Aβ(1–40) Prevents Heparanase-catalyzed Degradation of Heparan Sulfate Glycosaminoglycans and Proteoglycans in Vitro

A ROLE FOR HEPARAN SULFATE PROTEOGLYCAN TURNOVER IN ALZHEIMER’S DISEASE*

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Alzheimer’s disease is characterized by senile plaques composed of polymeric fibrils of beta amyloid (Aβ), a 39–42-amino acid peptide formed after proteolytic processing of the amyloid precursor protein (βAPP). Heparan sulfate proteoglycans have been shown to colocalize with Aβ in Alzheimer’s disease brain, and experimental evidence indicates that the interactions between the proteoglycan and the peptide are important for the promotion, deposition, and/or persistence of the senile plaques. Studies in rat brain indicated that both the core protein and the heparan sulfate glycosaminoglycan chains are required for amyloid fiber formation and deposition in vitro (Snow, A. D., Sekiguchi, R., Nochlin, D., Fraser, P., Kimata, K., Mizutani, A., Arai, M., Schreier, W. A., and Morgan, D. G. (1994) Neuron 12, 219–234), suggesting that one mechanism to prevent the formation of Aβ-heparan sulfate proteoglycan complexes that lead to deposition of amyloid would be to degrade the proteoglycan. Normally, heparan sulfate proteoglycans are internalized and degraded to short glycosaminoglycans by intracellular heparanases. These reactions occur in the endosomal-lysosomal pathway, which is the same intracellular location where βAPP is processed to Aβ. Using partially purified heparanase activities from Chinese hamster ovary cells we examined whether Aβ(1–40) affects the catabolism of Chinese hamster ovary heparan sulfate glycosaminoglycans and proteoglycans in vitro. Aβ(1–40) binds to both the long heparan sulfate glycosaminoglycans attached to core proteins and the short, heparanase-derived chains in a concentration-dependent and pH-dependent manner. When Aβ(1–40) is added to heparanase assays, it prevents the partially purified activities from releasing heparan sulfate chains from core proteins and degrading them to short glycosaminoglycans; however, a large molar excess of the peptide to heparan sulfate is required to see the effect. Our results suggest that normally the levels of Aβ in the endosomal pathway are not sufficient to interfere with heparanase activity in vivo. However, once the level of Aβ-peptides are elevated, as they are in Alzheimer’s disease, they could interact with heparan sulfate proteoglycans and prevent their catabolism. This could promote the formation and deposition of amyloid, since the binding of Aβ to the proteoglycan species will predominate.

Histochemical and immunocytochemical studies have shown that heparan sulfate (HS) proteoglycans (HSPGs) and glycosaminoglycans colocalize with β-amyloid protein (Aβ) in the senile plaques characteristic of Alzheimer’s disease (1). Although the precise role of the proteoglycans in the process of amyloidosis is not known, experimental evidence suggests that they may promote amyloid formation, deposition, and/or persistence (1) by binding to Aβ (2–5). HSPGs are anionic molecules consisting of one or more HS glycosaminoglycan chains covalently attached to a core protein (6). Aβ binds to HSPGs with high affinity, and interestingly, it interacts with both the core protein and the glycosaminoglycan chains (3, 4). Experiments by Snow et al. (1) suggest that both interactions are necessary for the formation of fibrillar Aβ-amyloid, since coinfusion of Aβ(1–40) and HSPGs into rat brain produced deposits, whereas coinfusion of Aβ(1–40) and HS glycosaminoglycans did not. This is somewhat surprising, since in vitro, HS chains alone can cause both Aβ(1–28) and Aβ(1–40) to aggregate (3), and heparin converts Aβ(11–28) into macrofibrils (2). It may be that in vivo, stabilization of the aggregates and decreased amyloid susceptibility to proteolysis require Aβ to interact with both the core protein and HS chains.

Production of Aβ from the amyloid precursor protein is thought to occur primarily in the endosomal-lysosomal pathway (7–9). HSPGs are initially degraded to short glycosaminoglycan chains in the same intracellular location (10–12), so it is possible that this is where the Aβ-HSPG interaction necessary for plaque formation occurs. In endosomes, HS glycosaminoglycans are released from core proteins and cleaved to short oligosaccharides by enzymes called heparanases (10–13). Catabolism of HSPGs by heparanases may be important for preventing the formation of Aβ-proteoglycan complexes that result in the production of amyloid deposits. After heparanases act on the proteoglycan, the glycosaminoglycan and core protein binding sites will have been physically separated. Aβ may still be able to bind to the core protein, but the short HS glycosaminoglycans remaining on the protein may not be sufficient to stabilize the interactions necessary for fiber formation and deposition.

Recent studies show Aβ peptide is protected from proteases by virtue of its association with HS glycosaminoglycans (14). It may be that a reciprocal arrangement exists, where the association of Aβ with the polysaccharide prevents heparanases from degrading the HS chain. Inhibiting heparanase action may promote the formation of amyloid plaques since the bind-

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The abbreviations used are: HS, heparan sulfate; HSPG, heparan sulfate proteoglycan; Aβ, β-amyloid; CHO, Chinese hamster ovary; CHAPS, 3-[3-Cholamidopropyl]dimethylammonio]-1-propanesulfonic acid; βAPP, β-amyloid precursor protein; CPC, cetylpyridinium chloride.
ing of Aβ to intact proteoglycans will predominate. Using partially purified heparanase activities from Chinese hamster ovary (CHO) cells, we have examined whether Aβ(1–40) peptide affects the catabolism of HS glycosaminoglycans and proteoglycans in vitro. Our results suggest that the association of Aβ with HS protects the glycosaminoglycan from degradation by heparanases.

EXPERIMENTAL PROCEDURES

Cell Culture—CHO-K1 cells were obtained from the American Type Culture Collection (CCL-61; Rockville, MD). Cells were maintained in Ham's F-12 medium (Life Technologies, Inc.) supplemented with 5.0% (v/v) bovine serum (Life Technologies, Inc.), 100 µg/ml streptomycin sulfate and 100 units/ml penicillin G (Sigma). Sulfate-free medium (defined F-12 medium) was used for all radioactive labeling experiments and was prepared as described previously (15). Cells were grown at 37 °C ± 0.2 °C in an atmosphere of 5% CO₂ in air and 100% relative humidity. They were subcultured every 3–4 days with 0.125% trypsin, and after 15–20 passages, fresh cells were revived from frozen stocks stored in liquid nitrogen.

Aβ Peptide—Aβ(1–40) (Bachem California, Torrance) was used in all studies. The peptide was resuspended in water to a concentration of approximately 6 mg/ml, aliquoted, frozen, and stored at −20 °C. For each experiment, an aliquot of Aβ was thawed, diluted with buffer and stored on ice. In most cases, the peptide was thawed only once; in some instances the peptide was frozen and thawed one additional time.

HS Glycosaminoglycan and Proteoglycan Isolation—[35S]HS glycosaminoglycans were isolated from confluent cell monolayers that had been incubated for 8–24 h in defined F-12 medium containing 50 µCi/ml [35S]H₂SO₄ and purified by anion exchange and gel filtration chromatography as described previously (11). Briefly, proteoglycans in the cell extract were isolated by DEAE-Sephacel chromatography and applied to a DEAE-Sephacel column (Pharmacia Biotech Inc.), equilibrated in 0.01 M HEPES (pH 7.6), 0.1 M NaCl, 1 mM glutathione (Buffer A). After the column was washed with Buffer A, heparanase was eluted by a gradient of NaCl (Buffer B). The peptide was resuspended in water to a concentration of 50–100 µl of the citrate phosphate buffer used to make up the reaction mixture. Aβ concentrations varied from 0.01 to 0.50 µM, and the pH values tested ranged from pH 4.0 to 7.0. After 30 min, vacuum was applied to the apparatus to pull any remaining solution through the nitrocellulose membrane. Each assay “dot” was washed three times with 50–100 µl of the citrate phosphate buffer to ensure that all of the unbound [35S]HS was removed. The membrane was removed from the apparatus and the assay dots cut out and placed in scintillation vials. Ultima Gold XR (Packard) was added to the vial, and the samples were assayed for radioactivity by scintillation counting. Heparin glycosaminoglycans, used to examine the specificity of the Aβ-CHO HS interaction, were purchased from Sigma or Seikagaku America, Inc. (Ijamsville, MD). The carboxyl-reduced heparin was a gift from Dr. H. E. Conrad (University of Illinois).

Affinity Coelectrophoresis—The affinity of the interaction between Aβ and CHO HS chains was determined by affinity coelectrophoresis (18). Briefly, [35S]HS samples were applied into a horizontal slot in a 1% agarose gel (FMC BioProducts) and electrophoresed through precast 42-mm lanes containing different concentrations of Aβ. The gels were prepared using 24 ml of 1× Tris-citrate buffer, 0.5% CHAPS and run in the same buffer without the detergent. Electrophoresis was performed for 110–130 min at 70 V (constant voltage). The gels were dried under vacuum and subject to autoradiography to visualize the migration of the [35S]HS. Radioactive bands were analyzed by a laser densitometer (ipi, Inc.; Huntington Station, NY), and the apparent dissociation constants were determined from relative retardation of HS by Aβ (18).

Partial Purification of Heparanase Activity from CHO Cells—The heparanase activity used in these experiments was partially purified from CHO cells. Cells were grown to confluence in 150-mm-diameter plates and detached with 0.125% trypsin at 37 °C. Trypsinization was stopped with soybean trypsin inhibitor, and the cells were released from the plate with a pipette. Cells were sedimented in a clinical centrifuge, resuspended in 5 mM HEPES (pH 7.6), containing 1 µl/ml leupeptin, 0.5 µg/ml pepstatin, 10 mM N-ethylmaleimide, and 0.2 mM phenylmethyl-sulfonl fluoride (100 µl/plate of cells), and stored at −20 °C until enough cell protein had been collected to begin the purification (around 240 150-mm dishes). Frozen cells were thawed, fresh protease inhibitors and glutathione (1 mM final) were added to the suspension, then the cells were broken with a Teflon homogenizer. The cell homogenate was centrifuged for 10 min at 15,000 × g to pellet nuclei and large organelles. Detergent and salt were added to the 15,000 × g supernatant to a final concentration of 0.1% Triton X-100 and 0.5 mM NaCl, then the mixture was centrifuged at 100,000 × g for 60 min to release heparanase activity from microsomal membranes. The 100,000 × g supernatant was diluted to bring the salt concentration below 0.1 M and applied to a DAE-Sephacel column (Pharmacia Biotech Inc.), equilibrated in 0.01 M HEPES (pH 7.6), 0.1 mM NaCl, 1 mM glutathione (Buffer B). After the column was washed with Buffer A, heparanase activity was eluted from the resin by increasing the NaCl concentration. Fractions containing heparanase activity (determined by the glycosaminoglycan degradation assay) were pooled, dialyzed against Buffer A, and applied to a heparin-FF-Gel column (Bio-Rad) equilibrated in 0.01 M sodium acetate (pH 5.5), 0.1 mM NaCl, 1 mM glutathione (Buffer B). The column was washed with Buffer B, then heparanase was eluted by a NaCl gradient buffer. The fractions containing heparanase activity were pooled, dialyzed against Buffer A, and stored at −20 °C until used in experiments.

Heparanase activity was purified over 100-fold with these steps; however, there were still at least 20 silver-stained bands on an SDS-polyacrylamide gel (data not shown). Characterization of the partially purified activity suggest the Km of the enzyme(s) for the long, 81-kDa CHO heparan sulfate substrate is 10–20 nM. The activity has been shown to be endogluconidase (13), and there is no exoglycosidase activity associated with the partially purified heparanase.

Nitrocellulose Retention Assay—Purified nascent or short [35S]HS glycosaminoglycans (5,000 cpm, 2 ng/µl) were mixed with varying concentrations of Aβ(1–40) peptide in 50 mM citrate, 100 mM sodium phosphate (15-µl total volume) and applied to nitrocellulose membranes in a Bio-Dot apparatus. The nitrocellulose membranes had been saturated with the same citrate phosphate buffer to make up the reaction mixture. Aβ concentrations varied from 0.01 to 0.50 µM, and the pH values tested ranged from pH 4.0 to 7.0. After 30 min, vacuum was applied to the apparatus to pull any remaining solution through the nitrocellulose membrane. Each assay “dot” was washed three times with 50–100 µl of the citrate phosphate buffer to ensure that all of the unbound [35S]HS was removed. The membrane was removed from the apparatus and the assay dots cut out and placed in scintillation vials. Ultima Gold XR (Packard) was added to the vial, and the samples were assayed for radioactivity by scintillation counting. Heparin glycosaminoglycans, used to examine the specificity of the Aβ-CHO HS interaction, were purchased from Sigma or Seikagaku America, Inc. (Ijamsville, MD). The carboxyl-reduced heparin was a gift from Dr. H. E. Conrad (University of Illinois).
acetic acid (pH 5.5). Under these conditions, uncleaved glycosaminoglycans precipitate, while cleaved chains remain in the supernatant (11). The 35S counts in the CPC supernatant were assayed by liquid scintillation counting and divided by the total number of 35S radioactivity added to the assay to determine the percentage of CPC-soluble [35S]HS.

To examine the size of the heparanase-cleaved glycosaminoglycans by gel filtration chromatography, the reaction was instead stopped with 75 μL of 0.1 M Tris (pH 8.0), 0.5 M NaCl, and the entire mixture was applied to a TSK 3000 gel filtration column (7.5 × 30 mm, TosohHaas) equilibrated in 0.1 M KH2PO4 (pH 7.0) 0.5 M NaCl, 0.2% Zwittergent 3–12 (11). [35S]HS chains were eluted from the column at a flow rate of 0.5 mL/min (11). The pH of the running buffer was pH 7.0 to dissociate 3–12 (11). [35S]HS chains were eluted from the column at a flow rate of 0.5 mL/min (11). The pH of the running buffer was pH 7.0 to dissociate

**RESULTS**

**CHO HS Binds Aβ(1–40)—** Before we could test whether Aβ(1–40) could prevent heparanases from degrading HS, we needed to determine whether the peptide would bind to the CHO cell glycosaminoglycans that we use for our heparanase studies. Long, nascent CHO [35S]HS chains were mixed with Aβ(1–40) and applied to nitrocellulose membranes. HS will not bind to nitrocellulose since both the membrane and the glycosaminoglycans are negatively charged. However, 35S radioactivity will be retained on the membrane if the glycosaminoglycan binds specifically to a protein that can bind nitrocellulose. With increasing concentrations of Aβ(1–40), the long [35S]HS chains are retained on the membrane (Fig. 1), indicating that the CHO glycosaminoglycan binds the peptide. Binding is specific for Aβ(1–40), since long [35S]HS chains are not retained on the membrane when mixed with either 2.5 mM or 5.0 mM bovine serum albumin (data not shown). The short [35S]HS glycosaminoglycans produced by heparanases are also retained on nitrocellulose by interacting with Aβ(1–40) (Fig. 1), and as with the long glycosaminoglycans, the binding of short HS chains is dependent on the peptide concentration. The shorter HS chains do not appear to bind to Aβ(1–40) as well as the longer glycosaminoglycans; however, this may be an artifact based on the size difference. Nascent CHO HS chains are approximately 14 times longer than the short, heparanase products (13, 19), thus the binding of one long HS molecule will retain more 35S radioactivity on the membrane than the binding of one short glycosaminoglycan chain.

As others observed for Aβ(1–28) (3), the interaction of Aβ(1–40) with the either long or short CHO HS glycosaminoglycans is pH-dependent, with most of the binding occurring at pH 6.0 or lower (Fig. 2). Long HS glycosaminoglycans interact with the peptide better than the short chains at the pH values found in endosomal compartments (pH 5.0–6.5, Ref. 20), which again may reflect that the size of the glycosaminoglycan influences the 35S counts retained on the membrane. Alternatively, because of their length, the nascent HS chains may be able to form more stable complexes with Aβ(1–40) at these pH values than the heparanase-degraded glycosaminoglycans. Although the long HS chains bind Aβ(1–40) well at pH 5.5, we chose to do the remaining binding and heparanase studies at pH 5.0 to maximize the binding of both long and short HS species to the Aβ(1–40) peptide. Since CHO heparanase activities have a broad pH optimum between pH 5.0 and 6.0 (data not shown), the results we obtain at pH 5.0 should be comparable to what would occur at the higher pH values.

When heparin is added to the incubation mixture, the unla- beled polysaccharide is able to compete with the labeled glycosaminoglycan for binding Aβ(1–40) (Fig. 3). This is not surprising, since heparin and HS have similar structures (21). Heparin chains that have been completely desulfated are no longer able to prevent the association of Aβ(1–40) with long [35S]HS chains, while carboxyl-reduced heparin is just as effective as the native glycosaminoglycan in inhibiting the retention of [35S]-chains on nitrocellulose (Fig. 3). These results indicate that sulfate groups, rather than carboxyl groups, are essential for the Aβ-heparan sulfate interaction, which is not unexpected since Fraser et al. (2) showed that sulfate ions alone can cause Aβ peptides to associate into macrofibers. Heparin chains that lack only N-sulfate groups (Fig. 3, No NS) compete more effectively for the peptide than heparin chains that only lack O-sulfate groups (Fig. 3, No OS), suggesting that the position of sulfate groups on the polysaccharide plays a role in the interaction of the glycosaminoglycan with Aβ(1–40). Short HS chains derived from heparanases are also able to compete with the long [35S]-glycosaminoglycans for binding to the peptide. When increasing concentrations of unlabeled, short CHO HS chains were included in the mixture, the amount of long [35S]HS chains retained on the nitrocellulose membrane decreased (Fig. 4), indicating that the shorter chains were dis-
The affinity of the long CHO HS glycosaminoglycans for the Aβ(1–40) polymerized in an agarose matrix was determined by affinity coelectrophoresis (18). Since basic fibroblast growth factor, which is known to bind specifically to HS glycosaminoglycans (19), would affect the catabolism of the polysaccharides, Heparanase activities, partially purified from CHO cells, degrade long 81-kDa HS chains to short 6-kDa glycosaminoglycans (19). The short glycosaminoglycan products can be separated from the substrate based on their solubility in the detergent CPC; long HS chains precipitate in the detergent, whereas the short heparanase-degraded glycosaminoglycans remain soluble. When long [35S]HS chains are incubated with partially purified CHO heparanase for 20 hours, over 80% of the substrate is converted to CPC-soluble [35S]-species (Fig. 6A). If increasing concentrations of Aβ(1–40) are added to the assay, the formation of the soluble [35S]HS chains is prevented (Fig. 6A). Inhibition is observed with as little as 25 μM Aβ(1–40), and the formation of short, CPC-soluble glycosaminoglycans is completely prevented by 150 μM Aβ(1–40) (Fig. 6A).

A large molar excess of Aβ(1–40) peptide is required to prevent catabolism of HS chains by heparanases in vitro. Using the CPC assay to measure inhibition, 50 μM Aβ(1–40) is necessary to inhibit the formation of soluble HS species by 50% (Fig. 6A), which corresponds to a ratio of 4,500 μM Aβ(1–40) to 1 mol of HS. This ratio is much greater than what we observed in similar studies with basic fibroblast growth factor, where only a 30–60 molar excess of the growth factor was required to completely inhibit heparanase activities from degrading CHO HS in vitro (19). Since basic fibroblast growth factor is known to bind specifically to HS glycosaminoglycans...
chains, the reaction mixture was applied to a TSK 3000 gel filtration column (Fig. 6B). The column was run in phosphate buffer at pH 7.0 to disrupt any pH-dependent interactions between Aβ(1–40) and the glycosaminoglycans (Fig. 2). In the absence of Aβ(1–40), partially purified CHO heparanase converts the [35S]HS substrate from 81 ± 5.4 kDa chains that elute at the void volume of the column to 7–10-kDa species that elute with K_v values greater than 0.5 (Fig. 6B). When Aβ(1–40) is included in the reaction mixture, the formation of the short [35S]HS species is prevented, and, as with the CPC assay, the inhibition of heparanase-catalyzed degradation is dependent on the concentration of Aβ(1–40) in the reaction mixture (Fig. 6B). These results confirm that the presence of Aβ(1–40) prevents heparanases from degrading the free HS glycosaminoglycans.

In addition to degrading long glycosaminoglycans to short products, heparanases are also responsible for cleaving the glycosaminoglycan chain from the core protein (11). To determine whether the presence of Aβ(1–40) peptide would affect the release of HS chains from proteoglycans, we repeated the heparanase assays, using [35S]HS glycosaminoglycans as the substrate and analyzed the reaction products on a TSK 4000 gel filtration column. The presence of Aβ in the reaction mixture also inhibited heparanase-catalyzed degradation of HS glycosaminoglycans substrates at a concentration range similar to that found for the free glycosaminoglycans (Fig. 6C). Thus, Aβ(1–40) interacts with HS chains covalently bound to core proteins as well as free glycosaminoglycans, and in both cases the interaction prevents heparanases from degrading the polysaccharide.

**DISCUSSION**

Alzheimer’s disease is characterized by extracellular amyloid deposits, or senile plaques, composed of polymeric fibrils of Aβ, a 39–42-amino acid peptide formed after proteolytic processing of the amyloid precursor protein (APP) (25). A number of other macromolecules colocalize with Aβ in the plaque, including HSPGs (1, 14). Over the years a body of experimental evidence has accumulated which indicates HSPGs play a role in the promotion, deposition, and persistence of the fibrillar form of Aβ present in plaques (1–3, 14). HS glycosaminoglycans induce Aβ aggregation (3), cause Aβ fibers to form bundles (2), and protect the fibrillar Aβ from proteases (14). Interestingly, although only the glycosaminoglycan is required for these interactions in vitro, both the HS chain and the core protein are required for amyloid fiber formation and deposition in vivo (1). Since Aβ has been shown to bind to both the glycosaminoglycan and core protein (4, 5), it may be that both interactions are required. Alternatively, fibril formation and deposition of Aβ may only require HS chains, but to create an environment that stabilizes the Aβ-HS interactions, the glycosaminoglycans must be physically clustered on a core protein.

If intact proteoglycans are important macromolecular species for formation of senile plaques in vivo, then one would predict that their normal catabolism may play an important role in preventing Alzheimer’s disease. HS glycosaminoglycans are cleaved from the core protein and degraded to short oligosaccharides by heparanases (10, 11). Our in vitro studies show Aβ(1–40) prevents partially purified heparanase activities from degrading long HS chains to short glycosaminoglycans. The large molar excess of Aβ(1–40) to HS required to inhibit chain degradation by heparanases suggests that multiple molecules of the peptide must bind the glycosaminoglycan. Soreghan et al. (24) showed that Aβ(1–40) and higher Aβ species could form aggregates due to the hydrophobic amino acids at the carboxyl terminus. The critical concentration of the peptide necessary to form these aggregates is 25 μM (24), which is the concentration of Aβ(1–40) where we first detect inhibi-
tion of heparanase activity (Fig. 6A). This suggests that beginning at this concentration, Aβ(1–40) forms associations with itself and with HS chains that result in the glycosaminoglycan being protected from heparanases. Based on their studies, Soreghan et al. (24) proposed that Aβ fibrils are organized as “tubular micelles” with the polar domain of the Aβ peptide (residues 1–28) forming the outer wall and the hydrophobic amino acids in the center. HS chains may lie along the outside of the fibril because of interactions between sulfate groups on the polysaccharide and the histidine or lysine side chains in the polar domain of Aβ (3, 14). Presumably, once the HS chain is associated with the fibril its normal helix structure (26) is altered so that the glycosaminoglycan is no longer recognized by heparanases. At lower Aβ(1–40) concentrations, it may be that only small aggregates or fibrils are formed, so a portion of the HS chains is still susceptible to the enzyme (Fig. 6). However, once the concentration of the peptide is increased to 150 μM the entire glycosaminoglycan associates with the fibrils and is protected from degradation (Fig. 6). At higher Aβ concentrations, the HS glycosaminoglycans may also promote interactions between Aβ fibrils, leading to the aggregates observed in Alzheimer’s disease.

If the association of Aβ with glycosaminoglycan chains prevents heparanases from degrading HSPGs in vivo, how might this contribute to Alzheimer’s disease? There is evidence that once βAPP reaches the cell surface it is internalized and cleaved by proteases to generate peptides that contain the Aβ sequence (8, 25). Pulse-chase experiments suggest the production of Aβ-containing fragments occurs in an acidic compartment near the cell surface, presumably endosomes (7, 8, 25). This is the same intracellular compartment where heparanases begin degrading HSPGs (10–12). Under normal conditions the concentration of Aβ-containing fragments in endosomes (high pm to low nm range, Ref. 25) will be too low to affect catabolism of the proteoglycans. However, once the levels of Aβ fragments are elevated in Alzheimer’s disease, the peptides could aggregate into fibrils and interact with the HSPGs in this compartment. The association of Aβ with the proteoglycan would prevent heparanases from degrading the HS glycosaminoglycans on the core protein to short oligosaccharides. This will result in an increased level of HSPGs, which could stabilize the aggregates and prevent them from being degraded by proteases (1, 14).

This hypothesis is supported by experiments that suggest that Aβ peptides stimulate the accumulation of amyloidogenic fragments of βAPP inside cells (9). When Aβ(1–42) is added to the media of cultured cells, it is internalized in a concentration-dependent manner (9, 27) and accumulates in an aggregated, protease-resistant form within late endosomes or secondary lysosomes (27). Even at low concentrations of Aβ(1–42) in the medium (100 μg/ml), more than 10 pg of peptide was internalized per cell (27), suggesting that the concentration of Aβ(1–42) inside cells is more than sufficient to affect HSPG catabolism by heparanases. Interestingly, if the cells were transfected with βAPP to increase the levels of expressed precursor, the exogenously added Aβ(1–42) stimulated the production of Aβ-containing fragments from the precursor protein, which also accumulated inside the cell (9). It was postulated that the amyloidogenic fragments produced from βAPP by normal processes interacted with the Aβ(1–42) aggregates, and this association allowed them to escape the normal degradation pathways (9).

The researchers in these studies did not examine whether the Aβ(1–42) aggregates were associated with other macromolecules (27), but it is possible that they were resistant to proteases, in part, because of interactions with HSPGs (14). If the Aβ(1–42) aggregates prevented the degradation of cell-associated HSPGs, the proteoglycans would then be available to interact with the other Aβ fragments and convert them to species that are resistant to proteolysis. It would be interesting to examine the ratio of HSPGs to free glycosaminoglycins in cells transfected with βAPP and see if this ratio changes when the cells are cultured in the presence of Aβ(1–42). If the accumulation of Aβ-containing fragments is dependent on decreased catabolism of proteoglycans, then there should be a higher proportion of cell-associated HSPGs when the cells are cultured with Aβ(1–42). An increase in the concentration of intracellular HS because of its interaction with Aβ peptides may also play a role in the formation of the neurofibrillary tangles present in Alzheimer’s disease. When tau protein is incubated with HS or heparin in vitro, paired helical filaments, similar to neurofibrillary tangles, are formed (28). Immunocytochemical examination of Alzheimer’s disease brain tissue suggests that accumulation of HS may be the initial event in the process (28). Thus, it is possible that alterations in HS catabolism may be a common link to the formation of both plaques and tangles.

The inhibition of heparanase activity may have another effect on the formation of stable Aβ deposits. We showed that Aβ(1–40) could bind to both the long HS chains on core proteins and the short glycosaminoglycans produced by heparanases (Fig. 1) and that the short HS could compete with the long chains for the peptide (Fig. 4). The studies by Snow et al. (1) would suggest that complexes formed between Aβ-containing peptides and short HS chains do not lead to amyloid deposits, and others have reported that small polysulfated molecules can displace HS glycosaminoglycans or proteoglycans bound to Aβ(1–28) affinity columns (29) or disassemble preformed Aβ(1–40) fibrils (30). Thus, in addition to degrading the proteoglycan species that would otherwise be found in senile plaques, heparanases may be generating glycosaminoglycan species that act to prevent Aβ aggregation and deposition.

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