Rapid isolation of monosialogangliosides from bovine brain gangliosides by selective-overload chromatography

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Summary A procedure for rapid isolation of monosialogangliosides from purified bovine brain gangliosides has been developed. It utilizes the selective difference in association between monosialogangliosides and polysialogangliosides for the ion-exchange resin Q-Sepharose. When the ion-exchange column is overloaded with a bovine brain ganglioside mixture in the proper ganglioside to column bed-volume ratio, the polysialogangliosides are selectively retained by the column while the monosialogangliosides emerge with the void volume without the use of salt for elution. With the critical ganglioside to bed-volume ratio (1 g:8.32 ml), and an appropriate column bed-height to column radius ratio of 6.9, monosialogangliosides are reproducibly obtained in high purity with greater than 90% yield. The method has been used at both the analytical and preparative scale. We call this separation technique selective-overload chromatography.—**Koul, O., M. Prada-Maluf, R. H. McCluer, and M. D. Ullman.** Rapid isolation of monosialogangliosides from bovine brain gangliosides by selective-overload chromatogrpahy. *J. Lipid Res.* 1991. **32:** 1712–1715.

Supplementary key words GM_1 • Q-Sepharose

Gangliosides, the acidic glycosphingolipids with one or more sialic acid residue(s), are relatively abundant components of the nervous system and are thought to play an

Abbreviations: BBG, bovine brain gangliosides; HPTLC, high performance thin-layer chromatography; SOC, selective-overload chromatography.

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important functional role in differentiation and developmental events. Lately, the possibility of their therapeutic value, especially for monosialogangliosides (1–3), has led to intense research in basic and clinical areas. An increase in demand for pure monosialogangliosides in bulk has made it desirable to develop a quick and reliable procedure for their preparation.

The preparative separation of underivatized ganglioside mixtures into pure individual fractions based on their sialic acid content has been a challenge (4, 5). Consequently, various chromatogrpahic techniques (5, 6) have been developed with varying degrees of success. The quest has been to identify a high capacity column support and a procedure that would provide individual components in high yield and minimal contamination. We describe here a rapid procedure for the bulk isolation of monosialogangliosides from bovine brain gangliosides. The procedure uses selective differences in association between the ganglioside species and the ion-exchange resin. When a Q-Sepharose ion-exchange column is overloaded with a bovine brain ganglioside (BBG) mixture in the appropriate BBG to bed-volume ratio, polysialogangliosides are selectively retained by the column while the monosialogangliosides emerge with the void volume. With the appropriate BBG:bed-volume ratio, and a column bed-height to column radius ratio described in this report, the monosialogangliosides can be isolated in high purity and in greater than 90% yield by a one-step procedure that is rapid and does not use salt for isolating the fraction. We have tested the method with milligram to gram quantities of BBG and have found it highly satisfactory both in yield and purity of the monosialogangliosides.

MATERIALS AND METHODS

A mixture of bovine brain gangliosides (BBG) was provided by Angio-Medical Corp., New York, NY. Q-Sepharose fast-flow ion exchange resin was purchased from Pharmacia-LKB (Piscataway, NJ); glass-backed HPTLC plates were from E. Merck (Germany); and solvents and other chemicals were from Fisher Chemical Co. (Medford, MA).

Q-Sepharose was converted to the acetate form essentially according to Hirabayashi et al. (6). Briefly, the resin was batch-washed three times with 5 volumes of solvent B, chloroform–methanol–4 M sodium acetate 30:60:8 (v/v). Each time, the resin was swirled and suspended in the solvent, allowed to stand for 1 h, and the supernatant was discarded. It was left overnight in solvent B at room temperature and then batch-washed three times with 5 volumes of solvent A, chloroform–methanol–water 30:60:8 (v/v). The resin (in solvent A) was poured into the column, washed again with 2–3 volumes of solvent A, and then loaded with BBG in solvent A.

Clean glass columns with sintered discs or sand-overlayered glass-wool retainers at the bottom were used for chromatography. The volume of Q-Sepharose used per gram of BBG loaded was 8.32 ml. The resin suspended in solvent A was poured into a column of appropriate diameter to obtain column bed-height to radius ratio of 6.9. In order to prepare a 1% solution of BBG in solvent A, 1 g of BBG was slowly dissolved at room temperature with continuous stirring in 8.2 ml of deionized water followed by successive addition of methanol (61.2 ml) and chloroform (30.6 ml). The clear solution was loaded on the column and the void volume effluent was collected as monosialogangliosides. The column was further washed with 4 column volumes of solvent A collected separately.

The retained gangliosides were either stripped off the column with 0.5 M sodium acetate in methanol to obtain a mixed polysialoganglioside fraction or eluted successively as di-, tri-, or tetrasialogangliosides with increasing salt concentrations. Ganglioside fractions were chromatographed on HPTLC in chloroform–methanol–0.25% $CaCl_2$ 50:40:10 (v/v), and visualized with resorcinol–HCl or sulfuric acid charring spray. The plates were scanned with a densitometric scanner (CAMAG) to determine the relative proportion of various bands.

In a typical run for a 1.2 cm i.d. column of 4.6 ml bed-volume and 4.15 cm bed-height (height/radius = 6.9) the flow rate was between 2 and 2.5 ml/min. In a preparative column of 6 cm i.d., 584 ml bed-volume and height/radius = 6.9, the flow rate was between 10 and 12 ml/min. At a bed-volume of 416 ml for a 50-g load the flow rate was held between 9 and 11 ml/min.

RESULTS

The ideal bed-volume of 8.32 ml Q-sepharose per gram of BBG was empirically determined to retain polysialogangliosides and allow monosialogangliosides to elute in the void volume. To achieve consistency, a relationship between bed-volume, bed-height, and column radius was determined and expressed as column bed-height to radius ratio of 6.9. Therefore, for a given BBG load, a column of specified bed-volume, bed-height, and radius would be needed to keep all the parameters including the flow rate within acceptable range.

Under optimal conditions of bed-volume to loaded BBG, most of the monosialogangliosides appear in the void volume without any other contaminating polysialogangliosides (**Fig. 1A**). Deviations led to contamination or lower yield of the monosialoganglioside fractions. When the ratio of resin to BBG was lower than ideal, disialogangliosides appeared in the void volume along with

the monosialogangliosides which were subsequently eluted in extra washes of solvent A along with some disialogangliosides (Fig. 1C). The flow rate was 2–2.5 ml/min.

In a scaled-up version of the procedure, we isolated pure monosialogangliosides from 70.7 g of BBG. At a bed-height of 20.7 cm we used 584 ml of resin in a column with a diameter of 6 cm. BBG dissolved in 6 liters of solvent A, was loaded and 500-ml fractions of the effluent were collected. The yield of pure monosialogangliosides was 90%. We have standardized this procedure for isolation of monosialogangliosides from 50-g lots of BBG by using 1% solutions in solvent A, with a BBG to resin ratio of 1:8.32 ml and height/radius of 6.9 as described in Materials and Methods. Under these conditions, successively, eight fractions of 500 ml each and four fractions of 250 ml each of solvent A effluent void volume were collected. The first two fractions did not have any significant quantities of gangliosides. The last fraction had some disialogangliosides along with the monosialogangliosides. Yields between 90 and 95% of the theoretical estimates for pure monosialogangliosides have been obtained. A densitometric scan of monosialogangliosides obtained from such a column-run on an HPTLC plate, visualized after charring with sulfuric acid, showed 95% to be GM_1 and the remainder mostly GM_2 with barely detectable amounts of GM_3 (**Fig. 2**).

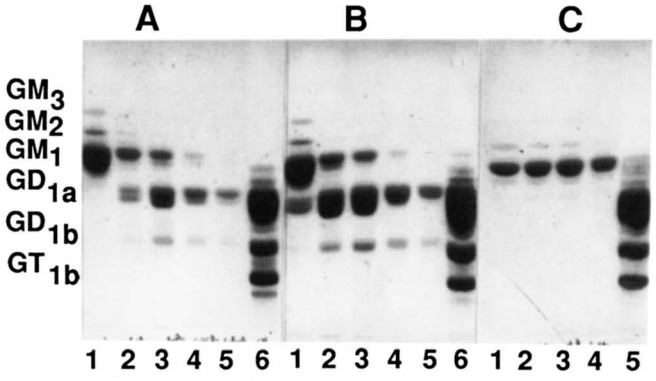

Fig. 1. HPTLC of Q-Sepharose column fractions. The fractions were obtained from 4.6-ml columns with varying loads of BBG. BBG was loaded on the column in solvent A. Monosialogangliosides were obtained in solvent A and the polysialogangliosides were eluted with 0.5 M sodium acetate in methanol. The plates were developed in chloroform–methanol–0.25% $CaCl_2$ 5:4:1 and gangliosides were detected with resorcinol spray. Under near-ideal SOC conditions (panel A) most of the monosialogangliosides appeared in the void volume without any contamination. Increased column load (panel B) contaminated monosialogangliosides in the void volume, and decreased load (panel C) resulted in retention of most of the monosialogangliosides requiring additional washes with solvent A to elute them from the column. The HPTLC plates were intentionally grossly overloaded. Panel A: BBG load 600 mg; lane 1: solvent A fraction (void vol.); lanes 2–5: solvent A washes; lane 6: 0.5 M sodium acetate fraction. Panel B: BBG load 700 mg; lane 1; solvent A fraction (void vol.); lanes 2–5: solvent A washes; lane 6: 0.5 M sodium acetate fraction. Panel C: BBG load 460 mg; lane 1: solvent A load fraction (void vol.); lanes 2–4: solvent A washes; lane 5: 0.5 M sodium acetate fraction.

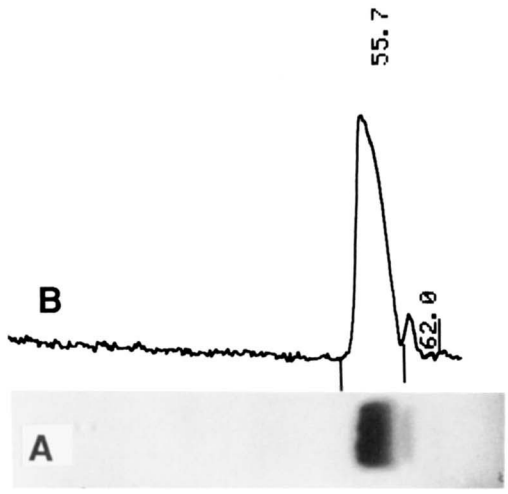

Fig. 2. HPTLC and densitometer scan of monosialogangliosides. The monosialoganglioside fraction obtained in the void volume with solvent A from the Sepharose column was run on an HPTLC plate. Q-Sepharose to BBG ratio was 8.32 ml:1 g. Column height to radius ratio was 6.9. Column load was 1% solution of 50 g BBG in solvent A. The plate was developed as described for Fig. 1 and charred with sulfuric acid before scanning. Panel A: HPTLC Plate. GM_1 is the major and GM_2 the minor band. Panel B: Densitometer scan of the plate in panel A; 95% of the fraction is GM_1. The number above the scan represents relative band mobility.

DISCUSSION

We have described a procedure of selective-overload chromatography (SOC) for the rapid isolation of monosialogangliosides from purified BBG (21% mono-, 53% di-, 26% tri-, and tetrasialogangliosides) obtained from Angio-Medical Corporation (New York, NY). The technique allows reproducible isolation of pure monosialogangliosides in high yield. The procedure takes advantage of the selective association of ganglioside classes with the ion-exchange resin. Therefore, critical overloading of the column leads to selective retention of polysialogangliosides by binding to the available ionic sites. The monosialogangliosides do not bind, or bind minimally, and hence pass-along in the void volume.

The technique has several advantages over methods currently used for isolation of monosialogangliosides. *1*) The single pass method is rapid and simple. *2*) The method can reliably be used for preparation of milligram or gram quantities of monosialogangliosides. *3*) No salt is used during elution of monosialogangliosides, hence no desalting step is required. *4*) SOC uses about fourfold less resin than that used in ion-exchange methods and lower quantities of solvents are used, hence it is cost effective. *5*) Pure monosialogangliosides are obtained in high yield. *6*) The column can be re-used after regeneration with salt. *7*) Finally, the retained polysialogangliosides can be eluted either successively in separate fractions or in bulk as one fraction.

The phenomenon of SOC was initially observed during ion-exchange chromatography of BBG on Q-Sepharose. The inferred competition between solute components of the mixture for binding sites on the column matrix and displacement of the least charged molecules in the absence of any salt was utilized to define the conditions for rapid isolation of monosialogangliosides. We have also observed the elution of monosialogangliosides in the void volume from aminopropyl columns with markedly lower column efficiency and product purity.

We call this chromatographic technique selective-overload chromatography (SOC). This name is proposed because the critical solute to bed-volume ratio allows selective retention of a specific class of compounds (in this case di-, tri-, and tetrasialogangliosides) while eluting the others (monosialogangliosides). The mechanism underlying selective-overload chromatography has not been studies. However, SOC is distinct from frontal analysis in that the desired component obtained in the void volume is pure and the yield approaches 90%. The technique is not the classical displacement chromatography as no external displacer follows the loaded sample (7, 8). As no ions are used to elute the monosialogangliosides from the column, SOC cannot be classified as ion-exchange chromatography. Mechanisms for component separation in binary mixtures on HPLC under overload conditions, e.g., blockage effect (9) and/or sample self-displacement effect (10, 11) have been proposed based on mathematical prediction and experimental results. SOC, in any event, is the first description of a preparative method that can be used to rapidly and selectively isolate biological components under overload conditions. The technique is unique and powerful.

This work was supported by Angio-Medical Corporation, New York, NY. The authors thank Drs. Reza Kamarei and Mehran Vajdi of Angio-Medical Corporatoin (New York) for BBG samples and helpful discussions, and Mr. Matthew Evans for help in HPTLC. We would further like to acknowledge the work done by Drs. Koul and Vajdi with aminopropyl columns. Ms. Thomassina Shavers and Ms. Denise Brescia helped with their expert assistance in preparation of the manuscript.

Manuscript received 9 April 1991 and in revised form 8 July 1991.

REFERENCES

1. Mahadik, S. P., and S. K. Karpiak. 1988. Gangliosides in treatment of neural injury and disease. *Drug Dev. Res.* **15:** 337–360.
2. Tettamanti, G., S. Sonnino, R. Ghidoni, M. Masserini, and B. Venerando. 1985. Chemical and functional properties of gangliosides: their possible implication in the membrane-mediated transfer of information. *In* Physics of Amphiphiles:Micelles, Vesicles and Microemulsions. Soc. Italiaine di Pisica, Bologna, Italy. 607–638.
3. Yates, A. J., J. D. Walters, C. L. Wood, and J. D. Johnson. 1989. Ganglioside modulation of cyclic AMP-dependent protein kinase and cyclic nucleotide phosphodiesterase in vitro. *J. Neurochem.* **53:** 162–167.
4. Ullman, M. D., and R. H. McCluer. 1985. Quantitative analysis of brain gangliosides by high performance liquid chromatography of their perbenzoyl derivatives. *J. Lipid Res.* **26:** 501–506.
5. Smid, F., V. Bradava, O. Mikes, and J. Sedlackova. 1986. Rapid ion-exchange separation of human brain gangliosides. *J. Chromatogr.* **377:** 69–78.
6. Hirabayashi, Y., T. Nakao, M. Matsumoto, K. Obata, and S. Ando. 1988. Improved method for large-scale purification of brain gangliosides by Q-Sepharose column chromatography. *J. Chromatogr.* **445:** 485–499.
7. Scott, R. P. W. 1976. Contemporary liquid chromatography. *In* Techniques of Chemistry. Volume XI. John Wiley & Sons, New York. 13–16.
8. Horvath, C., J. Frenz, and Z. El Rassi. 1983. Operating parameters in high performance displacement chromatography. *J. Chromatogr.* **255:** 273–293.
9. Eble, J. E., R. L. Grob, P. E. Antle, G. B. Cox, and L. R. Snyder. 1987. Simplified description of high performance liquid chromatographic separation under overload conditions, based on the Craig distribution model IV. *J. Chromatogr.* **405:** 31–50.
10. Newburger, J., and G. Guiochon. 1989. Experimental Utilization of a displacement effect for the optimization of the separation of a two component mixture. *J. Chromatogr.* **484:** 153–166.
11. Golshan-Shirazi, S., and G. Guiochon. 1990. Band migration, profile, and separation in overload liquid chromatography. *Am. Biotech. Lab.* **8:** 24–31.