Short Communication

The Use of Sep-Pak™ C₁₈ Cartridges During the Isolation of Gangliosides

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Abstract: The use of Sep-Pak™ C₁₈ reverse-phase cartridges to adsorb gangliosides from aqueous solutions was studied. When upper phases formed from chloroform-methanol tissue extracts or aqueous salt solutions containing gangliosides are rapidly passed through the cartridges, the lipids are retained and the non-lipid components can be washed through. Gangliosides and other retained lipids can subsequently be eluted with methanol or chloroform-methanol. Key words: Reverse-phase chromatography—Dialysis.

Gangliosides are glycosphingolipids that contain at least one sialic acid moiety. The classical method (Folch et al., 1957) used for the isolation of gangliosides from tissue involves a chloroform-methanol extraction, followed by the partitioning of the gangliosides into an aqueous upper phase. To remove salts and other low-molecular-weight, water-soluble substances, the upper phase can be dialyzed exhaustively (Folch et al., 1957; Svennerholm, 1972) and then concentrated by rotary evaporation and lyophilization. However, loss of ganglioside has been shown to occur (Kanfer and Spielvogel, 1973) when the upper phase is dialyzed without prior removal of organic solvents (Ghidoni et al., 1978). Another approach to ganglioside isolation is the use of a total lipid extract and column chromatography. Gangliosides can be retained on DEAE-Sephadex (Ledeen et al., 1973) and eluted with solvents containing sodium acetate. The resulting fraction can then be subjected to mild alkaline hydrolysis and desalted by dialysis or Sephadex G50 (Ueno et al., 1978) and further purified by chromatography on Unisil. For TLC examination of gangliosides from small tissue samples, a chloroform-methanol total lipid extract can be passed over a silica acid column. Neutral lipids are eluted first and gangliosides can then be eluted with chloroform/methanol/water (50:50:15, by vol.) (Irwin and Irwin, 1979).

In this work, we have investigated the use of reverse-phase chromatography for the isolation of gangliosides. We report here that gangliosides from upper-phase fractions can be desalted and concentrated in a short period of time with a minimum of steps using the Sep-Pak™ C₁₈ cartridge.

MATERIALS AND METHODS

Sep-Pak C₁₈ cartridges were obtained from Waters Associates, Milford, Massachusetts. Small three-way disposable stopcocks were purchased from Pharmaseal Inc., Toa Alto, Puerto Rico. Disposable (B-D) 10-ml syringes, 30-ml glass syringes, and all reagent grade solvents were from Fisher Scientific, Pittsburgh, Pennsylvania. Unisil (100–200 mesh) was purchased from Clarkson Chemical Co., Williamsport, Pennsylvania. Sephadex G 25 was obtained from Pharmacia Fine Chemicals, Piscataway, New Jersey.

Sep-Pak C₁₈ Procedure

The Sep-Pak cartridge is fitted with a three-way stopcock. Samples and solvents are applied to the cartridge with a syringe. Before sample application the Sep-Pak is washed alternately with 10 ml methanol and 20 ml of chloroform-methanol-water (3:48:47) (Folch et al., 1957).
chloroform-methanol (2:1), three times. A final wash of 10 ml methanol is followed by an equilibration wash of the cartridge with 10 ml of TUP containing 0.1 M-KCl.

Combined upper phases from the partition of a chloroform-methanol tissue extraction (Folch et al., 1957) are brought to a final concentration of 0.1 M-KCl and applied to the Sep-Pak cartridge with slight pressure. The eluate is collected and reapplied to the column twice. Salts are washed from the column with 10 ml of water. Gangliosides can then be eluted with a minimum of 15 ml of methanol or chloroform-methanol (2:1) and concentrated on a rotary evaporator under vacuum.

Sep-Pak C₁₈ cartridges may be reused up to ten times when washed with 10 ml methanol and equilibrated with

**FIG. 1.** Thin-layer chromatographs of mouse brain gangliosides isolated by various methods (see text for details): (A) Sephadex G-25 isolation; (B) Unisil; (C) dialysis of Folch et al. (1957) upper phase; (D) Sep-Pak C₁₈ procedure. Visualization was as follows: (1) resorcinol reagent; (2) ninhydrin spray; (3) orcinol; (4) phosphorus spray. All plates were chromatographed with chloroform/methanol/0.25% CaCl₂ (60:35:8, by vol.). The areas marked (x) on plates 1 and 3 were neither resorcinol- nor orcinol-positive, respectively. These spots correspond to the phosphorus spots seen on plate 4, and they behave as phosphatidyl serine.
10 ml TUP (theoretical upper phase) containing 0.1 m-KCl.

Qualitative Comparison of Four Ganglioside Isolation Procedures

Four C57 BL/6J adult mouse brains were extracted according to Folch et al., 1957. The total lipid extract was divided into four equal portions and treated as follows. (A) Total lipid extract was passed over Sephadex G 25 according to Kemp and Stoolmiller (1976). The resulting lipid fraction was dried and taken up in chloroform-methanol (2:1) and partitioned. The upper phases were combined and dried down. (B) Total lipid extract was passed over a Unisil column and washed with chloroform/methanol/water (50:50:15, by vol.) (Irwin and Irwin, 1979). (C) Total lipid extract was partitioned and the combined upper phases were dialyzed overnight against four changes of distilled water (Folch et al., 1957). The retentate was then lyophilized. (D) Total lipid extract was partitioned and the upper phases were combined and passed over a Sep-Pak C18 cartridge as described above.

The resulting ganglioside fraction from each method was taken up in 1 ml chloroform-methanol (2:1). Equal amounts of each sample were spotted on four TLC plates (Merck SG 60, 250 µm). The plates were developed in chloroform/methanol/0.25% CaCl₂ (60:35:8, by vol.). The plates were visualized with resorcinol, orcinol, ninhydrin, and phosphorus sprays.

Quantitative Recovery of Gangliosides with the Sep-Pak Procedure

A highly purified total rat brain ganglioside mixture, which had been labeled with tritium (Schwarzmann, 1978), was used for the quantitative recovery studies. TLC-autoradiography revealed that all ganglioside species were labeled. Triplicate samples were utilized for each of the tested conditions. Aliquots of [3H]ganglioside and carrier (0.05 pmol of ganglioside sialic acid) were added to: (1) water, (2) aqueous 0.1 m-KCl, (3) TUP, and (4) TUP containing 0.1 m-KCl. The samples were applied to the Sep-Pak C18 cartridge as described above. Duplicate aliquots of the methanol fractions were counted to obtain total ganglioside recoveries. Triplicate column blanks were processed at the same time, labeled gangliosides were added to the methanol fractions, and duplicate aliquots were taken to determine the total number of counts applied to the Sep-Pak cartridge.

RESULTS

The qualitative comparison of the Sep-Pak procedure to the three other methods tested is seen in Fig. 1. As seen from Fig. 1 (plates 3 and 4), there is a phospholipid and a glycolipid contaminant in all of the preparations. Preparation B also contained several ninhydrin-positive components (Fig. 1, plate 2). The gangliosides isolated by the Sep-Pak procedure appear to be identical to those obtained by the other methods. The ganglioside fractions from all of the procedures tested contained non-ganglioside components, and further purification would be necessary to remove these materials. These procedures were used for a qualitative comparison of ganglioside recovery.

Table 1 shows the percentage recovery of [3H]gangliosides from the Sep-Pak cartridge. The highest recovery (94%) was obtained when gangliosides were applied to the cartridge in aqueous 0.1 m-KCl. However, recoveries of 87% and 89% from TUP or TUP containing 0.1 m-KCl, respectively, are also within experimental limits, thereby indicating that it is not necessary to remove the methanol from the upper phase prior to adsorption of the gangliosides onto the reverse-phase material.

In addition to the two experiments reported here, other parameters have also been investigated. The KCl concentration was varied from 0.05 m to 0.2 m. There was a slight increase in recovery up to 0.1 m followed by a leveling off or slight decrease at 0.2 m. The effects of sodium and calcium chlorides on the adsorption of ganglioside to Sep-Pak were also measured. Sodium chloride at a concentration of 0.1 m in TUP appeared to be similar in its action to potassium, with a recovery of about 88%. On the other hand, 0.1 m-CaCl₂ diminished the recovery of gangliosides to about 60%. The capacity of the cartridge was estimated by the application of an excess of rat brain gangliosides (10 µmol) in upper phase. The upper phase was applied to Sep-Pak, and gangliosides were eluted with methanol as described above. The sialic acid content of the methanol fraction was determined colorimetrically with the resorcinol procedure. Approximately 5 µmol of ganglioside sialic acid was recovered from the cartridge. Radiolabeled (14C) nucleotide sugars (UDP-galactose and CMP-NeuNac) were taken up in TUP containing 0.1 m-KCl and processed over a Sep-Pak cartridge. Less than 0.5% of the nucleotide sugar was adsorbed onto the cartridge and recovered in the methanol fraction (UDP-)

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<tr>
<th>TABLE 1. Percent recovery of [3H]ganglioside mixture from Sep-Pak C18 cartridges</th>
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<tr>
<td>Initial solvent</td>
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<tr>
<td>1. H₂O</td>
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<td>2. 0.1 m-KCl</td>
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<td>3. TUP</td>
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<td>4. 0.1 m-KCl in TUP</td>
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* Column blanks were processed to determine the c.p.m. applied to the Sep-Pak cartridge. (See text for details.)
galactose. 0.42%; CMP-NeuNAc, 0.39%). We have found that additional water washes and inclusion of the unlabeled sugars in the washes will further reduce the amount of labeled nucleotide sugars retained by the Sep-Pak.

There is some contamination from the cartridge, which interferes with the resorcinol assay; however, this can be reduced to a minimum (particularly if methanol is used as the eluting solvent) by thoroughly washing the cartridge before its initial use.

**DISCUSSION**

The Sep-Pak C18 reverse-phase cartridge provides a useful alternative to dialysis during the isolation of gangliosides. All lipids in the TUP are retained by the reverse-phase support and non-lipid components are washed through. There appears to be no selective loss of any one ganglioside, and samples can be processed rapidly with a minimum of steps, resulting in a 90–94% recovery of 0.05 μmol of sialic acid. On a larger scale, 50 ml of upper phase at a time, containing up to 5 μmol ganglioside sialic acid, can be conveniently applied to the Sep-Pak cartridge. The ganglioside solutions are passed rapidly through the cartridge with the aid of a syringe, and reapplication of the sample, as described, is necessary to obtain high recoveries. When smaller quantities of gangliosides are processed, recoveries from the Sep-Pak appear to be adequate (approximately 80% recovery with 0.005 μmol of sialic acid), but losses of ganglioside during other purification and handling steps become significant, and we have relied upon the use of isotope dilution measures for quantitative analysis of gangliosides in the picomole range as reported for the HPLC analysis of perbenzoylated monosialoganglioside (Bremer et al., 1979). Thus, the cartridge is useful in the isolation of gangliosides from either small tissue samples or large-scale preparations.

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**REFERENCES**


