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Regional differences in the lipid composition and fluidity of rat colonic brush-border membranes

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The lipid composition and fluidity of brush-border membranes prepared from rat proximal and distal colonocytes were determined. Fluidity, as assessed by steady-state fluorescence polarization techniques using the fluorophores 1,6-diphenyl-1,3,5-hexatriene, DL-2(9-anthroyl)stearic acid and DL-12(9-anthroyl)stearic acid, was decreased in distal compared to proximal plasma membranes. This pattern was similar to that previously described for both antipodal plasma membranes in rat enterocytes of the small intestine. The decrease in fluidity of the distal as compared to the proximal membranes resulted from an increase in cholesterol content, cholesterol/phospholipid molar ratio and degree of saturation of the fatty acid residues in the distal membranes. The specific activities of total alkaline phosphatase and cysteine-sensitive alkaline phosphatase, enzymes previously shown to be functionally dependent on the physical state of the colonic brush-border membrane's lipid, were also significantly lower in distal as compared to proximal clonic plasma membranes. These studies, therefore, demonstrate that differences in the lipid fluidity, lipid composition and certain enzymatic activities exist in brush-border membranes prepared from rat proximal and distal colonocytes. the regional variation in rat colonic luminal membrane lipid fluidity and composition may, at least partially, be responsible for differences in these enzymatic activities as well as in sodium and water absorption along the length of this organ.

Introduction

The enterocyte, the predominant cell type lining the small bowel, is differentiated along the length of the small intestine for specialized functions. Corresponding to this functional specialization, regional differences in the composition and the 'lipid fluidity' ** of the rat enterocyte luminal membranes has been used in different ways by various authors. Several investigators have used it in a general sense to express the relative motional freedom of the lipid molecules or substituents thereof, combining in one term the concepts of both extent of movement and rates of movement [30]. As evaluated by steady-state fluorescence polarization of lipid fluorophores, 'fluidity' is assessed via the fluorescence anisotropy, r, without further resolution of the components which determine r. Prior studies with the rod-like fluorophore diphenylhexatriene, have demonstrated that the rotations of this probe are hindered in both artificial and biological membranes [41–45]. Therefore, the fluorescence anisotropy of such a fluorophore is not adequately described by the Perrin equation [30] but by a modified relationship [17,18], $r = r_\infty + (r_0 - r_\infty)[T_c/(T_c + T_f)]$ (see text). Heyn [17] and Jähnig [18] have suggested that the term fluidity be applied only to changes in the rate of

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** The term 'lipid fluidity' as applied to anisotropic bilayer

(microvillus) and contraluminal (basolateral) membranes have been demonstrated [1–5]. Both antipodal membranes of the distal, ileal mucosa contain more cholesterol [3,4] and are less fluid than their proximal counterparts.

The colonocyte, like the enterocyte, is also differentiated along the length of the large intestine for specialized functions such as sodium and water absorption [6–9]. Recently, our laboratory has isolated brush-border and basolateral membranes from rat colonocytes and examined the lipid dynamics and lipid-protein interactions of these membranes [10–12]. Basic similarities in composition and lipid dynamics between these colonic antipodal membranes and those isolated from rat enterocytes were shown [11,12].

The present studies were undertaken to determine whether brush-border membranes of colonocytes varied in lipid composition and fluidity along the length of the large intestine as well as to more completely characterize these membranes in the proximal and distal colonic segments. The results of these experiments demonstrate that distal luminal membranes have a lower lipid fluidity than their proximal counterparts. Furthermore, it appears that differences in the lipid composition of these membranes explain the variations in membrane fluidity between colonic segments. These findings as well as a discussion of their functional significance serve as the basis for the present report.

Materials and Methods

Isolation of colonic epithelial cell brush-border membranes. Male albino rats of the Sherman strain weighting 250–300 g were fasted 18 h with water ad libitum before being killed. The animals were killed rapidly by cervical dislocation and their colons excised. the cecum was discarded, the remaining large intestine divided into two parts: 'proximal and distal' [13]. Epithelial cells relatively devoid of goblet cells, were then obtained from each segment using a technique which combined chelation of divalent cations with mild mechanical dissociation as described [10].

The cells from each segment were pooled separately and used to isolate brush-border membranes as previously described [12]. Purity of the membrane preparations was assessed by the marker enzymes total alkaline phosphatase (*p*-nitrophenylphosphatase) and cysteine-sensitive alkaline phosphatase [12]; specific activity ratios ((purified membrane)/(original homogenates)) ranged from 15 to 20 for these enzymes as described previously [12]. The corresponding values for NADPH-cytochrome *c* reductase, succinate dehydrogenase and sodium-potassium dependent adenosine triphosphatase ($(Na^+ + K^+)$-ATPase), marker enzymes for microsomal, mitochondrial and basolateral membranes, respectively, ranged from 0.50 to 2.00 in all membrane preparations [12]. Protein was estimated by the method of Lowry et al. [14]. Liposomes were prepared from extracted lipids as described in Ref. 15.

Fluorescence polarization studies. Three fluorophores were used: 1,6-diphenyl-1,3,5-hexatriene, DL-2-(9-anthroyl)stearic acid (2-AS) and DL-12-(9-anthroyl)stearic acid (12-AS). All compounds were obtained from aldrich chemical Co. or Molecular Probes Inc. Steady-state fluorescence polarization studies were performed using a Perkin-Elmer 650-40 spectrofluorometer adapted for fluorescence polarization. The methods used to load the membranes and liposomes and the quantification of the polarization of fluorescence have been described [2,5,16]. the results were obtained according to the modified Perrin relationship [17,18]: $r = r_\infty + (r_0 - r_\infty)[T_c/(T_c + T_f)]$, where r_0 is the maximal limiting anisotropy, taken as 0.365 for diphenylhexatriene and 0.285 for the anthroyloxy probes [2], r_∞ is the limiting hindered anisotropy, T_c is the correlation time and T_f is the mean lifetime of the excited state. Values for r_∞ for DPH were calculated from r values as previously described by Van Blitterswijk et al. [19]. The static component of membrane fluidity was assessed by an order parameter, S, where $S = (r_\infty/r_0)^{1/2}$ as described previously [17–19]. The temperature dependence of the anisotropy, r, of diphenylhexatriene, was determined over the range of

rotation as assessed by T_c, while changes in r_∞ are related to an order parameter. Changes in r, due to T_c, r_∞, or both, in many biological applications, appear to have significance and a broad term is needed to designate them. In this report, therefore, fluidity is used in a general sense to denote the lipid's 'motional freedom'.

0–40°C for membranes and liposomes as described in Ref. 15. The logarithm of the anisotropy was plotted against $1/T$ (K^{-1}) as described [15] to detect thermotropic transitions. No changes in the excited-state lifetimes, as assessed by total fluorescence intensity, were demonstrated using each probe in each membrane or liposome preparation [5].

Composition studies. Total lipids were extracted from the membranes by the method of Folch et al. [20]. Cholesterol and phospholipids were measured by the methods of Zlatkis et al. [11] and Ames and Dubin [22], respectively. the phospholipid composition of the extracts was further examined by thin-layer chromatography according to the procedure of Katz et al. [23]. Fatty acids of the total lipid extracts were derivatized as described by Gartner and Vahouny [24]. Fatty acid methyl esters were determined on a Hewlett-Packard 5790A gas-liquid chromatography equipped with a flame ionization detector and interfaced with a Hewlett-Packard 3390A integrator, using authentic fatty acid methyl esters to identify retention times [24].

Statistical methods. All results are expressed as mean values ± S.E. Paired or unpaired *t*-tests were used for all statistical analysis. A p value < 0.05 was considered significant.

Materials. Fatty acids, methyl esters, GLC columns and lipid standards were all purchased from Applied Science Comp. and/or Supelco. All other materials were obtained from either Fisher Chemical Co. or Sigma Chemical Co. unless otherwise indicated.

Results

Fluorescence polarization studies

The lipid fluidity of distal colonic brush-border membranes, as assessed by steady-state fluorescence polarization techniques using all three fluorophores, was significantly lower than in membranes prepared from the proximal colonic segment (Table I). The fluorophores differ in structure and shape and localize in different domains of the bilayer [25–28]. It should also be noted that in biological and artificial membranes, the structural organization of the lipid bilayer appears to limit the extent of rotation of diphenylhexatriene, therefore, r_∞ values for this probe are high and largely determine r [19]. Other probes such as the anthroxyl derivatives of stearic acid (2-AS and 12-AS) yield relatively low values of r_∞ in bilayer membranes and their r values reflect mainly t_c, i.e. the speed of rotation [29,30]. In the present studies both the static and dynamic components of membrane lipid fluidity, as assessed by r_∞ and S of diphenylhexatriene and r values of 2-AS and 12-AS, were found to be lowest in brush-border membranes prepared from distal colonocytes (Table I). Differences in fluidity were also seen in liposomes

TABLE I

FLUORESCENCE POLARIZATION STUDIES OF BRUSH-BORDER MEMBRANES OF PROXIMAL AND DISTAL COLONOCYTES

Values presented are means ± S.E. of eight separate preparations of each membrane and four separate preparations of each liposome. DPH, diphenylhexatriene. BBM, brush-border membrane.

Probe	Preparation	Anisotropy, r at 25°C	Limiting hindered anisotropy, r_∞, at 25°C	Order parameter, S, at 25°C	Transition temp. (°C)
DPH	Proximal BBM	0.217 ± 0.003	0.189 ± 0.003	0.720 ± 0.006	24.1 ± 0.6
	Proximal liposomes	0.173 ± 0.005	0.131 ± 0.006	0.599 ± 0.010	23.2 ± 0.5
	Distal BBM	0.239 ± 0.003 [a]	0.219 ± 0.003 [a]	0.775 ± 0.005 [a]	27.4 ± 0.9 [a]
	Distal liposomes	0.191 ± 0.005 [a]	0.155 ± 0.005 [a]	0.651 ± 0.009 [a]	26.3 ± 0.7 [a]
2-AS	Proximal BBM	0.109 ± 0.002	–	–	–
	Distal BBM	0.115 ± 0.001 [a]	–	–	–
12-AS	Proximal BBM	0.100 ± 0.001	–	–	–
	Distal BBM	0.105 ± 0.001 [a]	–	–	–

[a] $p < 0.05$ or less compared to proximal value.

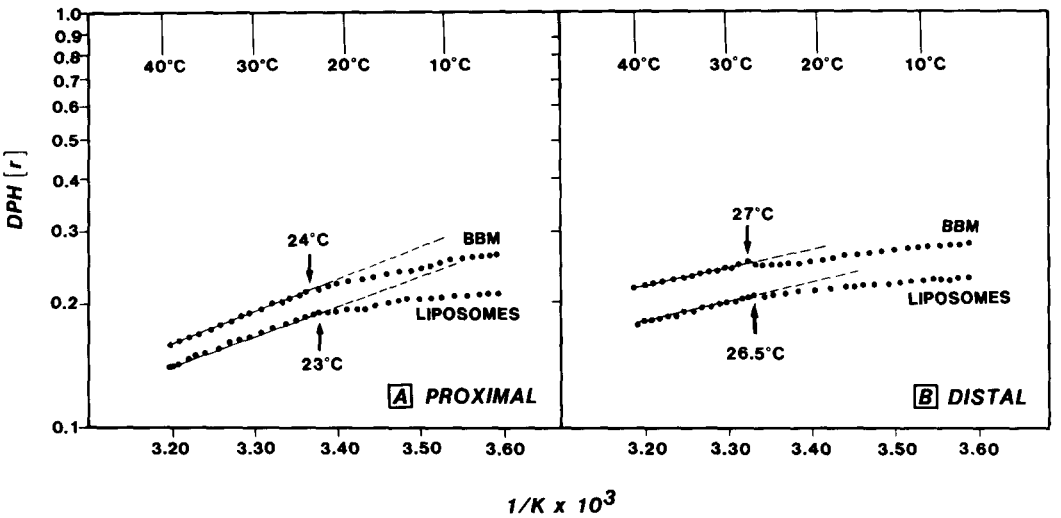

Fig. 1. Representative Arrhenius plots of the anisotropy values of diphenylhexatriene in a sample of isolated brush-border membranes (BBM, upper curve) and in liposomes prepared from a lipid extract of these membranes (lower curve) prepared from (A) proximal rat colonic epithelial cells; (B) distal rat colonic epithelial cells. Numbers of preparations tested and breakpoint temperatures for the individual experiments are listed in Table I. Linear plots shown were determined by the method of least squares.

prepared from these membranes using diphenylhexatriene (Table I).

To determine whether the foregoing regional differences in fluidity were temperature-dependent, Arrhenius plots of the diphenylhexatriene r values against 1/K were examined and the results for both intact membranes and liposomes are illustrated in Fig. 1. The r value of the distal membranes or liposomes exceeded that of the corresponding proximal preparations throughout the temperature range of 0–40°C. As shown in Table I, the breakpoints for proximal preparations (membranes and liposomes) were observed at approximately 23–24°C while for distal preparations they occurred at 26–27°C. As previously described [16], these breakpoints appear to correspond to the lower critical temperatures of broad lipid thermotropic transitions. In agreement with the present results, prior studies have correlated a higher transition temperature with a decreased membrane lipid fluidity [39,40]. Furthermore, values of r of intact membranes consistently exceeded those of the corresponding liposomes, a pattern observed previously in antipodal membranes of rat enterocytes [2–5,16] and ascribed to the effects of the membrane proteins on lipid fluidity.

TABLE II

COMPOSITIONAL ANALYSIS OF LIPID EXTRACTS OF BRUSH-BORDER MEMBRANES PREPARED FROM PROXIMAL AND DISTAL COLONOCYTES

Values are means ± S.E. of eight preparations of each membrane.

Parameter	Proximal	Distal
Cholesterol/phospholipid (mol/mol)	0.80 ± 0.05	0.99 ± 0.03 [a]
Sphingomyelin/phosphatidylcholine (mol/mol)	0.41 ± 0.04	0.50 ± 0.06
Protein/lipid (w/w)	0.89 ± 0.11	0.79 ± 0.09
Saturation index [b]	0.35 ± 0.02	0.39 ± 0.01 [a]
Cholesterol/protein (w/w)	0.32 ± 0.03	0.42 ± 0.02 [a]
Phospholipid-protein (w/w)	0.80 ± 0.03	0.84 ± 0.03

[a] $p < 0.05$ or less compared to proximal values.

[b] Calculated by dividing the total saturated acyl chains by the sum of each unsaturated chain multiplied by the number of double bonds.

Membrane compositional studies

As assessed by steady-state fluorescence polarization, distal rat colonic brush-border membranes appeared to possess a lower lipid fluidity than their proximal counterparts. Prior studies in model bilayers and natural membranes have correlated differences in lipid fluidity with variations in their lipid and protein composition [31–34]. It was, therefore, of interest to examine these composi-

TABLE III

COMPOSITION OF LIPID EXTRACTS OF RAT COLONIC BRUSH-BORDER MEMBRANES

Values presented are means ± S.E. for lipid extracts for eight preparations each of proximal and distal colonic brush-border membranes.

Component	% (w/w) total lipid of brush-border membranes	
	Proximal	Distal
Cholesterol	22.03 ± 1.22	25.48 ± 1.11 [a]
Cholesterol esters	2.73 ± 1.04	2.34 ± 0.99
Triacylglycerols	3.29 ± 2.20	4.07 ± 0.67
Fatty acids	16.68 ± 1.14	16.88 ± 0.98
Phosphatidylcholine	25.57 ± 2.04	21.92 ± 2.16
Lysophosphatidylcholine	1.00 ± 0.10	0.91 ± 0.10
Sphingomyelin	9.86 ± 0.85	10.91 ± 0.87
Phosphatidylethanolamine	18.85 ± 0.87	17.41 ± 0.91

[a] $p < 0.05$ compared to proximal value.

tional parameters in both plasma membranes. As can be seen in Table II, distal brush-border membranes possessed a significantly greater molar ratio of cholesterol/phospholipid and a higher fatty acyl saturation index than proximal membranes. The ratios of sphingomyelin/phosphatidylcholine in (mol/mol) and protein/lipid (w/w) were not statistically different in both membranes.

As shown in Table III, the higher cholesterol/phospholipid molar ratio of the distal membranes was due to a higher cholesterol content. The total phospholipid content was similar in both membranes. the greater saturation index of distal membranes appeared to be due largely to a higher content of palmitic acid (16 : 0) and a lower content of linoleic acid (18 : 2) in the distal membranes when compare to proximal membranes (Table IV). As can be seen in Table III, both membranes were apparently contaminated with about 6% non-membrane lipid (cholesterol esters and triacylglycerols).

Enzymatic activity studies

Prior studies in our laboratory have demonstrated that total alkaline phosphatase and cysteine-sensitive alkaline phosphatase activities were influenced by the physical state of rat colonic brush-border membrane lipid [12]. The specific activities of these enzymes in proximal membranes were found to be 331.2 ± 10.9 and 281.6 ± 8.7 nmol · min^{-1} · (mg protein)$^{-1}$, respectively, whereas, in distal membranes these activities were 257.4 ± 12.6 and 218.5 ± 9.9 nmol · min^{-1} · (mg protein)$^{-1}$ ($N = 8$). Thus, both activities were approximately 30% higher ($p < 0.05$) in membranes that were about 10% more fluid, i.e., the proximal membranes. In agreement with prior studies [12], the v_{max} of these enzymatic activities but not their K_m was higher in proximal membranes (data not shown) as assessed by double-reciprocal plots [35].

To further examine the relationship between fluidity and these membrane enzymatic activities, experiments were performed using benzyl and methyl alcohol. In this regard, prior studies [5,46] have shown that benzyl but not methyl alcohol could increase the fluidity of plasma membranes and, in turn, affect the activities of several membrane-bound enzymes. This ability of alcohols to alter fluidity has been shown to correlate well with their lipid/water partition coefficient [47].

As shown in Table V, 25 mM and 50 mM concentrations of benzyl alcohol increased the fluidity of colonic brush-border membranes by 10 and 15%, respectively, as assessed by diphenylhexatriene r values at 25°C and 37°C, and increased the specific activities of both enzymes by 27% and 45%. At the same concentrations, how-

TABLE IV

COMPOSITIONAL ANALYSIS OF TOTAL FATTY ACIDS OF BRUSH-BORDER MEMBRANES OF RAT PROXIMAL AND DISTAL COLONOCYTES

Values are means ± S.E. for lipid extracts from five preparations of membranes from each cell population.

Fatty acid	% by mass	
	Proximal	Distal
14 : 0	0.40 ± 0.06	0.47 ± 0.11
14 : 1	0.59 ± 0.08	0.79 ± 0.06 [a]
16 : 0	23.17 ± 0.81	25.11 ± 0.41 [a]
16 : 1	1.74 ± 0.28	1.80 ± 0.27
18 : 0	15.54 ± 1.50	16.28 ± 0.56
18 : 1	25.11 ± 0.83	25.82 ± 0.31
18 : 2	18.92 ± 0.91	13.13 ± 0.22 [a]
20 : 4	12.62 ± 1.48	13.78 ± 0.20

[a] $p < 0.05$ or less compared to proximal value.

TABLE V

INFLUENCE OF BENZYL AND METHYL ALCOHOL ON THE FLUIDITY AND ACTIVITIES OF TOTAL ALKALINE PHOSPHATASE AND CYSTEINE-SENSITIVE ALKALINE PHOSPHATASE OF RAT COLONIC BRUSH-BORDER MEMBRANES

Values presented means ± S.E. of four separate experiments. DPH, diphenylhexatriene.

Preparation	Anisotropy, r_∞ of DPH at 25°C	Anisotropy, r of DPH at 37°C	Total alkaline phosphatase (nmoles·(mg protein)$^{-1}$·min^{-1}) at 37°C	Cysteine-sensitive alkaline phosphatase (nmoles·(mg protein)$^{-1}$·min^{-1}) at 37°C
Control	0.226 ± 0.004	0.158 ± 0.004	290.1 ± 10.2	246.3 ± 8.4
25 mM benzyl alcohol	0.205 ± 0.003 [a]	0.1453 ± 0.003 [a]	368.0 ± 10.3 [a]	312.4 ± 9.1 [a]
25 mM methyl alcohol	0.225 ± 0.003	0.158 ± 0.003	287.2 ± 9.8	244.4 ± 11.3
50 mM benzyl alcohol	0.192 ± 0.004 [a]	0.134 ± 0.004 [a]	421.5 ± 16.0 [a]	360.6 ± 12.4 [a]
50 mM methyl alcohol	0.224 ± 0.005	0.157 ± 0.004	296.1 ± 11.2	251.3 ± 10.6

[a] $p < 0.05$ or less compared to control values.

ever, methyl alcohol failed to influence fluidity or alter these enzymatic activities. These findings further support the contention that the differences in fluidity of these membranes may, at least partially, be responsible for the variation in enzyme activities noted.

Discussion

The present results demonstrate that regional differences in lipid fluidity and composition exists in brush-border membranes of rat colonocytes. Both the static and dynamic components of membrane lipid fluidity were lowest in brush-border membranes prepared from distal colonocytes. Several differences in composition accounted for the decreased fluidity of the distal membranes. The cholesterol content and cholesterol/phospholipid ratio of the lipid increased distally. The saturation index of the fatty acids also increased in the distal membranes. The latter increased, owing largely to a reduction in linoleic acid and an increase in palmitic acid. Each of these compositional differences would be expected to lead to a decrease in fluidity in the distal membranes [31–34].

The higher content of linoleic acid in proximal brush-border membranes, deserves further comment. Proximal rat colonic basolateral membranes [11] as well as both proximal antipodal membranes of rat enterocytes of the small intestine [4] have been shown to have higher levels of this fatty acid than their distal counterparts. The higher content of linoleic acid in these membranes may result from the ingestion of this fatty acid in the diet [36]. Recent studies in our laboratory have, in fact, demonstrated that a diet highly enriched in linoleic acid elevated the content of this acyl residue in plasma membranes of all segments of the gut including basolateral membranes of the colon and eliminated the regional differences in its content [36].

In the present experiments the specific activities of the brush-border membrane enzymes, total alkaline phosphatase and cysteine-sensitive alkaline phosphatase, were significantly higher in the proximal as compared to the distal colonic mucosa. This is interesting in view of prior studies in our laboratory which demonstrated that these activities varied concordantly with colonic brush-border membrane fluidity [12]. It bears emphasis, however, that the number of enzyme units was not monitored in the present experiments. Therefore, the differences in specific activities of these enzymes could result from changes in the number of units and/or from alterations in the function of each unit by fluidity. The present experiments utilizing benzyl and methyl alcohol, however, do lend further support to the contention that differences in fluidity may at least partially, be responsible for these enzymatic alterations in these membranes. Resolution of this issue will require further examination but regardless of the mechanisms involved, the present data demonstrate that

regional differences in these enzymatic activities of brush-border membranes exist in rat colon.

The difference in lipid fluidity of these colonic luminal membranes may also be related to the well-recognized functional specialization of the colon for net absorption of sodium [6–9]. Passive permeability of sodium in the proximal colon is approximately 2–3-times higher than in the distal rat colon [7–9]. In this regard, prior investigations have correlated an increase in fluidity with an increase in permeability to sodium [37,38]. Recently, our laboratory has also correlated increases in fluidity with increases in water permeability in rat colonic brush-border membrane vesicles (unpublished observations). While further studies will be necessary to clarify this issue, it would seem reasonable to suggest that, in addition to other factors [6–9], the regional variation in colonic luminal membrane fluidity may be responsible for differences in sodium and water absorption along the length of this organ.

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