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Chapter 4

GLYCOSAMINOGLYCANS IN PRION AND PRION-LIKE DISEASES

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ABSTRACT

Glycosaminoglycans (GAGs) comprise an important group of polysaccharides that are found inside and outside animal cells and that act as tissue organizers, modulate growth factor activity, and regulate neurite outgrowth, among many other functions. Amyloid deposits were first found to contain polysaccharides in 1854 by Virchow, but the association of GAGs with amyloid deposits gained much attention after the 1980s, when GAGs were implicated in many conformational diseases (prion diseases, Alzheimer's disease, Parkinson's disease, and others). The role of GAGs in prion-like diseases and amyloidoses has been related to amyloid fiber nucleation and formation *via* a passive role as a component of amyloid deposits and an active role by promoting aggregation-prone protein conformations. In the case of prion pathology, GAGs are important cofactors and receptors in prion infections. These molecules may serve as cofactors by acting as adjuvants for prion conversion, whereas others demonstrated that these molecules serve as inhibitors of conversion. Alternatively, GAGs may serve as the primary cell surface receptor for the pathological isoform of the cellular prion protein (PrP^C), although some studies reported contrasting results. GAGs play paradoxical roles in prion and prion-like diseases. In this chapter, we discuss how these molecules regulate protein conformation to provide crucial guidance for the development of therapeutic drugs for many amyloid diseases. Recent studies have shown that the interaction of low-molecular-weight heparin (LMWHep) with the N- or C-terminal domain of PrP^C affects the extent and kinetics of PrP^C fibrillization. These findings explain the protective effect of heparin in different models of prion and prion-like neurodegenerative diseases and establish the foundation for developing therapeutic strategies based on GAGs.

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INTRODUCTION

Transmissible spongiform encephalopathies (TSEs) have either a genetic, infectious or sporadic nature and have been found in several species such as humans (kuru and Creutzfeldt-Jakob disease), sheep (scrapie) and cattle (bovine spongiform encephalopathy, also known as "mad cow disease") [1]. Although rare, these diseases are unavoidably fatal. The primary TSE symptoms are paralysis, ataxia and dementia [1]. A '*prion*', which is a term created as a contraction of '*proteinaceous infectious particle*' lacks the codifying nucleic acid [1]. Although this particle alone accounts for the transmission of these diseases [2], cofactors have been suggested to catalyze, initiate or modulate the conversion of PrP^{C} to PrP^{Sc} [3, 4]: nucleic acids [5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15], lipids [14, 16, 17, 18, 19] or polysaccharides [9, 20, 21, 22, 23, 24].

Mature PrP^{C} comprises 208-209 amino acids and is attached to the outer leaflet of the plasma membrane through a glycosylphosphatidylinositol (GPI) anchor [25]. The first half of the PrP^{C} protein is an intrinsically disordered peptide containing four repeats of an eight-amino acid sequence (PHGGGWGQ) termed the octapeptide or octarepeat domain, which is related to copper binding [26] as well as to the binding of glycosaminoglycans and nucleic acids, especially RNA [12]. The C-terminal region of PrP^{C} is globular and contains three alpha-helices at positions 144-154, 173-194 and 200-228. A disulfide bond is formed between cysteine residues 179 and 214 [27]. The conversion of PrP^{C} to its misfolded isoform, PrP^{Sc}, is characterized by an increase in beta sheet content [1]. PrP^{Sc} has a high propensity to form protease-resistant aggregates, which tends to accumulate in the brains of TSE patients.

Here, we review the role of GAGs in TSEs and prion-like diseases. GAGs appear to play paradoxical roles by either attenuating or stimulating prion conversion. We review how GAGs affect protein misfolding and the potential of GAGs as therapeutic agents against these diseases and discuss the protective effects of low-molecular-weight heparin (LMWHep) against prion and prion-like neurodegenerative diseases.

PROTEOGLYCANS AND GLYCOSAMINOGLYCANS

Proteoglycans (PGs) are a group of glycoconjugates comprising a protein portion and an unbranched polysaccharide portion termed as glycosaminoglycan (GAG). GAGs comprise the largest group of sulfated polysaccharides found in animal tissues. These compounds possess a negative charge due to the presence of carboxylated and/or sulfated sugar residues. In these carboxylated polysaccharides, one sugar (a hexuronic acid) alternates *via* an O-glycosidic bond with an amino sugar (one hexosamine), forming repeating disaccharide units along the chain [28, 29].

PGs act as tissue organizers, influence the growth and maturation of cells from specialized tissues, perform functions such as biological filtration, modulate the growth factor activity, regulate collagen fibrillogenesis, affect tumor cell growth and invasion, regulate neurite growth, attract cations, and mediate blood coagulation and pressure variation absorption. GAG chains are primarily responsible for the functions of PGs [28, 29, 30].

GAGs are classified according to their structural organization (disaccharide units) and to the extent and nature of their modifications after polymerization (N-sulfation, O-sulfation and

epimerization). According to the Jeanloz (1960) nomenclature [31], naturally occurring GAGs can be divided into the following groups: hyaluronic acid (HA), dermatan sulfate (DS)/chondroitin sulfate (CS), keratan sulfate (KS), and heparan sulfate (HS)/heparin (Hep).

HA shows greater simplicity in its structural composition. The disaccharide units of HA comprise a glucuronic acid (GlcA) connected to an N-acetylglucosamine (GlcNAc) linked β 1 → 4. HA is not sulfonated; therefore, the anionic property of HA is provided only by its carboxyl groups [32]. In contrast to other GAGs, HA is not covalently attached to the protein chain and is therefore simply classified as a GAG. HA forms high-molecular-weight polymers that are important extracellular matrix components [32] and plays important functional roles in signaling during embryonic morphogenesis, cell repair, and joint lubrication, among others [32].

CS and DS are considered as GAGs belonging to the same family, also referred to as galactosaminoglycans [33], due to their identical disaccharide units of N-acetylgalactosamine (GalNAc). CS contains a disaccharide unit comprising a GalNAc linked to GlcA as β 1 → 4 or β 1 → 3, and its GalNAc may be sulfonated at position 4 or 6, forming chondroitin 4 (chondroitin A) or 6 (chondroitin C) sulfate, respectively. DS contains alternating GalNAc residues with a variable number of GlcAs and iduronic acid (IdoA) residues; these residues are formed from the epimerization of GlcA during polymerization and the subsequent sulfation of GalNAc at position 4. DS is considered as a variant of chondroitin 4 sulfate and is also referred to as B chondroitin. Additionally, DS is often sulfated at position 2 of IdoA and is occasionally sulfated at position 6 of GalNAc [34]. CS and DS play roles in central nervous system development, tissue repair, infection, growth factor signaling, morphogenesis and cell division, among others [34].

KS has β 1 → 3-linked disaccharide units comprising GlcNAc and the neutral sugar galactose (Gal) in place of hexuronic acid. This polysaccharide is found in a wide variety of sizes and sulfation patterns and is present in the cornea, cartilage, dermis and zona pellucida [35].

HS and Hep GAGs have closely related structures. They form another group of GAGs in which the amino sugar is glucosamine with a hexosamine bond in the α 1 → 4 configuration. Glucosamine can be N- or 6-sulfated ($GlcNSO_3$), N-acetylated (GlcNAc), or N-free ($GlcNH_2$). The hexuronic acid may be GlcA or IdoA, O-sulfated at position 2 [36, 37]. Approximately 40-50% of the glucosamines in HS are N-sulfated [37]. Approximately 80% of the glucosamines in Hep are sulfated, and this polysaccharide primarily comprises trisulfated disaccharides [38].

Notably, Hep can be isolated from different animal tissues, including those of vertebrates and invertebrates [39]. In mammals, Hep is synthesized and stored in cytoplasmic granules of mast cells and is released as protein-free chains following mast cell activation and degranulation. HS is synthesized by a variety of cell types, is secreted as either a free GAG or a PG chain, and is found on the cell surface and basement membrane [40, 41].

Depending on its localization, HS interacts with several proteins. These interactions are physiologically important as they modulate the function of various proteins such as growth factors, extracellular matrix proteins, and lipolytic enzymes, among others, to regulate different biological activities such as cell signaling, cytoskeletal organization, migration, inflammation, metastasis and synapse formation [41]. In addition to its known anticoagulant activity, Hep displays activities such as of lipid metabolism regulation and antiproliferative activity [41].

GAGS AND AMYLOIDS

Protein aggregation has been suggested as a generic feature of the polypeptide chain [42]. This aggregation can occur *via* two processes, one of which involves the formation of species with well-ordered morphological and structural features such as amyloid fibers, and the other one involves the formation of disordered structures such as amorphous aggregates [43]. Aggregation can occur slowly *via* an orderly process in which partially unfolded proteins combine to form a stable core. This core then acts as a template by recruiting other intermediates that are then added to the growing aggregated protein filament (protofibril). The sequential addition of intermediates leads to the formation of a highly structured insoluble protein structure known as an amyloid fiber. The kinetics of amyloid fiber formation include a lag phase, which involves the formation of a stable nucleus, and an elongation phase [44, 45, 46].

Many diseases, including Parkinson's (PD) and Alzheimer's diseases (AD), TSEs, systemic AA amyloidosis (AA) and type II diabetes, are directly associated with extracellular or intracellular aggregate formation. In each disease, these aggregates are primarily formed by a major protein such as Aβ and Tau in AD, α-synuclein in PD, prion scrapie or resistant prion protein (PrP^{Sc} or PrP^{res}, respectively) in TSEs, serum amyloid A (SAA) in AA, and islet amyloid polypeptide (IAPP) in type II diabetes.

PrP^{Sc} is able to convert the cellular prion (PrP^{C}) form into the misfolded protein form and to seed PrP fibrillization. Acting as a template, PrP^{Sc} is able to transmit its pathological structural information. Over the last decade, many authors have shown that aggregated proteins associated with diseases other than TSEs show the same characteristics; therefore, these diseases have been termed prion-like diseases [47, 48].

Amyloid deposits were first found to contain polysaccharides in 1854 by Virchow [49]. GAGs and PGs have been implicated in many conformational diseases and have been detected in different types of amyloid deposits. Furthermore, the importance of these molecules is not restricted to the deposition space but also to time [50] and induction of conformational changes [20] in amyloidogenesis.

Among all GAGs, HS and DS are predominantly found in amyloid deposits [51]. In addition to several extracellular amyloid deposits, GAGs are associated with intracellular proteins, which form deposits such as Lewy bodies in patients with PD [52]. The role of GAGs in amyloidoses is related to the nucleation and formation of amyloid fibers. These molecules stabilize mature amyloid fibers from dissociation [53] and proteolytic degradation [54] and interact with various amyloid proteins to facilitate fiber formation [55]. HS/Hep were shown to promote the fibrillization of transthyretin [56] and the polymerization of amyloidogenic light chain (AL) [57], IAPP [58], Aβ peptide [59], Tau protein [60] and α-synuclein [52]. GAGs may play an active role in amyloid deposition by promoting protein conformations that are likely to fibrillize [52, 55].

GAGS IN PRION METABOLISM

GAGs, especially HS, have been the subject of many prion disease studies. Snow et al., first demonstrated the presence of heparan sulfate in prion-based amyloid plaques in diseases

such as Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome, and scrapie [61, 62]. Since then, studies have focused on the function of these carbohydrates in the pathogenesis of TSEs.

Much evidence shows the interaction of GAGs with PrP^C both in its soluble form and on the cell surface [20, 63, 64, 65, 66, 67, 68]. These findings emphasize two primary roles for these molecules in prion pathology: as receptors and as cofactors.

GAGs as Receptors

PrP^C is primarily localized to the cell surface *via* its attachment to a glycosylphosphatidylinositol (GPI) anchor [25] in lipid raft microdomains [69]; in this environment, PrP^C likely interacts with cell surface proteoglycans (HS proteoglycans (-HSPGs) such as syndecans and glypicans) and the extracellular matrix (Figure 1). The ability of PrP^C to interact with GAGs appears to be involved in not only prion pathogenesis but also the normal functions of PrP^C and HS [70, 71, 72]. The GPI-anchored GAG glypican-1 (Gpc-1) mediates PrP^C localization to lipid rafts [73]; thus, HSPG and PrP^C trafficking are closely related. PrP^C controls Gpc-1 internalization and self-processing, although Gpc-1 expression does not influence PrP^C endocytosis [72]. Additionally, PrP^C loaded with copper (Cu(II)) is able to sustain Gpc-1 autodegradation catalyzed by nitric oxide *in vitro* [71], and sulfated polysaccharides and Cu(II) ions stimulate PrP^C endocytosis [74, 75].

PrP^C conversion likely occurs at a location at which the two PrP isoforms can physically interact. At present, the specific intracellular compartment in which this event occurs remains controversial [76, 77, 78, 79]. In some studies, the entry of PrP^{Sc} into cells was shown to be an essential event for its conversion, because the inhibition of endocytosis prevented its formation [80, 81], whereas others showed that the plasma membrane is the initial site of prion conversion and is able to act alone to support this event [82, 83].

Although PrP^C must be present at the cell surface to enable infection [82, 84], other molecules have been indicated as receptors or co-receptors of PrP^{Sc} [85, 86, 87, 88, 89]. Many studies have suggested that HS may be the primary receptor of PrP^{Sc} on the cell surface [74, 85, 86, 90], whereas others [91] have not, representing a paradoxical effect of HS (Figure 1). HS is responsible for the binding and uptake of prion rods (PrP 27-30 purified from prion-infected hamster tissues) in neurons [85] and of PrP^{Sc} from hamster-infected brain homogenates in Chinese hamster ovary (CHO) cells [86]. The specific depletion of Gpc-1 reduced the formation of PrP^{Sc} by 55% in persistently infected N2a cells [73, 90]. Moreover, sheep strain propagation in mice was suggested to be internalized in epithelial Rov cells independent of HS binding, although HS was important for infection during the steps after uptake [91]. These controversial results may be due to differences in prion strains, PrP^{Sc} preparations and cell lineages analyzed and due to the presence of components other than PrP^{Sc} in brain homogenates. Thus, a single mechanism of PrP^{Sc} uptake (Figure 1) must not exist, depending on factors that remain unknown.

During the last decade, many authors have shown that other aggregated proteins such as amyloid-β, Tau protein, and α-synuclein are able to seed the aggregation of the corresponding soluble protein [92, 93, 94, 95]. This capacity was also shown for other proteins that are not related to neurodegenerative diseases, including p53, a protein related to cell cycle control, the aggregation of which appears to be associated with cancer development [96, 97, 98, 99,

100]. Aβ peptide forms extracellular aggregates, but proteins that form intracellular aggregates must enter the cell to propagate. HSPG mediates the uptake of Tau and α-synuclein aggregates but not of huntingtin fibrils in cell culture [93]. Intracellular Aβ accumulation induces neurotoxic and inflammatory activity, and its uptake is cooperatively mediated by HSPG and low-density lipoprotein receptor-related protein 1 (LRP1) [94].

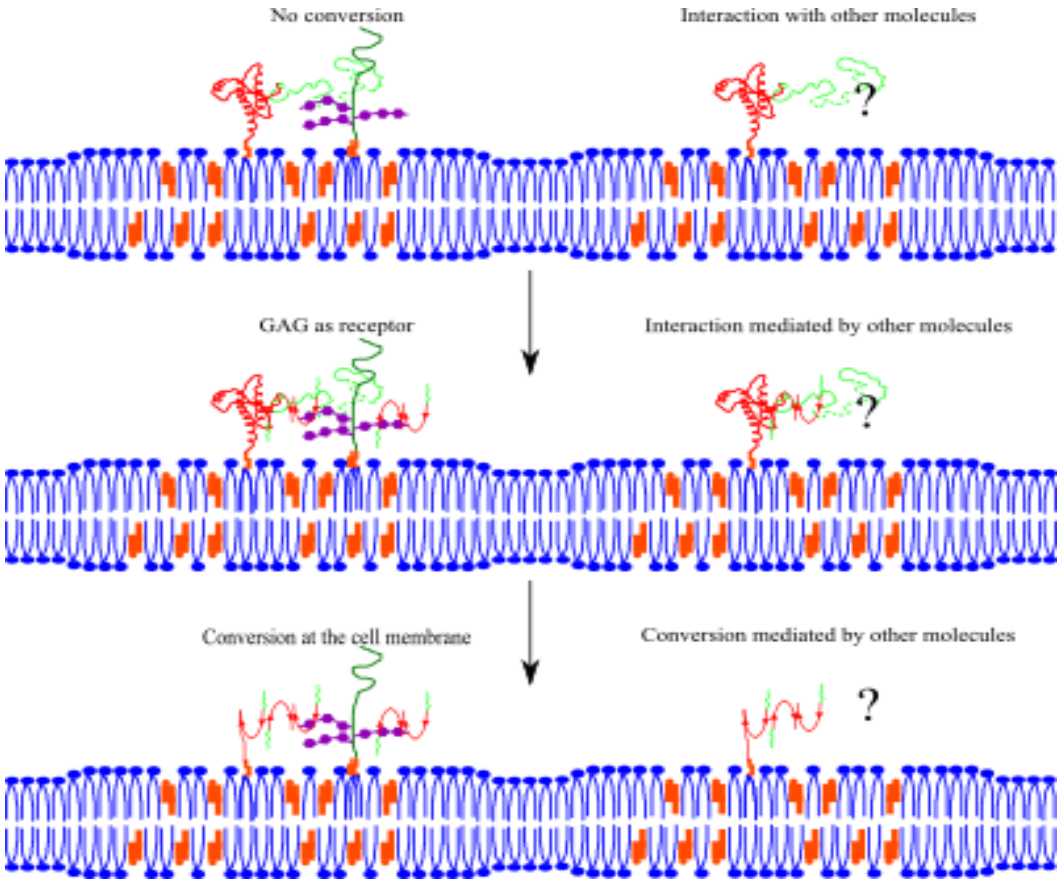

Figure 1. Cell surface PG may interact with PrP^{C}. This interaction is insufficient to trigger prion conversion, but PG may act as a receptor of PrP^{Sc}, thereby bringing isoforms together. Because PrP^{Sc} is able to convert PrP^{C}, this conversion may occur at the cell surface. However, PG is not the only molecule at the cell surface that interacts with PrP^{C}. Thus, other ligands may interact with PrP^{Sc} to mediate its conversion.

GAGs As Cofactors

The "protein-only" hypothesis is the most accepted theory for prion protein conversion [1]. Nevertheless, brain homogenates and polyanions (including HS) have been shown to generate more efficiently prion conversion than purified prion proteins [9, 101, 102, 103]. Thus, other molecules may promote important changes in protein structure that induce or facilitate prion conversion. The following classes of molecules have been shown to act as an

adjuvant factor for prion conversion: nucleic acids [5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15], lipids [14, 16, 17, 18, 19] and polysaccharides [9, 20, 21, 22, 23, 24].

As GAGs are significant components of amyloid aggregates and share the PrP^{C} microenvironment, these molecules serve as excellent candidate receptors and cofactors. Using a cell-free conversion assay, Wong et al., [20] suggested that sulfated carbohydrates induce conformational changes in PrP^{sen} and stimulate conversion to the PrP^{res} isoform. Moreover, in another study, exogenous GAGs were able to reconstitute PrP^{Sc} formation in cells in association with a large reduction in cell membrane-associated GAGs [22]. HSPG also stimulated the amplification of PrP^{res} *in vitro* [9], and Hep enhanced the amplification of variant CJD seeds *via* protein misfolding cyclic amplification (PMCA) [24], increased prion protein fragment (PrP185-208) aggregation and induced toxic amyloid aggregate formation in neurons [104, 105]. In contrast, studies have shown that sulfated polysaccharides suppressed PrP^{res} accumulation in scrapie-infected cells [63, 106, 107, 108] and inhibited its endocytosis [109]. GAGs inhibited the polymerization of synthetic prion peptides into amyloid fibrils, thereby decreasing their neurotoxicity [110], and Hep significantly inhibited lipid-induced PrP (106-126) fibrillization [111]. Moreover, intraventricular pentosan polysulfate (iPPS) infusion was shown to modify the accumulation of PrP oligomers in the brains of patients with prion diseases [112] and to extend the life of mice infected with bovine spongiform encephalopathy [113]. Additionally, HS mimetics decreased PrP^{res} accumulation in infected mice and prolonged infected hamster survival [108]. This contradiction in the literature regarding the ability of sulfated polysaccharides to stimulate PrP^{C} conversion represents another paradoxical effect of these molecules.

One hypothesis explaining the protective effect of these sulfated polysaccharides postulates that the interaction of PrP (PrP^{C} and/or PrP^{Sc}) with endogenous GAGs is necessary for PrP^{Sc} propagation and that exogenous GAGs act as inhibitors to block the interaction of PrP with endogenous PGs (Figure 2B). Thus, distinct from endogenous GAGs, exogenous polysaccharides alone may not play an important role in facilitating PrP^{Sc} formation/propagation (Figure 2A).

The suggestion that GAGs may promote changes in PrP^{C} structure to induce prion conversion have primarily been derived from *in vitro* studies using spectroscopic techniques [20, 114], but protein aggregation renders the interpretation of these results difficult. We showed that the interaction of PrP^{C} with LMWHep leads to protein aggregation but that this effect is transitory and highly influenced by the salt concentration and pH [67]. LMWHep showed a dissociation constant for PrP^{C} of 31.5 nM at pH 5.5 and 120.3 nM at pH 7.4 [67]. After disaggregation, a complex was observed, and *via* nuclear magnetic resonance (NMR) we found that LMWHep alone was insufficient to induce prion conversion [67].

The interaction of PrP with Hep was shown to protect PrP from aggregation induced by other known cofactors (Figure 3A). The soluble complex LMWHep-PrP was shown to be resistant to RNA-induced aggregation [67]. Similarly, Hep inhibited lipid-induced PrP(106-126) fibrillization [111]. The binding of PrP to LMWHep modulates PrP fibrillization thermodynamics (decreasing the amount of fibrils) and kinetics (affecting fibril nucleation and growth) [68]. LMWHep inhibits PrP seeding by Syrian hamster ShaPrP(109-149) aggregates and modulates the seeding activity of mouse- and hamster-infected brain homogenates in real-time quaking-induced conversion (RT-QuIC) assays [68] (Figure 3A). LMWHep-PrP also failed to form amyloid fibrils under denaturing conditions [68].

Stabilization of the PrP monomer may modulate its tendency to aggregate [68], and this activity may explain the protective effects of GAGs.

This paradoxical effect was also observed for molecules other than PrP. Although HS and CS promoted Aβ peptide fibrillization [59], they protected neuronal cultures from the neurotoxic effects of Aβ [115, 116]. The same result was observed for the interaction of HS or Hep with IAPP [58, 117]. CS but not HS showed an inhibitory effect on AL fibrillization [57].

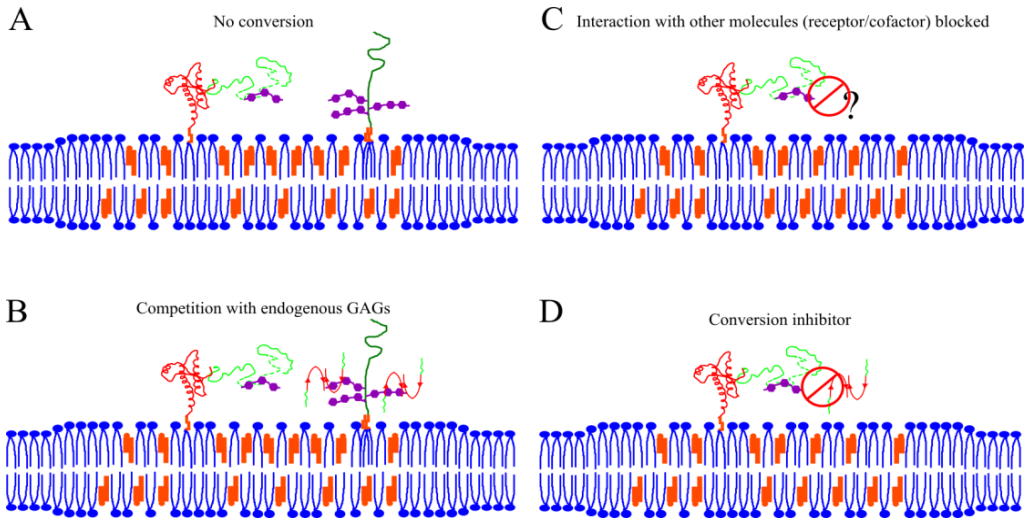

Figure 2. Exogenous GAGs exert protective effects in different models. These molecules have not been shown to facilitate PrP^{Sc} formation alone (A). Their protective effect may be explained by different mechanisms: these molecules may act as inhibitors to block the interaction of PrP^{C} with endogenous PG (B), may compete with other adjuvant cofactors (C), and/or may promote the stabilization of the PrP^{C} monomer.

GAGs at the cell surface in the form of PGs are immobilized on the cell membrane as PrP^{C}. Aggregation induced by these molecules in this environment may be a sporadic event that is rare due to spatial restriction and the influence of ions (Figure 1). Monsellier et al., [118] collected data from articles in PubMed showing the kinetic data of protein aggregation in the presence of GAGs under detailed experimental conditions. They developed a mathematical aggregation prediction tool, which determined that the sulfation state of the GAG, the solute molarity and the protein/GAG molar ratio are determinants of the effect of the GAG on amyloid formation. They found a correlation between the sulfation degree and accelerated amyloid formation, with no effect caused by the molecular weight of the GAG. At salt concentrations identical to those in the extracellular space, GAGs weakly affected aggregation. The protein/GAG molar ratio showed a bell-shaped dependency, as a low or a high molar ratio (as found at the basement membrane and cell surface) exerted a small effect. Therefore, GAGs may not directly convert and/or aggregate PrP or other amyloidogenic proteins in the cellular context (Figure 1). However, these polysaccharides are able to interact with not only well-folded proteins but also misfolded proteins, oligomers and fibrils, and these interactions may be important for the establishment of related diseases (Figure 1). The protective effects of exogenous GAGs may arise from their competition for binding with endogenous PGs that may facilitate protein aggregation (Figure 2B). Exogenous GAGs may

also compete with other cofactors (Figure 2C); for example, Hep might block the interaction of PrP with membrane lipids, thereby decreasing its amyloid toxicity [111]. The increase in protein stability observed for the LMWHep-PrP interaction [68] may also explain the protective effect of this molecule (Figure 2D). Evaluating whether interaction with PrP^{Sc} stabilizes the scrapie isoform remains necessary.

HEP/HS BINDING SITES

GAGs are negatively charged molecules due to the presence of sulfate and carboxylic groups. For this reason, its interactions with proteins are primarily ionic, with basic amino groups. This interaction, although appearing random, shows specificity for certain sequences. Cardin and Weintraub in 1989 [119] were the first to demonstrate the presence of certain patterns in Hep-binding domains in four investigated proteins. They characterized two consensus sequences, XBBXBX and XBBBXXBX, where B is a basic residue and X is any amino acid. Sobel et al., [120] proposed a third consensus sequence, XBBBXXBBBXXBBX. These motifs are not always found in characterized Hep-binding regions; therefore, a distinct spatial pattern might also be important in determining the ability of a protein to interact with Hep [121, 122, 123]. Torrent et al., when analyzing the protein structures bound to Hep in the Protein Data Bank, recently reported a novel motif referred to as a cation-polar-cation (CPC) clip-like structure, which contained two cationic residues and a polar residue displaying conserved distances between the α carbons and the side chains [123].

Lysine and arginine are basic amino acids that show the highest affinity for Hep, although the affinity of arginine is 2.5-fold higher than that of lysine [124]. Alternatively, histidine residues are important for the interaction of Hep with some proteins, which was strongly dependent on pH and cation binding [125, 126, 127].

Prion proteins contain a conserved region in their N-terminal domain termed an octapeptide region with the consensus sequence PHGGGWGQ [26]. This region binds to Cu(II), and repetitions of this region are associated with prion diseases [128, 129]. Although this region does not contain a Hep consensus sequence, some studies have indicated this motif as the Hep-binding region of prion proteins. This interaction may be mediated by divalent ions in histidine side chains [21, 130, 131]. Synthetic peptides comprising amino acids 53-93 interact with HS/Hep, as measured by biosensor analyses [65] and NMR [131]. Experiments using peptides cannot always be extrapolated to the entire protein. Using the full-length protein in fast kinetic experiments, we found that the number of binding sites in PrP^{C} for LMWHep was two at pH 5.5 and one at pH 7.4 [67]. Using a PrP mutant in which amino acids 51-90 were deleted, we observed no interaction at pH 7.4 but did find an interaction at pH 5.5 at a stoichiometry of 1:1 (PrPΔ51-90:LMWHep), suggesting that the octarepeat is the binding site for LMWHep at pH 7.4 and that a second region is responsible for LMWHep binding at pH 5.5 [67]. The interaction with the octarepeat at the N-terminus was shown to be important for the LMWHep-mediated modulation of PrP conversion and fibrillization induced by infected seeds [68].

In addition to the octapeptide region, Warner et al., [65] identified two additional synthetic peptides comprising amino acids 23-52 and 110-128 that independently interact with Hep/HS. The first region contains a Cardin-Weintraub sequence with four basic residues,

KKRPK. Pan et al., [66], using the synthetic peptide 23-35, identified this region as the only site that was able to interact with GAGs. Subsequently, they found that deleting the first 12 amino acids in the prion protein was sufficient to abolish its interaction with GAGs. Another Cardin-Weintraub sequence is present in the N-terminus of PrP comprising amino acids 112-121, which are included in the third sequence identified by Warner et al., [65]. The 185-208 amino acid fragment of the prion protein was also able to interact with Hep/HS, and this interaction led to amyloid aggregate formation [104, 105].

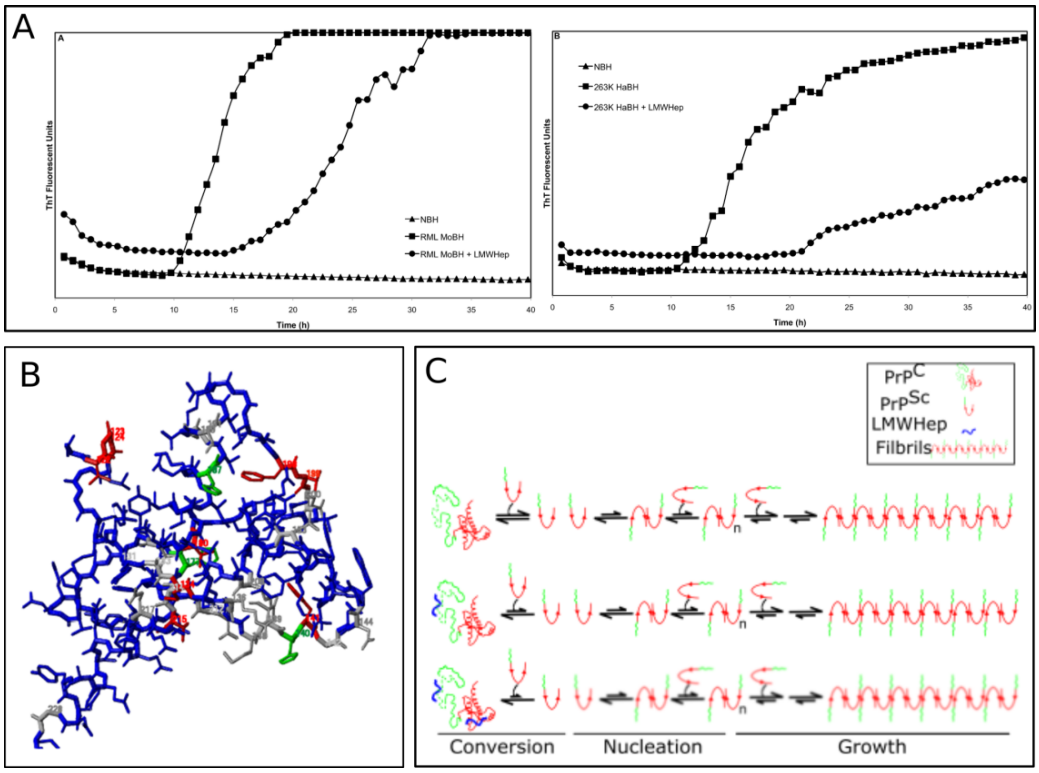

Figure 3. LMWHep was shown to protect PrP from aggregation induced PrP^{Sc} seeds from mouse- and hamster-infected brain homogenates in real-time quaking-induced conversion (RT-QuIC) assays. Modified from [68] (A). LMWHep was shown to bind PrP C-terminal near histidine 187 in the tertiary structure of PrP, between helix 2 (H2) and helix 3 (H3) at acid pH. Modified from [67] (B). The binding of LMWHep to PrP at acidic pH, likely at the octarepeat and H2H3 regions, impaired PrP conversion and fibrillization. Modified from [68] (C).

The conformational properties of PrP are modulated by pH [132, 133]. The presence of a binding site for LMWHep only at acidic pH [67] might be justified by changes causing increased exposure of the N-terminus and/or motifs that are typically hidden from the solvent. Another possible reason for this pH dependence is the protonation state of histidine residues. Histidine is the only amino acid with a side chain that can function as either an acid or a base at physiological pH; thus, these side chains may be responsible for this interaction. The pK(a) of His155 and His187 is approximately 5.5 [133, 134]. We showed that PrP treatment with DEPC (known to modify histidine side chains) blocks its interaction with LMWHep at pH 5.5 [67]. In NMR experiments, we also observed chemical shift differences from the PrP C-terminus at pH 5.5, which occurred near histidine 187 in the tertiary structure of PrP,

suggesting a second binding site near helix 2 (H2) and helix 3 (H3) at this pH (Figure 3B). The binding of LMWHep to PrP at acidic pH, likely at the octarepeat and H2H3 regions, impaired PrP conversion and fibrillization [68].

GAGS AS THERAPEUTIC DRUGS FOR MANY AMYLOID DISEASES

A number of therapeutic strategies have been used to identify effective treatments against amyloidosis. In this setting, the therapeutic agent should be effective and eliminate protein accumulation, targeted to the sites affected within a specific time window, and tolerated by the body. Because the aggregates accumulate and the resulting damage occurs prior to the clinical signs of the disease, developing an effective therapy is a major challenge. Compounds that affect the amyloid nucleation and growth steps are termed true inhibitors [135]. Preventing the formation of oligomeric species is a very interesting effect because several studies have demonstrated the toxic effect of soluble species, pre-fibrillar dimers and trimers, or other oligomers produced during the nucleation step of amyloid fiber formation [136]. Affecting the kinetics of these steps might be important for halting the progression of these diseases.

Sulfated sugars, which were originally used as antivirals, were the first drugs used against TSEs that were demonstrated to prolong the incubation period of the disease and to prevent symptoms when prophylactically administered in mice [137, 138, 139, 140]. As noted above, these molecules show protective effects *in vitro* and *in vivo* [63, 106, 107, 108, 109, 110, 111, 112, 113]. PPS has been the main glycan used as treatment for prion diseases in clinical trials. iPPS treatment of patients with vCJD showed no definite clinical benefit in a 39-year-old female [141], but was suggested to prolong survival of a 22-year-old male [142]. 11 Japanese patients receiving long-term iPPS including familial CJD, iatrogenic CJD and sporadic CJD also showed no clinical improvements and extended survival [143]. The explanation for the longer survival observed in different patients [142, 143, 144, 145] still has to be uncovered. A case report of sporadic CJD suggested that prolonged survival might be associated with a decrease on the level of protease-resistant PrP [145]. However, brain examination of vCJD treated patients showed no neuropathological changes [146].

In the setting of neurodegenerative diseases, although intraventricular infusion was shown to be safe, complication from the surgical procedure may arise [144]. Hence, the therapeutic agent must be able to cross the blood-brain barrier (BBB); therefore the size and charge of the drug are issues. Many sulfated polysaccharides cannot cross the BBB [147]. For this reason, LMW drugs have been developed to overcome this challenge [148]. LMWHep was shown to cross the BBB, inhibit Aβ precursor protein secretion [149] and play a protective role against AD and AA in animal models [150, 151]. LMWHep limited SAA [151], Aβ [151] and PrP [68] fibril formation *in vitro*. We showed that LMWHep acts as a true inhibitor to modulate PrP fibrillization kinetics and thermodynamics; these results are very interesting regarding disease progression. As PGs act as receptors in many of these diseases, the putative inhibitory activity of these exogenous polysaccharides displays therapeutic potential.

Hep has been used for a century, primarily as an anticoagulant drug. Hep is obtained from animal tissues, and LMWHep is essentially prepared *via* the controlled depolymerization of unfractionated Hep. After the Hep crisis in 2008, due to its contamination with an oversulfated CS that exerted severe side effects, new approaches for obtaining synthetic Hep became urgent [152]. Recently, a chemoenzymatic process was successfully developed to synthesize Hep using *Escherichia coli* [153]; this method represents a good strategy for the near future.

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