Lipid composition and membrane fluidity of monkey small intestinal brush border membrane: Regional differences

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Brush border membrane vesicles (BBMV) isolated from various regions of monkey small intestine were compared for lipid composition, membrane fluidity and sodium-dependent D-glucose transport. Total phospholipid content was same in all regions whereas cholesterol content was high in duodenum and jejunum as compared to ileum resulting in higher cholesterol/phospholipid molar ratios. Regional differences in individual phospholipid subclasses and fatty acids in total lipids were also observed. Fluidity measurements showed that the membranes of duodenum and jejunum were less fluid than ileum. The change in BBMV fluidity appears to be due to alteration in cholesterol/phospholipid ratio and phospholipid composition. The sodium dependent D-glucose uptake was more in duodenum and jejunum as compared to ileum. These results suggest that there is a regional difference in the lipid composition and fluidity of BBMV in monkey small intestine which may influence their function.

The small intestine is a highly specialized organ and is differentiated along the length of the intestine for carrying out various functions. Enterocytes, the absorptive cells of the small intestinal mucosa, are characterized by the presence of brush border, controlling the passage of dietary products after intraluminal digestion. The morphology of the brush border is fairly constant along the small intestine, although the role played during absorption by the three parts of the intestine namely duodenum, jejunum and ileum is quite different. The membrane lipids play an important role in modulating the function including enzyme activity and transport1-2 often by altering the membrane fluidity3. Studies have shown that, BBMV proteins can be classified functionally and structurally as intrinsic or extrinsic to the membrane. BBMV hydrolases like, disaccharidases and leucineaminopeptidase are extrinsic proteins since their activities are not influenced by the membrane lipids. On the other hand, alkaline phosphatase and D-glucose transporter are intrinsic proteins because their activities are linked structurally and functionally with the physical state of the membrane lipids4-5. Studies have shown that rodent BBMV lipid composition and fluidity change along the length of the small intestine and colon6-8. The present study looks at the lipid composition and membrane fluidity of BBMV isolated from various region of the monkey small intestine and their influence on the D-glucose uptake.

Materials and Methods

Tris, Heps, bovine serum albumin (BSA), standard lipids, pyrene and 1,6-diphenyl hexatriene (DPH) were all purchased from Sigma Chemical Company, St. Louis, Missouri (USA). [14C]-D-glucose was obtained from Bhabha Atomic Research Centre, Bombay. All other chemicals used were of analytical grade.

Isolation of BBMV—Overnight fasted monkeys (Macaca radiata) were killed by over-dose of pentobarbitone injection. Small intestine was removed, washed with ice cold saline and duodenum, jejunum and ileum were separated. Mucosa was scraped using a glass slide and BBMV were prepared by divalent cation precipitation6. The final pellet was suspended in 10 mM Heps/Tris buffer pH 7.5 containing 300 mM mannitol. Purity of the isolated BBMV was checked by enrichment of marker enzymes alkaline phosphatase and sucrase9,10. Protein was measured using BSA as standard12.

Lipid analysis—Lipids were extracted from BBMV by the method of Bligh and Dyer13. Neutral lipids were separated on silica gel G plates using the solvent system n-hexane : diethyl ether : acetonic acid (80:20:1, V/V). Spots were identified after exposure to iodine,
scraped, eluted and dried using dry nitrogen. Cholesterol and di and triglycerides were estimated as described14-15. Free fatty acids were converted to methyl esters and quantitated after separation using gas chromatograph fitted with 5% EGSS-X column. Heptadecanoic acid was used as internal standard. Total phospholipids were estimated by phosphate estimation after acid digestion16. Individual phospholipids were separated on silica gel H plate using the solvent system chloroform : methanol : acetic acid : water (25:15:4:2 V/V). Fluorescence polarization studies—Fluorescence measurements were done by Shimadzu RF-5000 spectrophotometer. Both steady state fluorescence polarization using DPH and excimer fluorescence intensity using pyrene were used to estimate relative membrane fluidity17. For steady state fluorescence, a 2 mM stock solution of DPH in tetrahydrofuran was prepared and stored protected from light at −20°C. Just prior to use, an aqueous suspension of the probe was prepared by diluting DPH stock solution in 2000 volumes of 0.02 M phosphate buffered saline (pH 7.4) and stirred vigorously for 2-3 hrs at 25°C until no odour of tetrahydrofuran could be detected. The resulting suspension of 2 µM DPH was clear and devoid of fluorescence. BBMV equivalent to 150 µg protein was incubated in 3 ml of DPH suspension at 37°C for 1 hr. Fluorescence emission intensities (excitation wavelength 360 nm, emission wavelength 430 nm) were recorded and expressed as fluorescence anisotropy (r) determined from the equation

\[ r = (I_{22} - I_1) / (I_{22} + 2I_1) \]

where \( I_2 \) and \( I_1 \) are fluorescence intensities parallel and perpendicular to the excitation plane.

Excimer fluorescence intensity was studied using pyrene as the probe. Pyrene 100 µM (final concentration) was incubated with BBMV corresponding to 2 mg protein in 0.1 M Tris-HCl buffer pH 7.1 for 2 hr at 37°C. After incubation, it was centrifuged at 48000 × g for 30 min and the pellet was washed twice with 10 mM Tris-HCl buffer, pH 7.1 and resuspended in the same buffer. An aliquot corresponding to 100 µg protein in a total volume of 2 ml of 0.1 M Tris-HCl buffer pH 7.1 was used for fluorescence measurements. Fluorescence intensity of the incorporated pyrene was measured at an excitation wavelength of 340 nm, the monomer fluorescence was measured at 390 nm and the excited dimer or excimer fluorescence at 495 nm. Estimation of excimer/monomer fluorescence intensity ratio provides a convenient index of fluidity.

Measurements of D-glucose uptake—D-glucose uptake was done by rapid filtration technique using Millipore filter (pore size 0.45 µm)18. Uptake was initiated using 40 µl of BBMV corresponding to 100 µg protein. This was incubated with 160 µl uptake buffer (150 mM NaSCN, 10 mM Hepes/Tris buffer, pH 7.5) containing 50 µM [14C]-D-glucose (0.8 µCi). At various time intervals the uptake was stopped by adding 3 ml of ice cold stop buffer (150 mM NaCl, 10 mM Hepes/Tris buffer pH 7.5) and filtered rapidly through membrane filter under constant vacuum. The filter was washed three times with 5 ml of stop buffer and counted using LKB Rackbeta scintillation counter.

Results

BBMV isolation

Nearly 15-18 fold purification was achieved on the BBMV prepared from various regions of the monkey small intestine as judged by marker enzymes alkaline phosphatase and sucrase. The marker enzyme enrichment was almost identical in all the three regions suggesting that BBMV prepared from three regions are comparable. The activities of the BBMV marker enzymes were slightly high in duodenum and jejunum compared to ileum (data not shown).

BBMV lipid composition

The lipid composition of the BBMV isolated from various regions of the monkey small intestine is shown in Table 1. The total phospholipid was almost identical in all the three regions but the cholesterol (both free and ester) was high in duodenum and jejunum as compared to ileum. This resulted in higher cholesterol/phospholipid ratio in duodenum and jejunum than ileum. Di- and triglycerides remained unchanged. Nonesterified fatty acids formed a considerable portion of BBMV lipids and their level was higher in the distal intestine as compared to the proximal part. Fig. 1 shows the fatty acid compo-

<table>
<thead>
<tr>
<th>Lipid Class</th>
<th>Duodenum (µg/mg protein)</th>
<th>Jejunum (µg/mg protein)</th>
<th>Ileum (µg/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phospholipids</td>
<td>624 ± 13.1</td>
<td>636 ± 24.3</td>
<td>614 ± 19.1</td>
</tr>
<tr>
<td>Free cholesterol</td>
<td>391 ± 22.3</td>
<td>396 ± 20.6</td>
<td>323 ± 28.3</td>
</tr>
<tr>
<td>Cholesterol ester</td>
<td>20 ± 0.3</td>
<td>16 ± 0.2</td>
<td>9 ± 0.1</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>33 ± 0.9</td>
<td>36 ± 0.2</td>
<td>37 ± 0.2</td>
</tr>
<tr>
<td>Dihydrated</td>
<td>35 ± 0.2</td>
<td>38 ± 0.3</td>
<td>31 ± 0.2</td>
</tr>
<tr>
<td>Free fatty acids</td>
<td>243 ± 17.8</td>
<td>266 ± 16.4</td>
<td>292 ± 22.9</td>
</tr>
<tr>
<td>C/P ratio</td>
<td>0.658</td>
<td>0.654</td>
<td>0.541</td>
</tr>
</tbody>
</table>

*p < 0.001 as compared to jejunum and duodenum. *p < 0.05 as compared to jejunum and duodenum. C/P ratio, Cholesterol/Phospholipid molar ratio.
major fatty acids were palmitic, stearic, oleic, linoleic and arachidonic acids. Content of stearic acid was higher and oleic acid lower in duodenum and jejunum as compared to ileum and this resulted in higher unsaturation in ideal lipids. Composition of BBMV phospholipids from various regions are shown in Fig. 2. The major phospholipids were phosphatidylcholine and phosphatidylethanolamine. Among the individual phospholipids, ileum had lower phosphatidylethanolamine and slightly higher phosphatidylcholine as compared to duodenum and jejunum. Other phospholipids were similar in all regions.

**Membrane fluidity**

Membrane fluidity was evaluated using the lipid soluble probes DPH which is a probe for rotational mobility and pyrene which is a probe for lateral mobility. As shown in Table 2, the fluorescence anisotropy of DPH was high in duodenum and jejunum as compared to ileum suggesting that the BBMV isolated from ileum were more fluid. The ratio of excimer/monomer intensities of pyrene was found to be more in ileum again suggesting that membranes isolated from ileum were more fluid than duodenum and jejunum.

**Table 2—Fluorescence anisotropy of DPH and excimer/monomer ratio of pyrene in BBMV prepared from various regions of small intestine**

<table>
<thead>
<tr>
<th>Region</th>
<th>Fluorescence anisotropy with DPH</th>
<th>Excimer/Monomer ratio of pyrene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum</td>
<td>0.255±0.003</td>
<td>0.214±0.006</td>
</tr>
<tr>
<td>Jejunum</td>
<td>0.256±0.003</td>
<td>0.214±0.006</td>
</tr>
<tr>
<td>Ileum</td>
<td>0.231±0.006*</td>
<td>0.233±0.006*</td>
</tr>
</tbody>
</table>

*p <0.001* as compared to duodenum and jejunum.

**D-glucose transport**

D-glucose uptake along the various regions of the monkey small intestine showed rapid over-shoot phenomenon in all the regions (Fig. 3). However, the uptake was more in duodenum and jejunum as compared to ileum.

**Fig. 1—Fatty acid composition of BBMV lipids in various regions of the monkey small intestine. Each value represents mean ±SD of three separate estimations. Values shown for fatty acids 18:0 and 18:1 of ileum are significantly different compared to duodenum and jejunum (p <0.01). ( (): duodenum; ( (): jejunum; ( (): ileum.)**

**Fig. 2—BBMV phospholipid composition in various regions of the monkey small intestine. Each value represents mean ±SD of three separate estimations. SM—Sphingomyelin; PC—Phosphatidylcholine; PE—Phosphatidylethanolamine; PS—Phosphatidylserine; PI—Phosphatidylinositol; LPC—Lysophosphatidylcholine. Values shown for PC and PE of ileum are significantly different compared to duodenum and jejunum (p <0.01). ( (): duodenum; ( (): jejunum; ( (): ileum).**

**Fig. 3—Comparison of D-glucose uptake by BBMV prepared from various regions of the monkey small intestine. Each value represents mean ±SD of three separate estimations. ( (): duodenum; ( (): jejunum; ( (): ileum).**
Discussion

The results presented here demonstrate regional difference in the lipid composition, membrane fluidity and sodium dependent D-glucose transport of BBMV isolated from monkey small intestine. Earlier studies have shown species variation in BBMV lipid composition and membrane fluidity in different regions. In the case of rat and rabbit, membranes of distal intestine were less fluid due to increased content of cholesterol, a greater cholesterol/phospholipid ratio and higher degree of saturation in the phospholipid acylchain. Studies with human small intestine have shown that the distal portion was more fluid mainly due to regional change in cholesterol content. The present results with monkey intestine agree with that of human studies. It has been shown in rat that endogenous cholesterol synthesis is greater in enterocytes of distal intestine as compared to the proximal intestine which was suggested to be responsible for high cholesterol content of the distal BBMV. This may also be true in the case of monkey where high cholesterol content in duodenum and jejunum may be due to increased biosynthesis. Normally very little nonesterified fatty acids (NEFA) are present in biomembranes and BBMV isolated from monkey small intestine showed higher content of NEFA as part of the total lipids. This may be unique to intestinal membranes since a similar high content of NEFA has been reported earlier. The high content of NEFA in intestinal BBMV protect these membranes from lipid peroxidation.

Physical properties of the membrane such as fluidity are influenced by the lipid composition and in the monkey BBMV, regional difference in the lipid composition was associated with changes in membrane fluidity. Studies with artificial and biological membranes indicated an inverse relationship of cholesterol/phospholipid ratio with fluidity. Membrane fluidity is also determined by the phospholipid composition and degree of saturation of fatty acyl side chain of the phospholipids. The higher fluidity of the ileal BBMV may be due to lower cholesterol/phospholipid ratio or high content of unsaturated fatty acids in phospholipid acyl chain or low content of phosphatidylethanolamine. But studies have shown that there is no simple relationship between number of fatty acid double bonds and membrane fluidity. It is likely that the lower cholesterol phospholipid ratio and phosphatidylethanolamine content of ileal BBMV may be the major factors responsible for its higher fluidity.

The regional alteration in lipid composition and membrane fluidity may influence the absorptive function of BBMV. Earlier studies with the small intestine have shown dependence of several enterocyte membrane function on membrane fluidity. In vitro fluidization of BBMV by n-aliphatic alcohol alters the sodium dependent D-glucose transport. In vivo dietary trisley-glycerol induced modification of BBMV lipid composition and fluidity also alters the sodium dependent D-glucose transport. It has been demonstrated that the regional change in intestinal calcium transport and lipid permeability correlate with regional difference in membrane fluidity. In conclusion this study has shown regional differences in the lipid composition and fluidity of BBMV in monkey small intestine and the regional differences seen in sodium dependent D-glucose transport may be due to these alterations.

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