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Effect of structural modifications of ganglioside GM2 on intra-molecular carbohydrate-to-carbohydrate interaction and enzymatic susceptibility

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Summary

The effect of inter-molecular carbohydrate-to-carbohydrate interaction on basic cell biological processes has been well documented and appreciated. In contrast, very little is known about the intra-molecular carbohydrate-to-carbohydrate interaction. The presence of an interaction between the GalNAc and the Neu5Ac in GM2 detected by NMR spectroscopy represents a well-defined intra-molecular carbohydrate-to-carbohydrate interaction. This intriguing interaction is responsible for the GM2-epitope, GalNAc β 1 Π 4(Neu5Ac α 2 Π 3)Gal-, to exhibit a rigid and compact conformation. We hypothesized that this compact conformation may be the cause for both the GalNAc and the Neu5Ac in GM2 to be refractory to enzymatic hydrolysis and the GM2 activator protein is able to interact with the compact trisaccharide GM2-epitope, rendering the GalNAc and the Neu5Ac accessible to β -hexosaminidase A and sialidase. We have used a series of structurally modified GM2 to study the effect of modifications of sugar chains on the conformation and enzymatic susceptibility of this ganglioside. Our hypothesis was borne out by the fact that when the GalNAc β 1 Π 4Gal linkage in GM2 was converted to the GalNAc β 1 Π 6Gal, both the GalNAc and the Neu5Ac became susceptible to β -hexosaminidase A and sialidase, respectively, without GM2 activator protein. We hope our work will engender interest in identifying other intra-molecular carbohydrate-to-carbohydrate interactions in glycoconjugates.

Keywords

GM2; Tauro-GM2; GM2-analogs; GM2-activator protein; Tay-Sachs disease; intra-molecular carbohydrate-to-carbohydrate interaction

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1. Introduction

Ganglioside GM2 is inseparable from Tay-Sachs Disease (TSD) and vice versa. GM2 was first identified as the main ganglioside accumulated in TS brain by Svennerholm in 1962 [1]. The complete structure of GM2 was proposed by Makita and Yamakawa in 1963 [2] and was subsequently confirmed by Ledeen and Salsman in 1965 [3]. While elucidating the structure of GalNAc-GD1a (Fig. 1), the terminal GalNAc of this ganglioside was found to be resistant to jack bean β -hexosaminidase; however, after removing the sialic acids from this ganglioside, the terminal GalNAc became enzymatically susceptible [4]. As shown in Fig. 1, GalNAc-GD1a contains a terminal and an internal GM2-epitope, GalNAc β 1 Π 4(Neu5Ac α 2 Π 3)Gal-. The resistance of the GalNAc in the terminal GM2-epitope of GalNAc-GD1a prompted us (Y.-T. Li and S.-C. Li) to initiate the study of the catabolism of GM2. We found that although the GalNAc in GM2 could be hydrolyzed by the crude human hepatic β -hexosaminidase preparation, it was refractory to the highly purified β -hexosaminidase A (Hex A) isolated from human liver and urine. A heat stable and non-dialyzable fraction obtained from the crude human liver β -hexosaminidase preparation was found to stimulate the hydrolysis of GalNAc from GM2 by the highly purified Hex A, but not Hex B, isolated from both human liver and urine [5]. This stimulating factor, GM2 activator protein (GM2AP), was subsequently purified from human kidney, liver and brain [6–8]. The clinical importance of GM2AP was revealed by the findings that this activator protein was absent in variant AB TSD [8–10]. To understand the catabolism of GM2 and the pathogenesis of TSD, the molecular cloning of the gene encoding GM2AP [11–13] and its expression [14,15], as well as the targeted disruption of this gene in mice [16], played center stage of TSD associated research during the late 20th century. Although the crystal structure of GM2AP has been solved [17], the mechanism of action of this protein cofactor is still not well understood. Two models for the mode of action of GM2AP have been put forth: model 1 proposes that the activator extracts a single GM2 molecule from a membrane or micelle to form a water soluble 1:1 protein-lipid complex to present the GM2 to the water soluble Hex A, whereas model 2 proposes the binding of GM2AP to one molecule of membrane-bound GM2, lifting it out of the membrane, and the activator-lipid complex is recognized and cleaved by the water soluble Hex A [18]. The authors of these two models clearly stated that “there is no definite knowledge of whether the GM2AP, in vivo, acts according either to these two models” [18]. Although these two models appear simple and reasonable, they cannot explain why the water soluble GM2-oligosaccharide (OM2) derived from GM2 is refractory to Hex A or Hex B in the presence or absence of GM2AP [15]. We found that in addition to efficiently stimulate the degradation of GM2 carried out by Hex A, GM2AP also stimulated the following reactions [15]: (a) conversion of GM2 to GA2 by clostridial sialidase; (b) hydrolysis of GalNAc from dipalmitoylphosphatidylethanolamine- Π^3 NeuAcGgOse₃ by Hex A; and (c) liberation of Gal from GM1 by β -galactosidase at a high activator concentration. Thus, this activator does not differentiate between GM2 and phosphatidylethanolamine- Π^3 NeuAcGgOse₃ or between Hex A and clostridial sialidase. These results together with the fact that the Neu5Ac in GM3 is readily hydrolyzed by sialidases, suggest that the action of GM2AP is not limited to solubilize the lipid substrate GM2. We hypothesized that the effectiveness of GM2AP in stimulating the hydrolysis of GM2 may be due to its ability to recognize the specific trisaccharide structure of the GM2-epitope, and to modify the GalNAc-Neu5Ac interaction in this structure [15]. The GalNAc-Neu5Ac interaction in GM2 represents a well-defined carbohydrate-to-carbohydrate interaction. The presence of GalNAc-Neu5Ac interaction in GM2 has also been supported by the NMR studies which revealed the existence of through the space interactions between these two sugar residues [19]. To understand the catabolism of GM2, we have used a series of structurally modified GM2 to study the effect of modifications of sugar chains in this ganglioside on the GalNAc-Neu5Ac interaction and the enzymatic susceptibility. The structures of GM2 and the four structurally modified GM2 are shown in Fig 2.

2. Effect of mutating the linkage between the GalNAc and the Gal in GM2 on intra-molecular carbohydrate-carbohydrate interaction and enzymatic susceptibility

2.1. Synthesis of a regioisomer of GM2 (6'GM2)

To show that the resistance of the GalNAc and the Neu5Ac in GM2 to enzymatic hydrolysis is due to the specific rigid conformation of the GM2-epitope, we have synthesized a regioisomer of GM2, called 6'GM2, in which the GalNAc is linked β 1,6 to the Gal, as shown in Fig. 2 [20]. We hypothesized that alteration of the linkage between the terminal GalNAc and the penultimate Gal in GM2 may affect the GalNAc-Neu5Ac interaction.

2.2. NMR analysis of the effect of mutating the GalNAc linkage in GM2 from β 1 Π 4Gal to β 1 Π 6Gal on the GalNAc-Neu5Ac interaction

By high resolution NMR spectroscopy, the pattern of interresidual NOE interaction on GM2 revealed a single prevalent spatial arrangement of the trisaccharide in the GM2-epitope (Fig. 3A). Due to the interaction of the GalNAc with the Neu5Ac residue, the GalNAc-Gal bond also shows a limited flexibility [21]. In sharp contrast, a considerable spatial flexibility is found for the GalNAc residue of 6'GM2. As shown in Fig. 3B, the NOE interactions across the GalNAc β 1 Π 6Gal linkage of 6'GM2 consist uniquely of the contacts between the anomeric proton of the GalNAc and the protons on the C6 of the Gal; the intensities of the two interactions are almost equal. Another prominent inter-residual interaction detected on the GalNAc of 6' GM2 is between the GalNAc-NH and the Glc-OH3 (Fig. 3B). The Monte Carlo calculations corroborate and strengthen the results of NMR analyses and also enable comparison of the lowest energy conformations of the oligosaccharide moieties of GM2 (Fig. 4A) and 6'GM2 (Fig. 4B and 4C). The conformational studies of 6'GM2 and GM2 reveal that the modification of the GalNAc linkage in GM2 from β 1 Π 4Gal to β 1 Π 6Gal dramatically alters the dynamics of the sugar chain. The overall effect of this modification is to give the oligosaccharide head group of 6'GM2 an "open" spatial arrangement (Fig. 4B and 4C) in which the GalNAc and the Neu5Ac residues are freely accessible to external interactions. The GalNAc β 1 Π 4-(Neu5Ac α 2 Π 3)Gal- (GM2-epitope) found in GM2, on the other hand, exists in one preferred special arrangement which is defined by a number of experimentally detected interactions especially between the GalNAc and Neu5Ac, as shown in Fig. 4A. The results of conformational analysis show that the specific trisaccharide structure of GM2-epitope in which the attachment of both the GalNAc β 1 Π 4 and the Neu5Ac α 2 Π 3 to the penultimate Gal residue can render these two sugar residues resistant to enzymatic hydrolysis. The disruption of this rigid structure by mutating the GalNAc linkage from GalNAc β 1 Π 4Gal to GalNAc β 1 Π 6Gal makes the GalNAc susceptible to Hex A and the Neu5Ac to sialidase.

2.3. Susceptibility of GM2 and 6'GM2 to Enzymatic Hydrolysis

It is remarkable that after mutating the GalNAc linkage in GM2 from β 1 Π 4Gal to β 1 Π 6Gal, the GalNAc and the Neu5Ac in 6'GM2 becomes susceptible to Hex A and clostridial sialidase, respectively, without the assistance of GM2AP [21]. As shown in Fig. 5a, the GalNAc in GalNAc β 1 Π 4(Neu5Ac α 2 Π 3)Gal- of GM2 is resistant to Hex A. In contrast, the GalNAc in GalNAc β 1 Π 6(Neu5Ac α 2 Π 3)Gal- of 6'GM2 is readily hydrolyzed by Hex A in the absence of GM2AP (Fig. 5b). Similarly the hydrolysis of the Neu5Ac in GM2 by clostridial sialidase requires the assistance of GM2AP, whereas the Neu5Ac in 6'GM2 could be hydrolyzed by clostridial sialidase without GM2AP [21]. Thus, the mutation of the GalNAc linkage from GalNAc β 1 Π 4 to GalNAc β 1 Π 6Gal abolished the requirement of GM2AP for the enzymatic hydrolysis of the GalNAc and the Neu5Ac in 6'GM2.

2.4. Enzymatic susceptibility of the oligosaccharide derived from GM2 (OGM2) and 6'GM2 (6' OGM2) and GA2 (OGA2)

To eliminate the possible influence of the ceramide moiety of the GM2-related glycolipids on the enzymatic hydrolysis of the GalNAc residue, the ceramide was removed from GM2, 6' GM2 and GA2 using ceramide glycanase [22] to yield the oligosaccharides OM2, 6'OGM2 and OA2, respectively. As shown in Fig. 6, both 6'OGM2 and OA2 are readily hydrolyzed by Hex A alone without GM2AP. In sharp contrast, OM2 is resistant to Hex A [21] and also refractory to clostridial sialidase [21]. These results further support the presence of the interaction between the GalNAc and the Neu5Ac in GM2. It should be pointed out that GM2AP does not stimulate the enzymatic hydrolysis of GalNAc or Neu5Ac from OM2, indicating that GM2AP requires the lipid moiety of the substrate to exert its stimulatory activity.

2.5. Effect of methyl esterification and reduction of the carboxyl group of Neu5Ac on enzymatic hydrolysis of GM2

To understand the mechanism of action of GM2AP, we have chemically methyl esterified (Me-GM2) and also reduced (HO-GM2) the carboxyl group of the Neu5Ac in GM2 [23]. The structures of Me-GM2 and HO-GM2 are shown in Fig. 2. As shown in Fig. 7, both Me-GM2 and HO-GM2 are resistant to the GM2AP assisted hydrolysis. This resistance is not due to the changes in the critical micellar concentration resulting from the chemical modifications. At concentrations ranging from 5 to 50 μ M, the results remain the same: in the activator assisted enzymatic hydrolysis, only GM2 is susceptible to Hex A while both Me-GM2 and HO-GM2 are resistant. However, in the presence of a detergent, sodium taurodeoxycholate, Me-GM2 is hydrolyzed by Hex A at a rate comparable to that of GM2. Under the same condition, HO-GM2 is hydrolyzed faster than GM2 or Me-GM2 (Fig. 8). These results indicate that the carboxyl function of Neu5Ac in GM2 is involved in the interaction between Hex A/or GM2AP for bringing about the hydrolysis of GalNAc. The fact that both Me-GM2 and HO-GM2 can be hydrolyzed by Hex A in the presence of sodium taurodeoxycholate indicates that the action of this detergent is different from that of GM2AP and the main function of GM2AP is not merely to serve as a detergent to solubilize GM2. It can recognize the GalNAc and the Neu5Ac residues in GM2.

3. Enzymatic susceptibility of four novel GM2 analogs

3.1. Chemical synthesis of four novel GM2 analogs

We found that if a lipid other than ceramide attached to the sugar chain of GM2 (OM2) was sufficiently hydrophobic, the nature of the lipid was not critical for the GM2AP assisted hydrolysis of GalNAc by Hex A [15]. We therefore synthesized four novel GM2 analogs in which the sugar chains are attached to the synthetic lipid, 2-(tetrahexyl)hexadecanol, instead of ceramide [24]. The structures of these four GM2 analogs are shown in Fig. 9. In analog I, the OM2 is attached to 2-(tetrahexyl)hexadecanol through a β -linkage. In analog II, the GM2-epitope is linked through β 1 Π 3Glc instead of β 1 Π 4Glc, as that found in analog I. In analog III, the internal Glc in analog II is replaced by Gal. In analog IV, the GM2-epitope is directly attached to 2-(tetrahexyl)hexadecanol through a β -linkage without any sugar spacer.

3.2. Enzymatic susceptibility of four novel GM2 analogs

The enzymatic susceptibility of analog I mirrors that of the native GM2: the GalNAc of analog I is hydrolyzed by Hex A in the presence of GM2AP; without GM2AP this analog is refractory to Hex A. These results support our previous finding that the nature of the lipid attached to OM2 is not critical for the GM2AP assisted hydrolysis of GalNAc [24]. Similarly, the GalNAc in analog II and III, in which the GM2-epitope is linked to the 3-OH of the spacer Glc and Gal, respectively, is also hydrolyzed by Hex A in the presence of GM2AP. However, the GalNAc

of analog IV is completely refractory to Hex A with or without GN2AP. These results suggest that GM2AP/Hex A requires one spacer sugar between the GM2-epitope and the lipid moiety to carry out the hydrolysis of the terminal GalNAc in the GM2-epitope and that the linkage and the nature of the spacer sugar may not be important for the GM2AP assisted hydrolysis of the GalNAc from GM2-epitope.

4. Tauro-GM2

Recently, a novel GM2-derivative called “tauro-GM2” (Fig. 1), has been detected in Tay-Sachs brain samples [25]. Tauro-GM2 is a new GM2-derivative in which the carboxyl group of Neu5Ac is amidated by taurine, one of the major free amino acids in the brain. To understand the pathophysiological significance of tauro-GM2, we have performed the physicochemical analyses of this unusual ganglioside derivative. By NMR spectroscopy, molecular modeling studies, and laser light scattering measurements, we found that the oligosaccharide chain of tauro-GM2 is much more flexible than that of GM2.

4.1. Dynamic properties of tauro-GM2

The direct evaluation of the NMR NOE interactions, determined for the oligosaccharide chain of the ganglioside monomers in dimethylsulfoxide, provides information regarding the distances between protons in spatially close proximity [26] for building the NOE distance maps. The ganglioside oligosaccharide conformation can be portrayed as a set of conventional glycosidic angle pairs $H1'-C1'-O1'-Cx$ (F) and $C1'-O1'-Cx-Hx$ (Y). For the sialic acid, these are defined by $C1'-C2'-O2'-Cx$ (F) and $C2'-O2'-Cx-Hx$ (Y) [27]. Comparing the NOE contacts of GM2 and tauro-GM2, some differences are immediately apparent (Table I). In fact, in tauro-GM2, the simultaneous presence of cross-peaks for the Gal-H3 proton with the H3ax and the H8 of taurine-conjugated Neu5Ac suggests a considerable spatial flexibility for the tauro-Neu5Ac-Gal disaccharide (Fig. 10) with no or reduced interactions between the tauro-Neu5Ac and the GalNAc. No interresidue NOE within the glycerol side chain of the Neu5Ac and the GalNAc for a typical “blocked trisaccharide” sequence of GalNAc-Gal-Neu5Ac was detected. Modeling of tauro-GM2 confirmed that amidation of the carboxylic acid function of sialic acid with taurine dramatically alters the dynamics of the oligosaccharide chain with respect to GM2. The most populated family of conformations (76%) features ϕ values spanning in a large well between -120° and -70° . A second well, with $\phi = -170^\circ$, accounts for 12% of the total population. These two sets of conformations appear to be in fast dynamic equilibrium. The simulations also sample a third family of conformers at ϕ ranging between $+70^\circ$ and $+140^\circ$, which, due to the exoanomeric effect [28], is normally not seen in sialic acid glycosides (Fig. 11). This effect was never observed before in our analysis of other ganglioside models [29]. The first conformation ($-120^\circ \leq \phi \leq -70^\circ$, 76%) corresponds to the type-B conformer, typically found in GM4, GM3 and 6'-GM2, a GM2 analogue in which the GalNAc is linked to the position 6 of Gal, rather than the natural 4 position. This conformation, however, is normally not populated by GM2 [29]. The second conformation ($\phi = -170^\circ$, 12%) is the type-C conformer typically populated by GM2. The presence of a third conformation ($+70^\circ \leq \phi \leq +140^\circ$) could not be confirmed experimentally, because it is not expected to give rise to visible NOE effects. However, this result is intriguing and suggests that the taurine-conjugated sialic acid may have a distinct conformational behavior, which deserves further studies. The overall effect of this modification is the loss of the typical rigidity of core trisaccharide, and a major flexibility of the taurine chain.

4.2. Aggregation and geometrical properties of tauro-GM2

It is well established that due to their marked amphiphilic properties, gangliosides aggregate in aqueous media to form micelles, with the exception of GM4 and GM3, that are highly hydrophobic due to the short oligosaccharide chain and form interdigitated vesicles [30,31].

The addition of a GalNAc residue to GM3 provides enough hydrophilicity to GM2 to form a big micellar aggregate of oblate structure having a ratio between the two axes of 3.1 (Table II).

The aggregation and geometrical properties of tauro-GM2 (shown in Table II) are in good agreement with the high dynamics of tauro-GM2. The molecular mass and hydrodynamic radius for tauro-GM2 micelles were found to be 560,000 and 64.5 Å, respectively, with a variance of 7%. Under the same condition, the values of the molecular mass and the hydrodynamic radius for GM2 micelles were 630,000 and 66 Å, respectively. Whereas, the surface area occupied by the monomer of tauro-GM2 inserted into the aggregate increased from 92 to 93.3 Å. This confirms that the presence of taurine bonded to the sialic acid residue makes the oligosaccharide head group bigger than GM2, which is in agreement with the dynamic of the taurine oligosaccharide chain. The hydrophilic portion of tauro-GM2 is very flexible and the charge properties of the sulfonic acid group are probably responsible for these drastic differences.

4.3. Enzymatic susceptibility of tauro-GM2

As in the case of Me-GM2 and HO-GM2, amidation of the carboxyl group of Neu5Ac of GM2 with taurine renders this taurine-conjugated sialic acid refractory to sialidases in the presence or absence of GM2AP. However, in contrast to Me-GM2 and HO-GM2, the GalNAc in tauro-GM2 is hydrolyzed by Hex A in the presence of GM2AP at a rate comparable to that of GM2. Without GM2AP, the GalNAc in tauro-GM2 is refractory to Hex A. Based on the enzymatic susceptibility of tauro-Neu5Ac and GalNAc in tauro-GM2, it can be concluded that taurine conjugation of the carboxyl group of Neu5Ac in GM2 does not completely abolish the interaction between the GalNAc and the Neu5Ac in this ganglioside derivative.

Conclusion

Although GM2AP has been the subject of intensive studies for over thirty years, the function and the mechanism of action of GM2AP are still not well understood. We have taken the approach of using chemical modifications of GM2-epitope to study the mechanism of action of GM2AP. The results of our NMR analysis support the presence of an interaction between the GalNAc and the Neu5Ac in GM2-epitope. This interaction represents a well-defined intramolecular carbohydrate-to-carbohydrate interaction that renders GM2 molecule to exhibit a compact and rigid conformation. We hypothesized that the role of GM2AP is to interact with the GM2-epitope to overcome this rigid conformation. Our studies summarized in this review suggest that: i) GM2AP recognizes the GalNAc/NeuAc interaction in the GM2 epitope as well as the lipid moiety of GM2; ii) the enzymatic hydrolysis of the GalNAc in GM2 in the presence of GM2AP requires at least one sugar unit (a spacer sugar) between the GM2-epitope and the lipid moiety. The spacer sugar can be either a Glc or a Gal, possibly other sugar units as well; iii) the nature of lipid moiety attached to the sugar chain is not crucial for the action of Hex A and GM2AP, but the hydrophobicity of the lipid moiety needs to be higher than that of an octanol; and iv) the carboxyl function of the Neu5Ac in GM2 should be devoid of any modifications. Recently we found that Tay-Sachs brain contained an unusual GM2-derivative, tauro-GM2, in which the carboxyl group of Neu5Ac in GM2 was amidated by taurine. Since tauro-GM2 is less rigid and more hydrophilic than GM2, it is conceivable that a detoxification mechanism may exist in Tay-Sachs brain to alleviate the toxicity of the accumulated GM2 by converting GM2 into tauro-GM2. It is hoped that this brief review will engender interest in this fascinating protein cofactor intimately associated with the catabolism of GM2.

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Fig. 1.
Structures of GalNAc-GD1a and the two units of GM2-epitope in this ganglioside.

Fig. 2. Structures of ganglioside GM2 and four structurally modified gangliosides derived from GM2: 6'GM2, HO-GM2, Me-GM2 and tauro-GM2. Structural modifications are highlighted in red.

Fig. 3. Conformational analysis of GM2 and 6'GM2 by NMR spectroscopy. Two-dimensional ROESY sections shown for GM2 (A) and 6'GM2 (B) contain three regions of the two dimensional maps: the GalNAc-H1(*III-H1*), the Neu5Ac-OH8 (*A-OH8*), and GalNAc-NH(*III-NH*) regions. *I*, Glc; *II*, Gal; *III*, GalNAc; *A*, Neu5Ac. The interactions critical for the definition of the oligosaccharide conformation are marked. Note the loss of GalNAc/Neu5Ac interactions in 6'GM2. (Adapted from Ref. [21] with permission.)

Fig. 4.

The spatial arrangements of the oligosaccharide moieties in GM2 (A) and 6'GM2 (B and C). Representative conformers are shown for GM2 and 6'GM2 as calculated by molecular mechanics. Conformers are taken from the lowest energy conformations within 8 kJ/mol. The computational results were validated by comparing the calculated distances with the experimental (NOE-derived) values. The calculated distances were obtained as $r = (r_i^{-6})^{-1/6}$ where (r_i^{-6}) is the Boltzman average of r_i^{-6} of the individual conformations within 10 kJ/mol from the global minimum. The GalNAc β 1 Π 4(Neu5Ac α 2 Π 3)Gal-trisaccharide (GM2-epitope) of GM2 shows one preferred spatial arrangement which is defined by a number of experimentally detected interactions (*arrows*). The GalNAc β 1 Π 6(Neu5Ac α 2 Π 3)Gal- of 6' GM2, on the contrary, shows a fairly "opened" spatial arrangement, with the GalNAc-Gal bond sampling the two conformations. The conversion of GM2 to 6'GM2 dramatically alters the spatial disposition of the Neu5Ac residue because of the loss of its interactions with the GalNAc that exist in the very compact GalNAc β 1 Π 4(Neu5Ac α 2 Π 3)Gal-trisaccharide structure of GM2. (Adapted from Ref. [21] with permission.)

Fig. 5. Thin layer chromatograms showing the hydrolysis of GM2 (a) and 6'GM2 (b) by Hex A. Hex, Hex A; h, hour; m, minute; 6'M2, 6'GM2. For the hydrolysis of GM2, 0.25 unit of Hex A was used. For the hydrolysis of 6'GM2, only 0.06 unit of Hex A was used since this ganglioside was completely hydrolyzed by 0.25 unit of Hex A in less than 5 min. Detailed incubation conditions are described in Ref. [21]. (Adapted from Ref. [21] with permission.)

Fig. 6. A thin layer chromatogram showing the susceptibility of the GalNAc in OM2, OA2, and 6'OM2 to Hex A. OM2, OA2, and 6'OM2 are the oligosaccharides derived from GM2, GA2, and 6'GM2, respectively. SL, sialyllactose; Hex, Hex A; h, hour; Lac, lactose. Detailed incubation conditions are described in Ref. [21]. (Adapted from Ref. [21] with permission.)

Fig. 7. Hydrolysis of GM2, Me-GM2, and HO-GM2 by Hex A in the presence of GM2AP. The detailed assay conditions are described in Ref. [23]. (Adapted from Ref. [23] with permission.)

Fig. 8. Hydrolysis of GM2, Me-GM2, and HO-GM2 by Hex A in the presence of sodium taurodeoxycholate. The detailed assay conditions are described in Ref. [23]. (Adapted from Ref. [23] with permission.)

Fig. 9.
Structures of four GM2 analogs in which the sugar chains are attached to 2-(tetrahexyl) hexadecanol.

Fig. 10. Section of Tauro-GM2 ROESY interaction maps. A: H3(Gal)-H3ax(Tauro-Neu5Ac); B: H3(Gal)-H8(Tauro-Neu5Ac).

Fig. 11.

Low-energy conformations calculated for tauro-GM2. A: A/B-type conformer, $\phi = -104$, $\psi = +23$ (A-type= $\phi -70$ and B-type= $\phi -120$). B: C-type conformer, $\phi = -163$, $\psi = -18$. C: new conformer, $\phi = +59,3$; $\psi = -2$.

TABLE 1

Experimental and calculated inter-proton distances (Å) of Tauro-GM2 and GM2*

	Tauro-GM2			GM2**	
	MC+MC/SD	EXP. DIST. 100ms	EXP. DIST. 180ms	MC+MC/SD	EXP. DIST.
H1(III)-H3(II)	3.96	3.23	3.52		
H1(III)-H4(II)	2.36	2.62	2.5	2.2	2.3
H1(III)-H5(II)	4.65	nd	nd		
NH(III)-H2(II)		3.88	3.62	2.9	3.4
NH(III)-H1/1' (I)	6.03; 6.13	3.77			
NH(III)-H1/1' (I)	6.03; 6.13	4.34	3.8		
H3(III)-H1/1' (I)	4.58; 3.92	nd	nd		
H5(III)-H1/1' (I)	4.50; 3.87	nd	nd		
CH3(III)-H2(II)		3.67	3.56	3.4	3.6
CH3(III)-H7(N)		nd	nd		
NH(I)-H3(III)	3.98	3.83	4.15		
NH(I)-NH(III)		3.9	4.41		
NH(I)-H1(III)	2.68	3.09	3.17		
NH(I)-H3(II)	2.78	3.84	4.21		
NH(I)-H5(III)	3.9	2.85	2.96		
NH(I)-H4(N)		2.85	2.96		
H3a(N)-H3(II)	2.95	2.61	2.63	2.3	2.4
H3e(N)-H3(II)	3.48	nd	nd	3.6	nd
H3a(N)-H4(II)	3.85	3.57	3.64		nd
H3e(N)-H4(II)	4.64	nd	nd		nd
H8(N)-H3(II)	3.48	2.65	2.72	3.7	2.7
H8(N)-H1(III)	4.92	nd	nd	2.8	nd
H1(II)-H4(I)	2.32	2.67	2.68	2.3	ov
H1(II)-H6(I)		3.44	3.64	3.1	3
H1(II)-H6(I)		3.61	4.58	3.1	ov
H1(II)-H5(I)		2.55	2.46		
H1(II)-H3(I)		nd	nd	3.5	ov
CH3(III)-H1(I)		4	4.27		
CH3(III)-H1(I)		3.73	3.54	4.6	3.8
OH8(N)-NH(III)		nd	nd	2.9	3.3
OH8(N)-H5(III)		nd	nd		

* MS, Monte Carlo conformational search. SD, Stochastic Dynamics; ms, millisecond; I, Glc; II, Gal; III, GalNAc; t, taurine; N, Neu5Ac; nd, not detected; ov, distances not calculated due to signal overlapping.

** Data from ref. [29]

TABLE 2

Aggregation and geometrical properties of GM2 and Tauro-GM2*

	R_h	N	M	R_g/R_h	a_0	P
GM2	66.0	529	740	3.1	92.0	0.440
Tauro-GM2	64.5	380	560	2.8	93.3	0.438

* R_h (Å) is the hydrodynamic radius, N is the aggregation number, M (kDa) is the molecular mass and R_g/R_h represents the axial ratio of the GM2 and tauro-GM2 micelles; a_0 (Å²) is the surface area, and P is the packing parameter of the monomers in the aggregate. Vesicles, liposomes and, in general, membrane-like structures are formed when $0.5 < P < 1$, micelles are formed when $0.33 < P < 0.5$, $P = V/a_0l$, where a_0 is the surface area, l is the maximum hydrophobic chain length and V the hydrophobic volume of monomer in the aggregate.