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Mechanism of folate transport across the human colonic basolateral membrane

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Dudeja, P. K., A. Kode, M. Alnounou, S. Tyagi, S. Torania, V. S. Subramanian, and H. M. Said. Mechanism of folate transport across the human colonic basolateral membrane. *Am J Physiol Gastrointest Liver Physiol* 281: G54–G60, 2001.—Previous studies from our laboratory have demonstrated the existence of a folate transporter in the human colonic apical membranes. The current studies were undertaken to examine the possible presence and function of a folate carrier in the human colonic basolateral membrane vesicles (BLMV). BLMV were purified from mucosal scrapings of colons of organ donors by a Percoll-density gradient centrifugation technique, and uptake studies were performed using a rapid filtration technique. Our results on [³H]Pte-Glu uptake are summarized as follows: 1) uptake was sensitive to osmolarity of the incubation medium; 2) Na⁺ removal from the incubation medium did not affect folate uptake into BLMV; 3) uptake was significantly increased with decreasing incubation buffer pH from 8 to 4; 4) uptake demonstrated saturation kinetics with an apparent Michaelis constant of $9.6 \pm 0.48 \mu\text{M}$ and a maximal velocity of $8.10 \pm 0.36 \text{ pmol} \cdot \text{mg protein}^{-1} \cdot 10 \text{ s}^{-1}$; 5) uptake was markedly inhibited by the structural analog methotrexate (inhibitory constant = $8.28 \pm 1.0 \mu\text{M}$); 6) uptake into BLMV demonstrated a *trans*-stimulation phenomenon; 7) anion exchange inhibitors DIDS and SITS significantly inhibited folate uptake; and 8) uptake was potential-insensitive, as voltage clamping of vesicles or making them inside positive with K⁺/valinomycin failed to influence folate uptake. Western blot analysis using purified human colonic basolateral membrane preparations and specific polyclonal antibodies against the human reduced folate carrier (hRFC) has shown expression of the hRFC protein at this membrane domain. These data demonstrate the existence of a pH-dependent, DIDS-sensitive, electro-neutral, carrier-mediated mechanism for folate transport across the human colonic basolateral membranes.

human colon; colonic folate uptake; uptake mechanism; colonic membrane vesicles

FOLATE IS AN ESSENTIAL micronutrient for normal cellular functions, growth, and development (1). Folate deficiency is one of the most common causes of nutritional anemia (1, 14, 17) and occurs in a variety of conditions, including congenital defect in the uptake process, drug

interaction, alcohol consumption, etc. (4, 5, 7, 9–11, 25, 26). Humans and other mammals cannot synthesize folate and thus must obtain the vitamin from exogenous sources. The intestine is exposed to folate from the following two sources: 1) dietary source, which is absorbed mainly in the proximal region of the small intestine, and 2) bacterial source, where the vitamin is synthesized by normal microflora of the large intestine and is absorbed in that region (please see for review Refs. 14 and 20 and references therein). The mechanism of absorption of dietary folate in the small intestine has been the subject of intense investigation over the past three decades. Much less information is available regarding the mechanism of transport of the bacterially synthesized folate in the large intestine (16). Additionally, the colon may also be exposed to folates arising from the cell turnover.

Recent studies from our laboratory with purified colonic apical membrane vesicles (AMV; see Ref. 6) and cultured colonic epithelial NCM460 cells (13) have characterized the mechanism and regulation of folate transport across the apical membrane (AM) domain of the functionally polarized human colonic epithelial cells. These results have shown the involvement of an efficient carrier-mediated, pH-dependent uptake system that appears to be under the regulation of an intracellular protein- and tyrosine kinase-mediated pathway (6, 13). To date, however, no information is available about the exit process of folate out of colonocytes, i.e., transport across the colonic basolateral membrane (BLM) domain. Such information is of significant physiological importance for detailed understanding of the folate transport process across the polarized colonocytes. In this study, we have examined the mechanism of folate uptake by purified basolateral membrane vesicles (BLMV) isolated from colonic mucosa of organ donors. The results demonstrated the involvement of a pH-dependent, DIDS-sensitive, carrier-mediated uptake system that transports folate across the human colonic BLM via an electroneutral process.

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MATERIALS AND METHODS

Materials

[3,5,7,9-³H]Pte-Glu, sodium salt (sp. act. 25.2 Ci/mmol), was purchased from American Radiolabeled Chemicals (St. Louis, MO). Cellulose nitrate filters (0.65- μ m pore size) were obtained from Sartorius (Hayward, CA). Unlabeled Pte-Glu, methotrexate (amethopterin), SITS, DIDS, amiloride, bumetanide, furosamide, and acetazolamide were obtained from Sigma Chemical (St. Louis, MO). The radiochemical purity of [³H]Pte-Glu used in this study was >97%. All other materials were obtained from either Fisher Scientific (Fairlawn, NJ) or Sigma Chemical, unless otherwise stated, and were of the highest purity available.

Methods

Preparation of human colonic BLMV. These investigations were approved by the Human Investigations Committee of the West Side Veterans Affairs Medical Center and the Institutional Review Board of the University of Illinois at Chicago. Colons from six to eight healthy adult organ donors were obtained immediately after harvest of transplantation organs (provided by Regional Organ Bank of Illinois, Chicago). The cecum was discarded, and the remaining large intestine was divided into the following two equal parts: proximal and distal. The mucosa was scraped from the seromuscular layer of the proximal colon and stored at -80°C. Purified BLMV were prepared using a differential centrifugation method followed by separation on a continuous Percoll gradient as previously described by us (24). All of the steps were carried out on ice to minimize any cellular activity or metabolism. The purity of the membrane vesicles and the degree of contamination with intracellular organelles were assessed by appropriate marker enzyme (24). The specific activity ratios (purified BLMV/crude homogenate) for the BLMV marker enzyme Na⁺-K⁺-ATPase were ~7–11 in all of the membrane preparations. The corresponding values for succinate dehydrogenase, NADPH cytochrome *c* reductase, and cysteine-sensitive alkaline phosphatase, the marker enzymes for mitochondrial and microsomal membranes and AM, ranged from ~0.5 to 2.0 in all membrane preparations. For loading vesicles with various constituents, the desired intravesicular medium buffer was used in the last two centrifugation steps and in all of the resuspension steps for the purification procedure. After the final suspension, the vesicles were used for uptake studies either within 1–2 h of purification or frozen at -80°C for latter use. Membrane protein was assessed as described by Bradford (2) using bovine plasma gamma globulin as standard.

[³H]Pte-Glu uptake studies. [³H]Pte-Glu uptake was measured at 25°C by a rapid filtration technique (12) as described by us previously (6, 18, 19, 24). The membrane vesicles were preloaded with a buffer of 280 mM mannitol and 20 mM Tris-HEPES, pH 7.5. Experiments were started by addition of 80 μ l incubation medium containing [³H]Pte-Glu (final concentration, 0.25 μ M), 280 mM mannitol, and 20 mM Tris-MES, pH 5.0, to 20 μ l of the membrane vesicles (50–70 μ g of membrane protein). The reaction was terminated by the addition of 5 ml of ice-cold stop solution containing 280 mM mannitol and 20 mM Tris-HEPES, pH 7.5, after designated periods of time. The sample was immediately filtered through a 0.65- μ m Sartorius nitrocellulose filter using a Millipore manifold filtration assembly. Filters were then washed three times with 5 ml of ice-cold stop solution and dissolved in Filtercount, and the radioactivity was measured in a Packard TR-1500 liquid scintillation counter. All values

were corrected for [³H]Pte-Glu binding to filters and/or vesicles by subtracting radioactivity present in *time 0* vesicle blanks.

Antibodies and Western blot analysis. The anti-human reduced folate carrier (hRFC) polyclonal antibodies were raised against a synthetic peptide in rabbits by a commercial vendor (Alpha Diagnostic, San Antonio, TX). For designing of the antigenic peptide, the protein sequence of the hRFC was searched for any similarities with other known proteins using the Blast-p algorithm and Swissport database. Because the recently cloned human thiamine transporter has been reported to have some degree of homology with reduced folate carrier (RFC; see Ref. 8), the sequence of that protein was therefore aligned alongside the corresponding RFCs of human, mouse, rat, and hamster (for review, see Ref. 23 and references therein) using the Clustal W algorithm to detect the regions of lowest homology. The regions of human RFC protein showing the lowest homology were then analyzed for hydrophilicity (Kyte-Doolittle hydrophathy plot), antigenicity (Hopp/Woods and Protrusion Index Antigenicity profiles), and accessibility. A specific region of RFC that corresponded to amino acids 1–20 of the human sequence (MVPSSPAVEKQVPVEPGDPP) was determined to be specific for human RFC and therefore was used for raising the polyclonal antibodies. We also confirmed the uniqueness of the selected peptide using the Swissport database to avoid any cross-reactivity of the resulting antibodies with other proteins. The selected peptide was synthesized as keyhole limpet hydrolysate-conjugate and was used for immunization in two rabbits.

Apical membrane and BLM proteins were isolated from human colonic and jejunal mucosa as described previously (6, 19, 18, 24) in the presence of 1 mM phenylmethylsulfonyl fluoride, 1 μ g/ml aprotinin, and 0.5 μ g/ml leupeptin. Protein (150 μ g) was treated with Laemmli sample buffer and resolved on a 10% SDS-polyacrylamide gel. After electrophoresis, the proteins were electroblotted onto an Immun-Blot polyvinylidene difluoride Membrane (Bio-Rad, Hercules, CA) overnight. The blots were washed two times in PBS-Tween 20 for 10 min, blocked with 5% dried milk in PBS-Tween 20 for 1 h at room temperature, and washed with PBS-Tween 20. They were then probed with anti-human RFC polyclonal antibodies (1:3,000 diluted in PBS-Tween 20) for 1 h at room temperature, washed two times in PBS-Tween 20 (Sigma), and reacted with goat anti-rabbit IgG conjugated to horseradish peroxidase (1:3,000 diluted in PBS-Tween 20; Sigma) for 1 h at room temperature. The blots were finally washed two times in PBS-Tween 20 for 10 min each time, and the color was developed using an enhanced chemiluminescence kit (Amersham). Specific bands were quantitated using Eagle Eye Software (Stratagene).

Statistical Analysis

Experiments were performed using three or four freshly isolated membrane preparations from colons of different organ donors. Results are expressed as means \pm SE. Paired or unpaired Student's *t*-tests were used in statistical analysis as appropriate. A *P* value of <0.05 was considered statistically significant.

RESULTS

Effect of Incubation Buffer pH

Folate transport along the human intestinal tract has been shown to have a pH optimum in the acidic range of 5.0–6.0 (14, 20). Therefore, studies were per-

formed to determine [^3H]folate (0.25 μM) uptake (at 15 s; initial rate, see below) by the human colonic BLMVs at different incubation buffer pHs. The pH of the incubation buffer was modified over the range of 4.0–8.0 by changing the concentrations of Tris/MES/HEPES in the incubation buffer. Intravesicular pH was maintained at a pH of 7.5. As shown in Fig. 1, there was a marked increase in the rates of folate uptake into the colonic BLMV upon decreasing incubation buffer pH. Maximum uptake was observed at the pH range of 4–5.

Time Course and Na^+ Dependence of Folate Uptake

In this study, we examined the [^3H]Pte-Glu (0.25 μM) uptake into the BLMV as a function of time both in the presence or absence of an inwardly directed Na^+ gradient (in the absence of Na^+ , K^+ was used). As shown in Fig. 2, the uptake of folate increased linearly with time for up to 30 s. There was no difference in the transport rate of folate in the presence of inwardly directed Na^+ or K^+ gradient at any incubation time examined (Fig. 2).

Additionally, to differentiate between Pte-Glu transport into the intravesicular space from nonspecific binding to membranes, the effect of increasing incubation medium osmolarities on the [^3H]Pte-Glu uptake into the vesicles was examined. Osmolarities were altered by varying the mannitol concentrations in the incubation medium. The results showed a decrease in Pte-Glu uptake with increasing incubation medium osmolarity, and a linear relationship existed between the uptake and the reciprocal of osmolarity (data not shown), suggesting that Pte-Glu uptake was sensitive

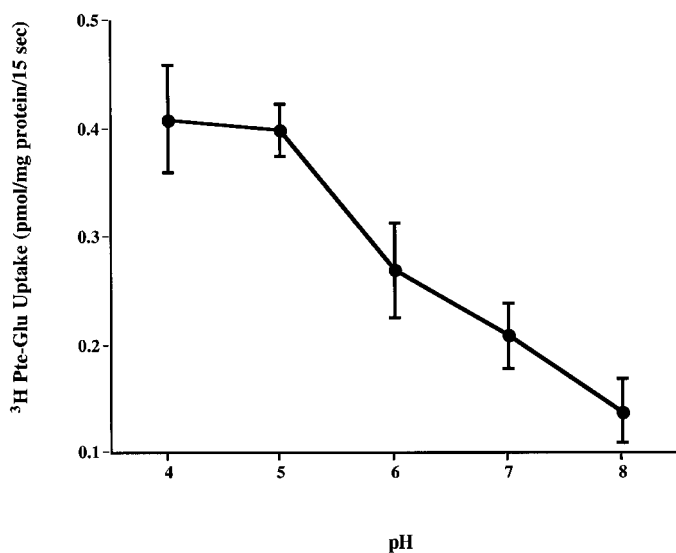


Fig. 1. Effect of extravesicular medium pH on [^3H]Pte-Glu uptake. Colonic basolateral membrane vesicles (BLMV) preloaded with 280 mM mannitol and 20 mM Tris-HEPES, pH 7.5, were incubated with buffers containing 280 mM mannitol and either 20 mM Tris-HEPES (for pH 7.0 and 8.0) or 20 mM Tris-MES (for pH 4.0, 5.0, and 6.0) and 0.25 μM [^3H]Pte-Glu (final concentration). Incubation was performed at 25°C for 15 s. Values represent means \pm SE of 4 separate preparations.

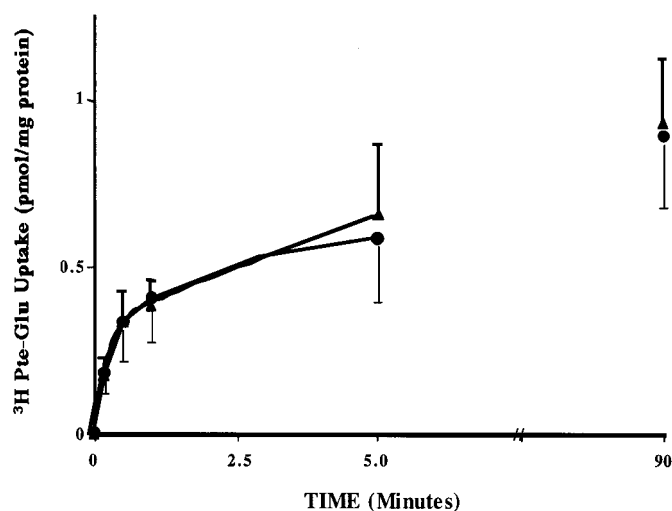


Fig. 2. Time course of the effect of inwardly directed Na^+ and K^+ gradients on [^3H]Pte-Glu uptake. Colonic BLMV preloaded with 280 mM mannitol and 20 mM Tris-HEPES, pH 7.5, were incubated with buffers containing 80 mM mannitol, 20 mM Tris-MES, pH 5.0, 0.25 μM [^3H]Pte-Glu (final concentration), and either 100 mM sodium gluconate (\blacktriangle) or 100 mM potassium gluconate (\bullet) for the indicated time periods at 25°C. Values represent means \pm SE of 3 separate membrane preparations.

to changes in medium osmolarity and therefore was taken up into the closed intravesicular space with minimal binding (<10%) to the surface.

Trans-Stimulation Phenomenon

In these studies, vesicles were preloaded with [^3H]Pte-Glu, and its efflux was measured in the presence and absence of excess extravesicular unlabeled folate (100 μM). The efflux rate of folate, which is determined by [^3H]Pte-Glu remaining in the vesicles after dilution into the incubation media, would be expected to be higher in the presence of excess cold folate in the media compared with control or the absence of folate, if the folate uptake is carrier mediated. As shown in Fig. 3, [^3H]Pte-Glu remaining in the

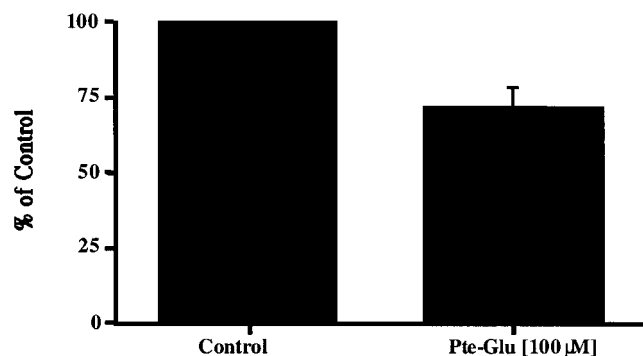


Fig. 3. Demonstration of *trans*-stimulation phenomenon. Colonic BLMV were preloaded with 1 μM [^3H]Pte-Glu, and its efflux was measured at early time point (15 s) in the presence and absence of excess cold extravesicular folate (100 μM). [^3H]Pte-Glu remaining in the vesicles was assessed at 25°C, indicative of the rate of [^3H]Pte-Glu efflux from vesicles diluted in the presence of excess cold folate (100 μM) or the absence of folate (control). Values are means of 6 observations from 2 separate preparations.

vesicles was significantly ($P < 0.01$) less in vesicles diluted in the presence of excess unlabeled folate compared with the absence of folate, demonstrating an increased rate of [^3H]Pte-Glu efflux. These findings suggest the presence of a carrier-mediated folate transport process in these vesicles.

Kinetics of [^3H]Pte-Glu Uptake

Saturation kinetics is also a notable characteristic feature of a carrier-mediated transport process. To examine the kinetics of the folate transport process in the human colonic BLMV, kinetic studies were performed in the presence of increasing concentrations of the substrate (0.1–10 μM). As shown in Fig 4A, uptake of [^3H]Pte-Glu showed saturation as a function of increasing the substrate concentration in the incubation media with an apparent Michaelis constant (K_m) of $9.6 \pm 0.48 \mu\text{M}$ and a maximal velocity (V_{max}) of $8.10 \pm 0.36 \text{ pmol} \cdot \text{mg protein}^{-1} \cdot 10 \text{ s}^{-1}$ (calculated by a Lineweaver-Burke plot; Fig. 4B).

Dose Response of Methotrexate (Amethopterin) Inhibition

Folate transport has been shown to be inhibited by its structural analogs, including methotrexate, in a variety of cell types (reviewed in Refs. 14 and 20). For this reason, we examined the effect of different concentrations of methotrexate on the initial rate of folate (0.25 μM) uptake into human colonic BLMV. The results in Fig. 5 show that methotrexate caused a dose-dependent inhibition in folate uptake with an inhibitory constant (K_i) value for methotrexate calculated at $8.28 \pm 1.0 \mu\text{M}$.

Effect of Transport Inhibitors

Previous studies with small intestinal and colonic AMV (6, 14, 18, 20, 22) have demonstrated that one of the possible mechanisms of folate uptake is via an anion exchange process. Thus, in the present study, we also examined the effect of anion transport and other inhibitors (all at 0.5 mM) on the uptake of Pte-Glu (0.25 μM). The study was performed in the presence of an inwardly directed pH gradient (i.e., pH 7.5 in and 5.0 out). As shown in Fig. 6, the anion transport inhibitors SITS and DIDS markedly inhibited folate uptake, whereas the other transport inhibitors, namely amiloride, acetazolamide, furosemide, and niflumic acid, failed to cause significant inhibition in folate uptake. These data suggest that this folate transporter is DIDS and SITS sensitive and could represent an anion exchanger.

Effect of Transmembrane Electrical Potential

In these studies, we examined the effect of changing the transmembrane electrical potential difference on uptake of the anionic Pte-Glu into the human colonic BLMV. Uptake of 0.25 μM [^3H]Pte-Glu into the BLMV was examined under the following conditions: 1) inside negative potential: K^+ inside = 100 mM, K^+ out = 0

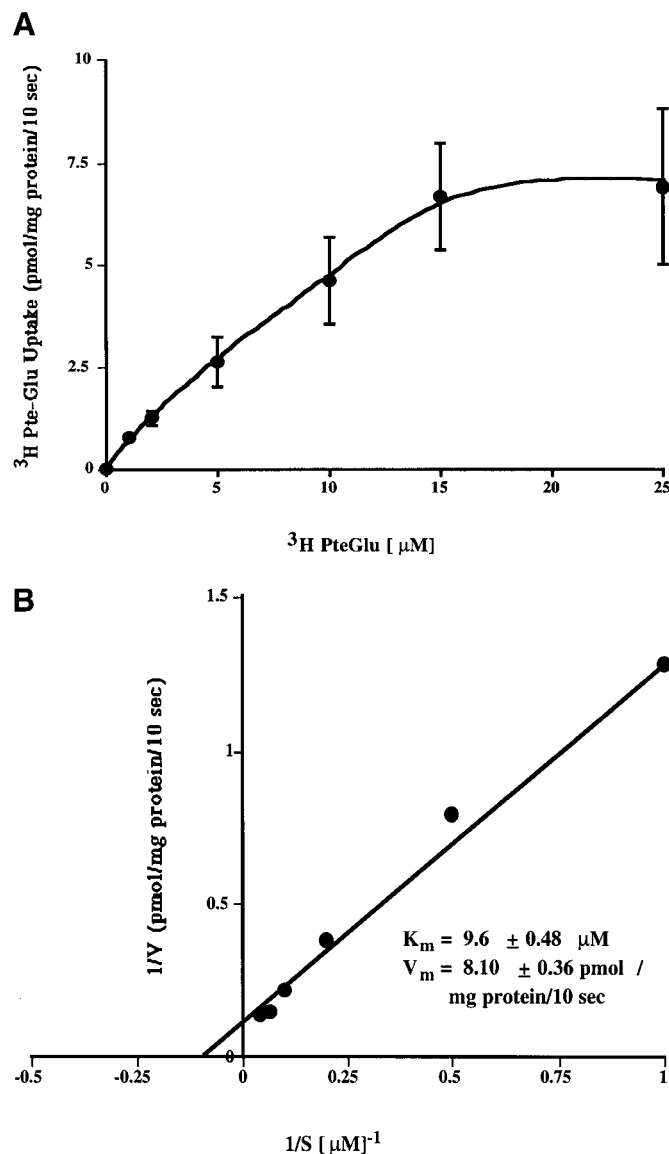


Fig. 4. A: kinetics of [^3H]Pte-Glu uptake. [^3H]Pte-Glu uptake at increasing extravesicular concentrations of Pte-Glu (0.1–10 μM). Colonic BLMV preloaded with 280 mM mannitol and 20 mM Tris-HEPES, pH 7.5, were incubated with buffers containing 280 mM mannitol and either 20 mM Tris-MES, pH 5.0, or 20 mM Tris-HEPES, pH 7.5, and increasing concentrations of [^3H]Pte-Glu (0.1–10 μM final concentrations). [^3H]Pte-Glu uptake at 25°C for 10 s was determined. Results shown are representative of 4–6 separate membrane preparations. The actual uptake values were corrected by subtracting the uptake in the absence of a pH gradient, i.e., pH 7.5_{in}/7.5_{out}. B: Lineweaver-Burke plot of [^3H]Pte-Glu uptake. A representative Lineweaver-Burke plot demonstrated a straight line with a Michaelis constant (K_m) for [^3H]Pte-Glu of $9.6 \pm 0.48 \mu\text{M}$ and a maximal velocity (V_{max}) of $8.10 \pm 0.36 \text{ pmol} \cdot \text{mg protein}^{-1} \cdot 10 \text{ s}^{-1}$.

mM + 20 μM valinomycin (K^+ ionophore); 2) inside positive potential: K^+ inside = 0 mM, K^+ out = 100 mM + 20 μM valinomycin; 3) voltage-clamped condition: K^+ inside = 100 mM, K^+ out = 100 mM + 20 μM valinomycin. As shown in Table 1, creation of a negative or positive intravesicular potential by using a K^+ /valinomycin system failed to influence [^3H]Pte-Glu uptake into these vesicles. These observations suggest

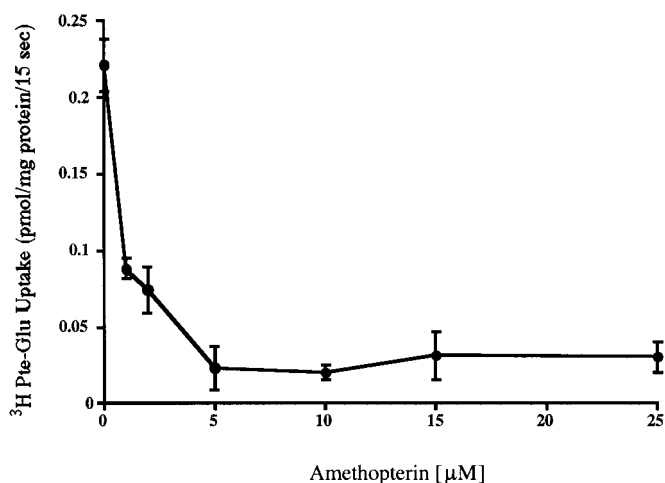


Fig. 5. Effect of methotrexate on $[^3\text{H}]\text{Pte-Glu}$ uptake: dose-response curve. Colonic BLMV preloaded with 280 mM mannitol and 20 mM Tris-HEPES, pH 7.5, were incubated with buffers containing 280 mM mannitol, 20 mM Tris-MES, pH 5.0, and 0.25 μM $[^3\text{H}]\text{Pte-Glu}$ (final concentration) with indicated concentrations of methotrexate for 15 s at 25°C. Values represent means \pm SE of 3 separate membrane preparations. Inhibitory constant for methotrexate = $8.28 \pm 1.0 \mu\text{M}$.

that the folate uptake process into these vesicles was potential-insensitive or electroneutral in nature.

Expression of hRFC Protein in Human Colon and Jejunum

The expression of the RFC protein in human colonic and jejunal AM and BLM was investigated by Western blot analysis. Specific polyclonal antibodies raised against a synthetic peptide of the human reduced folate carrier (hRFC) protein and purified colonic and jejunal apical and BLM preparations (150 μg of each) were used in the study (please see MATERIALS AND METHODS). The results (Fig. 7) showed that the hRFC protein is expressed at both the jejunal and colonic apical and

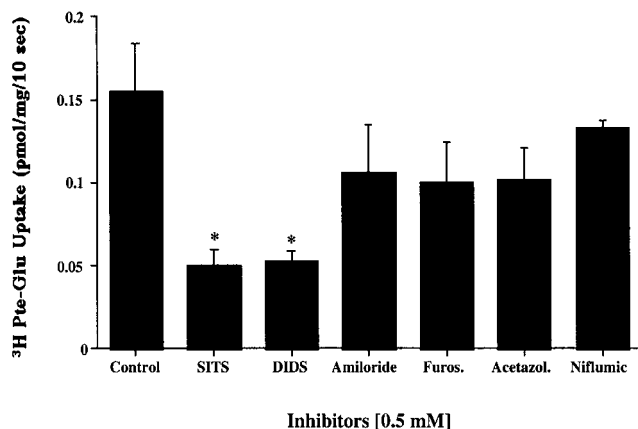


Fig. 6. Effect of transport inhibitors on $[^3\text{H}]\text{Pte-Glu}$ uptake. Colonic BLMV preloaded with 280 mM mannitol and 20 mM Tris-HEPES, pH 7.5, were incubated with buffer containing 280 mM mannitol, 20 mM Tris-MES, pH 5.0, and 0.25 μM $[^3\text{H}]\text{Pte-Glu}$ (final concentration) with 0.5 mM concentrations of inhibitors for 10 s at 25°C. Values represent means \pm SE of 3 separate membrane preparations. * $P < 0.05$ compared with control values.

Table 1. Effect of membrane potential on $[^3\text{H}]\text{Pte-Glu}$ uptake into the human colonic BLMV

Groups	Conditions	$[^3\text{H}]\text{Pte-Glu}$ Uptake, $\text{pmol}\cdot\text{mg}^{-1}\cdot 10 \text{ s}^{-1}$
Voltage clamped	$K_{\text{out}}^+ = K_{\text{in}}^+ + \text{Valinomycin}$	0.29 ± 0.06
Inside negative	$K_{\text{out}}^+ = K_{\text{in}}^+ + \text{Valinomycin}$	0.32 ± 0.07
Inside positive	$K_{\text{out}}^+ = K_{\text{in}}^+ + \text{Valinomycin}$	$0.24 \pm 0.04^*$

Values are means \pm SE of 3 independent preparations. BLMV, basolateral membrane vesicles. *Nonsignificant compared with voltage-clamped values.

BLM domains. The relative density of the bands were as follows: jejunal BBMV = 389,792; jejunal BLMV = 53,140; for colon AMV = 48,007; for colon BLMV = 6,702 (mean of 2 representative blots). As is evident from the relative density data, the highest expression was found at the jejunal AM, followed by jejunal BLM, colonic AM, and then by colonic BLM. In both regions of the intestinal tract, hRFC protein expression was found to be higher at the AM domain compared with the BLM domain of the epithelial cells. Specificity of the observed bands ($\sim 85 \text{ kDa}$) was confirmed by preincubating the polyclonal antibodies with the free synthetic peptide (against which the polyclonal antibodies were raised), which competed out the specific band (data not shown).

DISCUSSION

The normal microflora of the large intestine synthesize considerable amounts of folate, and a significant portion of this folate exists in the lumen in the absorbable monoglutamate form. In vivo studies have shown that the colon is indeed capable of absorbing some of this luminal folate (23, 25). The mechanism involved, however, was not clearly understood. Recent studies from our laboratories using purified AMV isolated from colons of organ donors (6), and cultured human-derived, nontransformed colonic epithelial NCM460 cells (13), have demonstrated the involvement of a pH-dependent, carrier-mediated system in folate uptake across the luminal membrane domain of the polarized colonic epithelial cells. This system was found to be DIDS sensitive and transports the vitamin via an elec-

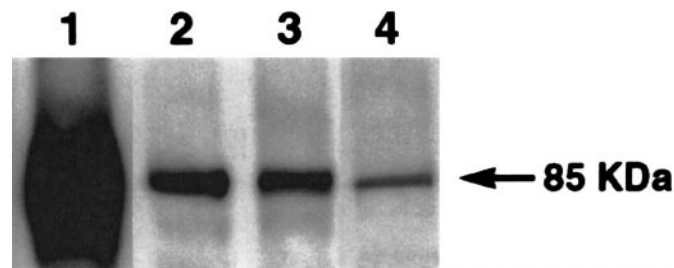


Fig. 7. Western blot analysis of human reduced folate carrier (hRFC) protein in human jejunal and colonic apical and basolateral membranes (BLM). Analysis was performed using 150 μg of each membrane preparation as described in *Methods*. Lanes 1, 2, 3, and 4 refer to human jejunal brush-border membranes, jejunal BLM, colon apical membranes, and colon BLM, respectively. Data shown are representative of 3 separate sets of experiments.

troneutral process (6, 13). To date, however, nothing is known about the mechanism of exit of folate out of the human colonocytes, i.e., transport across the BLM domain. In this study, we used BLMV preparations isolated from the colon of organ donors to address this issue. The purity of this membrane preparation was established by measuring the enrichment/depurification of marker enzyme activities. Our results showed uptake of Pte-Glu by colonic BLMV to be pH dependent and increased with decreasing incubation buffer pH. The Pte-Glu uptake, however, was Na^+ independent in nature.

The mechanism of uptake of folate across the human colonic BLM was found to be carrier mediated in nature. Evidence includes the saturation in the vitamin uptake as a function of concentration, the *trans*-stimulation of [^3H]Pte-Glu, i.e., exit from preloaded vesicles by unlabeled Pte-Glu in the incubation medium, and the dose-dependent inhibition of [^3H]Pte-Glu uptake by the folate analog methotrexate. The observation that the apparent K_i for inhibition of Pte-Glu uptake by methotrexate of $8.28 \pm 1.0 \mu\text{M}$ is similar to the apparent K_m of Pte-Glu uptake by human colonic BLMV of $9.6 \pm 0.48 \mu\text{M}$ suggests that the involved carrier has similar transport affinity for both substrates. The folate carrier was also found to be sensitive to the effect of anion transport inhibitors SITS and DIDS but not to the effect of other inhibitors. In addition, the uptake of folate by the identified carrier system appears to be electroneutral in nature, as alteration in transmembrane potential was found to have no significant effect on the uptake of the anionic folate. This finding together with the above-described findings of increased uptake of folate upon increasing the outwardly directed OH^- gradient, i.e., upon decreasing incubation buffer pH, and the ability of the anion transport inhibitors SITS and DIDS in inhibiting the uptake process raises the possibility of involvement of an anion exchange mechanism in folate uptake across the human colonic BLMV. A similar conclusion was drawn previously for folate uptake across the human colonic AM (6) and the human and rabbit small intestinal brush-border membrane (BBM; see Refs. 18 and 22). In this regard, previous studies of Zimmerman (25) using organ culture of the human colonic tissue showed that the uptake of folate in the human colon was via a facilitated diffusion process. In their studies, although the initial uptake was not affected by luminal pH, folate accumulation in colonic mucosa was much higher at a luminal pH of 5.5 compared with 7.5, and the uptake showed inhibition by methotrexate. Our findings of the uptake of folate by the human colonic AMV (6) and BLMV agree in this report with respect to the inhibition by analogs and mediation via a folate carrier but are different with respect to sensitivity of the initial uptake rates to the luminal pH (as seen in our studies). The reason for this difference is not clear but may possibly be due to differences in the tissue preparation used in that study compared with our studies of direct demonstration of the transport mechanism using purified plasma membranes.

Comparison of the transport characteristics of Pte-Glu across the human colonic BLM observed in the present study with those previously reported for the vitamin transport across the human colonic AM (6) showed many similarities with regard to pH dependence, K_m , DIDS sensitivity, and electroneutrality. The two processes, however, showed some difference with regard to uptake capacity of the carrier system involved. This is indicated by the difference in the capacity (V_{max}) of the two uptake processes, which was reported at 8.10 ± 0.36 and $19.8 \pm 2.9 \text{ pmol} \cdot \text{mg protein}^{-1} \cdot 10 \text{ s}^{-1}$ for human colonic BLMV and AMV, respectively. Similarly, folate transport across jejunal BLM was found in a previous study in our laboratory to be similar in many aspects to that across jejunal BBM but was different in terms of uptake capacity (18, 19, 21). Because the characteristics and molecular size of the human colonic BLMV folate transporter appear to be similar to its apical counterpart, one possibility could be that the activity in the BLMV may be due to possible cross contamination of this membrane by BLMV. This, however, does not appear to be the case. As we have previously shown, our purified BLMV demonstrate minimal contamination by microsomal, mitochondrial, and AM, as assessed by their respective marker enzyme activities (24), and clearly indicate that these preparations originate predominantly from the BLM region of the human colonocytes and are substantially pure. Additionally, our recent preliminary immunoblotting studies have further shown that Na^+/H^+ exchanger (NHE)-3 (an NHE isoform known to be expressed in AM of the mammalian intestine) and downregulated in adenoma (DRA; known to be localized to mammalian colonic AM) proteins could not be detected in these human colonic BLM preparations by using antipeptide antibody against NHE3 (Alpha Diagnostics) and DRA, whereas NHE3 and DRA could be detected only in the colonic AM (unpublished observations). These findings further substantiate that our human colonic BLM were relatively free of contamination from the AM.

To further establish whether the human colonic BLMV folate carrier is identical in molecular mass to apical colonic or small intestinal folate carrier, we performed Western blot analysis using purified colonic BLM preparations and specific polyclonal antibodies raised against a synthetic peptide of the hRFC protein. We also examined and compared the level of expression of the hRFC protein at the AM domain of the human colonocytes and at the BBM and BLM domains of the human jejunal enterocytes. Purified membrane preparations isolated from the specified area of the intestinal tract by established procedures were used in the study (6, 19, 18, 24). The results showed that hRFC protein is expressed at the colonic BLM and at all other membrane domains examined. The highest level of expression of the protein was found at the jejunal BBM domain followed by jejunal BLM and colonic AM domain, followed by colonic BLM domain. Also, the level of expression at the AM domain of both colonic and jejunal epithelial cells was found to be markedly

higher than that at the BLM domain of these cells. The latter finding is in agreement with previous observations of a higher capacity (V_{\max}) for folate uptake by jejunal and colonic AMV preparations compared with their BLMV counterparts (please see above). The findings of expression of the hRFC protein at the human colonic apical and BLM domain are in line with the previous observation of expression of mRNA transcripts of hRFC in the human colonic mucosa (15). These observations together with the functional data on folate uptake presented in this report and previously (6) lend further support to the understanding that reduced folate uptake is the transporter responsible for folate uptake in the intestinal tract (3, 15).

In summary, the current study demonstrates for the first time the existence of a carrier-mediated mechanism for folate transport across the human colonic BLM. These data, in conjunction with our previous demonstration of the existence of a specialized folate uptake mechanism across the AM of human colonocytes, further support the belief that bacterially synthesized folate in the large intestine may be nutritionally important (16). Furthermore, absorption of folate in the large intestine may represent an important "reserve" capacity for absorption of the vitamin in cases of small intestinal disease.

The laboratories of P. K. Dudeja and H. M. Said have contributed equally to this work.

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