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RABBIT SMALL INTESTINAL BRUSH BORDER MEMBRANE

PREPARATION AND LIPID COMPOSITION

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Summary

1. Rabbit intestinal brush border membrane vesicles were prepared either from frozen or fresh tissue and the lipid composition was analysed.

2. Lipids extracted from membranes prepared by the Ca^{2+}-precipitation method (Schmitz, J., Preiser, H., Maestracci, D., Ghosh, B.K., Cerda, J.J. and Crane, R.K. (1973) Biochim. Biophys. Acta 323, 98—112; Kessler, M., Acuto, O., Storelli, C., Murer, H., Müller, M. and Semenza, G. (1978) Biochim. Biophys. Acta 506, 136—154) had exceptionally high levels of lysophospholipids and free fatty acids. An intrinsic, Ca^{2+}-activated phospholipase A is responsible for the lipid decomposition.

3. It was necessary to modify the preparation of brush border membranes. Essentially, EGTA is used to keep the free Ca^{2+} concentration low so that phospholipases are inactivated, and Mg^{2+} instead of Ca^{2+} is employed to aggregate selectively contaminating membranes. The modified procedure gives membranes suitable for lipid analysis.

4. The molar ratio of neutral, phospholipid and glycolipid is about 1 : 1 : 1. The major neutral lipids are free cholesterol and fatty acids. The major phospholipids are phosphocholine-containing (approx. 45%) and phosphatidylethanolamine (approx. 40%). The remainder (15—20%) is made up of acidic phospholipids, mainly phosphatidylserine and phosphatidylinositol. The major glycolipid is ceramide monohexoside.

Abbreviations: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N'*-tetraacetic acid.

5. The major fatty acids of total lipids and phospholipids are palmitic, stearic, oleic and linoleic acids. Individual phospholipids are characterised by distinct fatty acid compositions and differ markedly in the ratio of unsaturated-to-saturated fatty acid.

Introduction

Work in the past has established that the brush border membrane is involved in both digestion and absorption of nutrients. As a result of this dual function, the membrane contains a number of hydrolytic enzymes [1—4] and transport (carrier) systems [5]. A general question of prime importance is the structure-function relationships in this membrane. A first step towards this final goal is the analysis and characterization of the main components of the membrane, lipid and protein. Here we describe the lipids contained in rabbit intestinal brush border membrane. This information is essential for an understanding of the fluidity of the lipid bilayer part of the membrane and of how the lipid environment controls the function of membrane-bound enzymes and transport systems.

The method which we used for the preparation of rabbit intestinal brush border membranes is based on the Ca^{2+}-precipitation method originally introduced by Schmitz et al. [6] and recently modified by Kessler et al. [7]. It gives reproducible brush border membrane vesicles which are essentially free of contamination by basolateral plasma membranes and other subcellular components and which, therefore, are suitable for a more detailed examination of the lipid composition. However, a modification of the preparation was necessary to prevent extensive decomposition of phospholipids by highly active membrane-bound phospholipases [8]. The need to control these intrinsic phospholipases is obvious from previous work on the lipid composition of brush border membranes of different origin [9—12]: high levels of both free fatty acids and lysophospholipids indicative of lipolytic activity have been reported.

Materials and Methods

Preparation of brush border membrane vesicles. Rabbit brush border membranes were prepared either from freshly removed small intestine or tissue kept frozen at −20°C, following the procedure of Refs. 6 and 7 or the modified MgEGTA procedure described in Results. Routinely, 25 g of frozen small intestine processed as described in Ref. 7 were used as the starting material. If fresh small intestine was used, the animal fed a normal diet was killed by a gun shot at the base of the skull, the small intestine removed immediately, rinsed with 0.15 M NaCl and cut with scissors into 1 g pieces. The fragmented intestine (34 g wet wt.) was immersed in 60 ml of 12 mM Tris-HCl buffer, pH 7.1, containing 300 mM D-mannitol and the preparation continued as described in Ref. 7 or in Results. Rat brush border membrane vesicles were prepared according to Ref. 13 starting from small intestine freshly removed from six to ten rats.

Extraction of lipids. Brush border membrane vesicles obtained from 25 g frozen tissue were dispersed in 2 ml buffer (2 mM Tris-HCl, pH 7.1, 5 mM EGTA, 50 mM D-mannitol). Lipids were extracted from this suspension by blending with 7.5 vols. $CHCl_3/CH_3OH$ (2 : 1, v/v) and keeping at 40°C for 1 h. The mixture was centrifuged at 48 000 × *g* for 15 min and the lower $CHCl_3$-rich phase was collected. The pellet was re-extracted with 15 ml $CHCl_3/CH_3OH$ (2 : 1, v/v) as described above, and the combined extracts were washed twice with 0.2 vol. 0.15 M KCl and evaporated in a rotary evaporator. The residue of total lipids thus obtained amounted to 14.3 ± 1.5 mg (from 25 g starting material) and was dissolved in 5 ml $CHCl_3/CH_3OH$ (2 : 1, v/v). Further extractions of the pellet with CH_3Cl/CH_3OH (2 : 1, v/v) did not yield any more lipid. Alternatively, lipids were extracted according to the procedure of Bligh and Dyer [14].

Characterisation of brush border membrane vesicles. Each preparation of brush border membrane vesicles was characterised by determining the lipid and protein content, the sucrase activity and K^+-stimulated phosphatase activity, the D-glucose transport and the electrophoretic mobility. The total lipid content was determined by lipid extraction as described above and dry weight analysis. The total amount of phospholipids in the lipid extract was determined by phosphorus analysis [15]. Protein determination was carried out by using the method of Lowry et al. [16] using crystalline bovine serum albumin as the standard. Sucrase and K^+-stimulated phosphatase were measured according to Refs. 17 and 18, respectively. The uptake of D-glucose by brush border membrane vesicles was measured as described in Refs. 7 and 13. For uptake measurements, vesicles prepared by Ca^{2+} precipitation [6,7] and equilibrated in 10 mM Tris-Hepes, pH 7.5, containing 50 mM D-mannitol were incubated in the same buffer containing 0.06—1 mM D-[1-^{3}H]glucose and 0.1 M NaSCN ($[Na^+]$ = 0.1 M outside, $[Na^+]$ = 0 inside). If brush border membrane vesicles were prepared by Mg^{2+} precipitation in the presence of EGTA (see Results), the suspension in 10 mM Tris-Hepes buffer, pH 7.5, containing 50 mM D-mannitol was diluted 25-fold with the same buffer containing 20 mM choline chloride. After incubation for 60 min at room temperature, the membranes were spun down and resuspended in the same buffer (without choline chloride). D-Glucose uptake was measured after adding D-[1-^{3}H]glucose (0.06 - 1 mM) and NaSCN (0.1 M).

Electrophoretic mobilities (ζ-potentials) of brush border membrane vesicles in 2 mM Tris-HCl buffer (pH 7.1) + 50 mM D-mannitol with or without 5 mM EGTA were measured at 25 ± 1°C in a particle electrophoresis apparatus (Mk II, from Rank Brothers, Cambridge, U.K.) (cf. Ref. 19).

Separation and estimation of phospholipids and neutral lipids. Phospholipids extracted as described above were separated either by single-dimensional thin-layer chromatography (TLC) on silica gel 60 F/254 plates (from Merck) using $CHCl_3/CH_3OH/H_2O$/acetic acid (65 : 50 : 4 : 1, v/v) or two-dimensional TLC as described by Bowyer and King [20]. Lipids were made visible by exposing the TLC plate to I_2 vapour and phospholipids by spraying to reveal phosphorus [21]. They were identified by comparison with known standards. Phospholipid spots were scraped into Pyrex test tubes, digested with perchloric acid, diluted with H_2O and analysed for phosphorus after removal of the silica

gel by centrifugation [15]. Neutral lipids were separated and estimated by two-dimensional TLC as described in Ref. 20.

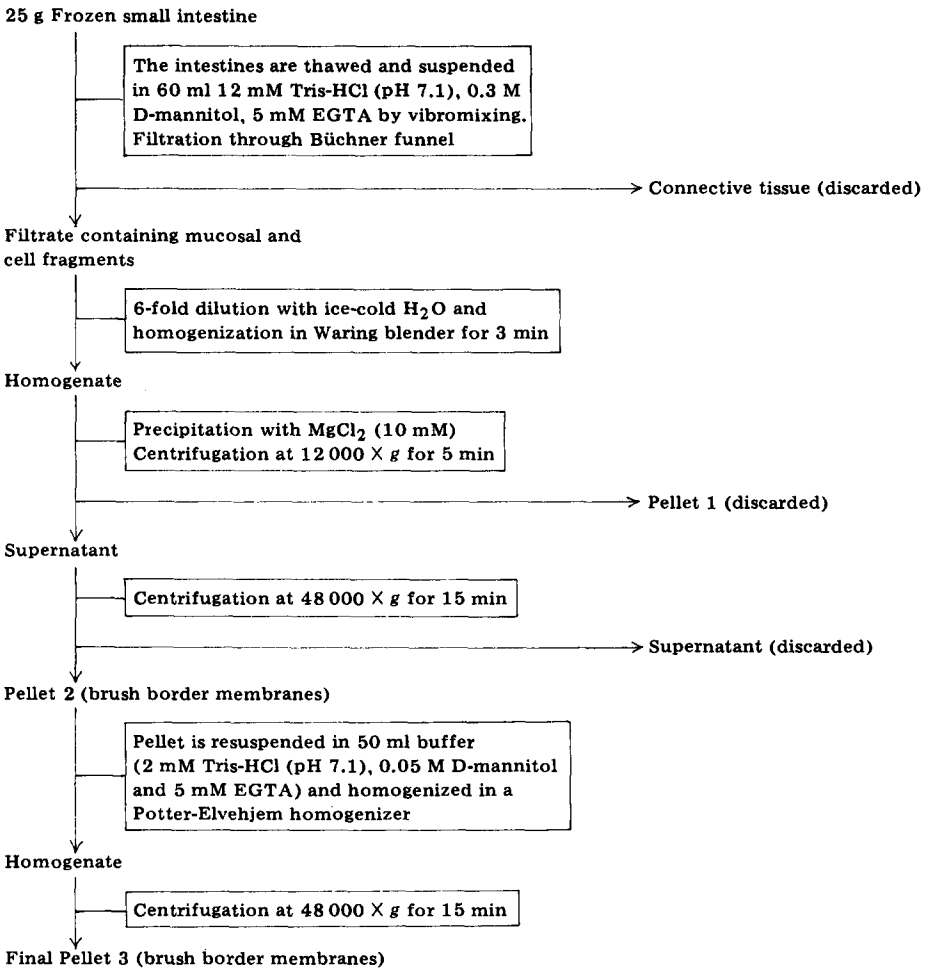
The composition of the phospholipids was examined in more detail by using chemical degradation techniques as described by Dawson [22]. In this procedure, acylated phospholipids were degraded to water-soluble intermediaries by using alkaline alcoholysis. The residual phospholipids were then catalytically hydrolysed using $HgCl_2$ to break down the plasmalogens, leaving the sphingomyelin and glycerol ether phospholipids which can be separated by TLC.

For the fatty acid analysis, lipids separated by single-dimensional TLC were scraped off and refluxed for 1 h with 6% KOH in 95% ethanol. After cooling, the alkaline digest was washed with petroleum ether (40–60°C) to remove non-saponified lipids. The aqueous phase was then acidified with HCl and the free fatty acids extracted into petroleum ether. They were then converted to their methyl esters by reacting them with diazomethane. The methyl esters were analysed using a Pye Series 104 gas chromatograph together with a column of 10% poly(ethylene glycol adipate) absorbed on diatomite CQ. The column was run at 18°C with a gas flow of 60 ml/min argon.

Results

Preparation of brush border membrane vesicles

The preparation of brush border membranes by the procedure described in Refs. 6 and 7 is based upon the use of $CaCl_2$ to aggregate nuclei, microsomes, mitochondria and basolateral membranes so that they can be readily separated from brush border membranes by centrifugation at low speed. The brush border membrane vesicles thus obtained are essentially free of contamination by other membranes. The characterisation of these vesicles by monitoring the activity of marker enzymes and D-glucose transport gave results consistent with those reported by Kessler et al. [7]. The average lipid-to-protein weight ratio was 0.5 (approx. 30% lipid; cf. Table I). However, when the lipid extract of brush border membranes prepared by the Ca^{2+}-precipitation method was analysed, exceptionally high levels of lysophospholipids and free fatty acids were found, for instance, the lysophosphatidylcholine and lysophosphatidylethanolamine content amounted to 16 ± 2% and 26 ± 2% of the total lipid phosphorus, respectively. This level of lysocompounds far exceeds the trace concentrations of these cytotoxic and lytic substances usually found in biological membranes, suggesting that substantial lipid decomposition had occurred. From this it is clear that the determination of the lipid composition of brush border membrane prepared by Ca^{2+} precipitation is unsatisfactory. On the assumption that an intrinsic Ca^{2+}-activated phospholipase A was responsible for the lipid decomposition [8], a number of variations of the preparation were tried. Reducing the incubation time with $CaCl_2$ and shortening the centrifugation time by using increased *g* values were ineffective. There was also no difference in lysophospholipid content, regardless of whether fresh or frozen small intestine was used as the starting material. This observation indicates that during storage of the small intestine at temperatures equal to or less than −20°C, the rate of lipid hydrolysis is negligible. $MgCl_2$ was found to be a good replacement for $CaCl_2$ in precipitating membranes apart from the brush borders



Scheme I. Flow diagram of the procedure used to prepare brush border membranes for lipid analysis.

[29] while $LaCl_3$ precipitated indiscriminately. The use of Mg^{2+} instead of Ca^{2+} together with the addition of EDTA (approx. 20 mM) to all buffer solutions used after the precipitation (removal) of contaminating membranes did reduce the level of lysophospholipid but was only partly effective, probably due to Ca^{2+} bound to brush border membranes. When brush border membranes spun down at 27 000 × *g* from the supernatant of the Ca^{2+} precipitate were resuspended in 12 mM Tris-HCl buffer, pH 7.1, containing 20 mM EDTA and boiled for 5 min, lipid degradation could not be prevented.

The modified procedure eventually adopted to prepare brush border membranes suitable for lipid analysis is summarized in the flow-diagram of Scheme I. Frozen small intestine (25 g) was cut into small pieces (approx. 1 g) which were thawed in 60 ml buffer (12 mM Tris-HCl, pH 7.1, 0.3 M D-mannitol, 5 mM EGTA) for 30 min. The suspension at 2°C was vibrated for 90 s with a Vibromixer (100 Hz) and filtered through a Büchner funnel with 1-mm holes. The filtrate was diluted with 300 ml ice-cold water and homogenized

for 3 min in a Waring blender. $MgCl_2$ was added to give a final concentration of 10 mM and the blending continued for 1 min. The suspension was centrifuged at 12 000 × *g* for 5 min. The pellet containing nuclei and various contaminating membranes and cell debris was discarded. The supernatant was centrifuged at 48 000 × *g* for 15 min. The pellet of brush border membranes was resuspended in 50 ml buffer (2 mM Tris-HCl buffer, pH 7.1, 0.05 M D-mannitol and 5 mM EGTA) and homogenized in a Potter-Elvehjem homogenizer (10 strokes). The homogenate was centrifuged at 48 000 × *g* for 15 min and the pellet resuspended in the same buffer.

Characterisation of brush border membrane vesicles prepared by the MgEGTA procedure. Compared to all other preparative procedures tested, the one outlined above (cf. Scheme I) appeared to produce brush border membrane suitable for quantitative lipid analysis. The lysophosphatidylcholine content in the lipid extract of that preparation was minimal as compared to lipid extracts from previous membrane preparations, particularly those involving the addition of Ca^{2+} as precipitating agent. The lysophosphatidylcholine content in lipids extracted from fresh membranes prepared as described above was 1.8% ± 0.9 (mean ± S.D. of ten measurements). When brush border membranes were stored for more than 24 h at room temperature before lipid extraction, the lysophospholipid content exceeded the above value significantly. The lipid and protein yield and some characteristic properties of brush border membranes prepared as described above are summarized in Table I. Both methods of extraction gave similar results.

As observed with brush border membranes of other sources [10,11], glycolipids make up a large proportion. The specific activity of sucrase was similar to that of brush border membranes prepared by Ca^{2+} precipitation [7]; however, the specific activity of K^+-stimulated phosphatase, which is a marker enzyme for contaminating basolateral membrane, was clearly below the level of 19 mU/mg protein usually found in Ca^{2+}-precipitated preparations [7]. This indicates that brush border membranes prepared as described here are less contaminated with basolateral membranes.

The ability of the Mg^{2+}-precipitated vesicles to accumulate D-glucose against

TABLE I

CHARACTERISATION OF BRUSH BORDER MEMBRANE VESICLES PREPARED AS DESCRIBED IN SCHEME I

Values are the mean ± S.D. of 10—12 experiments.

Total lipid (mg/mg protein)	0.5 ± 0.15
Lipid phosphorus * (μg/mg protein)	8 ± 2 (range 6—12)
Cholesterol (μg/mg protein)	50 ± 5
Neutral lipid/phospholipid/glycolipid (molar ratio)	1 : 1.2 : 1.1
Phospholipid/monohexoside (molar ratio)	2.2 ± 0.2
Specific activity sucrase	1.7 ± 0.7 units/mg protein, range 0.8—2.5 units/mg
K^+-stimulated phosphatase	8 ± 3 mU/mg protein, range 6—12 mU/mg
Electrophoretic mobility $cm^2/V \cdot s$	$-1.6 \pm 0.15 \times 10^{-4}$, range -1.4 to $-1.8 \cdot 10^{-4}$

* From the average phospholipid composition (Table II and the fatty acid composition (Table IV)), the effective phospholipid molecular weight was calculated as 720. Using this value, the total phospholipid content is 0.19 ± 0.05 mg/mg protein (range 0.14—0.28 mg/mg protein). The large standard deviation and range obtained for the lipid content when referred to protein are due to variations in the protein content of different preparations.

a concentration gradient was similar to Ca^{2+}-precipitated vesicles [7]. In the presence of an NaSCN gradient ($[NaSCN]_{outside} = 0.1$ M, $[NaSCN]_{inside} = 0$), the transient accumulation of D-glucose at its maximum led to an internal D-glucose concentration approx. 30-times larger than the external one. This is consistent with the D-glucose uptake into vesicles prepared by Ca^{2+} precipitation [23,24]. For the Na^+ gradient to be fully effective, it was necessary to incubate 'MgEGTA' membranes with excess choline chloride. This treatment ensured the removal, from the internal vesicle space, of Na^+ resulting from the EGTA in the buffer.

The average electrophoretic mobilities of brush border membrane vesicles prepared by the new method and dispersed in 2 mM Tris-HCl (pH 7.1), 50 mM D-mannitol in the presence and absence of 5 mM EGTA were $(-1.7 \pm 0.15) \cdot 10^{-4}$ cm^2/V · s (range -1.4 to $-1.9 \cdot 10^{-4}$ cm^2/V · s) and $(-1.6 \pm 0.2) \cdot 10^{-4}$ cm^2/V · s (range -1.4 to $1.8 \cdot 10^{-4}$ cm^2/V · s), respectively. These values are practically equal to that measured for brush border membrane vesicles prepared by Ca^{2+} precipitation.

The phospholipid composition as determined by techniques given in Materials and Methods is given in Table II. The major phospholipids in both rabbit and rat brush border membrane are choline- and ethanolamine-containing. The former, consisting largely of phosphatidylcholine and sphingomyelin, make up about 45% (40% in rat brush border membrane) of the total lipid phosphorus, the latter about 38--40%. As significant proportion (approx. 8--9%) of phosphatidylethanolamine is present in the plasmalogen form (only determined for rabbit brush border membrane). The remainder of the phospholipids

TABLE II

PHOSPHOLIPID COMPOSITION OF RABBIT AND RAT BRUSH BORDER MEMBRANE

Results are expressed as % of total lipid phosphorus and are presented as the mean ± S.D. of 12 experiments. n.d., not determined. (Column 1) A possible explanation for the higher lysophospholipid content found by two-dimensional TLC is that in this case the lipid extracts in $CHCl_3/CH_3OH$ (2 : 1, v/v) were subjected to a Folch-wash with 0.2 vol. of 0.025 M $CaCl_2$. Samples analysed by one-dimensional TLC were Folch-washed with 0.2 vol. 0.15 M NaCl. Data in column 2 were corrected assuming that the inherent lysophospholipid content is that determined immediately by one-dimensional TLC.

Phospholipid	Rabbit				Rat
	Two-dimensional TLC (1)		Two-dimensional TLC (after correction) (2)	One-dimensional TLC (3)	One-dimensional TLC (4)
Phosphatidic acid	1.2 ± 0.5		1.2	n.d.	n.d.
Phosphatidylethanolamine	23.3 ± 1.4				
Ethanolamine plasmalogen	8.5 ± 1	35.0	35.6	35.2 ± 3	38.3 ± 2
Alkylacylglycerophosphoethanolamine	3.2 ± 1.5				
Phosphatidylserine	7.5 ± 0.8		7.4		
		11.1		9.8 ± 2	13.0 ± 2
Lysophosphatidylethanolamine	3.6 ± 1		2.3	(2.3)	
Phosphatidylcholine	27.3 ± 1.7		33.3	32.1 ± 3	32.6 ± 3
Alkyl and alkenylglycerophosphocholine	3.0 ± 1.7	30.3			
Lysophosphatidylcholine	3.1 ± 1.5		1.8	1.8 ± 1	1.0 ± 0.5
Phosphatidylinositol	8.3 ± 1		8.2	9.5 ± 1	8.7 ± 1
Sphingomyelin	10.5 ± 0.4		10.3	11.2 ± 1.6	6.4 ± 1

TABLE III

NEUTRAL LIPIDS OF RABBIT SMALL INTESTINAL BRUSH BORDER MEMBRANES

Results are given as mol% and molar ratios of neutral lipid-to-phospholipid as mean ±S.D.

Lipid	mol%	Molar ratio neutral lipid-to-phospholipid
Cholesterol	58.0	0.50 ± 0.03 range 0.48—0.53
Cholesterol ester	≤1.0	0.01 ± 0.01
Diglyceride	—	0.01 ± 0.02
Triglyceride	≤2.0	0.03 ± 0.04
Fatty acid	40.0	0.35 ± 0.12 range 0.27—0.43

(approx. 15—20%) consists of acidic phospholipids, mainly phosphatidylinositol (8—10%) and phosphatidylserine (7—8%).

The phospholipid analysis by one-dimensional and two-dimensional TLC differs mainly in the content of lysophospholipids. The larger content of these lipids in the two-dimensional TLC analysis (column 1, Table II) is probably due to a preliminary Folch-washing of the lipid extract in $CHCl_3/CH_3OH$ before TLC with 0.025 M $CaCl_2$. This might activate any residual phospholipase A present in the lipid extract. Lysophosphatidylethanolamine comigrates with phosphatidylserine on one-dimensional TLC. With the value for phosphatidylserine of 7.5% obtained from two-dimensional TLC, an estimate for lysophosphatidylethanolamine of 2.3% is derived from one-dimensional TLC (column 3 of Table II; the value is given in brackets to indicate that it was not determined directly). Correcting the values in column 1 of Table II to the lysophospholipid content of column 3 gives the values of column 2. For

TABLE IV

FATTY ACID COMPOSITION OF RABBIT INTESTINAL BRUSH BORDER MEMBRANE LIPIDS AND VARIOUS PHOSPHOLIPID FRACTIONS

Values represent the mean of duplicate experiments. Several minor components contributing 1% or less are not given.

Fatty acid	Total lipids	Phosphoglycerides		Sphingomyelin	Phosphatidyl inositol	Phosphatidylserine and lysophosphatidyl ethanolamine
		Choline	Ethanolamine			
16 : 0	26.3	42.6	6.9	36.1	9.3	4.4
16 : 1	3.4	—	1.1	—	1.7	1.6
18 : 0	16.9	16.8	33.1	9.1	33.0	35.8
18 : 1	16.1	9.6	19.7	—	20.5	23.3
18 : 2	24.4	16.2	27.2	—	15.0	12.3
18 : 3	3.7	1.1	2.5	—	—	1.0
Polyunsaturated long chain fatty acids (mainly C20, C22)	3.5	12.0	6.7	28.8	16.1	19.4
26 : 0	—	—	—	17.4	—	—
Weight ratio unsaturated to saturated fatty acid	1.09	0.65	1.45	1.00	1.30	1.40

comparison, the phospholipid composition of rat intestinal brush border membrane was determined (column 4, Table II).

The composition of neutral lipids, expressed as molar ratios referred to phospholipid phosphorus, is summarized in Table III. The main components of the neutral lipid fraction are cholesterol and free fatty acids (58 ± 2% and 40 ± 6%, respectively). Triglycerides and cholesterol esters are present in trace quantities. The molar (weight) ratio of cholesterol-to-phospholipid is 0.50 ± 0.03 (wt. ratio 0.28 ± 0.02).

The major fatty acids of the total lipids and of the major phospholipid fractions of rabbit brush border membrane were palmitic, stearic, oleic and linoleic acids (Table IV). Sphingomyelin and the acidic phospholipids, phosphatidylinositol and phosphatidylserine, contain a large proportion of polyunsaturated, long-chain fatty acids with 20 and 22 carbon atoms. It is noteworthy that individual phospholipids have distinct fatty acid compositions (Table IV). Table IV also shows that the ratio of unsaturated-to-saturated fatty acid is highest for ethanolamine-containing phospholipids followed by the acidic phospholipids.

Discussion

In general, our lipid analysis agrees with those previously presented for intestinal brush border membranes of other species [10–12]. The major neutral lipids are cholesterol and fatty acid. The high cholesterol content is typical for plasma membranes, but the molar ratio of cholesterol-to-phospholipid found in the rabbit appears to be lower than that of 1.26, 1.0 and 0.73 reported for the rat [10], mouse [11] and guinea-pig (Coleman, R., personal communication) intestinal brush border membranes, respectively. In spite of the measures taken to prevent phospholipid hydrolysis, large quantities of free fatty acid are present. Similar high levels of fatty acids have been reported for brush border membranes from the mouse [11], rat [10] and hamster [9]. The origin of these fatty acids is unknown.

Glycosphingolipids make up a significant proportion of the total lipids. The major components of the glycosphingolipids are monohexose ceramides (about 50–70%) (Table I). Breimer et al. [30,31] have reported that glycosphingolipids of rabbit small intestine consist of ceramides with one to seven sugar residues. A detailed analysis of the glycolipid fraction of rabbit brush border membrane is in progress and will be the subject of a separate report.

The phospholipid analysis is also similar to those previously presented for mouse [11], rat [10] and chicken [12]. No ethanolamine plasmalogen had previously been detected in rat brush border membrane [10], but more recent studies on guinea-pig (Coleman, R., personal communication) gave a value of 7%, similar to our value of 8.5%. Phosphatidylinositol is a major component of the acidic phospholipids. While Forstner et al. [10] were unable to detect phosphatidylinositol in rat brush border membranes, the values found for mouse (9–11%, Ref. 11) and guinea-pig (12.6%, Coleman, R., personal communication) are similar to our values for rabbit and rat. It is worth mentioning in this connection that intestinal mucosa contains a very active Ca^{2+}-requiring phosphodiesterase which is specific for phosphatidylinositol [25].

In brush border membranes prepared by Ca^{2+} precipitation, the free fatty acid content is exceptionally high and, not surprisingly, lysophospholipids are substantial components present at levels exceeding by far the usual low concentrations of these compounds. This finding suggests that much decomposition had occurred. High levels of lysophosphatidylcholine have been reported for other intestinal brush border membranes [11,12]. The intestinal mucosa is one of the richest sources of phospholipases [26]; both phospholipase A_2 and B are present [8,27] whereby phospholipase A_2 has been shown to have the highest specific activity in the brush border membrane [8]. Both enzymes can be activated by fatty acid [8,28] so that if some fatty acid is produced during the isolation of brush border membranes the decomposition of phospholipids can become autocatalytic. A further disadvantage with respect to the lipid analysis is that the phospholipase activity in a mucosal homogenate [9] appears to continue both at low and high temperatures.

It is unlikely that lysophospholipids and fatty acids found in excessive amounts in Ca^{2+}-precipitated preparations are genuine membrane components. Brush border membrane vesicles prepared by the Ca^{2+} method are sealed and intact as inferred from the D-glucose transport against a concentration gradient. It is unlikely that the structural integrity of the membrane is preserved in the presence of large quantities of lysolecithin. One possible explanation is that the integrity of the membrane controls the phospholipase activity and only when the membrane is disintegrated by the $CHCl_3/CH_3OH$ (2 : 1, v/v) extraction is the phospholipase activated. The amount of Ca^{2+} required for the activation seems to be always present in brush border membranes, presumably as membrane-bound Ca^{2+}.

The following modifications in the preparation of brush border membranes were necessary to reduce lipid degradation to a minimum. Firstly, the presence of 5 mM EGTA in all buffers ensures that the concentration of free Ca^{2+} is low, preventing the activation of phospholipases. Secondly, in the presence of EGTA, Mg^{2+} appears to be as effective as Ca^{2+} in the selective precipitation of all other membranes apart from brush borders (cf. Ref. 29).

In conclusion, the method of preparation we used gives brush border membranes suitable for lipid analysis: (i) the phospholipase activity of the membrane is kept under control (Tabel II), and (ii) the contamination with subcellular membranes is negligible. The contamination by small amounts of basolateral membrane cannot be ruled out at present. However, considering the error of our lipid analysis, contamination of the brush border membrane by a few percent basolateral membrane will not seriously affect the data presented.

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