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Transepithelial Transport of α-Lipoic Acid across Human Intestinal Caco-2 Cell Monolayers

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α-Lipoic acid (LA) is used in dietary supplements or food with antioxidative functions. The mechanism for the intestinal absorption of α-lipoic acid was investigated in this study by using human intestinal Caco-2 cell monolayers. LA was rapidly transported across the Caco-2 cell monolayers, this transport being energy-dependent, suggesting transporter-mediated transport to be the mechanism involved. The LA transport was strongly dependent on the pH value, being accelerated in the acidic pH range. Furthermore, such monocarboxylic acids as benzoic acid and medium-chain fatty acids significantly inhibited LA transport, suggesting that a proton-linked monocarboxylic acid transporter (MCT) was involved in the intestinal transport of LA. The conversion of LA to the more antioxidative dihydrolipoic acid was also apparent during the transport process.

KEYWORDS: **Caco-2; α-lipoic acid; dihydrolipoic acid; monocarboxylic acid transporter; intestinal absorption; transepithelial transport**

INTRODUCTION

Naturally occurring α-lipoic acid (LA) is an essential cofactor for mitochondrial respiratory enzymes and exerts a powerful antioxidative effect (*1*, *2*). This effect is due to direct radical scavenging, recycling of other antioxidants, accelerating glutathione (GSH) synthesis, and modulating the transcription factor activity, especially that of NF-κB. Synthetic LA has long been used as a therapeutic agent in the treatment of diabetic neuropathy (*2*, *3*), and its recent use in dietary supplements or foods with antioxidative functions has been markedly increasing.

The excretion and biotransformation of LA have been investigated, following a single dose of ^{14}C-labeled LA to mice, rats, and dogs and of unlabeled LA to humans (*2*, *4*, *5*). More than 80% of the radioactivity given was renally excreted, and 12 metabolites were identified. These results suggest that LA would be efficiently absorbed in the intestines and metabolized in the body.

There are two possible pathways for the intestinal transport of such low molecular weight compounds as LA: a transporter-mediated intracellular transport pathway and a paracellular pathway between the intercellular junctions. The former involves energy-dependent active transport, whereas the latter is passive diffusion. The intestinal absorption of such nutrients as glucose, amino acids, dipeptides, and water-soluble vitamins (*6*) is mainly via the transporter-mediated route, whereas the paracellular pathway plays an important role in the intestinal absorption of such minerals as calcium (*7*). The paracellular transport of nutrients such as glucose and amino acids has also been reported (*8*). It would be important to identify the mechanism for the intestinal transport of orally administered LA to control its intestinal absorbability, although this mechanism remains obscure.

Prasad et al. (*9*) and Balamurugan et al. (*10*) have reported that LA inhibited in a dose-dependent manner the uptake of such soluble vitamins as biotin and pantothenic acid, which were transported by a sodium-dependent multivitamin transporter (SMVT) in placental (*9*) or renal (*10*) epithelial cells. Although this implies that LA would be transported by SMVT, there is no direct evidence for this.

We investigated in this study the mechanism for the intestinal absorption of LA by using human intestinal Caco-2 monolayers as an in vitro model of the intestinal epithelium. Caco-2 cells express a variety of nutrient transporters and are frequently used to analyze transporter functions (*11*). The paracellular transport of a food-derived bioactive peptide has also been characterized by using Caco-2 cell monolayers (*12*). We found from the present study that proton-dependent transporter-mediated transport was the main pathway for the transepithelial transport of LA. The metabolic change of LA during the absorption process in Caco-2 cells was also studied.

MATERIALS AND METHODS

Materials. The human colon adenocarcinoma cell line, Caco-2, was obtained from the American Type Culture Collection (Rockville, MD). Dulbecco's modified Eagle's medium (DMEM) was purchased from Kohjin Bio Co. (Saitama, Japan), and fetal bovine serum (FBS) was

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from Gemini Bio-Products (Woodland, CA). Penicillin−streptomycin (10000 units/mL and 10 mg/mL in 0.9% sodium chloride, respectively) and nonessential amino acids (NEAA) were purchased from Gibco (Gaithersburg, MD). The type-1 collagen solution was purchased from Nitta Gelatin (Osaka, Japan), Hanks' balanced salt solution (HBSS) was from Sigma (St. Louis, MO), and phosphate-buffered saline (PBS) was from Nissui Pharmaceutical Co. (Tokyo, Japan). α-Lipoic acid, dihydrolipoic acid, and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) were purchased from Sigma (St. Louis, MO); sodium azide, biotin, and pantothenic acid were from Kanto Chemical Co. (Tokyo, Japan); and glycyl-sarcosine was from Bachem (Bubendorf, Switzerland). DL-Lactic acid, sodium benzoate, sodium acetate, sodium butyrate, sodium hexanoate (caproate), and sodium octanoate (caprylate) were purchased from Kanto Chemical Co. (Tokyo, Japan). All other chemicals used were of reagent grade.

Cell Culture. The Caco-2 cells were cultured at a density of 2 × 10^5 cells in 100-mm plastic dishes with a culture medium containing DMEM, 10% FBS, 100 μM NEAA, 100 units/mL of penicillin, and 100 μg/mL of streptomycin. The cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2 in air, the culture medium being renewed on alternate days. When the cells had reached confluence (6−7 days after seeding), they were passaged by trypsinization with 0.1% trypsin and 0.02% EDTA in PBS. All of the cells used in this study were between passages 50 and 60. A transepithelial transport experiment was performed by using Caco-2 cells that had been cultured at a density of 2 × 10^5 cells per well in 12-well Transwell inserts (12-mm diameter and 0.4-μm pore size; Corning Costar, NY) that had been precoated with collagen. The cell monolayers for all experiments were used after 14 days of culture.

Measurement of the Transepithelial Electrical Resistance (TER). The integrity of the cell layer was evaluated by measuring TER with Millicell-ERS equipment (Millipore, MA). Monolayers with TER of >500 Ω/cm^2 were used for the experiments. TER of the monolayers was measured before and after an assay sample was added to the insert (apical side).

Transepithelial Transport Experiments. The apical side of Caco-2 cell monolayers that had been cultured in the 12-well Transwell inserts and the basal chambers were washed twice with 0.5 mL of HBSS. The insert and basal chamber were, respectively, filled with 0.5 and 1.5 mL of HBSS and then incubated at 37 °C for 1 h. After HBSS in the inserts had been removed, 0.5 mL of 0.5 mM LA in HBSS (pH adjusted to 5.0−8.0) was added to the insert and then incubated at 37 °C for 2 h. The LA concentrations in the apical and basal solutions were measured by high-performance liquid chromatography (HPLC).

Competition Experiments on LA Transport. After the Caco-2 cell monolayers had been washed and equilibrated with HBSS as just described, HBSS in the inserts was removed. A 500-μL amount of 0.5 mM LA in HBSS adjusted to pH 7.4 or 6.0 and containing an assay sample was then added to the insert and incubated at 37 °C for 15 min. The assay samples used were water-soluble vitamins (biotin and pantothenic acid), monocarboxylic acids (lactic acid, benzoic acid, acetic acid, butyric acid, hexanoic acid, and octanoic acid), and a peptide (glycyl-sarcosine). The concentration of each assay sample was 10 mM, except that biotin was 2 mM because of its low solubility. After the incubation, the basal solution was taken and the concentration of LA measured by HPLC.

Competition Experiment on Fluorescein Transport. This experiment was performed in a manner similar to that just described. Briefly, 25 μM fluorescein, in the presence or absence of 0.5 mM LA, was added to the apical solution of the Caco-2 cell monolayers and then incubated at 37 °C for 15 min. The fluorescence of the basal solution was measured with a Fluoroskan Ascent CF fluorescence microplate reader (Labsystems, Helsinki, Finland), detecting the emission at 485 nm with excitation at 544 nm.

HPLC Analysis of LA and Dihydrolipoic Acid (DHLA). A Gulliver HPLC system (Jasco International Co., Japan) consisting of a PU-2080 Plus intelligent HPLC pump, a UV-1575 intelligent UV−vis detector, and an 807-IT integrator was used. Chromatographic separation was performed in a C18 column (Cosmosil $5C_{18}$-MS-II, 4.6 × 150 mm; Nacalai Tesque, Kyoto, Japan). The mobile phase was a 70% methanol solution containing 0.02% acetic acid at a flow rate of 1.0 mL/min. LA was monitored at UV 334 nm and quantified from its peak area. DHLA was monitored by a Nanospace SI-2 electrochemical detector (ECD; Shiseido Co., Tokyo, Japan), the applied potential being 900 mV versus Ag/AgCl.

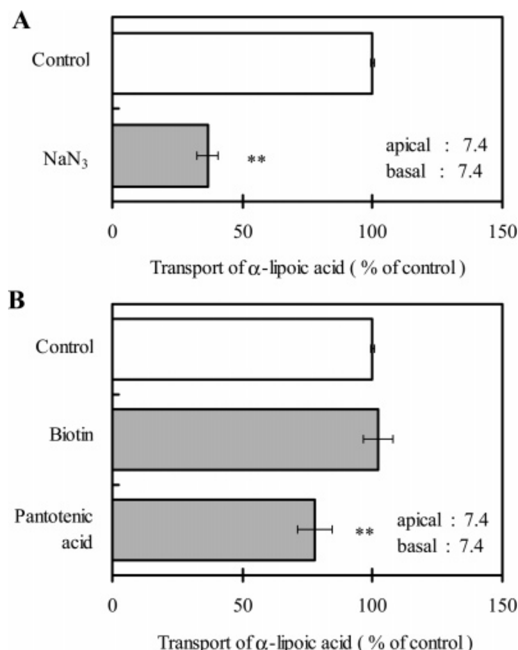

Figure 1. Effect of NaN_3 and soluble vitamins on the transepithelial transport of α-lipoic acid across the Caco-2 cell monolayers. The amount of LA in the basal chamber was determined after incubation of the Caco-2 cell monolayers at 37 °C for 15 min. The initial concentration of LA added to the apical solution was 0.5 mM. The concentrations of sodium azide (**A**) and pantothenic acid (**B**) were 10 mM, whereas that of biotin (**B**) was 2 mM because of its low solubility. The pH value was adjusted to 7.4 on both sides. Data are expressed as a percentage of the control and are presented as the mean ± SD ($n = 3$). **, significantly lower than the control value ($P < 0.01$).

Inhibition of Glutathione Reductase. Caco-2 cells were monolayer-cultured in a 12-well microplate for 2 weeks. The cells were washed with HBSS (pH adjusted to 6.0) and then incubated in the same buffer containing 100 μM LA in the presence or absence of BCNU (0−100 μM). The amount of DHLA in the culture solution after a 60-min incubation was measured by HPLC-ECD.

Statistical Analysis. Each result is expressed as the mean ± standard deviation (SD). A statistical analysis was conducted by Dunnett's test to identify significant differences between the control and test groups.

RESULTS

Transepithelial Transport of LA Was Energy-Dependent. After 15 min of incubation, >10% of LA added to the apical solution (at pH 7.4) had been transported to the basal chamber. This transport was strongly inhibited by pretreating the Caco-2 cells with the metabolic inhibitor, sodium azide (**Figure 1A**), suggesting that the transport was via an energy-dependent pathway. To reveal whether this transport was mediated by the multivitamin transporter (SMVT), as has been suggested in previous work (*9*, *10*), the competitive effect of such SMVT substrates as biotin and pantothenic acid on the LA transport was examined. The SMVT substrates generally had no effect on the transport of LA, except that pantothenic acid in the apical solution (pH 7.4) significantly decreased the transport of LA (**Figure 1B**). This suggests that SMVT was not principally involved in the transepithelial transport of LA across the Caco-2 cell monolayers.

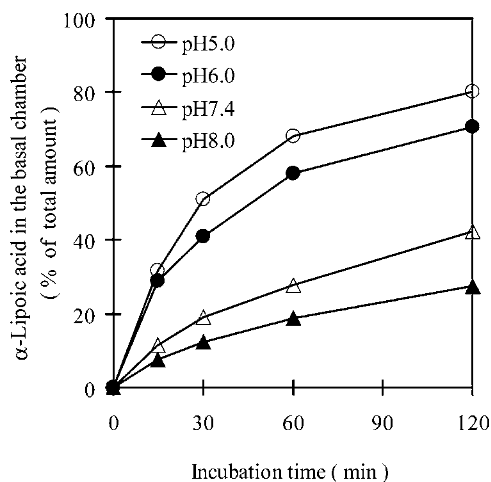

Figure 2. Effect of pH value on the transepithelial transport of α-lipoic acid across the Caco-2 cell monolayers. The amount of LA in the basal chamber was determined as a function of time after incubation of the Caco-2 cells at 37 °C. The initial concentration of LA added to the apical solution was 0.5 mM. The pH value of the apical solution was adjusted within the range of 5.0–8.0, and that of the basal solution was fixed at 7.4. Data are expressed as a percentage of the total amount of LA and are presented as the mean ± SD ($n = 3$).

Transepithelial Transport of LA Was Proton-Dependent. The transepithelial transport of LA from the apical to basal chamber across the Caco-2 cells increased as a function of time. The transport of LA was also strongly pH-dependent (**Figure 2**). When the apical solution was adjusted to the acidic pH range, the transport rate for LA was significantly higher than that at the neutral pH level. At pH 5.0, >60% of LA added to the apical chamber had been transported to the basal solution within 60 min. After a 120-min incubation, almost 80% of LA was in the basal solution, indicating that the basal LA concentration was already higher than the apical LA concentration. In contrast, the transport of LA at an apical pH level of 8.0 was 30% lower than the control value (pH 7.4). These results suggest that a proton-linked transporter was involved in the LA transport. Addition of LA did not affect the monolayer integrity of Caco-2 cells at any pH between 5 and 8, because no change in the transepithelial electrical resistance was observed (data not shown).

Transepithelial Transport of LA Was Not Na^+-Dependent. Because many transporters are dependent on the Na^+ concentration, the Na^+ dependence of the LA transport was examined. LA (0.5 mM) was added to the apical side of the Caco-2 cell monolayers in the presence of NaCl (0, 35, 70, or 140 mM) and incubated at 37 °C for 60 min. The LA content in the basal solution was then measured by HPLC. The transport rate of LA from the apical to basal chamber across the Caco-2 cell monolayers remained unchanged, irrespective of the Na^+ concentration in the apical and basal solutions at pH 7.4 (**Figure 3A**). This Na^+ independence was also apparent when the apical pH value was 6.0 (**Figure 3B**).

Peptide Transporter Was Not Involved in the Transport of LA. Because peptide transporter 1 (PepT1) expressed in the intestinal epithelium is proton-dependent, showing a higher transport rate in the acidic pH range (*6*), PepT1 may have been involved in the LA transport. However, adding glycyl-sarcosine, a typical PepT1 substrate with high affinity, to the apical solution had no effect on the transport of LA (data not shown), indicating that PepT1 was not involved in the LA transport.

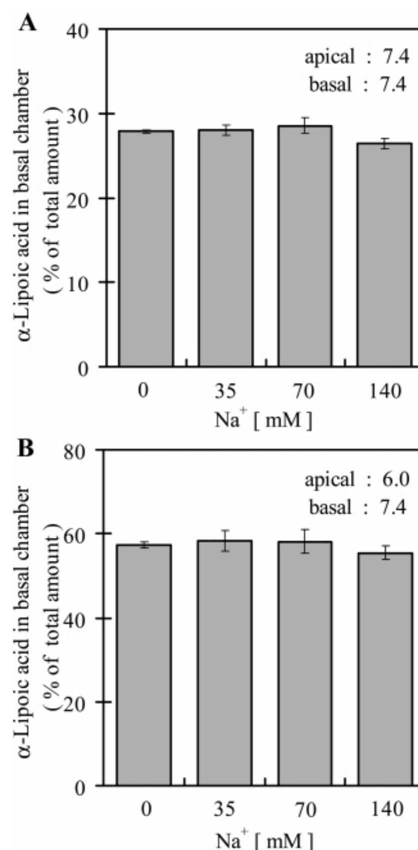

Figure 3. Effect of Na^+ on the transepithelial transport of α-lipoic acid across the Caco-2 cell monolayers. The amount of LA in the basal chamber was determined after incubation of the Caco-2 cells at 37 °C for 60 min. The initial concentration of LA added to the apical solution was 0.5 mM, and that of Na^+ was from 0 to 140 mM on both sides. The pH value of the apical solution was adjusted to 7.4 (**A**) or 6.0 (**B**), and that on the basal side was fixed at 7.4. Data are expressed as a percentage of the total amount of LA added to the apical chamber and are presented as the mean ± SD ($n = 3$).

Transepithelial Transport of LA Was Inhibited by Monocarboxylic Acids. The effect of monocarboxylic acids on the LA transport was then examined to learn whether the monocarboxilic acid transporter (MCT), another proton-dependent transporter, was involved in the LA transport. DL-Lactic acid and acetic acid, the good substrates of MCT-1, scarcely affected the transport of LA. On the other hand, benzoic acid, butyric acid, hexanoic acid, and octanoic acid all significantly inhibited the transport of LA, the inhibitory activity being dependent on the carbon chain length (**Figure 4**). The effect of long-chain fatty acids, such as lauric acid, myristic acid, and palmitic acid, was not evaluated because they had low solubility.

LA Did Not Affect the Transport of Fluorescein, an MCT Substrate. We have previously reported fluorescein as one of the MCT substrates (*13*, *14*). However, the transepithelial transport of fluorescein from the apical to basal chamber across the Caco-2 cell monolayers was not inhibited by LA. The accumulation of fluorescein in the Caco-2 cells and the amount of fluorescein remaining in the apical solution were also not affected by LA (data not shown).

Dihydrolipoic Acid Was Produced during Transport in the Caco-2 Cells. An HPLC analysis using a UV−vis detector could detect only the peak of LA (**Figure 5A**). On the other hand, an electrochemical detector made it possible to detect the peaks of both LA and DHLA, demonstrating that DHLA was produced during the transport experiment (**Figure 5B**). DHLA

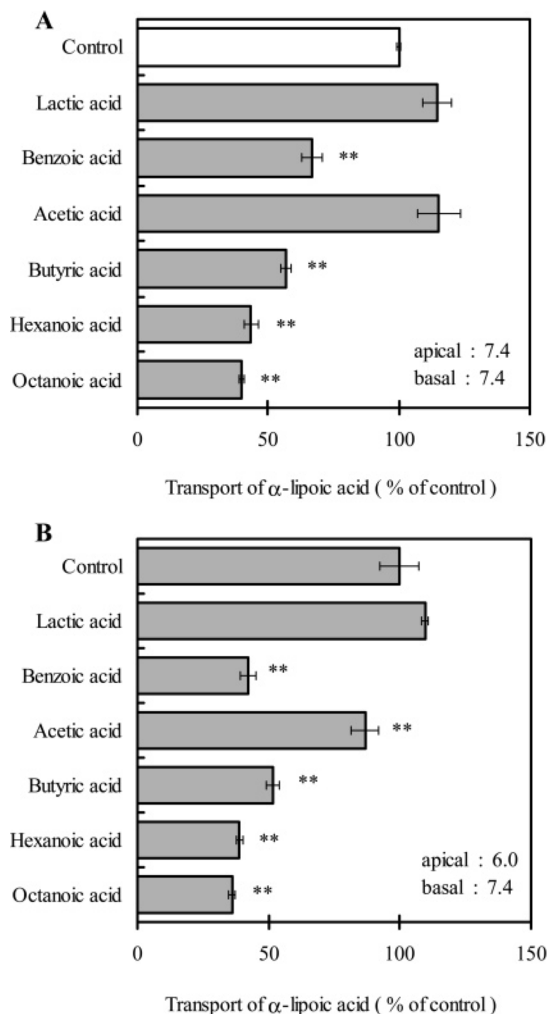

Figure 4. Effect of monocarboxylic acids on the transepithelial transport of α-lipoic acid across the Caco-2 cell monolayers. The amount of LA in the basal chamber was determined after incubation of the Caco-2 cells at 37 °C for 15 min. The initial concentration of LA added to the apical solution was 0.5 mM, and that of each competitor was 10 mM on the apical side. The pH value of the apical solution was adjusted to 7.4 (**A**) or 6.0 (**B**), and that on the basal side was fixed at 7.4. Data are expressed as a percentage of the total amount of LA and are presented as the mean ± SD ($n = 3$). ∗∗, significantly lower than the control value ($P < 0.01$).

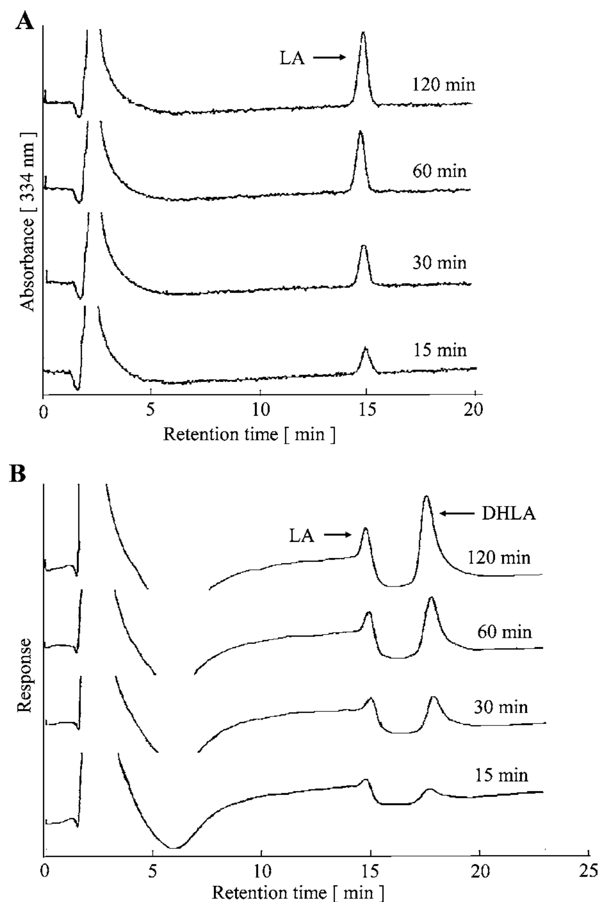

Figure 5. HPLC chromatograms of α-lipoic acid and dihydrolipoic acid. LA and DHLA in the basal chamber were monitored by using a UV–vis detector (**A**) and ECD (**B**). The mobile phase was a 50% methanol solution containing 0.02% acetic acid to separate LA and DHLA, and the flow rate was 1.0 mL/min.

in the basal chamber increased as a function of time, reaching an amount corresponding to 7% of the total LA in 120 min (**Figure 6A**). DHLA was also detected on the apical side, the amount reaching 3% of the total LA after 30 min of incubation, although the amount of LA in the apical chamber remained unchanged during the subsequent 90 min (**Figure 6A**). These results suggest that about 10% of the total LA was converted to DHLA in the Caco-2 cells, 30% of which was countertransported to the apical side and 70% transported to the basal side (**Figure 6A**).

Glutathione Reductase Was Likely To Be Responsible for the Production of DHLA. Constantinecsu et al. (*15*) have reported that LA is metabolized to DHLA in the erythrocyte by such reducing enzymes as glutathione reductase (GR) and thioredoxin reductases. Expression of several reducing enzymes including GR in Caco-2 cells has also been reported by Baker and Baker (*16*). Involvement of the reducing enzymes in the DHLA production by Caco-2 cells was therefore studied. When Caco-2 cells were treated with BCNU, a specific GR inhibitor, the production of DHLA in Caco-2 cells was markedly inhibited (**Figure 7**), suggesting that GR was responsible for DHLA production in Caco-2 cells.

DISCUSSION

The transepithelial transport of LA across the Caco-2 cell monolayers was observed to be energy-dependent, suggesting transporter-mediated transport to be the major mechanism for intestinal LA absorption. Because the involvement of SMVT in the intestinal transport of LA has been suggested in previous studies (*9*, *10*) and SMVT was expressed in Caco-2 cells (*17*), we had initially assumed that LA would be transported via SMVT. However, the LA transport was proton-dependent, the transport rate being increased by reducing the apical pH value (**Figure 2**). The transport of LA was also found to be independent of the Na^+ concentration (**Figure 3**) and not affected by SMVT substrates (**Figure 1B**), indicating that SMVT was not involved in the LA absorption.

Another possibility was the transport of LA via a peptide transporter. The peptide transporter, PepT1, is proton-dependent and has a wide range of substrate specificity, recognizing di- and tripeptides (*18*). Other compounds such as β-lactam antibiotics and synthetic peptide analogues have also been reported to be PepT1 substrates (*6*). However, LA transport across the Caco-2 cell monolayers was not affected by the PepT1 substrate, glycyl-sarcosine (data not shown). Döring et al. (*19*) have reported that octanoic acid, the structure of which

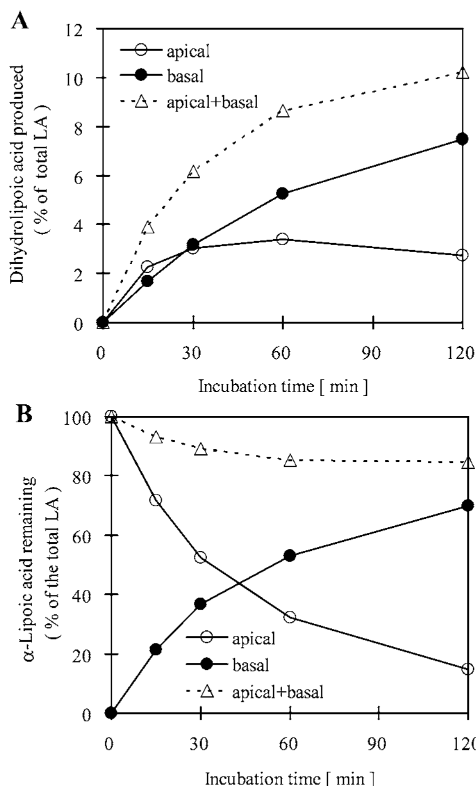

Figure 6. Production of dihydrolipoic acid in Caco-2 cells during the transport experiments. The amounts of DHLA produced (**A**) and LA remaining (**B**) as a function of time were determined after incubation of the Caco-2 cell monolayers at 37 °C. The initial concentration of LA was 0.5 mM in the apical solution, the respective pH values of the apical and basal solutions being adjusted to 6.0 and 7.4. Data are expressed as a percentage of the total amount of LA and are presented as the mean ± SD ($n = 3$).

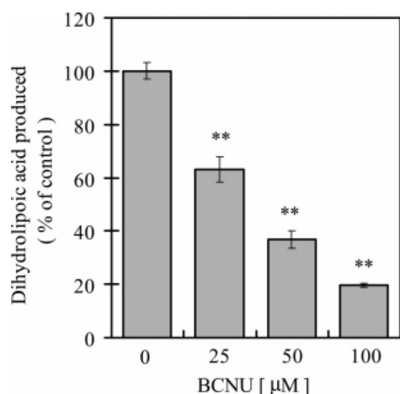

Figure 7. Effect of a glutathione reductase inhibitor on the production of dihydroplipoic acid. The amount of DHLA secreted into the culture solution was measured after incubation of the Caco-2 cells at 37 °C. The cells were incubated in HBSS for 60 min with 100 μM LA and BCNU of indicated concentrations. Data are expressed as a percentage of the amount of DHLA produced in control cells (without BCNU) and are presented as the mean ± SD ($n = 3$). **, $P < 0.01$ versus control.

resembles that of LA, could not be a PepT1 substrate. Taking these observations together, PepT1 is unlikely to have been involved in the LA transport.

It is well-known that long-chain fatty acids (an alkyl chain of C10 or longer) are transported by fatty acid transporters (FATP). The FATP family consists of six members which

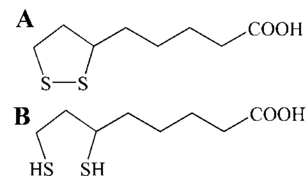

Figure 8. Chemical structures of α-lipoic acid (**A**) and dihydrolipoic acid (**B**).

represent a group of evolutionarily conserved proteins that are involved in the cellular uptake and metabolism of long- and very-long-chain fatty acids (*20*). Among them, FATP4 is located in the intestines and is expressed to a greater extent than that of the other members (*21*). However, it has been reported that FATP did not contribute to the transport of medium- and short-chain fatty acids with alkyl chains of shorter than C8 (*21*, *22*). Considering the result that the LA transport was inhibited by octanoic acid, it is unlikely that FATP would be involved in the transport of LA in Caco-2 cells, although the inhibitory effect on LA transport of fatty acids with alkyl chains of longer than C10 was not examined in the present study because of their very low solubility in HBSS.

LA is a derivative of octanoic acid with a dithiolan ring (**Figure 8**). It would therefore be possible for a MCT to be involved in the transport of LA. The transport rate of LA was significantly reduced in the presence of monocarboxylic acids as shown in **Figure 4**, although lactic and acetic acids did not inhibit LA transport. The inhibitory activity of those monocarboxylic acids having a hydrocarbon chain was correlated with the chain length (**Figure 4**).

MCTs (SMCT1 = SLC5A8, SMCT2 = SLC5A12) that are involved in the transport of such short-chain fatty acids as lactate and pyruvate have recently been identified (*23*, *24*). The contribution of these transporters to LA transport can also be discounted, because the transport by SMCTs was found to be a sodium-dependent process.

The MCT family, other than SMCTs, now comprises 14 members (**Table 1**), of which only the first four (MCT1−MCT4) have already been demonstrated experimentally to catalyze the proton-dependent transport of metabolically important monocarboxylates such as lactate, pyruvate, and ketone bodies (*25*). MCT1 is known to be expressed in the human intestines on the apical side of the epithelial cells, whereas MCT4 and MCT5 are expressed in the basal membrane (*26*). Buyse et al. (*27*) have reported that MCT1 was expressed in the apical membrane of Caco-2 cells. The mRNA expression for MCT1 and MCT4−MCT7 in Caco-2 cells has also been confirmed (*28*). However, the types of ligand have only been identified for six members (i.e., monocarboxylates for MCT1−4 and aromatic amino acid derivatives for MCT8 and MCT10). The functions and substrate specificities for MCT5−7, MCT9, and MCT11−14 remain to be determined (*29*). Because lactate, a good substrate for MCT1−4, did not inhibit the LA transport, other members of the MCT family might be involved in LA transport. Although the expression and properties of most MCTs in the intestines have not yet been fully characterized, it is most probable that LA is transported via some of the MCTs, because of their proton-dependent characteristics (**Figure 2**) and the inhibition of LA transport by monocarboxylic acids (**Figure 4**).

Konishi et al. (*13*) have demonstrated fluorescein to be one of the MCT substrates. They have also found that such phenolic acids as ferulic, benzoic, and coumaric acids reduced the transepithelial transport of fluorescein by competitively inhibiting its transporter-mediated transport (*14*). However, LA did

Table 1. Characteristics of the Monocarboxylic Acid Transporter (MCT) Family (*25*, *29*)

human gene	protein	aliases	predominant substrates	tissue distribution	sequence accession ID
SLC16A1	MCT1		lactate, pyruvate, ketone body	ubiquitous	NM_003051
SLC16A2	MCT8	XPTC, MCT7	T3, T4	brain, liver, kidney, heart, thyroid, eye, pituitary, other	NM_006517
SLC16A3	MCT4	MCT3	lactate, pyruvate, ketone body	ubiquitous	NM_004207
SLC16A4	MCT5	MCT4	unknown	multiple	NM_004696
SLC16A5	MCT6	MCT5	unknown	multiple	NM_004695
SLC16A6	MCT7	MCT6	unknown	eye, melanocytes, brain, other	NM_004694
SLC16A7	MCT2		lactate, pyruvate, ketone body	kidney, other	NM_004731
SLC16A8	MCT3	REMP	unknown	eye, other	NM_013356
SLC16A9	MCT9		unknown	kidney, eye, other	NM_194298
SLC16A10	TAT1		aromatic amino acids (W, Y, F)	kidney, placenta, brain, intestine, liver, spleen, thymus, other	NM_018593
SLC16A11	MCT11		unknown	skin, kidney, thyroid, brain, other	NM_153357
SLC16A21	MCT12		unknown	kidney, placenta, other	NM_213606
SLC16A13	MCT13		unknown	kidney, eye, brain, other	NM_201566
SLC16A14	MCT14		unknown	brain, lung, breast, other	NM_152527

not affect the transport of fluorescein (data not shown), suggesting that the MCTs for LA transport would be different from those for phenolic acid transport.

The present results strongly suggest that LA is transported via an MCT which is responsible for the transport of medium-chain fatty acids, although this type of MCT has not yet been identified. The identification and characterization of this transporter are therefore important subjects for future study.

The sum of the amount of LA remaining in the apical solution and that transported to the basal solution did not account for the total amount of LA that had been added to the apical chamber, the value being reduced with increasing incubation time (**Figure 6B**). This suggests that the remaining LA had been accumulated in the cells or metabolized to different compounds. Because the amount of LA inside the cells was not detectable, we thought that part of the LA could have been converted to a different compound, such as DHLA (*1*), a reduced form of LA. An HPLC analysis with an electrochemical detector (ECD) revealed the peak of DHLA to have appeared in the basal solution and to increase during incubation (**Figure 5B**). As the production of DHLA was markedly suppressed by a GR inhibitor, LA transported into the Caco-2 cells would have been rapidly reduced by GR and then transported out of the cells (**Figure 7**). DHLA has higher antioxidative activity than LA. The conversion of LA to DHLA in Caco-2 cells therefore strongly suggests that the intestinal epithelium acts as an amplifier of dietary antioxidants, increasing the antioxidative activity of food during the absorption process.

In conclusion, it was observed that LA was transported across the intestinal Caco-2 cell monolayers very rapidly in a proton-dependent manner. The transport of LA was inhibited by monocarboxylic acids and, particularly, by medium-chain fatty acids, suggesting that LA and the medium-chain fatty acids shared a common transporter in the intestinal epithelium. The production of DHLA, a reduced form of LA, in the cells during the transport process was also apparent. LA in a dietary supplement or in a functional food product would be efficiently absorbed in the intestines and be expected to express antioxidative functions in the body.

ABBREVIATIONS USED

BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; DHLA, dihydrolipoic acid; ECD, electrochemical detector; FATP, fatty acid transporter; GR, glutathione reductase; HBSS, Hanks' balanced salt solution; LA, α-lipoic acid; MCT, monocarboxylic acid transporter; PepT1, peptide transporter 1; SLC, solute carrier; SMCT, sodium-dependent monocarboxylic acid transporter; SMVT, sodium-dependent multivitamin transporter; TER, transepithelial electrical resistance.

LITERATURE CITED

(1) Packer, L. α-Lipoic acid: a metabolic antioxidant which regulates NF-κB signal transduction and protects against oxidative injury. *Drug Metab. Rev.* **1998**, *30*, 245−275.

(2) Moini, H.; Packer, L.; Saris, NE. Antioxidant and prooxidant activities of α-lipoic acid and dihydrolipoic acid. *Toxicol. Appl. Pharmacol.* **2002**, *182*, 84−90.

(3) Packer, L.; Kraemer, K.; Rimbach, G. Molecular aspects of lipoic acid in the prevention of diabetes complications. *Nutrition* **2001**, *17*, 888−895.

(4) Schupke, H.; Hempel, R.; Peter, G.; Hermann. R.; Wessel, K.; Engel, J.; Kronbach, T. New metabolic pathways of α-lipoic acid. *Drug Metab. Dispos.* **2001**, *29*, 855−862.

(5) Harrison, E. H.; McCormick, D. B. The metabolism of DL-(1,6-^{14}C)lipoic acid in the rat. *Arch. Biochem. Biophys.* **1974**, *160*, 514−522.

(6) Tsuji, A.; Tamai, I. Carrier-mediated intestinal transport of drugs. *Pharm. Res.* **1996**, *13*, 963−977.

(7) Karbach, U. Paracellular calcium transport across the small intestine. *J. Nutr.* **1992**, *122*, 672−677.

(8) Turner, J. R. Show me the pathway! Regulation of paracellular permeability by Na^+-glucose cotransport. *Adv. Drug Deliv. Rev.* **2000**, *41*, 265−281.

(9) Prasad, P. D.; Wang, H.; Kekuda, R.; Fujita, T.; Fei, Y. J.; Devoe, L. D.; Leibach, F. H.; Ganapathy, V. Cloning and functional expression of a cDNA encoding a mammalian sodium-dependent vitamin transporter mediating the uptake of pantothenate, biotin, and lipoate. *J. Biol. Chem.* **1998**, *273*, 7501−7506.

(10) Balamurugan, K.; Vaziri, N. D.; Said, H. M. Biotin uptake by human proximal tubular epithelial cells: cellular and molecular aspects. *Am. J. Physiol. Renal Physiol.* **2005**, *288*, F823−F831.

(11) Satsu, H.; Watanabe, H.; Arai, S.; Shimizu, M. Characterization and regulation of taurine transport in Caco-2, human intestinal cells. *J. Biochem.* **1997**, *121*, 1082−1087.

(12) Satake, M.; Enjoh, M.; Nakamura, Y.; Takano, T.; Kawamura, Y.; Arai, S.; Shimizu, M. Transepithelial transport of the bioactive tripeptide, Val-Pro-Pro, in human intestinal Caco-2 cell monolayers. *Biosci., Biotechnol., Biochem.* **2002**, *66*, 378−384.

(13) Konishi, Y.; Hagiwara, K.; Shimizu, M. Transepithelial transport of fluorescein in Caco-2 cell monolayers and use of such transport in *in vitro* evaluation of phenolic acid availability. *Biosci., Biotechnol., Biochem.* **2002**, *66*, 2449−2457.

(14) Konishi, Y.; Shimizu, M. Transepithelial transport of ferulic acid by monocarboxylic acid transporter in Caco-2 cell monolayers. *Biosci., Biotechnol., Biochem.* **2003**, *67*, 856−862.

(15) Constantinescu, A.; Pick, U.; Handelman, G. J.; Haramaki, N.; Han, D.; Podda, M.; Tritschler, H. J.; Packer, L. Reduction and transport of lipoic acid by human erythrocytes. *Biochem. Pharmacol.* **1995**, *50*, 253−61.

(16) Baker, S. S.; Baker, R. D. Antioxidant enzymes in the differentiated Caco-2 cell line. *In Vitro Cell Dev. Biol.* **1992**, *28A*, 643−647.

(17) Balamurugan, K.; Ortiz, A.; Said, H. M. Biotin uptake by human intestinal and liver epithelial cells: role of the SMVT system. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2003**, *285*, G73−G77.

(18) Fei, Y. J.; Kanai, Y.; Nussberger, S.; Ganapathy, V.; Leibach, F. H.; Romero, M. F.; Singh, S. K.; Boron, W. F.; Hediger, M. A. Expression cloning of a mammalian proton-coupled oligopeptide transporter. *Nature* **1994**, *368*, 563−566.

(19) Döring, F.; Will, J.; Amasheh, S.; Clauss, W.; Ahlbrecht, H.; Daniel, H. Minimal molecular determinants of substrates for recognition by the intestinal peptide transporter. *J. Biol. Chem.* **1998**, *273*, 23211−23218.

(20) Stahl, A. A current review of fatty acid transport proteins (SLC27). *Pfluegers Arch.* **2004**, *447*, 722−727.

(21) Stahl, A.; Hirsch, D. J.; Gimeno, R. E.; Punreddy, S.; Ge, P.; Watson, N.; Patel, S.; Kotler, M.; Raimondi, A.; Tartaglia, L. A.; Lodish, H. F. Identification of the major intestinal fatty acid transport protein. *Mol. Cell* **1999**, *4*, 299−308.

(22) Gimeno, R. E.; Ortegon, A. M.; Patel, S.; Punreddy, S.; Ge, P.; Sun, Y.; Lodish, H. F.; Stahl, A. Characterization of a heart-specific fatty acid transport protein. *J. Biol. Chem.* **2003**, *278*, 16039−16044.

(23) Martin, P. M.; Gopal, E.; Ananth, S.; Zhuang, L.; Itagaki, S.; Prasad, B. M.; Smith, S. B.; Prasad, P. D.; Ganapathy, V. Identity of SMCT1 (SLC5A8) as a neuron-specific Na^+-coupled transporter for active uptake of L-lactate and ketone bodies in the brain. *J. Neurochem.* **2006**, *98*, 279−288.

(24) Srinivas, S. R.; Gopal, E.; Zhuang, L.; Itagaki, S.; Martin, P. M.; Fei, Y. J.; Ganapathy, V.; Prasad, P. D. Cloning and functional identification of slc5a12 as a sodium-coupled low-affinity transporter for monocarboxylates (SMCT2). *Biochem. J.* **2005**, *392*, 655−664.

(25) Halestrap, A. P.; Meredith, D. The SLC16 gene family−from monocarboxylate transporters (MCTs) to aromatic amino acid transporters and beyond. *Pfluegers Arch.* **2004**, *447*, 619−628.

(26) Gill, R. K.; Saksena, S.; Alrefai, W. A.; Sarwar, Z.; Goldstein, J. L.; Carroll, R. E.; Ramaswamy, K.; Dudeja, P. K. Expression and membrane localization of MCT isoforms along the length of the human intestine. *Am. J. Physiol. Cell Physiol.* **2005**, *289*, C846−C852.

(27) Buyse, M.; Sitaraman, S. V.; Liu, X.; Bado, A.; Merlin, D. Luminal leptin enhances CD147/MCT-1-mediated uptake of butyrate in the human intestinal cell line Caco2-BBE. *J. Biol. Chem.* **2002**, *277*, 28182−28190.

(28) Hadjiagapiou, C.; Schmidt, L.; Dudeja, P. K.; Layden, T. J.; Ramaswamy, K. Mechanism(s) of butyrate transport in Caco-2 cells: role of monocarboxylate transporter 1. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2000**, *279*, G775−G780.

(29) Friesema, E. C.; Jansen, J.; Milici, C.; Visser, T. J. Thyroid hormone transporters. *Vitam. Horm.* **2005**, *70*, 137−167.

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