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Anion antiport mechanism is involved in transport of lactic acid across intestinal epithelial brush-border membrane

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Abstract

Intestinal epithelial membrane transport of L-lactic acid was characterized using rabbit jejunal brush-border membrane vesicles (BBMVs). The uptake of L-[^{14}C]lactic acid by BBMVs showed an overshoot phenomenon in the presence of outward-directed bicarbonate and/or inward-directed proton gradients. Kinetic analysis of L-[^{14}C]lactic acid uptake revealed the involvement of two saturable processes in the presence of both proton and bicarbonate gradients. An arginyl residue-modifying agent, phenylglyoxal, inhibited L-[^{14}C]lactic acid transport by the proton cotransporter, but not by the anion antiporter. The initial uptakes of L-[^{14}C]lactic acid which are driven by bicarbonate ion and proton gradients were inhibited commonly by monocarboxylic acids and selectively by anion exchange inhibitor 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid and protonophore carbonylcyanide *p*-trifluoromethoxyphenylhydrazone, respectively. These observations demonstrate that L-lactic acid is transported across the intestinal brush-border membrane by multiple mechanisms, including an anion antiporter and a previously known proton cotransporter.

Keywords: Anion antiport; Proton cotransport; L-Lactic acid; Monocarboxylic acid; Intestinal absorption; Brush-border membrane vesicle

1. Introduction

Monocarboxylic acids such as lactic acid and short-chain fatty acids are well absorbed from the small intestine and the underlying mechanisms are considered to include both carrier-mediated transport and passive diffusion according to the pH-partition hypothesis [1,2]. We have previously shown that monocarboxylic acids such as acetic acid, nicotinic acid and the xenobiotic benzoic acid cross the intestinal epithelial apical membrane via pH-dependent mechanisms, including proton cotransport and anion antiport, using isolated membrane vesicles and

Abbreviations: BBMV, brush-border membrane vesicle; BBM, brush-border membrane; DIDS, 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid; FCCP, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone; DEPC, diethylpyrocarbonate; PCMBS, *p*-chloromercuribenzenesulfonic acid; PGO, phenylglyoxal; PLP, pyridoxal-5′-phosphate; HEPES, 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid; MES, 2-(*N*-morpholino)ethanesulfonic acid

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a human intestinal adenocarcinoma cell line, Caco-2 [3–9]. Furthermore, the monocarboxylate transporter MCT has been demonstrated to exist in intestinal epithelial cells and to facilitate pH-dependent transport of pyruvic acid and lactic acid, especially at the basolateral membrane [10,11]. In addition, we found that MCT1 protein is also present at the brush-border membrane, where it may participate partially in the absorption of monocarboxylic acids, causing an apparent pH-dependent transport phenomenon in the small intestine [12–14].

In renal epithelial cell membranes, anion exchange transport for monocarboxylic acids operates [15] and other organic anions also cross the renal epithelial cell membrane via exchange transport mechanisms, e.g., via the organic anion/dicarboxylic acid exchanger OAT [16]. Lactic acid was also suggested to participate as one of the counter-anions to be exchanged with urate at the renal epithelial apical membrane [17]. Furthermore, we demonstrated that monocarboxylic acids are transported by an anion-exchange transporter, AE2 [18], which is a well-known chloride/bicarbonate exchanger expressed in a variety of tissues [19], including intestinal epithelial brush-border membrane [20]. We also found that transport of lactic acid across Caco-2 cells could involve a bicarbonate/lactic acid antiport mechanism [21]. These observations suggest that lactic acid might be transported via an anion antiport mechanism in intestinal epithelial brush-border membrane in parallel with a proton-cotransport mechanism.

The purpose of the present study was to examine whether a pH-dependent anion antiport system participates in lactic acid transport across the intestinal epithelial brush-border membrane, in addition to with a proton-cotransport mechanism, using rabbit jejunal brush-border membrane vesicles (BBMVs).

2. Materials and methods

2.1. Chemicals

L-[^{14}C]Lactic acid (5.55 GBq/mmol) was purchased from American Radiolabeled Chemicals (St. Louis, MO). Valinomycin, *p*-chloromercuribenzenesulfonic acid (PCMBS) and diethylpyrocarbonate (DEPC) were purchased from Sigma Chemical (St. Louis, MO). Pyridoxal-5′-phosphate (PLP) was purchased from Boehringer-Mannheim (Germany). All other chemicals were of reagent grade or the highest purity available.

2.2. Membrane vesicle preparation and uptake study

The present study was performed according to the Guidelines for the Care and Use of Laboratory Animals in Takara-machi Campus of Kanazawa University and was approved by the Committee on Animal Experimentation of Kanazawa University, Takara-machi Campus. Jejunal BBMVs of male rabbits weighing 2.5–3.0 kg (Japan SLC, Shizuoka, Japan) were isolated by the magnesium precipitation method as described previously [8] and used on the day of preparation. BBMVs were suspended in the medium specified in 25 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES)-Tris buffer (pH 7.5) containing either 30 mM $KHCO_3$ or 30 mM K-gluconate, to give a concentration of membrane protein of 28.9 ± 3.7 mg/ml ($n = 10$, mean ± S.E.). The purified membrane preparation resulted in an alkaline phosphatase enrichment of 10.0 ± 0.8-fold compared with the crude mucosal homogenate ($n = 10$, mean ± S.E.) [22]. The enrichment of (Na^+,K^+)-ATPase activity was less than unity [23].

Uptake measurements were carried out by a rapid filtration technique as described previously [6]. All media containing bicarbonate were gassed for 1 h with a 95% O_2-5% CO_2 mixture and the pH was adjusted immediately before experiments. Other media were gassed with 100% O_2 as a control for any nonspecific effects of gassing. Uptake was initiated by adding a 90 μl aliquot of incubation medium containing L-[^{14}C]lactic acid to a 10 μl aliquot of BBMV suspension. Uptake of L-[^{14}C]lactic acid was determined at 37°C by incubating in 25 mM 2-(*N*-morpholino)ethanesulfonic acid (MES)-Tris buffer (pH 6.0) or 25 mM HEPES-Tris buffer (pH 7.5) containing 30 mM K-gluconate and was terminated by the addition of 1 ml of ice-cold stop solution, followed immediately by filtration on a 0.45 μm filter (HAWP, Millipore, Bedford, MA). The filter was washed twice with 4 ml of ice-cold stop solution (25 mM HEPES-Tris buffer, pH 7.5, 30 mM K-gluconate and the appropriate concentration of mannitol to be osmotically equivalent to the incubation medium).

Initial uptake was evaluated in terms of the uptake at 10 s.

The amount of L-[^{14}C]lactic acid taken up was determined from the radioactivity. The filters trapping L-[^{14}C]lactic acid were transferred to counting vials and dissolved in 4 ml of scintillation fluid, Clear-sol I (Nacalai Tesque, Kyoto, Japan) for quantitation in a liquid scintillation counter. Protein was measured by the method of Bradford [24] using a Bio-Rad protein assay kit (Bio-Rad, Richmond, CA) with bovine serum albumin as a standard.

2.3. Effect of an anion antiport inhibitor, a protonophore and amino acid modifiers

The membrane vesicles were preloaded with 0.1 mM or 1 mM 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS), or 0.02 mM carbonylcyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) in 25 mM HEPES-Tris buffer (pH 7.5) containing either 30 mM $KHCO_3$ (bicarbonate gradient), 30 mM KCl (chloride gradient) or 30 mM K-gluconate (proton gradient and no gradient) at room temperature for 20 min. As the control, membrane vesicles were similarly incubated without corresponding reagents. After the incubation, the mixture was diluted and centrifuged at $27\,000\times g$ for 30 min. The final pellet was suspended in experimental buffer. BBMVs were treated with DEPC according to the method of Bindslev and Wright [25]. The membrane vesicles, suspended in a buffer consisting of 100 mM mannitol and 20 mM phosphate buffer (pH 6.5), were mixed with 0.5 mM DEPC and then incubated at room temperature for 10 min. The reaction of BBMVs with 1 mM PCMBS and 25 mM phenylglyoxal (PGO) was performed according to the method of Miyamoto et al. [26]. The treatment with PCMBS was performed in 20 mM phosphate buffer (pH 7.5) containing 300 mM mannitol and an equal concentration of ethylenediaminetetraacetic acid with PCMBS in order to chelate free mercury, and then the mixture was incubated at room temperature for 10 min. The treatment with PGO was performed in the 20 mM phosphate buffer (pH 8.7) containing 280 mM mannitol and the mixture was incubated at room temperature for 30 min. The reaction of membrane vesicles with 25 mM PLP was done as previously reported [27]. The membrane vesicles were incubated with buffer containing 20 mM $MgCl_2$, 60 mM imidazole and 20 mM NH_4Cl (pH 6.8) at room temperature for 15 min.

2.4. Data analysis

The uptakes were represented as uptake coefficient (μl/10 s/mg protein), uptake rate (pmol/10 s/mg protein) or vesicle/medium ratio obtained by dividing the uptake amount of L-lactic acid by the L-lactic acid concentration in the incubation medium (μl/mg protein). The kinetic parameters for the uptake of L-lactic acid by BBMVs were estimated by solving the following equations, consisting of both saturable and apparently nonsaturable linear terms, using the nonlinear least-squares regression analysis program MULTI [28].For a single saturable process and one nonsaturable process:

$$v = V_{\max}\times[s]/(K_t + [s]) + k_d\times[s]$$

and for two saturable processes and one nonsaturable process:

$$v = V_{\max_1}\times[s]/(K_{t_1} + [s]) + V_{\max_2}\times[s]/(K_{t_2} + [s]) + k_d\times[s]$$

where v and $[s]$ represent the apparent uptake rate and the concentration of L-lactic acid, respectively. $V_{\max}$ and K_t are the maximum uptake rate and the apparent Michaelis constant for a saturable process, respectively, and k_d is the first-order rate constant for the apparently nonsaturable component, which was estimated from the uptake determined in the absence of a bicarbonate gradient at pH 7.5.

3. Results

3.1. Effect of bicarbonate and/or proton gradients on L-lactic acid uptake

Fig. 1 shows the time courses of uptake of L-[^{14}C]lactic acid in the presence and absence of proton and/or bicarbonate gradients. In the presence of an inward-directed proton or outward-directed bicarbonate gradient, uptake of L-[^{14}C]lactic acid showed an overshoot phenomenon, whereas in the absence of any ion gradient, no significant increase of uptake

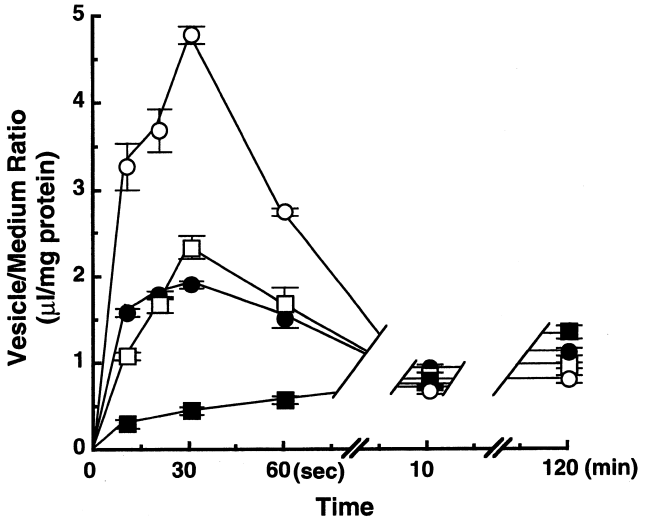

Fig. 1. Time courses of L-[^{14}C]lactic acid uptake by rabbit jejunal BBMVs. BBMVs were preloaded with 25 mM HEPES-Tris buffer (pH 7.5) containing either 30 mM K-gluconate (closed symbols), or 30 mM $KHCO_3$ (open symbols, for bicarbonate gradient). The uptake of L-[^{14}C]lactic acid (15 μM) was performed at 37°C by incubating in 25 mM MES-Tris buffer (pH 6.0, circles, for proton gradient) or 25 mM HEPES-Tris buffer (pH 7.5, squares) containing 30 mM K-gluconate. Each solution contained an appropriate concentration of mannitol to be isotonic. All experiments were performed in a medium containing 10 μM valinomycin and 1% ethanol (final concentration). Each point represents the mean ± S.E. of three to four experiments.

was observed. The simultaneous imposition of the two ion gradients, bicarbonate and proton gradients, induced very efficient uptake with a 10-fold increased transient overshoot uptake over the uptake at the steady state. The initial uptake measured at 10 s (3.33 ± 0.22 μl/mg protein) was larger than the sum of the uptakes dependent on proton (1.59 ± 0.07 μl/mg protein) and bicarbonate (1.09 ± 0.06 μl/mg protein) gradients.

3.2. Concentration dependence of L-lactic acid uptake

Fig. 2 shows the saturation kinetics of initial uptake of L-lactic acid dependent on bicarbonate and/or proton gradients, expressed as Eadie-Hofstee plots after correction for nonsaturable uptake. Apparently two saturable processes were involved in the presence of both proton and bicarbonate gradients. The nonlinear least-squares analysis of the results yielded K_{t_1} and K_{t_2} values of 61.2 ± 58.8 mM and 2.1 ± 1.8 mM, and V_{max_1} and V_{max_2} values of 60.9 ± 24.3 and 7.4 ± 5.7 nmol/10 s/mg protein, respectively. In the presence of a proton or bicarbonate gradient alone, a single saturable component was observed. The K_t and V_{max} values for proton and bicarbonate gradient-driven uptakes were 43.1 ± 4.7 mM and 60.3 ± 5.6 nmol/10 s/mg protein and 11.3 ± 1.5 mM and 16.0 ± 1.1 nmol/10 s/mg protein, respectively. The nonsaturable uptake rate, k_d, estimated in the absence of a bicarbonate gradient at pH 7.5 was 0.274 ± 0.008 μl/10 s/mg protein.

3.3. Anion specificity of the antiport process in L-lactic acid uptake

Table 1 shows the anion dependence of L-[^{14}C]lactic acid transport at the intra- and extravesicular pH of 7.5. Intravesicular chloride ion enhanced L-lactic acid uptake as well as bicarbonate ion, whereas the bivalent anion sulfate ion did not show such an effect. The enhancement of L-lactic acid

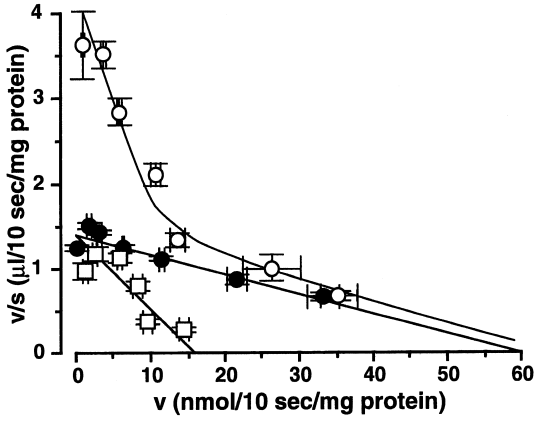

Fig. 2. Eadie-Hofstee plots for concentration dependence of L-lactic acid uptake by rabbit jejunal BBMVs. BBMVs were preloaded with 25 mM HEPES-Tris buffer (pH 7.5) containing either 30 mM K-gluconate (closed symbols), or 30 mM $KHCO_3$ (open symbols, for bicarbonate gradient). The incubation medium contained 0.1–50 mM L-lactic acid, 30 mM K-gluconate, 25 mM MES-Tris buffer (pH 6.0, circles, for proton gradient) or 25 mM HEPES-Tris buffer (pH 7.5, squares). Each solution contained an appropriate concentration of mannitol to be isotonic. All experiments were performed under identical conditions to those described in the legend to Fig. 1. Each point represents the mean ± S.E. of three to four experiments after correction for nonsaturable transport estimated from the uptake at pH 7.5 in the absence of both bicarbonate and gradients.

Table 1
Effect of intravesicular anion and anion transport inhibitors on L-[^{14}C]lactic acid uptake by rabbit jejunal BBMVs

Driving force	Relative uptake (% of control)
Gluconate (control)	100
Bicarbonate	166.2 ± 9.3*
+1 mM DIDS	88.9 ± 4.6*,**
Chloride	168.6 ± 11.6*
+1 mM DIDS	115.0 ± 16.2**
Sulfate	112.5 ± 6.7

BBMVs were preloaded with 25 mM HEPES-Tris buffer (pH 7.5), containing 30 mM $KHCO_3$ (for bicarbonate gradient), KCl (for chloride gradient), K_2SO_4 (for sulfate gradient), or K-gluconate (for control). The preload condition with DIDS is mentioned in Section 2. The uptake of L-[^{14}C]lactic acid was performed by incubating in HEPES-Tris buffer (pH 7.5) and adding 30 mM K-gluconate, except for the sulfate gradient, for which 60 mM K-gluconate was added to adjust the K^+ concentration at either side of the membrane. Each solution contained an appropriate concentration of mannitol to be isotonic. All experiments were performed under identical conditions to those described in the legend to Fig. 1. Each value represents the mean ± S.E. of three to four experiments.
*Significantly different from the uptake value in the absence of any gradient by Student's *t*-test ($P < 0.05$).
**Significantly different from the uptake value in the absence of DIDS under each condition by Student's *t*-test ($P < 0.05$).

transport was reduced by DIDS, an anion exchange inhibitor.

3.4. Effect of an anion antiport inhibitor, a protonophore and amino acid modifiers on L-lactic acid uptake

Table 2 shows the effect of an anion antiport inhibitor, protonophore and chemical modifiers of the amino acid residues that are functionally important for L-lactic acid transport. The uptake of L-[^{14}C]lactic acid was inhibited by 0.1 mM of DIDS only in the presence of bicarbonate gradient, and by a protonophore, FCCP, at the concentration of 0.02 mM only in the presence of proton gradient. The uptake of L-[^{14}C]lactic acid was inhibited by histidyl (DEPC), thiol (PCMBS) and lysine (PLP) residue-modifying agents both in the presence of bicarbonate ion and proton gradients, whereas PGO, an arginyl residue-modifying agent, inhibited only proton cotransport of L-[^{14}C]lactic acid, but not an anion antiport.

3.5. Inhibitory effects of various compounds on lactic acid uptake

The substrate specificity of lactic acid transport by bicarbonate antiport was examined by the inhibitory effects of various compounds and compared with that of proton cotransport. As shown in Table 3, L-[^{14}C]lactic acid uptake facilitated by a bicarbonate gradient was reduced by various monocarboxylic acids, but not by acetamide, succinic acid or citric acid. In addition, stereoselectivity in the inhibition of L-[^{14}C]lactic acid uptake by L- (36.3% of the control) and D-lactic acid (68.6%) was observed. In the presence of proton gradient, L-[^{14}C]lactic acid uptake was reduced by various carboxylic acids but not by acetamide. However, the observations of the significant inhibition by succinic acid and citric acid and the absence of stereoselectivity in the inhibitory effect of L- and D-lactic acids are distinct from the characteristics of bicarbonate ion-dependent lactic acid transport.

Table 2
Effects of a anion antiport inhibitor, a protonophore and amino acid modifiers on the initial uptake of L-[^{14}C]lactic acid

Inhibitors (conc.)	Relative uptake (% of control)	
	Bicarbonate gradient	Proton gradient
DIDS (0.1 mM)	74.1 ± 2.4*	101.6 ± 1.7
FCCP (0.02 mM)	92.9 ± 6.9	29.5 ± 0.7*
DEPC (0.5 mM)	41.3 ± 1.1*	86.9 ± 1.3*
PCMBS (1 mM)	55.5 ± 2.9*	51.6 ± 2.7*
PLP (25 mM)	51.2 ± 3.8*	56.7 ± 2.1*
PGO (25 mM)	97.5 ± 6.6	38.8 ± 2.0*

The preload condition with DIDS, FCCP and amino acid modifiers is mentioned in Section 2. The uptake of L-[^{14}C]lactic acid was performed at 37°C by incubating in 25 mM HEPES-Tris buffer (pH 7.5) containing 30 mM K-gluconate for the bicarbonate gradient-dependent uptake, or 25 mM MES-Tris buffer (pH 6.0) containing 30 mM K-gluconate for the proton gradient-dependent uptake. Each solution contained an appropriate concentration of mannitol to be isotonic. All experiments were performed under identical conditions to those described in the legend to Fig. 1. Each value represents the mean ± S.E. of three to four experiments.
*Significantly different from the control value by Student's *t*-test ($P < 0.05$).

Table 3
Inhibitory effects of various carboxylic acids on the initial uptake of L-[^{14}C]lactic acid

Carboxylic acids	Relative uptake (% of control)	
	Bicarbonate gradient	Proton gradient
Acetic acid	11.3 ± 1.9*	45.5 ± 1.9*
D-Lactic acid	68.6 ± 7.8*,**	54.0 ± 3.8*
L-Lactic acid	36.3 ± 1.1*,**	50.6 ± 2.4*
Nicotinic acid	1.5 ± 2.2*	38.7 ± 3.3*
Valproic acid	ND*	33.5 ± 1.5*
Acetamide	81.8 ± 6.6	90.7 ± 1.9
Citric acid	98.8 ± 4.1	77.3 ± 3.5*
Succinic acid	107.4 ± 2.0	53.8 ± 5.5*

BBMVs were preloaded with 25 mM HEPES-Tris buffer (pH 7.5) containing either 30 mM $KHCO_3$ for bicarbonate gradient or 30 mM K-gluconate for proton gradient. Each solution contained an appropriate concentration of mannitol to be isotonic. The concentration of each inhibitor was 20 mM. All experiments were performed under identical conditions to those described in the legend to Fig. 1. Each value represents the mean ± S.E. of three to four experiments as a percentage of the control uptake. Uptake coefficients of the control studies for bicarbonate exchange and proton cotransport, corrected for the uptake in the absence of any ion gradient, were 1.07 ± 0.049 and 1.36 ± 0.083 (μl/10 s/mg protein, mean ± S.E.), respectively.
*Significantly different from the control value by Student's *t*-test ($P < 0.05$).
**Significant difference between isomers by Student's t-test ($P < 0.05$).

4. Discussion

Although it has long been believed that lactic acid is absorbed from the small intestine mainly by passive diffusion [29], precise studies on membrane transport mechanisms have demonstrated the involvement of carrier-mediated transport mechanisms driven by sodium and/or proton gradients across the intestinal epithelial brush-border membrane [30–32]. Since the finding of proton/lactic acid cotransporter MCTs [10,11], we suggested the partial role of MCT1 as the intestinal monocarboxylic acid transporter [12–14]. However, the results of our membrane physiological studies of monocarboxylic acids indicated that an anion antiport mechanism might also be involved in the brush-border membrane transport of monocarboxylic acids in the small intestine [4,8,9,18]. In the present study, using isolated membrane vesicles, it was demonstrated that an anion antiport and proton cotransport mechanism does participate in the transport of lactic acid across the intestinal brush-border membrane.

In the presence of outward-directed bicarbonate and/or inward-directed proton gradients, the uptake of L-lactic acid by isolated rabbit jejunal BBMVs showed an overshoot uptake (Fig. 1). Uptake of L-lactic acid in the presence of both bicarbonate ion and proton gradients appeared to involve multiple transport systems, while Eadie-Hofstee plot analysis in the presence of either a bicarbonate or a proton gradient suggested that only a single mechanism functions in each case (Fig. 2). In addition, bicarbonate ion-dependent uptake of L-lactic acid was inhibited by an anion exchange inhibitor, DIDS, but not by a protonophore FCCP (Table 2). Furthermore, the absence of stereoselectivity in the inhibitory effects of L- and D-lactic acid and the significant reduction of L-lactic acid uptake in the presence of succinic acid and citric acid were observed only in the proton gradient-dependent uptake of L-lactic acid (Table 3). These results strongly demonstrated that there are multiple pathways for L-lactic acid transport that function independently in the intestinal epithelial brush-border membranes, namely a bicarbonate/lactic acid antiporter and a proton-lactic acid cotransporter. An arginyl residue was suggested to be involved in the functional domain of the proton cotransporter, but not of the anion antiporter, since an arginyl residue-modifying agent, PGO, inhibited only proton-dependent transport of L-[^{14}C]lactic acid. This specific inhibitory effect provides additional evidence that lactic acid is transported by an anion antiport mechanism, which is distinct from the proton cotransport mechanism.

Kinetic analysis of the proton gradient-dependent uptake of L-lactic acid transport at the extravesicular pH of 6.0 gave a K_t of 43.1 mM, and this system seems to correspond to the low-affinity component (K_{t_2}, 61.2 mM) observed in the presence of both proton and bicarbonate gradients. Accordingly, the higher-affinity component (K_{t_1}, 2.1 mM) obtained at pH 6.0 in the presence of a bicarbonate gradient is thought to represent the bicarbonate gradient-dependent uptake of L-lactic acid. The K_t value at pH 7.5 in the presence of a bicarbonate gradient alone (11.3 mM) was comparable with the reported K_t value for the anion antiport of L-lactic acid across Caco-2 cells (10.9 mM) [21]. The obtained K_t value

(11.3 mM) is apparently larger than the higher-affinity component K_{t_1} 2.1 mM in the presence of both bicarbonate and proton gradients. The smaller K_t value (2.1 mM) may be explained by the pH dependence of the anion antiport process. Because anion antiport of monocarboxylic acids could be facilitated at acidic pH 6.0 compared with that at neutral pH as observed previously for the transport of other monocarboxylic acids in isolated BBMVs [8]. So, the K_{t_1} is expected to represent the Michaelis constant of the anion antiporter at acidic pH. In addition, the initial uptake measured at pH 6.0 in the presence of an outward bicarbonate ion gradient (3.3 μl/mg protein/10 s) was larger than the sum of the uptakes (2.68) observed in the presence of proton (1.59) and bicarbonate (1.09) gradients. These results strongly suggest an activation of the anion antiporter at the physiological (acidic) pH.

The inhibitory effect of various compounds suggested that several monocarboxylic acids might be substrates for the common anion antiporter with L-lactic acid (Table 3). It was also indicated that chloride ion can behave as a counter-anion for L-lactic acid uptake in BBMVs, the same as bicarbonate ions for the anion antiporter. Previously, we showed that uptake of monocarboxylic acids mediated by an anion exchange transporter AE2 was inhibited by extracellular chloride ion, bicarbonate ion and DIDS, and was activated by extracellular acidification [18]. We preliminarily observed the expression of uptake activity of L-[^{14}C]lactic acid by AE2-expressing cells (data not shown). So, since there is one report of the presence of AE2 in the intestinal brush-border membrane [20], AE2 or related transporter might be involved as anion antiporter for lactic acid. Recently, another anion exchanger, DRA, which is expressed in the brush-border membranes of intestine, highly in colon, was demonstrated [33,34]. Functionally DRA is characterized by transporting sulfate ions by anion exchange mechanism [35,36]. Since uptake of lactic acid was not enhanced by preloading sulfate ions in the present study (Table 1), it is unlikely that DRA is involved in the lactic acid/anion antiporter in rabbit small intestine. Further studies are essential for the molecular identification of anion antiporter for L-lactic acid in the intestinal epithelial brush-border membrane.

Proton gradient-dependent transport of L-lactic acid was clearly demonstrated to be involved independently from the anion antiport as discussed above. We have previously demonstrated that monocarboxylic acid transporter MCT1 is partially contributed to the intestinal absorption of various compounds [12–14]. However, several observations in the present study, including the K_m value (Fig. 2), absence of stereospecificity for L- and D-lactic acids, and apparent inhibition by di- and tricarboxylic acid (Table 3), were not completely consistent with previously known MCT1 [7,8,37–39], although rabbit MCT1 has not been functionally characterized yet. So, it is probable that unknown transporters such as MCT isoforms or others might be involved as the proton cotransporter for lactic acid.

Cheeseman et al. [40] demonstrated that a lactic acid transporter in the rat intestinal basolateral membrane operates as an anion exchanger. They showed that several monocarboxylic acids are transported in a similar manner, which was influenced by a bicarbonate gradient, but not by a pH gradient or by DIDS. The anion antiporter and the proton cotransporter that are presumed to exist both in brush-border and basolateral membranes may regulate the overall membrane transport process of lactic acid in the intestine.

In conclusion, the present study demonstrated that an anion antiporter is involved in the transport of L-lactic acid across the intestinal brush-border membrane in parallel with a proton cotransporter. Since intracellular bicarbonate ions and intestinal luminal protons are supplied by the cytoplasmic carbonic anhydrase and by microclimate pH due to sodium/proton antiport [41], respectively, these transporters are expected to function efficiently for the intestinal absorption of L-lactic acid.

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