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SEPARATION OF GANGLIOSIDES BY ANION-EXCHANGE CHROMATOGRAPHY ON MONO Q™

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SUMMARY

A new type of strong anion-exchange resin, Mono Q™, has been used in the separation of brain gangliosides. The resin consists of monodisperse particles (9.8 μm) and was used in prepacked columns with a bed volume of 1 ml. The gangliosides were separated into mono-, di-, tri- and tetrasialoganglioside fractions by a discontinuous gradient of potassium acetate in methanol. The separation was complete in a volume of 50 ml. The major advantages of the new procedure compared to conventional methods are the shorter separation time, higher loading capacity and recovery of separated ganglioside fractions in small solvent volumes. The procedure was applied to the separation of gangliosides from normal human and GM2-gangliosidosis brain.

INTRODUCTION

Gangliosides are complex acidic lipids that are normal constituents of all mammalian cell membranes, the highest concentrations being found in neuronal cell membranes[1]. Their acidic properties make them suitable for separation by anion-exchange chromatography. Several different anion-exchange resins have been tried, the most commonly used being DEAE-Sephadex[2,3], DEAE-Sepharose[4], Spherosil-DEAE-Dextran[5], DEAE-Sephacel[6] and DEAE-silica gel[7]. All these procedures were carried out at atmospheric pressure. A new type of monodisperse high-performance anion-exchange resin, Mono Q™[8], has recently become available, the low back pressure of which makes possible chromatography at relatively high flow-rate (1–4 ml/min) with only moderate applied pressures (1–4 MPa). This paper gives a detailed description of the separation of gangliosides on the new type of resin and its application to different human brain tissues.

MATERIALS AND METHODS

Equipment

The high-performance ion-exchange chromatography was performed with the

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FPLC system from Pharmacia (Pharma Fine Chemicals, Uppsala, Sweden). The system consists of a GP-250 gradient programmer, two P-500 reciprocating pumps, a V7 valve for the introduction of sample onto the column via a 10-ml Superloop and a FRAC-100 fraction collector. The column used was a prepacked Mono Q HR, 5/5 (50 × 5 mm), with a bed volume of 1 ml (Pharmacia). The resin is a strong anion exchanger consisting of monodisperse particles (9.8 μm).

Chemicals

High performance thin-layer plates, Kieselgel, 60, 20 × 10 cm, and thin-layer plates, Kieselgel 60, 20 × 20 cm, were from Merck (Darmstadt, F.R.G.). Iatrobead GRS-8060 was from Iatron Laboratories (Tokyo, Japan). Dialysis tubing was obtained from Union Carbide (Chicago, IL, U.S.A.). Methanol (Pronalys quality) was purchased from May and Baker (Dagenham, U.K.). All other solvents and chemicals were of analytical quality.

Tissue material

Autopsy brain materials used in this study were from a human control aged 80 years and a 3-year-old child with Tay-Sachs disease. For the elaboration of the procedure, human brain tissue was pooled from several brains of different ages.

Isolation and purification of gangliosides

A crude ganglioside brain extract was obtained by the procedure of Svennerholm and Fredman[9]. The brain tissue was homogenized in 3 volumes of water, and methanol and chloroform were subsequently added during continued homogenization to give a final chloroform–methanol–water ratio of 4:8:3 (v/v/v). After centrifugation (1500 *g*), the tissue was re-extracted with 10 volumes of the same solvent, chloroform–methanol–water (4:8:3). The extracts were pooled and evaporated to dryness. The residue was dissolved in 1 volume of chloroform–methanol (2:1, v/v) per gram original tissue and left overnight (16 h). A precipitate appeared which was removed by centrifugation. Methanol and water were added to the clear solution to give a final chloroform–methanol–water ratio of 1:2:1.4 (v/v/v). After phase separation, methanol and 0.01 *M* aqueous potassium chloride were added to the lower phase to give a chloroform–methanol–0.01 *M* aqueous potassium chloride ratio of 2:1:0.7 (v/v/v). The combined upper phases were evaporated to dryness, saponified overnight at room temperature with 0.5 *M* potassium hydroxide in methanol–water (1:1, v/v), neutralized and dialysed for 2 days. The crude ganglioside extract was then separated on an Iatrobead silica gel column with 1 g gel per g original tissue. After evaporation, the gangliosides were dissolved in 0.5 ml chloroform–methanol–water (65:25:4, v/v/v) per g tissue and applied to the column. Fatty acids, alkali-resistant phosphoglycerides and neutral glycolipids were eluted with 10 volumes of chloroform–methanol–water (65:25:4, v/v/v). The gangliosides were then eluted with 15 volumes of chloroform–methanol–water (50:40:10, v/v/v). The ganglioside extract was evaporated to dryness, dissolved in 1 ml of water per g tissue and dialysed for 2 days. After evaporation the gangliosides were dissolved in chloroform–methanol (1:2, v/v) to give a final concentration of 1 μmol sialic acid per ml.

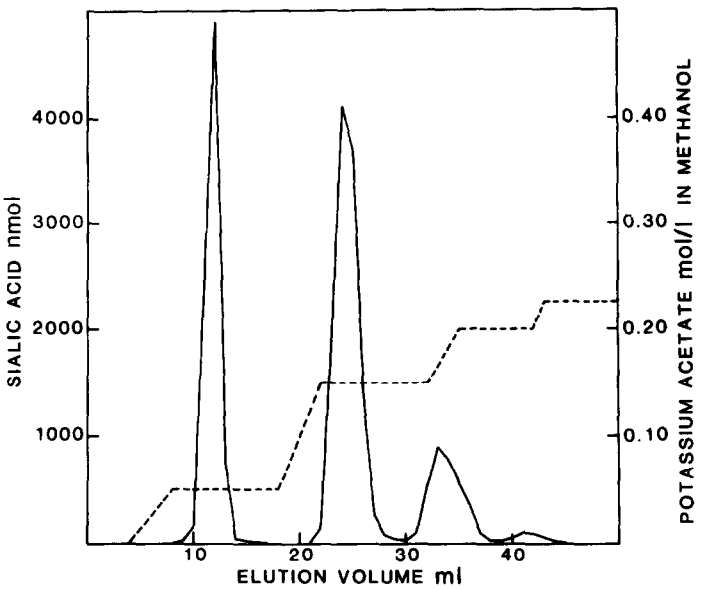

Fig. 1. Separation of pooled human brain gangliosides, 25 μmol of sialic acid, on a Mono Q anion-exchange column. The dotted line shows the concentration of potassium acetate in methanol.

Assay methods

The gangliosides were quantified by the resorcinol assay[10]. The patterns of individual gangliosides were determined by densitometry at 620 nm of high-performance thin-layer plates developed with resorcinol after separation in the solvents 1-propanol–0.25% potassium chloride (3:1, v/v) or chloroform–methanol–0.25% aqueous KCl (50:40:10, v/v/v).

High-performance anion-exchange chromatography

The Mono Q column was washed before use with 10 ml of methanol, 50 ml of 1 *M* potassium acetate in methanol and 10 ml of methanol, at a flow-rate of 1 ml/min. After each analysis the column was washed with 5 ml of 1 M potassium acetate in methanol and 20 ml of methanol. The ganglioside sample, dissolved in chloroform–methanol (1:2, v/v), was introduced to the column at a flow-rate of 1 ml/min. The gangliosides were eluted with a stepwise gradient from 0 to 0.225 *M* potassium acetate in methanol (Fig. 1), with a flow-rate of 2 ml/min. Fractions of 1 ml were collected and the total elution volume was 50 ml. The collected fractions were assayed by analysing 2–5 μl of each fraction by high-performance thin-layer chromatography in 1-propanol–0.25% aqueous potassium chloride (3:1, v/v), and the gangliosides were visualized by use of the resorcinol reagent. Fractions corresponding to mono-, di-, tri- and tetrasialogangliosides were pooled, dialysed against water and assayed as described under *Assay methods*.

RESULTS

Optimization of the method

Several different solvent mixtures containing chloroform–methanol,

TABLE I

DISTRIBUTION AND RECOVERY OF GANGLIOSIDES FROM POOLED HUMAN BRAINS AFTER HIGH-PERFORMANCE ANION-EXCHANGE CHROMATOGRAPHY ON MONO Q

In each separation experiment, 25 μmol of ganglioside-NeuAc were applied to the column. The values are the means from six different separations.

Fraction	*N-Acetylneuraminic acid (%)*		
	Anion-exchange chromatography		*Scanning of total extract*
	Mean	*S.D.*	
Monosialogangliosides	37	0.8	37
Disialogangliosides	47	0.7	45
Trisialogangliosides	14	0.3	16
Tetrasialogangliosides	2	0.3	2
Recovery	98	2.0	

chloroform–methanol–water, isopropanol–water and isopropanol–hexane–water were tested for the application of the ganglioside mixtures on the anion-exchange column. Chloroform–methanol (1:2, v/v) was chosen as the most suitable solvent, since water-containing mixtures gave a break-through of about 5% of the applied ganglioside sialic acid. It was also essential to remove all low-molecular-weight contaminants from the lipid extract before the ion-exchange chromatography. This was done either by dialysis or chromatography on Sephadex G-25. A loading of 1 μmol sialic acid per ml was found optimal, and the sample could be applied at a rates of up to 1.5 ml/min. The flow-rate finally chosen was 1 ml/min. When natural brain ganglioside mixtures were used, the maximum column loading was 25 μmol of ganglioside-NeuAc. The reproducibility of the separation and the recovery of gangliosides were excellent when the column was loaded with this amount (Table I).

The elution order of the gangliosides is shown in Fig. 2. As is seen in Fig. 2 and Table II, the major portion of ganglioside GQ1b was eluted together with the monosialogangliosides. The pooled tetrasialoganglioside fraction was a mixture of approximately equal amounts of GT1b and GQ1b and a small amount of disialo-

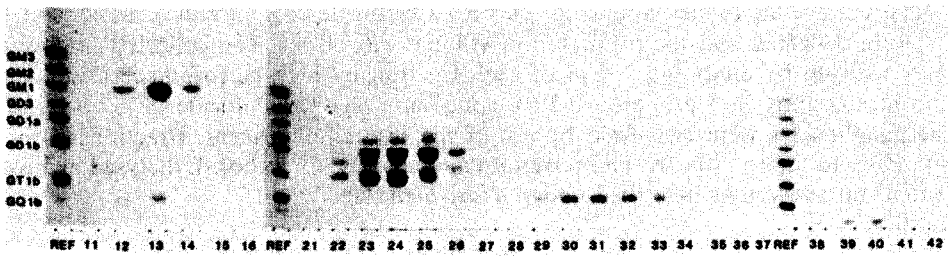

Fig. 2. Elution pattern of gangliosides from a human adult forebrain separated by anion-exchange chromatography on a Mono Q column and assayed by HPTLC. The fraction numbers correspond to the elution volume in ml. HPTLC solvent: 1-propanol–0.25% aqueous KCl (3:1, v/v). The spots were visualized by use of the resorcinol reagent.

TABLE II

GANGLIOSIDE PATTERN OF HUMAN ADULT FOREBRAIN

The column was loaded with 24.9 μmol of NeuAc from the forebrain of an 80-year-old adult, containing 2.15 μmol ganglioside-NeuAc per g fresh tissue. The recovery of ganglioside-NeuAc was 97%. The GM3 fraction was a mixture of GM3 and sialosylgalactosylceramide.

*Ganglioside**	*nmol NeuAc/g fresh tissue*				*NeuAc (%)*	
	Pooled fractions				*Total ganglioside extract*	*FPLC separated fractions*
	Mono	*Di*	*Tri*	*Tetra*		
GM3	48				2	2
GM2	26				1	1
GM1	216				12	11
GD3		114			4	5
GD1a		467		3	21	23
GD2		91			4	4
GD1b		467	18	3	26	26
GT1b			585	10	28	28
GQ1b	32		6	10	2	2

* The gangliosides have been designated according to Svennerholm[13].

gangliosides. The retention of disialogangliosides may be due to non-specific adsorption to the resin caused by oxidation of the ceramide portion of these gangliosides. To investigate further the co-elution of GQ1b with monosialogangliosides, monosialoganglioside fractions from several separations of adult forebrain were pooled and rechromatographed. The chromatography was performed at two different sample loadings, 2.75 and 7.75 μmol NeuAc, respectively. At the lower sample loading, 100% of ganglioside GQ1b appeared in the tetra fraction, but at the higher sample loading 45% of the GQ1b was still eluted in the mono fraction. When a tetrasialoganglioside fraction was rechromatographed, part of the GQ1b was eluted in the mono fraction.

The elution characteristics of several Mono Q columns were tested with the ganglioside mixture from pooled human brains. The differences between individual columns were extremely small. To obtain the standard elution profile, only very small modifications of the gradient had to be performed. For some columns, monosialogangliosides were eluted with 0.06 *M* instead of 0.05 *M* potassium acetate, and trisialogangliosides with 0.19 *M* instead of 0.20 *M* potassium acetate. The maximum lifetime of Mono Q columns has not yet been fully investigated, but the same column was used daily for 6 months without worsening of the separation.

Application of the separation procedure

A ganglioside extract from human adult forebrain corresponding to 25 μmol of NeuAc was separated on the Mono Q column. The total NeuAc and the ganglioside pattern in each fraction were determined (Table II). The ganglioside patterns before and after the FPLC separation were very similar. Similar results were found when 5 μmol or 25 μmol of the same extract were chromatographed. Different ratios between the major brain gangliosides did not have any negative effect on the sepa-

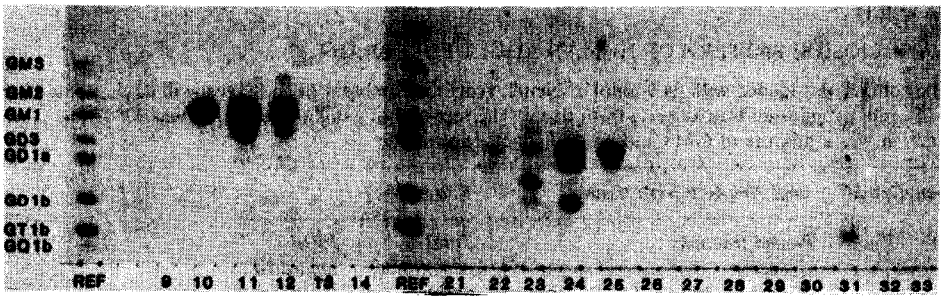

Fig. 3. Elution pattern of gangliosides from a Tay-Sachs brain separated by anion-exchange chromatography on a Mono Q column and assayed by HPTLC. Details as in Fig. 2.

ration ability of the column. In the GM2-gangliosidosis, one single monosialoganglioside, GM2, constituted 85% or more of the total sialic acid, but this ganglioside was also completely eluted in the monosialoganglioside fraction without any tailing in the disialoganglioside fraction (Table III and Fig. 3).

DISCUSSION

The major advantage of the Mono Q anion-exchange resin compared to DEAE-Sepharose, DEAE-Sephacel and Spherosil-DEAE-Dextran resins is its much higher binding capacity for gangliosides. It could be loaded with 25 μmol of ganglioside-NeuAc per ml resin without break-through, while the corresponding value

TABLE III

GANGLIOSIDE PATTERN OF TAY-SACHS BRAIN

The column was loaded with 24.4 μmol of NeuAc from the brain of a 5-year-old child with diagnosed Tay-Sachs disease, containing 14.4 μmol ganglioside NeuAc per fresh tissue. The recovery of gangliosides was 99%.

Ganglioside	*nmol NeuAc/g fresh tissue*			*NeuAc (%)*
	Pooled fractions			*FPLC separated fractions*
	Mono	*Di*	*Tri + tetra*	
GM3	211			1
GM2	12034		9	85
GM1	750			5
GM1-GalNAc	158			1
GD3		97	9	<1
GD1a		411	17	3
GD1a-GalNAc		160		<1
GD2		102		<1
GD1b		158	11	1
GT1b			109	<1
GQ1b			29	<1

for DEAE-Sepharose is 2–2.5 μmol/ml[4,5], for DEAE-Sephacel 3 μmol/ml[6] and for Spherosil-DEAE-dextran 5 μmol/ml[5]. This high binding capacity was also valid when investigations were made on samples from GM2-gangliosidosis brains which contained more than 90% of monosialogangliosides. The high loading capacity of the Mono Q resin also makes it possible to recover the gangliosides in smaller solvent volumes than with the conventional gels. The bed volume of the Mono Q column is independent of salt concentration and solvent composition due to the rigid structure of the resin. Restricted flow caused by shrinkage of the gel may be a problem when using DEAE-Sepharose or DEAE-Sephadex.

When different samples were analysed with the new procedure the recovery of gangliosides varied between 93 and 99%. The yields of total gangliosides after FPLC separation are in good agreement with reported values for Spherosil-DEAE-Dextran and DEAE-Sepharose[5] and DEAE-silica[7,11]. The small proportion of gangliosides lost on the column was mainly due to adsorption of gangliosides with an oxidized ceramide portion.

The major disadvantage of using the Mono Q anion-exchange resin for separation of gangliosides is the elution of part of ganglioside GQ1b among the monosialogangliosides. Such an elution profile has never been observed with any of the anion-exchange resins previously used in our laboratory nor from any other laboratory[2–7]. It may be explained by the spontaneous formation of lactones from a part of ganglioside GQ1b on the column. We have previously observed that the terminal sialic acid in a disialosyl linkage easily forms lactone, but the present finding suggests that one of the inner sialic acids also occurs in the lactone form.

Recently, Blaszczyk *et al.*[12] reported the use of Mono Q for the isolation of gangliosides from colon adenocarcinomas. In their procedure the gangliosides were eluted with a linear gradient of ammonium acetate. No data were, however, given on loading and recovery.

In conclusion, the use of Mono Q for anion-exchange chromatography of gangliosides is a fast procedure with quantitative recovery of the gangliosides in small solvent volumes. Further work is now in progress in our laboratory using 0.50-ml columns for small scale preparations and 8-ml columns for large scale preparations.

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