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Binding of Amyloid β to Ganglioside Micelles is dependent on Histidine 13

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Abbreviations used: HSQC, heteronuclear single-quantum correlation; TOCSY, total correlation spectroscopy

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Summary

The amyloid β-peptide Aβ is a major component of plaques in Alzheimer's disease, and formation of senile plaques has been suggested to originate from regions of neuronal membrane rich in gangliosides. Here we demonstrate using NMR on ^{15}N-labelled Aβ(1-40) and Aβ(1-42) that the interaction with ganglioside GM1 micelles is localised to the N-terminal region of the peptide, particularly residues His13 to Leu17, which become more helical when bound. The key interaction is with His13, which undergoes a GM1-specific conformational change. The sialic acid residue of the ganglioside headgroup is important for determining the nature of the conformational change. The isolated pentasaccharide headgroup of GM1 is not bound, suggesting the need for a polyanionic surface. Binding to heparin confirms this suggestion, since binding is of similar affinity but does not produce the same conformational changes in the peptide. A comparison of Aβ(1-40) and Aβ(1-42) indicates that binding to GM1 micelles is not related to oligomerisation, which occurs at the C-terminal end. These results imply that binding to ganglioside micelles causes a transition from random coil to helical in the N-terminal region, leaving the C-terminal region unstructured.

Keywords: amyloid; ganglioside; sialic acid; fibril; NMR

INTRODUCTION

The brains of Alzheimer's patients are characterised by amyloid plaques, whose main constituent is the amyloid β-peptide Aβ, which forms 'cross-β' fibrils [1]. This peptide ranges from 40 to 43 residues in length, with the difference being at the C-terminal end. Longer peptides are much more fibrillogenic [2]. All adult brains contain amyloid plaques, but in most individuals these are 'diffuse' and not apparently harmful; by contrast, in Alzheimer's sufferers, the plaques are fibrillar and are associated with dystrophic neurons. This, together with many other results, has suggested that it is the conversion from diffuse to fibrillar form that dictates disease progression, and has focussed efforts on identifying the 'seed' from which fibrils are propagated in what appears to be a nucleation-dependent process that involves a change in the conformation of the peptide [3]. Interestingly, the diffuse plaques are predominantly Aβ(1-42) and the neuritic plaques predominantly Aβ(1-40) [4].

A major debate has focused on whether the seed for fibril formation is formed in solution (possibly as an oligomer [5]) or on membrane surfaces. In recent years, much attention has focussed on interactions between Aβ and gangliosides, particularly GM1 (Figure 1), which is one of the most abundant gangliosides in the brain, constituting approximately 20% of brain gangliosides [6]. Gangliosides are sialic acid-containing glycosphingolipids with a role in synaptic transmission and signalling, and are found in high concentration in neural cell membrane, particularly in synaptic membrane [7]. The finding that GM1-bound Aβ is generated in human brain [8] has stimulated further studies in this area, including the recent results that ganglioside micelles stimulate aggregation and fibrillisation of Aβ [9], that regional deposition of Aβ in the brain is induced by the local gangliosides [10], and that Aβ-GM1 binding in living cells takes place in a seed-dependent manner and directly induces cytotoxicity [11], and may be a mechanism common to many amyloidoses [12]. A particularly interesting line of research locates GM1-rich membranes in cholesterol-rich lipid rafts [13, 14], thereby suggesting how the amyloid deposits could affect neuronal membranes and signalling, and also why cholesterol and apolipoprotein E (which redistributes cholesterol in the brain) might be linked to Alzheimer's disease [15].

In this paper, we describe NMR experiments aimed at characterising the interactions between Aβ and ganglioside micelles. We identify a small part of the peptide, residues 13-17, that we demonstrate to be most crucial for substrate-specific interaction. A comparison of Aβ(1-40) and Aβ(1-42) suggests that Aβ(1-42) forms oligomers in solution by interactions at the C-terminus, but that these are not related to GM1 binding. The relationship to seeding of amyloid plaques is discussed.

EXPERIMENTAL

Uniformly ^{15}N-labelled Aβ(1-40) and Aβ(1-42) were purchased from rPeptide (Athens, GA), and gangliosides were purchased from Alexis Biochemicals (now Axxora (UK) Ltd), Nottingham, UK. The purity of the gangliosides is quoted as >98% by the manufacturer, but was not checked. All other reagents were from Sigma-Aldrich. The heparin used was the sodium salt (H4784).

Aβ(1-40) solutions were prepared following [16]: the peptide was dissolved at a concentration of 200 μM in 10 mM NaOH with 1 min sonication, and immediately frozen if required. Subsequently, the pH was adjusted to 7.2 with a minimal amount of 0.1 M HCl, and D_2O was added to make approximately 200 μM peptide solution containing 10% D_2O. Solutions prepared in this way were stable and showed no sign of aggregation for at least a week. By contrast, solutions made up in buffer aggregated much faster, often being almost entirely precipitated after 24 hr. Aβ(1-42) was pre-treated by dissolution in hexafluoroisopropanol and lyophilisation, before dissolving in 10 mM ammonium hydroxide, followed by adjustment of the pH to 7.2. These solutions started to aggregate and form fibrils immediately and were only usable for 2-3 days after solution. Solution of the commercial material directly into sodium hydroxide resulted in no NMR signal whatsoever, indicating significant aggregation using this method. Stock solutions of gangliosides or heparin were prepared at high concentration (approximately 100-200 mM), adjusted to pH 7.2 and added direct to the NMR tube. All NMR experiments were carried out on a Bruker DRX-500 equipped with a cryoprobe, and operated at 13 °C. ^{15}N HSQC experiments used gradient selection for water suppression and water flip-back pulses to minimise loss of magnetisation through exchange and relaxation processes. The 3D TOCSY-HSQC spectrum incorporated solvent suppression using gradients, with a 35 ms spin-lock at

8.3 kHz decoupler power. Processing of NMR data used FELIX (Accelrys Inc., San Diego, CA), and titration data were analysed using home-written scripts. Cross-peak intensities were measured within FELIX, transferred to a text file, and fitted to an exponential decay using a Marquardt nonlinear least-squares fitting based originally on a Numerical Recipes© algorithm. Binding constants were obtained by fitting to a standard equation using Excel (Microsoft).

RESULTS

The HSQC spectrum of Aβ(1-40) was assigned based on assignments described and kindly provided by Dr M. Zagorski [16]. Assignments were confirmed using a 3D TOCSY-HSQC experiment, and proved very similar to published assignments [16]. The HSQC spectrum is shown in Figure 2. Almost all backbone signals are resolved. At 13 °C, all signals can be found except for the N-terminal residue and His6. His14 gives a weak signal, as expected because of its rapid amide exchange under these conditions [17]. The reason for the absence of His6 in our HSQC spectra is less clear, but it was also not observable by Hou *et al.* [16]. On the basis of the chemical shifts, we concur with other authors [16, 18] that the peptide is random coil in aqueous solution. The majority of HSQC peaks in Aβ(1-42) were assigned in the same way, but the assignment is somewhat less complete due to its rapid aggregation, which limits the time available for 3D NMR experiments. The signals from Aβ(1-42) have very similar chemical shifts to those of Aβ(1-40) except at the C-terminus, implying no significant conformational differences between them.

GM1 forms micelles with a critical micelle concentration in the low μM range [19]. Thus, at concentrations >100 μM, as used here, it is essentially 100% micellar. On titration of GM1 micelles into Aβ(1-40), chemical shift changes were seen in the NMR spectrum, as shown in Figure 3. The chemical shift changes are small, but are reproducible and specific, since many residues have essentially no change in shift. Smaller chemical shift changes have been demonstrated to be biologically relevant on many previous occasions (eg [20, 21]). Almost all the chemical shift changes were in the N-terminal half of the peptide, and were close to potentially positively charged residues: Glu3-Arg5 (close to Arg5 and His6) and Val12-Leu17 (close to His13, His14 and Lys16). However, the pK values of the three histidines are all

approximately 6.5 [22]. Hence at the pH of our measurements, 7.2, most of the histidines will be unprotonated. We therefore expect that the only residues significantly positively charged at this pH will be Arg5 and Lys16, together with Lys28. This makes it unlikely that the chemical shift changes are due only to coulombic interactions with the single negative charge in the GM1 headgroup, the sialic acid. In order to confirm this, a further titration was carried out at approximately physiological salt concentration (150 mM NaCl), which should markedly reduce purely coulombic interactions in water. The results (Figure 4) show reduced chemical shift changes, indicating a loss of affinity. Residues 14-17 still shift, but the changes seen in residues 3-13 are much reduced. This implies that although some of the binding and the chemical shift changes are primarily coulombic in origin, others (and in particular the changes seen in residues 15-17) are much less so. We note that other authors have concluded that binding to GM1 is not *primarily* coulombic, although again there are clearly coulombic interactions[23-25].

A further titration of Aβ(1-40) was carried out, using micelles of asialo-GM1 (Figure 1). The results are shown in Figure 5, in which the chemical shift ranges used in the plot are 75% of those used for Figure 3, and show that chemical shift changes with asialo-GM1 are similar to those seen for GM1 but reduced in magnitude for equivalent concentrations by approximately 25%. A reduction is expected in the magnitude of the shift change, because asialo-GM1 is known to bind less tightly than GM1 to Aβ. The extent of the difference has been reported differently. Choo-Smith *et al.* report no binding at all to asialo-GM1 [23], while a factor of 2 has also been reported [26]. Our data are in better agreement with the latter result. The overall similarity of the chemical shift changes for GM1 and asialo-GM1 implies that the binding interactions are similar, although with some differences in the region of His13-Gln15. This result therefore also implies that the interaction is not dominated by coulombic forces, since asialo-GM1 has no charge in the headgroup.

In a further experiment, Aβ(1-40) was titrated with heparin (Figure 6). The chemical shift changes again affect very similar residues to those affected by GM1, although the size and direction of the change is in several cases markedly different (eg Arg5, His13 and His14). Heparin is a polyanionic polysaccharide (consisting mainly of 2-deoxy-2-sulphamino-α-D-glucose 6-sulphate, α-L-iduronic acid 2-sulphate, 2-acetamido-2-deoxy-α-D-glucose, β-D-glucuronic acid, and α-L-iduronic acid in

random order), but otherwise has little structural similarity to GM1 micelles. Despite its completely different covalent structure, and presumably its three-dimensional structure, it causes similar chemical shift changes in Aβ to those caused by ganglioside micelles. Hence, many of the changes must reflect a general conformational propensity of the peptide on binding to a surface, especially to one carrying a negative charge (although the overall similarity of results from asialo-GM1 imply that a negative charge is not essential). However, the exact structure adopted when bound, indicated by the direction of the chemical shift changes for residues Phe4, Arg5, Val12 and His13 in particular, depends on the substrate.

By contrast, Aβ(1-40) was also titrated with the pentasaccharide headgroup of GM1. No chemical shift changes could be observed at all (data not shown), implying a lack of interaction, and therefore the requirement for an extended surface for efficient binding. A similar observation has been made before [27].

The chemical shift changes for Gln15, Lys16 and Leu17 on titration are in all cases similar, and show a reduction in chemical shift (ie, an upfield shift) for both ^{1}H and ^{15}N. These changes are those expected for a change from random coil to α-helix, and in the opposite direction for those expected on going from random coil to β-sheet [28, 29]. The data therefore imply that this region of the peptide becomes more helical on binding, but that the interacting region N-terminal to this sequence has a more complicated and substrate-specific conformational change.

The affinity of Aβ(1-40) for GM1 micelles was estimated by fitting the chemical shift changes to a standard saturation curve [30]. The numerical result requires an assumption as to the number of GM1 molecules in a micelle. Here we have assumed an aggregation number of 310 [31], which produces a dissociation constant for a micelle of approximately 5 μM, in approximate agreement with values produced by others for binding to vesicles containing GM1 [13, 14, 23]. This result implies that the fairly weak binding to GM1 does not act to concentrate Aβ directly (and therefore increase the local concentration of Aβ which might encourage fibrillisation); rather, it acts to fix Aβ into a fibrillogenic conformation. When the dissociation constant is calculated for GM1 monomers, it is much weaker at approximately 1 mM. The dissociation constant for heparin (per disaccharide repeat) is similar, implying that the binding to gangliosides is not particularly strong, in agreement with results from other groups [32]. The calculations also imply that at 8

equivalents of GM1 (the maximum shown here), the shift changes are approximately 30% of maximal. Thus, ^{1}HN chemical shift changes on 100% binding to GM1 micelles are estimated to be approximately 0.06 ppm for residues 15-17, implying that the bound structure is not fully helical, which would produce shift changes of approximately 0.2 ppm [28].

During the course of the titration, reductions in peak intensity and increases in linewidth were seen in the HSQC spectrum. Such changes are expected due to the increased correlation time arising from binding to a large micelle, which reduces T_2 and therefore reduces intensity in multipulse experiments such as HSQC. For most protons, the increased linewidth and reduced intensity seen are consistent with a fast-exchange limit equilibrium between the free and micelle-bound forms: fast exchange is expected from the the relatively weak binding affinity, which is in the low μM range. Thus, for the heparin titration, intensity changes were small and showed no clear variation along the sequence (except that the two C-terminal residues showed a lesser intensity reduction, implying little or no restriction in motional freedom arising from binding of the peptide to heparin). However, for the titrations with GM1, intensity changes were large and markedly non-uniform along the sequence (Figure 7). One would expect the changes to be larger than with heparin because of the much longer correlation time of the GM1 micelles. The non-uniformity is consistent either with greater motional restriction at some sites, or with exchange broadening. The latter explanation is less likely because the affinity is too weak to cause significant exchange broadening. As a way of handling the data, the intensities were fitted to an exponential curve (intensity against amount of GM1 added), the results of which are shown in Figure 8. For both GM1 and asialo-GM1, the most dramatic decrease in intensity is for residues 26-28, despite the fact that these residues show only very small chemical shift changes. There is also faster broadening at the C-terminal end, around Gly37. Loss of intensity can be caused by a large number of factors, all of which imply a change in conformation or environment. We therefore conclude that binding to GM1 micelles does affect the C-terminal part of the peptide, specifically around residues 27 and 37, even though no or only small chemical shift changes are seen here.

There are also marked decreases in intensity around His13 and Ser8 in the N-terminal end of the peptide on binding GM1, which are smaller or absent on titration

with asialo-GM1. This implies a reduced interaction at these locations in asialo-GM1, an observation that is consistent with the chemical shift changes described above, which were smaller and different for asialo-GM1 for Ser8, Gly9, His13 and Gln15. Thus, both chemical shift changes and line-broadening imply an interaction with the sialic acid group in the region of residues 8-15.

HSQC experiments were also carried out on Aβ(1-42). This peptide differs from Aβ(1-40) only by the presence of Ile41 and Ala42. Immediately after separation of aggregates by treatment with hexafluoroisopropanol, followed by lyophilisation, sonication at high pH and adjustment to pH 7.2, HSQC spectra already indicated the presence of aggregated species, which increased in intensity with time (Figure 9). These signals were sharp and apparently in slow exchange with monomer, and only affected C-terminal residues (from G33 onwards). We therefore conclude that the peptide forms small well-defined aggregates, probably dimers because of their sharpness, in a time-dependent manner, centred on the C-terminus. The sharpness of the oligomer signals is inconsistent with their being as large as hexamers.

Titrations of Aβ(1-42) with GM1 showed chemical shift changes. The slow exchange between monomer and oligomer meant that we were able to monitor chemical shift changes for some of the C-terminal residues in both monomer and oligomer. We were therefore able to identify binding interactions for monomer and oligomer from the same solution. There were no significant chemical shift changes in either monomer or oligomer, for any of the residues showing signal splitting (ie, G33 onwards), implying that the C-terminal end of the peptide does not interact with GM1 micelles, either as monomers or oligomers. The lack of binding of the oligomer is of interest in the light of recent reports that the fibrillogenic form of Aβ is soluble oligomers [5]. By contrast, residues in the N-terminal end show shift changes comparable to those observed for Aβ(1-40), with the largest changes being for Y10, V12, K16 and L17. (We note that shift changes for H13 and Q15 were not measurable due to signal overlap and poor solubility.) We therefore conclude that Aβ(1-42) binds to GM1 micelles in a similar way to Aβ(1-40), and particularly in the region 10-17; and that it also undergoes a dimerisation or aggregation at the C-terminal end, which is independent of any binding to GM1.

DISCUSSION

There have been a large number of conformational studies of Aβ. Many of these are in unphysiological solvents or in SDS or lipid micelles, which are well known to have a tendency to push peptides towards helical structures. It is therefore not surprising to find that these studies tend to report helical conformations for Aβ. However, studies in water have suggested random coil states [16, 18], a conclusion with which we agree.

In this study we demonstrate binding in the low μM range to GM1, asialo-GM1 and heparin, but no measurable binding to the isolated GM1 headgroup. Several experiments (both ours and those of others [33]) have demonstrated that although there may be a coulombic element to the binding, the interactions are not limited to coulombic ones. Our results are therefore relevant to the physiological situation where the salt concentration is higher and coulombic interactions are weaker.

The chemical shift changes reported here demonstrate binding of Aβ to GM1 micelles, which is localised to the N-terminal half of the peptide. We confirm that binding to asialo-GM1 is weaker, specifically with differences around residues 12-15. [This conclusion is radically different from one resulting from another NMR study [34]. However, that study was carried out using SDS micelles, which may be the reason for the different results.] Binding to heparin also involves the same residues, although the chemical shift changes (and therefore presumably the interactions and conformational changes on binding) are different. Our results therefore agree with the consensus of opinion, which is that high affinity binding is directed to the N-terminal end of the Aβ peptide [26]. For binding to heparin, the region 12-17 has previously been identified [33, 35], and this region has also been demonstrated to be important for attachment to microglial cells and for neurotoxicity [36]. There is no binding to isolated pentasaccharide headgroups. The results therefore imply that Aβ binds to a range of surfaces in a similar way and with similar affinity, the binding being at the N-terminal end of the peptide.

Chemical shift changes (particularly of ^{15}N and ^{1}HN) are notoriously difficult to relate to specific conformational changes. However, there is wide agreement that helical regions have higher field ^{15}N and ^{1}HN shifts than random coil. The results reported here therefore imply that the short region from Gln15 to Leu17 becomes

more helical on binding, to GM1, asialo-GM1 and heparin. It is perhaps significant that this is exactly the same region identified above as being important for neurotoxicity [36]. The region C-terminal to Leu17 has little or no observable conformational change. Intensity changes did however suggest some interaction in this part of the peptide, particularly around residues 27 and 37.

A comparison of chemical shift changes in Aβ on addition of GM1 micelles compared to asialo-GM1 or heparin implies that His13 interacts specifically with the sialic acid moiety. Because previous studies have identified the sialic acid as being important for the growth of amyloid fibres [10, 13, 37], the results imply that His13 binding may play an important role in fibrillogenesis. Indeed, His13 has previously been identified as crucial for rapid fibrillisation [38]. It is relevant to note that rats, which differ in their Aβ from humans in only three positions (R5G, Y10F and H13R), do not form cerebral Aβ amyloid [39].

Comparison of chemical shift changes in Aβ(1-40) and Aβ(1-42) implies that binding to GM1 micelles is located at the N-terminal end, while the C-terminal end plays no part in binding, but does lead to oligomerisation in solution. We note that residues 1-28 alone are sufficient for formation of structured aggregates, but do not form fibrils [40]. The results therefore suggest that the N-terminal binding of the peptide to GM1 may provide the initial fibrillogenic seed, but that the C-terminus is required for propagation into fibrils.

This raises the question of what this study implies about the formation of amyloid fibres *in vivo*. The study is consistent with the seeding/nucleation amyloid cascade model of Alzheimer's. By contrast, it implies that soluble Aβ aggregates, currently a very popular topic of study [5], may be off-pathway intermediates with no direct involvement in plaque formation. There is however a recent report [41] that Aβ polymers are found associated with lipid rafts in mouse brain, but that oligomers are found particularly at axon terminals. It is therefore possible that both modes of polymerisation operate at the same time, in different regions of the brain. Multiple assembly pathways would be no surprise [42].

The study also implies that the fibrillogenic seed nucleus involves an interaction of His13 with the sialic acid moiety of GM1. Aβ can bind to other non-fibrillogenic surfaces (heparin, for example), but without inducing the same structural change in Aβ. This implies that binding of Aβ in other conformations may merely

lead to buildup of Aβ without the formation of fibrils – in other words, to diffuse plaques. Indeed, this could provide an explanation of why Aβ(1-42), which is less soluble than Aβ(1-40) and speeds up fibrillogenesis [38], surprisingly tends to be found in diffuse rather than neuritic plaques [4]: we suggest that it binds and aggregates so rapidly that it does not have time to rearrange into the correct conformation to form the required seed. This suggestion is consistent with our observation that oligomerisation of Aβ(1-42) is rapid and independent of GM1 binding, and occurs in a different region of the peptide. It could also provide an explanation of the observation that the amount of soluble Aβ oligomer correlates with synaptic loss better than does the amount of insoluble Aβ [43]: it may be that deposition of Aβ in the correct conformation requires multiple equilibration between soluble and insoluble forms. The formation of fibrils is thus seen to be a fine balance between over-rapid deposition (leading to diffuse plaques) and inadequate deposition (leading to small and non-aggressive plaques). In partial support of this argument, we note that treatment of transgenic mice (which develop Alzheimer's-like symptoms) with anti-Aβ antibody not only leads to a reduction in plaques (implying reversible binding of Aβ to plaques *in vivo*), but also gives a reduction in neuritic damage, implying that the neuritic damage is a consequence of the plaques [44].

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REFERENCES

1 Kirschner, D. A., Abraham, C. and Selkoe, D. J. (1986) X-Ray diffraction from intraneuronal paired helical filaments and extraneuronal amyloid fibers in Alzheimer disease indicates cross-β conformation. Proc. Natl Acad. Sci. USA **83**, 503-507

2 Jarrett, J. T., Berger, E. P. and Lansbury, P. T. (1993) The carboxy terminus of the β-amyloid protein is critical for the seeding of amyloid formation. Biochemistry **32**, 4693-4697

3 Huang, T. H. J., Yang, D. S., Fraser, P. E. and Chakrabartty, A. (2000) Alternate aggregation pathways of the Alzheimer β-amyloid peptide. J. Biol. Chem. **275**, 36436-36440

4 Esiri, M. M. (2001) The neuropathology of Alzheimer's disease. In Neurobiology of Alzheimer's disease (Allen, S. J., ed.), pp. 33-53, OUP, Oxford

5 Cleary, J. P., Walsh, D. M., Hofmeister, J. J., Shankar, G. M., Kuskowski, M. A., Selkoe, D. J. and Ashe, K. H. (2005) Natural oligomers of the amyloid-protein specifically disrupt cognitive function. Nature Neuroscience **8**, 79-84

6 Kracun, I., Rösner, H., Cosovic, C. and Stavljenic, A. (1984) Topographical atlas of the gangliosides of the adult human brain. J. Neurochem. **43**, 979-989

7 Nagai, Y. (1995) Functional roles of gangliosides in bio-signaling. Behavioural Brain Res. **66**, 99-104

8 Yanagisawa, K., Odaka, A., Suzuki, N. and Ihara, Y. (1995) GM1 ganglioside-bound amyloid β-protein (Aβ): a possible form of preamyloid in Alzheimer's disease. Nature Med. **1**, 1062-1066

9 Yamamoto, N., Hasegawa, K., Matsuzaki, K., Naiki, H. and Yanagisawa, K. (2004) Environment- and mutation-dependent aggregation behavior of Alzheimer amyloid β-protein. J. Neurochem. **90**, 62-69

10 Yamamoto, N., Hirabayashi, Y., Amari, M., Yamaguchi, H., Romanov, G., van Nostrand, W. E. and Yanagisawa, K. (2005) Assembly of hereditary amyloid β-protein variants in the presence of favorable gangliosides. FEBS Letts. **579**, 2185-2190

11 Wakabayashi, M., Okada, T., Kozutsumi, Y. and Matsuzaki, K. (2005) GM1 ganglioside-mediated accumulation of amyloid β-protein on cell membranes. Biochem. Biophys. Res. Comm. **328**, 1019-1023

12 Gellermann, G. P., Appel, T. R., Tannert, A., Radestock, A., Hortschansky, P., Schroeckh, V., Leisner, C., Lutkepohl, T., Shtrasburg, S., Rocken, C., Pras, M., Linke, R. P., Diekmann, S. and Fandrich, M. (2005) Raft lipids as common components of human extracellular amyloid fibrils. Proc. Natl. Acad. Sci. USA **102**, 6297-6302

13 Kakio, A., Nishimoto, S., Yanagisawa, K., Kozutsumi, Y. and Matsuzaki, K. (2002) Interactions of amyloid β-protein with various gangliosides in raft-like membranes: Importance of GM1 ganglioside-bound form as an endogenous seed for Alzheimer amyloid. Biochemistry **41**, 7385-7390

14 Kakio, A., Nishimoto, S., Yanagisawa, K., Kozutsumi, Y. and Matsuzaki, K. (2001) Cholesterol-dependent formation of GM1 ganglioside-bound amyloid β-protein, an endogenous seed for Alzheimer amyloid. J. Biol. Chem. **276**, 24985-24990

15 Wolozin, B. (2001) A fluid connection: Cholesterol and Aβ. Proc. Natl Acad. Sci. USA **98**, 5371-5373

16 Hou, L. M., Shao, H. Y., Zhang, Y. B., Li, H., Menon, N. K., Neuhaus, E. B., Brewer, J. M., Byeon, I. J. L., Ray, D. G., Vitek, M. P., Iwashita, T., Makula, R. A., Przybyla, A. B. and Zagorski, M. G. (2004) Solution NMR studies of the Aβ(1-40) and Aβ(1-42) peptides establish that the Met35 oxidation state affects the mechanism of amyloid formation. J. Amer. Chem. Soc. **126**, 1992-2005

17 Bai, Y. W., Milne, J. S., Mayne, L. and Englander, S. W. (1993) Primary structure effects on peptide group hydrogen exchange. Proteins: Struct. Funct. Genet. **17**, 75-86

18 Zhang, S., Iwata, K., Lachenmann, M. J., Peng, J. W., Li, S., Stimson, E. R., Lu, Y., Felix, A. M., Maggio, J. E. and Lee, J. P. (2000) The Alzheimer's peptide Aβ adopts a collapsed coil structure in water. J. Struct. Biol. **130**, 130-141

19 Basu, A. and Glew, R. H. (1985) Characterization of the activation of rat liver β-glucosidase by sialosylgangliotetraosylceramide. J. Biol. Chem. **260**, 3067-3073

20 Morrison, J., Yang, J. C., Stewart, M. and Neuhaus, D. (2003) Solution NMR study of the interaction between NTF2 and nucleoporin FxFG repeats. J. Mol. Biol. **333**, 587-603

21 Laguri, C., Phillips-Jones, M. K. and Williamson, M. P. (2003) Solution structure and DNA binding of the effector domain from the global regulator PrrA (RegA) from *Rhodobacter sphaeroides*: insights into DNA binding specificity. Nucleic Acids Res. **31**, 6778-6787

22 Ma, K., Clancy, E. L., Zhang, Y. B., Ray, D. G., Wollenberg, K. and Zagorski, M. G. (1999) Residue-specific pK_a measurements of the β-peptide and mechanism of pH-induced amyloid formation. J. Amer. Chem. Soc. **121**, 8698-8706

23 Choo-Smith, L. P., GarzonRodriguez, W., Glabe, C. G. and Surewicz, W. K. (1997) Acceleration of amyloid fibril formation by specific binding of Aβ-(1-40) peptide to ganglioside-containing membrane vesicles. J. Biol. Chem. **272**, 22987-22990

24 McLaurin, J., Franklin, T., Fraser, P. E. and Chakrabartty, A. (1998) Structural transitions associated with the interaction of Alzheimer β-amyloid peptides with gangliosides. J. Biol. Chem. **273**, 4506-4515

25 Bokvist, M., Lindstrom, F., Watts, A. and Gröbner, G. (2004) Two types of Alzheimer's β-amyloid (1-40) peptide membrane interactions: Aggregation preventing transmembrane anchoring Versus accelerated surface fibril formation. J. Mol. Biol. **335**, 1039-1049

26 Ariga, T., Kobayashi, K., Hasegawa, A., Kiso, M., Ishida, H. and Miyatake, T. (2001) Characterization of high-affinity binding between gangliosides and amyloid β-protein. Arch. Biochem. Biophys. **388**, 225-230

27 Choo-Smith, L. P. and Surewicz, W. K. (1997) The interaction between Alzheimer amyloid β(1-40) peptide and ganglioside G_{M1}-containing membranes. FEBS Letts. **402**, 95-98

28 Williamson, M. P. (1990) Secondary structure dependent chemical shifts in proteins. Biopolymers **29**, 1428-1431

29 Xu, X. P. and Case, D. A. (2002) Probing multiple effects on ^{15}N, $^{13}C\alpha$, $^{13}C\beta$, and $^{13}C'$ chemical shifts in peptides using density functional theory. Biopolymers **65**, 408-423

30 Charlton, A. J., Baxter, N. J., Khan, M. L., Moir, A. J. G., Haslam, E., Davies, A. P. and Williamson, M. P. (2002) Polyphenol/peptide binding and precipitation. J. Agric. Food Chem. **50**, 1593-1601

31 Cantu, L., Corti, M., Del Favero, E., Muller, E., Raudino, A. and Sonnino, S. (1999) Thermal hysteresis in ganglioside micelles investigated by differential scanning calorimetry and light-scattering. Langmuir **15**, 4975-4980

32 Kakio, A., Nishimoto, S. I., Kozutsumi, Y. and Matsuzaki, K. (2003) Formation of a membrane-active form of amyloid β-protein in raft-like model membranes. Biochem. Biophys. Res. Comm. **303**, 514-518

33 McLaurin, J. and Fraser, P. E. (2000) Effect of amino-acid substitutions on Alzheimer's amyloid-β peptide-glycosaminoglycan interactions. Eur. J. Biochem. **267**, 6353-6361

34 Mandal, P. K. and Pettegrew, J. W. (2004) Alzheimer's disease: NMR studies of asialo (GM1) and trisialo (GT1b) ganglioside interactions with Aβ(1-40) peptide in a membrane mimic environment. Neurochem. Res. **29**, 447-453

35 Brunden, K. R., Richter-Cook, N. J., Chaturvedi, N. and Frederickson, R. C. A. (1993) pH-dependent binding of synthetic β-amyloid peptides to glycosaminoglycans. J. Neurochem. **61**, 2147-2154

36 Giulian, D., Haverkamp, L. J., Yu, J. H., Karshin, M., Tom, D., Li, J., Kazanskaia, A., Kirkpatrick, J. and Roher, A. E. (1998) The HHQK domain of β-amyloid provides a structural basis for the immunopathology of Alzheimer's disease. J. Biol. Chem. **273**, 29719-29726

37 Matsuzaki, K. and Horikiri, C. (1999) Interactions of amyloid β-peptide (1-40) with ganglioside-containing membranes. Biochemistry **38**, 4137-4142

38 Kirkitadze, M. D., Condron, M. M. and Teplow, D. B. (2001) Identification and characterization of key kinetic intermediates in amyloid β-protein fibrillogenesis. J. Mol. Biol. **312**, 1103-1119

39 Selkoe, D. J. (1989) Biochemistry of altered brain proteins in Alzheimer's disease. Ann. Rev. Neurosci. **12**, 463-490

40 Yip, C. M. and McLaurin, J. (2001) Amyloid-β peptide assembly: A critical step in fibrillogenesis and membrane disruption. Biophys J. **80**, 1359-1371

41 Kokubo, H., Kayed, R., Glabe, C. G., Saido, T. C., Iwata, N., Helms, J. B. and Yamaguchi, H. (2005) Oligomeric proteins ultrastructurally localize to cell processes, especially to axon terminals with higher density, but not to lipid rafts in Tg2576 mouse brain. Brain Research **1045**, 224-228

42 Goldsbury, C., Frey, P., Olivieri, V., Aebi, U. and Müller, S. A. (2005) Multiple assembly pathways underlie amyloid-β fibril polymorphisms. J. Mol. Biol. **352**, 282-298

43 Walsh, D. M., Klyubin, I., Fadeeva, J. V., Rowan, M. J. and Selkoe, D. J. (2002) Amyloid-β oligomers: their production, toxicity and therapeutic inhibition. Biochem. Soc. Trans. **30**, 552-557

44 Brendza, R. P., Bacskai, B. J., Cirrito, J. R., Simmons, K. A., Skoch, J. M., Klunk, W. E., Mathis, C. A., Bales, K. R., Paul, S. M., Hyman, B. T. and Holtzman, D. M. (2005) Anti-Aβ antibody treatment promotes the rapid recovery of amyloid-associated neuritic dystrophy in PDAPP transgenic mice. J. Clin. Invest. **115**, 428-433

Figure legends

Figure 1 The structure of gangliosides GM1 and asialo-GM1.

Figure 2 HSQC spectrum of ^{15}N-labelled Aβ(1-40) in water, pH 7.2, 13 °C.
The small unlabelled peak at (^{1}H = 7.2, ^{15}N = 119 ppm) is probably an arginine sidechain NH.

Figure 3 Chemical shift changes in Aβ(1-40) on addition of GM1 to 200 μM peptide in water.
Each box shows results for a different residue, with ^{1}H shifts horizontal (increasing left to right, total range ± 0.02 ppm) and ^{15}N shifts vertical (increasing bottom to top, total range ± 0.1 ppm). This representation therefore resembles the change seen in an HSQC spectrum, except that the directions of the axes are reversed. The start of the titration is indicated with a filled circle, and subsequent titrations are 1, 2, 4 and 8 equivalents of GM1. The size of the circles approximates the experimental uncertainty. No data are shown for His6 because it could not be observed.

Figure 4 Chemical shift changes in Aβ(1-40) on addition of GM1 to 200 μM peptide in 150 mM NaCl.
Only the N-terminal residues are shown, because there were effectively no changes in the C-terminal residues. Titrations are 0, 1, 2, 4 and 6.6 equivalents of GM1. Other conditions are as for Figure 3.

Figure 5 Chemical shift changes in Aβ(1-40) on addition of asialo-GM1 to 200 μM peptide in water.
Other conditions are as for Figure 3, except that the chemical shift ranges in each box are ± 0.015 ppm for ^{1}H and ± 0.075 ppm for ^{15}N.

Figure 6 Chemical shift changes in Aβ(1-40) on addition of heparin to 200 μM peptide in water.
Other conditions are as for Figure 3, except that the chemical shift ranges are ± 0.05 ppm for ^{1}H and ± 0.3 ppm for ^{15}N.

Figure 7 Intensity changes of HSQC peaks on addition of GM1 micelles to Aβ(1-40).

The horizontal scale shows the molar ratio of GM1 to Aβ, with for each residue a range of 0 to 40 molar equivalents: the data are shown for 0, 1, 2, 4, 8, 12, 16, 20, 24, 28, 32, and 36 equivalents. The vertical scale shows the peak intensity relative to that in pure peptide, on a range from 0 to 100%. The intensity changes are fitted to an exponential decay.

Figure 8 Loss of intensity during the titrations of (A) GM1 and (B) asialo-GM1 into Aβ(1-40).

The bars show the decay constant from the exponential fits of the experimental data (Figure 7), with the estimated fitting error. The numerical values of the decay constants therefore have no direct meaning, but serve as a measure of the extent of intensity loss during the titration. Data for His14 are not shown in B, because the intensity of His14 is too weak to allow fitting of the data.

Figure 9 Titration of GM1 into Aβ(1-42).

HSQC spectra of Aβ(1-42) alone (left), with 8 mole equivalents of GM1 (centre) and 16 mole equivalents (right). The spectra were acquired sequentially, about 6 hours apart. The signals for glycines 9 and 29 are barely affected. The signal for glycine 38 is also not affected by the addition of GM1, but there is an independent and time-dependent loss of the signal from monomer (marked m) and increase of a signal from an oligomeric species (marked o). All signals are starting to decrease in the final spectrum because of precipitation of aggregates.

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