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A novel heptasialosyl c-series ganglioside in embryonic chicken brain: its structure and stage-specific expression

Megumi Saito*, Hisayo Kitamura, Kiyoshi Sugiyama

Department of Clinical Pharmacology and Therapeutics, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, 422-8526 Shizuoka, Japan

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Abstract

A ganglioside of unknown structure (ganglioside X) was purified from chicken brain at embryonic day 12 (E12) and characterized for its structure. Ganglioside X was reactive with a monoclonal antibody A2B5 and migrated below GH1c on thin-layer chromatography (TLC). Extensive treatment of ganglioside X with *Clostridium perfringens* sialidase produced a single ganglioside product. This ganglioside was identified as GM1 based upon its chromatographic mobility and reactivity to cholera toxin B subunit and anti-GM1 antibody. Partial hydrolysis of ganglioside X by sialidase generated several degradation products including GH1c, GP1c, and GQ1c. Electrospray ionization (ESI)-mass spectrometry (MS) of the permethylated derivative of ganglioside X produced a triple-charged parent ion peak at *m/z* 1355, which corresponded with the gangliotetraose oligosaccharide structure having seven sialic acids and ceramide with the molecular mass of 566 (as non-methylated form). Collision-induced dissociation (CID)-MS^2 showed fragment ions including those at *m/z* 1066 and 1931; these two ions matched the structures of $(NeuAc)_3$-Gal-Glc-Cer and $(NeuAc)_4$-Gal-GalNAc, respectively. These structures were confirmed by CID-MS^3 of the corresponding peaks. Based upon these findings, the structure of ganglioside X was identified as NeuAc-NeuAc-NeuAc-NeuAc-Galβ1–3GalNAcβ1–4(NeuAc-NeuAc-NeuAcα2–3)Galβ1–4Glcβ1-1′ Cer. This ganglioside was designated as GS1c. A developmental study demonstrated that GS1c was expressed in chicken brain during a period from E6 to E13 and thereafter decreased rapidly in its concentration. The present study suggests that GS1c may play a specific role in early development of chicken brain.

Keywords: Glycolipid; Ganglioside; C-series ganglioside; A2B5; Chicken; Brain; Development

1. Introduction

C-series gangliosides are characterized by a trisialosyl residue at the inner galactose of the hemato- or ganglio-type oligosaccharide structure. They are enriched in adult brain of bony fish such as cod fish [1,2] and cartilaginous fish including dogfish [3] and skate fish [4]. The presence of 9-*O*-acetyl derivatives of GT2 and GT3 in cod fish brain were also reported [5,6]. The expression of c-series gangliosides is not restricted to certain fish species. We recently examined fish brain gangliosides and demonstrated that c-series gangliosides are widely distributed in different bony fish species [7]. In higher animals, c-series gangliosides are expressed in brain tissues at certain embryonic stages, but hardly detected at adult ages [8–15]. C-series gangliosides have also been detected in extraneural tissues including hog kidney [16], human lung [17], bovine butter milk [18], cat erythrocytes [19], pancreatic tissues and islet cells of several species [20,21], and different types of cancer cells and tissues [22–25]. We have recently demonstrated the wide distribution of c-series gangliosides in adult rat tissues [26].

In higher animals, c-series gangliosides constitute minor ganglioside components of tissues and cells and often are difficult to separate from major ganglioside species on thin-layer chromatography (TLC). Accordingly, specific antibodies directed toward c-series gangliosides have been developed. The antibody A2B5 was originally prepared by immunizing chicken embryonic retinal cells [20]. While there has been some controversy about its specificity, recent studies have suggested the specific reactivity of A2B5 to c-series gangliosides [14,27–29]. We have recently investigated the reactivity of the antibody to gangliosides and neutral glycolipids and demonstrated its strict specificity to

Abbreviations: ESI, electrospray ionization; MS, mass spectrometry; CID, collision-induced dissociation; TLC, thin-layer chromatography; ECL, enhanced chemiluminescence; HPLC, high-performance liquid chromatography; DMB, 1,2-diamino-4,5-methylenedioxy-benzene

* Corresponding author. Tel.: +81-54-264-5763; fax: +81-54-264-5764.
E-mail address: saitom@ys2.u-shizuoka-ken.ac.jp (M. Saito).

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c-series gangliosides [7]. The epitope for the antibody was assumed to include the trisialosyl residue connected the inner galactose of the hemato or gangliotetraose oligosaccharide structure. Subsequently, we examined gangliosides in embryonic chicken brain using this antibody and found an A2B5-reactive ganglioside of unknown structure below GH1c on TLC. The presence of ganglioside with similar chromatographic mobility was previously reported, though its structure has remained to be determined [30,31].

In the present study, we characterized the structure of this A2B5-reactive ganglioside and showed that this ganglioside was a novel c-series gangliotetraose ganglioside having a tetrasialosyl residue at the outer galactose moiety. We also demonstrated the restricted expression of this ganglioside at early embryonic stages of chicken brain.

2. Materials and methods

2.1. Materials

Fertilized eggs and 5-day-old chickens (white Leghorn) were obtained from a local chicken farm. A monoclonal antibody A2B5-producing hybridomas CRL 1520 were obtained from American-Type Culture Collection (Manassas, VA, USA) and cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. A culture medium containing A2B5 (IgM type) was used for experiments [7]. A polyclonal antibody directed toward GM1 (IgG type) was prepared by immunizing New Zealand white rabbits with purified lipid. This antibody has been successfully employed for specific detection of GM1 [32]. GM1 was purified from bovine brain in our laboratory. Four different c-series gangliosides, i.e., GT3, GQ1c, GP1c, and GH1c, were purified from bonito fish brain [7]. Gangliosides were isolated from adult rat brain and used as standard gangliosides for TLC. Other reagents were purchased from the following companies: *Clostridium perfringens* sialidase, peroxidase-conjugated cholera toxin B subunit, and peroxidase-conjugated anti-mouse IgM or anti-rabbit IgG antibody (Sigma, St. Louis, MO, USA), high-performance TLC plates (nanoplate, Merck KGaA, Damstadt, Germany), and enhanced chemiluminescence (ECL) Western blotting detection kits (Amersham Pharmacia Biotech, Buckinghamshire, England).

2.2. Isolation of unknown ganglioside in embryonic chicken brain

Twenty-seven gram of brain tissue were obtained from 100 chicken embryos at embryonic day 12 (E12). Total lipids were extracted with 20 volumes of chloroform/methanol (1:1) and applied to a DEAE-Sephadex column equilibrated with chloroform/methanol/H_2O (30:60:8). While neutral lipids passed through the column, acidic lipids were retained on the column and eluted with eight column volume of chloroform/methanol/1.6 M sodium acetate (30:60:8). Using this solvent, the recovery of c-series gangliosides (e.g., GP1c) increased by 30% as compared with the solvent containing 0.8 M sodium acetate [33]. To hydrolyze coexisting phospholipids, acidic lipids were incubated in 0.2 M methanolic NaOH at 37 °C for 1 h, followed by neutralization with acetic acid. Purified gangliosides, including a ganglioside of unknown structure (designated as ganglioside X), were obtained by desalting the neutralized sample on Sephadex LH-20 column chromatography using methanol as the solvent.

Ganglioside X in E12 chicken brain gangliosides was isolated by high-performance liquid chromatography (HPLC) with a silica gel column based upon a method reported previously with modifications [34]. Gangliosides were applied to the column (TSKgel Silica-60, 4.6×250 mm, Tosoh, Tokyo, Japan) and eluted using a isocratic solvent system of acetonitrile/isopropanol/50 mM tetramethylammonium chloride (10:35:55). Ganglioside peaks were monitored by absorbance at the wave length of 205 nm.

2.3. Overlay analysis of gangliosides

Overlay analysis of gangliosides with a specific ligand was carried out based upon a method reported previously [35]. In brief, gangliosides were developed on TLC. After coating with a 0.4% polyisobutylmethacrylate solution, the plate was treated successively with anti-ganglioside antibody and peroxidase-conjugated second antibody at room temperature for 1.5 h. The reactive band(s) were detected using the ECL method. After removing the organic polymer from the plate with hexane/chloroform (1:1), gangliosides were visualized with resorcinol-HCl reagent [36]. Densitometric analysis of gangliosides was carried out using a densitometric image analyzer (Atto Densitograph AE-6920M, Atto Co., Tokyo, Japan).

Alternatively, gangliosides on TLC were analyzed using peroxidase-conjugated cholera toxin B subunit [37]. Detection of reactive bands was carried out, as described above.

2.4. Treatment of gangliosides with sialidase in test tube reaction

A ganglioside was dissolved in 0.1 M sodium acetate buffer (pH 4.8) and incubated with *C. perfringens* sialidase at 37 °C in two different conditions: 0.5 U/ml for 12 h (extensive hydrolysis) and 0.1 U/ml for 30 min (partial hydrolysis). The reaction product(s) were desalted by Sephadex LH-20 chromatography, developed on TLC, and analyzed using ganglioside-specific ligands (see above).

2.5. Electrospray ionization (ESI)-mass spectrometry (MS) of gangliosides

The structure of ganglioside was analyzed by ESI-MS using an LCQ ion-trap mass spectrometer equipped with an

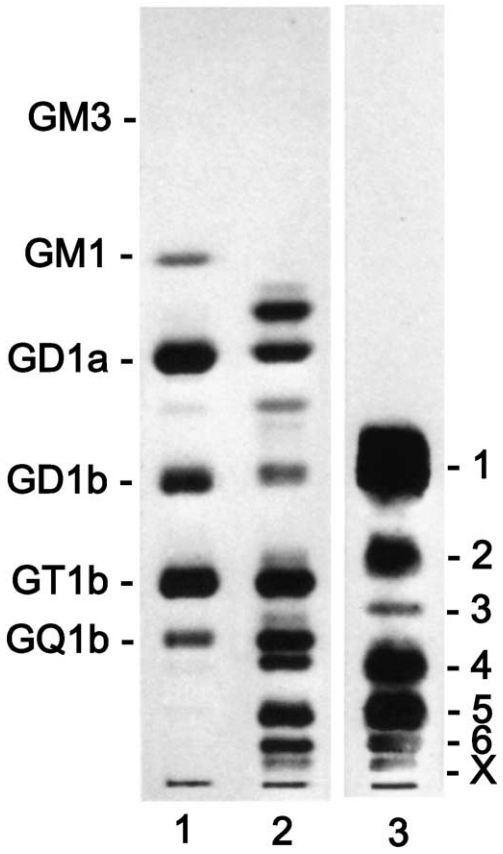

Fig. 1. Major and c-series gangliosides of E12 chicken brain. Gangliosides were isolated from E12 chicken brain and developed on high-performance thin-layer chromatography (TLC) with a solvent system of chloroform/methanol/0.2% $CaCl_2 \cdot 2H_2O$ (40:40:11) until the solvent front reached at the position of 14 cm from the bottom of the plate. Gangliosides on the plate were visualized with resorcinol-HCl reagent (lane 2, 3 μl sialic acid per lane) or immunostained using a monoclonal antibody A2B5 (lane 3, 0.5 μl sialic acid). Lane 1: standard gangliosides visualized with resorcinol-HCl reagent.

ESI source (Finnigan MAT, USA). A ganglioside was permethylated using a method reported previously [38]. The permethylated derivative formed a single band on TLC. The permethylated lipid was then dissolved in methanol at a concentration of 10 pmol/μl and introduced into the electrospray needle by mechanical infusion at a flow rate of 3 μl /min. The ESI capillary was kept at a voltage of +4 V at 200 °C. The tube lens offset was set at −30 V. The collision-induced dissociation (CID)-MS^2 and CID-MS^3 spectra were taken using helium as the collision gas. The relative collision energy scale was set at +2.5 eV. Mass spectra were averaged over 10 scans.

2.6. Fluorometric HPLC analysis of sialic acids

Lipid-bound sialic acids were analyzed using a method reported previously [39]. In brief, gangliosides were first treated in 25 mM sulfuric acid at 80 °C for 1 h. The hydrolysate was then incubated with a 1,2-diamino-4,5-methylenedioxy-benzene (DMB) reagent at 60 °C for 2.5 h. The fluorescent derivatives of sialic acids were analyzed by HPLC with an ODS column (Mightysil RP 18, 4.6×250 mm, Kanto Chemicals, Tokyo, Japan). The excitation and emission wave lengths were 373 and 448 nm, respectively. Quantitation was carried out based upon the standard curve obtained with *N*-acetylneuraminic acid solutions of different concentrations.

3. Results

3.1. A2B5-reactive ganglioside of unknown structure in embryonic chicken brain

Gangliosides were isolated from E12 chicken brain and characterized using a monoclonal antibody A2B5. At least seven A2B5-reactive gangliosides were observed (Fig. 1). Among them, gangliosides 1–6 were identified as GT3, GT2, GT1c, GQ1c, GP1c, and GH1c, respectively, based upon their chromatographic mobility on TLC and reactivity with A2B5. The structure of GH1c was also confirmed by

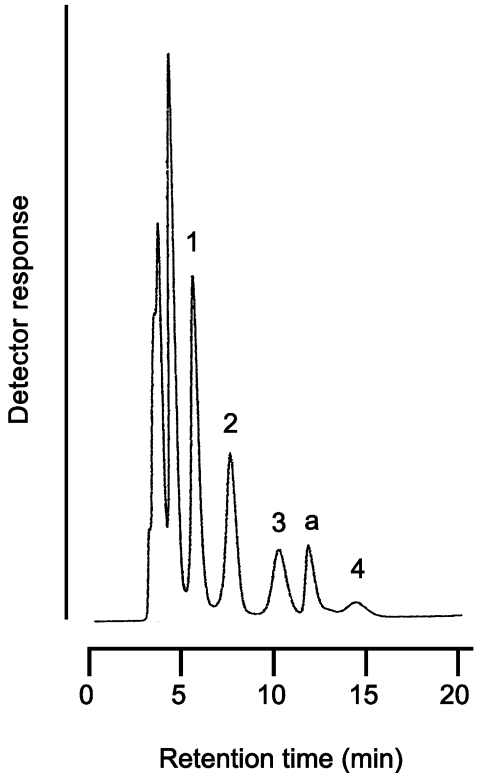

Fig. 2. High-performance liquid chromatography (HPLC) of embryonic day 12 (E12) chicken brain gangliosides. Purified gangliosides from E12 chicken brain were applied to a silica gel column and eluted with a isocratic solvent system of acetonitrile/isopropanol/50 mM tetramethylammonium chloride (10:35:55) at a flow rate of 0.5 ml/min. Ganglioside peaks were monitored by absorbance at 205 nm. Peak 1, GQ1b+GQ1c; 2, GP1c; 3, GH1c; and 4, ganglioside X. Peak a was an unknown peak containing no sialic acid.

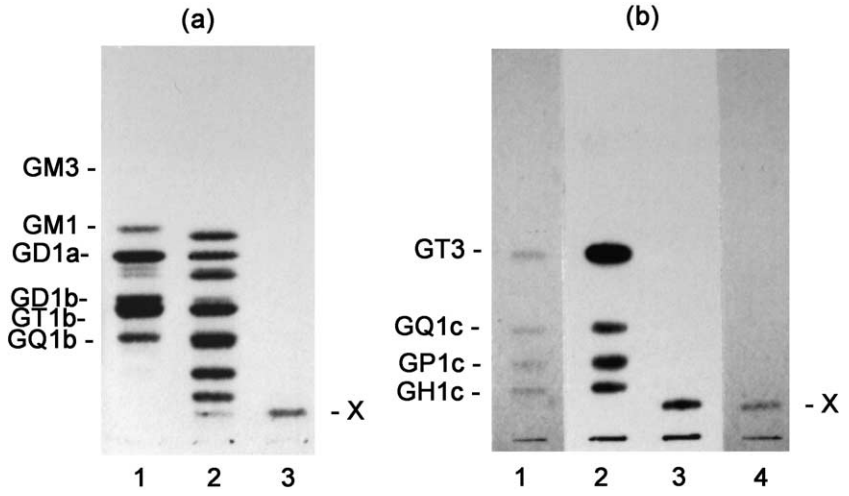

Fig. 3. Immunostaining of ganglioside X in E12 chicken brain. An unknown ganglioside (designated as ganglioside X) in E12 chicken brain was purified and developed on TLC using a two-step developing technique. The first development was carried out with a solvent system of *n*-propanol/2.5 M NH_4OH (7:3) to 7 cm from the bottom of the plate. After drying the plate, the second development was done with a solvent system of chloroform/methanol/0.2% $CaCl_2 \cdot 2H_2O$ (45:40:10) to 9 cm from the bottom. (a) Ganglioside X (0.1 μg sialic acid) was visualized with resorcinol-HCl reagent (lane 3). Lane 2, E12 chicken brain gangliosides (2 μg sialic acid). Lane 1, standard gangliosides. (b) Ganglioside X (0.1 μg sialic acid) was immunostained with A2B5 (lane 3). Authentic c-series gangliosides (i.e., GT3, GQ1c, GP1c, and GH1c) were also immunostained with the antibody (lane 2). Lanes 1 and 4: authentic c-series gangliosides and ganglioside X, both of which were respectively visualized with resorcinol-HCl reagent.

ESI-MS and CID-MS^2 (data not shown). In addition to these c-series gangliosides, an A2B5-reactive ganglioside of unknown structure was detected below GH1c on TLC. This ganglioside (designated as ganglioside X) was purified from E12 chicken brain gangliosides by HPLC with a silica gel column (Fig. 2). The obtained preparation of ganglioside X gave a single band on TLC (Fig. 3a). The A2B5 reactivity of the purified ganglioside was confirmed through immunostaining (Fig. 3b).

3.2. Product analysis of ganglioside X after sialidase treatment

Ganglioside X was treated with sialidase, and the reaction product(s) were analyzed. Extensive treatment of ganglioside X with *C. perfringens* sialidase generated a single ganglioside product that had the same chromatographic mobility of GM1 on TLC (Fig. 4a). This ganglioside was identified as GM1 by positive reactivity with

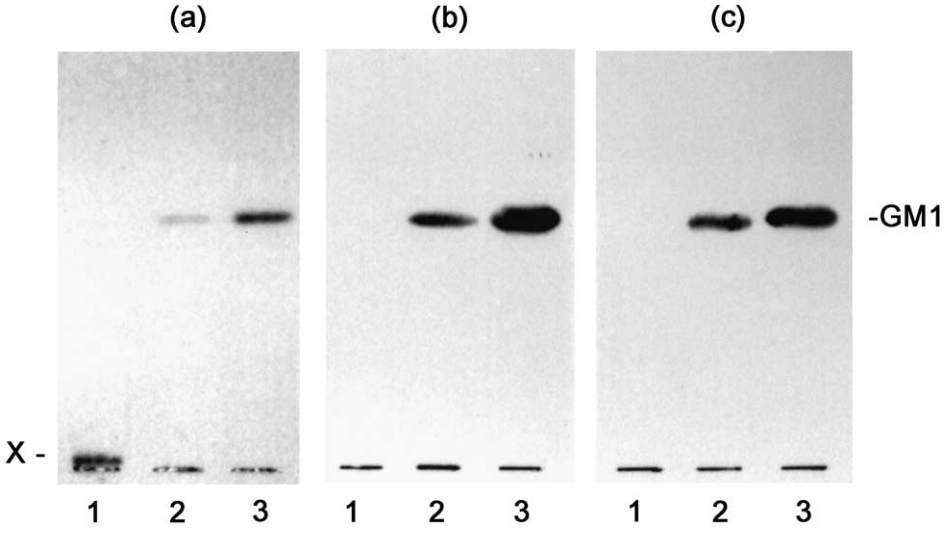

Fig. 4. Product analysis of ganglioside X after extensive hydrolysis with sialidase. Ganglioside X (0.2 μg sialic acid) was treated with Cl. perfringens sialidase (0.5 U/ml of 0.1 M sodium acetate buffer, pH 4.8) at 37 °C for 12 h. The reaction product was desalted by LH-20 column chromatography, developed on TLC, and visualized with resorcinol-HCl reagent (a) or overlaid with cholera toxin B subunit (b) or with anti-GM1 antibody (c). In each panel: lane 1, ganglioside X; 2, reaction product from ganglioside X after sialidase treatment; and 3, authentic GM1.

cholera toxin B subunit (Fig. 4b) or anti-GM1 antibody (Fig. 4c). Based upon these results, it was concluded that ganglioside X shared the same oligosaccharide structure with GM1. Partial hydrolysis of ganglioside X with the enzyme generated several ganglioside products (Fig. 5). One reaction product migrated immediately above ganglioside X on TLC and was identified as GH1c based upon its chromatographic mobility on TLC and A2B5 reactivity. Thus, it was suggested that ganglioside X contained the same structure of GH1c with one or more additional sialic acid residues. The reaction products also included GP1c and GQ1c as well as GM1.

Sialic acid analysis by fluorometric HPLC revealed that *N*-acetylneuraminic acid was the sole sialic acid molecular species in ganglioside X (data not shown).

3.3. ESI-MS of ganglioside X

The structure of ganglioside X was further characterized by ESI-MS using the permethylated derivative of the compound [40]. The mass spectrum of ganglioside X showed a triple-charged ion of $[M+2+3Na]^{3+}$ at *m/z* 1355; this parent mass ion corresponded with a gangliotetraose oligosaccharide structure having seven sialic acids and a ceramide with the molecular mass of 566 (as non-methylated structure) (Fig. 6a). The CID-MS^2 of this ion produced fragment ions including those at *m/z* 1066 and 1931; they corresponded with the structures of $[3NeuAc+Gal-Glc-Cer+2Na]^{2+}$ and $[4NeuAc+Gal-GalNAc - H_2O+Na]^+$, respectively. Other fragment ions at *m/z* 398, 759, 1163, 1230, 1291, 1471, 1482, 1652, and 1833 matched with $[NeuAc - H_2O+Na]^+$, $[2NeuAc - H_2O+Na]^+$, $[M - Cer+3Na]^{3+}$, $[6NeuAc+Gal-GalNAc-Gal-Glc-Cer+3Na]^{3+}$, $[3NeuAc+Gal-GalNAc-Gal-Glc-Cer+2Na]^{2+}$, $[4NeuAc+Gal-GalNAc-Gal-Glc-Cer+2Na]^{2+}$, $[4NeuAc - H_2O+Na]^+$, $[5NeuAc+Gal-GalNAc-Gal-Glc-Cer+2Na]^{2+}$, and $[6NeuAc+Gal-GalNAc-Gal-Glc-Cer+2Na]^{2+}$, respectively (Fig. 6b). Among these fragment ions, the ions at *m/z* 1066 and 1931 were further characterized by CID-MS^3. The CID-MS^3 spectrum of the ion at *m/z* 1066 showed fragment ions that corresponded with the partial structures of (hexa-methylated NeuAc)-(penta-methylated NeuAc)$_2$-(di-*O*-methylated-Gal)-(tri-*O*-methylated-Glc)-(di-methylated Cer); the ions at *m/z* 576, 745, 878, 1011, 1033, 1311, and 1372 matched with [di-methylated Cer - $H_2O+H]^+$, [(penta-methylated NeuAc)$_2$ - $H_2O+Na]^+$, [(penta-methylated NeuAc)$_2$-(di-*O*-methylated-Gal)-(tri-*O*-methylated-Glc)-(di-methylated Cer)+$2Na]^{2+}$, [(di-*O*-methylated-Gal)-(tri-*O*-methylated-Glc)-(di-methylated Cer)+$Na]^+$, [(di-*O*-methylated-Gal)-(tri-*O*-methylated-Glc)-(di-methylated Cer) - $H+2Na]^+$, [(hexa-methylated NeuAc)-(penta-methylated NeuAc)$_2$-(di-*O*-methylated-Gal) - $H_2O+Na]^+$, and [(penta-methylated NeuAc)-(di-*O*-methylated-Gal)-(tri-*O*-methylated-Glc)-(di-methylated Cer)+$Na]^+$, respectively (Fig. 6c). The CID-MS^3 spectrum of the ion at *m/z* 1931 contained fragment ions that accorded with the partial structures of (hexa-methylated NeuAc)-(penta-methylated NeuAc)$_3$-(tri-*O*-methylated-Gal)-(tri-methylated GalNAc); the ions at *m/z* 760, 834, 990, 1122, 1195, 1556, and 1704 corresponded with [(hexa-methylated NeuAc)-(penta-methylated NeuAc) - $H_2O+Na]^+$, [(penta-methylated NeuAc)-(tri-*O*-methylated-Gal)-(tri-methylated GalNAc) - $H_2O+Na]^+$, [(penta-methylated NeuAc)-(tri-*O*-methylated-Gal) - $H+2Na]^+$, [(hexa-methylated NeuAc)-(penta-methylated NeuAc)2 - $H_2O+Na]^+$, [(penta-methylated NeuAc)$_2$-(tri-*O*-methylated-Gal)-(tri-methylated GalNAc)-$H_2O+Na]^+$, [(penta-methylated NeuAc)$_3$-(tri-*O*-methylated-Gal)-(tri-methylated GalNAc) - H_2O+Na]+, and [(hexa-methylated NeuAc)-(penta-methylated NeuAc)$_3$-(tri-*O*-methylated-Gal)+$Na]^+$, respectively (Fig. 6d). These results indicated that ganglioside X had the gangliotetraose oligosaccharide structure with two separate polysialosyl residues, i.e., a non-branched trisialosyl residue at the inner galactose and a non-branched tetrasialosyl residue at the outer galactose molecule.

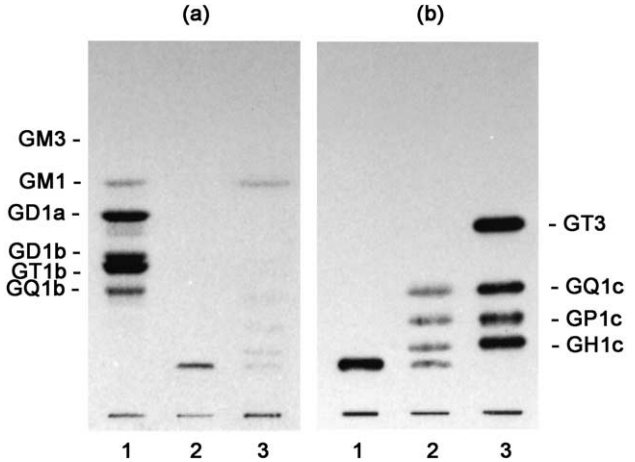

Fig. 5. Product analysis of ganglioside X after partial hydrolysis with sialidase. Ganglioside X (0.2 μg sialic acid) was treated with Cl. perfringens sialidase (0.1 U/ml of 0.1 M sodium acetate buffer, pH 4.8) at 37 °C for 30 min. The reaction products were desalted by LH-20 column chromatography, developed on TLC, and visualized with resorcinol-HCl reagent (a) or immunostained with A2B5 (b). (a) Lane 1, standard gangliosides; 2, ganglioside X; and 3, reaction products after sialidase treatment. (b) Lane 1, ganglioside X; 2, reaction products after sialidase treatment; and 3, standard c-series gangliosides.

Based upon the findings described above, the structure of ganglioside X was determined to be NeuAc-NeuAc-NeuAc-NeuAc-Galβ1−3GalNAcβ1−4(NeuAc-NeuAc-NeuAcα2−3)Galβ1−4Glcβ1-1′ Cer. This ganglioside was designated as GS1c, in which "S" is the initial of the word "septem", the Latin number seven.

3.4. Developmental changes of GS1c and other c-series gangliosides in chicken brain

The developmental expression of GS1c and other c-series gangliosides in chicken brain was examined using A2B5. As shown in Fig. 7, GS1c exhibited a specific

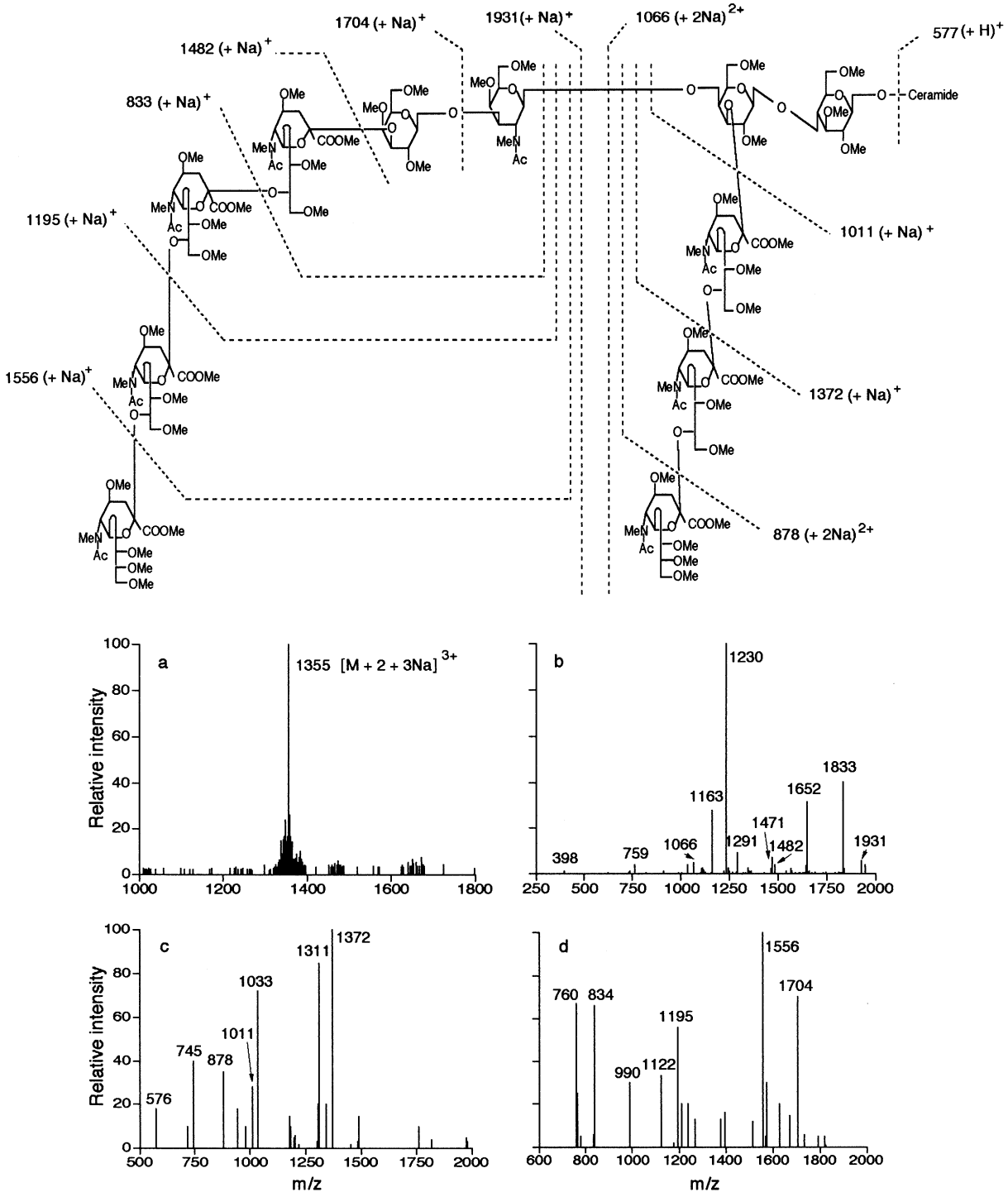

Fig. 6. Electrospray ionization (ESI)-mass spectrometry (MS) of ganglioside X. The structure of ganglioside X was analyzed by ESI-MS after permethylation with methyl iodide. (a) ESI-MS spectrum of ganglioside X; (b) collision-induced dissociation (CID)-MS^2 spectrum of the ion peak at *m/z* 1355 in panel a; (c) CID-MS^3 spectrum of the ion peak at *m/z* 1066 in panel b; and (d) CID-MS^3 spectrum of the ion peak at *m/z* 1931 in panel b.

developmental pattern. it was characterized by higher expression at early embryonic stages, after which a gradual reduction led to an almost zero level by postnatal day 5. In contrast, other gangliosides such as GT3, GT1c, GQ1c, GP1c, and GH1c showed different developmental profiles in which higher concentrations at early developmental stages reduced after E9, and maintained nearly constant levels up to postnatal day 5. Expression of ganglioside GT2

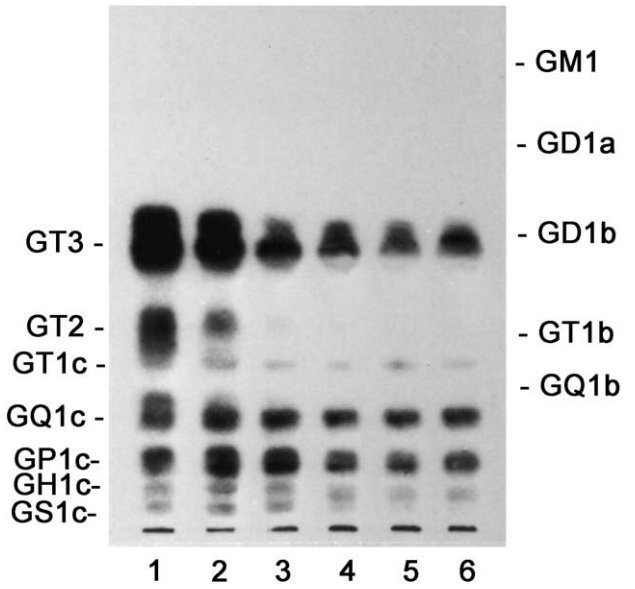

Fig. 7. Developmental profiles of GS1c and other c-series gangliosides in chicken brain. Gangliosides were isolated from chicken brain, developed on TLC, and immunostained using A2B5. Lanes 1, embryonic day 6 (E6); 2, E9; 3, E13; 4, E16; 5, E20; and 6, postnatal day 5. The amount of gangliosides per lane was equivalent to 0.4 μg sialic acid.

was more restricted; the ganglioside was found only in E6 and E9 chicken brain.

4. Discussion

The presence of c-series gangliosides was suggested by earlier studies on fish brain gangliosides [41]. In 1979, Ando and Yu [1] identified the structure of GQ1c in cod fish brain. Subsequently, they characterized the structures of GT3, GT2, and GP1c in cod fish brain and proposed the metabolic pathway of c-series gangliosides [2]. They also recognized a ganglioside of unknown structure, G″H″, which migrated below GP1c. We recently characterized this ganglioside in bonito fish brain and determined its structure as (NeuAc-NeuAc-NeuAc)-Galβ1–3GalNAcβ1–4(NeuAc-NeuAc-NeuAcα2–3)Galβ1–4Glcβ1-1′ Cer, i.e., GH1c [7]. Therefore, in all, the five different c-series gangliosides, i.e., GT3, GT2, GT1c, GQ1c. GP1c, and GH1c, has structurally been identified to date, except for their *O*-acetyl derivatives.

In the present study, we examined E12 chicken brain gangliosides using a monoclonal antibody A2B5 and detected a novel A2B5-reactive ganglioside (ganglioside X). The positive reactivity to A2B5 strongly suggests that this ganglioside is one of c-series gangliosides or a compound having a closely related structure. Further characterization of ganglioside X revealed its structure to be NeuAc-NeuAc-NeuAc-NeuAc-Galβ1–3GalNAcβ1–4(NeuAc-NeuAc-NeuAcα2–3)Galβ1–4Glcβ1-1′ Cer. The partial structure, Galβ1–3GalNAcβ1–4(NeuAcα2–3)Galβ1–4Glcβ1-1′ Cer, was identified using GM1-specific ligands including cholera toxin B subunit [42]. Fluorometric HPLC analysis demonstrated that all sialic acid residues of ganglioside X were *N*-acetylneuraminic acid although it is possible that they or a part of them may originally exist in alkali-labile derivatives (e.g., *O*-acetylated forms) in tissues. The structures and positions of the tri- and tetrasialosyl residues were successfully determined by ESI-MS. Since GQ1c was produced from partial hydrolysis of ganglioside X, the tetrasialosyl residue was assumed to be connected to the outer galactose through an α2–3 linkage. Although the linkage of sialic acids in the polysialosyl resides was not determined, it is reasonably assumed that sialic acids are mutually connected through α2–8 linkage, as observed in other natural sialoglycoconjugates [43]. Previously, Rosner examined embryonic chicken brain gangliosides and found a ganglioside that migrated below G″H″. This ganglioside was demonstrated to contain seven sialic acid molecules per sphingosine and called as G″S″ [30,31]. It is likely that ganglioside X and this ganglioside are the same ganglioside.

The ganglioside GS1c was found to have a unique developmental pattern in chicken brain; it contrasts with other known c-series gangliosides. This finding suggests that GS1c may play a specific role in early development of chicken brain. Regarding the developmental profiles of c-series gangliosides, Hirabayashi et al. [12] reported developmental changes of GT3, GT2, GT1c, GQ1c, and GP1c in embryonic chicken optic lobe using TLC immunostaining with specific monoclonal antibodies M6704 and M7103. When their data are recalculated and the amount of each ganglioside is expressed on the basis of the amount of total gangliosides (e.g., per μg sialic acid), the developmental profiles of these gangliosides are similar to our results, except that GT2 decreases at a slower rate than that in the present study. While the reason for this discrepancy is not known, it may represent topographical difference in the composition and metabolism of brain gangliosides [44].

In summary, we identified a novel c-series ganglioside GS1c in chicken brain and demonstrated its stage-specific expression. Regarding the distribution of GS1c in other animal species, we recently found that cod fish brain contains a ganglioside corresponding to GS1c (Saito and Sugiyama, unpublished data). No information is available about mammalian brain. To clarify this issue, a further study on the phylogenetic distribution of GS1c in different tissues is being planned.

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