

● PERSPECTIVE

Enzymatic remodeling of heparan sulfate: a therapeutic strategy for systemic and localized amyloidoses?

In 1854, Rudolf Virchow introduced the term “amyloid” to indicate white waxy deposits that stained positive for iodine and that were found in many organs of patients with chronic inflammatory diseases. He observed that these deposits stained pale blue after treatment with iodine and became dark blue or black after subsequent addition of sulfuric acid, in a similar manner to that of cellulose or carbohydrate. He concluded that these deposits were carbohydrate in nature and termed the substances “amyloid,” a name that was derived from the Latin *amy-lum* and the Greek *amylon*, meaning starch (Virchow, 1854). However, in 1859, Friedrich and Kekule used protein-staining dyes and showed that these deposits were in fact protein in nature (Friedrich and Kekule, 1859). The term “amyloidosis” today refers to diseases in which amyloidogenic proteins deposit as insoluble fibrils in many tissues and organs, consistent with the conclusion of Friedrich and Kekule. These protein fibrils are characterized by having a β -structure, resistance to proteolysis, and specific binding to histochemical dyes such as Congo red and thioflavin that are used to identify amyloid deposits in tissues. Thus far, many *in vivo* observations revealed that amyloid deposits contain, besides protein fibrils, carbohydrate and other protein components derived from intra- and extracellular spaces. These non-amyloid components may be involved in the pathogenesis and pathology of amyloidosis, such as amyloid formation and amyloid-induced tissue damage. In the 1980s, Snow and Kisilevsky found that glycosaminoglycans (GAGs) were associated with tissue amyloid deposits (Snow and Kisilevsky, 1985). They identified the GAG as heparan sulfate (HS), which is a component of heparan sulfate proteoglycan (HSPG) and a member of the GAG family. HS is now known to be associated with different types of amyloid in systemic and localized amyloidoses. From this perspective, we will discuss here the possible roles of HS and its highly sulfated domains in the pathogenesis and pathology of amyloidosis.

HS is a sulfated polysaccharide comprising repeating disaccharide units of glucosamine (GlcN) and glucuronic acid or iduronic acid (IdoA). Physiologically, HS is present in a proteoglycan form, in which one or more HS chains are covalently bound to the core protein; this HS form is ubiquitous at the cell surface and in the extracellular matrix. HS structure varies in length and modification. The HS backbone typically contains 25–250 disaccharide units. After stepwise additions and substitutions on the core protein, HS chains can be modified *via* certain consecutive steps, such as N-deacetylation and N-sulfation of GlcN residues, and 2-O-sulfation and 6-O-sulfation at various disaccharide positions. These modifications are quite strictly regulated. The structural diversity resulting from these modifications confers a superior selectivity for HS-protein interactions. One of these modifications, 6-O-sulfation is particularly important for interaction between HS and certain proteins such as bone morphogenetic protein, and growth factors including fibroblast growth factor, glial cell line-derived neurotrophic factor, and vascular endothelial growth factor (VEGF).

With regard to amyloidosis, we found that lipoprotein lipase (LPL), which acts as a bridging molecule between lipoproteins and HSPG, bound to amyloid β (A β) that is the amyloidogenic peptide in Alzheimer’s disease (AD), and promoted A β cellular uptake in a GAG-dependent manner in mouse primary astrocytes (Nishitsuji et al., 2011). We also showed that this enhancement of cellular uptake of A β depended on the positions of sulfation modifications within GAGs of HS and chondroitin sulfate. A β that had been internalized *via* bridging with GAGs by LPL underwent degradation through a lysosomal pathway (Nishitsuji et al., 2011). Thus, GAGs and their sulfate moieties at the cell-surface regulate the cellular uptake of LPL-A β complex, which could contribute to pathogenesis of AD *via* regulating extracellular level of A β . We also recently reported that apolipoprotein A-I (apoA-I) fibrils interacted with heparin (Kuwabara et al., 2015). ApoA-I is a major component of high-density lipoproteins, and specific mutations in apoA-I cause systemic amyloidosis, *i.e.*, ApoA-I amyloidosis. ApoA-I

fibrils showed cytotoxicity against Chinese hamster ovary (CHO) cells, but not against the HS deficient variant cells. HS and heparin, which is a structural analogue of highly sulfated HS, competitively inhibited interaction of apoA-I fibrils with CHO cells and subsequent cytotoxicity, whereas hyaluronic acid, which is the only non-sulfated member of the GAG family, did not. As CHO cells do not express heparin, exogenously added HS is sufficient for competing the cell-surface HS with cellular interaction of apoA-I fibrils. Furthermore, treating cells with sodium chlorate that inhibits sulfation of HS abolished the cellular interaction and cytotoxicity of apoA-I fibrils. Our results strongly suggest that sulfate moieties of HS are critical for cellular interaction and cytotoxicity of apoA-I fibrils (Kuwabara et al., 2015) (Figure 1). These results emphasize the importance of HS modifications, especially sulfation, for selective and specific interaction of HS and its interacting proteins, such as amyloid fibrils.

Certain structural domains within HS are highly sulfated. These domains consist of clusters of a trisulfated disaccharide, [-IdoA(2-OSO₃)-GlcNSO₃(6-OSO₃)-], and often play an essential role in interactions between HS and its protein ligands including growth factors and cytokines. To distinguish these domains from other HS domains, phage display antibodies that recognize specific HS domains were established. One such antibody, RB4CD12, recognized HS domains containing two or more consecutive trisulfated disaccharides (Dennissen et al., 2002). We previously showed that RB4CD12 epitopes were present in the basement membrane of brain vessels. The function of the RB4CD12 epitopes in the brain vessel basement membrane remains to be elucidated, but these epitopes may act as a storage site for growth factors, especially VEGF. We also reported another important finding: RB4CD12 epitopes accumulated in senile plaques or amyloid plaques in brains of patients with AD and in brains of transgenic AD mouse models (Hosono-Fukao et al., 2012). This leads to an idea that an abundance of VEGF in amyloid plaques in AD brains could induce depletion of VEGF, which is detrimental to neurons. These lines of evidences strongly support the notion that GAGs and their sulfate moieties play a role in pathogenesis and pathology of amyloidosis beyond a role as mere co-accumulating molecules. Possible roles of RB4CD12 epitopes include the following: (1) enhance amyloid formation by A β , (2) promote interaction between A β amyloid and cells and tissues, and (3) stimulate or inhibit signaling pathways of growth factors. In fact, GAGs and their sulfate moieties were reportedly critical for enhancing A β fibril formation (Castillo et al., 1999). Although cell-surface HSPG reportedly mediated cell A β internalization and cytotoxicity (Sandwall et al., 2010), we still do not know how RB4CD12 epitopes affect accumulation and/or cellular interaction of A β and A β fibrils in the brain. The possibility that RB4CD12 epitopes may be deposited with other amyloid proteins in various amyloidoses is also of interest.

HSulf-1 and HSulf-2, which are human extracellular endosulfatases, removes 6-O-sulfates from highly sulfated domains of HS and heparin, and thereby diminishes the reactivity of these subdomains to RB4CD12. We previously reported that HSulfs degraded RB4CD12 epitopes in an enzyme-linked immunosorbent assay and in cultured cells (Hossain et al., 2010). *Via* regulation of highly sulfated domains in HS, these HSulfs affect various signaling pathways including those of growth factors. A knockout study suggested that activity of Sulfs contributed to the generation of tissue- and organ-specific sulfation patterns of HS including that in the brain (Nagamine et al., 2012). RB4CD12 epitopes that were previously shown to accumulate in cerebral amyloid plaques of AD model mice were degradable by HSulfs (Hosono-Fukao et al., 2012). These results suggest that HSulfs may play a role in the pathology of AD by regulating extracellular levels of highly sulfated domains of HS. As noted above, HSulfs may have three distinct roles, *via* such regulation of highly sulfated domains of HS, in the pathogenesis or pathology of AD or other amyloidoses. As an interesting finding, a knockout study also showed that Sulf was involved in expression of specific sulfation patterns in the kidney, which is a major site of amyloid deposition in different systemic amyloidoses (Nagamine et al., 2012).

To elucidate the role of highly sulfated domains of HS, we used CHO cells stably expressing HSulf-1 or HSulf-2 (CHO-HSulf-1 or CHO-HSulf-2 cells). The RB4CD12 epitopes were expressed in CHO cells, and their cell-surface level was reduced by stably expressing HSulfs to 25–50% of those in mock-transfected cells (Hossain et al., 2010). HSulfs enzymatically remodeled cell-surface HS in a cell-based assay system. By using these cells, we found that cellular interaction of apoA-I fibrils

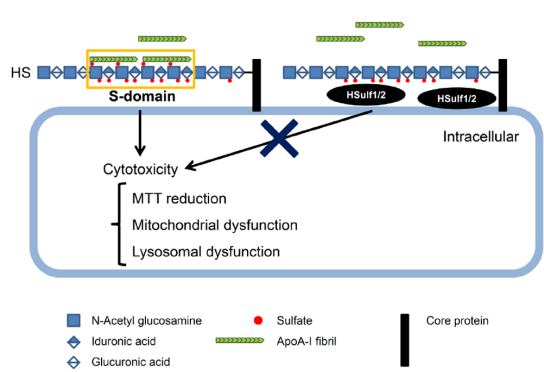


Figure 1 Possible roles of highly sulfated domains of heparan sulfate (HS) in amyloidoses.

Highly sulfated domains of cell-surface heparan sulfate mediate cellular interaction and cytotoxicity of apolipoprotein A-I (apoA-I) fibrils. In the presence of highly sulfated domains of HS, apoA-I fibrils accumulated on the cell surface and caused mitochondrial and lysosomal dysfunction. When highly sulfated domains of HS were degraded by HSulf-1 or HSulf-2, such cellular dysfunction was ameliorated (see Kuwabara et al., 2015). MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

depended, at least partly, on highly sulfated domains of cell-surface HS (Kuwabara et al., 2015). Furthermore, cytotoxicity of apoA-I fibrils was significantly attenuated in CHO-HSulf-1 and CHO-HSulf-2 cells. These results suggest that highly sulfated domains of HS at least partly mediate cellular interaction and cytotoxicity of apoA-I fibrils. These lines of evidence suggest that sulfate moieties and highly sulfated domains of HS are involved in biological and/or pathological actions of various amyloids. Additional studies to confirm the role of highly sulfated domains of HS are needed. As suggested by others, sulfated GAGs such as HS promoted aggregation or fibril formation of non-pathogenic form of different amyloidogenic proteins including A β , transthyretin, muscle acylphosphatase and so on, by acting as a scaffold for fibril formation (Castillo et al., 1999; Motamedi-Shad et al., 2009). Elucidating the effect of highly sulfated domains of HS, *i.e.*, the RB4CD12 epitopes, on aggregation or fibril formation of amyloidogenic proteins is a future challenge (Figure 2).

As described above, HSulf-1 and HSulf-2 degraded RB4CD12 epitopes that had accumulated in amyloid plaques of AD model mice (Hosono-Fukao et al., 2012). HSulf-1 and HSulf-2 also interfered with the cellular interaction and cytotoxicity of apoA-I fibrils (Kuwabara et al., 2015). An *ex vivo* study showed that amyloid plaques were retained after degradation of RB4CD12 epitopes (Hosono-Fukao et al., 2012), so whether these “post-treated” amyloid plaques are unstable or vulnerable to proteolytic degradation and/or microglial clearance requires clarification. These results suggest a novel concept that enzymatic remodeling of HS may be a therapeutic strategy for systemic and localized amyloidoses. We previously showed that sulfate moieties of HS affected cytotoxicity and degradation of amyloid or amyloidogenic proteins by regulating their cellular interactions. For better understanding the role of the highly sulfated domains of HS, further studies to elucidate whether these highly sulfated domains could be superior scaffolds for various amyloidogenic proteins and actually co-accumulating as a non-amyloid component of tissue/organ amyloid deposits in various amyloidoses besides AD. Additional studies to elucidate the roles of highly sulfated domains of HS in amyloid formation, interaction of amyloid with cells in various tissues and organs, and subsequent cytotoxicity or degradation are ongoing.

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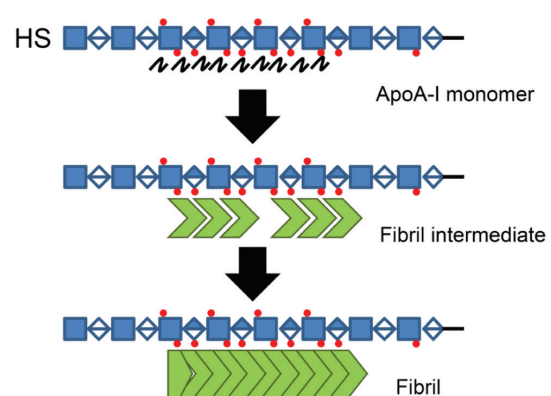


Figure 2 A possible mechanism by which highly sulfated domains promote amyloid fibril formation.

Interaction between monomers or oligomers of an amyloidogenic protein and sulfate moieties of cell-surface heparan sulfate (HS) increases local concentration and/or changes conformation and/or orientation of the amyloidogenic protein, which are favorable for amyloid fibril formation. In this model, highly sulfated domains act as a scaffold for aggregation.

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References

- Castillo GM, Lukito W, Wight TN, Snow AD (1999) The sulfate moieties of glycosaminoglycans are critical for the enhancement of β -amyloid protein fibril formation. *J Neurochem* 72:1681-1687.
- Dennissen MA, Jenniskens GJ, Pieffers M, Versteeg EM, Petitou M, Veerkamp JH, van Kuppevelt TH (2002) Large, tissue-regulated domain diversity of heparan sulfates demonstrated by phage display antibodies. *J Biol Chem* 277:10982-10986.
- Friedrich N, Kekule A (1859) Zur amyloidfrage. *Virchows Arch Pathol Anat Physiol* 16:50-65.
- Hosono-Fukao T, Ohtake-Niimi S, Hoshino H, Britschgi M, Akatsu H, Hossain MM, Nishitsuji K, van Kuppevelt TH, Kimata K, Michikawa M, Wyss-Coray T, Uchimura K (2012) Heparan sulfate subdomains that are degraded by Sulf accumulate in cerebral amyloid β plaques of Alzheimer's disease: evidence from mouse models and patients. *Am J Pathol* 180:2056-2067.
- Hossain MM, Hosono-Fukao T, Tang R, Sugaya N, van Kuppevelt TH, Jenniskens GJ, Kimata K, Rosen SD, Uchimura K (2010) Direct detection of HSulf-1 and HSulf-2 activities on extracellular heparan sulfate and their inhibition by PI-88. *Glycobiology* 20:175-186.
- Kuwabara K, Nishitsuji K, Uchimura K, Hung SC, Mizuguchi M, Nakajima H, Mikawa S, Kobayashi N, Saito H, Sakashita N (2015) Cellular interaction and cytotoxicity of the Iowa mutation of apolipoprotein A-I (apoA-I-Iowa) amyloid mediated by sulfate moieties of heparan sulfate. *J Biol Chem* 290:24210-24221.
- Motamedi-Shad N, Monsellier E, Chiti F (2009) Amyloid formation by the model protein muscle acylphosphatase is accelerated by heparin and heparan sulphate through a scaffolding-based mechanism. *J Biochem* 146:805-814.
- Nagamine S, Tamba M, Ishimine H, Araki K, Shiomi K, Okada T, Ohto T, Kunita S, Takahashi S, Wismans RG, van Kuppevelt TH, Masu M, Keino-Masu K (2012) Organ-specific sulfation patterns of heparan sulfate generated by extracellular sulfatases Sulf1 and Sulf2 in mice. *J Biol Chem* 287:9579-9590.
- Nishitsuji K, Hosono T, Uchimura K, Michikawa M (2011) Lipoprotein lipase is a novel amyloid β (A β)-binding protein that promotes glycosaminoglycan-dependent cellular uptake of A β in astrocytes. *J Biol Chem* 286:6393-6401.
- Sandwall E, O'Callaghan P, Zhang X, Lindahl U, Lannfelt L, Li JP (2010) Heparan sulfate mediates amyloid-beta internalization and cytotoxicity. *Glycobiology* 20:533-541.
- Snow AD, Kisilevsky R (1985) Temporal relationship between glycosaminoglycan accumulation and amyloid deposition during experimental amyloidosis. A histochemical study. *Lab Invest* 53:37-44.
- Virchow R (1854) Zur Cellulose-frage. *Virchows Arch Pathol Anat Physiol* 6:416-426.