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DEMONSTRATION OF COMPLEXITY OF THE GLYCOSPHINGOLIPID FRACTION OF RAT SMALL INTESTINE

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SUMMARY

A total neutral (non-acid) glycolipid fraction has been isolated from rat small intestine. By silicic acid column chromatography of the acetylated glycolipid derivative, 7 different partly purified fractions were obtained. Thin-layer chromatography of both the acetylated and native glycolipid fractions revealed a highly complex pattern with at least 30 different glycolipid bands having a thin-layer mobility as for mono- to dodecaglycosylceramides. Mass spectrometry of the permethylated and permethylated-reduced ($LiAlH_4$) derivatives showed the presence of several glycolipid species not known before, including oligohexosylceramides with 4, 5, 6 and 7 sugar residues and a tetraglycosylceramide with a blood group A determinant. This is the first report on such a complex glycolipid composition of a single organ.

INTRODUCTION

McKibbin (1) and Suzuki et al. (2) were probably the first to show that small intestine is a relatively rich source of blood group fucolipids (3). It has later been shown that the fucolipids are confined to the epithelial cells (4,5). Although a study of rat small intestine (6) revealed fucose in the glycolipid fractions from a microvillus membrane preparation, a more recent investigation on epithelial cells of different maturity showed a very simple glycolipid pattern and fucolipids were not noted (7). As a part of a systematic investigation on epithelial cells of small intestine including novel techniques of analysis (8,9), the present paper will demonstrate that the complexity of glycolipids of rat small intestine is much higher than earlier revealed by conventional methods.

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MATERIALS AND METHODS

An inbred strain of black and white (hooded) rats was used. Small intestine was rapidly taken out after ether anaesthesia and sacrificing by decapitation. Lyophilized tissue was cut into small pieces and extracted in two steps in a Soxhlet apparatus, one day with chloroform-methanol 2:1 (by vol.) and one day with chloroform-methanol 1:9 (by vol.). The combined extracts were evaporated and subjected to mild alkaline degradation, dialysis, DEAE and silicic acid chromatography as described (10). To free the non-acid glycolipid fraction of alkali-stable phospholipids, acetylation and silicic acid chromatography were used (11).

The pure non-acid glycolipids were then fractionated as acetylated derivatives on a column of silicic acid using methanol in chloroform as eluant, and together 7 fractions were eluted (fig. 1). These were de-acetylated in KOH in methanol and dialysed and parts of the fractions obtained were subjected to immunological analysis and to direct inlet mass spectrometry after permethylation and after permethylation and $LiAlH_4$-reduction (12).

RESULTS

At least 30 different non-acid glycolipid bands (all coloured green with the anisaldehyde reagent) were discernable on thin-layer chromatography of rat small intestine glycolipids, fractionated as acetylated derivatives and de-acetylated (fig. 1). All seven fractions (B_1-B_7) were analysed by mass spectrometry in form of the two derivatives, and the spectra obtained from permethylated-reduced derivatives of two of these (B_4 and B_5) are reproduced (figs. 2 and 3, respectively).

As shown by the formulas on top of the spectrum of fraction B_4 (fig. 2), three glycolipids containing only hexoses were interpreted, namely tri-, tetra- and pentahexosylceramides. As is the rule for these derivatives (8,9,12), relatively abundant ions are obtained with all sugars and the fatty acid. For the major tetrahexosylceramide these are shown at *m/e* 1128, 1156, 1184, 1212 and 1240, corresponding to 4 hexoses and normal fatty acids with 16, 18, 20, 22 and 24 carbon atoms, respectively (compare top formula). Similarly, the pentahexosyl derivative produced the ion series at *m/e* 1332, 1360, 1388, 1416 and 1444, and the trihexosyl derivative with 24:0 fatty acid is indicated at *m/e* 1036. The peak at *m/e* 1302 may correspond to four hexoses, one fucose and 16:0 fatty acid. Some sequence ions are explained by the formulas, and additional information from the only permethylated derivative (spectrum not shown) supported the formulas proposed.

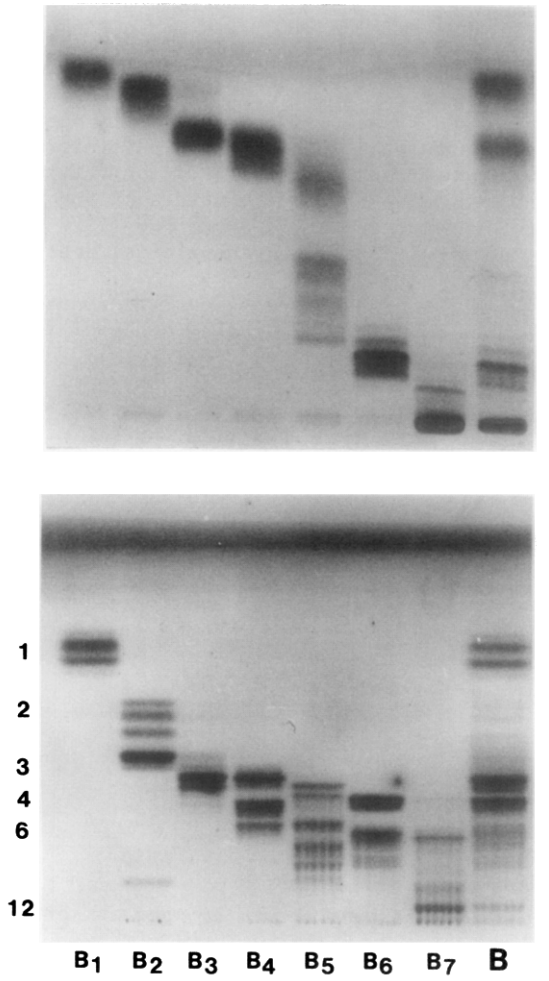
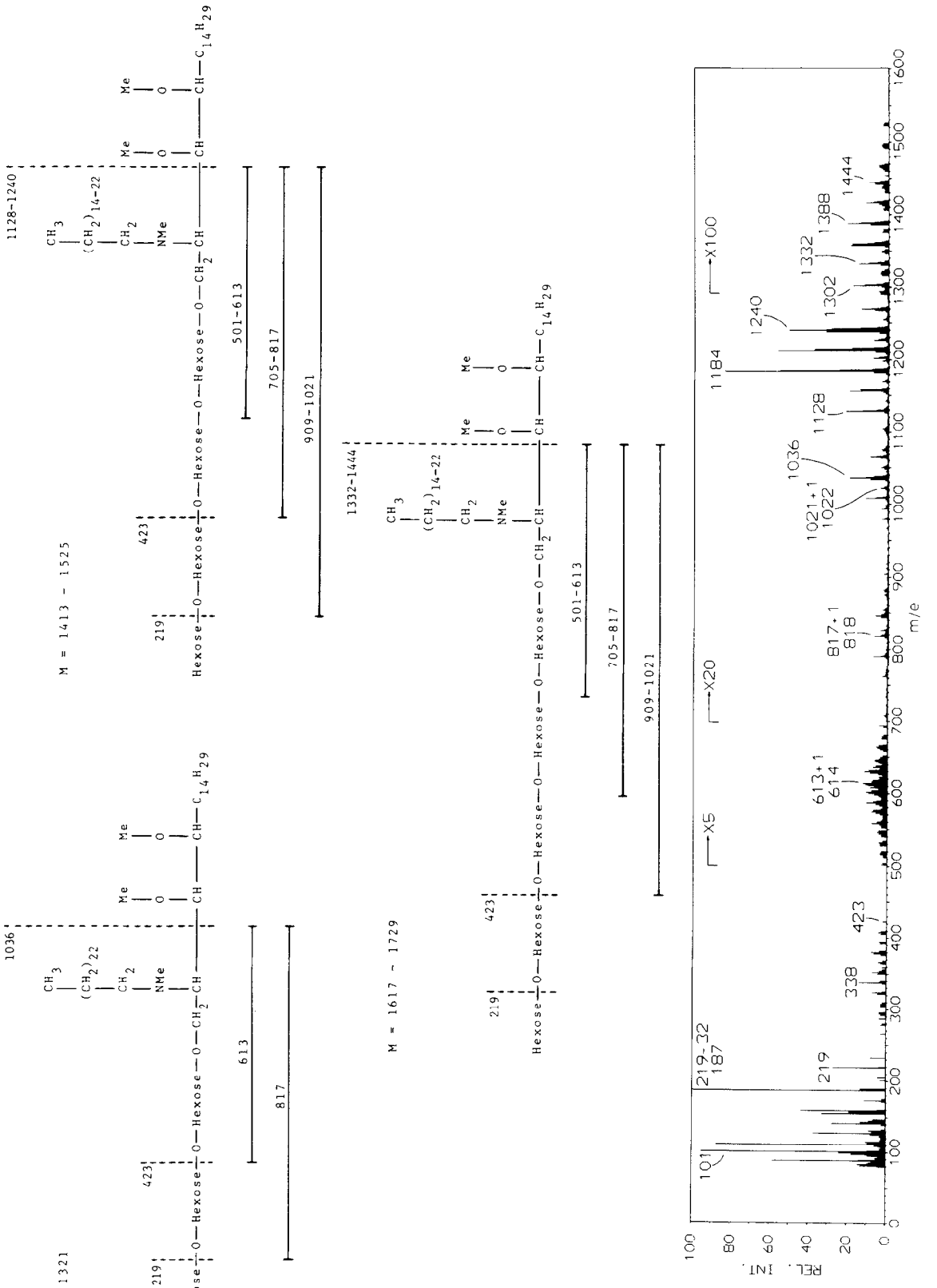
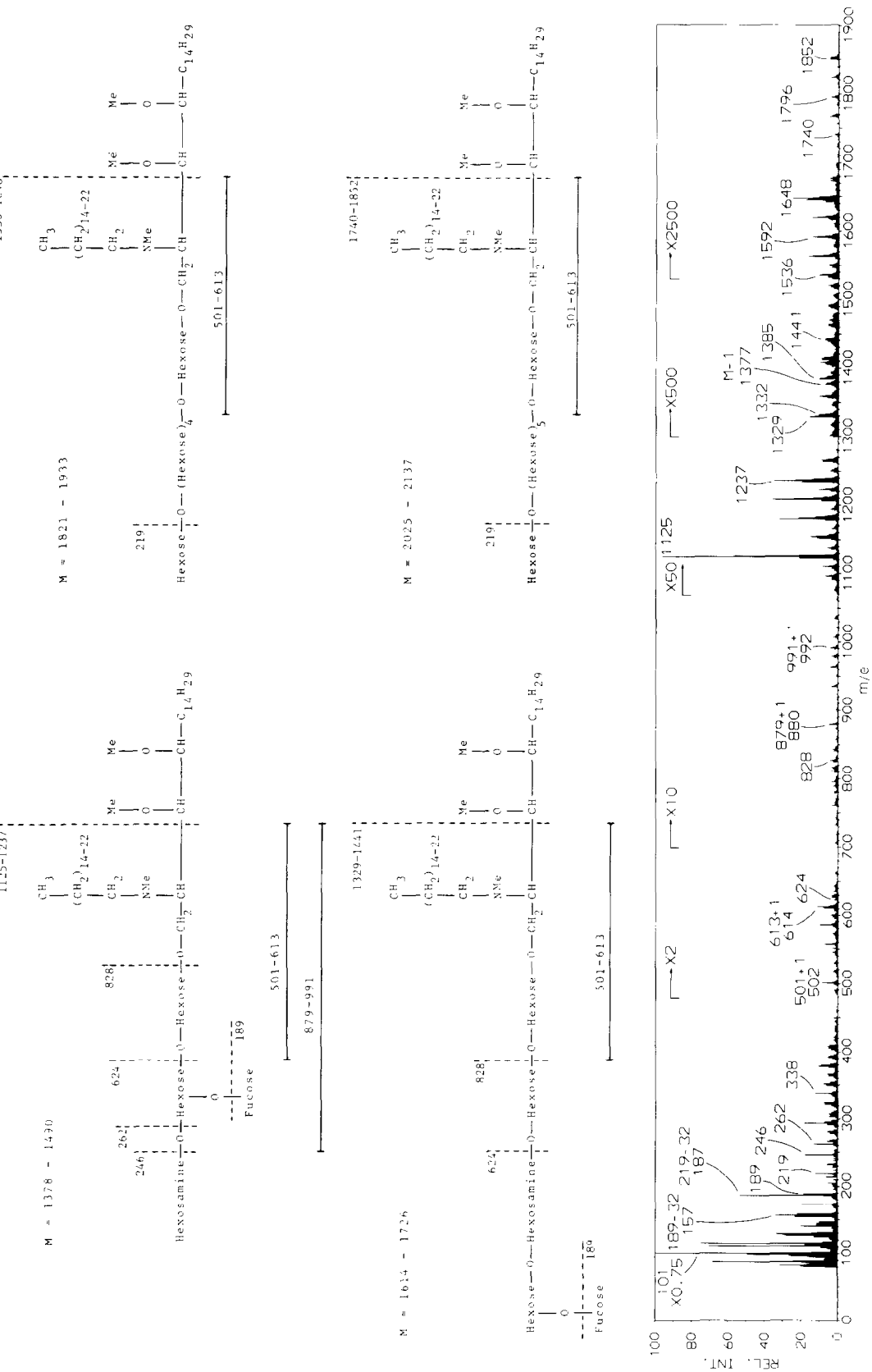


Fig. 1. Thin-layer chromatogram of acetylated (top) and native (bottom) non-acid glycolipid fractions obtained by silicic acid chromatography of acetylated total glycolipids (B). About 40 µg of the total and 10 µg of the separated fractions were applied. HPTLC (Merck) plates were developed with chloroform-methanol 96:4 (by vol.) for acetylated derivatives and chloroform-methanol-water 60:35:8 (by vol.) for native glycolipids. Anisaldehyde was used for detection (10).

Analogously, the spectrum of the reduced derivative of fraction B_5 may be interpreted for at least four glycolipids shown in the top formulas of fig. 3. Oligohexosylceramides with six and seven sugars are shown with sugar and fatty acid ion series in the intervals at *m/e* 1536-1648 and 1740-1852,

Fig. 2. Mass spectrum of the permethylated-reduced ($LiAlH_4$) derivative of fraction B_4 of Fig. 1. The amount used for analysis was 60 µg. Further conditions: electron energy 36 eV, acceleration voltage 4 kV, trap current 500 µA, ion source temperature 300°C, and probe temperature 260°C.


M = 1321
M = 1413 - 1525
M = 1617 - 1729
Hexose
O
CH3
(CH2)22
(CH2)14-22
CH2
NMe
Me
CH
C14H29
1036
1128-1240
1332-1444
219
423
613
817
501-613
705-817
909-1021
REL. INT.
m/e
X5
X20
X100
101
219-32
187
219
338
423
613+1
614
817-1
818
1021+1
1022
1036
1128
1184
1240
1302
1332
1388
1444


M = 1378 - 1430
1125-1237
M = 1821 - 1933
1536-1648
M = 1614 - 1726
1329-1441
M = 2025 - 2137
1740-1852
Hexosamine
Hexose
Fucose
501-613
879-991
REL. INT.
m/e
X0.75
X2
X10
X50
X500
X2500
1125
1237
1329
1332
1377
1385
1441
1536
1592
1648
1740
1796
1852

respectively. The major glycolipid appeared to be a blood group A tetraglycosylceramide with a terminal hexosamine, and there was also an H type pentaglycosylceramide. Again, additional sequence ions to those indicated in fig. 3 were obtained from the only permethylated derivative (spectrum not shown). The presence of the A glycolipid was supported by blood group A activity of the native fraction B_5.

From the interpretation of the mass spectra it was possible to ascribe the chemical identity of the separate bands on the chromatogram of fig. 1. Thus, the three bands of fraction B_4 were tri-, tetra- and pentahexosylceramides, in order of decreasing mobility. In an analogous way all other bands detected have been identified (papers in preparation), including the slow-migrating component of fraction B_7, which was a 12-sugar blood group A glycolipid.

DISCUSSION

The combined use of fractionation of acetylated glycolipids and mass spectrometry of permethylated and permethylated-reduced derivatives is apparently a potent technique for revealing complexity of glycolipid fractions. In addition to earlier known species several unknown structures were definitely established. The separation into groups of glycolipids, in part based on the presence or absence of amino sugars, is a clear advantage for succeeding analysis by mass spectrometry. A further development of mass spectrometry was recently published (8,9), where mixtures of glycolipid derivatives were fractionated by distillation in the ion source, thus significantly validating the identity even of minor separate components.

The interesting complexity of rat small intestine concerning glycolipids is at present subject of a more detailed study. The composition of isolated epithelial cells (7,13) and non-epithelial residue are being compared. Also,

Fig. 3. Mass spectrum of the permethylated-reduced ($LiAlH_4$) derivative of fraction B_5 of Fig. 1. The amount used for analysis was 180 µg. Further conditions: electron energy 38 eV, acceleration voltage 4 kV, trap current 500 µA, ion source temperature 300°C and probe temperature 295°C.

epithelial cells of different maturity (7,13) are being defined concerning enzyme and glycolipid characteristics.

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