NMR characterization of the interactions between lyso-GM1 aqueous micelles and amyloid β

Maho Yagi-Utsumia,b, Tomoshi Kamedac, Yoshiki Yamaguchid, Koichi Katoa,b,e,f,*

a Graduate School of Pharmaceutical Sciences, Nagoya City University, 3-1 Tanabe-dori, Mizhuo-ku, Nagoya 467-8603, Japan
b Institute for Moleculer Science and Okazaki Institute for Integrative Bioscience, National Institutes of Natural Sciences, 5-1 Higashiyama Myodaiji, Okazaki 444-8787, Japan
c Institute for Molecular Science and Okazaki Institute for Integrative Bioscience, National Institutes of Natural Sciences, 5-1 Higashiyama Myodaiji, Okazaki 444-8787, Japan
d RIKEN, Advanced Science Institute, Chemical Biology Department, Systems Glycobiology Research Group, Structural Glycobiology Team, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan
e Glycoscience Institute, Ochanomizu University, 2-1-1 Ohtsuka, Bunkyo-ku, Tokyo 112-8610, Japan
f GLYENCE Co., Ltd., 2-22-8 Chikusa, Chikusa-ku, Nagoya 474-0858, Japan

A R T I C L E   I N F O

Article history:
Received 17 December 2009
Revised 27 December 2009
Accepted 5 January 2010
Available online 12 January 2010

Edited by Sandro Sonnino

Keywords:
Nuclear magnetic resonance
Ganglioside
Lyso-GM1
Spin label
Amyloid β

A B S T R A C T

Gangliosides are targets for a variety of pathologically relevant proteins, including amyloid β (Aβ), an important component implicated in Alzheimer’s disease (AD). To provide a structural basis for this pathogenic interaction associated with AD, we conducted NMR analyses of the Aβ interactions with gangliosides using lyso-GM1 micelles as a model system. Our NMR data revealed that the sugar–lipid interface is primarily perturbed upon binding of Aβ to the micelles, underscoring the importance of the inner part of the ganglioside cluster for accommodating Aβ in comparison with the outer carbohydrate branches that provide microbial toxin- and virus-binding sites.

© 2010 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

1. Introduction

Glycosphingolipids, including gangliosides, are known to play a variety of normal physiological and pathological roles in animal cells, including as receptors for microbial toxins, mediators of cell adhesion and modulators of signal transduction [1]. Recently growing evidence indicates that gangliosides interact with amyloid β (Aβ) and thereby promote its assembly, which is considered as a crucial step in Alzheimer’s disease (AD) [2,3]. In 1995, Yanagisawa et al. have reported a unique Aβ species in cerebral cortices from AD patients, that is tightly associated with GM1 ganglioside [4]. Subsequently, a series of in vitro studies have indicated that GM1-bound Aβ possesses an extremely high potential to facilitate Aβ assembly [5,6], which prompted the hypothesis that this Aβ species acts as a seed for Aβ fibrillogenesis in the brain [4,7,8]. Furthermore, it has been suggested that a variety of gangliosides can interact with Aβ and its heredity variants resulting in their pathogenic assembly and deposition [2,3]. Hence, the ganglioside–Aβ interaction systems are potential therapeutic targets in AD treatment. To better understand the underlying mechanisms of the pathological functions of gangliosides in AD, it is highly desirable to obtain detailed structural information of these interaction systems.

Based on circular dichroism (CD) data, Kakio et al. demonstrated that Aβ undergoes conformational transition from an α-helix-rich structure to a β-sheet-rich structure when Aβ density increases on liposomes containing gangliosides such as GM1 [6]. Their CD data, along with fluorescence spectroscopic data, also indicated that GM1 micelles provided a binding platform for Aβ, closely mimicking the ganglioside-containing raft-like membranes. Using CD and nuclear magnetic resonance (NMR) data, we have recently shown that lyso-GM1 micelles can serve as small mimics of GM1 micelles, and offer an environment to accommodate Aβ(1–40), where this peptide assumes a conformation and topology similar to that exhibited in GM1 micelles [9].

Abbreviations: Aβ, amyloid β; AD, Alzheimer’s disease; CD, circular dichroism; HSQC, heteronuclear single-quantum coherence; MTSL, (1-oxy-2,2,5,5-tetramethyl-2-pyrroline-3-methyl)methanethiosulfonate; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect; NOESY, nuclear Overhauser effect spectroscopy; PRE, paramagnetic relaxation enhancement; TOCSY, total correlation spectroscopy

* Corresponding author. Address: 5-1 Higashiyama Myodaiji, Okazaki 444-8787, Japan. Fax: +81 564 59 5224.
E-mail address: kkatonmr@ims.ac.jp (K. Kato).
These findings provide a unique opportunity for obtaining detailed structural information of the interaction mode of the ganglioside cluster with Aβ by NMR spectroscopy. In addition, recently emerging ultra-high field NMR techniques have enabled us to perform structural analyses at the atomic level for huge, complex carbohydrate–protein interaction systems [10]. In this report, we address the conformational characterization of the ganglioside environment surrounding Aβ using 920 MHz NMR spectroscopy.

2. Materials and methods

2.1. Preparation of micelles

Powdered lyso-GM1 was purchased from Takara Bio Inc. Powdered GM1 and GM2 were purchased from Sigma–Aldrich. Lyso-GM1, GM1 and GM2 were dissolved in methanol. Subsequently, the solvent was removed by evaporation. The residual ganglioside was suspended at a concentration of 12 mM in 10 mM potassium phosphate buffer (pH 7.2) containing 99% (v/v) 2H2O, and then mixed by vortexing.

2.2. Preparation of Aβ(1–40)

Expression and purification of recombinant Aβ(1–40) was performed as described previously [9]. Aβ(1–40)-Cys peptide with an extra cysteine residue at its C terminus (Aβ(1–40)-Cys) was constructed by standard polymerase chain reaction (PCR) and genetic engineering techniques. It was expressed and purified by the protocol used for wild-type Aβ(1–40) with slight modifications.

The reaction of Aβ(1–40)-Cys with the nitroxide spin label MTSL (1-oxy-2,2,5,5-tetramethyl-pyrroline-3-methyl)methanethiosulfonate (Toronto Research Chemicals) was carried out as described previously [11] with slight modifications. Briefly, lyophilized Aβ(1–40)-Cys peptides were dissolved in 0.1% (v/v) trifluoroacetate. Subsequently, a free sulfhydryl group was reacted with a five-fold molar excess of MTSL solubilized in acetone at 4 °C for 16 h. Unreacted spin label was removed by size exclusion chromatography (PD-10 column, GE Healthcare), and Aβ(1–40)-Cys spin labelled with MTSL (Aβ(1–40)-Cys-MTSL) was purified by reverse-phase chromatography using an octadecysilane column (TSKgel ODS-80TM, TOSOH).

2.3. NMR measurements

Lyso-GM1 was dissolved at a concentration of 6 mM in 10 mM potassium phosphate buffer (pH 7.2) containing either 99% (v/v) 2H2O or 10% (v/v) 2H2O. NMR spectral measurements were obtained in the presence or absence of 0.2 mM Aβ(1–40) using a JEOL EC-920 spectrometer with a GORIN application [12] unless otherwise stated. The probe temperature was set to 37 °C. Resonances originating from lyso-GM1 micelles were assigned using 1H–13C heteronuclear single-quantum coherence (HSQC), 1H–13C HSQC-TOCSY, 1H–13C HSQC-NOESY, 1H–1H NOESY and 13C-edited NOESY. NMR spectra were processed and analyzed using the program nmr-
Pipe/Sparky. Inter-proton distances were calculated from nuclear Overhauser effect (NOE) build-up curves with mixing times of 40–200 ms, using the intra-residue distance of 2.75 Å calculated based on the intensities of GalIII H-1 and GalIII H-3 crosspeaks as a reference. For saturation transfer experiments, $^1$H-$^{15}$N HSQC spectra were recorded using 6 mM lyso-GM1 in 10 mM potassium phosphate buffer containing 10% (v/v) $^2$H$_2$O with on-resonance irradiation at 1.31 ppm (the acyl chains of lyso-GM1) and at 4.69 ppm (H$_2$O) along with off-resonance irradiation at 40.0 ppm by a continuous wave technique with an irradiation duration of 2.5 s. The strength of the irradiation field was adjusted to 66 Hz for the saturation of water and acyl chains [9].

To observe paramagnetic relaxation enhancement (PRE) effects of the spin label, lyso-GM1 was dissolved at a concentration of 6 mM in 10 mM potassium phosphate buffer (pH 7.2) containing 99% (v/v) $^2$H$_2$O in the presence of 0.2 mM $\text{A}^\beta$ (1–40)-Cys-MTSL and subjected to $^1$H–$^{13}$C HSQC measurement. The unpaired electron of MTSL was subsequently reduced using 4 mM ascorbic acid. PRE effects were measured from the peak intensity ratios between two HSQC spectra of lyso-GM1 acquired in the presence and absence of the nitroxide radical of $\text{A}^\beta$(1–40)-Cys-MTSL.

2.4. Structure determination

The 3D structure of the lyso-GM1 glycan was determined using molecular dynamics simulation with a distance restraint. An initial structure of the pentasaccharide was obtained from the GLYCAM biomolecular builder server [13]. A total of 49 distance constraints derived from the NOE data were employed for calculations using the AMBER10 package [14] with a spring constant of $k = 15$ kcal/mol Å$^2$. To sample a broad range of the conformational space of the carbohydrate, we carried out 1000 annealing calculations with different initial velocities: 100-ps calculations with 1-fs time steps were performed with temperature gradually decreasing from 1000 to 0 K. The GLYCAM force field was used for carbohydrate interactions [15]. To validate the calculated conformations, we used the penalty function $P$ as a criterion:

![Diagram](image-url)
\[ P = \sum_{ij} NOESY_{ij} \left( r_{ij}^{\text{NOESY}} \right)^2, \]

where \( r_{ij}^{\text{NOESY}} \) is the distance for constraints.

2.5. Dynamic light scattering

Lyso-GM1 was dissolved at a concentration of 6 mM in 10 mM potassium phosphate buffer (pH 7.2) in the presence or absence of 0.2 mM \( \alpha\beta(1–40) \). Measurements were taken at 37 °C using a DynaPro Titan (Wyatt technology).

2.6. CD measurements

CD spectra of \( \alpha\beta(1–40) \) were measured as described previously [9].

3. Results and discussion

3.1. Spectral assignments and conformational characterization of lyso-GM1 micelles

To analyze the \( \alpha\beta \)-binding mode of lyso-GM1 micelles in aqueous solution, we first made spectral assignments of lyso-GM1 (Fig. 1A) that formed approximately 60-kDa micelles in aqueous solution by inspection of scalar and NOE connectivities. The assignments for the chemical shifts of \(^1\)H and \(^{13}\)C, as well as \(^{15}\)N, are summarized in Table S1.

The \(^1\)H–\(^1\)H NOESY datasets also provided distance constraints for conformational analyses of the lyso-GM1 carbohydrate moiety (Table S2). From molecular dynamics calculations using these distance constraints as pseudoenergies, we determined the 3D structure of the lyso-GM1 carbohydrate moiety. For validation, we scored the calculated structures using a penalty function (summation of pseudoenergies, see Section 2). Views of the superpositions of the 10 lowest-penalty structures are shown in Fig. 1B, which demonstrate that an NOE-based conformational calculation converged to a well-defined bouquet-like structure of the carbohydrate moiety.

For additional structural validation, solvent accessibilities of the amide groups of GalNAcIII and NeuAc residues were estimated by saturation transfer experiments because proton exchange rates sensitively reflect conformational properties of oligosaccharides [16,17]. The selective saturation of H2O resonance resulted in attenuation of the amide peaks, while irradiation of the lyso-GM1 acyl chains (CH2) perturbed these peaks minimally (Fig. S1). The effect of water saturation was more pronounced for the NeuAc amide than for the GalNAcIII amide. These results indicate that the amide group of NeuAc is more exposed to the aqueous environment, and therefore, undergoes a more rapid hydrogen exchange than the GalNAcIII amide group; this is consistent with the NOE-derived 3D structure of the glycan (Figs. 1B and 3B). Our determined structure of the carbohydrate moiety of the lyso-GM1 aqueous micelles agreed well with those reported for GM1 dissolved in dimethyl sulfoxide and GM1-derivatives that form aqueous micelles (Fig. S2) [18–21].

3.2. The \( \alpha\beta \)-binding mode of lyso-GM1 aqueous micelles

We next observed the NMR spectral changes of lyso-GM1 micelles upon interaction with \( \alpha\beta(1–40) \). Spectral measurements

![Fig. 3. PRE effects observed for lyso-GM1 micelles in the presence of spin-labeled \( \alpha\beta(1–40) \). (A) Plots of the intensity ratios of the CH peaks of lyso-GM1 in the presence of \( \alpha\beta(1–40) \)-Cys-MTSL before and after radical quenching. The lower intensity ratios are indicative of closer distance with the unpaired electron of the spin label. Intensity ratios are the mean ± S.D. of three independent experiments. (B) Mapping of non-exchangeable hydrogens showing the PRE effects on the lowest-penalty 3D model of the carbohydrate moiety of lyso-GM1 with a linear colour gradient (from red to white). Other atoms are coloured in yellow, except for the amide protons of GalNAcIII and NeuAc that are shown in blue.](image-url)
were conducted using 6 mM lyso-GM1 in the presence or absence of 0.2 mM Aβ(1–40). The dynamic light scattering data showed that the lyso-GM1 micelles exhibited a polydispersity of about 10% and a hydrodynamic radius of 3.5 nm, irrespective of the presence or absence of Aβ(1–40), indicating that lyso-GM1 formed highly monodisperse micelles of constant size under the solution conditions employed. Aβ(1–40) binding caused small but significant chemical shift changes in the peaks originating from R-1a, R-1b, R-2, R-3, R-4 and R-5 (Fig. 2 and Table S1). These data indicate that the sugar–lipid junction of lyso-GM1 was responsible for binding to Aβ(1–40), although it should be noted that the observed chemical shift changes presumably reflected an ensemble average for the excess amount of lyso-GM1 molecules that transiently interacted with Aβ(1–40).

For more sensitive analyses of this interaction, we made a site-specific spin label of Aβ(1–40) as a source of distance information. The interaction between a specifically attached paramagnetic nitroxide radical and nearby (<approximately 25 Å) protons causes broadening of their NMR signals due to an increased transverse relaxation rate with an r−6 dependence on the electron–proton distance [22]. Upon addition of Aβ(1–40)-Cys-MS-TSL, the peak intensities originating from Gln and GalB, as well as those from the head group of the lyso-GM1 lipid moiety, exhibited significant line broadening due to PRE effects (Fig. 3A and Fig. S3). The observed effects were mapped onto the 3D structure of the lyso-GM1 carbohydrate moiety (Fig. 3B). These results indicated that the sugar–lipid interface was primarily perturbed upon interaction of Aβ with the micelles. This was consistent with the abovementioned chemical shift perturbation data and our previous observation that Aβ(1–40) exhibited amphiphilic up-and-down topology on GM1 and lyso-GM1 micelles [9].

Our findings underscore the importance of the inner part of the ganglioside cluster for accommodating Aβ in comparison with the outer carbohydrate branch, which is involved in the interactions with cholera toxin and simian virus 40 [23,24]. Because the structure of the inner part is common among gangliosides [20,21,25], Aβ(1–40) would be expected to interact with various ganglioside micelles. Aβ(1–40) has indeed been shown to bind to a number of gangliosides [6,26]. Our CD data indicated that GM2 micelles induced an α-helical conformation of Aβ(1–40) similar to that induced by GM1 micelles (Fig. S4). In vitro binding studies indicated that the outer branches of sugar chains of gangliosides contribute to their affinities for Aβ(1–40) [5,26]. It is possible that outer carbohydrate branches play specific roles during the association phase to form encounter complexes with Aβ, resulting in slight differences in the apparent Kd values.

Our present data, while offering new insights into the interaction of gangliosides with Aβ, raise questions regarding the structural basis of specific environments for binding coupled with a conformational transition of Aβ molecules leading to their formation of amyloids. For example, growing evidence indicates that Aβ fibril formation is significantly enhanced by the presence of cholesterol [3,27]. Thus, it is intriguing to address whether or not the interaction modes between ganglioside clusters and Aβ are altered depending on their assembly states. In our forthcoming studies, systematic NMR analyses of ganglioside-Aβ interactions will be performed using various ganglioside-assembling systems, including mixed micelles [18] and ganglioside-embedded bicelles [28] with different ganglioside densities, surface curvatures and co-existing lipids.

Acknowledgements

We wish to acknowledge Dr. Katsuhiro Yanagisawa (National Institute for Longevity Sciences) and Dr. Naoki Yamamoto (Ritsumeikan University) for their useful discussions on the pathological aspects of the GM1–Aβ interaction. We thank Ms. Michiko Nakano (IMS) and Dr. Takumi Yamaguchi (IMS) for their help with measurements using the 920 MHz NMR spectrometer. We thank Dr. Mario Schubert (ETH) and Dr. Olivier Serve (IMS) for their useful discussions on carbohydrate conformational analyses. We thank Ms. Kiyomi Senda (NCU) and Ms. Kumiiko Hattori (NCU) for their help with the preparation of recombinant proteins. This work was supported, in part, by the Nanotechnology Network Project and Grants in Aid for Scientific Research (20023033 and 20107004) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and by the CREST project from the Japan Science and Technology Agency. M.Y.-U. is a recipient of a Japan Society for the Promotion of Science Research Fellowship for Young Scientists.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2010.01.005.

References


