SPECIFIC PATTERN OF GLYCOSPHINGOLIPIDS ENRICHED IN A MUCOSA SCRAPING OF HUMAN SMALL INTESTINE

Karl-Erik FALK+, Karl-Anders KARLSSON*, Hakon LEFFLER and Bo E. SAMUELSSON

+*Department of Biochemistry, University of Göteborg and Chalmers Institute of Technology, S-412 96 Göteborg* and *Department of Medical Biochemistry, University of Göteborg, S-400 33 Göteborg, Sweden*

Received 6 March 1979

1. Introduction

Epithelial cells of small intestine offer a unique system for the study of differentiation of eukaryotic cells (see [1]). Cells of different maturity levels can now be prepared by a gentle washing of the mucosa, cells being eluted in the order of decreasing differentiation [2]. By use of this technique interesting results have been obtained concerning the activity of some rat glycosyltransferases during differentiation [3].

Cell-surface carbohydrates exist in two forms, glycoproteins and glycolipids. Developmental changes have been found for both classes of substance [4,5], but no solid information has yet been obtained to prove the functional importance of surface carbohydrates [6].

A relatively high fucolipid content has been shown in whole small intestine of man [7]. Immunofluorescence studies on dog small intestine further indicated that Le^b-like activity (based on fucolipid) was associated with epithelial cells, while Forssman activity was found in lamina propria [8]. Interesting differences in lipophilic components of these two glycolipids were later shown by mass spectrometry [9].

The purpose of the present investigation was to define the general glycolipid composition of epithelial cells (mucosa scraping) compared with the intestinal wall (residue after mucosa scraping), a prerequisite for a more detailed and laborious study on epithelial cells fractionated according to the level of maturity [1,2].

* To whom correspondence should be addressed

2. Material and methods

Small intestine (jejunum and ileum) of a 31-year-old blood group A human male was obtained at autopsy. The intestine was slit longitudinally and rinsed gently with tap water. The mucosa was prepared by scraping with a spoon along the whole intestine. The mucosa preparation and the rest of the intestine were then lyophilized and extracted in two steps with chloroform–methanol in a Soxhlet apparatus, one day with 2:1 (v/v) and one day with 1:9 (v/v). The combined extracts were subjected to mild alkaline degradation, dialysis, DEAE-cellulose and silicic acid chromatography as in [10]. To obtain a pure non-acid glycolipid fraction free of contaminants, acetylation, silicic acid chromatography and deacetylation were used [11]. The total non-acid glycolipid fraction from the residue after scraping was further fractionated into groups of glycolipids by successive elution with increasing proportions of methanol in chloroform from a silicic acid column. These fractions were analyzed by direct inlet mass spectrometry as in [12]. A slow-moving major glycolipid with 5 sugars was isolated by column and preparative thin-layer chromatography and analyzed by mass spectrometry and NMR spectroscopy [13].

3. Results

The yields of glycolipids of the two tissues are collected in table 1, and thin-layer chromatogram of acid and non-acid glycolipids is shown in fig.1. The

Table 1
Quantitative data for the preparation of glycosphingolipids of small intestine of man

Tissue	Dry wt (g)	Total non-acid glycolipids (mg/g)	Crude acid glycolipids (mg/g)	Purified penta-glycosylceramide (mg)
Mucosa scraping	7	4.7	5.2	8
Remaining tissue after scraping	100	1.9	2.1	21

number of sugars of the non-acid glycolipids as indicated in the margin of the chromatogram was obtained by mass spectrometry of methylated and methylated-reduced derivatives [12]. The monoglycosylceramides were mainly galactosylceramides with only small amounts of glucosylceramides, as shown by thin-layer chromatography on a borate-containing layer [14]. The di-, tri- and tetra-glycosylceramides were dihexosylceramides, trihexosylceramides and globoside, respectively.

The isolated pentaglycosylceramide produced mass spectra that were identical with the spectra of a Le^a-active pentaglycosylceramide characterized in [7]. In NMR spectra of the 2 derivatives, 5 anomeric protons were identified [13], 1 α and 4 β resonances. The chemical shift of 5.0 ppm is specific for 1→4-linked α-fucose and differs from α-fucose in 1→2- or 1→3-linkage. In a type 1 chain (Gal1→3GlcNAc) but not in a type 2 chain (Gal1→4GlcNAc) the βGal signal increases its chemical shift upon reduction due to a deshielding from the vicinal amine. In fig.2, middle and below, the β resonance admixed to the αFuc signal at 5.0 ppm is therefore evidence for a type 1 chain [13]. The Le^a activity (liposomes of 2 μg glycolipid, 50 μg sphingomyelin, 50 μg lecithin and 25 μg cholesterol per ml inhibited completely a 4+ hemagglutination reaction by an equal volume of goat antiserum (Behring) diluted 1:4) is further evidence that the glycolipid is identical with the glycolipid earlier

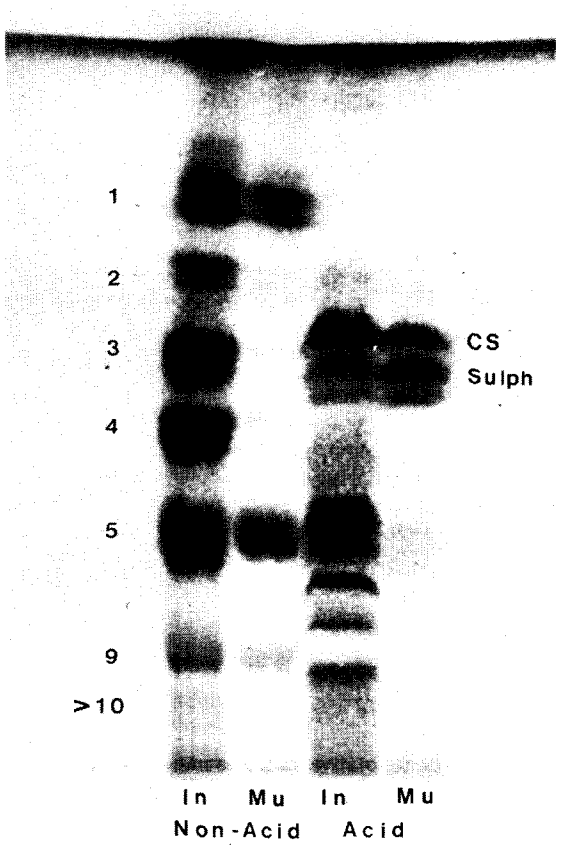

Fig.1. Thin-layer chromatogram of non-acid and crude acid glycosphingolipids of a mucosa scraping (Mu) and whole intestine after mucosa scraping (In). The amounts applied were for (In) one part and for (Mu) two parts out of 2000. The figures to the left of the non-acid intestinal fraction indicate the number of sugars present in the glycolipids. The designations to the right mean cholesterol sulphate (CS) and sulphatides (Sulph). In the non-acid fractions, all bands were coloured green, and were therefore glycolipids. In the acid fractions, cholesterol sulphate was coloured blue-violet, and sulphatides (double-band) and most of the slow-moving bands (gangliosides) were coloured green. A 0.15 mm layer of silica gel G (Fluka AG, Buchs, Switzerland) was coated on 20×20 cm glass plates. Chloroform–methanol–water 60:35:8 (v/v/v) was used as solvent and the anisaldehyde reagent (see [10]) for the detection.

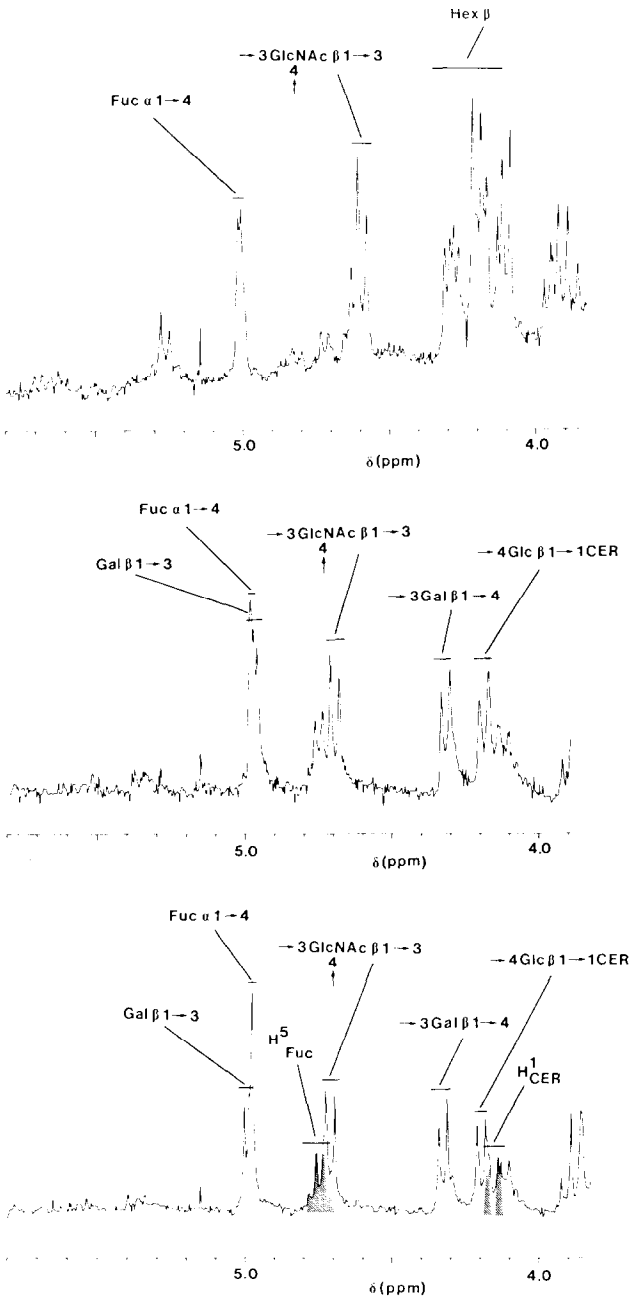

Fig.2. Top: NMR spectrum of permethylated Le^a active pentaglycosylceramide, 2 mg in 0.5 ml chloroform, 1100 pulses at 40°C. Middle and bottom: Spectra of permethylated-reduced Le^a-active pentaglycosylceramide, 2 mg in 0.5 ml chloroform, 600 pulses at 26°C (middle) and 1000 pulses at 40°C (bottom). The two temperatures were used to improve interpretation [13].

characterized by us and having the structure Galβ1→3GlcNAc(4←1αFuc)β1→3Galβ1→4Glc1→1Cer [7].

The more slow-moving material has not yet been analyzed in detail, but the 9-sugar glycolipid is probably also a Le^a-type glycolipid. Interestingly, blood group A-type glycolipids with 6 and 7 sugars, identified before in the small intestine of man [15,16], are apparently lacking and blood group A activity existed only below the 9-sugar band.

As shown in fig.1 for the non-acid glycolipids, the mucosa preparation practically lacked glycolipids with 2–4 sugars. Mass spectra of the partially purified fractions from the intestinal residue after scraping showed that these glycolipids (2–4 sugars) contained only sphingosine (dihydroxy base) and non-hydroxy fatty acids. Glycolipids with 1 and with ≥ 5 sugars contained, however, mostly phytosphingosine (trihydroxy base) and exclusively 2-hydroxy fatty acids. This clearly indicates a difference in ceramide structure between epithelial cells and non-epithelial tissue of small intestine, the more hydroxylated ceramides being confined to the epithelial cells. This is in support of our earlier results from dog intestine [9].

These findings and the probable content in the mucosa scraping of cells primarily from the villus tip, make it very likely that only a part (~25% as judged from the plate in fig.1 and from table 1) of the mucosa cells was scraped off, the remaining part being included in the residue after scraping. Glycolipids with 1 sugar and with >4 sugars are therefore probably exclusively located in the epithelial cells.

The major glycolipid of the crude acid fraction of mucosa was identified as sulphatides (ceramide galactose 3-sulphate). As for the blood group substances, sulphatides are most probably located in the epithelial cells. Gangliosides, on the other hand, are apparently more concentrated in the non-mucosa (see also [17]). The band moving just before sulphatides (see indication in fig.1) was probably cholesterol sulphate, identified earlier in large amounts in starfish tissues [18].

4. Discussion

The present results show that small intestinal glycolipids with 1 sugar (galactosylceramides and

sulphatides) and with > 4 sugars (blood group glycolipids) are probably exclusively located in the epithelial cells, and that di-, tri- and tetra-glycosylceramides, and most of the gangliosides, are present in the non-mucosa. The two tissue compartments are further characteristically different in their ceramide composition, the more hydroxylated ceramides being parts of the epithelial glycolipids. A hypothesis, based on lateral hydrogen bonding, for the functional significance (membrane stability) of this ceramide difference has been put forward [19].

The finding of sulphatides as one of the major glycolipids in epithelial cells of small intestine is not unexpected, as these cells have a high level of Na^+ transport [20], and we have shown before a stoichiometric relation of this lipid and Na^+-K^+-dependent ATPase (see [19]). A specific role for sulphatides as a K^+ receptor has been postulated [19].

The large amounts of blood group glycosphingolipids in epithelial cells of human small intestine, as shown here and in [7], are difficult to explain. Future studies may show if these rapidly renewing cells [1] with a large-surface contact with foreign substances and with a variety of microorganisms have a special need for self- and non-self discrimination mechanisms, of which glycolipids are a part.

Acknowledgements

We thank Karin Nilson for important technical assistance. The work was supported by grants from the Swedish Medical Research Council (no. 3967), the Swedish Natural Science Research Council (no. F2214-021) and the Medical Faculty, University of Göteborg.

References

[1] Lipkin, M. (1973) Physiol. Rev. 53, 891–915.
[2] Weiser, M. (1973) J. Biol. Chem. 248, 2536–2541.
[3] Weiser, M. (1973) J. Biol. Chem. 248, 2542–2548.
[4] Warren, L., Fuhrer, J. P., Tuszynski, G. P. and Buck, C. A. (1974) Biochem. Soc. Symp. 40, 147–157.
[5] Hakomori, S. (1975) Progr. Biochem. Pharmacol. 10, 167–196.
[6] Hughes, R. C. (1975) Essays Biochem. 11, 1–36.
[7] Smith, E. L., McKibbin, J. M., Karlsson, K.-A., Pascher, I., Samuelsson, B. E., Li, Y.-T. and Li, S.-C. (1975) J. Biol. Chem. 250, 6059–6064.
[8] Hiramoto, R. N., Smith, E. L., Ghanta, V. K., Shaw, J. F. and McKibbin, J. M. (1973) J. Immunol. 110, 1037–1043.
[9] Smith, E. L., McKibbin, J. M., Karlsson, K.-A., Pascher, I., and Samuelsson, B. E. (1975) Biochim. Biophys. Acta 388, 171–179.
[10] Karlsson, K.-A., Samuelsson, B. E. and Steen, G. O. (1973) Biochim. Biophys. Acta 316, 317–335.
[11] Handa, S. (1963) Japan. J. Exp. Med. 33, 347–360.
[12] Karlsson, K.-A. (1976) in: Glycolipid Methodology (Witting, L. A. ed) pp. 97–122, Am. Oil Chem. Soc., Champaign, IL.
[13] Falk, K.-E., Karlsson, K.-A. and Samuelsson, B. E. (1979) Arch. Biochem. Biophys. 192, in press.
[14] Karlsson, K.-A., Samuelsson, B. E. and Steen, G. O. (1973) Biochim. Biophys. Acta 306, 317–328.
[15] Smith, E. L., McKibbin, J. M., Karlsson, K.-A., Pascher, I., and Samuelsson, B. E. (1975) Biochemistry 14, 2120–2124.
[16] McKibbin, J. M., Smith, E. L., Månsson, J.-E. and Li, Y.-T. (1977) Biochemistry 16, 1223–1228.
[17] Keränen, A. (1975) Biochim. Biophys. Acta 409, 320–328.
[18] Björkman, L. R., Karlsson, K.-A., Pascher, I. and Samuelsson, B. E. (1972) Biochim. Biophys. Acta 270, 260–265.
[19] Karlsson, K.-A. (1977) in: Structure of Biological Membranes (Abrahamsson, S. and Pascher, I. eds) pp. 245–274, Plenum, New York.
[20] Ganong, W. F. (1969) Review of Medical Physiology, Lange Medical Publications, Los Altos, CA.