



Responsibility of lipid compositions for the amyloid β assembly induced by ganglioside nanoclusters in mouse synaptosomal membranes

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Abstract

The assembly of amyloid β protein (A β) on ganglioside-enriched microdomains in presynaptic neuronal membranes is involved in the onset of Alzheimer's disease. We previously found that a highly enriched ganglioside domain generated in a reconstituted lipid bilayer composed of synaptosomal lipids functioned as an A β -sensitive ganglioside nanocluster (ASIGN) that may induce a spherical A β assembly. In the present study, the major lipid components of a detergent-resistant membrane microdomain (DRM) fraction of synaptosomes prepared from an aged mouse brain were identified, and we demonstrate that the proportion of A β -insensitive gangliosides such as GM3 directly affects the A β assembly in synaptosomal membranes. Liquid chromatography coupled to mass spectrometry analyses indicated that ganglio a-series gangliosides (GM1, GM2, GM3, and GD1a) were abundant in DRM fractions. In addition, the GM3/GM1 ratio and the cholesterol content in the synaptosomal fraction differed from those in the non-synaptosomal fraction, in which GM1 and GM3 are sensitive and insensitive gangliosides, respectively. The highly enriched ganglioside nanocluster was identified by the surface topography of ternary mixed lipid bilayers composed of gangliosides, sphingomyelin, and cholesterol. Our results indicate that the composition of gangliosides is responsible for the characteristics of the nanocluster generated in the presynaptic neuronal membranes.

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Introduction

Senile plaques and brain atrophy are pathological hallmarks of Alzheimer's disease (AD), and amyloid β protein (A β) assemblies such as oligomers, protofibrils, and fibrils have been implicated in both hallmarks [1]. A β with 40–43 residues is generated from amyloid precursor proteins by the cleavage of proteases on cell membranes, in which A β (1–40) is abundant and A β (1–42) has high toxicity [2, 3]. A β spontaneously assembles into oligomers and fibrils [4], and this assembly is accelerated in the presence of gangliosides [5, 6] and metal ions [7].

A β fibril formation is accelerated by a ganglioside-bound A β (GA β) complex on neuronal membranes, in which GA β acts as an endogenous seed for A β assembly [5]. Gangliosides are abundant in the nervous system, and their behavior is often followed by cholera toxin B (CTB) subunit, which binds to GM1 [8]. Over the past two decades, evidence for ganglioside-induced A β assembly has been accumulating. We previously showed that a spherical A β assembly was generated on lipid membranes

composed of lipid extracts from the synaptosomes of aged mouse brains [9]. A reconstituted lipid membrane composed of the lipids isolated as the detergent-resistant membrane microdomain (DRM) fraction of synaptosomes was prepared, and A β interactions were investigated using atomic force microscopy (AFM). We found that a highly enriched GM1 domain generated in the membrane is an A β -sensitive ganglioside nanocluster (ASIGN) [10]. ASIGN was able to be labeled by CTB, but the lipid components of ASIGN were not identified, except GM1 [9].

We previously investigated the ability of gangliosides such as GM1, GM2, GM3, GD1a, GD1b, GT1b, and GQ1b to initiate A β assembly [10], and it was found that the generation and elongation of A β fibrils are dependent on the oligosaccharide structures of gangliosides. Since gangliosides are abundant in synaptosomal membranes, several kinds of gangliosides in ASIGN have the potential to be responsible for A β assembly. In the present study, the molecular species of gangliosides in the synaptosomal DRM lipids were identified using liquid chromatography coupled to mass spectrometry (LC-MS) analyses. Four types of gangliosides, GM1, GM2, GM3, and GD1a, were detected in synaptosomal DRM lipids, and the GM3/GM1 ratio was lower in the synaptosomal fraction than in the non-synaptosomal fraction. Furthermore, the contents of GM1, sphingomyelin (SM), and cholesterol (chol) in the DRM lipids were assessed by a TLC analysis. To construct a highly enriched ganglioside nanocluster that mimics the characteristic features of ASIGN, GM1-containing membranes were prepared by the accumulation of ternary mixed monolayers composed of GM1, SM, and chol. AFM images of the surface topography of the GM1-containing membrane indicated the formation of the nanometer-sized domain with several SM/chol ratios, and a highly enriched ganglioside nanocluster was identified by ganglioside cluster-binding peptides (GCBPs). These results indicate that a limited SM/chol ratio is required for membrane microdomain formation, and the composition of gangliosides, such as their GM3/GM1 ratio, is critical for the generation of ASIGN, which induces A β assembly.

Experimental procedures

Synaptosomal DRM lipids from an aged mouse brain

DRM lipids extracted from the synaptosomes and non-synaptosomes of an aged mouse were obtained as previously described [9]. Briefly, the DRM fraction (1.5 mL) that was obtained by sucrose density-gradient centrifugation in MES buffer saline was mixed with

chloroform/methanol (2:1), and DRM lipids were extracted into the organic phase. After the evaporation of the organic phase, a white lipid powder containing sucrose was obtained (DRM lipids). The white powder was dissolved in 0.5 mL of chloroform/methanol (1:1) and stored in a freezer until use.

LC-MS analysis of DRM lipids for ganglioside identification

The DRM lipids dissolved in chloroform/methanol were adsorbed to a discovery DSC-NH₂ SPE cartridge (Supelco, Sigma-Aldrich). After washing the cartridge with chloroform, neutral lipids were eluted with methanol. Acidic lipids were subsequently eluted with methanol/acetic acid/triethylamine (90:4:6, v/v/v).

Acidic lipids were desalted using a Sep-Pak C18 column (Waters) and analyzed by LC-MS as described previously [11]. An electrospray ionization/ion trap mass spectrometer (Esquire 3000 plus, Bruker Daltonics) was coupled online with an Agilent 1100 series LC system (Agilent Technologies). Desalted lipids (5 μ L in methanol) were loaded onto a 2.0 \times 150 mm UK-silica column (Imtakt Corp., Kyoto, Japan) that had been equilibrated with solvent A [chloroform/methanol/50 mM triethylamine–acetate buffer (pH 4.2) (83:16:1, v/v/v)] and were eluted with a 0–100% linear gradient of solvent B [methanol/50 mM triethylamine–acetate buffer (pH 4.2) (3:1, v/v)] for 45 min at a flow rate of 0.1 mL/min. The scan range was 100–2500 *m/z*. MS and MS² spectra (top two peaks) were collected in the AutoMS mode.

TLC analysis for GM1, SM, and chol identification

An aliquot of DRM lipids was spotted onto a high-performance thin-layer chromatography plate and developed with chloroform/methanol/0.2% CaCl₂ aq. (5:4:1, v/v/v). The quantification of lipids was performed by comparisons with commercially available GM1, SM, and chol standards (Sigma, St. Louis, MO, USA) developed on the same plates.

The level of GM1 was estimated by a TLC overlay assay [12, 13]. Briefly, the dried TLC plate was immersed in a solution of 0.4% poly(isobutyl methacrylate)/hexane for 30 s and blocked with 0.1% bovine serum albumin/phosphate-buffered saline (PBS) for 2 h. GM1 was labeled with a horseradish peroxidase (HRP)-conjugated CTB, and the band was visualized using a Konica Immunostain HRP-1000 Kit (Konica Minolta). The chromatograms were scanned using a CanoScan LiDE 600 F scanner (Canon, Inc., Tokyo, Japan) and analyzed using ImageJ software (National Institutes of Health).

To detect SM, the plate was sprayed with 10% CuSO_4 /8% H_3PO_4 solution and heated at 180 °C for 10–20 min. In the case of chol, the DRM lipids were developed with chloroform/hexane/diethyl ether/acetic acid (50:42:7.5:0.5, v/v/v/v), and chol was visualized with 10% H_2SO_4 . The target TLC bands were quantified with a CS-9300 chromatoscanner (Shimadzu Ltd., Kyoto, Japan).

Regarding the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis, the lipids of a target spot on the TLC plate were collected before staining. These lipids were analyzed using a 2,5-dihydroxybenzoic acid matrix in 0.1% trifluoroacetic acid in the reflectron negative ion mode [14].

Preparation of ganglioside-containing membranes and GCBP incubation

To prepare ganglioside-containing membranes, lipid bilayers were prepared on mica, as previously described [10]. Briefly, a 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) lipid monolayer at the air–water interface of a Langmuir–Blodgett trough was transferred to freshly cleaved mica (1×1 cm) by horizontal deposition at a surface pressure of 35 mN m^{-1} (POPC-coated mica). A mixed lipid monolayer composed of GM1/SM/chol (molar ratio) was loaded onto POPC-coated mica by horizontal deposition at a surface pressure of 30 mN m^{-1} to obtain a lipid bilayer (GM1-containing membrane). After washing three times with PBS, the membrane was analyzed by AFM.

To identify the ganglioside nanocluster, the GM1-containing membrane was incubated with a GCBP solution (10 μM) for 10 min prior to AFM measurements [15].

AFM measurements of ganglioside-containing membranes

To investigate the surface topography of GM1-containing membranes, AFM measurements were conducted using an SPM-9600 microscope (Shimadzu Co.), as previously described [10]. A topographic image (typically 1×1 μm) was recorded in the dynamic mode at a scanning rate in the range of 1–2 Hz by using a 38- μm -long soft cantilever with integrated pyramidal silicon nitride tips (spring constant of 0.1 N m^{-1}).

In the assessment of the GCBP-bound area, the AFM image was binarized based on the heights of the membrane, and pixels were counted using the image processing software GIMP portable (GNU Image Manipulation Program). The white area of an image binarized by thresholding at 5 nm from the bottom was identified as the GCBP-bound area on the membrane.

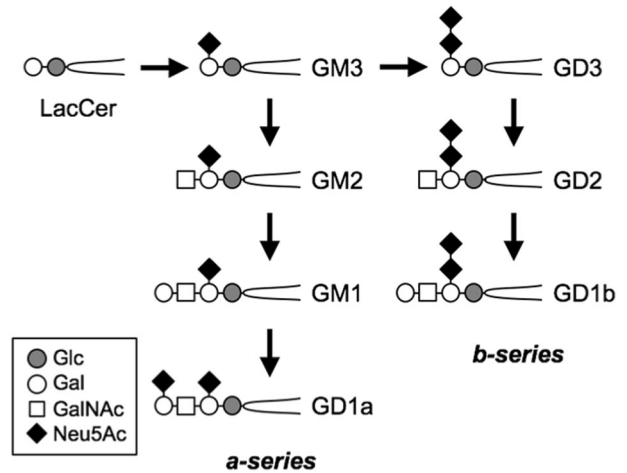


Fig. 1 Structure and biosynthetic pathway of a-series and b-series gangliosides. Glc glucose, Gal galactose, GalNAc *N*-acetylglactosamine, Neu5Ac *N*-acetylneuraminic acid

Results

Identification of gangliosides in synaptosomal DRM lipids by LC-MS analysis

Gangliosides (Fig. 1) are enriched in membrane microdomains, and a conformational change in $\text{A}\beta$ is induced by an ASIGN of neuronal membranes [9]. To identify $\text{A}\beta$ assembly-related lipid molecular species in ASIGN, DRM lipids extracted from aged mouse synaptosomes were analyzed by LC-MS [16]. Prior to the LC-MS analysis, acidic lipids were separated from neutral lipids.

Acidic lipids were analyzed by LC using a silica column, and five gangliosides with d18:1–C18:0 and/or d20:1–C18:0 ceramides in synaptosomal and non-synaptosomal DRM lipids were detected in the extracted ion chromatograms (EICs) (Fig. 2 and Figure S1). Based on the MS^2 spectra of each peak, GM3, GM2, and GM1 were identified (Figure S2 and Table S1). The structure of the peak at m/z 917.5, which corresponded to the $[\text{M}-2\text{H}]^{2-}$ ion of GD1x, and 32.2 min was not able to be identified from the MS^2 spectra, and thus, standard GD1a and GD1b were analyzed. Based on the retention times of the standard GD1a and GD1b peaks (32.5 and 31.2 min, respectively), the peak at m/z 917.5 at 32.2 min was assigned to GD1a (d18:1–C18:0) as the $[\text{M}-2\text{H}]^{2-}$ ion (Figure S3 and Table S2). The peak at m/z 1544.8 and 32.7 min was assigned to a post-source decay fragment of GD1a with d18:1–C18:0 ceramide as the $[\text{M}-\text{H}]^-$ ion. In all gangliosides detected, the major molecular species of ceramides were d18:1–C18:0 and d20:1–C18:0 (e.g., m/z 1544.9 and 1573.0 for GM1). These results indicated that a-series gangliosides, such as GM1, GM2, GM3, and GD1a, were abundant in both synaptosomes and non-synaptosomes.

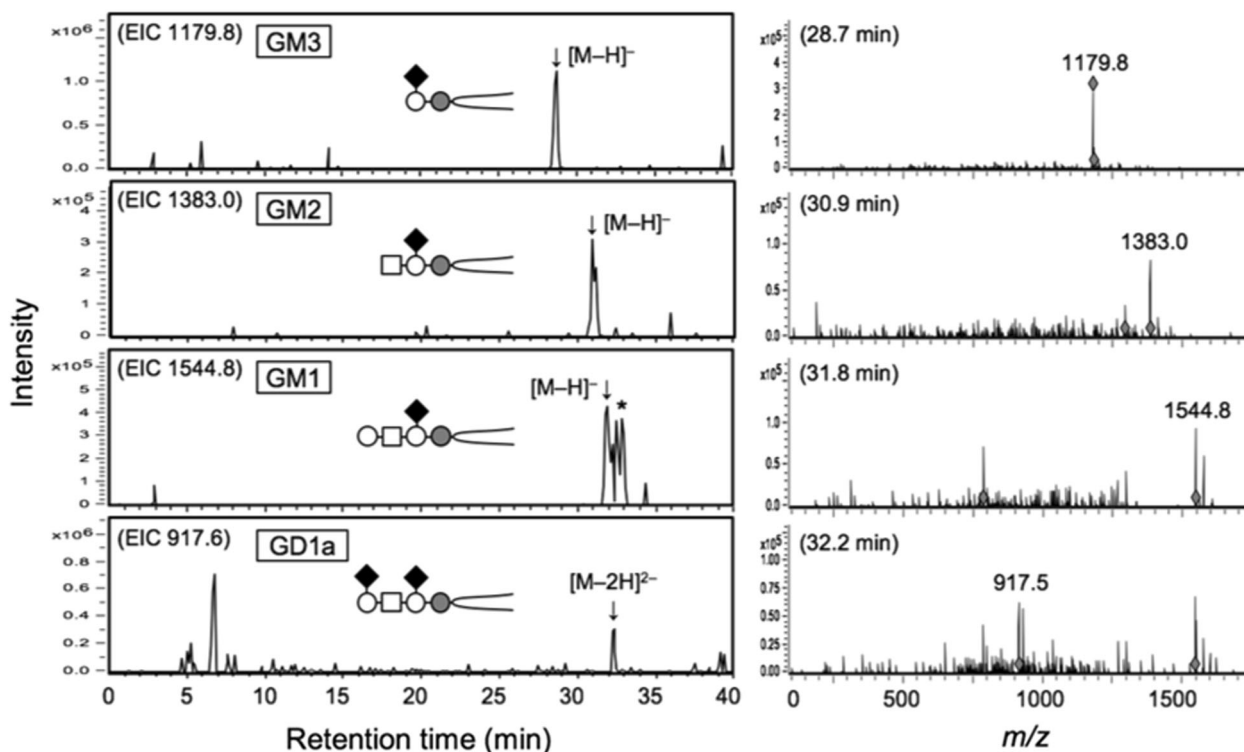


Fig. 2 Identification of gangliosides in the DRM lipids from mouse synaptosomal membranes by LC-MS. Extracted ion chromatograms (EICs, left) and mass spectra (right) of an acidic fraction of

synaptosomal DRM lipids. GM3, GM2, GM1, and GD1a composed of d18:1–C18:0 ceramides are shown. *, Post-source decay fragment of GD1a. Red diamonds, selected precursor ions for MS²

Table 1 Peak areas of major gangliosides (d18:1–C18:0) assessed by LC-MS

Ganglioside ^a	Ion type	<i>m/z</i> (calcd.)	Area (×10 ⁶)		Area ratio (ganglioside/GM1)	
			SPS	nSPS	SPS	nSPS
GM3	[M–H] [–]	1179.8	18.4	76.2	1.7	3.4
GM2	[M–H] [–]	1382.9	5.7	8.4	0.53	0.37
GM1	[M–H] [–]	1544.9	10.8	22.5	1.0	1.0
GD1a	[M–2H] ^{2–}	917.5	4.9	8.4	0.45	0.37

SPS synaptosome, nSPS non-synaptosome

^a Sphingosine–fatty acid (d18:1–C18:0)

Based on the peak area of GM1 in the EICs, a marked change was not observed in the composition of gangliosides between synaptosomes and non-synaptosomes, except for GM3 (area ratio of gangliosides to GM1, Table 1). The proportion of GM3 in synaptosomal DRM lipids (GM3/GM1 ratio, 1.7) was less than that in non-synaptosomal DRM lipids (3.4).

Assessment of the levels of GM1, SM, and chol by TLC analysis

Since CTB exhibits high affinity for the GM1 pentasaccharide [8, 17], the level of ganglioside GM1 in DRM

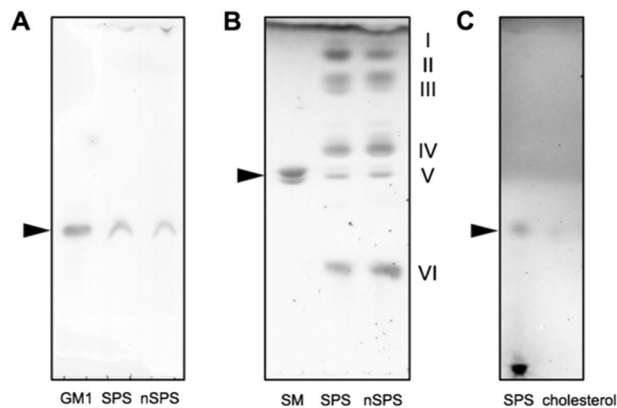


Fig. 3 TLC chromatograms of GM1, sphingomyelin, and cholesterol in DRM lipids isolated from synaptosomes. **a** Detection of GM1 by a TLC overlay assay with CTB-HRP. An aliquot of DRM lipids was separated by TLC in chloroform/methanol/0.2% CaCl₂ (5:4:1, v/v/v), and GM1 was then visualized by the binding of CTB-HRP. Lane GM1, GM1 standard (10 nmol). **b** Detection of SM by TLC. An aliquot of DRM lipids was separated by TLC in chloroform/methanol/0.2% CaCl₂ (5:4:1, v/v/v), and lipids were then visualized with 10% CuSO₄ in 8% H₃PO₄. Lane SM, SM standard (10 nmol). Assignment of bands I–VI is discussed under “Results”. **c** Detection of cholesterol by TLC. An aliquot of DRM lipids was separated by TLC in chloroform/hexane/diethyl ether/acetic acid (50:42:7.5:0.5, v/v/v/v), and then lipids were visualized with 10% H₂SO₄. Lane cholesterol, cholesterol standard (1 nmol). Arrows indicate the locations of GM1, SM, and cholesterol. Lane SPS, DRM lipids from the synaptosomal fraction (60 μL); lane nSPS, DRM lipids from the non-synaptosomal fraction (60 μL)

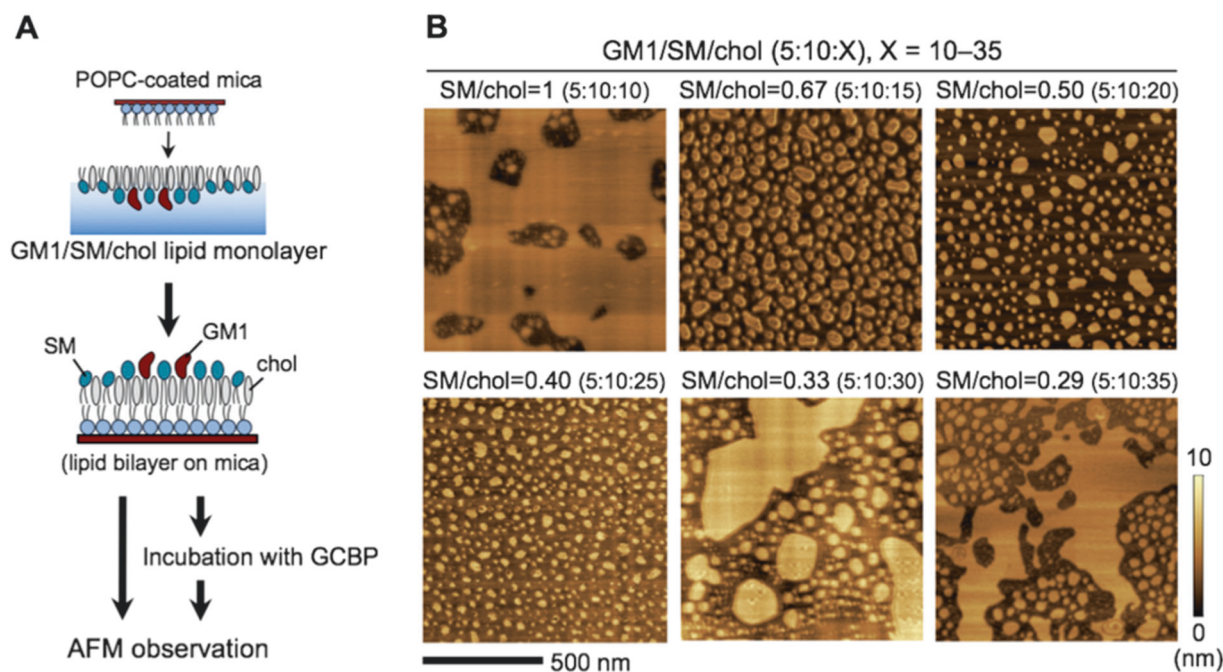


Fig. 4 Surface topography of GM1-containing membranes (GM1/SM/cho) depending on the SM/cho ratio. **a** Preparation of GM1-containing membranes for AFM measurements. A lipid monolayer of GM1/SM/cho was loaded onto POPC-coated mica to obtain a lipid bilayer (GM1-containing membrane). **b** AFM images of GM1-

containing membranes composed of GM1/SM/cho (5:10: X , $X = 10-35$). The molar ratios of SM/cho are 1 (10:10), 0.67 (10:15), 0.50 (10:20), 0.40 (10:25), 0.33 (10:30), and 0.29 (10:35). AFM images suggested that GM1 is enriched in the higher area

lipids was estimated by the TLC overlay assay using the CTB-peroxidase conjugate (CTB-HRP) [13]. GM1 levels in synaptosomal and non-synaptosomal DRM lipids were estimated to be 0.87 and 0.62 nmol/mL, respectively (Fig. 3a and Figure S4A). As reported previously, no significant differences were noted in the GM1 levels between the synaptosomal and non-synaptosomal fractions [9, 18].

The major components of the membrane microdomain (membrane rafts) are chol and sphingolipids including SM [19–21]. All lipid components of DRM lipids were developed, and six major bands were colored by CuSO_4 staining (Fig. 3b). The lipids of each band were extracted, and band V was found to be SM with a d18:1-C18:0 ceramide ($[\text{M} + \text{H}]^+$, m/z 732.0) identified by MALDI-TOF MS analysis (data not shown). SM levels were assessed from the intensity of band V, and 40–47 nmol of SM was found to be contained in 1 mL of the DRM fractions (Figure S4B). Different from the assignment of standard lipids, bands I–IV and VI were assigned to phospholipids and sucrose, respectively (data not shown) [22]. On the other hand, H_2SO_4 staining showed that synaptosomal and non-synaptosomal DRM lipids contained 86 and 26 nmol/mL chol, respectively. The TLC results indicated that the content of chol was threefold higher in synaptosomes than in non-synaptosomes (Table S3).

Based on these results, the GM1/SM/cho ratios in synaptosomal and non-synaptosomal DRM lipids were

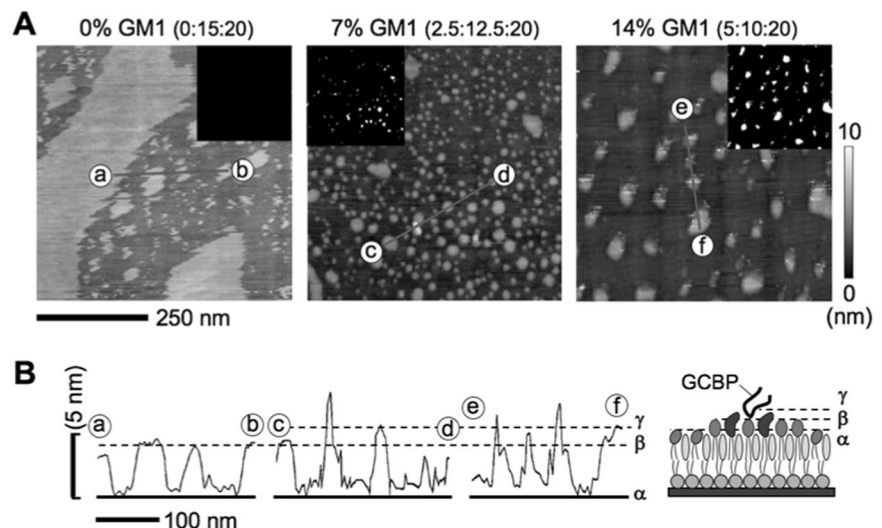
1:46:99 and 1:76:42, respectively (Table S3). The SM/cho ratio of 0.47 in synaptosomes was markedly different from that of 1.8 in non-synaptosomes.

Influence on the ratio of SM to chol for domain formation

LC-MS and TLC results indicated that no significant differences existed in the molecular species of gangliosides between the DRM lipids extracted from synaptosomes and non-synaptosomes. To clarify the influence of the lipid proportion on the formation of ganglioside clusters, the molecular ratio of SM to chol (SM/cho ratio) was changed, and the domain formation in the membrane was observed by AFM [20].

The GM1-containing membrane was prepared by the accumulation of the ternary lipid monolayer composed of GM1/SM/cho (5:10: X , $X = 0, 10, 15, 20, 25, 30$, and 35) (molar ratio) on POPC-coated mica, and the surface topography of the bilayer was observed by AFM (Fig. 4a) [10]. The surface topographies of the GM1/SM/cho membranes markedly changed depending on the SM/cho ratio, and domains of 10–100 nm in diameter were found in the membranes at an SM/cho ratio of 0.67–0.40 (Fig. 4b). These domains were clearly observed in the GM1/SM/cho (5:10:20) membrane, in which this composition corresponded to the SM/cho ratio (0.47) in

Fig. 5 Surface topography of GM1-containing membranes after the incubation of GCBP. **a** AFM images of 0–14 mol% GM1-containing membranes (GM1/SM/chol; 0:15:20, 2.5:12.5:20, and 5:10:20). Binarized AFM images (threshold, 6 nm) indicate the GCBP-bound area (inset). **b** Section analysis plots (a–b, c–d, and e–f). The height of domain γ (GM1-enriched domain) was higher than that of domain β in the SM/chol membrane. The binding of GCBP with >6 nm was observed on the γ domain



synaptosomal DRM lipids in the TLC analysis (Table S3).

Detection of ganglioside clusters in GM1/SM/chol membranes by GCBP labeling

GCBP [15] has the ability to detect the high-density GM1 sites on the presynaptic neuritic terminus of PC12 cells [9, 18]. To mimic the high-density GM1 site in presynaptic membranes, GM1/SM/chol membranes with compositions of 0:15:20 (without GM1), 2.5:12.5:20 (7% GM1), and 5:10:20 (14% GM1) were prepared. In the absence of GM1, an SM/chol (15:20) membrane showed two phases (Fig. 5a). Domain β , which had a height of 4 nm, was distinguished from the surrounding phase (area α) (section analysis, Fig. 5b).

In the presence of GM1 (GM1/SM/chol, 5:10:20), domain γ with a height of 5–6 nm was observed and presumed to be the GM1-enriched phase (Fig. 4b) [9, 10]. The binding of GCBP was observed on domain γ , and the area of GCBP that bound depended on the GM1 content (Fig. 5a, inset). The section analysis indicated that the binding of GCBP was observed at a height of >6 nm as small dots (Fig. 5b). These results indicate that ganglioside clusters were generated in SM/chol membranes.

Discussion

We previously reported A β assembly on lipid membranes composed of the DRM lipid extracts from the synaptosomes of aged mouse brains by AFM [9]. GM1, SM, chol, and phospholipid levels in DRM lipids were measured [18]; however, other lipid components were not identified. In the present study, the ganglioside species in DRM lipids were

identified by LC-MS analysis, and GM1, GM2, GM3, and GD1a were found in both synaptosomal and non-synaptosomal DRM lipids (Fig. 2 and Figure S3). We initially focused on the fatty acids and sphingosines of ceramides because Taki and co-workers reported that the level of d18:1 sphingosine-containing GM1 isolated from the brain of AD patients was higher than that of d20:1 GM1 [23]. In addition, the ratio of GD1b (d20:1–20:0) to GD1b (d20:1–18:0) has been shown to influence A β assembly in an amyloid-bearing precuneus in the human brain [24]. However, no significant differences were observed in ceramide species among gangliosides in both synaptosomal and non-synaptosomal DRM lipids (Table S1). These results indicated that the ganglioside species and variety of ceramides are not directly responsible for the characteristics of membranes composed of DRM lipids.

The GM1, SM, and chol levels in the DRM lipids were subsequently assessed by TLC analysis. As reported previously, no significant differences were observed in the GM1 or SM levels of DRM lipids between synaptosomes and non-synaptosomes (Fig. 3 and Table S3) [9, 18]. On the other hand, the content of chol was threefold higher in synaptosomal DRM lipids than in non-synaptosomal DRM lipids (Table S3). Apolipoprotein E4 (apoE4) knock-in mice showed a twofold higher content of chol than wild-type mice, and the inheritance of apoE4 was identified as a risk factor for the development of AD [25]. The membrane microdomain is composed of SM and chol [21]. The chol-dependent formation of domains was investigated by AFM of GM1/SM/chol (5:10:X, X = 10–35) membranes, and the results obtained indicated that a high content of chol (low SM/chol ratio) was largely responsible for domain formation (Fig. 4). The membrane microdomain was clearly observed in chol-enriched membranes (chol > SM), and the formation of

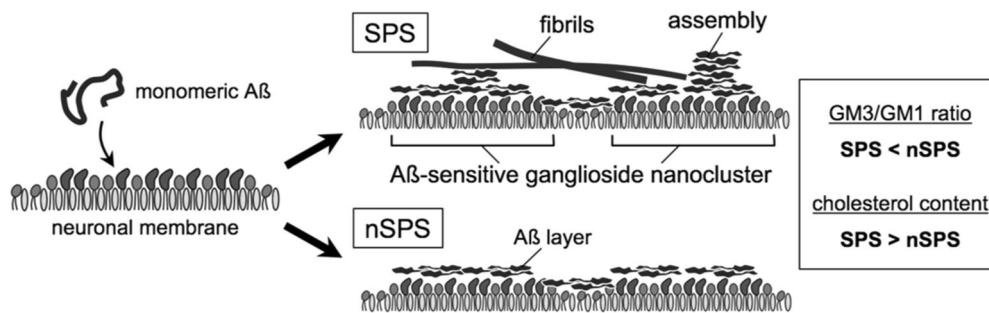


Fig. 6 A proposed model for the formation of ganglioside-induced A β assembly on ganglioside nanoclusters in the aged mouse brain. An A β -sensitive ganglioside nanocluster (ASIGN) containing GM1, GM2, GM3, and GD1a induces A β assembly and fibril formation. The GM3/

GM1 ratio of the synaptosomal membrane (SPS) is less than that of the non-synaptosomal membrane (nSPS), in which GM3 is insensitive for A β assembly

ganglioside clusters was assessed in the presence of GM1 by the binding of GCBP (Fig. 5).

In terms of the levels of gangliosides, changes were noted in the proportion of GM3 in DRM lipids; the area ratios of GM3 to GM1 in the DRM lipids of synaptosomes and non-synaptosomes were 1.7 and 3.4, respectively (Table 1). We recently showed that GM1, GM2, GD1a, GD1b, and GT1b induce the formation of A β fibrils on ganglioside-containing membranes, whereas GM3 is A β -insensitive [10]. These findings suggest that less A β assembly on the membranes of non-synaptosomal DRM lipids was caused by an increase in GM3 (Fig. 6). Due to their small contents in DRM lipids, GD1b and GT1b were not detected by the LC-MS analysis.

Conclusion

To elucidate the reasons for the characteristic features of ASIGN, the lipid components of DRM lipids from synaptosomes and non-synaptosomes were examined by LC-MS and TLC analyses. The content of chol and the ratio of GM3/GM1 in synaptosomal DRM lipids differed from those in non-synaptosomal DRM lipids. The non-synaptosomal membrane contains high contents of A β -insensitive GM3, suggesting that GM3 suppresses the A β assembly. These results indicate that the compositions of SM, chol, and gangliosides in the domain are critical to the feature of ASIGN that induces A β assembly, and these results may contribute to the development of novel anti-AD therapies.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Sipe JD, Cohen AS. Review: history of the amyloid fibril. *J Struct Biol*. 2000;130:88–98.
- Burdick D, Soreghan B, Kwon M, Kosmoski J, Knauer M, Henschen A, Yates J, Cotman C, Glabe C. Assembly and aggregation properties of synthetic Alzheimer's A4/beta amyloid peptide analogs. *J Biol Chem*. 1992;267:546–54.
- Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science*. 2002;297:353–6.
- Nichols MR, Moss MA, Reed DK, Cratic-McDaniel S, Hoh JH, Rosenberry TL. Amyloid-beta protofibrils differ from amyloid-beta aggregates induced in dilute hexafluoroisopropanol in stability and morphology. *J Biol Chem*. 2005;280:2471–80.
- Yanagisawa K. Pathological significance of ganglioside clusters in Alzheimer's disease. *J Neurochem*. 2011;116:806–12.
- Yanagisawa K, Odaka A, Suzuki N, Ihara Y. GM1 ganglioside-bound amyloid beta-protein (A β): a possible form of pre-amyloid in Alzheimer's disease. *Nat Med*. 1995;1:1062–6.
- Bush AI, Pettingell WH, Multhaup G, d Paradis M, Vonsattel JP, Gusella JF, Beyreuther K, Masters CL, Tanzi RE. Rapid induction of Alzheimer A beta amyloid formation by zinc. *Science*. 1994;265:1464–7.
- Merritt EA, Sarfaty S, van den Akker F, L'Hoir C, Martial JA, Hol WG. Crystal structure of cholera toxin B-pentamer bound to receptor GM1 pentasaccharide. *Protein Sci*. 1994;3:166–75.
- Matsubara T, Iijima K, Yamamoto N, Yanagisawa K, Sato T. Density of GM1 in nanoclusters is a critical factor in the formation of a spherical assembly of amyloid beta-protein on synaptic plasma membranes. *Langmuir*. 2013;29:2258–64.
- Matsubara T, Nishihara M, Yasumori H, Nakai M, Yanagisawa K, Sato T. Size and shape of amyloid fibrils induced by ganglioside nanoclusters: role of sialyl oligosaccharide in fibril formation. *Langmuir*. 2017;33:13874–81.
- Wang Y, Kumazawa T, Shiba K, Osumi K, Mizuno M, Sato T. Glycosylation of Nalpha-lauryl-O-(beta-d-xylopyranosyl)-l-serinamide as a saccharide primer in cells. *Carbohydr Res*. 2012;361:33–40.
- Wu GS, Ledeen R. Quantification of gangliotetraose gangliosides with cholera toxin. *Anal Biochem*. 1988;173:368–75.

13. Muthing J. High-resolution thin-layer chromatography of gangliosides. *J Chromatogr A*. 1996;720:3–25.
14. Colsch B, Woods AS. Localization and imaging of sialylated glycosphingolipids in brain tissue sections by MALDI mass spectrometry. *Glycobiology*. 2010;20:661–7.
15. Matsubara T, Iijima K, Nakamura M, Taki T, Okahata Y, Sato T. Specific binding of GM1-binding peptides to high-density GM1 in lipid membranes. *Langmuir*. 2007;23:708–14.
16. Ikeda K, Shimizu T, Taguchi R. Targeted analysis of ganglioside and sulfatide molecular species by LC/ESI-MS/MS with theoretically expanded multiple reaction monitoring. *J Lipid Res*. 2008;49:2678–89.
17. Harder T, Scheiffele P, Verkade P, Simons K. Lipid domain structure of the plasma membrane revealed by patching of membrane components. *J Cell Biol*. 1998;141:929–42.
18. Yamamoto N, Matsubara T, Sato T, Yanagisawa K. Age-dependent high-density clustering of GM1 ganglioside at pre-synaptic neuritic terminals promotes amyloid beta-protein fibrillogenesis. *Biochim Biophys Acta*. 2008;1778:2717–26.
19. Sonnino S, Prinetti A, Mauri L, Chigorno V, Tettamanti G. Dynamic and structural properties of sphingolipids as driving forces for the formation of membrane domains. *Chem Rev*. 2006;106:2111–25.
20. Prinetti A, Chigorno V, Tettamanti G, Sonnino S. Sphingolipid-enriched membrane domains from rat cerebellar granule cells differentiated in culture. A compositional study. *J Biol Chem*. 2000;275:11658–65.
21. Simons K, Ikonen E. Functional rafts in cell membranes. *Nature*. 1997;387:569–72.
22. Iwabuchi K, Handa K, Hakomori S. Separation of “glycosphingolipid signaling domain” from caveolin-containing membrane fraction in mouse melanoma B16 cells and its role in cell adhesion coupled with signaling. *J Biol Chem*. 1998;273:33766–73.
23. Valdes-Gonzalez T, Goto-Inoue N, Hirano W, Ishiyama H, Hayasaka T, Setou M, Taki T. New approach for glyco- and lipidomics—molecular scanning of human brain gangliosides by TLC-Blot and MALDI-QIT-TOF MS. *J Neurochem*. 2011;116:678–83.
24. Oikawa N, Matsubara T, Fukuda R, Yasumori H, Hatsuta H, Murayama S, Sato T, Suzuki A, Yanagisawa K. Imbalance in fatty-acid-chain length of gangliosides triggers Alzheimer amyloid deposition in the precuneus. *PLoS ONE*. 2015;10:e0121356. 10
25. Hayashi H, Igbavboa U, Hamanaka H, Kobayashi M, Fujita SC, Wood WG, Yanagisawa K. Cholesterol is increased in the exofacial leaflet of synaptic plasma membranes of human apolipoprotein E4 knock-in mice. *Neuroreport*. 2002;13:383–6.