Biochimica et Biophysica Acta, 728 (1983) 11–19
Elsevier Biomedical Press

BBA 71523

ISOLATION AND PARTIAL CHARACTERIZATION OF BASOLATERAL MEMBRANES FROM RAT PROXIMAL COLONIC EPITHELIAL CELLS *

THOMAS A. BRASITUS and ROGER S. KERESZTES

Department of Medicine, Columbia University, College of Physicians and Surgeons, New York, NY 10032 (U.S.A.)

(Received June 28th, 1982)
(Revised manuscript received October 18th, 1982)

Key words: Membrane lipid; Membrane fluidity; ($Na^+ + K^+$)-ATPase; (Rat colon basolateral membrane)

The isolation of basolateral membranes from rat proximal colonic epithelial cells is described. Cells were harvested using a technique combining chelation of divalent cations with mechanical dissociation. After homogenization, differential centrifugation yielded a 'crude' membrane fraction which was further purified using sucrose density centrifugation. The final membrane fraction was enriched 10–14-fold over homogenate in ouabain-sensitive sodium-potassium dependent adenosine triphosphatase and ouabain-sensitive potassium-dependent phosphatase specific activities. SDS-polyacrylamide gel electrophoresis of this membrane revealed at least 18 protein bands with molecular weights of 14 600–200 000. Phosphatidylcholine, phosphatidylethanolamine, sphingomyelin, free cholesterol and fatty acids were the major lipid components of this membrane. The predominant fatty acids were palmitic (16:0), oleic (18:1), stearic (18:0) and linoleic (18:2) acid. Membranes and their liposomes were studied, using the lipid soluble fluorophore 1,6-diphenyl-1,3,5-hexatriene (DPH), by steady-state fluorescence polarization. The fluorescence anisotropy was greater in the intact membranes compared to their liposomes, indicating greater fluidity in the liposomes. Compositional studies suggested that the high fluidity of this membrane was due to its low ratios of protein/lipid (w/w), cholesterol/phospholipid (mol/mol), and sphingomyelin/phosphatidylcholine (mol/mol).

Introduction

Methods for the isolation of the plasma membranes of small intestinal enterocytes have been reported and the structure [1–3], composition [3–9], transport [7] including ionic conductances [10, 11] of the basolateral and brush border membranes described.

While the colon is also recognized as an important organ in regard to both physiological and pathological processes, little is known about the composition of plasma membranes of colonic epithelial cells. Attempts to isolate these membranes have been hindered by the large quantities of mucus [12] and heterogeneity of cell types present in this organ [13,14]. To date, only two studies have been published on the isolation of plasma membranes from mammalian colonic epithelium. Jackson et al. [15], using mucosal scrapings as starting materials, described a procedure for the isolation of lumenal and contralumenal mem-

* Supplementary data to this article are deposited with, and can be obtained from, Elsevier/North-Holland Biomedical Press B.V., BBA Data Deposition, P.O. Box 1345, 1000 BH Amsterdam, The Netherlands. Reference should be made to BBA/DD/241/71523/728 (1983) 11–19. The supplementary information includes: Two tables which show the distribution of marker enzyme activities for each fraction obtained during the fractionation procedure.

branes from 'normal' and malignant rat colonic mucosa. Since goblet cells comprise 20–40% of cells in the rat colonic mucosa, however, the membranes obtained undoubtly originated from both goblet and epithelial cells. Recently, Gustin and Goodman [12] isolated and partially characterized lumenal plasma membranes from rabbit descending colon epithelial cells.

In the present report, using a modification of methods previously validated for rat small intestine [16] and colon [17], an enriched population of epithelial cells was first isolated from rat proximal colon and used as starting material for subsequent purification and partial characterization of basolateral membranes.

Methods and Materials

Cell fractionation

Male albino rats of the Sherman strain weighing 250–300 g were fasted 18 h with water ad libitum before being sacrificed. The animals were killed rapidly by cervical dislocation and their colons excised. The cecum was discarded, the remaining large intestine divided equally into two parts: 'proximal' and 'distal'. The proximal segment was then rinsed thoroughly with a solution containing 0.015 M NaCl and 0.001 M dithiothreitol, and tied at one end. The sac was filled, tied at the other end and shaken at 37°C in a 250 ml siliconized glass Erlenmeyer flask containing 50 ml of phosphate-buffered saline, pH 7.2 (buffer 1), at 75 oscillations per min, sequentially, with the following solutions: 10% fetal calf serum in 1.6% Joklik's modified minimal essential medium (GIBCO: Grand Island, NY) $\times 10$ min (twice); citrate buffer (0.027 M sodium citrate, 0.0015 M KCl, 0.096 M NaCl, 0.0056 M KH_2PO_4, (pH 7.3)) $\times 15$ min; 0.1 M phosphate-buffered saline, pH 7.2, containing 0.0015 M EDTA and 0.0005 M dithiothreitol $\times 15$ min (twice).

Each solution was collected, filtered through a single layer of Nitex (Tetko, Inc., Elmsford, NY) with a pore diameter of 100 μm, then each was brought up to 40 ml with buffer 1 (containing 0.001 M Ca^{2+} and 0.001 M Mg^{2+}) and centrifuged at $500 \times g$ for 10 min. The cell pellets were washed twice in the same buffer, and kept on ice until used (see below).

Membrane preparation

The cells collected from all five incubations were combined, pelleted, washed and brought up in 50 ml of sucrose/histidine/imidazole/EDTA buffer (0.25 M sucrose, 0.005 M histidine, 0.005 M imidazole, 0.0005 M EDTA, (pH 7.0)) (buffer 2). The suspension of cells was homogenized $\times 20$ strokes, using a tight fitting Dounce homogenizer (pestle A), filtered through a single layer of Nitex (Tetko, Inc., Elmsford, NY) with a pore diameter of 100 μm to remove debris and unbroken cells, and washed with an additional 10–20 ml of buffer 2. The homogenate was centrifuged at $300 \times g$ for 10 min in a Sorvall Centrifuge (Dupont Instruments, Model RC-5B) to obtain an initial pellet (P_1) and supernatant (S_1). S_1 was collected and kept on ice while P_1 was brought up in 10 ml of buffer 3 (0.005 M histidine, 0.0005 M imidazole, 0.005 M EDTA, (pH 7.0)), dounced $\times 10$ strokes and spun at $300 \times g$ for 10 min (S_2, P_2). S_2 was collected on ice. P_2 was brought up in 10 ml of the same buffer, dounced $\times 5$ strokes and centrifuged at $360 \times g$ for 10 min (P_3, S_3). P_3 was discarded. $S_1 - S_3$ were combined and centrifuged at $1085 \times g$ for 15 min in a Sorvall centrifuge at 0–5°C (P_N, S_4). P_N was discarded and the supernatant (S_4) centrifuged at $22\,000 \times g$ for 15 min. The 'fluffy white portion' of the pellet (P_M) was collected, brought up in 5 ml of 50% sucrose/buffer 2 (w/w) and dounced $\times 5$ strokes. 4 ml of this material was placed at the bottom of a '$5/8 \times 3$' cellulose nitrate tube (Beckman, Palo Alto, CA) and a discontinuous gradient formed by successively applying 3 ml of 40% sucrose/buffer 2, 3 ml of 30% sucrose/buffer 2 and filling the remainder of the tube with 20% sucrose/buffer 2. The discontinuous gradient was centrifuged at $140\,000 \times g$ for 120 min in a Beckman SW 36 rotor using a Beckman L5-50 ultracentrifuge (Palo Alto, CA). Seven fractions were collected (20%, 30%, 40%, 50% and three interfaces), diluted with distilled H_2O and pelleted by ultracentrifugation, using a Beckman 40 rotor, at $105\,000 \times g$ for 60 min. Each pellet was then brought up in 1 ml of buffer 2, pH 7.4 and analyzed immediately or quick frozen with acetone and dry ice and stored at −70°C.

Enzyme assays

Assay conditions were chosen for each enzyme

to assure linear kinetics with respect to time and protein. Ouabain-sensitive sodium-potassium dependent adenosine triphosphatase ($(Na^+ + K^+)$-ATPase) was quantitated by the method of Siegel and Goodwin [18]. NADPH-cytochrome *c* reductase, succinate dehydrogenase and alkaline phosphatase (*p*-nitrophenylphosphatase) were assayed as previously described [2,3,19]. The latter enzyme was also assayed using the fluorogenic substrate 4-methylumbelliferyl phosphate [12]. Acid phosphatase, ouabain-sensitive and insensitive potassium-dependent phosphatases were assayed as previously described [12] using *p*-nitrophenyl phosphate as substrate.

Exogeneous galactosyltransferase activity was measured according to the method of Kim et al. [20] as modified by Podolsky and Weiser [21], using 727 μM UDP[^{3}H]galactose (spec. act. 1.14 Ci/mmol) prepared to a final specific activity of 0.076 Ci/mmol by the addition of unlabeled UDPgalactose (Sigma Chemical Co.). Ovalbumin (Sigma Chemical Co.) was used as exogeneous acceptor.

Slab gel electrophoresis

Gel electrophoresis was performed using gradient gels PAA 4/30 (Pharmacia Fine Chemicals, Piscataway, NJ) following general procedures as previously described [22,23]. At the end of the electrophoresis, gels were stained for proteins with Coomassie blue [22,23].

Fluorescence polarization studies

Four lipid-soluble fluorophores were used: 1,6-diphenyl-1,3,5-hexatriene (DPH), trimethylamino-DPH (TMA-DPH), DL-2-(9-anthroyl)stearic acid (2-AS) and DL-12-(9-anthroyl)stearic acid (12-AS). The fluorophores were obtained from Aldrich Chemical Co., Sigma Chemical Co., or Molecular Probes Inc. The methods used to load the membranes and the quantification of the polarization of fluorescence, using an SLM polarization spectrofluorometer, have previously been described [2,3,19]. Liposomes were prepared as previously described [19]. The polarization of fluorescence was expressed as the fluorescence anisotropy, *r*, and as the anisotropy parameter, $((r_0/r) - 1)^{-1}$. The anisotropy parameter varies directly with the rotational relaxation time of the probe and hence is related inversely to the lipid fluidity *. All anisotropy values were determined at 25°C. Possible changes in the excited state lifetimes of the probes were monitored by calculations of the total fluorescence intensity. No changes in the total fluorescence intensity could be demonstrated, using each probe, in either basolateral membranes or liposomes preparations.

Compositional studies

Total lipids were extracted from basolateral membranes by the method of Folch et al. [24]. The composition of total neutral and phospholipid extracts of these membranes were examined by thin-layer chromatography according to the method of Katz et al. [29]. Additionally, phospholipid and cholesterol content was measured using the methods of Ames and Dubin [30] and Zlatkis et al. [31], respectively. Fatty acids of the total lipid extract of membranes were derivatized as previously described by Gartner and Vahouny [32]. Fatty acid methylesters were determined on a Joel JGX-20K gas chromatograph equipped with a plasma ionization detector and interfaced with a Hewlett-Packard 3390A intergrator [32]. Authentic fatty acid methylesters were utilized to identify retention times. Protein was estimated by the method of Lowry et al. [33] using bovine serum albumin as standard.

Electron microscopy

The membrane fractions were prepared for positive staining by fixing in glutaraldehyde (3.0 g/100 ml) in 0.1 M cacodylate buffer, pH 7.5

* The term 'lipid fluidity' as applied to natural membranes is used to express the relative motional freedom of the lipid molecules or substituents thereof. It bears emphasis, however, that this term is broad and includes different types of motion, e.g., rotational or lateral diffusion of a molecule in an array, movements of substituent groups of a molecule, and flow of molecules under a pressure gradient accord with a fluidity which is 1/(viscosity of the molecular array) [25,26]. In this report 'lipid fluidity' was assessed by the steady-state fluorescence polarization of lipid soluble probes. The anisotropy parameters so obtained are probe dependent and reflect the overall motional freedom of these molecules without distinguishing the specific mechanism affecting its motion such as viscous drag of the environment, anisotropic rotations and hindered motions due to structural factors [3,27,28].

TABLE I

DISTRIBUTION OF ENZYME MARKERS DURING FRACTIONATION PROCEDURE

Values are averages of five experiments with a S.E. less than 6% for recovery. Specific activities (mean ± S.E.) are given in parentheses. All enzyme activities are given in nmol per mg protein per min except for the following: Succinate dehydrogenase and NADPH-cyto-

Fraction	Protein	$(Na^+ + K^+)$-ATPase	Ouabain-sensitive K^+-phosphatase	Alkaline phosphatase	NADPH-cytochrome *c* reductase
Homogenate	100% (0.92 ± 0.07)	100% (34.9 ± 3.8)	100% (4.4 ± 0.6)	100% (8.4 ± 0.4)	100% (27.5 ± 2.8)
'Crude' membrane fraction (PM)	11.2% (0.65 ± 0.11)	33.2% (103.3 ± 6.6)	25.8% (16.1 ± 1.4)	18.8% (16.4 ± 0.8)	11.2% (27.6 ± 2.8)
Basolateral membranes (30/40)	1.7% (0.45 ± 0.7)	14.6% (353.0 ± 25.5)	12.6% (56.5 ± 6.3)	2.8% (13.8 ± 0.6)	1.8% (30.3 ± 1.1)

for 2 h as previously described by Weiser [34] and examined with a electron microscope (Zeiss EMS-9, New York, NY).

Materials and general methods

All radioactive materials were purchased from New England Nuclear Company (Boston, MA). All other reagents were obtained from Fisher Chemical Company (Fairlawn, NJ) except for NCS tissue solubilizer which was obtained from New Amersham Company (Arlington Heights, IL). DNA content was measured using the method of Burton [35], as modified by Giles and Myers [36].

Statistical methods

All results are expressed as mean values ± standard error. Paired or unpaired *t*-tests were used for all statistical analysis. A P value < 0.05 was considered significant.

Results

Cell isolation

80–90% of the cells were found to exclude 0.1% Trypan blue dye. 5% of the cells were stained by mucicarmine or periodic acid-schiff reagent (i.e., goblet cells) *.

Isolation of basolateral membranes

The isolation of basolateral membranes from rat proximal colonic cells was carried out in two stages. After disruption of the cells by homogenization, the resulting organelles and membrane fragments were fractionated by differential centrifugation. This procedure yielded a 'crude' membrane fraction (PM). The PM fraction was not contaminated by intact brush borders or nuclei as assessed by phase microscopy. The distribution of nuclear, mitochondrial, microsomal, Golgi and lysosomal material in the various fractions was measured using DNA, succinate dehydrogenase, NADPH-cytochrome *c* reductase, galactosyltransferase and acid phosphatase, respectively. Apical plasma membrane contamination was assessed using alkaline phosphatase and ouabain-insensitive K^+-phosphatase activities. The PM fraction contained approx. 25% and 33% of the total ouabain-sensitive K^+-phosphatase and $(Na^+ +$

* Examination of sections prepared from the residual colonic surface after the last incubation, using light microscopy, revealed that the majority of cells remaining stained positively with periodic acid-Schiff reagent, i.e., were goblet cells, further indicating that this technique harvested mainly epithelial cells. Additional studies were also performed using ^{35}S-labeled Na_2SO_4 administered i.p. After completion of the five incubations the remaining mucosa from each segment was scraped. The specific activity of the scraped mucosa compared to the isolated cells was shown to be 1.5–2.0-times as high, again suggesting that most cells remaining were goblet cells.

chrome *c* reductase are in ΔA per mg protein per min; galactosyltransferase is in pmol per mg protein per min; protein is expressed in mg/ml; DNA is expressed in μg per mg protein.

Succinate dehydrogenase	DNA	Galactosyl-transferase	Ouabain-insensitive K^+-phosphatase	Acid phosphatase
100% (235.6 ± 13.1)	100% (189.2 ± 0.8)	100% (2.3 ± 0.1)	100% (2.9 ± 1.0)	100% (5.6 ± 1.1)
15.0% (471.9 ± 34.3)	7.3% (130.0 ± 10.1)	10.9% (16.1 ± 1.8)	14.3% (5.9 ± 1.1)	17.6% (7.5 ± 17.6)
2.5% (251.2 ± 27.8)	0.1% (5.8 ± 1.3)	1.8% (4.3 ± 0.4)	2.2% (9.2 ± 1.1)	2.8% (9.6 ± 1.8)

K^+)-ATPase activities present in the homogenate, respectively, whereas the % total activity for all other markers enzymes was below 20%. These two enzymes showed a 3–4-fold increase in specific activity compared to homogenate. DNA content was decreased, NADPH-cytochrome *c* reductase specific activity was unchanged and the specific activities of the other marker enzymes were slightly increased * (Table I).

The second stage of the purification scheme involved density gradient velocity sedimentation of the PM fraction using a discontinuous sucrose gradient. The specific activity and % yield of each of the marker enzymes, for the final membrane fraction (30/40) are presented in Table I.

The 30/40 fraction appeared maximally enriched in ($Na^+ + K^+$)-ATPase and ouabain-sensitive K^+-phosphatase demonstrating a 10–14-fold increase in specific activity compared to homogenate for both markers. This fraction also contained approx. 13–15% of the total activity of each of these enzymes. Contamination by other intracellular material, as assessed by appropriate marker enzymes, was minimal (Table I). Alkaline phosphatase and ouabain-insensitive K^+-phosphatase, were enriched 1.6- and 3.2-fold, respectively. Yields of these latter enzyme activities, however, averaged only 2–3% of the total homogenate values in contrast to the 13–15% yield seen with the basolateral membrane markers *.

Fluorescence polarization studies

The results of steady-state fluorescence polarization studies, using the lipid soluble fluorophore DPH, performed on intact basolateral membranes and their liposomes are shown in Table II. The anisotropy parameter, $((r_0/r) - 1)^{-1}$, at 25°C was approximately twice as high in the membranes as in the liposomes, indicating greater lipid fluidity in the liposomes. In agreement with previous studies performed on basolateral membranes of rat enterocytes [2,3] this indicates that lipid fluidity is significantly reduced in the presence of membrane proteins. The anisotropy and the anisotropy parameter at 25°C, obtained on intact membranes using the probes 2-AS, 12-AS, and TMA-DPH are also shown in Table II.

Basolateral membrane composition

Analysis of the protein pattern of the 30/40 fraction by SDS-polyacrylamide gel electrophoresis (Fig. 1) demonstrated at least 18 separate bands with molecular weights varying between 16400 and 196000, the majority in the range of 30000 to 100000. The neutral and phospholipid composition of this membrane was further analyzed by thin-layer chromatography. Expressed as percent

* BBA Data Deposition, see footnote on p. 11.

TABLE II

FLUORESCENCE POLARIZATION STUDIES

Values are means ± S.E.

Probe	Preparation	Number of preparations	Mean fluorescence anisotropy, r, at 25°C	Anisotropy parameter, $((r_0/r)-1)^{-1}$, at 25°C
Diphenylhexatriene (DPH)	BLM intact	10	0.23 ± 0.02	1.72 ± 0.08
	BLM liposomes	3	0.19 ± 0.02	1.06 ± 0.09
TMA-diphenylhexatriene (TMA-DPH)	BLM intact	3	0.26 ± 0.02	2.82 ± 0.03 [a]
2-Anthroylstearate (2-AS)	BLM intact	3	0.09 ± 0.01	0.45 ± 0.01 [b]
12-Anthroylstearate (12-AS)	BLM intact	3	0.06 ± 0.01	0.26 ± 0.3

[a] $P < 0.05$ compared to corresponding value for intact membrane obtained with DPH.
[b] $P < 0.05$ compared to corresponding value obtained for intact membrane with 12-AS.

by weight of total lipid (Table III), the major neutral lipids were cholesterol and free fatty acids. The major phospholipids were phosphatidylcholine (35.2%), phosphatidylethanolamine (26.9%) and sphingomyelin (12.4%). Analysis of the total fatty acids of this membrane by GLC (Table IV) revealed that the major fatty acids present were palmitic (16 : 0) (28.4%), oleic (18 : 1) (24.7%), stearic (18 : 0) (13.2%) and linoleic (18 : 2) (12.6%) acid.

Electron microscope appearance of the 30/40 fraction

The 30/40 fraction showed fairly uniform membrane vesicles with no significant contamination of structures or particles suggestive of other subcellular fractions. The vesicles appear surrounded by well preserved bilaminar membranes (not shown).

Discussion

The procedure described for the isolation of basolateral membranes from rat proximal colonic epithelium is simple, reproducible and rapid (requiring 8–9 h for completion). An approximate yield of 15% of this membrane is obtained, based on $(Na^+ + K^+)$-ATPase and ouabain-sensitive K^+-phosphatase recovery, with a 10–14-fold purification over homogenate.

TABLE III

COMPOSITION OF LIPID EXTRACTS OF RAT BASOLATERAL MEMBRANES

Values are means ± S.E. for lipid extracts of nine determinations using five preparations of basolateral membranes.

Component	% (w/w) of total lipid of basolateral membrane
Cholesterol	11.7 ± 0.8
Cholesterol esters	2.1 ± 0.4
Triacylglycerols	0.7 ± 0.4
Fatty acids	11.9 ± 1.8
Phosphatidylcholine	35.2 ± 0.3
Lysophosphatidylcholine	0.2 ± 0.1
Sphingomyelin	12.4 ± 2.4
Phosphatidylethanolamine	26.9 ± 2.5

TABLE IV

COMPOSITION OF TOTAL FATTY ACIDS OF RAT PROXIMAL COLONIC BASOLATERAL MEMBRANES

Values are means ± S.E. for lipid extracts from nine preparations of proximal basolateral membranes.

Fatty acid	% by mass	Fatty acid	% by mass
12 : 0	0.97 ± 0.21	18 : 1	24.69 ± 0.61
12 : 1	0.30 ± 0.06	18 : 2	12.64 ± 0.42
14 : 0	1.61 ± 0.17	20 : 0	0.53 ± 0.24
14 : 1	1.02 ± 0.13	20 : 1	0.34 ± 0.19
16 : 0	28.42 ± 0.66	20 : 3	2.12 ± 0.25
16 : 1	1.87 ± 0.17	20 : 4	9.31 ± 0.41
18 : 0	13.18 ± 0.57		

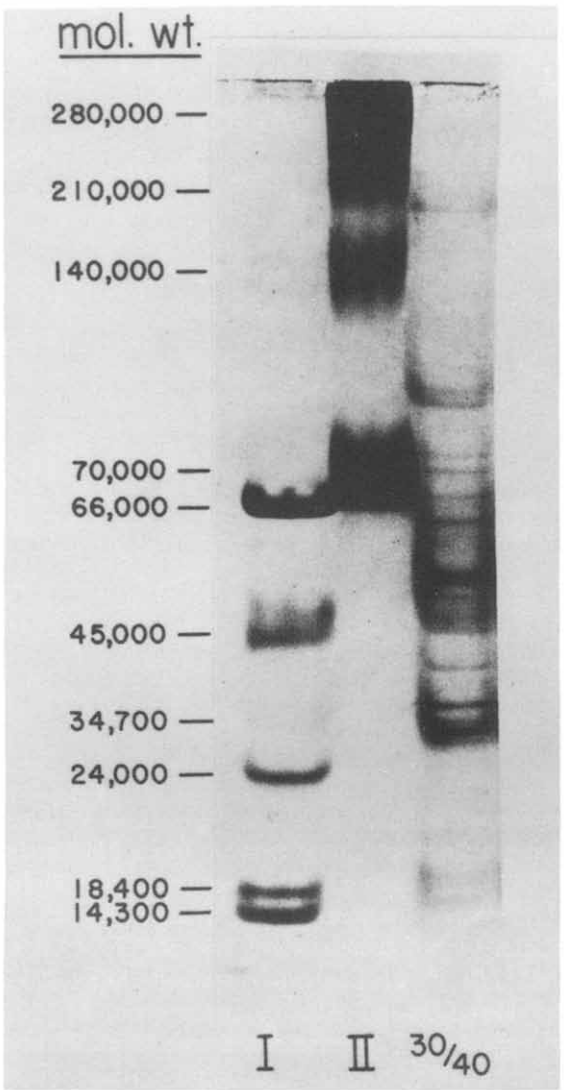

Fig. 1. SDS-polyacrylamide gel electrophoresis. Gels I and II refer to low molecular and high molecular weight standards, respectively. 30/40 refers to the basolateral membrane fraction. The molecular weights and arrows correspond to standard proteins. Further details are given in Materials and Methods.

The use of these enzyme activities as markers for the basolateral membrane appears valid. Ouabain-sensitive K^+-phosphatase has previously been shown to be a partial activity of ($Na^+ + K^+$)-ATPase and with a single exception [37], has been shown to be present only on the contralumenal membrane of all epithelial cells studied to date [38], including the rat small intestinal enterocyte. Additionally, using histochemical methods, Vengesa and Hopfer [39] have localized this enzyme exclusively to the basolateral membrane of rat and rabbit colonic epithelial cells.

A number of procedures for isolation of this membrane from rat enterocytes have used scraped mucosa as starting material. This was specificity avoided in the present procedure, since colonic mucosa contains a marked heterogenity of cell types [13,14]. The only previous published method [15], to date, for isolation of basolateral membranes from mammalian colon used scraped mucosa as starting material. The membranes obtained in that study most likely represented a mixture of membranes from goblet and epithelial rat colonic cells.

The successful isolation of an enriched population of epithelial cells in rat proximal colon, while avoiding enzymatic digestion, was an important prerequisite for the current studies. Enzymatic digestion has been utilized in a number of studies [40–44] for isolation of normal and malignant colonic epithelial cells. These agents are potentially injurious to the constituents of the surface membrane [45,46] and may alter important cellular activities [47–49]. While all cell dissociation methods perturb cell membranes to some extent, recent studies by Eade et al. [50], suggest that the present method employed appears to do so much less than other procedures *. This should certainly enhance the suitability of membranes prepared from such cells for compositional analysis.

Previous studies [2,3,51] have demonstrated the physiologic importance of lipid fluidity in both antipodal membranes of the rat enterocyte. The contralumenal membrane was shown to have a higher lipid fluidity than its apical counterpart [2,3], which appeared to be due to differences in membrane composition [2,3].

In the present report, steady-state fluorescence polarization studies using the fluorophore DPH, demonstrated anisotropy parameter values of 1.72 ± 0.08 and 1.06 ± 0.09 at 25°C for colonic basolateral membranes and their liposomes, respectively. These values are similar to those previously obtained [2] for rat enterocyte basolateral mem-

* We have previously demonstrated [17] that basal and NaF-stimulated adenylate cyclase activity is intact in these cells, further supporting this contention.

branes and their liposomes, indicating that colonic membranes also possess a high lipid fluidity. Furthermore, the ratio of anisotropy values for intact basolateral membranes/liposomes of colonic cells and enterocytes is 1.6 and 1.9, respectively, suggesting that each membrane's lipid fluidity is influenced approximately to the same extent by membrane proteins.

Earlier studies in model bilayers and natural membranes [52,53], including the plasma membranes of rat enterocytes [2,3], have correlated lipid fluidity with differences in composition. High molar ratios of cholesterol/phospholipid and sphingomyelin/phosphatidylcholine as well as increased ratio of protein/lipid (w/w) have all been associated with decreased lipid fluidity. Phosphatidylcholine, phosphatidylethanolamine and sphingomyelin together accounted for approx. 70% of the total neutral and phospholipid by weight of this membrane. This membrane is also enriched in free cholesterol and fatty acids. The lipid composition of these membranes is similar to published values for mouse [54] and rat [3] basolateral membranes isolated from enterocytes. Furthermore, the molar ratios of cholesterol/phospholipid (0.4 ± 0.1), sphingomyelin/phosphatidylcholine (0.4 ± 0.1) and protein/lipid ratio (w/w) (0.4 ± 0.1) are also close to those obtained for small intestinal basolateral membranes [2,3]. The predominant fatty acids of this membrane are the same as those small intestinal basolateral membranes (unpublished observations).

This membrane possesses a complex protein pattern on slab gel electrophoresis. Similar complex patterns, have been demonstrated for the contralumenal membranes of rat enterocytes [34,55].

In summary, basolateral membranes from an enriched population of rat proximal colonic epithelial cells have been isolated and partially characterized. A basic similarity between these membranes and contralumenal membranes from rat enterocytes is suggested. Further characterization of the lipid dynamics and composition of these membranes is now in progress in our laboratory.

Acknowledgements

This paper is dedicated to the memory of Dr. Daniel V. Kimberg. The authors are grateful to Dr. R.M. Glickman for his support and helpful criticisms of this work, as well as his critical review of this manuscript. The authors would also like to thank Drs. M. Lee and S. Chien for the excellent electron microscope photographs. The authors are grateful to Ms. Sandra McGriff for her excellent secretarial support. This investigation was supported by PHS grant number CA 28040 awarded by the National Cancer Institute, DHHS.

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