. Klintworth, F.S. Vogel, *Am. J. Pathol.* **1964**, *45*, 565–576.

194 A. Gardner, *Invest. Ophthalmol.* **1969**, *8*, 475–483.

195 K. Nakazawa, J.R. Hassell, V.C. Hascall, L.S. Lohmander, D.A. Newsome, J. Krachmer, *J. Biol. Chem.* **1984**, *259*, 13 751–13 757.

196 T.O. Akama, J. Nakayama, K. Nishida, N. Hiraoka, M. Suzuki, J. McAuliffe, O. Hindsgaul, M. Fukuda, M.N. Fukuda, *J. Biol. Chem.* **2001**, *276*, 16 271–16 278.

197 H. Wang, T. Toida, Y.S. Kim, I. Capila, R.E. Hileman, M. Bernfield, R.J. Linhardt, *Biochem. Biophys. Res. Commun.* **1997**, *235*, 369–373.

198 S.J. Wu, M.W. Chun, K.H. Shin, T. Toida, Y. Park, R.J. Linhardt, Y.S. Kim, *Thrombosis Res.* **1998**, *92*, 273–281.

199 E. Percival, R.H. McDowell, *Chemistry and Enzymology of Marine Algal Polysaccharides*, Academic Press, New York, **1967**.

200 T.J. Painter, *The Polysaccharides*, Academic Press, New York, **1983**.

201 M. Nagaoka, H. Shibata, I. Kimura-Takagi, S. Hashimoto, R. Aiyama, S. Ueyama, T. Yokokura, *BioFactors* **2000**, *12*, 267–274.

202 G.K. SeGall, W.J. Lennarz, *Dev. Biol.* **1979**, *71*, 33–48.

203 P.S.A. Mourão, I.G. Bastos, *Eur. J. Biochem.* **1987**, *166*, 639–645.

204 M.S. Patankar, S. Oehninger, T. Barnett, R.L. Williams, G.F. Clark, *J. Biol. Chem.* **1993**, *268*, 21770–21776.

205 G. Bernardi, G.F. Springer, *J. Biol. Chem.* **1988**, *263*, 75–80.

206 S. Colliec, A.M. Fischer, J. Tapon-Bretaudiere, C.P. Boisson, P. Durand, J. Jozefonvicz, *Thromb. Res.* **1991**, *64*, 143–154.

207 T. Nishino, T. Nagumo, *Carbohydr. Res.* **1992**, *229*, 355–362.

208 M.C. Mahony, S. Oehninger, G.F. Clark, A.A. Acosta, G.D. Hodgen, *Contraception* **1991**, *44*, 657–665.

209 M. Baba, R. Snoeck, R. Pauwels, E. DeClerq, *Antimicrob. Agents Chemother.* **1988**, *32*, 1742–1745.

210 C. Foxall, S.R. Watson, D. Dowbenko, C. Fennie, L.A. Lasky, M. Kiso, D. Hasegawa, D. Asa, B.K. Brandley, *J. Cell. Biol.* **1992**, *117*, 895–902.

211 L. Ramsden, C.C. Rider, *Eur. J. Immun.* **1992**, *22*, 3027–3031.

212 T. Kobayashi, K. Honke, T. Miyazaki, K. Matsumoto, T. Nakamura, I. Ishizuka, A. Makita, *J. Biol. Chem.* **1994**, *269*, 9817–9821.

213 S. Oehninger, G.F. Clark, A.A. Acosta, G.D. Hodgen, *Fertil. Steril.* **1991**, *55*, 165–169.

214 M.C. Mahony, S. Oehninger, G.F. Clark, A.A. Acosta, G.D. Hodgen, *Contraception* **1991**, *44*, 657–665.

215 K.E. Arfors, K. Ley, *J. Lab. Clin. Med.* **1993**, *121*, 201–202.

216 D.J. Stancioff, N.F. Stanley, *Proc. Int. Seaweed Symp.* **1969**, *6*, 595.

217 K.A. Pittman, L. Golberg, F. Coulston, *Fd. Cosmet. Toxicol.* **1976**, *14*, 85–93.

218 E. Booth, *Chemical Oceanography*, Academic Press, London, **1975**.

219 H.J. Schwarz, R.W. Kellermeyer, *Proc. Soc. Exp. Biol. Med.* **1969**, *132*, 1021–1024.

220 T.H. Noda, H. Amano, K. Arashima, S. Hashimoto, K. Nisizawa, *Nippon, Suisan, Gakkaishi* **1989**, *55*, 1265–1271.

221 G. Yu, H. Guan, A.S. Ioanoviciu, S.A. Sikkander, C. Thanawiroon, J.K. Tobacman, T. Toida, R.J. Linhardt, *Carbohydr. Res.* **2002**, *337*, 433–440.

222 J.F. Kennedy, C.A White, *Bioactive Carbohydrates*, Ellis Horwood Limited, New York, **1983**.

223 S.-I. Nishimura, N. Nishi, S. Tokura, *Carbohydr. Res.* **1986**, *156*, 286–292.

224 C.R. Rickets, K.W.W.H. Walton, US Patent 2715091, 1955.

225 A. Osol, *16th Remington's Pharmaceutical sciences*, Mack Publishing Co., Pennsylvania, **1980**.

226 M. Windholz, *The Merck Index, 9th Ed.*, Merck Publishing Co., Rahway, **1976.**

227 G. Oshima, *Thromb. Res.* **1988**, *49*, 353–361.

228 L.B. Jaques, L.M. Hiebert, S.M. Wice, *J. Lab. Clin. Med.* **1991**, *117*, 122–130.

229 G. Skjak-Braek, *Int. J. Biol. Macromol.* **1986**, *8*, 330–336.

230 M. Asada, M. Sugie, M. Inoue, K. Nakagomi, S. Hongo, K. Murata, S. Irie, T. Takeuchi, N. Tomizuki, S. Oka, *Biosci. Biotech. Biochem.* **1997**, *61*, 1030–1032.

231 R. Langer, R.J. Linhardt, M. Klein, P.M. Galliher, C.L. Cooney, M.M. Flanagan, *Biomaterials: Interfacial Phenomenon and Applications*, ACS Press, Washington D.C., **1982.**

232 D.L. Taylor, T.M. Brennan, C.G. Bridges, M.J. Mullins, A.S. Tyms, R. Jackson, A.D. Cardin, *Antiviral Res.* **1995**, *28*, 159–173.

233 F. Hawking, *Pharmacol. Chemother.* **1978**, *15*, 289–322.

234 G.T. Tan, A. Wickramasinghe, S. Verma, R. Singh, S.H. Hughes, J.M. Pezzuto, M. Baba, P. Mohan, *J. Med. Chem.* **1992**, *35*, 4846–4853.

235 R. Kisilevsky, L.J. Lemieux, P.E. Fraser, X. Kang, P.G. Hultin, W.A. Szarek, *Nature Med.* **1995**, *1*, 143–148.

236 K. Kasuraya, N. Ikushima, N. Takahashi, T. Shoji, H. Nakashima, N. Yamamoto, T. Yoshida, T. Uryu, *Carbodr. Res.* **1994**, *260*, 51–61.

237 B.K. Yeh, A.N. Plotnikov, A.V. Eliseenkova, D. Green, J. Pinnel, T. Polat, R.J. Linhardt, submitted *Molec. Cell Biol.* **2002**.

238 M.A. Mitchell, J.W. Wilks, *Ann. Reports in Medicinal Chemistry*, Academic Press Inc., San Diego, **1992**.

239 A. Gerloczy, T. Hoshino, J. Pitha, *J. Pharm. Sci.* **1994**, *83*, 193–196.

240 V. Ferro, C. Li, K. Fewings, M.C. Palemo, D. Podger, R.J. Linhardt, T. Toida, *Carbohydr. Res.* **2002**, *337*, 139–146.

241 C.R. Parish, C. Freeman, R.J. Brown, D.J. Francis, W.B. Cowlen, *Cancer Res.* **1999**, *59*, 3433–3441.

242 M.D. Hulett, C. Freeman, B.J. Hamdorf, R.T. Baker, M.J. Harris, C.R. Porish, *Nat. Med.* **1999**, *5*, 803–809.

243 G. Yu, N.S. Gunay, R.J. Linhardt, T. Toida, J. Fareed, D.A. Hoppensteadt, H. Shadid, V. Ferro, C. Li, K. Fewings, M.C. Polermo, D. Podger, *Eur. J. Med. Chem.* **2002**, in press.

244 M.F. Scully, M. Kumudini, K.M. Weerasinghe, V. Ellis, B. Djazaeri, V.V. Kakkar, *Thrombosis Res.* **1983**, *31*, 87–97.

245 F. Dol, P. Sie, D. Dupouy, B. Boneu, *Thromb. Haemostas.* **1986**, *56*, 295–301.

246 H.P. Wessel, *Tet. Lett.* **1990**, *31*, 6863–6866.

247 J. Giedrojc, K. Knipinski, H.K. Breddin, M. Bieawiec, *Polish J. Pharmacol.* **1996**, *48*, 317–322.

248 P.D. Ward, T.K. Tippin, D.R. Thakker, *Pharm. Sci. Tech. Today* **2000**, *3*, 346–358.

249 S.Y. Cho, J.S. Kim, H. Li, C. Shim, R.J. Linhardt, Y.S. Kim, *Arch. Pharmacol. Res.* **2002**, *25*, 86–98.

250 T.M. Rivera, A. Leone-Bay, D.R. Patow, H.R. Leipold, R.A. Baughman, *Pharm. Res.* **1997**, *14*, 1830–1834.

251 Y. Ichikawa, R. Monden, H. Kuzuhara, *Carbohydr. Res.* **1988**, *172*, 37–64.

252 K.M. Koeller, C.H. Wong, *Glycobiology* **2000**, *10*, 1157–1169.

253 M.D. Burkart, M. Izumi, E. Chapman, C.H. Lin, C.H. Wong, *Journal of Organic Chemistry* **2000**, *65*, 5565–5574.

254 P.H. Seeberger, W.-C. Haase, *Chem. Rev.* **2000**, *100*, 4349–4394.