Heparan sulfate found in the cerebral plaques of Alzheimer's disease binds to β-amylloid (Aβ) fibrils. This interaction has been proposed to enhance fibril deposition and mediate Aβ-induced glia activation and neurotoxicity. On the other hand, heparan sulfate augments signaling of fibroblast growth factor-2 (FGF-2), a neuroprotective factor that antagonizes the neurotoxic effects of Aβ. We defined structures in heparan sulfate from human cerebral cortex that bind Aβ fibrils. The minimal binding site is found in N-sulfated hexasaccharide domains and contains critical 2-O-sulfated iduronic acid residues. By contrast, binding of Aβ monomers requires, in addition, 6-O-sulfate groups on glucosamine residues. The binding specificity of fibrillar Aβ is shared by FGF-2, and we here show that cerebral heparan sulfate domains selected for binding to Aβ-(1–40) fibrils bind also to FGF-2. These data suggest that neurotoxic and neuroprotective signals may converge by competing for the same binding sites on the heparan sulfate chain.

Common Binding Sites for β-Amyloid Fibrils and Fibroblast Growth Factor-2 in Heparan Sulfate from Human Cerebral Cortex*

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Alzheimer's disease (AD) is characterized by amyloid accumulation in the brain parenchyma and vasculature. The main component of the amyloid plaques is the amyloid β peptide (Aβ), a 39–43-amino acid residue cleavage product from a larger membrane-associated amyloid precursor protein (1). The amyloid deposits are also rich in heparan sulfate (HS) and chondroitin sulfate proteoglycans (2, 3). The carbohydrate moieties of these macromolecules, particularly HS, have been attributed to a role in enhancing the fibrillation of Aβ peptides and tissue deposition of the fibrils (4, 5). Moreover, HS proteoglycans seem to function as Aβ receptors on microglia, Aβ recognition resulting in microglia activation, production of neurotoxic agents and neurone killing (6). On the other hand, HS binds and activates fibroblast growth factor (FGF)-2 (7), a neuroprotective protein that has been shown to specifically attenuate the neurotoxic effects of Aβ in cultured neurones (8, 9). In AD brain sections, FGF-2 immunoreactivity is enriched in heparitinase-sensitive sites of the amyloid deposits (10). FGF-2 and HS also appear to colocalize in other amyloid lesions such as dialysis-related amyloidosis (11).

In the present study, we undertook a structural characterization of the Aβ binding domain in HS from human cerebral cortex. The complex structure of HS derives from regioselective modifications of a glucuronic acid-N-acetylgalactosamine repeat (GlcNAc-GlcNAc), structure during biosynthesis of the polysaccharide. The modification is initiated by partial N-deacetylation and N-sulfation of the GlcNAc residues. The protein binding sites generally reside within regions of consecutive N-sulfated disaccharide units (NS-domains) (12, 13). NS domains are rich in IdoA (formed by C-5-epimerization of GlcA) and O-sulfate groups, that are most frequently found at C-2 of IdooA and C-6 of GlcNSO₃. The O-sulfate substitution pattern of NS domains varies between different HS species and determines the protein binding specificity of the polysaccharide (12). NS domains are spaced along the polymers by arrays of N-acetylated disaccharide units largely devoid of O-sulfate groups (NA/NS-domains) or by sequences of alternating N-sulfated and N-acetylated disaccharide units (NA/NA/NS domains) (13). Heparin, frequently used as a substitute for HS in experimental work, consists almost exclusively of highly modified NS domains and largely lacks the regioselective modification characteristic of HS.

We here define an Aβ binding domain in human cerebral HS and show that it is strikingly similar in composition to the previously characterized domain interacting with FGF-2. Both proteins are recognized by the same NS domains with critical IdoA(2-OSO₃) residues, indicating that Aβ and FGF compete for binding to the same HS binding sites.

MATERIALS AND METHODS

Aβ Peptide—Aβ-(1–40) was synthesized at the departmental peptide synthesis facility. A stock solution of 5 mg/ml was prepared by dissolving the lyophilized peptide in water and stored as aliquots at −20 °C. For experiments with nonfibrillar peptide, aliquots were thawed and used immediately. Fibrillar Aβ-(1–40) was prepared by 1:10 dilution of the peptide with 150 mM NaCl, 3 mM KCl, 1.7 mM CaCl₂, 0.9 mM MgCl₂ in 1.5 mM phosphate buffer, pH 6.5, followed by a 2-day incubation at room temperature on a shaking platform (14). Thioflavin T binding, as measured by fluorescence spectroscopy (15), was used to monitor fibril formation.

HS and Heparin Preparations—HS was prepared from autopsy specimens of human cerebral cortex (from an AD patient and an age-matched control subject) and radiolabeled, by partial N-deacetylation and re-N-[3H]acetylation as previously (16). Heparin from pig intestinal mucosa (Inolex Pharmaceutical Division, Park Forest South, IL) was radiolabeled by N-[3H]acetylation of N-unsubstituted GlcN residues (17). The specific radioactivities of the labeled preparations...
were estimated after quantification of the polysaccharides by the carboxyl reaction (18). Even-numbered, $^3$H-labeled heparin oligosaccharides and N-, 2-, O-, and 6-O-desulfated heparin species were prepared as described previously (17, 19).

**Preparation of NS Domains**—Fifty μg of cerebral HS (from AD cortex; see below) was N-deacetylated by hydrazinolysis as described previously and subjected to deaminative cleavage by treatment with HNO$_2$ at pH 3.9 (19). At this pH, the reagent cleaves the polymer at the N-unsubstituted GlcN residues generated by N-deacetylation. The NS domains will thus be recovered as heparaxasaccharide fragments, whereas NA/NS domains give rise to tetrasaccharides and NA domains to disaccharides. The cleavage products were end-labeled by reduction with 0.5 mM of NaB$_3$H$_4$ (Amersham Pharmacia Biotech) as described (16) and passed through a column of Sephadex G-15 (1 × 190 cm; Amersham Pharmacia Biotech) in 0.2 mM NH$_4$HCO$_3$. Fractions corresponding to NS domains were pooled, desalted and used in further studies. NS domains of defined sizes were prepared by chromatography on a column of Bio-Gel P10 (1 × 150 cm; Bio-Rad) in 0.5 mM NH$_4$HCO$_3$. The octameric NS domains used in Aβ/FGF-2 affinity chromatography experiments (see below) were further purified by a second gel chromatography step on a Superdex 30 fast protein liquid chromatography column (1.5 × 60 cm; Amersham Pharmacia Biotech), run in 0.5 mM NH$_4$HCO$_3$ at a flow rate of 1 ml/min.

**Affinity Fractionation of Saccharides**—Filter trapping experiments (17) with fibrillar Aβ-(1–40) were carried out in 50 mM Tris HCl, pH 7.4, 0.13 M NaCl (TBS) and with nonfibrillar Aβ-(1–40) in 10 mM sodium acetate, pH 5.5, 0.12 M NaCl, 2.7 mM KCl. For analytical studies, radiolabeled saccharides were incubated with Aβ-(1–40) in a volume of 200 μl at room temperature for 2 h, after which the mixtures were rapidly passed through a nitrocellulose filter (Sartorius, pore size 0.45 μm; diameter 25 mm), followed by washing of the filter with the appropriate buffer (pH 7.4 or 5.5). Proteins and protein-bound saccharides remain on the filter, whereas unbound saccharides pass through. Bound saccharides were released from the filters with 2 M NaCl and quantified by scintillation counting. Preparative incubations were performed in a volume of 2 ml with a larger filter (38 mm in diameter). Bound and unbound saccharides were desalted and subjected to further analysis.

Saccharides were also fractionated by Aβ and FGF-2 affinity chromatography. To prepare the Aβ affinity matrix, fibrillar Aβ-(1–40) in 1.5 mM phosphate buffer, pH 6.5, containing 240 mM NaCl, 3 mM KCl, 1.7 mM CaCl$_2$, and 0.9 mM MgCl$_2$ was centrifuged (16,000 × g for 90 min), and the pellet was resuspended in TBS. The fibrils (1–1 mg) were mixed with 0.5 ml of Sepharose CLAB (Amersham Pharmacia Biotech) gel in 10 mM Tris HCl, pH 7.4. This Aβ-(1–40) fibril-Sepharose CLAB mixture was applied on top of a 0.5-ml layer of Sepharose CLAB gel (without Aβ) that had been previously poured into a Poly-Prep column (Bio-Rad). Although no covalent immobilization of Aβ was used, the fibrils were unlikely to migrate in the gel because Aβ-(1–40) fibrils pelletted by centrifugation represent insoluble macrofibrils aggregated to huge meshes (20). To further ensure that fibrils did not leak from the column, the column outlet was covered with a nitrocellulose filter that was placed on the porous gel support of the column. Samples of $^3$H-labeled heparin dodecasaccharides were quantitatively bound to the column, whereas no binding occurred to a control column without Aβ fibrils (data not shown). To isolate Aβ binding HS domains, octameric [H]HNS-domains from cerebral HS (unfractionated or affinity fractionated on the FGF-2 matrix; see below) were applied to the column that was equilibrated with TBS. Saccharides bound to Aβ-(1–40) fibrils were eluted by a gradient of NaCl (0.13–2.0 M) in 50 mM Tris, pH 7.4. Fractions of 1 ml were collected and measured for radioactivity. In preparative experiments, the bound and unbound saccharide pools were desalted and subjected to FGF-2 chromatography or to compositional disaccharide analysis. Recombinant human FGF-2 (21) was covalently immobilized to 1 ml of a CH-Sepharose matrix (Amersham Pharmacia Biotech) according to the instructions of the manufacturer. Prior to immobilization, FGF-2 (200 μg) was mixed with a 5-fold molar excess of heparin to protect the heparin binding sites of the protein. The immobilization involves reaction of primary amino groups of the ligand with N-hydroxysuccinimide groups in the matrix. To avoid coupling of heparin to the matrix, potential free amino groups, i.e. N-unsubstituted GlcN units, were been previously destroyed by treatment of heparin with HNO$_2$ at pH 3.9 followed by recovery of high molecular weight species resistant to the cleavage. Unfractionated or Aβ affinity fractionated, octameric [H]HNS-domains were applied to the column in TBS, followed by elution of FGF-2 bound saccharides with a gradient (0.13–2.0 M) of NaCl in 50 mM Tris, pH 7.4.

**Analysis of Aβ-(1–40) Binding NS Domains**—Following isolation by preparative filter trapping, the Aβ-(1–40) bound NS domains were subjected to chromatography on a Superdex 30 column as described above. Fractions of 1 ml were collected and analyzed for radioactivity. The elution positions of the NS domains were compared with those of $^3$H-labeled heparin oligosaccharide standards. Fractions corresponding to 6–10-mers were pooled, desalted, and subjected to further analysis as described below.

For compositional disaccharide analysis, the Aβ bound and unbound NS domains were reacted with HNO$_2$ at pH 1.5, and the resultant disaccharide derivatives were radiolabeled by reduction with NaB$_3$H$_4$. The products were recovered by chromatography on Sephadex G-15 (1 × 190 cm) in 0.2 M NH$_4$HCO$_3$ and analyzed by chromatography on a Partisil-10 SAX HPLC column (16). The non-O-sulfated disaccharide species were incompletely resolved from $^3$H-labeled impurities on the SAX HPLC analysis, and were therefore quantified by high voltage paper electrophoresis (at pH 5.3) as described previously (22).

**RESULTS**

**HS from Human Cerebral Cortex Binds Aβ-(1–40) Fibris**—We first wanted to determine whether human cerebral HS species express domains capable of interacting with Aβ fibrils. For this purpose we used HS from autopsy specimens of cerebral cortex from an AD patient and an age-matched control subject. Our previously published structural characterization of cerebral HS indicated that, whereas this material was structurally distinct from other human HS species, there was no detectable difference in disaccharide composition between HS samples from AD and control brain (16). Increasing amounts of cerebral $^3$HHS were incubated with Aβ-(1–40) fibrils, followed by treatment of the formed protein-HS complexes on nitrocellulose filters and quantification of the filter-bound radioactivity. Parallel incubations were performed with $^3$H-heparin from pig intestinal mucosa. As shown in Fig. 1, AD and control brain HS species and heparin all bound to Aβ-(1–40) fibrils in a dose-dependent, saturable manner. Scatchard analysis (not shown) of the binding data in Fig. 1 suggested that HS from AD fibrils.
and control brain bound Aβ-(1–40) with similar affinity, $K_d$ in the range of 10–100 nM.

The Minimal Aβ Binding Domain Is a Hexasaccharide—To define the minimal size of heparin oligosaccharides required for binding to Aβ, 3H-labeled, even-numbered heparin oligomers were incubated with Aβ-(1–40) fibrils, and the binding was assessed with the filter trapping method. The smallest fragments capable of significant binding to Aβ fibrils were hexasaccharides (Fig. 2). Maximal binding, at less than twice the level observed for heparin, was reached with octasaccharides and longer oligosaccharides. To investigate the minimal length of Aβ binding NS domains from cerebral HS, such components were prepared and radiolabeled as described under “Materials and Methods” and incubated together with Aβ-(1–40) fibrils. The Aβ-bound NS domains were recovered using the filter trapping procedure in preparative mode, and further analyzed by gel chromatography on a column of Superdex 30 calibrated with standard heparin oligosaccharides (Fig. 2). The bound domains spanned a fairly narrow range of oligomer species, with a major proportion of octa/decasaccharides and some hexasaccharides (Fig. 2).

Nonfibrillar and Fibrillar Aβ Bind to Distinct Saccharide Structures—To characterize in more detail the structural requirements for HS binding to Aβ, NS domains were separated with regard to affinity for the fibrillar peptide, and the bound and unbound species were subjected to compositional analysis. Aβ bound NS hexamers (i.e. the smallest binding species), ~10% of the total fraction, were recovered by nitrocellulose filter trapping and were then cleaved with HNO$_2$ at pH 1.5. The resultant disaccharides were radiolabeled and separated by anion-exchange HPLC and paper electrophoresis (see “Materials and Methods”). Similar analysis was applied to the unbound hexamer fraction, and to NS octamers separated by affinity chromatography on immobilized Aβ fibrils. All samples yielded a major IdoA(2-OSO$_3$)-a-Man$_n$ disaccharide component (representing an IdoA(2-OSO$_3$)-GlcNSO$_3$ sequence in the intact NS domains), other disaccharides occurring in smaller amounts (Table I; a representative anion-exchange HPLC pattern is shown in Fig. 3). Whereas the IdoA(2-OSO$_3$)-GlcNSO$_3$ sequence was the only single disaccharide unit that approached or exceeded 1 mol/mol of each NS domain analyzed, total 6-O-desulfation reached comparable levels, such that total O-desulfation ranged from 2.1 to 4.0 residues/oligosaccharide (Table I). No consistent difference in composition between Aβ bound and unbound fractions was found. Whereas 2-O-sulfate groups thus appeared to be enriched in bound NS hexamer, 6-O-sulfation was more abundant in bound NS octamer. Notably, the Aβ unbound octamer used for disaccharide analysis had been subjected to repeated affinity chromatography and thus was depleted of binding species. The final Aβ unbound fraction corresponded to ~60% of the initial unfractionated NS domain species (data not shown). These findings suggest that Aβ fibril binding should be attributed to the positioning rather than the total abundance of sulfate groups, i.e. to the sequence of differently substituted disaccharide units.

To further assess the binding specificity, we tested the ability of selectively desulfated, unlabeled heparin preparations to inhibit binding of native [3H]heparin to fibrillar Aβ. Efficient inhibition was achieved with native heparin and with 6-O-desulfated heparin, whereas the 2-O- and N-desulfated/N-acetylated heparin preparations had little or no inhibitory activity (Fig. 4). These data indicate a critical role for 2-O-sulfated substituents in the HS-Aβ interaction and also point to the importance of N-sulfate groups, whereas 6-O-sulfate groups do not seem to be required for the binding. For comparison, we performed similar analysis using nonfibrillar Aβ-(1–40) as the protein ligand. Nonfibrillar Aβ binds heparin only at low pH (~5.5) (23), presumably because the interaction requires protonation of the His$^{14}$ and His$^{15}$ residues in the peptide. At pH 5.5, Aβ monomers bound heparin and cerebral HS in a dose-dependent and saturable manner, as measured by the filter trapping procedure (data not shown). None of the selectively desulfated heparin preparations was able to inhibit binding of [3H]heparin to nonfibrillar Aβ, whereas addition of native heparin resulted in complete inhibition of binding (Fig. 4). These data suggest that N-, 2-O- and 6-O-sulfate groups all participate in the interaction between heparin and Aβ monomers. Collectively, the findings from the inhibition studies indicate that the fibrillar and nonfibrillar forms of Aβ-(1–40) are recognized by distinct saccharide domains.

Aβ Fibrils and FGF-2 Bind the Same HS Domains—The above results suggest that the minimal NS domain structures recognizing Aβ-(1–40) fibrils are hexa-/octasaccharide sequences containing at least one IdoA(2-OSO$_3$)$_2$ residue. These features match those previously established for the FGF-2 binding NS domain (24), raising the possibility that the two proteins might share a common binding site in HS. We therefore fractionated 8-mer NS domains according to binding to Aβ or FGF-2 and then tested the bound and unbound saccharides for binding to the converse protein ligand. As shown in Fig. 5, the Aβ bound saccharides were quantitatively retained by the FGF-2 affinity column, whereas the unbound saccharides showed partial binding. Further, the majority of FGF-2-bound saccharide species bound also to Aβ whereas the unbound saccharides had no Aβ affinity. These data demonstrate that FGF-2 and Aβ binding NS domains colocalize in HS from human cerebral cortex, such that the Aβ binding NS domains interact quantitatively with FGF-2, whereas, conversely, a major fraction of the FGF-2 binding NS domains also binds Aβ fibrils. The Aβ binding NS structures thus represent a major subpopulation of FGF-2 binding domains.

DISCUSSION

We show that HS from human cerebral cortex binds Aβ-(1–40) fibrils via NS domains with 2-O-sulfated IdoA residues. By contrast, 6-O-sulfate groups, although present in Aβ binding NS domains, appear redundant to the interaction as shown by the ability of 6-O-desulfated heparin to efficiently compete with 6-O-desulfation (reaction with dimethyl sulfoxide) is accompanied by loss of ~30% of the 2-O-sulfate groups in heparin (19). This partial 2-O-desulfation may account for the somewhat decreased inhibitory effect of 6-O-desulfated as compared with native heparin.
with NaB₃H₄ as described under “Materials and Methods.” [³H]Disaccharides were incubated in TBS together with unlabeled native or selectively desulfated heparin preparations. The ability of the various disaccharide units in the NS domains were calculated by dividing the mol% values for each unit by the factor that corresponds to 1 mol/1 mol of NS domain (33 for hexasaccharides and 25 for octasaccharides).

FIG. 3. Compositional disaccharide analysis Aβ bound NS domain. Hexameric [³H]-labeled NS domains were fractionated according to binding to Aβ fibrils using the filter trapping procedure in preparative mode. The bound NS domain species were recovered and reacted with HNO₂ at pH 1.5 followed by radiolabeling of the cleavage products by anion-exchange HPLC using a stepwise gradient of KH₂PO₄ (---). For details, see “Materials and Methods.” [³H]-Disaccharide derivatives were recovered by gel chromatography and separated by anion-exchange HPLC using a stepwise gradient of KH₂PO₄ (---). The peaks are numbered as follows: 1, GlcA(2-OSO₃)-αMan(6-OSO₃)₂; 2, GlcA-αMan₆(6-OSO₃)₃; 3, IdoA-αMan₆(6-OSO₃)₃; 4, IdoA(2-OSO₃)-αMan₆(6-OSO₃)₃; and 5, IdoA(2-OSO₃)-αMan₆(6-OSO₃)₃. The peak marked with * represents tetrasaccharides, partly because of “anomalous” ring contraction.

**TABLE I**

| Sample | GlcA(2-OSO₃)-αMan(6-OSO₃) | GlcA-αMan₆(6-OSO₃)₃ | IdoA-αMan₆(6-OSO₃)₃ | IdoA(2-OSO₃)-αMan(6-OSO₃) | Overall degree of O-sulfation |
|--------|---------------------------|---------------------|---------------------|---------------------------|-----------------------------|
| Bound 6-mer | 0.1 | 0.4 | 0.2 | 1.4 | 0.4 | 1.9 | 1.0 | 2.9 |
| Unbound 6-mer | 0 | 0.3 | 0.3 | 0.9 | 0.3 | 1.2 | 0.8 | 2.1 |
| Bound 8-mer | 0.3 | 0.7 | 0.2 | 1.2 | 0.8 | 2.3 | 1.7 | 4.0 |
| Unbound 8-mer | 0.2 | 0.5 | 0.5 | 1.5 | 0.5 | 2.2 | 1.3 | 3.5 |

* The proportions of the various disaccharide species were determined by separation of the O-sulfated disaccharide units by SAX HPLC chromatography (see “Materials and Methods” and Fig. 3) after assessing the proportions of the nonsulfated HexA-αManR disaccharide units by high voltage paper electrophoresis (data not shown). The numbers of the various disaccharide units in the NS domains were calculated by dividing the mol% values for each unit by the factor that corresponds to 1 mol/1 mol of NS domain (33 for hexasaccharides and 25 for octasaccharides).

FIG. 4. Distinct sulfation requirements for binding of fibrillar and nonfibrillar Aβ-(1–40) to heparin. Aβ-(1–40) fibrils (5 μg) and [³H]heparin (20,000 dpm) were incubated in TBS together with unlabeled native or selectively desulfated heparin preparations. The ability of the unlabeled competitors to inhibit the Aβ-(1–40)-[³H]heparin interaction was assessed by the filter trapping method. The assays with nonfibrillar Aβ-(1–40) were performed similarly with the exception that the incubations were in 10 mM sodium acetate, pH 5.5, 120 mM NaCl, 2.7 mM KCl. ○—○, native heparin; ●—●, 6-O-desulfated heparin; ○—○, 2-O-desulfated heparin; △—△, N-desulfated heparin.

Intact heparin for binding to Aβ-(1–40) fibrils. These binding requirements resemble those described earlier for the neuroprotective factor FGF-2. Biochemical studies of FGF-2 binding NS domains (24) and x-ray crystallography of a FGF-2-heparin hexamer complex (25) suggest that a single IdoA(2-OSO₃) residue in a 5–6-mer NS domain would be sufficient for high affinity binding (although the two approaches point to different locations of that residue). Indeed, cross-fractionation of octameric [³H]NS domains were fractionated by affinity chromatography on immobilized Aβ-(1–40) fibrils and FGF-2 (top panels). The Aβ bound and unbound saccharide species (fractions 41–60 and 1–10, respectively, top left) were further chromatographed on a column of immobilized FGF-2. The FGF-2 bound and unbound octamers (fractions 63–84 and 1–10, respectively, top right) were subjected to Aβ affinity chromatography. The bound saccharides were eluted with a gradient of NaCl (---). For details, see “Materials and Methods”. Aβ affinity chromatography of FGF-2 bound fractions 41–62 (upper-right panel) gave a pattern similar to that illustrated in the middle left panel (not shown).

FIG. 5. Colocalization of Aβ-(1–40) and FGF-2 binding in human cerebral HS NS domains. Octameric [³H]NS domains were fractionated by affinity chromatography on immobilized Aβ-(1–40) fibrils and FGF-2 (top panels). The Aβ bound and unbound saccharide species (fractions 41–60 and 1–10, respectively, top left) were further chromatographed on a column of immobilized FGF-2. The FGF-2 bound and unbound octamers (fractions 63–84 and 1–10, respectively, top right) were subjected to Aβ affinity chromatography. The bound saccharides were eluted with a gradient of NaCl (---). For details, see “Materials and Methods”. Aβ affinity chromatography of FGF-2 bound fractions 41–62 (upper-right panel) gave a pattern similar to that illustrated in the middle left panel (not shown).
Aβ monomers bind HS/heparin at pH 5–6 (23, 26), suggesting that HS and Aβ may interact in the acidic milieu of intracellular compartments where newly synthesized or endocytosed amyloid precursor protein/Aβ and HS are both present (27). We now show that the low pH interaction with Aβ-(1–40) monomers is distinguished from the interaction with fibrils by the requirement for 6-O-sulfate groups in the saccharide ligand. These distinct specificities conform to the notion that the interaction of HS with Aβ fibrils involves composite binding sites dependent on fibril formation rather than sites expressed in individual monomers. Heparin/HS presumably enhance Aβ fibrillation by providing a scaffold with multiple Aβ binding sites, thereby promoting the association of early fibrils to more mature ones (14). Conversely, HS oligosaccharides encompassing a single Aβ fibril binding site could provide means of interfering with further fibrillization. Similar strategies could apply to other amyloidotic conditions where HS is known to interfere with fibril formation. For example, fibrillization of amylin, occurring in pancreatic lesions of type 2 diabetes patients, is efficiently promoted by HS/heparin. Both the N- and O-sulfate substituents of heparin appear important for this effect (28).

The functional significance of the low pH interaction with Aβ monomer remains unclear. Assuming that the aggregation of Aβ begins intracellularly (as has been suggested because the Aβ concentrations found in extracellular fluids are insufficient to nucleate the fibril formation (29)), it might be possible to impede the initial aggregation process by appropriate saccharides/mimetics. Indeed, one of the hydrophobic regions of Aβ-(1–40) involved in the oligomerization of the peptide (30) partially overlaps with the VHHQKL domain suggested to mediate interaction of HS with Aβ-(1–40) mono- mers found in extracellular fluids are insufficient to nucleate the fibril formation (29)). It might be possible to impede the initial aggregation process by appropriate saccharides/mimetics. Indeed, one of the hydrophobic regions of Aβ-(1–40) involved in the oligomerization of the peptide (30) partially overlaps with the VHHQKL domain suggested to mediate interaction of HS with Aβ-(1–40) to heparin (6, 14). Further detailed analysis of the various HS-Aβ interactions may promote the development of drugs capable of interfering, at different levels, with Aβ fibril formation, as well as with the Aβ-induced microglia activation (6).

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