A NOVEL MONOSIALOGANGLIOSIDE SYNTHESIZED BY A RAT BRAIN CYTIDINE-5'-MONOPHOSPHO-N-ACETYLNEURAMINIC ACID: GALACTOSYL-N-ACETYLGALACTOSAMINYL-GALACTOSYL-GLUCOSYLCERAMIDE SIALYLTRANSFERASE

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SUMMARY The present paper reports a cytidine-5'-monophospho-N-acetylneuraminic acid: galactosyl-N-acetylgalactosaminyl-galactosyl-glucosylceramide sialyltransferase in young rat brain. The enzymic product is a new monosialoganglioside containing a neuraminidase-labile neuraminic acid. The proposed structure for this novel monosialoganglioside is as follows: N-acetylneuraminyl(2→3)Galactosyl(β, 1→3)N-acetylgalactosaminyl(β, 1→4)Galactosyl(β, 1→4)Glucoayl(1→1)ceramide.

INTRODUCTION

Monosialoganglioside (GMla)[1] has been recognized as one of the major complex gangliosides in a variety of normal and pathological tissues (1, 2). The major occurance of GMla is in the brain; thus, this tissue has been emplosyed extensively for the metabolic studies of the complex gangliosides (3-9).

In rat brain, GMla is synthesized by a UDP-galactose: GM2 galactosyltransferase (4, 6), and is subsequently metabolized further to GDla by a CMP-NANA: GMla sialyltransferase (3). Another pathway for the biosynthesis of monosialoganglioside via the asialoganglioside has been proposed (10):

$$\text{Glucosylceramide} + \text{UDP-galactose} \xrightarrow{\text{galactosyltransferase (1)}} \text{GL-2a}$$

$$\text{GL-2a} + \text{UDP-N-acetylgalactosamine} \xrightarrow{\text{N-acetylgalactosaminyltransferase}} \text{Asialo-GM2}$$

$$\text{Asialo-GM2} + \text{UDP-galactose} \xrightarrow{\text{galactosyltransferase (2)}} \text{Asialo-GM1}$$

$$\text{Asialo-GM1} + \text{CMP-NANA} \xrightarrow{\text{sialyltransferase}} \text{Monosialoganglioside}$$

However, in rat brain, only the first three glycosyltransferases of the above pathway have previously been demonstrated, and the step leading to the formation of monosialoganglioside from the precursor, asialo-GM1, remains to be determined.

The present paper reports a CMP-NANA: Asialo-GM1 sialyltransferase in young rat brain, and the enzymic product is a new monosialoganglioside containing a neuraminidase-labile neuraminic acid.

MATERIALS AND METHODS

The asialo-GM1 was prepared by acid hydrolysis (0.1 N HCl) of bovine brain gangliosides for 1 hr at 100°C. The material was dialyzed and the dialysand lyophilized. The asialo-GM1 was purified by preparative thin layer chromatography as described previously (10). Gangliosides were isolated from ox brain according to Folch et al. (11). The individual gangliosides, GM1a and GD1a, were isolated from a silica gel G column by elution with the solvent chloroform-methanol-water (61:32:7, v/v/v) (12), and were further purified by preparative thin layer chromatography in chloroform-methanol-2.5 N NH_4OH (60:35:8, v/v/v) (13). The labelled CMP-NANA was purchased from New England Nuclear Corporation and the non-radioactive CMP-NANA was prepared by the method of Brunetti et al. (14) with the modification of Arce et al. (15).

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1 Abbreviations: GM1a, galactosyl-N-acetylgalactosaminyl-(N-acetylneuraminyl)-galactosyl-glucosylceramide; GM2, N-acetylgalactosaminyl-(N-acetylneuraminyl)-galactosyl-glucosylceramide; GD1a, N-acetylneuraminyl-galactosyl-N-acetyl-galactosaminyl-(N-acetylneuraminyl)-galactosyl-glucosylceramide; CMP-NANA, cytidine-5'-monophospho-N-acetylneuraminic acid; GL-2a, galactosyl-glucosyl-ceramide; Asialo-GM2, N-acetylgalactosaminyl-galactosyl-glucosylceramide; Asialo-GM1, galactosyl-N-acetylgalactosaminyl-galactosyl-glucosylceramide; GM3, N-acetylneuraminyl-galactosyl-glucosylceramide; GD3, N-acetylneuraminyl-N-acetylneuraminyl-galactosyl-glucosylceramide; GM1b, N-acetylneuraminyl-galactosyl-N-acetylgalactosaminyl-galactosyl-glucosylceramide.

TABLE I. Requirements of CMP-NANA : Asialo-GM1 sialyltransferase activity in young rat brain.

System	u mole	(^{14}C) NANA Incorporated (CPM)
Complete		1208
Complete, Boiled Enzyme		20
- Glycolipid		118
- Detergents		86
+ Mg^{++}	1.0	1152
+ Mn^{++}	1.0	1180
+ Ca^{++}	1.0	927
+ NANA	0.1	1158
+ UDP-gal	0.1	1158
+ UDP-glc	0.1	908
+ CMP-NANA	0.1	174

Sprague-Dawley rats were obtained from the laboratory of Dr. S. Roberts at the University of California School of Medicine at Los Angeles.

The complete incubation system for the CMP-NANA : Asialo-GM1 sialyltransferase assay contained 0.1 M cacodylate buffer (pH 6.5), 0.2 mg Triton CF-54 and 0.1 mg Tween 80, 0.75 mM asialo-GM1, 0.25 mM CMP-(^{14}C)NANA (specific activity 1.64 x 10^6 cpm/u mole), 0.03 ml enzyme preparation and water to a final volume of 0.1 ml. The enzyme preparation was prepared by homogenizing the young rat brain (1 to 5-day-old) in four volumes of a solution containing 0.32 M sucrose and 0.11% (w/v) 2-mercapto-ethanol. The enzymic reaction was stopped by the addition of 2 ml of chloroform-methanol (2:1, v/v). After shaking, the reaction mixture was transferred to a thin column (1 cm diameter) containing 0.8 g of Sephadex G-25 superfine previously equilibrated with chloroform-methanol-water (60:30:4.5, v/v/v). After collecting the filtrate, the column was

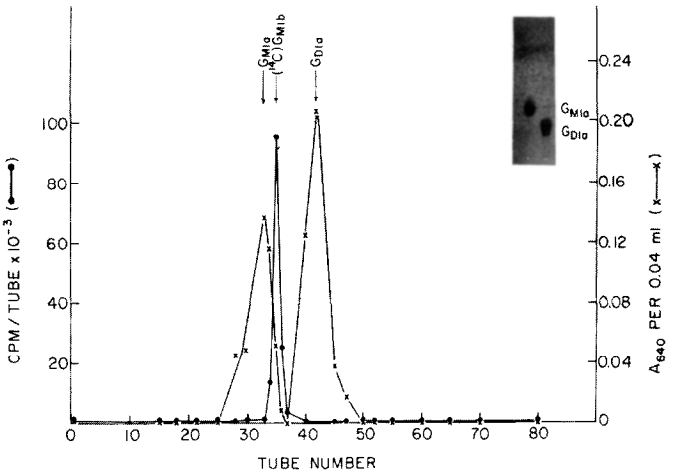

Figure I. The elution pattern of the radioactive product , GM1b , synthesized by rat brain CMP-NANA : Asialo-GM1 sialyltransferase in silica - gel G column. Purified GM1a and GD1a (3 mg each), as shown on a thin layer plate in the upper right-hand coner , were added to the radioactive product as carrier. The mixture was transferred with 4 ml of chloroform-methanol-water (60:30:4.5, v/v/v) to the silica-gel column (2.2 x 27 cm) previously equilibrated with the same solvent. After washing the column with 100 ml of the above solvent, elution was started with chloroform-methanol-water (61:32:7, v/v/v). Each fractions (3.6 ml/tube) were examined for radioactivity (• —— •) and the N-acetylneuraminic acid contents by resorcinol method (x —— x).

washed twice with 2.5 ml of the same solvent used for equilibration. The total filtrate was collected in a counting vial and dried under vacuum. The radioactivity was detected by a Packard Tri-Carb counter with Bray's solution as the scintillation fluid.

RESULTS AND DISCUSSION

The factors which affect the sialyltransferase reaction are illustrated in Table I. The glycolipid substrate, asialo-GM1, and the detergent mixture are required for the transferase reaction. The addition of either Mg^{++}, Mn^{++} or Ca^{++} to the incubation mixture has no significant effect to the enzymic activity. Among the non-radioactive nucleotide sugars tested, only the addition of CMP-NANA (100 mumole) affects the sialyltransferase reaction drastically, suggesting that CMP-NANA was the NANA donor.

Using similar enzyme preparation from young rat brain, monosialoganglioside (GMla), hematoside (GM3), and dihexosylceramide (GL-2a) were found to be active as glycolipid acceptors although asialo-GM1 was most active in the present system. The enzymic products have been identified as GDla, GD3 and GM3 when GMla, GM3 and GL-2a were used as glycolipid acceptors respectively. The above enzymic products have been demonstrated from studies using embryonic chick brain and young rat brain (3, 8, 15, 16).

The glycolipid product of the CMP-NANA : Asialo-GM1 sialyltransferase reaction is identified as monosialoganglioside with sugar analysis. The molar ratio of the carbohydrate moieties, as determined by enzymic and colorimetric methods (16), is 1:2:1:1 respectively to glucose, galactose, N-acetylgalactosamine and N-acetylneuraminic acid. However, this monosialoganglioside (GMlb) is a novel glycosphingolipid differing from the major brain monosialoganglioside (GMla) in that 1.) the migratory rate of the newly synthesized GMlb in silica-gel G column chromatography is slower than that of the authentic GMla standard (Fig. 1). Similar difference in mobility between GMlb and GMla is also observed by ascending thin-layer chromatography on silica-gel G plates in three solvent systems: (A) chloroform-methanol-2.5 M NH_4OH (60:35:8, v/v/v), (B) N-propanol-water (7:3, v/v) and (C) chloroform-methanol-water (60:35:8, v/v/v), and 2.) the N-acetylneuraminic acid moiety of GMlb is totally sensitive to enzymic hydrolysis by neuraminidase from Clostridium perfringens. Under identical conditions, the N-acetylneuraminic acid in GMla is completely resistant to enzymic hydrolysis (Table II). These results suggest that the N-acetyl-neuraminic acid in GMlb is covalently linked to the terminal galactose. The postulated structure for this new monosialoganglioside synthesized by the rat brain CMP-NANA : Asialo-GM1 sialyltransferase is :

TABLE II. Action of neuraminidase from Clostridium perfringens on (^{14}C)GM1b synthesized by the CMP-NANA: Asialo-GM1 sialyltransferase in young rat brain*.

Glycolid	Neuraminidase (U)	% of N-acetylneuraminic acid release	
		Resorcinol Method	(^{14}C)NANA
GD1a (0.06 u mole)	0.2	49	-
GD1a (0.06 u mole)	0.2 (Boiled)	6	-
GM1a (0.12 u mole)	0.2	0.95	-
GM1a (0.12 u mole)	0.2 (Boiled)	0.64	-
(^{14}C)GM1b (0.08 mumole)	0.2	-	96.40
(^{14}C)GM1b (0.08 mumole)	-	-	2.70
(^{14}C)GM1b (0.08 mumole) + GM1a (0.12 u mole)	0.2	-	97.10

* The experiment was performed according to the procedures of Arce et al. (9). GD1a, which is known to be converted to GM1a by losing the neuraminidase-labile N-acetylneuraminic acid under the above experimental procedures, was served as control.

N-acetylneuraminyl (2→3)galactosyl (β,1→3) N-acetylgalactosaminyl (β,1→4)galactosyl (β,1→4)glucosyl (1→1)ceramide

Recent studies in the in vivo incorporation of radioactive glucosamine into young rat brain glycolipids by Maccioni et al. (17) have indicated the labelling of an unknown glycolipid, possibly an undescribed monosialoganglioside containing a neuraminidase-labile neuraminic acid. If this undescribed monosialoganglioside is identical to GMlb, the asialoglycolipid pathway for the synthesis of monosialoganglioside proposed originally by Yip and Dain (10) is, therefore, likely to be operative in vivo. This fact is strengthened that all the glycosyl transferases involved in the proposed pathway are now completely demonstrated in vitro.

Although GMlb has yet to be proven as the natural component in brain tissue, the dramatic accumulation of its proposed precursor, asialo-GM1, in neural tissues from patients afflicted with generalized gangliosidoses (18) suggests that GMlb and its asialo-glycolipid may play an important and dynamic role in the metabolism of complex sphingoglycolipids.

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