Glycoconj J (2015) 32:105–112
DOI 10.1007/s10719-015-9587-5

MINI-REVIEW

Structural and dynamic views of GM1 ganglioside

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Received: 12 March 2015 / Accepted: 30 March 2015 / Published online: 1 May 2015

Abstract The ganglioside GM1 mediates various physiological and pathological processes mainly through the formation of GM1 clusters on cell surfaces. Therefore, detailed characterization of conformational properties of the glycan moiety of GM1 and the structures and interactions of this glycosphingolipid in membrane environments is necessary for better understanding of the clustering-coupled functional promotion. Nuclear magnetic resonance (NMR) spectroscopy has provided conformational information of GM1 in solution as well as in membrane-like environments. Recently, sophisticated paramagnetism-assisted NMR approaches combined with molecular dynamics simulations have enabled the quantitative exploration of conformational spaces of a series of gangliosides, including GM1, taking into account their minor conformations. NMR techniques have also been successfully applied to investigations of the dynamic interactions of GM1 clusters with amyloidogenic proteins such as amyloid β and α-synuclein associated with neurodegenerative disorders. Further integration of experimental and computational approaches will open up new possibilities to provide structural views of the more complicated heterogeneous systems exemplified by microdomains involving GM1.

Keywords Ganglioside GM1 · Nuclear magnetic resonance spectroscopy · Molecular dynamics simulation · Amyloid β · α-synuclein · Bicelle

Abbreviations

Aβ	amyloid β-peptide
αSN	α-synuclein
Chol	cholesterol
DHPC	1,2-dihexanoyl-*sn*-glycero-3-phosphocholine
DMPC	1,2-dimyristoyl-*sn*-glycero-3-phosphocholine
DMSO	determined in dimethyl-d_6-sulfoxide
DPC	dodecylphosphocholine
MD	molecular dynamics
NMR	nuclear magnetic resonance
NOEs	nuclear Overhauser effects
PCS	pseudocontact shift
PRE	paramagnetic relaxation enhancement
REMD	replica-exchange molecular dynamics
SM	sphingomyelin
3D	three-dimensional

Introduction

Glycosphingolipids were initially discovered by Thudichum who identified sphingosine as their primary part and named it after the mythological riddle of Sphinx because of its enigmatic nature [1]. At present, it is widely known that glycosphingolipids, including gangliosides, play physiological and pathological roles in animal cells as receptors for microbial toxins, mediators of cell adhesion, and modulators of signal transduction [2]. GM1, a ganglioside abundant in neuronal membranes, is one of the most extensively studied glycosphingolipids (Fig. 1a). This glycolipid serves as a target of growth-regulatory galectin-1 [3], the nerve growth factor

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receptor Trk [4], cholera toxin [5], and auto-antibodies that are associated with Guillain–Barré syndrome [6]. The various functions of gangliosides in physiological and pathological contexts are thought to be based on their assembling properties on cell surfaces. Recently, growing evidence has indicated that GM1 clusters on neuronal cell surfaces offer unique platforms for binding coupled with the conformational transition of amyloidogenic proteins involved in neurodegenerative diseases, *e.g.*, amyloid β (Aβ) in Alzheimer's disease [7, 8]. Therefore, GM1 can be a potential therapeutic target for various intractable diseases. For better understanding of the mechanisms underlying such a clustering-coupled functional promotion, it is highly desirable to obtain detailed conformational information of the carbohydrate moieties of this molecule and to provide in-depth descriptions of the structures and interactions of gangliosides on membranes.

Recently, sophisticated nuclear magnetic resonance (NMR) techniques typified by paramagnetism-assisted approaches along with computational methods have successfully provided structural information of gangliosides, including GM1, in solution as well as in membrane-like environments. This article outlines the cutting-edge views of conformations and interactions of GM1 ganglioside, highlighting its dynamic properties.

Dynamic structure of GM1 glycan

The conformations of several gangliosides have been traditionally characterized by NMR spectroscopy, primarily on the basis of nuclear Overhauser effects (NOEs) observed between sugar residues of the liberated pentasaccharides [9–11] as well as the entire molecules in aqueous micelles [12, 13] and organic solvents [14, 15]. The overall conformations of the GM1 glycan determined to date by inspecting the NMR data have been quite consistent with each other, except for that determined in dimethyl-d_6-sulfoxide (DMSO). Namely, the core -GalNAc^{III}-(NeuAc)-Gal^{II}- part exhibits a well-defined bouquet-like structure by the interaction of NeuAc with the GalNAc^{III} residue. This structure is consistent with the solvent accessibility of the amide groups of the sugar residues estimated by the inspection of the water saturation effects, indicating that the amide group of NeuAc is more exposed to the aqueous environment than the amide group of GalNAc^{III} [13].

On the other hand, crystallographic studies have visualized three-dimensional (3D) structures of the GM1 pentasaccharide recognized by cholera toxin, AB_5 toxin, and polyoma virus [5, 16, 17]. In these structures, the outer branch of the carbohydrate chain is involved in the interactions with these hazardous binders. The pentasaccharide conformations differ among these complexes and are deviated from the NOE-derived NMR structures, suggesting that GM1 glycan undergoes conformational alteration upon binding to proteins in a ligand-dependent manner.

Recently, a paramagnetism-assisted NMR technique has been developed for the conformational characterization of oligosaccharides in solution. This technique exploits paramagnetic probes such as lanthanide tags and spin labels attached to the reducing end of the oligosaccharide, which can induce paramagnetic effects such as pseudocontact shift (PCS) and paramagnetic relaxation enhancement (PRE) as NOE-independent sources of long-distance information [18–22]. In general, NMR data of flexible oligosaccharides should be interpreted as the population-weighted average derived from a dynamic conformational ensemble rather than a limited number of conformational states. Therefore, the paramagnetic NMR data were used for experimental validation of a conformational space proposed by theoretical approaches. A PCS-based NMR experiment in conjunction with molecular dynamics (MD) simulation was applied to conformational characterization of a series of ganglioside oligosaccharides, including the GM1 pentasaccharide [23–25]. Considerably long calculation times were necessary for exploration of the conformational spaces of these oligosaccharides by MD simulation with explicit water molecules. For example, replica-exchange MD (REMD) simulation was employed to obtain experimentally validated torsion angle density maps of the GM1 pentasaccharide [25]. Figure 1b compares the PCS-validated conformational spaces of oligosaccharides derived from three gangliosides, GM1, GM2, and GM3, indicating similarities between the GM1 pentasaccharide and the GM2 tetrasaccharide in terms of the conformational spaces of their common parts, *i.e.*, the Gal^{II}-Glc^{I}, NeuAc-Gal^{II}, and GalNAc^{III}-Gal^{II} glycosidic linkage conformations. In contrast, the GM3 trisaccharide exhibits a distinct conformational ensemble regarding the NeuAc-Gal^{II} glycosidic linkage. In the GM3 trisaccharide, the linkage conformation is most highly populated in the cluster $(\phi, \psi)=(45\pm11, -177\pm11°)$, whereas the corresponding conformational cluster is missing for the sialyl linkage of the GM1 and GM2 oligosaccharides. These data indicate that the GalNAc^{III} residue restricts the conformational freedom of the NeuAc-Gal^{II} glycosidic linkage in these branched glycans, whereas the outermost Gal^{IV} residue has no significant impact on the conformation of the remaining parts of the carbohydrate moiety in GM1.

Although the experimentally validated conformational landscape is qualitatively consistent with the previously reported φ-ψ maps regarding the major conformations, which correspond to the previously reported NOE-based structures, it offers more quantitative views of the individual conformer populations, including those of the minor conformers (Fig. 1b). The ligand-bound conformers of the GM1 pentasaccharide observed in crystal structures were all identified in the conformational ensemble thus obtained, suggesting that the specific conformations are selected from the dynamic ensemble upon binding (Fig. 1c and d).

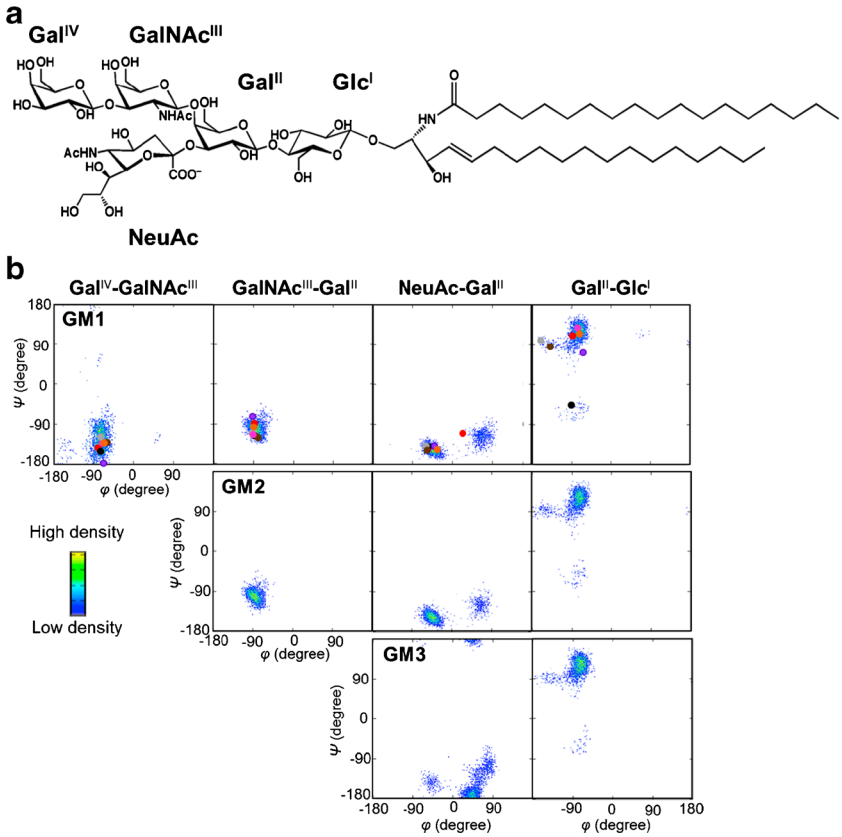

Fig. 1 **a** Structure of GM1 ganglioside. **b** Density map of glycosidic linkage torsion angles of the experimentally validated MD trajectory of the GM1, GM2, and GM3 oligosaccharides. The glycosidic torsions of the carbohydrate moieties of GM1 in DMSO calculated by NOE-based molecular mechanics [10] (●), the carbohydrate moieties of GM1 in aqueous solution calculated by NOE-based molecular modeling [9] (●), and GM1-acetyl in aqueous solution calculated by MD simulation [11] (●) with those of the GM1 pentasaccharide in complex with cholera toxin [PDB code: 2CHB (●) and 3CHB (●)]; polyoma virus [3BWR (●)]; AB_5 toxin [4L6T (●)]. The definitions of ϕ and ψ were used for the Gal^{IV}-$GalNAc^{III}$, $GalNAc^{III}$-Gal^{II}, and Gal^{II}-Glc^{I} linkage, (ϕ = O5-C1-O1-C'n, ψ=C1-O1-C'n-C'n-1) and for the NeuAc-Gal^{II} linkage (ϕ = O6-C2-O1-C'n, ψ = C2-O1-C'n-C'n-1). This figure was from Zhang *et al.* [25] and parts of this figure were originally reproduced from Yamamoto *et al.* [23] and Zhang *et al.* [24] with permissions from Springer, the Royal Society of Chemistry and MDPI, respectively. **c** Superimposition of 200 conformers extracted from the PCS-validated results of the replica exchange MD simulation of GM1 oligosaccharide (*left*) and Comparison of the 3D structures of the carbohydrate moieties of GM1 obtained by crystallographic analyses [PDB code: 2CHB (*brown*), 3CHR (*gray*), 3BWR (*black*), and 4L6T (magenta)] (*right*). Four structures are superimposed by fitting the ring atom of the reducing-terminal Glc^{I} residue. **d** Each crystal structure of the GM1 pentasaccharide superposed on the lowest-rmsd structure selected from the conformational ensemble

Structures and interactions of GM1 in membrane environments

NMR spectroscopy is potentially useful for the characterization of the structure and dynamics of the glycolipid assemblies if one could design and create appropriate membrane mimics suitable for sophisticated high-resolution spectral measurements. NMR studies indicated that the glycan conformation of GM1 displayed on its aqueous micelles is almost identical to that of the liberated pentasaccharide [12, 13]. Possible effects of the phospholipid membrane surface on the conformation of gangliosides embedded therein were examined by NMR spectroscopy using small mixed micelles composed of dodecylphosphocholine (DPC) and GM1 in aqueous solution [10, 26]. The results showed that the glycosidic linkage conformations of Gal^{II}-Glc^{I} and Gal^{IV}-$GalNAc^{III}$ are significantly altered in DPC micelles. MD simulation indicated that conformations of the Gal^{II}-Glc^{I} and Gal^{IV}-$GalNAc^{III}$ linkages are restricted in a dipalmitoylphosphatidylcholine bilayer, leaving the Glc^{I} residue fully extended away from the bilayer surface [27].

In addition, the conformation of GM1 embedded in membrane-mimicking phospholipid bicelles was studied by paramagnetism-assisted NMR experiments in conjunction with MD simulations [28]. In this study, PREs were measured for the membrane-embedded GM1 molecules employing both water-soluble and membrane-anchored paramagnetic probes. MD simulations based on the PRE data revealed that in GM1 in the membrane environment, the Gal^{IV} and NeuAc residues have higher flexibility than the other portions of the glycan. In addition, these two residues exhibited larger PRE effects by the water-soluble paramagnetic probe, indicating that they

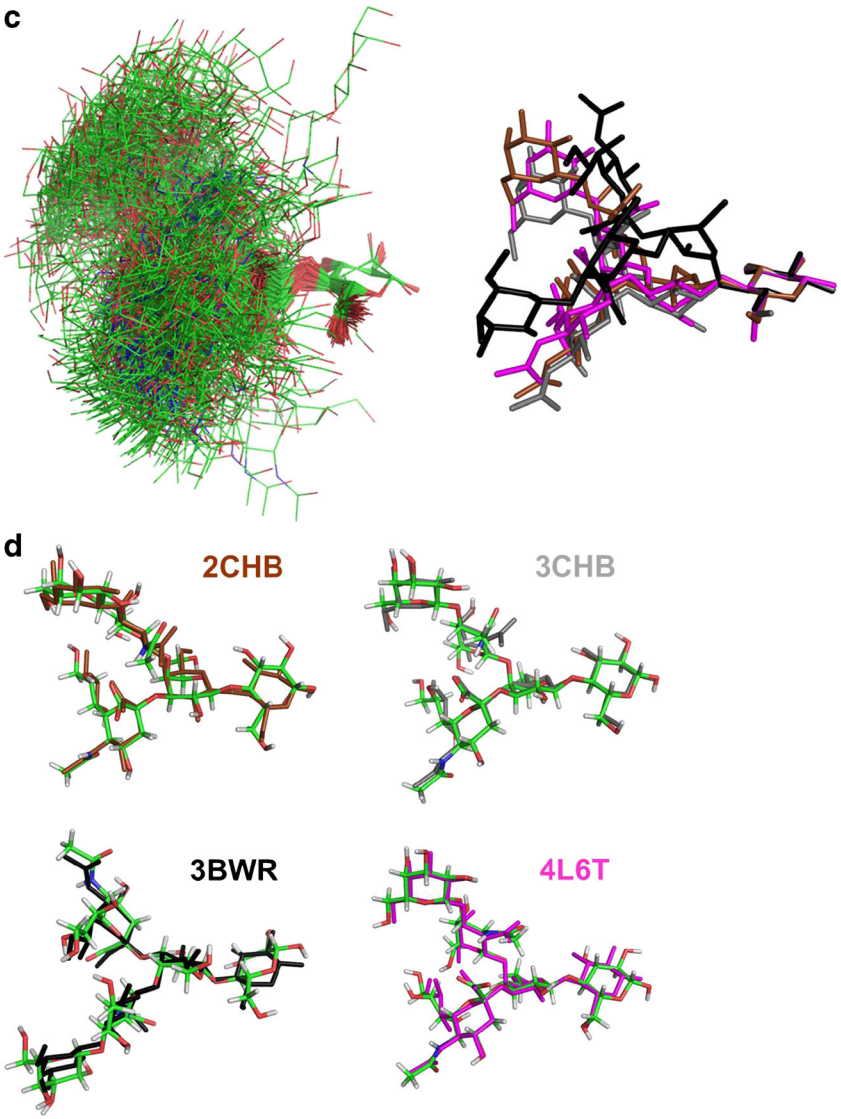

Fig. 1 (continued)

were most accessible in the membrane-embedded form of GM1, consistent with their involvement in interactions with the toxins and viruses as the primary acceptor sites.

A series of NMR studies have characterized the interactions of GM1 clusters with Aβ using aqueous micelles composed of GM1 or lyso-GM1 [13, 29, 30]. The PRE and chemical shift perturbation data underscore the importance of the sugar–lipid interface of the ganglioside clusters for accommodating Aβ [13]. The chemical shift titration and transverse relaxation-optimized spectroscopy-based saturation transfer data have indicated that the interaction between Aβ and the GM1 micelles involves multiple steps, including the initial encounter complex formation and the accommodation process for the two α-helices and the C-terminal Val39–Val40 dipeptide segment in the hydrophobic interior [29] (Fig. 2a). It was further demonstrated that Aβ exhibits a β-structure at higher densities on the GM1 micelles [30]. These findings suggest that the ganglioside clusters offer a unique platform at their hydrophobic/hydrophilic interface for binding coupled with α-helix formation of Aβ molecules, thereby restricting their spatial rearrangements to promote specific intermolecular interactions depending on the Aβ density on the gangliosidic clusters (Fig. 2b).

Recently, small ganglioside-embedding bicelles have been proposed as standardized nanoscale membrane models for detailed NMR analyses of the interactions between ganglioside clusters and biomolecules, including neuropeptides such as substance P and bradykinin, as well as intrinsically disordered proteins associated with neurodegenerative diseases [31–33]. Khatun *et al.* have shown that intrinsically unstructured neuropeptides strongly bound lipid bilayers and thereby attained a well-ordered secondary structure on the membrane containing GM1 or other gangliosides [32]. Moreover, experiments on ganglioside-containing bicelles have successfully

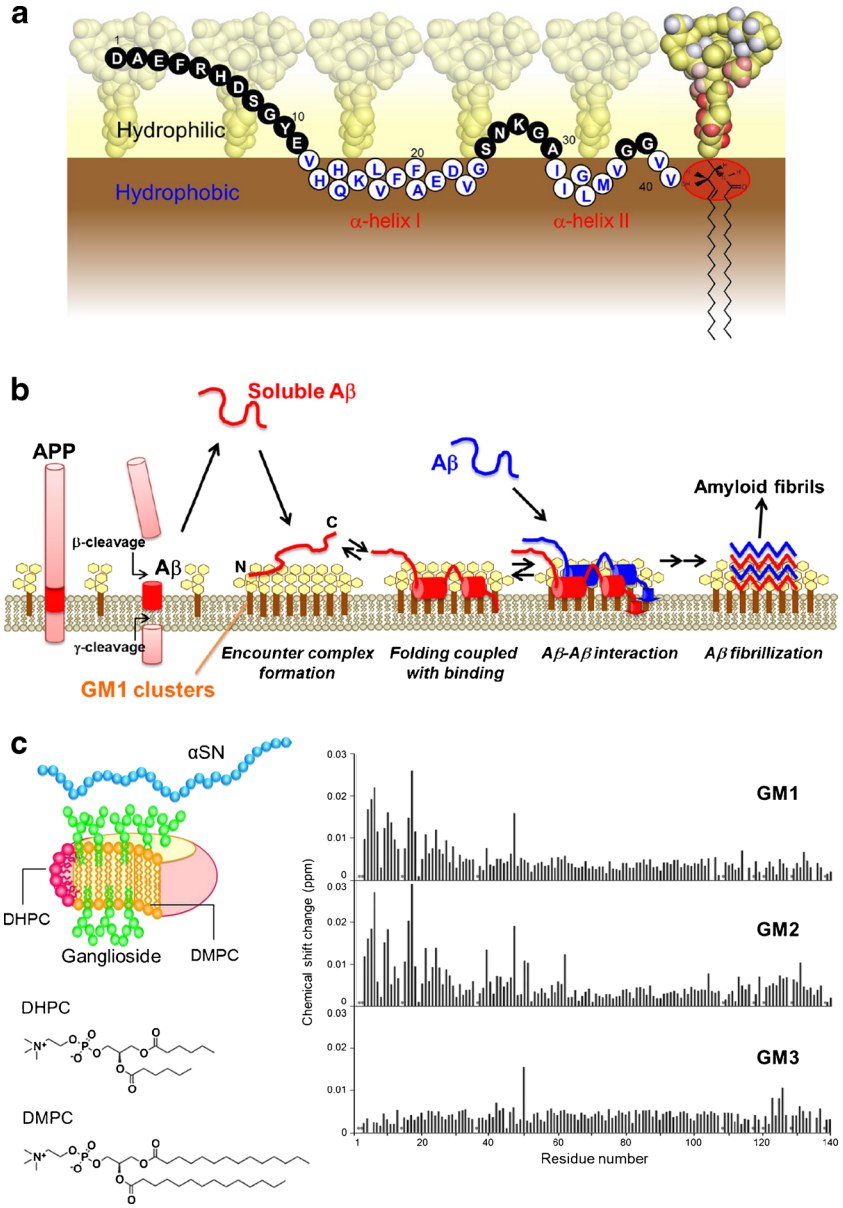

Fig. 2 **a** Schematic drawing of Aβ(1–40) positioned on the hydrophobic/hydrophilic interface of the ganglioside clusters. The amino-acid residues exposed to the hydrophilic and hydrophobic milieus are represented by close and open circles with single-letter codes. Mapping of nonexchangeable hydrogens showing the PRE effects observed for lyso-GM1 micelles in the presence of a C-terminally spin-labelled Aβ(1–40) on the lowest-penalty 3D model of the carbohydrate moiety. The linear color gradient (from *red to white*) indicates the intensity ratios of the CH peaks of lyso-GM1 before and after radical quenching of the paramagnetic probe. The carbohydrate hydrogen atoms are colored red, and the ceramide hydrogen atoms located within the red circle area exhibit lower peak intensity ratios, indicating that they are in close spatial proximity to the C-terminus of Aβ(1–40). Parts of this figure were originally reproduced from Utsumi *et al.* [29] and Yagi-Utsumi *et al.* [13] with permission from Springer and Elsevier, respectively. **b** Schematic drawing of the conformational transitions and intermolecular interactions of Aβ molecules promoted ganglioside clusters. Aβ forms a weak encounter complex with the ganglioside clusters through the interaction between its N-terminal region and the outer branch moieties and is subsequently accommodated on the hydrophilic/hydrophobic interface. The ganglioside clusters serve as a platform for binding coupled with the conformational transition of Aβ molecules, which restrict their spatial rearrangements to promote specific intermolecular interactions. **c** Schematic representation of the interaction of ganglioside-embedding bicelles with αSN (*left*) and chemical shift perturbation profiles of αSN upon interactions with the GM1-embedding, GM2-embedding, and GM3-embedding bicelles (*right*). The data are shown according to the equation $(0.04\Delta\delta_N^2 + \Delta\delta_H^2)^{1/2}$, where $\Delta\delta_N$ and $\Delta\delta_H$ represent the change in nitrogen and proton chemical shifts, respectively. Asterisks indicate proline residues or amino-acid residues that did not exhibit observable peaks in the spectrum because of severe broadening. Parts of this figure were originally reproduced from Yamaguchi *et al.* [33] with permission from the Royal Society of Chemistry

demonstrated that α-synuclein (αSN) is capable of interacting with GM1 and GM2 but not GM3 through its interaction hot spots located in the N-terminal segment [33] (Fig. 2c). In larger anionic membranes, this protein forms long α-helical segments that cannot be accommodated in the limited sizes of the bicelles [34–37]. These data highlight the utility of the small ganglioside-embedding bicelles for the characterization of the initial encounter complex formation of ganglioside clusters with their cognate proteins depending on their outer carbohydrate structures.

GM1 in microdomains: future issues

Accumulating evidence has indicated that gangliosides can form certain ordered phases known as microdomains in the presence of phospholipids, sphingomyelin (SM), and cholesterol (Chol). In particular, various physicochemical methods, including atomic force microscopy [38], F rester resonance energy transfer [39], and imaging mass spectrometry [40], have demonstrated the Chol-dependent cohesive phase separation of GM1 and the preferential colocalization of GM1 with Chol on lipid surfaces.

Recent studies have underscored the important physiological and pathological roles of the microdomains involving GM1 gangliosides in both physiological and pathological states [41]. Namely, GM1 microdomains mediate cell signaling and immune responses and are also involved in the onset and development of cancers and neurodegenerative diseases caused by the pathological protein aggregation of Aβ [38, 42–44], αSN [45], and prion proteins [46]. Therefore, atomic information regarding GM1 clustering depending on SM and Chol is crucial for further understanding of the mechanisms underlying disease progression.

MD simulations have shown that GM1 cluster formation is specifically induced in GM1/SM/Chol membranes, in which Chol critically suppresses membrane fluidity, establishing a hydrogen bond between the OH group of Chol and the oxygen atom at the Glc^{I}-sphingosine junction and keeping the sphingolipid moieties of GM1 molecules close to each other to facilitate cluster formation [44, 47]. However, the atomic-level experimental characterization of carbohydrate–carbohydrate and carbohydrate–protein interactions involving ganglioside clusters on microdomains remains largely unexplored. This is primarily because the experimental methodology has been undeveloped for such complicated heterogeneous systems composed of proteins, carbohydrates, as well as lipids. In addition, the altered acyl chain length of glycosphingolipid can affect the molecular assembly state and subsequent biological events [48–50]. To observe such enigmatic targets, it is obviously necessary to develop multilateral experimental approaches, including stable isotope-assisted solid-state NMR spectroscopy, using appropriately designed model systems [51]. The integration of biophysics-based experimental observations and theoretical approaches will be the key for answering the remaining Sphinx's riddle.

Acknowledgments This study was partly supported by a JSPS/MEXT KAKENHI Grant-in-Aid for Scientific Research on Innovation Areas (25102001 and 25102008), Grant-in-Aid for Challenging Exploratory Research (26560451), Research Funding for Longevity Sciences (25–19) from the National Center for Geriatrics and Gerontology, the Nanotechnology Platform Program of MEXT, and the Okazaki ORION project. We thank Drs. Hisashi Okumura, Satoru G. Itoh, and Takumi Yamaguchi for the useful discussion.

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

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