Journal of Chromatography, 153 (1978) 550–552

CHROM. 10,879

Note

Separation of glycolipids from neutral lipids and phospholipids

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(Received December 12th, 1977)

The most important functions of glycosphingolipids found in biological membranes are antigenicity and cell–cell recognition, which are manifested even at very low concentrations. The concentrations are mostly 5–10 times less than that of the accompanying phospholipids. The chromatographic separation of these substances is difficult because of their similar polarities and solubilities. In order to develop a satisfactory method of separating all glycosphingolipids quantitatively from the phospholipids in one step, use has been made of the ability of boric acid to form complexes with sugars. Using Kieselguhr impregnated with boric acid–disodium borate buffer, all glycosphingolipids except mono-, di- and triglycosylceramides and sulphatides were separated from phospholipids and neutral lipids[1]. In the preparative use of the method, boric acid–borax buffer was always found in the glycosphingolipid fractions and, as this hindered further use of the material, the buffer had to be separated by special purification procedures.

We have studied the possibilities of developing a simple and effective separation procedure. The above disadvantage could be avoided if the boric acid used for complexing were bound on a polymeric matrix. Fréchet and co-workers[2–4] have described the preparation of a resin that contains covalently bound phenylboronic acid held on a cross-linked polystyrene matrix. By the reaction of lithiated polystyrene with triethyl borate they achieved very high capacity of 2–3.5 mmole per gram of resin. The polarity of the non-polar polystyrene matrix was varied by adjusting the proportion of polar, hydrophilic phenylboronic acid. It seemed to us that this resin would be suitable for the separation of glycolipids, which contain both lipophilic and hydrophilic groups. In addition, the mechanical stability of the rigid, porous resin is superior to that of a cellulose[5] or a polyacrylamide support[6,7].

EXPERIMENTAL

The resin was prepared according to Farrall and Fréchet[2] and contained 3.7 mmole per gram of phenylboronic acid. A 7-g amount of resin was swollen in chloroform–methanol (10:2, v/v), the column being 16 cm long with an I.D. of 1.5 cm. A mixture of about 5 mg of phospholipids from egg and bovine brain together with cerebrosides, sulphatides and gangliosides from bovine brain was applied to the column. Elution was performed with chloroform–methanol (10:2, v/v) containing in-

creasing amounts of water (0, 0.3, 0.6, 1.2 and 3.0 ml of water per 100 ml of solvent) and fractions of 25 ml were collected.

For thin-layer chromatography (TLC) (Fig. 1), two corresponding fractions were combined and evaporated *in vacuo* at 40°. Chromatograms were developed as described previously[8] with tetrahydrofuran–water (5:1, v/v), 100 mg of potassium chloride being added per 100 ml of solvent mixture. Using pre-coated silica gel 60 HPTLC plates for nano-TLC (E. Merck, Darmstadt, G.F.R.), very sharp separations were obtained in a particularly short development time of 45 min. The developing solvent was removed completely from the plates in a vacuum oven at 130° before staining the glycosphingolipids with orcinol–sulphuric acid[9]. Phospholipids were detected using a molybdenum spray or by charring the orcinol–sulphuric acid-sprayed plates at 160°.

RESULTS AND DISCUSSION

Fig. 1 shows that all glycosphingolipids (fractions 2–10) were separated from phospholipids and neutral lipids (fraction 1) in a single step. The phospholipids and neutral lipids were stained only slightly by the orcinol–sulphuric acid spray. Less than 1% of the glycosphingolipids were lost in fraction 1. The separation of the glycosphingolipids from the phospholipids and the neutral lipids was accompanied by a fractionation of the glycosphingolipids according to the number of free hydroxyl groups. Sulphatides (fractions 2–5) were followed by monoglycosylceramides (fractions 6–9) and gangliosides (mainly fractions 9 and 10).

A large number of different column separations, of which Fig. 1 shows only one example, have demonstrated that the addition of water was necessary for the elution of the glycosphingolipids. In fact, even very small amounts of water added to the chloroform–methanol solvent liberated from the resin those glycosphingolipids which contain only a few sugar moities. Using solvent mixtures with minimal differences in water concentration, a further separation of glycosphingolipids might be achieved. However, the separation of brain gangliosides can possibly be obtained more easily by TLC as shown in Fig. 1.

The separation time can be increased or decreased according to the volume of water-free elution solvent used. If an additional fractionation of the glycosphingolipids is not required, the primary separation from the phospholipids can be achieved in 1–2 h. This short separation time is especially recommended for mixtures that contain gangliosides with several sialic acids in order to avoid hydrolysis of sialic acid residues. Some experiments showed that the capacity of the column used was sufficient to separate about ten times as much material as is indicated under Experimental.

An advantage of the method is the separation of all glycosphingolipids from neutral lipids and phospholipids and also from remaining proteins and colouring matter in a single step. Further, it is possible additionally to fractionate and concentrate the glycosphingolipids, which normally occur in only very small amounts, from relatively large amounts of biological material. Of special advantage is the fact that the resin can be regenerated and that no boric acid is eluted.

Hence, on the one hand our procedure might be helpful in obtaining larger amounts of purified glycosphingolipids for chemical studies, and on the other it could be used as an automated process in the routine determination of gangliosidoses.

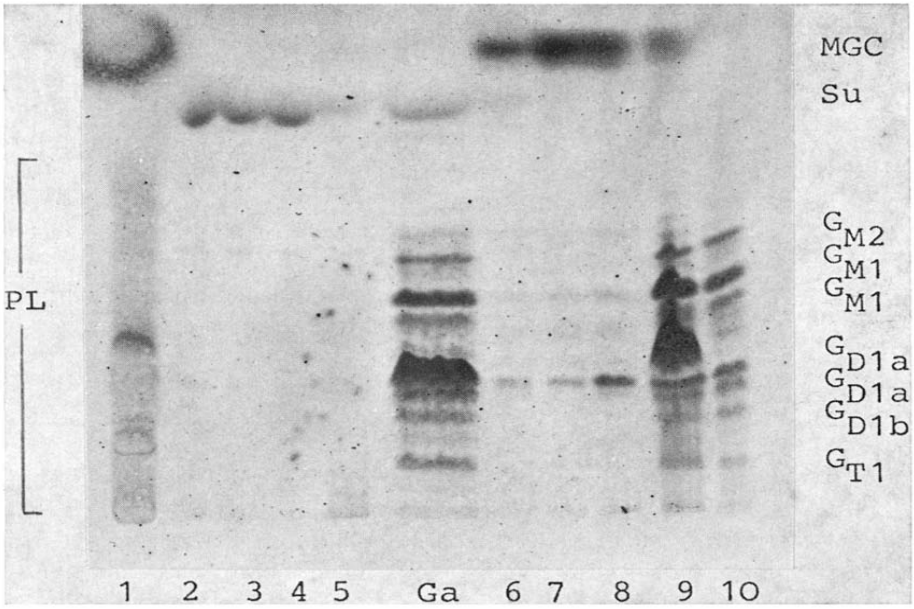

Fig. 1. Thin-layer chromatogram of lipids separated by column chromatography on polyphenylboronic acid. Adsorbent: pre-coated silica gel 60 HPTLC plate for nano-TLC (E. Merck). Solvent: tetrahydrofuran–water (5:1, v/v) containing 0.1% (w/v) of KCl. Spray: 0.2% orcinol in sulphuric acid, heated at 130° for 4 min. Samples: 1–10 = fractions from column chromatography; Ga = gangliosides from bovine brain. Abbreviations: MGC = monoglycosyl ceramide; Su = sulphatide; G_{M2}, G_{M1}, G_{D1a}, G_{D1b}, G_{T1} = gangliosides according to the Svennerholm nomenclature; PL = phospholipids.

ACKNOWLEDGEMENT

We thank Dr. Judith Gompertz for her assistance.

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