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COMPARATIVE CHROMATOGRAPHIC STUDY OF MODIFICATIONS OF BRUSH-BORDER MEMBRANE VESICLES INDUCED BY AN ESSENTIAL FATTY ACID-DEFICIENT DIET

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SUMMARY

The surface properties of small intestine brush-border membranes (BBMs) were examined by frontal affinity chromatography using three types of unsolubilized ligands: phlorizin polymer, immobilized lectins and linolenic acid bound to agarose gel. BBM vesicles were purified from piglets fed a corn oil diet (control diet) or a hydrogenated coconut oil diet. The second diet was representative of a deficient supply of essential polyunsaturated fatty acid (EPUFA). It induced a marked decrease in 18:2$n-6$ content in membrane choline phosphoglycerides and ethanolamine phosphoglycerides, whereas 20:3$n-9$ appeared in each class of phospholipids. Control and EPUFA-deprived BBM vesicles bound to phlorizin polymer, linolenic acid–agarose and wheat germ agglutinin (WGA) gel. In contrast, concanavalin A gel and *Lens culinaris* A gel exhibited a low binding capacity towards the two types of vesicles. EPUFA deficiency induced a slight decrease in binding on phlorizin polymer and a marked increase in binding on WGA gel, whereas the two types of vesicles similarly bound to linolenic acid–agarose. Desorption of phlorizin polymer-bound membranes was performed using several detergents with special regard to sodium deoxycholate (NaDOC) micelles. Sucrase activity recovery showed that the efficiency of NaDOC desorption was diminished in the case of EPUFA-deprived vesicles. EPUFA-deprived membrane domains involved in the binding would be less sensitive to the detergent attack. This assumption agrees with the putative decrease in membrane fluidity induced by the deficient diet. The possibility that fatty acid compositional changes induced by dietary lipids are extensive enough to alter some chromatographic properties of BBM vesicles is discussed.

INTRODUCTION

It has been well established that diet lipids are susceptible to modifying the membrane fatty acid composition, which is in turn correlated with alterations of

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numerous cellular functions. Many of these functional responses probably originate from dynamic and static structural changes of the lipid matrix in which functional membrane proteins such as enzymes, receptors or carriers are closely embedded (reviewed in refs. 1 and 2). According to the general meaning of $(n-9)$ trienoic acid appearance, it has been shown recently that a deficiency of essential polyunsaturated fatty acids (EPUFAs) in the diet induced an increase in the $20{:}3n-9/20{:}4n-6$ ratio in phospholipid fatty acids of the brush-border membrane (BBM) of piglet enterocyte [3]. In addition, increasing order of the BBM matrix structure and increasing mobility (or accessibility) of a spin-label bonded to the membrane–protein surface were observed with the EPUFA-deprived diet [4]. In order to assess the putative diet-induced modifications of the general properties of BBM surface components, a biochemical approach was sought. The behaviours of control and EPUFA-deprived BBM vesicles were compared in frontal affinity chromatography using three types of sorbent. Phlorizin polymer, linolenic acid–agarose and Ultrogel™ coupled with lectins were screened as potential unsolubilized ligands of glucose carrier, fatty acid binding membrane protein and lectin receptors, respectively. Suitable conditions were established to set up a general procedure for the chromatography of undamaged membranes and special emphasis was placed on the use of phlorizin polymer in the frontal chromatography of BBM dilute vesicles.

EXPERIMENTAL

Chemicals

All reagents were of analytical-reagent grade. Phlorizin, linolenic acid–agarose, octylthioglucoside and N-acetyl-D-galactosamine were purchased from Sigma (St. Louis, MO, U.S.A.) and sodium deoxycholate from Merck (Darmstadt, F.R.G.) *Lens culinaris* A (LcA), concanavalin A (ConA) and wheat germ agglutinin (WGA)–Ultrogel AcA 22 were obtained from Réactifs IBF (Villeneuve la Garenne, France). Sep-Pak columns were purchased from Waters Assoc. (Milford, MA, U.S.A.).

Animals, diets and preparation of biological materials

Six Large-White male piglets were divided into two groups one week after weaning (ten days old). They were fed ad libitum for six weeks a diet containing 7% lipids, which consisted of corn oil for the standard diet (control) and hydrogenated coconut oil for the EPUFA-deficient diet. Diets were prepared in the INRA center at Jouy-en-Josas (France).

The lipid supply of the control diet consisted of 16:0 (15%), $18{:}1n-9$ (33.3%), $18{:}2n-6$ (49.7%) and $20{:}1n-9$ (0.2%), whereas the EPUFA-deficient diet contained 8:0 (9.6%), 10:0 (9.5%), 12:0 (43.2%), 14:0 (13.5%), 16:0 (9.6%), 18:0 (6.4%), $18{:}1n-9$ (3.6%) and $18{:}2n-6$ (4.5%). Small intestines were rapidly

removed after slaughtering and jejunal fragments were sampled, washed with cold saline, frozen in liquid nitrogen and stored at −80°C until used.

Purification of BBM vesicles

The calcium precipitation method [5], slightly modified [6], was routinely used for the purification of BBM vesicles. The electrophoretic patterns of purified BBM vesicles with a major band corresponding to actin (Fig. 1) were very similar to those published elsewhere [7]. Purified BBM vesicles [15–20 mg ml^{-1} of protein in a buffer composed of 10 m*M* 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES), 7 m*M* *n*-butylamine acid and 500 m*M* sorbitol, adjusted to pH 7.4 with maleic acid] were stored in liquid nitrogen until analysis. Protein recovery [8] and the ratio of purified BBM protein to fresh tissue weight (w/w) were considered as identical for control and EPUFA-deprived piglets (2.3–2.5% of the homogenate prior to purification and 1.6–1.7 mg/g, respectively). Purification steps were controlled using sucrase activity [9] as a specific marker of BBMs. The specific activity of this enzyme was enriched about 12-fold in the final BBM vesicle suspension, whatever the nutritional status of the piglets. A specific sucrase activity of 0.23–0.3 μmol min^{-1} mg^{-1} of released glucose was

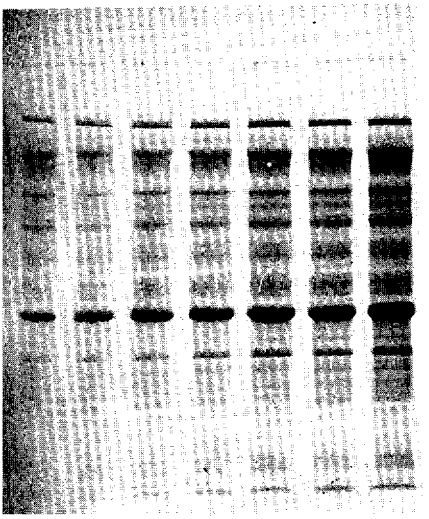

Fig. 1. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Samples of BBM proteins were analysed by SDS-PAGE carried out in a 1 mm thick, 16×18 cm slab gel using Tris-glycine-0.1% SDS buffer (pH 8.3) [27]. The separation gel consisted of linear polyacrylamide gradient [3 to 15% (w/v) with 30:0.8 acrylamide to bisacrylamide]. Proteins (27–180 μg) solubilized in 10 m*M* HEPES, 7 m*M* *n*-butylamine, 500 m*M* sorbitol (pH 7.4) were added with 0.3 m*M* dithiothreitol and 3% SDS and then heated for 3 min at 100°C before application to the gel. The electrophoresis was run at a total constant current of 50 mA. The proteins were stained with 0.25% Coomassie Blue in 50% acetic acid-methanol (9:91, v/v) solution.

found for control or EPUFA-deprived vesicles. Consequently, there was no noticeable difference in the purification procedure for the two types of BBM vesicles.

Polymerization of phlorizin

Copolymerization at 120°C of phlorizin (2 g), formaldehyde (65 ml) and urea (30 g) in the presence of acetic acid (1 drop) was performed as described by Lin and Kinne [10], according to whom the phlorizin molecules were interconnected by $-CH_2-O-$ and $-CH_2-NH-CO-NH$ chains. The white mass obtained (3.05% of phlorizin) was ground to fine particles and washed thoroughly with ethanol–water (50:50, v/v) to remove soluble materials. The particles were homogeneized by decantation, dried at 60°C and resuspended in phosphate buffer. Four columns were filled with different amounts of this suspension. The phlorizin content and the dimensions of columns (length×inner diameter) were 50 μmol (12 mm×11 mm), 150 μmol (5 mm×25 mm), 500 μmol (20 mm×25 mm) and 650 μmol (30 mm×25 mm), respectively. The columns were equilibrated with the appropriate buffer and stored at 4°C. After each chromatographic operation the polymer was vigorously resuspended in 0.1% Triton X-100 and washed thoroughly with ethanol–water (50:50, v/v).

General conditions of chromatography and expression of results

All chromatography was performed at 4°C using BBM vesicles with the same specific sucrase activity. One sucrase unit was defined as 1 nmol of glucose released per minute.

The amounts of BBM vesicles bound to immobilized ligand were determined from the difference between the loaded and eluted material expressed as amounts of total protein and total sucrase activities. The bound-to-loaded amounts ratio was defined as the binding ratio. With the phlorizin polymer, the bound and loaded amounts were reported relative to the molar amount of phlorizin so that comparison of BBM-binding ratios between columns became possible. In the same way, the detergent desorption yields obtained with each column were expressed as the ratio of total desorbed material (amount of protein or sucrase activity) to the molar amount of phlorizin.

Analysis of membrane phospholipid fatty acids

Total membrane lipids were extracted according to Folch et al. [11] and phospholipids were separated from neutral lipids and glycolipids by a Sep-Pak method [12]. Phospholipid classes were separated by high-performance liquid chromatography (HPLC) [13]. The fatty acid methyl esters, obtained after transmethylation [14] were analysed using a Packard Model 427 gas chromatograph equipped with a flame ionization detector and a CP WAX 52 CB bonded fused-silica capillary column (50 m×0.2 mm I.D.). Peaks were identified by comparison of equivalent chain lengths with those of authentic fatty acid methyl esters

[15]. Automatic data manipulation was performed by a microcomputer coupled with an integrator.

RESULTS

Compositional changes of BBM fatty acid induced by EPUFA deficiency

Table I reports the composition of the main fatty acids in serum and BBMs. The essential fatty acid deficiency was well evidenced by measurement of the $20{:}3n-9/20{:}4n-6$ ratio, which increased in the serum from 0.02 to 0.77. The EPUFA-deprived diet induced a marked decrease in the $18{:}2n-6$ content in membrane choline phosphoglycerides (CPGs) and ethanolamine phosphoglycerides (EPGs) and, to a lesser extent, in serine phosphoglycerides and inositol phosphoglycerides (data not shown). There was no marked modification of the membrane $20{:}4n-6$ contents whatever the phospholipid class considered, whereas $20{:}3n-9$, absent in the control membranes, appeared in each class of phospholipids of EPUFA-deprived vesicles. These fatty acid compositional changes typically induced by deficiency are probably responsible for the higher order of the lipid matrix structure as reported elsewhere [3,4].

Frontal chromatography of BBM vesicles on the phlorizin polymer

Fig. 2A shows a typical elution profile of a standard BBM vesicle dilute solution applied on the smallest phlorizin polymer column. The absorbance at 275 nm and the sucrase activity of the eluted fractions increased with elution volume but did not reach the plateau corresponding to the loaded solution levels as 32–36% of the vesicles remained bound to the sorbent. In fact, the binding ratio of BBM (loaded at the same concentration) increased with the amount of unsolubilized phlorizin contained in the columns (Fig. 3). Therefore, about 95% of membranes were trapped when the ratio of loaded vesicles to amount of unsolubilized phlorizin remained below 1 sucrase unit per micromole of phlorizin. The binding ratio decreased to 50% between 2 and 4 sucrase units μmol^{-1} and reached a plateau of 30–35% up to 30 sucrase units loaded per micromole of unsolubilized phlorizin.

Solubilization and desorption of polymer-bound membranes were performed using sodium deoxycholate (NaDOC) or octyl thioglucopyranoside, a non-ionic detergent recommended for membrane studies [16]. Eluted membranes were detected by protein determination and from the absorbance at 275 nm and the sucrase activity of the fractions (Figs. 2B and 4). The UV absorbance spectrum of optically transparent NaDOC-eluted material is shown in Fig. 5.

Examination of various chromatographic conditions on the highest binding capacity of phlorizin polymer showed that a noticeable binding defect occurred only in the presence of Triton X-100 or bovine serum albumin (Table II).

TABLE I

MAIN POLYUNSATURATED FATTY ACID CONTENTS [EXPRESSED AS A PERCENTAGE (w/w) OF THE TOTAL FATTY ACIDS] IN SERUM AND MEMBRANE PHOSPHOLIPIDS OF PIGLETS RECEIVING A STANDARD OR AN EPUFA-DEPRIVED DIET

DBI/SFA is the ratio of the double bond index to sum of saturated fatty acids. EPG represent 31% and 34% of the total BBM phospholipids in control and EPUFA-deprived vesicles, respectively. CPG represent 30% in control and 35% in EPUFA-deprived vesicles.

Fatty acids	Serum: total phospholipids				Brush border membrane											
	Control		EPUFA-deprived		Total phospholipids				EPG				CPG			
	Mean ($n=4$)	S.E.M.	Mean ($n=4$)	S.E.M.	Control		EPUFA-deprived		Control		EPUFA-deprived		Control		EPUFA-deprived	
					Mean ($n=2$)	S.E.M.	Mean ($n=2$)	S.E.M.	Mean ($n=3$)	S.E.M.	Mean ($n=2$)	S.E.M.	Mean ($n=3$)	S.E.M.	Mean ($n=2$)	S.E.M.
16:00	22.33	0.84	22.68	0.37	20.27	2.02	18.4	1.22	11.82	4.26	8.43	1.90	34.99	0.79	32.83	1.56
18:00	31.35	1.26	24.27	0.68	16.29	1.1	17.18	0.52	14.34	2.22	13.46	0.47	9.46	0.47	9.03	0.48
18:1$n-9$	13.48	0.93	21.06	0.61	17.81	0.09	23.58	0.07	15.15	0.72	18.38	0.00	18.85	1.27	29.12	0.25
18:2$n-6$	17.55	0.11	7.16	0.74	12.97	2.24	7.36	0.42	10.86	1.22	7.4	0.48	20.05	0.99	7.65	1.01
18:3$n-3$	0.49	0.03	0.15	0.01	0.54	0.11	0.26	0.11	0.59	0.32	0.37	0.04	0.66	0.06	0.38	–
20:3$n-9$	0.19	0.01	4.26	0.53	–	–	2.60	0.1	–	–	2.99	0.60	–	–	1.55	0.27
20:4$n-6$	10.88	0.17	5.57	0.44	8.45	0.46	7.54	1.27	10.8	2.74	14.03	1.11	4.9	0.69	4.76	0.71
22:5$n-6$	0.17	0.01	0.32	0.02	0.19	0.01	0.35	0.04	0.73	0.49	0.44	0.06	0.15	0.15	0.38	0.10
22:6$n-3$	4.34	0.29	0.77	0.06	1.75	0.54	1.06	0.22	3.71	1.10	1.2	0.00	0.64	0.02	0.23	0.06
DBI/SFA	3.12		1.87		1.86		1.62		2.80		2.77		1.76		1.49	
18:2/20:4	1.61		1.29		1.53		0.98		1.01		0.53		4.09		1.61	
20:3/20:4	0.02		0.77		–		0.35		–		0.22		–		0.34	
22:5/22:6	0.04		0.42		0.11		0.33		0.20		0.37		0.23		1.65	

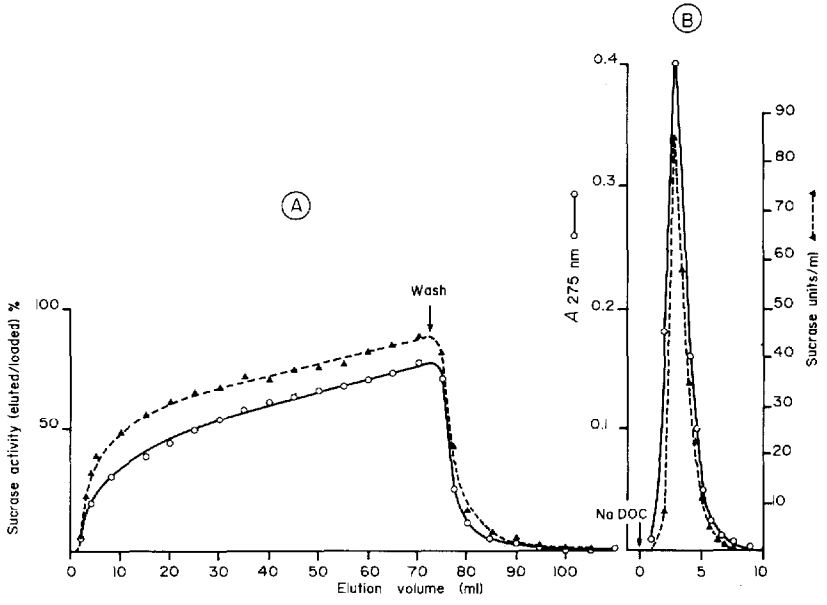

Fig. 2. (A) Frontal chromatography of a standard BBM suspension loaded in the unsolubilized phlorizin column (50 μmol of phlorizin, void volume = 2.6 ml). Purified BBM vesicles were diluted to 40 μg ml^{-1} of protein (10.7 sucrase units ml^{-1}, extinction at 275 nm = 0.23) in 0.1 *M* sodium phosphate, 1 m*M* EDTA, 0.1 m*M* phenylmethylsulphonyl fluoride, 0.01% sodium azide (pH 7.4) (phosphate buffer) and 73 ml of this suspension were loaded at 3 ml h^{-1} on the column equilibrated with the same buffer at 4 °C. The polymer was then washed with the phosphate buffer until the absorbance of eluted fraction reached the baseline. ○, Absorbance at 275 nm; ▲, eluted sucrase activity expressed as a percentage of those of the loaded suspension, 32% of the total loaded membrane proteins and 36% of the total loaded sucrase remained bound to the polymer after washing. (B) Polymer-bound membranes were recovered by flushing the column (12 ml h^{-1}) at 4 °C with a detergent-buffer composed of 0.01 *M* sodium phosphate, 0.16 *M* sodium chloride, 1 m*M* EDTA and 0.1% sodium deoxycholate. ▲, Sucrase units per millilitre of eluted fraction. Note that the sucrase activity of the peak was concentrated about eight-fold with respect to the loaded suspension. Yields of total (A+B) eluted proteins and sucrase were 85 and 89%, respectively.

Effect of EPUFA deficiency on BBM binding to phlorizin polymer

Control and EPUFA-deprived vesicles were loaded on four differently sized columns. The specific activity ratios of eluted to loaded sucrase were 0.93 (S.D. = 0.14) and 1.15 (S.D. = 0.35) for control and EPUFA-deprived BBM vesicles, respectively. The sucrase-specific activity of unbound membranes was not considered to be markedly different from that of the loaded solution, whatever the type of BBM vesicles used. Although no difference appeared between the control and EPUFA-deprived vesicles below 1 sucrase unit loaded per micromole of phlorizin (about 95% of binding, see Fig. 3), it can be clearly seen in Fig. 6 that the values of bound versus loaded sucrase units were distributed on two different straight lines. The two slopes representing the mean bound-to-loaded sucrase

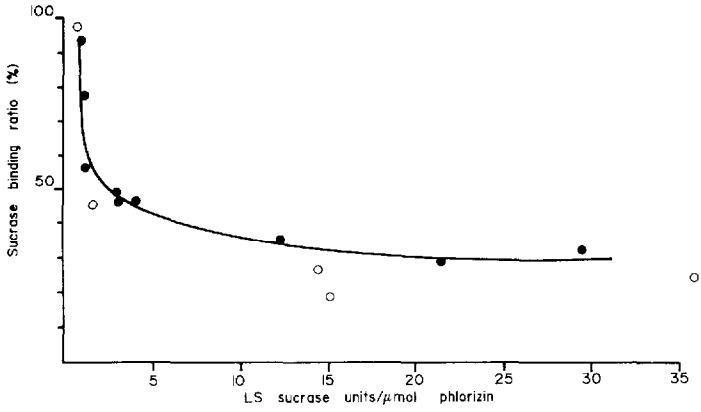

Fig. 3. Dilute BBM vesicles were loaded under the same conditions as in Fig. 2 on four differently sized columns packaged with increasing amounts of unsolubilized phlorizin. ●, Control vesicles, ○, EPUFA-deprived vesicles. LS: total sucrase loaded per micromole of phlorizin. A minimal value of 0.7 sucrase units μmol^{-1} and a maximal value of 29.5 sucrase units μmol^{-1} were obtained when 450 and 1475 sucrase units were loaded on the 650- and 50-μmol phlorizin column, respectively.

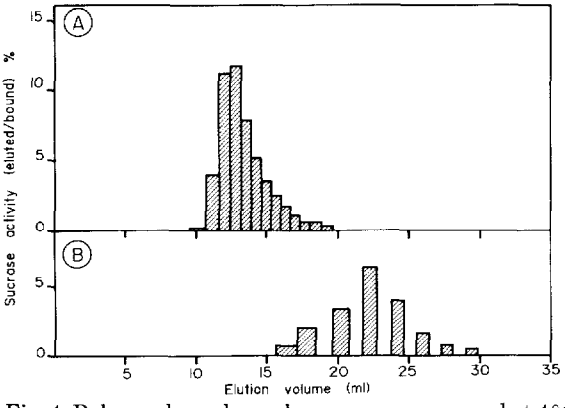

Fig. 4. Polymer-bound membranes were recovered at 4 °C using the deoxycholate procedure (see Fig. 2B) or at 20 °C using a 0–50 m*M* octyl thioglucoside linear gradient set in the phosphate buffer. (A) Peak of sucrase activity eluted with deoxycholate (yielding 50% of the total bound sucrase); (B) peak of sucrase activity eluted with octyl thioglucoside (yielding 39% of the total bound sucrase).

units ratio provide evidence that the highest and the lowest binding ratios were obtained with control and EPUFA-deprived vesicles, respectively. The slopes were equal to 0.32 (as measured by sucrase activity) or 0.355 (as measured by proteins, data not shown) for control vesicles, and to 0.234 (sucrase activity) or 0.203 (proteins) for EPUFA-deprived vesicles. These values allow the minimal binding capacity of membranes on the phlorizin polymer to be estimated as 32–36% for the control and 20–24% for EPUFA-deprived vesicles.

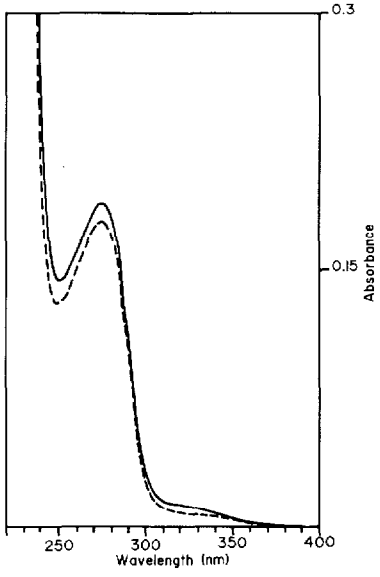

Fig. 5. Difference in UV absorption spectrum between NaDOC-eluted proteins (corresponding to the top of the peak in Fig. 2) and NaDOC-buffer (dotted line) or phosphate buffer (full line). Maximal absorption was recorded at 275 nm.

TABLE II

BBM VESICLE INTERACTIONS WITH UNSOLUBILIZED PHLORIZIN TESTED IN THE PRESENCE OF TRITON X-100, NaDOC, BOVINE SERUM ALBUMIN (BSA) OR LACTOSE

The 650 μmol phlorizin column was equilibrated with an appropriate buffer and frontal affinity chromatography of vesicles diluted in the same buffer was performed as described in Fig. 1. Binding ratios obtained (from 0.7 sucrase units loaded per micromole) were referred to those under standard conditions (sodium phosphate buffer) which are defined as 0% inhibition.

Elution buffer	Inhibition (%)
0.1 *M* sodium phosphate, 1 m*M* EDTA (pH 7.4)	0
0.01 *M* sodium phosphate, 0.16 *M* sodium chloride, 1 m*M* EDTA, 0.1% NaDOC	26
0.1 *M* sodium phosphate, 1 m*M* EDTA (pH 7.4), 0.1% Triton X-100	94
10 m*M* HEPES, 1 m*M* EDTA, 0.1 m*M* phenylmethylsulphonyl fluoride, 0.16 *M* sodium chloride, 1% BSA (pH 6.8)	81
0.1 *M* sodium phosphate, 1 m*M* EDTA (pH 7.4), 1 m*M* lactose	10

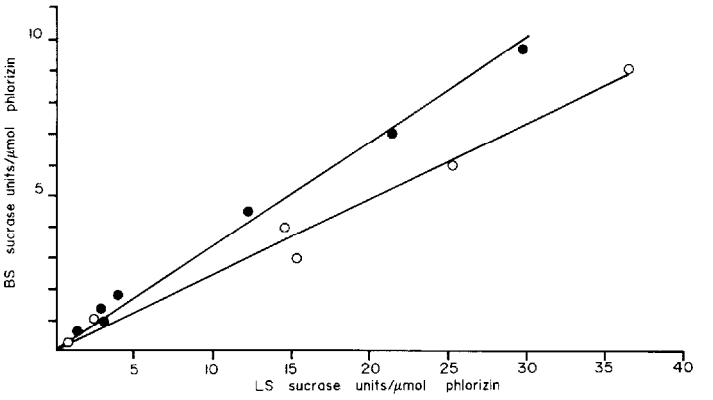

Fig. 6. BBM binding capacity on the phlorizin polymer. BS, total bound sucrase per micromole of phlorizin; LS, total sucrase loaded per micromole of phlorizin. Experimental conditions as in Figs. 2 and 3. ●, Control vesicles (slope = 0.32; $r = 0.997$); ○, EPUFA-deprived vesicles (slope = 0.234; $r = 0.989$).

Effect of EPUFA-deficiency on polymer-bound membrane desorption by sodium deoxycholate

A plot of amounts of detergent-eluted protein versus amounts of polymer-bound protein gave evidence that the desorption yield by NaDOC is constant and independent of the column and the type of vesicles (Fig. 7A). The slope led to a mean desorption yield of 47%. On the other hand, a plot of eluted material expressed as sucrase units (Fig. 7B) showed that the quality of desorption clearly depends on the type of vesicles. The sucrase desorption yield of control vesicles was constant and led to a mean value (57%) close to that obtained with proteins, whereas it was not constant but ranged from 14 to 33% with EPUFA-deprived vesicles. Taking the average of the results, it was concluded that the specific sucrase activities of the recovered material were increased by a factor of 1.2 and decreased by a factor of 0.3–0.7 for the control and EPUFA-deprived membranes, respectively.

Attempts at BBM binding to linolenic acid–agarose

The binding capacity of linolenic acid–agarose gel was estimated to be about 37% from the gel-bound/gel-loaded membrane ratio obtained after filtration of dilute BBM vesicle solutions in a single-sized column (Fig. 8). Measurements were made at 4 and 30 °C and no difference in binding appeared between the control and EPUFA-deprived vesicles.

Sorbent-bound membranes were eluted at 30 °C with a mixed micelle solution composed of NaDOC and sodium oleate in a molar ratio of 2.4:35. About 100% of the total gel-bound proteins were eluted with the micellar solution, whether the BBMs were EPUFA-deprived or not. The total recovery of sucrase activity was about 85% of the loaded solution.

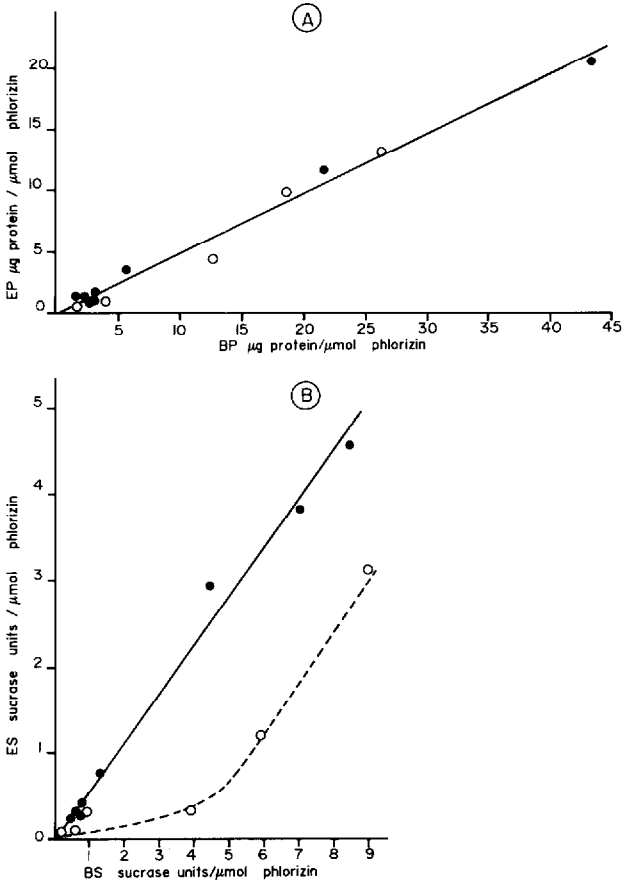

Fig. 7. Desorption of polymer-bound membranes by deoxycholate. Experimental conditions as in Figs. 2–4. ●, Control vesicles; ○, EPUFA-deprived vesicles. (A) Elution of proteins. EP, total NaDOC-eluted membrane proteins (μg) per micromole of phlorizin; BP, total polymer-bound proteins (μg) per micromole of phlorizin. Slope = 0.47; $r = 0.989$. (B) Elution of sucrase. ES, total NaDOC-eluted membrane sucrase (nmol min^{-1}) per micromole of phlorizin; BS, total polymer-bound sucrase (nmol min^{-1}) per micromole of phlorizin. A straight line (slope = 0.57; $r = 0.992$) was obtained with control vesicles only.

Attempts at BBM binding to immobilized lectins

Preliminary experiments showed that control and EPUFA-deprived BBM vesicles were poorly retained by LcA–Ultrogel and ConA–Ultrogel under the present conditions (Table III). In contrast, the binding capacity of a 5-ml WGA–Ultrogel column was estimated to be 25 and 54% of total loaded membranes for the control and EPUFA-deprived vesicles, respectively.

A first fraction of immobilized lectin-bound membrane was specifically eluted after washing with a solution of a competing sugar, i.e., α-methyl-D-mannose for LcA and ConA or N-acetyl-D-glucosamine for WGA. Non-eluted material was

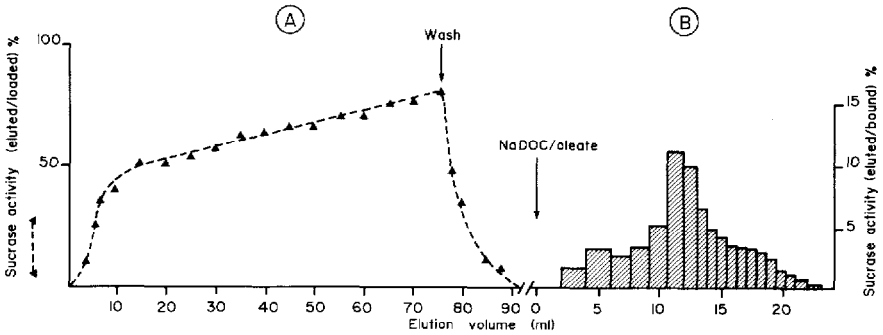

Fig. 8. Binding of BBM vesicles on a linolenic acid-agarose gel: a column (1.1 cm×5 cm) filled with 5 ml of gel was equilibrated at 4 or 30°C with previously degassed sodium phosphate buffer. (A) A 75-ml volume of control or EPUFA-deprived (not shown) BBM vesicles diluted to 35 μg ml^{-1} of protein in the same buffer was loaded at 3 ml h^{-1}. The gel was then washed with the phosphate buffer until the absorbance at 275 nm of the eluted fraction reached the baseline. Recoveries of proteins and sucrase activity led to a binding ratio of 35–40%. (B) Desorption of bound material performed at 30°C and 12 ml h^{-1} with a deoxycholate-oleate buffer composed of 0.01 *M* sodium phosphate, 0.16 *M* sodium chloride, 2.4 m*M* NaDOC, 35 m*M* sodium oleate, 1 m*M* EDTA and 0.1 m*M* phenylmethylsulphonyl fluoride (pH 8.5). Deoxycholate-oleate buffer removed about 100% of the bound proteins and 75% of the total bound sucrase activity were recovered in the eluted fractions. The gel was then washed with phosphate buffer and acetic acid–ethanol–water (1:10:9, v/v) to remove tightly bound material.

TABLE III

BINDING RATIO OF CONTROL AND EPUFA-DEPRIVED VESICLES IN LcA, ConA AND WGA GELS AS ESTIMATED FROM ELUTED-TO-LOADED TOTAL SUCRASE ACTIVITY RATIO

Chromatographic conditions for WGA gel are described in Fig. 7. For LcA and ConA gels, phosphate buffer was replaced with a Ca–Mn buffer composed of 10 m*M* HEPES, 7 m*M* *n*-butylamine, 500 m*M* sorbitol, 1 m*M* calcium chloride, 1 m*M* manganese chloride, 0.1 m*M* phenylmethylsulphonyl fluoride and 0.01% sodium azide, adjusted to pH 7.4 with maleic acid. Other conditions remained identical.

Vesicle	Binding ratio (%)		
	AcA22-WGA	AcA22-LcA	AcA22-ConA
Control	25	11	15
EPUFA-deprived	54	14	11

next removed by washing the gel successively with 0.1% NaDOC and 0.05 *M* hydrochloric acid. The competing sugar removed material from WGA-bound membranes and the total amounts eluted were higher for EPUFA-deprived vesicles (Fig. 9). Washing LcA and ConA gels with the competing sugar removed only traces of material and washing the WGA gel with 0.05 *M* hydrochloric acid did not remove any material when the vesicles were primarily loaded and eluted in the presence of N-acetyl-D-glucosamine (data not shown).

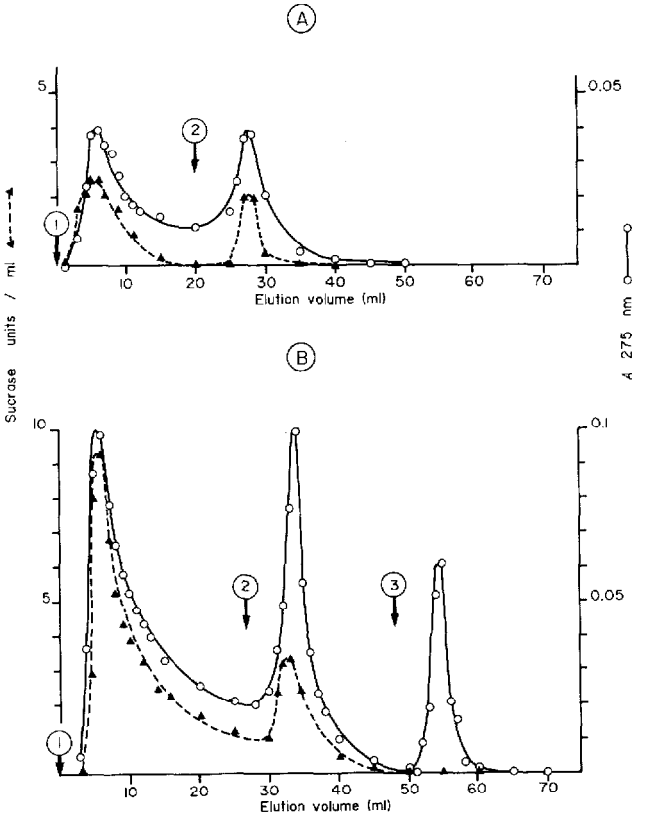

Fig. 9. Desorption of immobilized WGA-bound vesicles using competing sugar, deoxycholate or 0.05 *M* hydrochloric acid. A column (1.1 cm×5 cm) filled with 5 ml of WGA–AcA 22 gel was equilibrated at 4°C with sodium phosphate buffer (pH 7.4); 72 ml of control (A) or EPUFA-deprived (B) BBM vesicles diluted to 40 μg ml^{-1} of protein in the same buffer were loaded (3 ml h^{-1}) at 4°C. The gel was then washed with the phosphate buffer until the absorbance at 275 nm of the eluted fraction reached the baseline. Desorption of bound material was then performed at 12 ml h^{-1} using successively (1) 0.1 *M* sodium phosphate, 1 m*M* EDTA, 0.1 *M* N-acetyl-D-glucosamine, (2) 0.01 *M* sodium phosphate, 1 m*M* EDTA, 0.16 *M* sodium chloride, 0.1% NaDOC and (3) 0.05 *M* hydrochloric acid. The total yield of sucrase activity for control and EPUFA-deprived vesicles was 68 and 50%, respectively.

DISCUSSION

Affinity chromatography of detergent-solubilized membranes has been used extensively in purification and reconstitution studies of receptors and transport systems [17,18]. An undamaged membrane subfraction could be also isolated by affinity chromatography, as shown by Szamel et al. [19], who purified specific membrane domains by affinity chromatography of calf thymocytes on ouabain–Sepharose or ConA–Sepharose. The subfraction membrane isolated by this way (5–18% of the total loaded proteins) could be differentiated from the entire plasma

membrane on the basis of functional properties and phospholipid fatty acid composition. We undertook a different approach in which two populations of piglet BBM vesicles, differing in their phospholipid fatty acid composition, were obtained from controlled diets with the purpose of establishing the possible influence of membrane lipid components on the surface properties of vesicles. Three different sorbents, mimicking specific ligands of membrane receptors or carriers, were screened using a frontal chromatography test.

Phlorizin is known to be a high-affinity competitive inhibitor for sodium-dependent sugar transport. The isolation of sugar-binding proteins (lectins) and partial purification of hog kidney sodium–glucose cotransport system using batch chromatography on a phlorizin polymer have been described previously [20]. Using frontal chromatography of undamaged and extensively dilute membranes, we observed in this work that the BBM vesicle–phlorizin polymer complex displays strong interactions which are partly disrupted by detergents such as deoxycholate or octylthioglucoside. The lowest binding capacity of the polymer measured in the presence of serum albumin suggests that the high affinity of vesicles for unsolubilized phlorizin was mostly unspecific. In the presence of a non-ionic detergent such as Triton X-100, solubilized membrane proteins were weakly retained by the polymer. This is in agreement with the results of Lin et al. [20], who found that only 0.4% of Triton X-100-solubilized membrane proteins was retained and further released by washing the polymer with a D-glucose solution. This fraction was enriched with the cotransport system. Partial involvement of the glucose carrier in the interactions between entire vesicles and phlorizin polymer remains possible, as the physical properties of the resin allow free rotation of the sugar moiety of phlorizin and free access of the sugar-binding proteins [10]. Finally, it is likely that a number of membrane domains, consisting of glycoprotein sets, are involved in the interactions with the phlorizin polymer.

Using four differently sized columns, we found that the actual binding capacity depends closely on the ratio of loaded membrane to amounts of unsolubilized phlorizin. Depending on the existence of specific and non-specific components in the binding of phlorizin to isolated enterocytes [21], two possibilities could be differentiated: high binding capacity, when less than 1 sucrase unit was loaded per micromole of phlorizin, on the one hand, and low binding capacity, when more than 5 sucrase units were loaded per micromole of phlorizin on the other hand. No difference exists between control and EPUFA-deprived vesicles in the former instance (see Fig. 3), whereas a slight difference was found in the latter, viz., the EPUFA-deprived diet induced about a 12–16% decrease in the minimal binding capacity (Fig. 6). Several possibilities might be considered to explain this different behaviour of EPUFA-deprived BBM vesicles on phlorizin polymer: (i) a decrease in the total surface-membrane sites responsible for the non-specific interactions with the phlorizin polymer, (ii) a compositional change of the lipid matrix structure (see Table I) which reduces the accessibility of some surrounding lipid-sensitive binding sites or (iii) a conformational change of the whole

membrane lipid–protein structure leading to a lower accessibility of some membrane sites involved in the binding. Further accurate information about the nature of the interactions between membranes and unsolubilized phlorizin is now required to decide between these different possibilities.

The results for the desorption of polymer-bound membranes using deoxycholate raise other questions as a clear difference appeared between the control and EPUFA-deprived vesicles. The protein and sucrase activity recoveries were similar for control vesicles, whereas with the EPUFA-deprived vesicles the specific sucrase activity of NaDOC-eluted proteins was diminished with respect to the initial specific activity of the loaded solution. Now, the extent of membrane lysis induced by bile salts correlates with the degree of membrane fluidity: the more fluid the membrane, the more susceptible it is to lysis [22]. Hence it can be assumed that the "rigidification" of BBMs induced by the EPUFA-deprived diet [3] was responsible for the less efficient solubilization (and desorption) of some tightly bound proteins. It is also inferred from these observations that sucrase, or its near environment, could be closely involved in the interactions with the polymer binding sites. In other words, some of the membrane domains including sucrase involved in the interactions with the polymer, being less susceptible to detergent damage because of their altered lipid composition, were not incorporated into the deoxycholate micelles and remained bound to the sorbent. In contrast, unbound membrane proteins were quickly solubilized and ran with the deoxycholate micelles.

BBM vesicles bound on linolenic acid–agarose gel were almost completely recovered when the column was flushed with mixed micelles of deoxycholate and oleate. On the basis of the data cited above, this result could indicate a specific competing effect of oleate micelles, which were used to disrupt the putative interactions between immobilized linolenic acid and membrane fatty acid binding protein (see ref. 23). However, no difference in binding or desorption between the control and EPUFA-deprived vesicles appeared, based on preliminary results obtained with a single-sized linolenic acid-column.

Among the three immobilized lectin gels tested, only the WGA–Ultrogel exhibited a noticeable binding capacity when it was loaded with a dilute solution of BBM vesicles. Part of these interactions was highly specific, as N-acetyl-D-glucosamine alone was adequate to compete with WGA-bound membranes. However, lectin gels increasingly lost their binding capacity [24] and repeated experiments with the same column were not reproducible. The weak retention of BBM vesicles observed in LcA and ConA gels shows the non-existence of interactions between membranes and the Ultrogel matrix, and supports the assumption of specific binding in WGA–Ultrogel. The absence of interaction between BBM vesicles and LcA or ConA gels might be due to steric hindrance rather than to a lack of membrane receptors for these lectins (see the results obtained previously with ConA [25]). WGA receptors would be more accessible, especially with EPUFA-deprived vesicles (binding ratio of 54% compared with 25% for

control vesicles). It is noteworthy that during maturation of rat intestine, the WGA binding sites on microvillus membranes could be either lost or covered so that they became progessively inaccessible to WGA [26]. We do not know yet if the best binding capacity of piglet EPUFA-deprived vesicles on immobilized WGA, observed in a single-sized column, reflect a failure of cell maturation, but, taken together, the results focus interest on the eventuality of structural changes of the membrane surface components, namely glycoproteins of the glycocalyx, induced by the EPUFA-deficient diet. Thorough investigations in this area are necessary in order to evaluate the validity of this hypothesis.

In conclusion, frontal affinity chromatography of sólutions at low concentration could be profitably used to start any comparative study of undamaged membranes differing in the accessibility or distribution of their surface components.

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