Biochimica et Biophysica Acta, 733 (1983) 295–299
Elsevier

BBA Report

BBA 70118

THE SUBCELLULAR LOCALIZATION OF THE GLYCOSPHINGOLIPIDS IN THE EPITHELIAL CELLS OF RAT SMALL INTESTINE

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(Received February 1st, 1983)
(Revised manuscript received April 25th, 1983)

Key words: Glycosphingolipid; Subcellular localization; Brush-border membrane; Basolateral membrane; (Rat intestine)

Analysis of brush-border and basolateral membrane prepared from the epithelial cells of rat small intestine showed that the brush border contained less of some phospholipids and more glycosphingolipids per protein. The *N*-glycoloylneuraminosyllactosylceramide was enriched in the brush border, while cholesteryl sulphate and fucosyllactosylceramide were more prominent in the basolateral membrane. By periodate-NaB^{3}H$_4$ labelling of the intact small intestine, it was shown that *N*-glycoloylneuraminosyllactosylceramide is exposed at the luminal cell surface of the epithelial cell.

Glycosphingolipids have been implicated in connection with both cell-cell, and self-nonself interactions [1] as, for example, in the ABO, H, Lewis and P blood group systems [2] and bacteria interactions with a host [3,4]. We have studied glycosphingolipid structures in the small intestine of rat [5], their localization to epithelial cell and non-epithelial stroma [5] as well as their alteration with differentiation during epithelial cell migration from crypt to villus tip [6,7]. Quantitative changes were found similar to those reported by others [8], together with alterations in the ceramide moiety [7]. A fundamental question when discussing the functional role of glycosphingolipids in the epithelial cells of the small intestine is the subcellular localization of the different structures, whether they are situated in the brush border of the epithelial cell, and are thus in contact with the exterior of the individual, or in the basolateral membrane, and thus in contact with adjacent cells or the basal membrane.

Male rats of two (not strictly) inbred strains, one white (Sprague-Dawley) and one black-white (hooded), were used. Non-fasting animals were killed by decapitation during diethyl ether anaesthesia. The small intestine (jejunum-ileum) was taken out, cut open and washed in 0.9% saline, and the epithelial cells were isolated by gentle scraping. The cells were washed twice in 25 ml phosphate-buffered saline by centrifugation for 5 min at 250 × *g* and filtered through a nylon net to remove most of the mucus released.

Subcellular fractionation was done principally according to the methods of Forstner et al. [9] and Mircheff et al. [10,11]. The cells were suspended with 80 ml 5 mM EDTA (pH 7.2), homogenized by 40 strokes with a Dounce homogeniser (pestle B) and diluted with 160 ml 5 mM EDTA. The homogenate was centrifuged at 450 × *g* for 10 min, the supernatant was sucked off and the pellet was suspended in 80 ml 5 mM EDTA. This procedure was repeated another three times. The pellet was suspended in 30 ml 90 mM NaCl/0.8 mM EDTA and kept for 30 min before filtering through glass wool. The purified brush borders were obtained after two centrifugations at 500 × *g* for 10 min, with a washing in 5 mM EDTA. The first two supernatants (see above) were subjected to centri-

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fugation at 2500 × *g* for 15 min and the supernatant obtained was used for preparation of the other membrane fractions.

In one series of experiments used for the white rat, the supernatant was centrifuged 23 500 × *g* for 15 min, the pellet obtained was resuspended in 40 ml 250 mM sorbitol/histidine/imidazole buffer [10] and disrupted with 20 strokes of a glass-Teflon homogenizer [11]. 56 ml 65% sorbitol in histidine/imidazole buffer [10] were added. Discontinuous density gradients were made of 7 ml 50% sorbitol, 15 ml sample, 10 ml 30% sorbitol and 6 ml 20% sorbitol and centrifuged at 20 000 rpm for 23 h in a SW-27 rotor (Beckman). The density gradient medium was removed by dialysis or dilution-centrifugation. The interphase between 30 and 40% sorbitol was the basolateral membrane fraction.

The fractions obtained were analysed for protein [12], sucrase activity [13] and $(Na^+ + K^+)$-ATPase activity [14].

The pellets of the brush-border fraction, the 'mitochondrial' fraction and the 'heavy microsomal' fraction were separately extracted with methanol in tubes with Teflon-faced screw caps for 30 min at 70°C. The debris was spun down at 900 × *g* for 5 min and the supernatant decanted. The fractions were then extracted in the same way with chloroform/methanol (1 : 2, by vol.) another three times. The basolateral membrane fraction was lyophilized and extracted similarly. The total non-acid glycosphingolipid and acid lipid fractions were prepared essentially as described [6]. All thin-layer chromatograms were run on microanalytical plates (HPTLC Si 60, Merck, Darmstadt).

The luminal cell surface gangliosides were labelled by the periodate-NaB^3H_4 method [15]. In two different experiments, 20 cm of ileum were washed twice with 0.9% saline, filled with 0.5 mM or 2 mM $NaIO_4$ in phosphate-buffered saline (pH 7.3), and incubated 10 or 20 min, respectively, at 0°C. The small intestines were washed five times with phosphate-buffered saline, incubated with 0.1 M glycerol in phosphate-buffered saline for 5 min, washed three times with phosphate-buffered saline and incubated with 1 mCi NaB^3H_4 (Amersham International) for 10 or 30 min, respectively, at room temperature. The intestines were washed ten times and cut open, and the epithelial cells were scraped off. The epithelial cells were extracted, the extract was treated with mild alkali and dialysed and the glycolipids separated on silicic acid [6]. The gangliosides were bound on 2 ml DEAE-Sepharose (Pharmacia) and eluted with 10 ml 0.3 M ammonium acetate in methanol. The fractions obtained were analysed on a thin-layer plate and detected by the anisaldehyde reagent or autoradiographed (XAR-5, Kodak) 140 h at −80°C after treatment with EN^{3}HANCE spray (New England Nuclear).

Under the phase contrast microscope, the brush-border fraction contained only brush borders, including their fibrillar network. The specific activity of sucrase, a marker of brush-border membranes [10,16], was increased about 12-times, see Table I. The specific $(Na^+ + K^+)$-ATPase activity, a marker of the basolateral membrane [10,16], was increased in the basolateral fraction (Table I).

The total non-acid glycosphingolipids (left in

TABLE I

SUCRASE AND $(Na^+ + K^+)$-ATPase ACTIVITY OF HOMOGENATE, BRUSH-BORDER FRACTION, AND BASOLATERAL MEMBRANE FRACTION FROM WHITE RAT SMALL INTESTINE EPITHELIAL CELLS

All values mean of two preparations. 1 unit sucrase hydrolyzes 1 μmol sucrose per min [13].

Fraction	Sucrase		$(Na^+ + K^+)$-ATPase		Protein (mg)
	total activity (units)	specific activity (units/mg protein)	total activity (μmol P_i/h)	specific activity (μmol P_i/h per mg protein)	
Homogenate	160	0.9	178	1.0	181
Brush border	54	11	4	0.8	5.2
Basolateral membrane	1.7	0.4	42	9.3	4.5

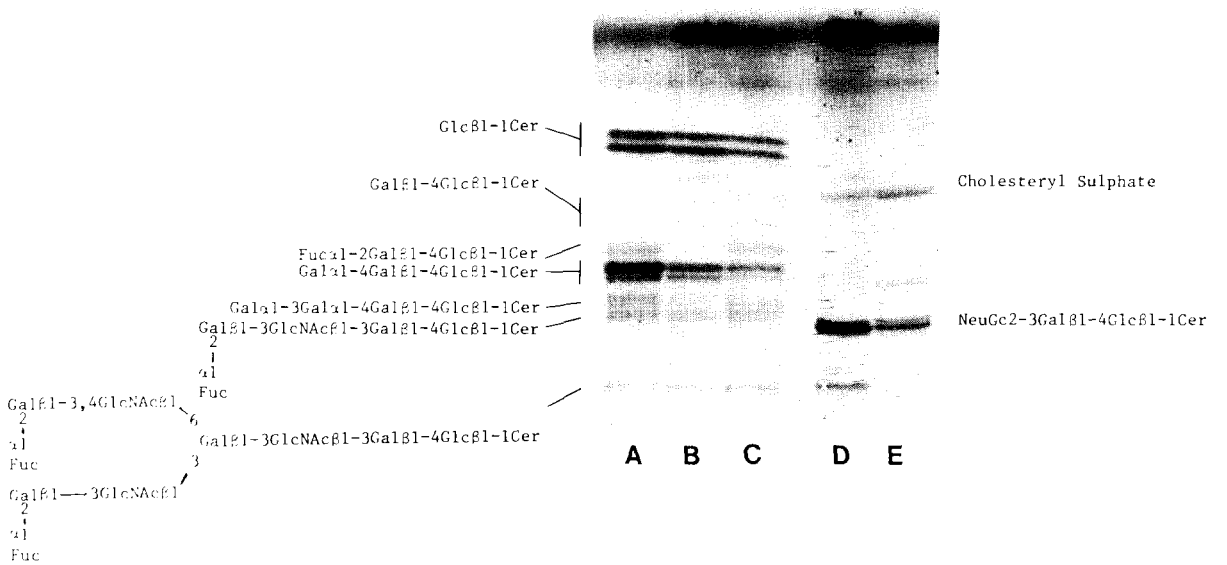

Fig. 1. Thin-layer chromatogram of the non-acid glycosphingolipids of the white rat from total epithelial cells (A), brush-border (B) and basolateral membrane (C). The acid alkali-stable lipids from the brush-border (D) and basolateral (E) membrane are shown on the right. The chemical structures of the bands are marked on the left and right. The amount of lipid applied for the brush-border and basolateral membrane corresponds to 0.4 mg protein for the non-acid and the 0.2 mg protein for the acid lanes. The solvent was chloroform/methanol/water (60:35:8, by vol.), and detection was done with the anisaldehyde reagent [17]. The two bands marked with a white cross were non-lipid contaminants.

Fig. 1) and the alkali-stable acidic lipids (right in Fig. 1) were prepared from the brush-border and basolateral membranes. The different glycolipids in the epithelial cells of white rat small intestine have been purified to homogeneity and structurally characterized [5]. Their structures are indicated for the various bands on the thin-layer chromatogram in Fig. 1. All lipids were found in both subcellular compartments. The brush-border membrane is slightly richer in glycosphingolipids (relative protein) than the basolateral membrane. This is more prominent for *N*-glycoloylneuraminosyllactosylceramide (haematoside), but also seen for glucosylceramide and globotriaosylceramide. The only structures having higher concentrations in the basolateral membrane are cholesteryl sulphate and fucosyllactosylceramide.

In another series of experiments for the black-white rat, a 'mitochondrial fraction' was obtained from the $2500 \times g$ supernatant (see above) by centrifugation at $12\,000 \times g$ for 15 min. The supernatant of the last run was subjected to another centrifugation at $25\,000 \times g$ for 15 min to obtain a so-called 'heavy microsomal' fraction. The total

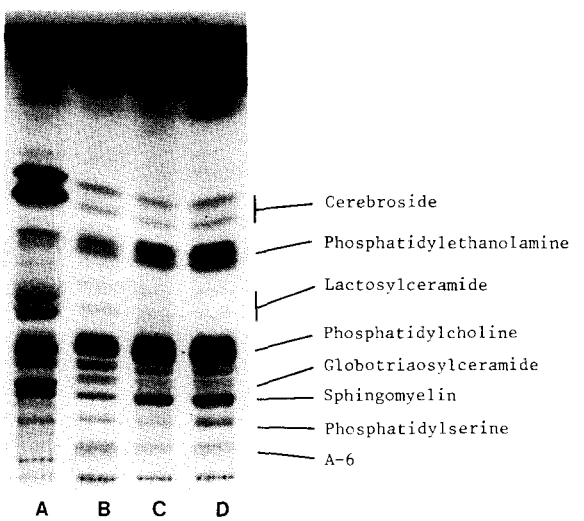

Fig. 2. Thin-layer chromatogram of total non-acid lipids from the black-white rat (before mild alkaline treatment); total lipid of human brain (A), brush border fraction (B), 'mitochondrial' fraction (C) and 'heavy microsomal' fraction (D). B–D correspond to 0.2 mg protein. The solvent was chloroform/methanol/water (65:25:4, by vol.) and detection with the anisaldehyde reagent [17]. A-6 means blood group A-active glycolipid with six sugars [5].

non-acid lipids (before mild alkaline treatment) of these fractions were analysed by thin-layer chromatography (Fig. 2). The 'mitochondrial' fraction is probably similar to the mitochondrial fraction of Lewis et al. [18]. The 'heavy microsomal' fraction has the highest specific activity for $(Na^+ + K^+)$-ATPase of these three fractions and thus is an enriched fraction of basolateral membrane. The brush-border fraction contained smaller amounts of the phospholipids per protein (phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine and sphingomyelin), especially when compared to the 'heavy microsomal' fraction, Fig. 2. This shows that the brush-border membrane contains less phospholipids than the basolateral membrane.

Cell surface labelling of the sialic acids on the luminal membrane of the epithelial cell was done by incubating the intact intestine with periodate followed by NaB^3H_4 reduction. The acid lipid fractions and the corresponding autoradiogram for two different experiments used are shown in Fig. 3. The only band seen on the autoradiogram with the shorter and less concentrated periodate treatment (right-hand gel B of Fig. 3) corresponds to labelled haematoside. The stronger labelling with the second protocol (right-hand gel A of Fig. 3) revealed additional bands probably due to minor unidentified gangliosides.

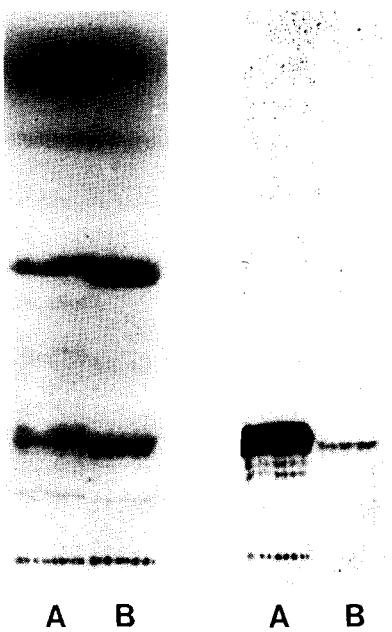

Fig. 3. Thin-layer chromatogram of acid lipids obtained after cell surface labelling of sialic acid (left) and corresponding autoradiogram (right). The intact small intestine was incubated with 2 mM $NaIO_4$ for 20 min in A and with 0.5 M $NaIO_4$ for 10 min in B at 0°C. The acid lipids applied on the thin-layer plate were 1/50 for the chromatogram revealed by the anisaldehyde reagent [17] shown on the left and 1/100 for A and 1/30 for B for the autoradiogram shown on the right. The solvent system was chloroform/methanol/water (60 : 45 : 8, by vol.).

The cell surface glycosphingolipids were also labelled by the galactose oxidase method [19]. The washed intact ileum was incubated with 10 units of galactose oxidase (Sigma) at 30°C for 45 min and reduced with NaB^3H_4. The non-acid glycolipids were prepared [6] and analysed by autoradiography as described above. No labelled glycolipid was detected. One explanation may be that the glycolipids are hidden below protecting glycoproteins and mucus.

From the results, it is evident that the brush-border membrane contains relatively small amounts of phospholipids and higher amounts of glycosphingolipids per protein. Forstner et al. [20] have estimated the enrichment of glycolipids in the brush-border membrane compared to total mucosa to be at least 4-fold. The sphingolipids are particularly plasma-membrane components [21] and a comparison of basolateral and brush-border membrane shows smaller differences. Brasitus and Schachter [22] have shown a 50% higher concentration of glycolipids and a 35% lower concentration of phospholipids in the brush-border membrane compared to the basolateral membrane. These results confirm the present results.

The haematoside was enriched in the brush-border membrane and was shown for the first time to be exposed at the lumen of the intestine. This glycolipid showed the highest increase in concentration during the epithelial cell migration from crypt to villus tip [6]. It was suggested that the increased sialylation could increase the cell-cell repulsion needed for cell extrusion at the villus tip [6]. The results shown here do not substantiate this suggestion, as one would expect a basolateral localization of haematoside according to this hypothesis. The non-acid glycosphingolipids do not show that much difference in concentration be-

tween the brush-border and basolateral membranes, except for fucosyllactosylceramide, which was enriched in the basolateral membrane.

The cholesteryl sulphate has a higher concentration in the basolateral membrane, the same subcellular site as the $(Na^+ + K^+)$-ATPase. The rat small intestine lacks sulphatide almost completely [5,23]. Sulphatide has been shown to have a stoichiometric relation to the $(Na^+ + K^+)$-ATPase in many tissues [21,24] and this lipid has been proposed to function to enrich K^+ at the cell surface [21]. The cholesteryl sulphate may have a similar function and replace sulphatide in this membrane.

By the present approach it is impossible to state that a certain glycolipid is found at only one surface membrane compartment and not at the other. A majority of the glycolipids are probably enriched in the brush-border membrane, where they cannot be involved directly in cell-cell interactions. However, the presence of a certain glycolipid in the luminal pool does not exclude the possibility that it may also be involved in cell-cell interaction phenomena at the basolateral membrane.

The enrichment of glycolipids at the brush border is interesting and may help to improve the cell membrane integrity by creating a more stable membrane [7,21]. The research interest in glycolipids at surfaces exposed to the body exterior has increased with the finding that glycolipids may be receptors for bacterial toxins [25] and bacteria [3,4]. The glycolipids may be considered to be involved in host interactions with normal and pathogenic bacteria.

The work was supported by grants from the Swedish Medical Research Council (No. 3967) and the Medical Faculty, University of Göteborg, Sweden.

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