Innovative and Emerging Technologies in GI Physiology and Disease

Functional involvement of RFVT3/SLC52A3 in intestinal riboflavin absorption

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Submitted 18 October 2013; accepted in final form 13 November 2013

Riboflavin (vitamin B2) is a water-soluble vitamin involved in a variety of metabolic reactions. It is converted to the active coenzyme forms, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which are indispensable for various redox reactions, including carbohydrate, lipid, and amino acid metabolism. A deficiency of riboflavin has been implicated as a risk factor for anemia, cancer, cardiovascular disease, and neurodegeneration (4, 18). Humans are not able to synthesize riboflavin and thus must obtain it as a nutrient via diet. Because riboflavin is highly expressed in the small intestine (31), it is considered to play an important role in the intestinal absorption of riboflavin.

RFVT3 consists of 469 amino acids with 11 transmembrane domains, exhibiting 41 and 44% identity to RFVT1 and RFVT2, respectively (33). Riboflavin transport via RFVT3 is pH-dependent and Na+-independent, whereas that by RFVT1 and RFVT2 is independent of pH and Na+ (30, 31). RFVT3 tagged with green fluorescent protein localizes in the apical membranes of polarized MDCK and Caco-2 cells (11, 26, 27).

In the present study, we investigated the functional involvement of RFVT3 in riboflavin absorption in the intestinal epithelial T84 cells and mouse small intestine. T84 cells expressed RFVT3 and conserved unidirectional riboflavin transport corresponding to intestinal absorption. Apical $[^{3}H]$riboflavin uptake was pH-dependent in T84 cells. This uptake was not affected by Na+ depletion at apical pH 6.0, although it was significantly decreased at apical pH 7.4. The $[^{3}H]$riboflavin uptake from the apical side of T84 cells was prominently inhibited by the RFVT3 selective inhibitor methylene blue and significantly decreased by transfection of RFVT3-small-interfering RNA. In the gastrointestinal tract, RFVT3 was expressed in the jejunum and ileum. Mouse jejunal and ileal permeabilities of $[^{3}H]$riboflavin were measured by the in situ closed-loop method and were significantly reduced by methylene blue. These results strongly suggest that RFVT3 would functionally be involved in riboflavin absorption in the apical membranes of intestinal epithelial cells.

RIBOFLAVIN (vitamin B2) is a water-soluble vitamin involved in a variety of metabolic reactions. It is converted to the active coenzyme forms, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which are indispensable for various redox reactions, including carbohydrate, lipid, and amino acid metabolism. A deficiency of riboflavin has been implicated as a risk factor for anemia, cancer, cardiovascular disease, and neurodegeneration (4, 18). Humans are not able to synthesize riboflavin and thus must obtain it as a nutrient via intestinal absorption. Previous studies using intestinal specimens, intestinal membrane vesicles, and cell lines have demonstrated that intestinal absorption of riboflavin is mediated by transporter(s) (9, 10, 13, 15, 20). The first mammalian riboflavin transporter was cloned in 2008 and has recently been named as RFVT1 (previous name RFT1, encoded by SLC52A1) (34). Thereafter, RFVT2 (RFT2, encoded by SLC52A2) and RFVT3 (RFT2, encoded by SLC52A3) have also been identified (30, 31).

MATERIALS AND METHODS

Cell culture and transfection. The human colorectal cancer-derived cell line T84 (CCL-248; American Type Culture Collection, Manassas, VA) was cultured in Dulbecco’s modified Eagle’s medium-Ham’s F-12 with i-Gln, sodium pyruvate, and HEPES (Nacalai Tesque, Kyoto, Japan) supplemented medium with 10% fetal bovine serum (Life Technologies, Carlsbad, CA) in an atmosphere of 5% CO2–95% air at 37°C. T84 cells were seeded on 24-well plates at a density of 20 × 104 cells/well or on microporous membrane filters (3.0-μm pores, 4.67 cm2) inside Transwell cell culture chambers (Costar, Cambridge, MA) at a cell density of 150 × 104 cells/filter. The cell monolayers grown on the plate or Transwell chamber were given complete medium every 2 days and were used on postculture day 6 for experiments. For the RNA interference system, duplicated Stealth RNAi small-interfering RNA (siRNA) (Life Technologies) was used. The target sequences of gene-specific siRNA for SLC52A3 coding...
hRFVT3 were as follows: hRFVT3-siRNA I targeting for sequence 5'-TCCTGCTAACAGGTCTCTGCTGTT-3'; hRFVT3-siRNA II for sequence 5'-ACCTGCGTCAATGTCACTGAGATAT-3'; and hRFVT3-siRNA III for sequence 5'-CCGGCGCACCTGGCCTTC-3'. A dose of 30 pmol siRNA was transfected into T84 cells at a density of 2 x 10^4 cells/well or poly-D-lysine-coated 12-well plates at 15 x 10^4 cells/well on the day before transfection. The cells were transfected with 0.2 μg of plasmid DNA using 1 μl of Lipofectamine 2000 Reagent (Life Technologies)/well for the 24-well plate and 0.4 μg of plasmid DNA using 2 μl of Lipofectamine 2000 Reagent/well for the 12-well plate. Forty-eight hours after the transfection, cells were used for the uptake experiments.

Isolation of total RNA and real-time PCR. Total RNA from T84 cells was isolated using the RNeasy Mini Kit (QIAGEN) according to the manufacturer’s instructions and reverse transcribed to yield cDNA. To determine the mRNA expression levels of hRFVT1, hRFVT2, and hRFVT3, real-time PCR was performed as described previously (31).

Transport study using T84 cell monolayers. Transepithelial transport of [3H]riboflavin (0.903 TBq/mmol; Moravek Biochemicals, Brea, CA) was studied using monolayer cultures grown in the Transwell chambers as previously described (24). Briefly, incubation medium containing [3H]riboflavin and D-[1-14C]mannitol (1.961 GBq/mmol; Moravek Biochemicals) was added to either the apical or basal side, whereas unlabeled incubation medium was added to the opposite side. D-[1-14C]mannitol, which is not transported by the cells, was used to calculate paracellular flux. An aliquot (100 μl) of the incubation medium was periodically taken from the opposite side. Radioactivity on the opposite side was measured.

Given complete medium every 2 days and were used on posttransfection day 6 for subsequent investigations.

Human embryonic kidney (HEK) 293 cells (CRL-1573; American Type Culture Collection) were cultured as previously described (24). For a transient expression system, pcDNA3.1/Hygro (+) containing human (h) RFVT1, hRFVT2, hRFVT3, mouse (m) RFVT2, and mRFVT3 were purified using the Hispeed Plasmid Purification system (QIAGEN, Hilden, Germany). For the uptake studies, HEK293 cells were seeded on poly-d-lysine-coated 24-well plates at 7.5 x 10^4 cells/well or poly-d-lysine-coated 12-well plates at 15 x 10^4 cells/well on the day before transfection. The cells were transfected with 0.2 μg of plasmid DNA using 1 μl of Lipofectamine 2000 Reagent (Life Technologies)/well for the 24-well plate and 0.4 μg of plasmid DNA using 2 μl of Lipofectamine 2000 Reagent/well for the 12-well plate. Forty-eight hours after the transfection, cells were used for the uptake experiments.

Fig. 1. Transcellular transport of [3H]riboflavin across the T84 cell monolayers. T84 cell monolayers were incubated at 37°C with 5 nM [3H]riboflavin added to the apical (closed circle, pH 6.0) or the basal (open circle, pH 7.4) side. The radioactivity on the opposite side was measured.
activity of the collected media was determined in ACS II (GE Healthcare, Little Chalfont, UK) by liquid scintillation counting. The transepithelial transport of [3H]riboflavin was calculated by subtracting the paracellular flux estimated by p-[1-14C]mannitol from the transepithelial flux of [3H]riboflavin. Transepithelial transport of unlabelled riboflavin, FMN, and FAD was also measured as described above with some modifications. An aliquot (100 μl) of the incubation medium was taken from the opposite side, and the concentrations of flavins were measured by HPLC as previously described (32).

The [3H]riboflavin uptake from the apical side was also measured in T84 cells grown on 24-well plates as previously described with some modifications (28). Briefly, the cells were preincubated for 10 min at 37°C with unlabeled incubation medium (pH 7.4). After the medium removal, cells were further incubated with incubation medium (pH 6.0) containing 5 nM [3H]riboflavin for 5 min at 37°C. Cells were then rapidly rinsed with ice-cold incubation medium and solubilized. The radioactivity of the solubilized cells was determined by a liquid scintillation counter. Concentration dependence of riboflavin uptake was fit by the combination of the Michaelis-Menten equation and a linear relationship: $V = V_{\text{max}}[S] / (K_m + [S]) + K_d[S]$, where $V$ is the transport rate, $V_{\text{max}}$ is the maximal transport rate, $[S]$ is the concentration of riboflavin, $K_m$ is the Michaelis-Menten constant, and $K_d[S]$ is the diffusion constant. Na⁺-free incubation buffer was prepared by replacing Na⁺ with choline, N-methyl-D-glucamine, or mannitol (pH 6.0 or 7.4). The protein content was determined by the method of Bradford with a Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Richmond, CA) with bovine γ-globulin as a standard.

Transport study using HEK293 cells transiently expressing RFVTs. Cellular uptake of [3H]riboflavin was measured with HEK293 cells transiently expressing RFVTs as described previously (31). The uptake experiments for unlabeled riboflavin, FMN, and FAD were carried out using transiently hRFVT-expressing HEK293 cells grown on poly-d-lysine-coated 12-well plates as previously described (33) with some modifications. Briefly, the cells were preincubated with incubation buffer (pH 7.4) for 20 min at 37°C. Next, the buffer was replaced with incubation buffer containing each compound. At the end of the incubation period (10 min), the buffer was aspirated, and the cells were rapidly washed with ice-cold incubation buffer. To determine the accumulation of unlabeled riboflavin, FMN, and FAD, 0.2 ml of mobile phase [10 mM phosphate buffer (pH 5.5)-methanol –

Fig. 3. Substrate recognition by T84 cells and RFVT3. A: the chemical structure of riboflavin, flavin mononucleotide (FMN), and flavin adenine dinucleotide (FAD). B: transepithelial transport of riboflavin, FMN, and FAD from the apical to basal side of T84 cell monolayers. T84 cell monolayers were incubated at 37°C with 1 μM of unlabeled riboflavin, FMN, or FAD added to the apical side. Concentrations of compounds on the basal side were measured by HPLC. Uptake of riboflavin (C), FMN (D), or FAD (E) by human (h) RFVT3. Human embryonic kidney (HEK) 293 cells were transfected with an empty vector or hRFVT3. The cells were incubated with 10 μM riboflavin, FMN, or FAD (pH 7.4) for 10 min at 37°C. Considering the endogenous flavins in HEK293 cells, the uptake values were calculated by subtracting the cellular concentration of each flavin before treatment from the concentration after treatment. Each point or column represents the mean ± SE of three wells. **P < 0.01, significantly different from vector-transfected cells.
65:35) was added to each well, and cells were scraped. Cells were then deproteinized by adding methanol and allowed to stand for 1 h at room temperature. After centrifugation, the supernatant was filtrated through a Cosmonice filter (0.45 μm; Merck Millipore, Billerica, MA), and the concentrations of flavins were determined by HPLC as described previously (32). Considering the endogenous flavins in HEK293 cells, the uptake values were calculated by subtracting the cellular concentration of each flavin before treatment from the concentration after treatment.

Animals. Animal experiments were conducted in accordance with the Guidelines for Animal Experiments of Kyoto University. All protocols were approved by the Animal Research Committee, Graduate School of Medicine, Kyoto University. C57BL/6 male mice of 10 and 15 wk of age (Japan SLC, Shizuoka, Japan) were housed in a temperature-controlled environment with an alternating 12:12-h light-dark cycle and fed on a standard diet and water ad libitum before experiments.

In situ hybridization. In situ hybridization was performed by the method of Genostaff (Tokyo, Japan) as previously described (17). The mouse small intestine was dissected after their perfusion, fixed with Tissue Fixative (Genostaff), and then embedded in paraffin by their proprietary procedures and sectioned at 6 μm. The sections were fixed with 4% paraformaldehyde in PBS for 15 min and then washed with PBS. The sections were treated with 8 μg/ml Proteinase K in PBS for 30 min at 37°C, refixed with 4% paraformaldehyde in PBS, and placed in 0.2 N HCl for 10 min. The sections were acetylated by incubation in 0.1 M triethanolamine hydrochloride, pH 8.0, and 0.25% acetic anhydride for 10 min. They were dehydrated through a series of ethanol. Hybridization was performed with sense or antisense probes for RFVT3 (sequence position; 1045–1645 in the open reading frame) at concentrations of 300 ng/ml in the Probe Diluent-1 (Genostaff) at 60°C for 16 h. After hybridization, the sections were washed and then in 50% formamide, 2× HybriWash at 60°C for 20 min, followed by RNase treatment. The sections were treated with anti-DIG AP conjugate (Roche, Basel, Switzerland) diluted 1:1000 with TBST for 2 h at RT. Coloring reactions were performed with NBT/BCIP solution (Sigma-Aldrich, St. Louis, MO) overnight and then washed with PBS. The sections were counterstained with Kernechtrot stain solution (Mutoh, Tokyo, Japan) and then mounted with CC/Mount (DBS, Pleasanton, CA).

In situ intestinal closed-loop experiment. The in situ intestinal closed-loop experiment was carried out as described previously with some modifications (25). Mice fasted overnight were anesthetized with pentobarbital sodium. Surgery was performed on each animal lying on a heating pad to maintain a constant body temperature. The abdominal cavity was opened via a midline incision, and the intestinal loops (length: 5 cm) were made at the jejunum area (2 cm distal to the ligament of Treitz) and ileum area (~1 cm proximal to the cecum). Intestinal contents were removed by slow infusion of saline and air. Phosphate-buffered solution (PBS, pH 6.5) containing 50 nM [3H]riboflavin was administrated in the intestinal loop. At 3 min following administration, the luminal solution in the loop was collected. The intestinal loops were isolated and washed with ice-cold PBS. The circumference and length of the loops were measured, and the intestinal mucosae were scraped. Radioactivity in the luminal solution and mucosal homogenates was measured by liquid scintillation counting. Permeability of [3H]riboflavin was evaluated in terms of the percentage of dose absorbed, i.e., subtracting the residual amount of [3H]riboflavin from the administered amount. The following equation was used to calculate the permeability as described previously with slight modifications (25):

\[
\text{Permeability (cm/s)} = \frac{V}{C \times L \times T} = \frac{\ln X_0 - \ln X}{CLT}
\]

where V is the volume of the solution applied to the loop, C and L are circumference and length of the intestinal loop, respectively. T is the incubation time (3 min), and X₀ and X are the administered and residual amounts of drug before and after incubation, respectively.

Statistical analysis. All values are expressed as means ± SE, and differences were statistically verified by the unpaired Student’s t-test. Multiple comparisons were performed by the Dunnett’s two-tailed test after a one-way analysis of variance using the GraphPad Prism (version 4.0a; GraphPad Software, San Diego, CA). Differences where P < 0.05 were considered statistically significant.

RESULTS

mRNA expression of hRFVT3 in T84 cells. The mRNA expression levels of hRFVTs in T84 cells were measured by real-time PCR. mRNAs of hRFVT2 and hRFVT3 were markedly detected in T84 cells, whereas hRFVT1 was at a negligible level. The mRNA expression levels of hRFVT2 and hRFVT3 were 7.17 ± 1.13 and 2.79 ± 0.69 (SE) amol/μg total RNA, respectively.

Fig. 4. Effects of methylene blue on [3H]riboflavin uptake by hRFVTs (A) and mouse (m) RFVTs (B). A: HEK293 cells transfected with hRFVT1 (open circle), hRFVT2 (open triangle), or hRFVT3 (closed circle) were incubated at 37°C for 1 min with 5 nM [3H]riboflavin in the presence of various concentrations of methylene blue. B: HEK293 cells transfected with mRFVT2 (open circle) or mRFVT3 (closed circle) were incubated at 37°C for 1 min with 10 nM [3H]riboflavin in the presence of various concentrations of methylene blue. mRFVT2 is an ortholog of both hRFVT1 and hRFVT2. The specific uptake of [3H]riboflavin by RFVTs was calculated by subtracting the uptake of [3H]riboflavin by HEK293 cells transfected with empty vector from the uptake with RFVTs. Each point represents the mean ± SE for three wells.
Table 1. *IC*50 values of methylene blue for [*3H*]riboflavin uptake by hRFVTs and mRFVTs

<table>
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<th><em>IC</em>50, µM</th>
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<tr>
<td>hRFVT1</td>
<td>5.143 ± 1.942</td>
</tr>
<tr>
<td>hRFVT2</td>
<td>7.303 ± 1.945</td>
</tr>
<tr>
<td>hRFVT3</td>
<td>6.7 ± 1.0</td>
</tr>
<tr>
<td>mRFVT2</td>
<td>8.392 ± 1.478</td>
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<tr>
<td>mRFVT3</td>
<td>8.1 ± 1.2</td>
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Each value represents the mean ± SE of three experiments. h, Human; m, mouse.

Functional characterization of riboflavin transport in T84 cells. We evaluated the transcellular transport direction of [*3H*]riboflavin in T84 cell monolayers grown on porous membrane filters. As shown in Fig. 1, the rate of transcellular transport of [*3H*]riboflavin was unidirectional, that is, from the apical to basal side. A saturable concentration-dependent [*3H*]riboflavin uptake was observed in the apical side of T84 cells (Fig. 2A). The apparent *K*<sub>m</sub> value was 53.2 ± 21.6 nM and *V*<sub>max</sub> was 1.8 ± 0.4 pmol·mg protein<sup>-1</sup>·min<sup>-1</sup>. Eadie-Hofstee plots of [*3H*]riboflavin uptake from the apical side of T84 cells were linear (Fig. 2A). The apical [*3H*]riboflavin uptake was markedly influenced by the extracellular pH with a maximal uptake at pH 5.5 (Fig. 2B). When Na<sup>+</sup> in the medium was replaced with choline, N-methyl-D-glucamine, or mannitol, this riboflavin uptake was unchanged at apical pH 6.0 (Fig. 2C), whereas it significantly decreased at apical pH 7.4 (Fig. 2D).

Substrate recognition by T84 cells and RFVT3. Riboflavin was a good substrate for hRFVT3. This transporter also slightly transported FMN, but not FAD.

Methylene blue as the selective inhibitor of RFVT3 (identification of RFVT3 selective inhibitor). We selected methylene blue as a candidate for RFVT3 selective inhibitor because it was reported to inhibit RFVT3-mediated riboflavin transport (11). To clarify whether the inhibitory effect was selective for RFVT3, we evaluated the concentration-dependent inhibitory effect of methylene blue on [*3H*]riboflavin uptake by RFVTs (Fig. 4). The *IC*50 values of methylene blue for [*3H*]riboflavin uptake by hRFVTs and mRFVTs are summarized in Table 1. Based on the *IC*50 values, the inhibitory effect of methylene blue on RFVT3 was about 1,000 times more potent than that on RFVT1 and RFVT2, indicating that methylene blue can be used as an RFVT3 selective inhibitor.

The functional involvement of hRFVT3 in T84 cells. The functional involvement of hRFVT3 in riboflavin uptake from the apical side of T84 cells was evaluated using methylene blue and hRFVT3-siRNAs. Apical [*3H*]riboflavin uptake was significantly inhibited by methylene blue (Fig. 5A). Next, the influence of hRFVT3-siRNAs on apical riboflavin uptake in T84 cells was examined. Real-time PCR analysis confirmed that hRFVT3-siRNA significantly reduced the expression of hRFVT3 mRNA in T84 cells (Fig. 5B). Knockdown of hRFVT3 by siRNA significantly reduced [*3H*]riboflavin uptake from the apical side of T84 cells (Fig. 5C).

Expression profile of mRFVT3 and functional involvement of mRFVT3 in riboflavin absorption in mouse small intestine. To visualize the expression profile of mRFVT3 in the mouse gastrointestinal tract, in situ hybridization was performed (Fig. 6). As a result, positive staining for mRFVT3 was observed in the jejunum and ileum, and its expression level was higher in the latter (Fig. 6, D and E). RFVT3 mRNA was almost negligible in the stomach, duodenum, and large intestine (Fig. 6, A, B, C, F, G, and H). Hybridization with a sense probe for mRFVT3...

Fig. 5. Effects of methylene blue and hRFVT3-small-interfering RNA (siRNAs) on [*3H*]riboflavin uptake from the apical side of T84 cells. A: T84 cell monolayers were incubated at 37°C for 5 min with 5 nM [*3H*]riboflavin in the presence of 100 µM methylene blue, and intracellular accumulation of [*3H*]riboflavin was measured. ***P < 0.001, significantly different from control cells. B: mRNA expression of hRFVT3 in T84 cells transfected with negative control siRNA or hRFVT3-siRNAs was determined by real-time PCR analysis. C: T84 cells transfected with hRFVT3-siRNA were incubated with 5 nM [*3H*]riboflavin at 37°C for 5 min, and intracellular accumulation of [*3H*]riboflavin was measured. Each column represents the mean ± SE for three wells. *P < 0.05 and ***P < 0.001, significantly different from negative control siRNA-transfected cells.
showed the absence of nonspecific binding to the jejunum and ileum (Fig. 6, I and J).

To evaluate the functional involvement of RFVT3 in the intestinal absorption of riboflavin, the effect of methylene blue on riboflavin intestinal absorption in mice was examined by the in situ closed-loop method. Permeabilities of \(^{3}H\)riboflavin in the mouse jejunum and ileum were significantly reduced by methylene blue (Fig. 7, A and B). The accumulation of \(^{3}H\)riboflavin in the luminal mucosa was also significantly decreased by methylene blue in both jejunum and ileum (Fig. 7, C and D). We confirmed that permeability and mucosal accumulation of \(\alpha\)-methyl-[\(^{14}C\)]glucopyranoside (negative control) were not influenced by methylene blue (data not shown).

**DISCUSSION**

The intestinal absorption of riboflavin is predominantly mediated by the active carrier-mediated transport processes (9, 10, 13, 15). In the present study, the functional involvement of RFVT3 in intestinal absorption of riboflavin was demonstrated using human intestinal epithelial T84 cells and mouse small intestine.

Human colonic-derived T84 cells were demonstrated to express RFVT3 at almost the same level as in the human small intestine (31) and conserved unidirectional riboflavin transport corresponding to intestinal absorption. Therefore, T84 cells could serve as a useful cell model for evaluating the role of...
RFVT3 in the intestinal absorption of riboflavin in vitro. The in vivo studies using T84 cells clearly demonstrated that riboflavin uptake from the apical side of cells was mediated by RFVT3, whose function was selectively inhibited by methylene blue and hRFVT3-siRNAs. When [3H]riboflavin was administrated in the mouse small intestine, the mucosal accumulation of this tritiated vitamin was significantly reduced by methylene blue, thus indicating that RFVT3 would be functionally involved in the apical membranes of intestinal epithelial cells. In fact, the functional characteristic of RFVT3 was in good agreement with previous observations using the intestinal brush-border membrane vesicle (6, 8, 11, 21, 30). In addition, cellular localization of RFVT3 appears to be dominant in the apical membranes of polarized epithelial cells (11, 26, 27). Taken together, RFVT3 would account for the major part of riboflavin transport in the apical membranes of intestinal epithelial cells.

Riboflavin in dietary sources exists as the form of FAD and FMN (19). It was believed that these coenzyme forms appeared to be unavailable for absorption and thus must be hydrolyzed to riboflavin before absorption (3, 10). However, this hypothesis has not been clarified from the viewpoint of substrate recognition in intestinal epithelial cells. The present study demonstrated that the apical-to-basal transports of FAD and FMN were lower than that of riboflavin in T84 cells. This result was in good agreement with the substrate recognition by RFVT3. These findings suggest that the absorption of vitamin B2 would occur primarily in the form of riboflavin attributable to substrate recognition by apical RFVT3.

The Na\(^+\) dependence of riboflavin intestinal absorption remains controversial. Previous studies have reported that intestinal riboflavin transport is Na\(^+\)-dependent (8, 9, 16, 20, 21), whereas some studies using intestinal brush-border membrane vesicle have reported the Na\(^+\)-independent riboflavin transport (6, 22). The present study clearly indicated that riboflavin uptake from the apical side of T84 cells was not affected by Na\(^+\) depletion when the apical pH was 6.0. On the other hand, Na\(^+\) depletion significantly decreased the apical riboflavin uptake under the neutral pH condition. In addition, the apical riboflavin uptake in T84 cells required H\(^-\)/H\(^+\) gradient. These functional characteristics suggest the possibility that Na\(^+\) might not directly affect RFVT3 but affect Na\(^+\)/H\(^+\) exchanger (NHE) that produces H\(^+\) gradient via Na\(^+\)/H\(^+\) (2, 14, 29). RFVT3 is also a pH-dependent and Na\(^+\)-independent transporter (31). RFVT3-mediated riboflavin transport in the intestinal epithelial cells could be partly activated by NHE, which requires a Na\(^+\) gradient. Further studies should be...
needed to clarify the contribution of NHE or additional factors to RFVT3-mediated riboflavin transport in intestinal epithelial cells.

In situ hybridization experiments revealed that RFVT3 was broadly distributed in the small intestine. Interestingly, the expression level increased higher along with more distal sites of the small intestine. Riboflavin absorptions in the jejunum and ileum were significantly reduced by methylene blue, suggesting that RFVT3 was responsible for the intestinal absorption of riboflavin in both sites. Riboflavin transport activity, similar to RFVT3 mRNA expression, was different at sites of the small intestine: the activity was higher in the distal than the proximal portion of the small intestine. The findings strongly indicate that RFVT3 would be extensively distributed and involved in riboflavin absorption in the small intestine. As a remaining question, the physiological meaning of RFVT3 distribution in the small intestine (ileum > jejunum) should be clarified in future studies.

In conclusion, RFVT3 functionally involves riboflavin absorption in the apical membrane of intestinal epithelial cells. RFVT3 would play an important role in maintaining riboflavin homeostasis.

REFERENCES


