Journal of Chromatography, 106 (1975) 425–427
© Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 8026

Note

Thin-layer chromatographic separation of gangliosides

KLAUS EBERLEIN and GÜNTHER GERCKEN
Institut für Organische Chemie und Biochemie, Universität Hamburg, Hamburg (G.F.R.)
(Received September 30th, 1974)

The thin-layer chromatographic (TLC) separation of complex mixtures of gangliosides has proved difficult owing to the relatively small differences in the fine structure of the strongly polar carbohydrate moieties, and the tendency to form micellar complexes. So far, the best separations have been obtained on silica gel G with the solvent systems chloroform–methanol–water (60:35:8), chloroform–methanol–2.5 *N* ammonia solution (60:35:8), *n*-propanol–water (7:3) and *n*-butanol–pyridine–water (3:2:1)[1]. However, these procedures require long development times or multiple developments, and fail to resolve all of the components of complex ganglioside mixtures. This paper describes a TLC system that gives better resolution in a single run with a short development time.

EXPERIMENTAL

Purified gangliosides from bovine brain (Type III; Sigma, St. Louis, Mo., U.S.A.), dissolved in chloroform–methanol (1:1), were applied to pre-coated silica gel 60 TLC plates (E. Merck, Darmstadt, G.F.R.), 20 cm high and previously activated at 120° for 12 h. The chromatograms were developed with tetrahydrofuran–water (5:1), a solvent system recently described for the separation of ceramide hexosides[2]. In order to obtain an optimal separation on calcium sulphate-free TLC plates, 100 mg of potassium chloride were added per 100 ml of solvent mixture according to the method of Van den Eijnden[1]. In a single run, the solvent front reached the upper edge of the plates within 160 min. Pre-coated TLC plates, purchased from Merck in 1974, required a development time of only 120 min and gave an even better separation of gangliosides than that shown in Fig. 1. Prior to rendering the gangliosides visible with orcinol–sulphuric acid spray[2,3], the developing solvent was completely removed from the plates in a vacuum oven at 130°.

Gangliosides were identified by their R_F values in several known TLC systems[1] and by gas–liquid chromatographic (GLC) determination of their molar carbohydrate ratios. For that purpose, the individual gangliosides, separated by TLC on a preparative scale and located with iodine, were extracted with *n*-propanol–water (1:1) from the adsorbent, evaporated and dried. After methanolysis[4] and re-N-acetylation[5], trimethylsilyl derivatives were prepared and analyzed by GLC[4].

For testing the capacity of the TLC system to resolve less complex

glycosphingolipids than gangliosides, total lipid extracts of rabbit and porcine erythrocytes were applied to a plate in concentrations as previously described[2].

RESULTS AND DISCUSSION

As shown in Fig. 1, bovine brain gangliosides are separated into at least nine distinct fractions by the TLC system. The four major components were identified as the gangliosides G_{M1}, G_{D1a}, G_{D1b} and G_{T1} (nomenclature according to the Svennerholm system[6]). The fraction running ahead of G_{M1} was found to be the Tay-Sachs ganglioside G_{M2}. With our TLC system, small sub-fractions, G_{M1}* and G_{D1a}*, could be separated from the gangliosides G_{M1} and G_{D1a}. Like the main fractions, they were found to contain galactose, glucose, galactosamine and neuraminic acid in the molar ratio of 2:1:1:1 and 2:1:1:2, respectively. We suggest, that G_{M1}* and G_{D1a}* contain sialic acids other than N-acetylneuraminic acid. Tettamanti *et al.*[7] reported evidence for the presence of small amounts of N-glycolylneuraminic acid in bovine brain gangliosides. In addition, it is known that N-glycolylneuraminyllactosylceramide with the solvent system chloroform–methanol–2.5 *N* ammonia solution has a lower R_F value than that of N-acetylneuraminyllactosylceramide[8].

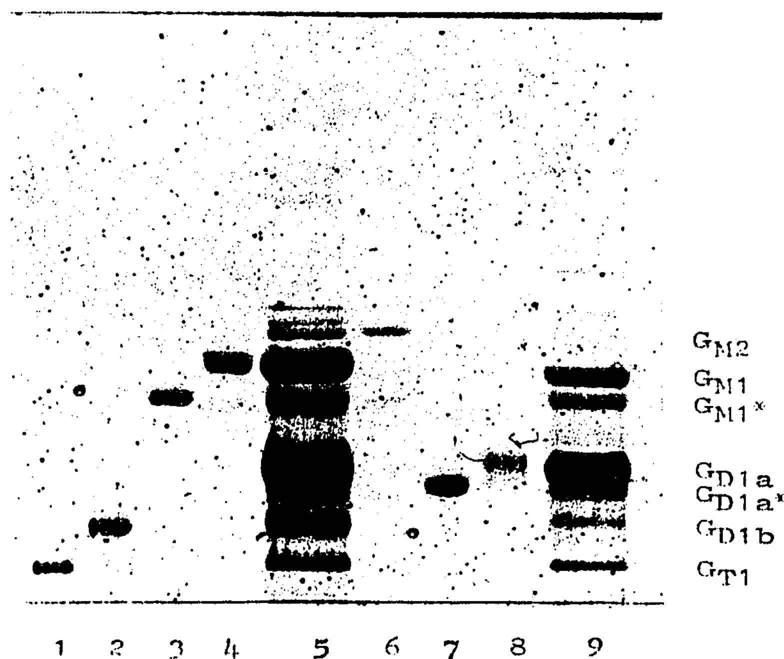

Fig. 1. Thin-layer chromatogram of bovine brain gangliosides on pre-coated silica gel 60 TLC plates. Solvent: tetrahydrofuran–water (5:1) with 0.1% (w/v) KCl. Spray: 0.2% orcinol in sulphuric acid, heated at 130° for 5 min. Samples: 5 and 9 = mixture of bovine brain gangliosides; 1–4, 6–8 = distinct gangliosides, isolated by preparative TLC; 1 = G_{T1}, 2 = G_{D1b}, 3 = G_{M1}*, 4 = G_{M1}, 6 = G_{M2}, 7 = G_{D1a}*, 8 = G_{D1a}.

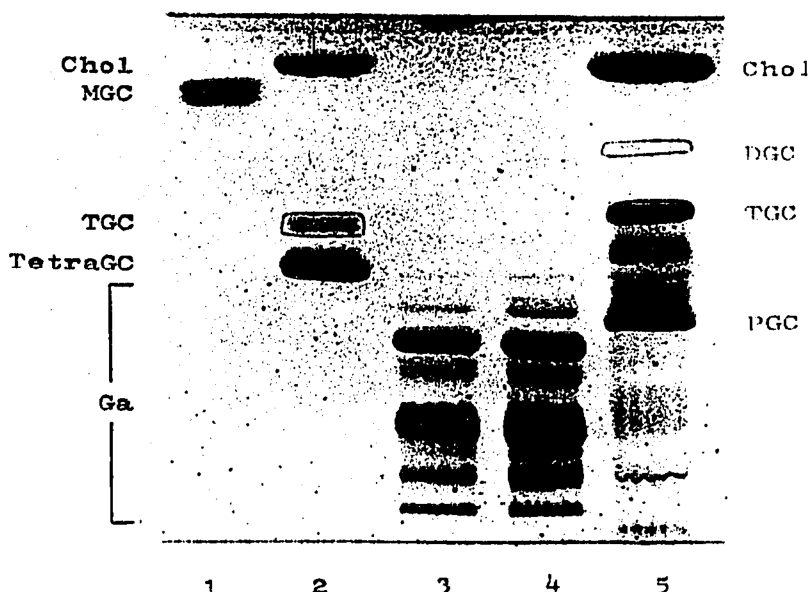

Fig. 2. Thin-layer chromatogram of glycosphingolipids. TLC plates, solvent and spray as in Fig. 1. Samples: 1 = monoglycosylceramides (cerebrosides) from bovine brain; 2 = total lipid extract from 0.2 ml of packed porcine erythrocytes; 3 and 4 = bovine brain gangliosides; 5 = total lipid extract from 1 ml of packed rabbit erythrocytes. Glycosphingolipids in total lipid extracts from erythrocytes were outlined with a needle after location with sugar-specific spray. Abbreviations: MGC = monoglycosylceramides; DGC = diglycosylceramides; TGC = triglycosylceramides; TetraGC = tetraglycosylceramides; PGC = pentaglycosylceramides; Ga = gangliosides; Chol = cholesterol.

It can be seen from Fig. 2 that the TLC system also separates less polar glycosphingolipids that are free from sialic acid. Good resolution is obtained for monoglycosylceramides (cerebrosides) from bovine brain (Koch-Light, Colnbrook, Great Britain), diglycosylceramides from rabbit erythrocytes, triglycosylceramides from rabbit and hog erythrocytes, tetraglycosylceramides (globosides) from hog erythrocytes and pentaglycosylceramides from rabbit erythrocytes.

REFERENCES

1 D. H. van den Eijnden, *Hoppe-Seyler's Z. Physiol. Chem.*, 352 (1971) 1601.
2 K. Eberlein and G. Gercken, *J. Chromatogr.*, 61 (1971) 285.
3 V. P. Skipski, A. F. Smolove and M. Barclay, *J. Lipid Res.*, 8 (1967) 295.
4 D. E. Vance and C. C. Sweeley, *J. Lipid Res.*, 8 (1967) 621.
5 R. E. Chambers and J. R. Clamp, *Biochem. J.*, 125 (1971) 1009.
6 L. Svennerholm, *J. Neurochem.*, 10 (1963) 613.
7 G. Tettamanti, L. Bertona, B. Berra and V. Zambotti, *Ital. J. Biochem.*, 13 (1964) 315.
8 R. Henning and W. Stoffel, *Hoppe-Seyler's Z. Physiol. Chem.*, 354 (1973) 760.