Nuclear Overhauser effect investigation on GM1 ganglioside containing N-glycolyl-neuraminic acid (II\(^3\)Neu5GcGgOse\(_4\)Cer)

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The conformational properties of the oligosaccharide chain of GM1 ganglioside containing N-glycolyl-neuraminic acid, \(\beta\)-Gal-(1-3)-\(\beta\)-GalNAc-(1-4)-[\(\alpha\)-Neu5Gc-(2-3)]-\(\beta\)-Gal-(1-4)-\(\beta\)-Glc-(1-1)-Cer, were studied through NMR nuclear Overhauser effect investigations on the monomeric ganglioside in dimethylsulfoxide, and on mixed micelles of ganglioside and dodecylphosphocholine in water. Several interresidual contacts for the trisaccharide core -\(\beta\)-GalNAc-(1-4)-[\(\alpha\)-Neu5Gc-(2-3)]-\(\beta\)-Gal- were found to fix the relative orientation of the three saccharides, while the glycosidic linkage of the terminal \(\beta\)-Gal- was found to be quite mobile as the \(\beta\)-Gal-(1-3)-\(\beta\)-GalNAc-disaccharide exists in different conformations. These results are similar to those found for two GM1 gangliosides containing N-acetyl-neuraminic acid and neuraminic acid [1].

**Keywords:** gangliosides, N-glycolyl-neuraminic acid, NMR, conformation, nOe

**Abbreviations:** Ganglioside nomenclature is in accordance with Svennerholm [23] and the IUPAC-IUB Recommendations [24]. GM3(Neu5Ac), II\(^3\)Neu5AcLacCer, \(\alpha\)-Neu5Ac-(2-3)-\(\beta\)-Gal-(1-4)-\(\beta\)-Glc-(1-1)-Cer; GM3(Neu5Gc), II\(^3\)Neu5GcLacCer, \(\alpha\)-Neu5Gc-(2-3)-\(\beta\)-Gal-(1-4)-\(\beta\)-Glc-(1-1)-Cer; GM1(Neu5Ac), II\(^3\)Neu5AcGgOse\(_4\)Cer, \(\beta\)-Gal-(1-3)-\(\beta\)-GalNAc-(1-4)-[\(\alpha\)-Neu5Ac-(2-3)]-\(\beta\)-Gal-(1-4)-\(\beta\)-Glc-(1-1)-Cer; GM1(Neu5Gc), II\(^3\)Neu5GcGgOse\(_4\)Cer, \(\beta\)-Gal-(1-3)-\(\beta\)-GalNAc-(1-4)-[\(\alpha\)-Neu5Gc-(2-3)]-\(\beta\)-Gal-(1-4)-\(\beta\)-Glc-(1-1)-Cer; GM1(Neu), II\(^3\)NeuGgOse\(_4\)Cer, \(\beta\)-Gal-(1-3)-\(\beta\)-GalNAc-(1-4)-[\(\alpha\)-Neu-(2-3)]-\(\beta\)-Gal-(1-4)-\(\beta\)-Glc-(1-1)-Cer; GD1a, IV\(^3\)Neu5AcGgOse\(_4\)Cer, \(\alpha\)-Neu5Ac-(2-3)-\(\beta\)-Gal-(1-3)-\(\beta\)-GalNAc-(1-4)-[\(\alpha\)-Neu5Ac-(2-3)]-\(\beta\)-Gal-(1-4)-\(\beta\)-Glc-(1-1)-Cer; GalNAc-GD1a, IV\(^3\)Neu5AcGgOse\(_4\)Cer, \(\alpha\)-Neu5Ac-(2-3)-\(\beta\)-Gal-(1-3)-\(\beta\)-GalNAc-(1-4)-[\(\alpha\)-Neu5Ac-(2-3)]-\(\beta\)-Gal-(1-4)-\(\beta\)-Glc-(1-1)-Cer; Neu, neuraminic acid; Neu5Ac, N-acetyl-neuraminic acid; Neu5Gc, N-glycolyl-neuraminic acid; Cer, ceramide.

**Introduction**

Gangliosides [2], sialic acid containing glycosphingolipids, are components of mammalian cell plasma membranes and form a heterogeneous family of compounds that differ in their sialic acid-, neutral oligosaccharide chain- and ceramide structures. They appear to play a role in specific interaction processes and in regulating membrane enzyme activities and cell functions [3–4]. The varying degree of ganglioside involvement is probably due to specific ganglioside-protein interaction within the membrane itself.

It should, however, be noted that fundamental parameters, like the geometrical properties of the ganglioside molecule, play a role in the microdomain organization of the membrane [5–6]. In fact the oligosaccharide chain bulky structure, tied to the primary and secondary structures, determines the spatial arrangement of the molecule in the hydrophilic layer [7].

Four main sialic acid structures have been found in gangliosides: the N-acetyl-, N-glycolyl, 9-O-acetyl-N-acetyl- and 4-O-acetyl-N-glycolyl- derivatives of neuraminic acid (5-amino-3,5-dideoxy-D-glicero-D-galacto-nonulosonic acid) [8]. Gangliosides containing N-acetyl- and N-glycolyl-neuraminic acid are widespread in
mammals but those containing N-glycoly neuraminic acid (Neu5Gc) have never been found in healthy human tissue [9], only in cancer tissue [10].

The purpose of the present work was to investigate the effect of the glycolic group of Neu5Gc on the conformational properties of the GM1 oligosaccharide chain [11], and make a comparison with what is already known about GM1(Neu5Ac) and GM1(Neu) conformations [1].

High resolution NMR investigations of the three dimensional (3D) structure of the GM1(Neu5Gc) oligosaccharide chain were made by evaluating nOe effects in both dimethylsulfoxide and water solution.

To overcome the difficulty of the high molecular mass of ganglioside aggregates the gangliosides were inserted into perdeuterated dodecylphosphocholine (DPC) micelles in a low molar ratio of one-ganglioside per micelle. This model system allowed the ganglioside to be kept in water solution and, moreover, mimics the natural phospholipid environment of membrane gangliosides. The DPC model system has already been used for detailed studies on the molecular properties of a number of gangliosides, GM1(NeuAc) [1], GD1a [12, 13], GalNAc-GD1a [13] and GM3 [14].

Materials and methods

Materials

Deuterated dimethylsulfoxide (DMSO-d6), >99.95% isotopically pure, was purchased from Merck (FRG) and dodecylphosphocholine-d38 was from MSD (Canada). Chelex 100 (100-200 mesh, sodium form) was from Biorad (USA). Ganglioside GM1 containing N-glycol neuraminic acid, GM1(Neu5Gc), was prepared by a semisynthetic procedure from GM1(Neu5Ac) [11, 15, 16].

After chromatographic purification GM1(Neu5Gc) was >99% pure. The structural characterization was confirmed by NMR as reported below.

NMR spectroscopy

GM1(Neu5Gc) was purified from cation contamination by passing a water solution of the ganglioside through a Chelex 100 cation exchange resin column.

Mixed micelles were prepared by dissolving the dried ganglioside and the dodecylphosphocholine (molar ratio 1:40) in deuterated potassium phosphate buffer (50 mM, 0.5 ml, pH 6) [17]. 1H-NMR and 13C-NMR spectra were respectively performed at 500 and 125 MHz, on a Bruker AM500 spectrometer, and analysed on a X32 Bruker satellite station equipped with the standard Bruker UXNMR software.

The chemical shift assignments were obtained by 1D- and 2D-HOHAHA, and 1H-13C heterocorrelated HSQC experiments [1, 12]. The nOe investigation was realized by 2D-ROESY experiments using an off-resonance spin lock procedure to avoid scalar transfer, as described elsewhere [1, 12, 13, 18].

2D 1H-1H and 1H-13C experiments were respectively acquired with 512 and 256 t1 increments, and 128 scans were collected for each t1. After zero filling and appropriate window function multiplication, the time domain spectra were transformed to give 2048 × 1024 point matrixes. Distance evaluation was based on the hypothesis of proportionality $V_{ij} \propto 1/r_{ij}^6$, where $V_{ij}$ is the cross-peak volume and $r_{ij}$ the proton-proton distance for proton pair H$_i$-H$_j$ [19]. The intraresidual fixed distances between syndiaxial (0.25 nm) and transdiaxial (0.31 nm) protons were used as internal references. For the DMSO sample five experiments were performed at different temperatures, varying the mixing time and the off-resonance lock frequency; the nOe distances derived from the five spectra were averaged to give the reported distances. Four experiments were performed on the D$_2$O sample and the results averaged.

Molecular mechanics calculations were performed on a Silicon Graphics IRIS 4D35GT workstation. Molecular modelling was carried out with an INSIGHT/DISCOVER package. The computation of the minimum energy conformations of the oligosaccharide moiety was performed by restrained molecular mechanics calculations using CVFF [20] with a dielectric constant e of 80 [13]. The nOe restraints were defined as harmonic forcing potentials.

Results and discussion

The complete proton and carbon chemical shift assignments for GM1(Neu5Gc) ganglioside in dimethylsulfoxide and water solution are reported in Tables 1 and 2, respectively.

The conformation of the trisaccharide fragment -β-GalNAc-(1→4)-[α-Neu5Gc-(2→3)]-β-Gal- is of particular interest since all the molecules investigated till now, GM1(Neu5Ac) [1], GD1a [12, 13] and GalNAc-GD1a [13], show this fragment to be fairly rigid. As a preliminary the potentially flexible glycerol sialic acid side chain, which shows a number of nOe interactions with the GalNAc residue, was analysed. It was found that vicinal coupling constants (Table 3) and nOe interactions along the side chain are in accordance with previous results for Neu5Ac linked to several gangliosides [1, 12, 13, 18] and Neu5Gc in GM3 ganglioside [14]. This suggests a rigid 3D structure for the glycerol sialic acid chain. Thus it can be deduced that neither substitution at the amino group, nor different aglicone structures, influences the sialic acid glycerol chain 3D structure. The trisaccharide fragment -β-GalNAc-(1→4)-[α-Neu5Gc-(2→3)]-β-Gal- is characterized by several interresidual nOe contacts (Table 5). The GalNAc residue has