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^{35}S-SULPHATE LABELLING OF GLYCOPROTEINS AND GLYCOLIPIDS IN RABBIT SMALL INTESTINAL BRUSH BORDERS

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Summary: Isolated brush borders become labelled within 7.5 hours following injection of $Na_2{}^{35}SO_4$ into rabbits. The label is present in glycoprotein (73%) and in an hydrophobic glycolipid fraction (24%). T.l.c. and DEAE-cellulose chromatography indicate the presence of one principal sulphated glycolipid in which the sugars are glucose, galactose and glucosamine (1:2:2). Sialic acid residues are absent from the glycoproteins and are found only in minor ganglioside components. Ester sulphate is considered to contribute significantly to the anionic character of the membrane.

Previous studies (1) have shown that the isolated small intestinal brush border in the rabbit is rich in both glycoproteins and glycolipids. Further studies showed that sialic acid is virtually absent from the glycoproteins and that sialic acid-bearing glycosphingolipids represent only a minor component (ca 10%) of the glycolipids (2). This observation is interesting because sialic acid is generally assumed to be ubiquitously present in mammalian plasma membranes (3). The present work considers evidence of sulphate ester groups being a source of the anionic character of these membranes.

Experimental

New Zealand white male rabbits (Hylyne Commercial Rabbits, Northwich, Cheshire) of 1.5 kg weight (6-8 weeks old) were injected intraperitoneally each with 100µCi of sodium ^{35}S-sulphate in 1 ml of distilled water and 7.5 hours later they were sacrificed by an intravenous injection of 1.5 ml of Nembutol (Pentobarbitone sodium B.P.; Abbot Laboratories, Queenborough, Kent). The small intestinal brush borders were then isolated and their glycoprotein, hydrophobic lipid and hydrophilic lipid fractions prepared as described previously (1).

DEAE-cellulose acetate column chromatography. Lipid solutions were separated into their acidic and neutral components by anion exchange

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chromatography. DEAE-cellulose acetate was prepared from DEAE-cellulose (Microgranular; Whatman Biochemicals Ltd., Maidstone, Kent, U.K.) by suspension overnight at room temperature in glacial acetic acid (3). The cellulose medium was washed free of acetic acid with methanol, packed in columns (5x0.6 cm) and equilibrated with chloroform-methanol (2:1). The lipid samples, dissolved in 1 ml of chloroform-methanol (2:1) were applied to the columns and eluted sequentially with 20 column volumes of chloroform-methanol (2:1), then 20 column-volumes of methanol, and finally 20 column-volumes of chloroform-methanol (2:1)- 50mM ammonium acetate. These fractions were collected separately and, where present, the ammonium acetate was removed by partitioning against 0.2 volumes of water and then 0.4 volumes of methanol-water (1:1). The organic solutions were concentrated under a stream of nitrogen and finally dissolved in 1 ml of chloroform-methanol (2:1).

Thin layer chromatography. Thin layer chromatographic separation of lipids on silica gel G using chloroform-methanol-2.5 Naq ammonia (60:35:8) and detection by iodine vapour was performed as described previously (2).

Protein Determinations. Samples (2-3 mg) were dissolved in 2 ml of 3% (w/v) aqueous sodium hydroxide on a boiling water bath for ten minutes and aliquots were used for the determination of protein as described by Lowry et al. (4).

Determination of Radioactivity. Measurements were made using a Beckman LS 200B liquid scintillation counter under conditions described previously (2).

Results and Discussion

The lipids and glycoproteins of rabbit small intestinal brush borders label significantly after the administration of $Na_2{}^{35}SO_4$ (Table 1). Approximately 73% of the incorporated label is present in the protein fraction and 27% in the lipids. (The lipid fraction was that extracted from the brush borders with chloroform-methanol while the insoluble residue was termed the protein fraction.) The lipid fraction was separated into two groups by partitioning in chloroform-aqueous solutions. The large majority of the ^{35}S incorporated into the overall brush border lipids is associated with the chloroform-soluble hydrophobic lipids (Table 1). Previous work on the lipid of the rabbit small intestinal brush border by Cooper and Kent (2) has shown that this hydrophobic fraction is rich in glycolipids, at least four being detectable by t.l.c. on silica gel G using chloroform-methanol-2.5 Naq ammonia (60:35:8) as the elutant. Thin layer chromatography

Table 1. Incorporation of label from ^{35}S-sulphate into the glycoproteins and glycolipids of the small intestinal brush border

Fraction	dpm/mg protein	% total incorporation in brush border
glycoprotein	4,600	73
hydrophobic lipids	1,500	24
hydrophilic lipids	190	3

of the ^{35}S-labelled hydrophobic lipids in this system shows one major and two minor radioactive areas (Fig. 1) while staining of the chromatogram with iodine shows the same four glycolipid bands described previously (2). The major radioactive component (Fig. 1B) corresponds to the chromatographically-fast acidic glycolipid (NL3) reported before (2). The anionic glycolipid (NL4) appears to be unlabelled in the present experiments. Of the minor components, the slowest moving radioactive component (Fig. 1. component A) co-chromatographs with sodium taurocholate and the chromatographically-fast radioactive component (Fig. 1C) is as yet unidentified but does not correspond to any of the glycolipid components of the previous work (2).

The hydrophobic ^{35}S-labelled lipids could also be resolved into their neutral and acidic components by chromatography on DEAE-cellulose. The neutral lipids are not retained by the column and are eluted by chloroform-methanol (2:1) and by methanol. The column retained 92% of the applied radioactivity and this was almost quantitatively recovered and acidic glycolipids eluted by chloroform-methanol (2:1) containing 50mM ammonium acetate (Table 2). The

Table 2. Recovery of ^{35}S following chromatography of the ^{35}S-sulphate-labelled hydrophobic glycolipids on DEAE-cellulose

Fraction	Elutant	dpm	% of recovered counts
neutral glycolipids NL1, NL2	chloroform-methanol (2:1)	792	2.65
	methanol	180	0.58
anionic glycolipids NL3, NL4	chloroform-methanol (2:1) containing 50mM ammonium acetate	29,960	96.85

Recovery from column, 92% of applied counts

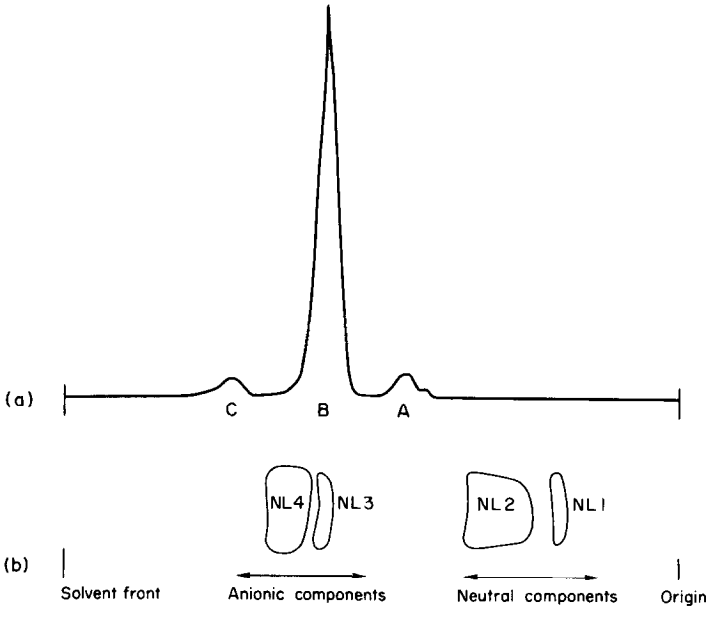

Fig. 1 CHROMATOGRAPHIC SEPARATION (t. l. c.) OF TOTAL ^{35}S-SULPHATED LABELLED BRUSH BORDER GLYCOLIPIDS (a) RADIOACTIVE SCAN (b) IODINE VAPOUR STAINED

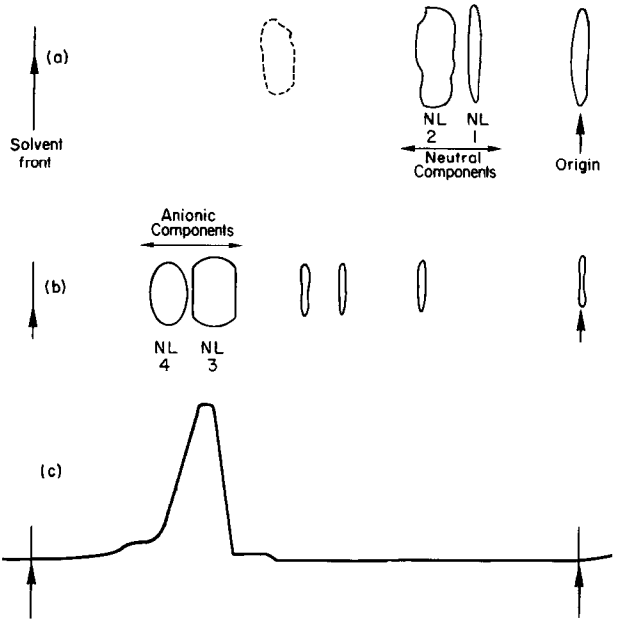

Fig. 2 CHROMATOGRAPHIC ANALYSIS (t.l.c.) OF NEUTRAL AND ANIONIC GLYCOLIPIDS AFTER DEAE-CELLULOSE SEPARATION.
(a) NEUTRAL GLYCOLIPIDS (I_2^- STAINED)
(b) ANIONIC GLYCOLIPIDS (I_2^- STAINED)
(c) ANIONIC GLYCOLIPIDS (RADIOACTIVE SCAN)

chloroform-methanol (2:1) and methanol fractions containing neutral fractions were pooled. The neutral and acidic fractions were then chromatographed (t.l.c.) on silica gel G using chloroform-methanol-2.5 Naq ammonia (60:35:8) as the elutant (Fig. 2). These results show that both of the chromatographically-fast glycolipids Nl3 and NL4 are retained by the DEAE-cellulose column and that the two chromatographically slow glycolipids NL1 and 2 are not. The major radioactive component chromatographed with NL3 provides further evidence that NL3 carries a sulphated label.

As has been found previously (2), two principal neutral glycolipids are present, NL1 containing glucose, galactose and glucosamine (1:2:1) and NL2 containing glucose and galactose (1:2).

Of the anionic glycolipids, NL4 is not radioactively labelled under the conditions used and contains glucose and galactose (1:1). The component NL3 containing glucose, galactose and glucosamine (1:2:2) on the other hand becomes substantially labelled as a sulphated derivative. In none of these hydrophobic or hydrophilic glycolipids isolated from brush border was there evidence of sialic acid residues, though the possibility exists of some loss during isolation.

The results strongly indicate that sulphate ester is present in both glycoprotein and glycolipid components of the brush border and that this is the major contributor to the anionic character of the membrane.

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