Glycoconjugate Journal (1994) **11**:249–256

Analysis of glycosphingolipid-derived oligosaccharides by high pH anion exchange chromatography

SHUTIAN RUAN and KENNETH O. LLOYD*
Immunology Program, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021, USA
Received 19 January 1994 and revised 15 February 1994

As an adjunct to existing thin layer and column chromatographic methods for the identification of glycolipids a method that utilizes the high pH anion chromatographic (HPAEC) analysis of the oligosaccharides released from the glycolipids by endoglycoceramidase has been developed. Using a Dionex Carbo Pak PA1 column and elution with a linear gradient of sodium acetate in 0.2 M NaOH, the elution times of eight neutral and fourteen acidic oligosaccharides derived from glycolipids were determined. Under these conditions the neutral oligosaccharides were well separated from each other but some of the acidic oligosaccharides had overlapping elution times. The ganglioside-derived oligosaccharides could be further identified by treating them with sialidase or by mild acid hydrolysis and reanalysing the products by HPAEC. The method was applied to the analysis of mixed bovine brain gangliosides. The procedure provides an additional approach for the initial identification of glycolipids by analysing the component oligosaccharides rather than the intact glycolipids.
Keywords: glycolipids, gangliosides, endoglycoceramidase, sialidase

Introduction

Glycosphingolipids are traditionally analysed and identified by thin layer chromatography on silica gel plates or more recently by high performance liquid chromatography (HPLC) [1, 2]. Although these methods are invaluable for the identification of glycolipids, additional techniques that would conveniently confirm the initial assignments are needed. The introduction of an enzyme (endoglycoceramidase) that liberates the oligosaccharide moieties from glycolipids [3, 4] and the development of methods for analysing oligosaccharides by high performance anion chromatography at high pH [5, 6] has opened up possible new approaches to glycolipid identification and analysis. Wang *et al.* [7] briefly described the combination of these two techniques to the analysis of G_{M1}, G_{D1a}, G_{D1b} and G_{T1b} gangliosides. We have extended this approach to demonstrate its general utility in identifying both neutral and acidic glycolipids by analysing the oligosaccharides released by endoglycoceramidase treatment and, in the case of gangliosides, the additional information that can be gained by analysing the neutral and acidic oligosaccharides formed by sialidase treatment or mild acid hydrolysis of the released acidic oligosaccharides.

* To whom correspondence should be addressed.

Materials and methods

Glycolipids and sugars

The following acidic and neutral glycolipids were purchased from Sigma Chemical Co. (St Louis, MO): G_{M3}, G_{M2}, G_{M1}, G_{D1a}, G_{D3}, G_{D2}, G_{D1b}, G_{T1b}, sulfatide, asialo-G_{M1}, asialo-G_{M2}, globoside, globopentaosylceramide, and ceramide dihexoside. Ceramide trihexoside was from Matreya, Inc., (Pleasant Gap, PA). All glycolipids were tested for purity by thin layer chromatography (TLC). NeuGc-G_{M3} and $(NeuGc)_2$-G_{D3} were isolated from horse [8] and cat erythrocytes [9], respectively. NeuGc-G_{M2} was a gift from Dr R. Yu (Virginia Medical College). Sialylparagloboside and disialylparagloboside were kindly provided by Dr D. M. Marcus (Baylor College of Medicine). Lacto-*N*-neotetraose was purchased from Oxford Glycosystems (Rosedale, NY). Lacto-*N*-tetraose was kindly provided by Dr A. Kobata (Tokyo). *N*-Acetylneuraminic acid and *N*-glycolylneuraminic acid were obtained from the Sigma Chemical Company.

Enzymes

Rhodococcus endoglycoceramidase was purchased from Genzyme Co. (Cambridge, MA) or from Sigma Chemical Co. (St Louis, MO). *Vibrio cholerae* neuraminidase was from Calbiochem Co. (San Diego, CA).

Release of oligosaccharides from glycolipids

Glycolipid (5–10 μg) was dried in a 6 × 50 mm glass culture tube under a stream of nitrogen. Twenty-five μl of detergent (Na tauroeoxycholate, 1 mg ml^{-1} in H_2O) was added and the sample was sonicated for 1 min. The dissolved glycolipid was then mixed with 25 μl of sodium acetate-acetic acid buffer, pH 6.0, containing 150 μU of endoglycoceramidase [3] and incubated at 37 °C for 24 h. The oligosaccharides released from gangliosides were isolated by adding chloroform (50 μl) to the reaction mixture and isolating the aqueous phase. The solution was evaporated to dryness in a Speed Vac and finally dissolved in 0.1 mM maltose in water (50 μl). For neutral glycolipids, the reaction mixture was diluted 10-fold with water, and passed several times through a C18 Sep-pak cartridge (Millipore/Waters, Milford, MA) that had been pretreated according to the manufacturers' instructions. Two ml of water was applied to the column, and pooled with the previous column fall-through fraction. After lyophilization of these pooled eluates, the resulting acidic oligosaccharide sample was redissolved in 0.1 mM maltose in water (50 μl). Aliquots (20 μl) of the neutral or acidic oligosaccharides were then analysed for acidic or neutral oligosaccharides by HPAEC.

Sialidase treatment

Approximately 5 μg of released oligosaccharides were treated with 15 mU of *Vibrio cholerae* neuraminidase in 50 μl volume of Na phosphate buffer (0.15 M, pH 5.5) at 37 °C overnight. The hydrolysed sample (20 μl) was analysed directly by HPAEC.

Partial acid hydrolysis of oligosaccharides

To release *N*-acetylneuraminic acid, the oligosaccharides were partially hydrolysed as follows: lyophilized oligosaccharides (5 μg) were dissolved in 200 μl of 0.1 N HCl and hydrolysed at 80 °C for 1 h. After evaporation in a Speed Vac the sample was redissolved in 50 μl of water and a 20 μl aliquot analysed by HPAEC.

High pH anion exchange chromatography

HPAEC analysis was performed in a Dionex BioLc System (Dionex Co. Synnyvale, CA) fitted with a Carbo Pak PA1 column (250 mm × 4 mm), a pulsed amperometric detector (PAD) and a Spectro-Physics 4270 Integrator. The column was eluted with 0.2 M NaOH for 5 min and then with a linear gradient from 0.2 M NaOH to 0.2 M NaOH with 0.4 M Na acetate (in CO_2-free water) over 40 min at a flow rate of 1.0 ml min^{-1}. The following pulse potentials on the PAD were used for detection: $E_1 = 0.05$ V; $E_2 = 0.60$ V; $E_3 = -0.60$ V. The range was normally set at 1000 nA.

Analysis of brain gangliosides oligosaccharides

In order to identify the oligosaccharides of bovine brain gangliosides a larger sample was treated with endoglycoceramidase and analysed: 100 μg of bovine brain ganglioside (Sigma Chemical Co.) was treated with 300 μU of endoceramidase in 100 μl of sodium taurodeoxycholate acetate buffer pH 6.0. After 24 h, the released oligosaccharides were isolated by chloroform-water partition, dried by evaporation, and redissolved in 20 μl of water. The oligosaccharide mixture was separated by HPAEC as described above except that the column eluate was collected in fractions (0.25 ml). Fractions corresponding to carbohydrate peaks were immediately pooled, neutralized with 1 M acetic acid and the samples were deionized by passing over a Dowex 50 (H^+) column (1 ml). The column was washed with water (2 ml) and the combined eluate and wash were lyophilized and the residue redissolved in water. Aliquots were partially hydrolysed or treated with sialidase as described above the rechromatographed by HPAEC to identify the neutral or partially desialylated oligosaccharides.

Results

Optimization of chromatographic conditions

To establish the optimal conditions for the separation of glycolipid-derived oligosaccharides, samples from CDH, CTH, G_{M3} and G_{D3} were prepared and analysed with a variety of gradients of increasing Na acetate in 0.2 M NaOH over different time periods. A program consisting of 0.2 M NaOH for 5 min, followed by a linear gradient from 0.2 M NaOH to 0.4 M Na acetate in 0.2 M NaOH over 40 min (Fig. 1A) was selected for further use. Under these conditions neutral oligosaccharides (CTH, peak **12**, Fig. 1B) and lactose (peak **10**, Fig. 1C) were eluted rapidly (6–12 min) whereas acidic oligosaccharides from G_{M3} (peak **19**) and G_{D3} (peak **27**) gangliosides were well retained on the column and eluted in 20–30 min (Fig. 1C). This chromatographic programme allows for separation both of neutral and acidic oligosaccharides in a single run. A number of non-glycolipid-related oligosaccharides were tested for their suitability as internal standard compounds, i.e. to be well separated from glycolipid-derived oligosaccharides; maltose was selected for this purpose (Figs. 1B and 1C).

Separation of the oligosaccharides released from glycolipids

Twenty-two oligosaccharides released from glycolipids were analysed by HPAEC. Table 1 shows absolute elution times and elution times relative to maltose of these oligosaccharides as well as the values for some relevant monosaccharides. In general, the compounds were well separated from each other. The compounds were distributed clearly in two groups. Monosaccharides, except NeuGc, and neutral oligosaccharides were totally eluted within 15 min. All oligosaccharides released from acidic glycolipids were eluted at later times (from 20.5 min for G_{M1} to 40.8 min for $(NeuGc)_2G_{D3}$). The eight neutral oligosaccharides examined were well separated from each other and could be

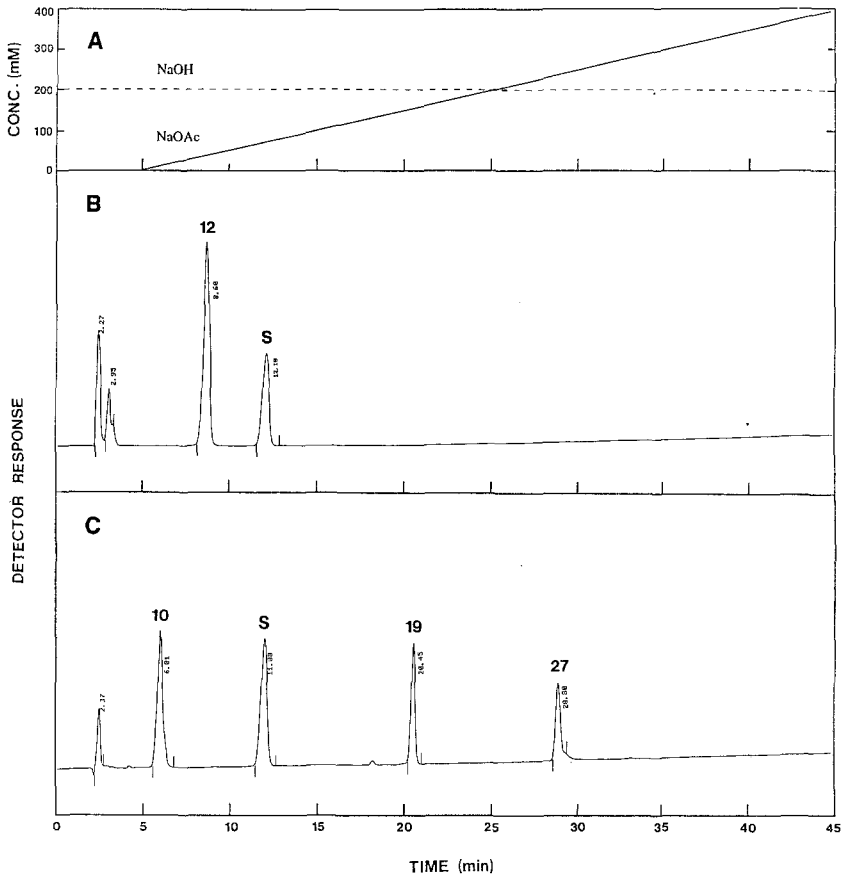

Figure 1. Separation by HPAEC of standard oligosaccharides produced from glycolipids by ceramidase treatment. Twenty pmol of each sample was treated wtih ceramidase and separated under the conditions described in the Materials and methods section. The peaks in this chromatogram are numbered according to Table 1. 1A: Gradient used for the elution; 1B: separation of oligosaccharides derived from CTH, **12** and maltose (internal standard, S); 1C: separation of oligosaccharides derived from CDH (**10**), G_{M3} (**19**), G_{D3} (**27**) and maltose (internal standard, S).

identified on the basis of their relative elution times. The elution times were approximately proportional to the length of the oligosaccharides, however structural features of the compounds also seemed to play important roles as demonstrated by the elution of $GgOse_3$ before $GbOse_2$ and $GbOse_5$ before $LcOse_4$. The number of substituent sialic acid residues was a major factor in determining the elution times of the acidic compounds. Thus, oligosaccharides from trisialogangliosides were eluted later than those from disialogangliosides, which in turn were eluted later than those from monosialogangliosides. Specifically, the order of the elution times was G_{M1}, G_{M2}, G_{M3}, G_{D1b}, G_{D2}, G_{D1a}, G_{D3} and G_{T1b}. NeuGc-containing oligosaccharides were eluted 7–8 min later than the corresponding NeuAc-derivatives (*cf* NeuGc-G_{M2} with G_{M2} and NeuAc-G_{M3} and G_{M3} in Table 1). Unlike the neutral oligosaccharides tested, which could be identified on the basis of their relative elution times alone, some of the acidic oligosaccharides showed identical or very close elution times (*cf* G_{M2} with G_{M3} and G_{D3} with diSPG). Additional information was therefore needed to identify these oligosaccharides.

Analysis of oligosaccharides released from acidic oligosaccharides by sialidase or mild acid hydrolysis

The endoglycoceramidase-released acidic oligosaccharides could be further identified on HPAEC by analysing the partially or completely hydrolysed oligosaccharides formed by sialidase treatment or acid hydrolysis (Table 2). For example, the oligosaccharides from G_{M3} (peak **19**, Fig. 2) and G_{M1} (peak **17**, Fig. 3) have almost identical elution times and could not readily be distinguished in the initial analysis of HPAEC. Following sialidase treatment, G_{M3} oligosaccharide was converted to two peaks identifiable as lactose (peak **10**) and NeuAc (peak **7**) as shown in Fig. 2B. A similar result was obtained by mild acid hydrolysis (Fig. 2C). These results identify the oligosaccharide as being derived from G_{M3}. On the other hand, treatment of G_{M1} oligosaccharide with sialidase did not result in any change in the elution profile (Fig. 3B). Mild acid hydrolysis of the oligosaccharide, however, yield peaks corresponding to $GgOse_4$ (peak **11**) NeuAc (peak **7**) and incompletely hydrolysed G_{M1} (peak **17**), as shown in Fig. 3C. As NeuAc substituted on internal residues are known to be resistant

Table 1. HPAEC elution parameters of oligosaccharides from glycolipids.

Designation	*Compound*	*Elution time (min)*[a]	*Relative elution time*[b,c]
	Mono- and oligosaccharides		
1	Fucose	2.98	0.25
2	Galactosamine	3.61	0.30
3	Glucosamine	4.10	0.34
4	Glucose	4.25	0.35
5	Galactose	4.33	0.36
6	Maltose	12.00	1.00
7	*N*-acetylneuraminic acid (NeuAc)	14.80	1.23
8	*N*-glycolylneuraminic acid (NeuGc)	26.70	2.22
	Oligosaccharides derived from glycolipids		
	I. From neutral glycolipids		
9	$GgOse_3$ (Asialo G_{M2}/G_{D2})	5.62	0.47
10	$GbOse_2$ (Lactosylceramide)	6.11	0.51
11	$GgOse_4$ (Asialo G_{M1})	7.14	0.60
12	$GbOse_3$ (Trihexosylceramide)	8.60	0.72
13	$GbOse_4$ (Globoside)	9.60	0.80
14	$nLcOse_4$ (Lacto-*N*-neotetraosylceramide)	9.96	0.83
15	$GbOse_5$ (Globopentaosylceramide)	10.90	0.91
16	$LcOse_4$ (Lacto-*N*-tetraosylceramide)	15.00	1.25
	II. From acidic glycolipids		
17	$II^3NeuAc\text{-}GgOse_4$ (G_{M1})	20.03	1.67
18	$II^3NeuAc\text{-}GgOse_3$ (G_{M2})	20.36	1.70
19	$II^3NeuAc\text{-}Lac$ (G_{M3})	20.46	1.71
20	$I^3SO_4\text{-}Gal$ (Sulfatide)	24.00	2.00
21	$IV^3NeuAc\text{-}LcnOse_4$ (Sialylparagloboside)	25.25	2.10
22	$II^3(NeuAc)_2\text{-}GgOse_4$ (G_{D1b})	25.60	2.13
23	$II^3(NeuAc)_2\text{-}GgOse_3$ (G_{D2})	26.50	2.20
24	$II^3NeuGc\text{-}GgOse_3$ (G_{M2}, NeuGc)	27.72	2.31
25	IV^3NeuAc, $II^3NeuAc\text{-}GgOse_4$ (G_{D1a})	27.84	2.32
26	$II^3NeuGc\text{-}Lac$ (G_{M3}, NeuGc)	28.70	2.39
27	$II^3(NeuAc)_2\text{-}Lac$ (G_{D3})	28.80	2.40
28	$IV^3(NeuAc)_2\text{-}LcnOse_4$ (Disialylparagloboside)	28.90	2.41
29	IV^3NeuAc, $II^3(NeuAc)_2\text{-}GgOse_4$ (G_{T1b})	32.60	2.72
30	$II^3(NeuGc)_2\text{-}Lac$ (G_{D3}, NeuGc)	40.80	3.40

[a] ±5%.
[b] Elution time relative to maltose.
[c] ±2%.

to *Vibrio cholerae* sialidase treatment, this result, in combination with the identification of $GgOse_4$, identifies the oligosaccharide as being derived from G_{M1}. NeuGc-containing oligosaccharides could be readily discriminated from NeuAc-containing oligosaccharides with similar elution times by the release of NeuGc following either sialidase or mild acid hydrolysis treatment.

Chromatography of oligosaccharides bovine brain gangliosides

The acidic fraction of bovine brain glycolipids is well known to contain a mixture of various gangliosides. Using thin layer chromatography, three major (G_{D1a}, G_{D1b}, and G_{T1b}) and two minor gangliosides (H_{M1} and G_{D2}) were detected by resorcinol-HCl spray (data not shown). To analyse the composition of bovine brain gangliosides a relatively large amount of the sample (100 μg) was treated with endoceramidase and separated by HPAEC. As shown in Fig. 4A, the sample was separated into six major peaks and several minor peaks. By comparison with the relative elution times of the stanard oligosaccharides (Table 1), the major peaks were found to correspond to G_{M2}/G_{M1} (peak **17**, **18**), G_{D1b} (peak **22**), G_{D1a} (peak **25**), and G_{T1b} (peak **29**) and minor peaks corresponding to G_{D2} (peak **23**) and G_{D3} (peak **27**). A peak eluting at 16.49 min could not be identified. In order to confirm the identity of these

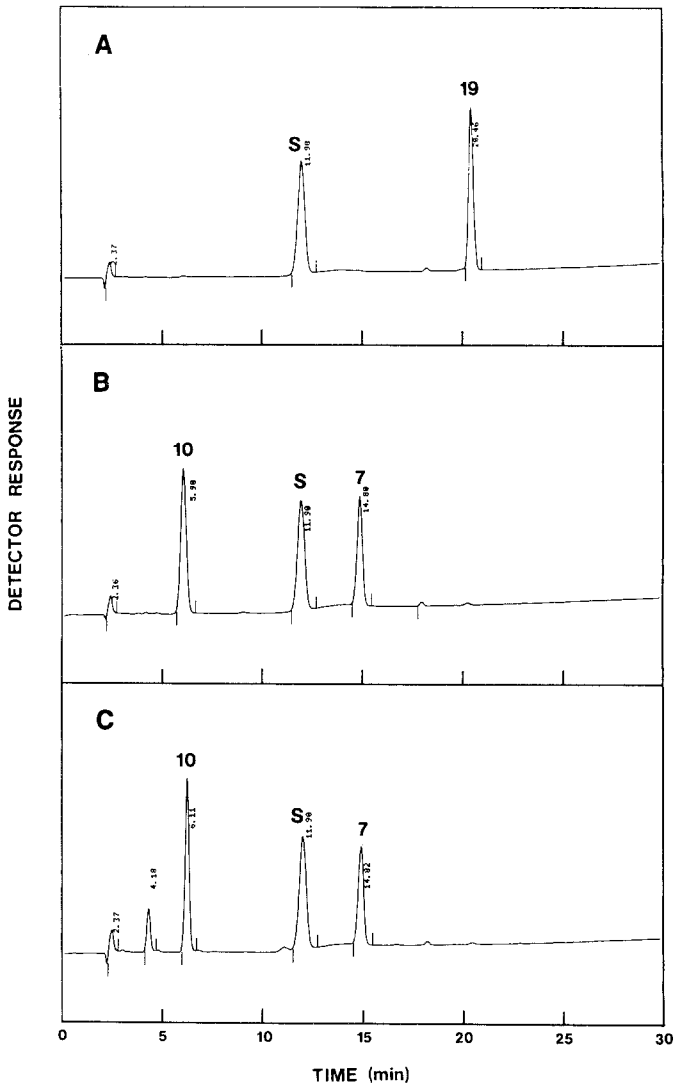

Figure 2. HPAEC of oligosaccharides from G_{M3} ganglioside before and after acid hydrolysis and sialidase treatment. 2A: G_{M3} oligosaccharide (**19**) released by ceramidase treatment; 2B: G_{M3} oligosaccharide after sialidase treatment (lactose, **10** and NeuAc **7**); 2C: G_{M3} oligosaccharide after mild HCl hydrolysis. Peak S: Internal stadnard (maltose).

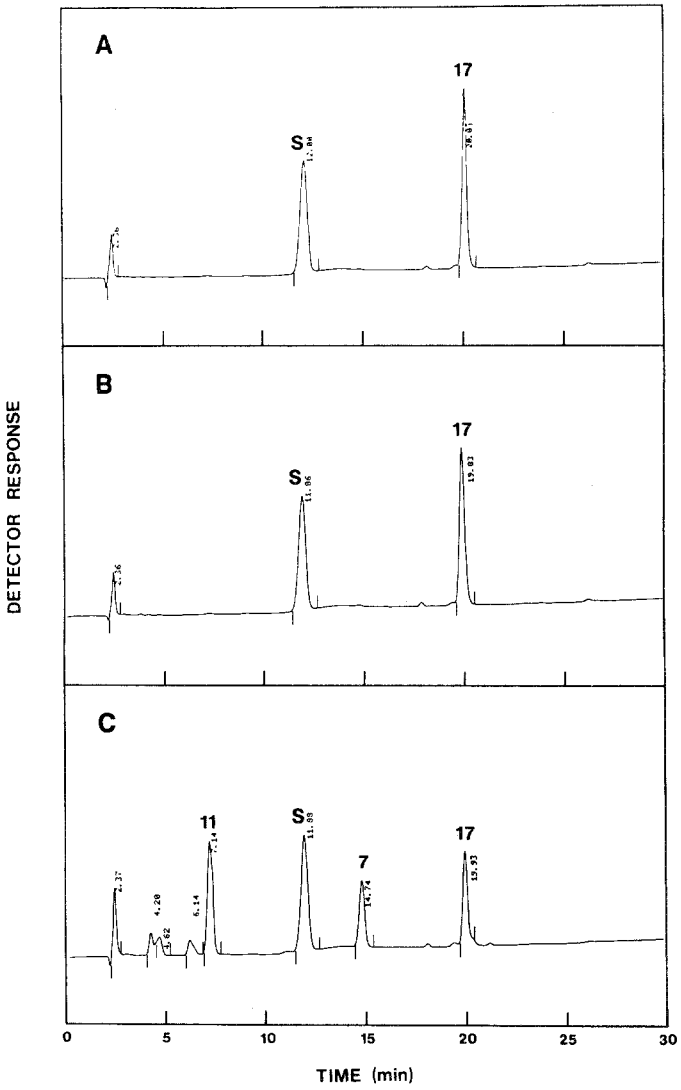

Figure 3. HPAEC of oligosaccharides from G_{M1} ganglioside before and after acid hydrolysis and sialidase treatment. 3A: G_{M1} oligosaccharide released with ceramidase (**17**); 3B: G_{M1} oligosaccharide after sialidase treatment (**17**); 3C: G_{M1} oligosaccharide after mild HCl hydrolysis (G_{M1} **17**, asialo G_{M1} **11**) and NeuAc (**7**). The peaks in the chromatogram are numbered according to Table 1. Peak S: internal standard (maltose).

compounds, fractions corresponding to the peaks were collected and the samples treated with dilute acid or sialidase as described above. Figure 4B shows the identification of peak **25** as G_{D1a} by partial acid hydrolysis to II^3NeuAc-GgOSe$_4$ (peak **17**; G_{M1} oligosaccharide), GgOse$_4$ (peak **11**) and NeuAc (peak **7**) and Fig. 4C shows that peak **29** is converted to $II^3(NeuAc)_2$-GgOse$_4$ (peak **22** G_{D1b} oligosaccharide), II^3NeuAc-GgOse$_4$, (peak **17**) and NeuAc (peak **7**) thus confirming its identity as G_{T1b}.

Discussion

Since its introduction in 1988 [5, 6], high pH anion exchange chromatography has been used for the analysis of monosaccharides [10, 11], oligosaccharides from *N*-linked [5, 6, 12, 13] and *O*-linked chains [13–15], and ganglioside-derived oligosaccharides [7]. We have now demonstrated its general utility in the analysis of both neutral and acidic oligosaccharides released from glycolipids by endoglycoceramidase. Using a simple gradient elution profile, all the monosaccharides and neutral oligosaccharides studied could be separated from each other (Table 1). Many of the acidic oligosaccharides, derived from gangliosides, could also be readily resolved. As would be expected for anion exchange chromatography, sialylated oligosaccharides were eluted at later times than the corresponding neutral compounds; substitution with one NeuAc delayed the

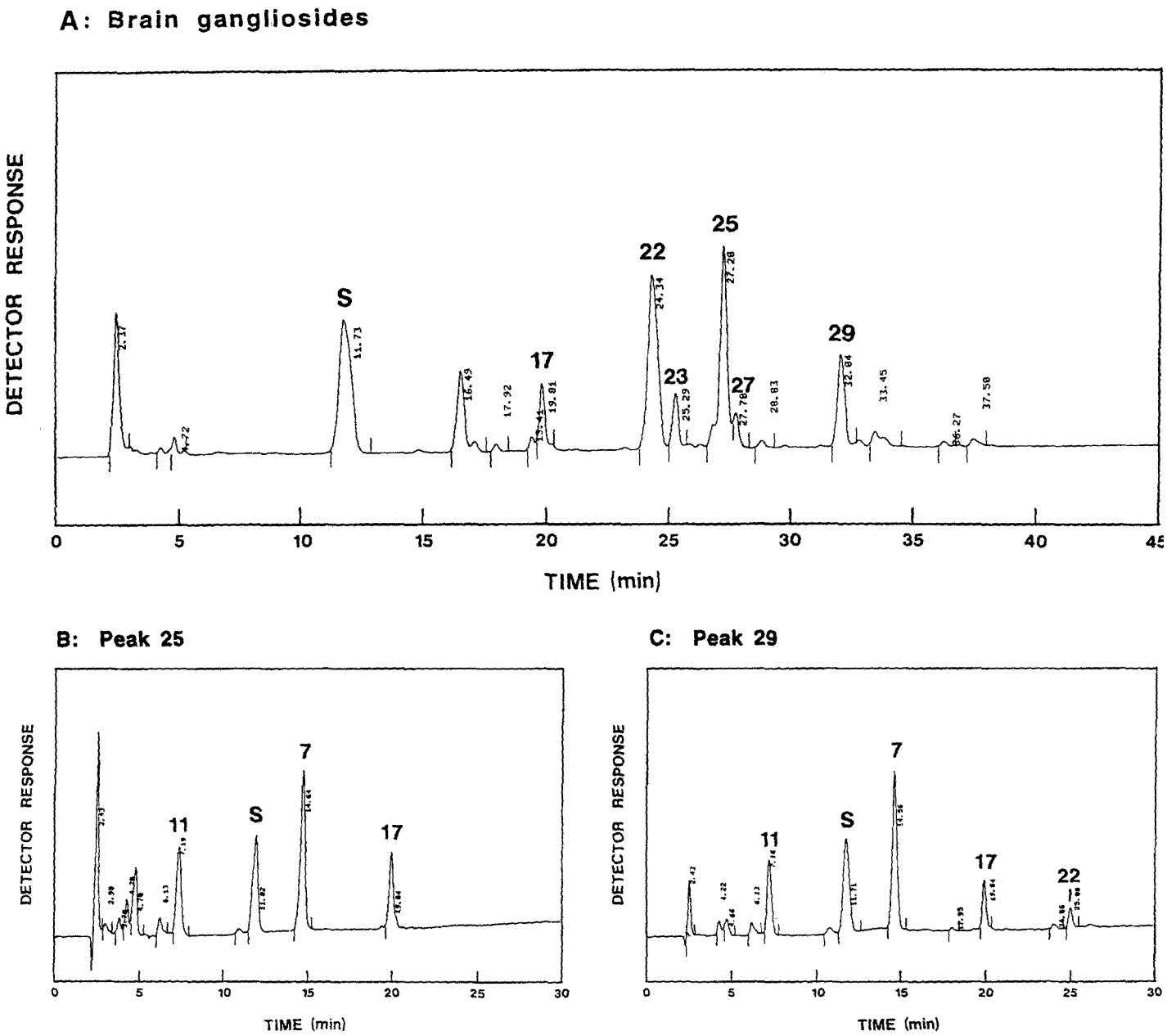

Figure 4. HPAEC of oligosaccharides produced from bovine brain gangliosides. 4A: Bovine brain gangliosides (100 μg) were treated with ceramidase and separated by HPAEC under the condition described in the Materials and methods section. 4B: HPAEC of partially acid hydrolysed peak **25**; 4C: HPAEC of partially acid hydrolysed peak **29**. The peaks in this chromatogram are numbered according to Table 1. Peak S: internal standard (maltose).

elution time by 13–17 min, while the substitution of a second NeuAc delayed the elution time by an additional 4–8 minutes. As reported previously [7, 14], NeuGc-containing oligosaccharides were eluted considerably later (7–8 min) than the corresponding NeuAc-containing compounds.

As some of the acidic oligosaccharides had identical or very similar elution times to each other, these compounds could not be firmly identified by their elution times. However, by mild acid hydrolysis or sialidase treatment to remove, or partially remove, sialic acid residues and rechromatography, the majority of the acid oligosaccharides studied could be distinguished (Table 2). Furthermore, the sialic acid moiety can be identified as NeuAc or NeuGc. Further structural information can be obtained by rechromatographing the sialidase or acid-treated oligosaccharides. As is well known, *Vibrio cholerae* sialidase or mild acid hydrolysis will completely remove terminal NeuAc residues whereas NeuAc residues substituted on internal sugars are more stable (16–18). Thus, analysis of the products gives additional data on the nature of the structure. Examples of this approach can be seen in Fig. 2.

Table 2. Relative elution times of ganglioside oligosaccharides and derived neutral oligosaccharides.

Ganglioside oligosaccharide	*Relative elution time (RET)*[a]	*Sialidase treatment*		*Acid hydrolysis*	
		Product	*RET*	*Product*	*RET*
G_{M1}	1.67	NC[b]	NC	$GgOse_4$	0.60
G_{M2}	1.70	NC	NC	$GgOse_3$	0.47
G_{M3}	1.71	$GbOse_2$	0.51	$GbOse_2$	0.51
G_{D1b}	2.13	G_{M1}	1.67	$GgOse_4$	0.60 and
				G_{M1}	1.67
G_{D2}	2.20	G_{M2}	1.70	$GgOse_3$	0.47 and
				G_{M2}	1.70
G_{D1a}	2.32	G_{M1}	1.67	$GgOse_4$	0.60 and
				G_{M1}	1.67
G_{D3}	2.40	$GbOse_2$	0.51	$GbOse_2$	0.51 and
				G_{M3}	1.71
G_{T1b}	2.72	G_{M1}	1.67	$GgOse_4$	0.60,
				G_{M1}	1.67 and
				G_{D1b}	2.13

[a] RET: Relative to the elution time of maltose (12.00 min).
[b] NC: No change.

Thus G_{M3} is converted to lactose and NeuAc by both acid hydrolysis and sialidase treatment (Fig. 2A). On the other hand, G_{M1} is stable to sialidase but dilute acid hydrolysis releases $GgOse_4$ and NeuAc (Fig. 2C). It is interesting to note that the hydrolysis conditions chosen (0.1 N HCl at 80 °C for 1 h), which are the standard conditions usually used to release sialic acid from complex carbohydrates, did not completely remove the NeuAc from G_{M1} (Fig. 2). By increasing the hydrolysis time most of the NeuAc could be released from G_{M1} but this leads to a lower yield of $GgOse_4$ and increasing amounts of smaller oligosaccharides (data not shown). A similar result was observed in the partial acid hydrolysis of G_{D1a} and G_{T1b} (Table 2) and the nature of these products provided additional data on the structure of the parent oligosaccharide. These results confirm and extend those made previously [17, 18] on the susceptibility of NeuAc residues to partial acid hydrolysis. As with sialidase treatment, sialic acid residues substituted on the internal galactose residue were less acid labile than those substituted on a terminal galactose residue. Moreover, even the $\alpha 2 \rightarrow 8$NeuAc in G_{D1b} and G_{T1b} is more resistant than the $\alpha 2 \rightarrow 3$Gal-linked, terminal sialic acid.

This HPAEC method is designed to complement standard TLC and HPLC methods for the identification of glycolipids. TLC of intact glycolipids is relatively insensitive and identification is sometimes ambiguous because of multiple bands resulting from heterogeneity in the ceramide portion of the glycolipids. HPLC methods can also give multiple peaks and derivatization is needed in some procedures. The analysis of released oligosaccharides by HPAEC provides an additional approch to glycolipid identification in which the parameters governing separation are quite different from those responsible for the chromatographic separations of intact glycolipids. The method described can be compared with two other published procedures for the analysis of endoglycoceramidase-relased oligosaccharides. Higashi *et al.* [19] described a two-dimensional HPLC mapping method based on the separation of p-aminobenzoic acid ethyl ester derivatives of the oligosaccharides. This method is effective and fairly sensitive but requires derivatization and two separate HPLC separations. An alternative approach described by Hansson *et al.* [20] utilizes gas chromatography of permethylated oligosaccharides and identification of the peaks by mass spectrometry. This is a powerful method but again requires derivatization and is not applicable to polysialosyl oligosaccharide.

An important advantage of the use of pulsed amperometric detection is that very small amounts of underderivatized compounds can be readily detected. In this study, we routinely treated 5–10 μg of glycolipid with endoglycoceramidase and analysed about half of the released oligosaccharide by HPAEC; however, much smaller amounts of glycolipid can be identified by increasing the sensitivity of the detector. The procedure can also be scaled up quite conveniently to a semi-preparative level. In analysing total brain ganglioside we separated oligosaccharides derived from 100 μg of glycolipids and isolated and reanalysed the individual peaks by partial acid hydrolysis. The method is also applicable to the analysis of oligosaccharides released from radiolabelled glycolipids. For instance, in a recent study (S. Ruan, K. O. Lloyd and K. Furukawa. Enzymatic analysis of melanoma cells stably

transfected with βGalNAc1 → 4transferase (G_{M2}/G_{D2} synthase) cDNA, submitted for publication) on the expression of a β1,4GalNAc-transferase we identified the products of the enzyme reaction using this HPAEC method. In this study the products resulting from the transfer of [^{14}C]CMP-NeuAc to G_{M3} or G_{D3}, were identified as G_{M2} and G_{D2}, respectively, by endoglycoceramidase treatment and coelution of the radioactive G_{M2}- and G_{D2}- derived oligosaccharides with unlabelled oligosaccharide standards.

Acknowledgements

This work was supported by grants from the National Health Service (CA 57485, CA C0680 and CA 08748). We thank Ms Claudette Bryant for her skilful secretarial assistance.

References

1. Ledeen RW, Yu RK (1982) *Methods Enzymol* **83**:139–91.
2. Hakomori S-I (1983) In *Sphingolipid Biochemistry*, (Kanfer JN, Hakomori S-I, eds) pp. 1–165. New York: Plenum Press.
3. Ito M, Yamagata T (1986) *J Biol Chem* **261**:14278–82.
4. Zhou B, Li S-C, Laine RA, Huang RTC, Li Y-T (1989) *J Biol Chem* **264**:12272–77.
5. Hardy MR, Townsend RR (1988) *Proc Natl Acad Sci USA* **85**:3289–93.
6. Chen L-M, Yet M-G, Shao M-C (1988) *FASEB J* **2**:2819–24.
7. Wang WT, Zopf D (1989) *Carbohydr Res* **189**:1–11.
8. Furukawa K, Thampoe IJ, Yamaguchi H, Lloyd KO (1989) *J Immunol* **142**:848–54.
9. Furukawa K, Chait BT, Lloyd KO (1988) *J Biol Chem* **263**: 14939–47.
10. Hardy MR, Townsend RR, Lee YC (1988) *Anal Biochem* **170**: 54–62.
11. Manzi AE, Diaz S, Varki A (1990) *Anal Biochem* **188**:20–32.
12. Lee YC (1990) *Anal Biochem* **189**:151–62.
13. Watson E, Bhide A, Kenney WC, Lin F-K (1992) *Anal Biochem* **205**:90–95.
14. Lloyd KO, Savage A (1991) *Glycoconjugate J* **8**:493–98.
15. Hayase T, Sheykhanazari M, Bhavanandan VP, Savage AV, Lee YC (1993) *Anal Biochem* **211**:72–80.
16. Wiegandt H (1967) *J Neurochem* **14**:671–74.
17. Ishiuzuka I, Wiegandt H (1972) *Biochim Biophys Acta* **260**: 279–89.
18. Ando S, Yu RK (1977) *J Biol. Chem* **252**:6247–50.
19. Higashi H, Ito M, Fukaya N, Yamagata S, Yamagata T (1990) *Anal Biochem* **186**:355–62.
20. Hansson GC, Li Y-T, Karlsson H (1989) *Biochem* **28**:6672–78.