Elimination of silica gel from gangliosides by using a reversed-phase column after preparative thin-layer chromatography

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Summary A simple and effective procedure has been developed for eliminating silica gel from gangliosides after preparative thin-layer chromatography. Gangliosides were extracted from the scraped silica gel with chloroform–methanol–water 10:10:3 (by volume) and dried. The residue was suspended in 0.1 M NaCl and centrifuged. After filtration through a sintered glass funnel, the supernatant was applied to a reversed-phase column. The column was washed with 0.1 M NaCl, water, and methanol–water 1:1 (v/v). Gangliosides were eluted from the cartridge with methanol with yields of 92% and 90% in terms of dry weight and sialic acid content, respectively. Almost all of the silica gel contamination was eliminated from gangliosides by this procedure. —**Kubo, H., and M. Hoshi.** Elimination of silica gel from gangliosides by using a reversed-phase column after preparative thin-layer chromatography. *J. Lipid Res.* 1985. **26:** 638–641.

Supplementary key words preparative thin-layer chromatography

Preparative thin-layer chromatography (TLC) is often adopted as an effective step for purifying various lipids. In the case of gangliosides, chloroform–methanol is generally used for extraction from the scraped silica gel. Higher concentrations of methanol in the extraction solvent are favorable for higher recoveries of gangliosides. However, the gangliosides are contaminated with more silica gel by using solvents containing more methanol. This dilemma limits the use of preparative TLC in ganglioside purification.

A simple procedure for eliminating salts and other nonlipid contaminants from gangliosides has recently been developed by using a short reversed-phase column, C_{18} Sep-Pak® cartridge (1, 2). We have examined whether a Sep-Pak cartridge is also useful for removal of silica gel from gangliosides. In this report, we present a simple and effective method for elimination of silica gel from gangliosides using a Sep-Pak cartridge after preparative TLC.

MATERIALS AND METHODS

Materials

C_{18} Sep-Pak cartridges were purchased from Waters Japan, Tokyo. A bovine brain ganglioside mixture was generously provided by Dr. A. Awaya, Mitsui Pharmaceuticals, Inc., Japan. TLC plates (Uniplate, 20 cm × 20 cm, silica gel G, 0.25-mm thick) were obtained from Analtech Inc., USA. High-performance thin-layer chromatography (HPTLC) plates (10 cm × 10 cm, silica gel 60, 0.2-mm thick) were purchased from E. Merck Co., West Germany. Solvents were freshly distilled before use. All the chemicals used were analytical grade.

Thin-layer chromatography

HPTLC and TLC plates were prewashed with methanol–ether 1:1 (v/v) prior to activation for 1 hr at 105°C. For analytical TLC, HPTLC plates were used with a combination of two consecutive runs at 38°C: the plates were first developed with the solvent containing chloroform–methanol–12 mM $MgCl_2$–15 M ammonium hydroxide 60:35:7.5:3 (by volume), dried, and then developed with chloroform–methanol–12 mM $MgCl_2$ 58:40:9 (by volume) (3). After development, gangliosides were visualized by Cu^{2+}-resorcinol reagent (4) and determined with a TLC densitometer (CS-910, Shimadzu Co.) at an analytical wavelength of 580 nm and a control wavelength of 710 nm (5). For preparative TLC, 2 mg of bovine brain gangliosides dissolved in 0.4 ml of chloroform–methanol 1:2 (v/v) was applied as a streak on a TLC plate. The plate was developed with chloroform–methanol–2.5 M ammonium hydroxide 60:35:8 (by volume), dried, and visualized with iodine vapor. The scraped silica gel was mixed with a half-weight of Dowex 50W-X8 (Na^+-form) (6). Gangliosides were extracted three times with 10 ml of chloroform–methanol–water 10:10:3 (by volume) (7) per 0.5 g of the silica gel (6) with mild sonication for 5 min and centrifugation at 2,500 rpm for 2 min. The supernatant was passed through a sintered glass funnel (6) and dried with a rotary evaporator. The residue thus obtained was suspended with mild sonication in 0.1 M NaCl at a concentration of approximately 300 μg of gangliosides per ml. After centrifugation at 3,500 rpm for 5 min, the supernatant was collected and the precipitate was further washed twice with the same volume of 0.1 M NaCl in the same way. The combined supernatant (approximately 100 μg of ganglioside per ml of 0.1 M NaCl) was applied to a Sep-Pak cartridge.

Sep-Pak cartridge

A Sep-Pak cartridge fitted with a 50-ml glass syringe was successively washed with 25 ml of chloroform–methanol 1:2 (v/v), 25 ml of methanol, and 50 ml of water before use (2).

Determination of sialic acid content

Sialic acid was estimated as previously reported (8)

Abbreviations: TLC, thin-layer chromatography; HPTLC, high performance thin-layer chromatography.

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TABLE 1. Elution of gangliosides from a Sep-Pak cartridge

	Solvent	% Total Applied Gangliosides
A:	M-W[a] 1:9	0.4
	2:8	0.1
	3:7	0.3
	4:6	0.5
	5:5	0.3
	6:4	6.0
	7:3	30.4
	8:2	34.0
	9:1	25.8
	Methanol	3.0
	C-M[b] 1:2	0
B:	M-W[a] 1:1	1.0
	Methanol	98.1
	C-M[b] 1:2	0

[a]Methanol-water, by volume.
[b]Chloroform-methanol, by volume.

with the bovine brain ganglioside mixture as a standard.

RESULTS AND DISCUSSION

Optimal conditions for application of gangliosides to a Sep-Pak cartridge

A mixture of bovine brain ganglioside (500 μg) was dissolved in 5 ml of solutions containing various concentrations of NaCl with mild sonication. After application of the ganglioside-containing salt solution, the cartridge was washed with 3 ml of 0.1 M NaCl and 25 ml of water with a little pressure by the aid of a plunger. The flow rate was approximately 1–2 ml/min. Break-through and wash fractions were combined. Then gangliosides were eluted from the column with 5 ml of methanol and 25 ml of chloroform-methanol 1:2 (v/v) (2). Recovery of gangliosides was calculated by sialic acid determination. When the concentration of NaCl was lower than 50 mM, gangliosides were not quantitatively retained by the cartridge. When the NaCl concentration was in a range of 50 to 200 mM, about 95% of applied gangliosides was adsorbed to the column. For unknown reasons, the highest recovery of gangliosides (96% of applied gangliosides) was obtained by using 0.1 M NaCl. This is consistent with the data previously reported (1). Thus, 0.1 M NaCl was used in subsequent experiments.

The effect of the pH of the NaCl solution on ganglioside adsorption by Sep-Pak was examined, because Kundu and Suzuki (2) used 0.1 M NaCl at pH 4.5. Freshly prepared 0.1 M NaCl gave a pH value of 5.8. Adjustment of pH of NaCl solution to 4.5 with HCl did not improve the recovery of gangliosides. Thus, 0.1 M NaCl was used without adjusting the pH in the following experiments.

Two ml of various concentrations of ganglioside solution in 0.1 M NaCl was applied to a Sep-Pak cartridge. The column was washed and eluted with 5 ml of methanol and 25 ml of chloroform-methanol 1:2 (v/v). No significant difference in the recovery of gangliosides was observed over the range of 25 to 1,000 μg/ml.

The adsorption capacity of the Sep-Pak cartridge was very high as reported (1); up to 10 mg of gangliosides could be quantitatively adsorbed by and recovered from the cartridge.

Elution of gangliosides from a Sep-Pak cartridge

Gangliosides (500 μg) dissolved in 0.1 M NaCl at 100 μg/ml were applied to a Sep-Pak cartridge. After being washed with 0.1 M NaCl and water, the column was further washed with 10 ml each of solvents as shown in **Table 1A.** No significant elution of gangliosides was observed until the concentration of methanol was increased up to 60% (v/v) (table 1A). Although most of the gangliosides were eluted by 90% (v/v) methanol, pure methanol was required to elute all of them (Table 1B). Thus as a standard procedure, the cartridge containing the adsorbed gangliosides was successively washed with 3 ml of

Scrape silica gel.
Add a half weight of Dowex 50W-X8 (Na^+-form).
Extract gangliosides three times with 10 ml of chloroform-methanol-water 10:10:3 (by volume) per 0.5 g of silica gel with mild sonication for 5 min.
Remove insoluble materials by centrifugation at 2,500 rpm for 2 min and filtration through a sintered glass funnel.
Evaporate the extract to dryness.
Suspend the residue in 0.1 M NaCl at approximately 300 μg of ganglioside per ml with mild sonication.
Centrifuge at 3,500 rpm for 5 min.
Wash the precipitate twice with the same volume of 0.1 M NaCl and centrifuge.
Charge the combined supernatant (approximately 100 μg of ganglioside per ml of 0.1 M NaCl) to a Sep-Pak cartridge.
Wash the cartridge with 3 ml of 0.1 M NaCl, 25 ml of water, and 10 ml of methanol-water 1:1 (v/v).
Elute gangliosides with 10 ml of methanol.

Fig. 1 Procedure for elimination of silica gel from gangliosides.

TABLE 2. Yield of gangliosides through the purification procedure

Step	A: Dry Weight	(% Yield[a])	B: Ganglioside[b]	(% Yield[a])	B/A
1. Gangliosides applied	2.00 mg	(100)	2.00 mg	(100)	1.00
2. Silica gel scraped	1.01 g				
3. C-M-W extract[c]	10.4 mg		1.94 mg	(97.0)	0.19
4. 0.1 M NaCl extract[d]			1.82 mg	(91.0)	
5. Gangliosides purified[e]	1.84 ± 0.04[f] mg	(91.8 ± 1.8[f])	1.80 ± 0.02[f] mg	(90.0 ± 0.8[f])	0.98

[a]Yield compared with the gangliosides applied on the TLC plate.
[b]Colorimetrically determined.
[c]Gangliosides extracted with chloroform–methanol–water 10:10:3 (by volume).
[d]Gangliosides extracted with 0.1 M NaCl followed by centrifugation.
[e]Gangliosides purified with a Sep-Pak cartridge.
[f]Standard deviation from triplicate experiments.

0.1 M NaCl, 25 ml of water, and 10 ml of methanol–water 1:1 (v/v), and then the gangliosides were eluted with 10 ml of methanol.

Elimination of silica gel from gangliosides

Fig. 1 shows our procedure for purification of gangliosides by preparative TLC followed by elimination of the contaminating silica gel. The overall recoveries of gangliosides in terms of dry weight and sialic acid content were 91.8 ± 1.8% and 90.0 ± 0.8%, respectively (**Table 2**). The problem with the standard protocol for extraction of gangliosides from preparative TLC is shown in Step 3 of Table 2. Note that less than 20% of the dry weight of the extracted material is ganglioside, the remainder being contaminants from the silica gel. This contamination interferes with the further analyses. By extracting this material with 0.1 M NaCl and passing the extract through Sep-Pak (Steps 4 and 5 in Table 2), we removed virtually all of the silica gel, and the gangliosides accounted for at least 98% of the dry weight (Table 2). The HPTLC patterns of gangliosides extracted from the scraped silica gel with chloroform–methanol–water 10:10:3 (by volume) and gangliosides purified with Sep-Pak were identical with the starting ganglioside mixture as shown in **Fig. 2.** In fact, the relative amounts of major gangliosides changed only slightly through this procedure (**Table 3**). From the data presented above, this procedure should be generally applicable for elimination of silica gel from various kinds of gangliosides after preparative TLC. Similar procedures may be applicable also to other polar lipids.

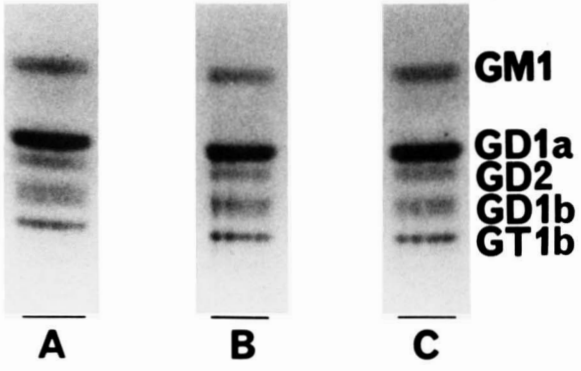

Fig. 2 HPTLC of bovine brain gangliosides before and after the Sep-Pak procedure. Six μg of the ganglioside was applied as an 8-mm streak on a HPTLC plate and developed consecutively with chloroform–methanol–12 mM $MgCl_2$–15 M ammonium hydroxide 60:35:7.5:3 (by volume) and chloroform–methanol–12 mM $MgCl_2$ 58:40:9 (by volume) (3). Gangliosides were detected by Cu^{2+}–resorcinol spray (4). The symbols GM1, GD1a, GD2, GD1b, and GT1b are in accordance with the Svennerholm system (9). A: Gangliosides before being loaded on a TLC plate; B: gangliosides extracted from the scraped silica gel with chloroform–methanol–water 10:10:3 (by volume); C: gangliosides purified with Sep-Pak.

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TABLE 3. Relative amounts of major gangliosides

Ganglioside	A Gangliosides before Being Loaded on TLC Plate	B Gangliosides Extracted with C-M-W 10:10:3	C Gangliosides Purified with Sep-Pak
GM1	1.00	1.00	1.00
GD1a + GD2	2.86	2.79	3.00
GD1b	1.45	1.46	1.21
GT1b	1.16	1.23	1.14

Relative amounts of major gangliosides were estimated by densitometric scanning of the HPTLC plate. The data are expressed as normalized to GM1.

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