Relationship between Structure and Permeability of Tryptophan Derivatives Across Human Intestinal Epithelial (Caco-2) Cells

Machiko Urakami, Rieko Ano, Yukitaka Kimura, Motohiro Shima, Ryuichi Matsuno, Tamio Ueno, and Miki Akamatsu*

Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan

Fax: +81-75-753-6489. E-mail: akamatsu@kais.kyoto-u.ac.jp

* Author for correspondence and reprint requests

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L-Trp and its derivatives were used as model compounds to clarify structural factors which influence the intestinal epithelial permeation and metabolism of amino-acid derivatives. Permeability of model compounds through Caco-2 cells was used as an *in vitro* absorption model for human intestinal epithelial cells. The influence of compound concentration, the effects of various transporter substrates on permeability coefficients, and pH dependency of permeability coefficients were investigated. The transcellular permeability of Trp and Trp-NH_2 in the direction from the apical side to the basolateral side, in which nutrients and drugs were ordinarily absorbed, declined with increasing concentration and saturated at more than 1 and 0.4 mM, respectively. The permeability coefficients for *N*-terminal protected Trp derivatives and Ac-Trp-NH_2 showed similar and constant values in both from the apical-to-basolateral and basolateral-to-apical directions. In addition, significant inhibition of the apical-to-basolateral permeation of Trp by Leu and Phe was observed. The permeability coefficient ratio at pH 6.3 to that at pH 7.3 was explained by the ratio of the ionic form to the neutral form of the compounds. Based upon these results and the partition coefficients in the 1-octanol/water system, possible absorption mechanism of Trp and its derivatives across Caco-2 cells was proposed.

Key words: Caco-2, Structure-Permeability Relationships, Tryptophan Derivatives

Introduction

Transport of amino acids into and out of cells is an essential part of intracellular amino acid homeostasis. It is also important for amino-acid related compounds such as L-DOPA (3-(3,4-dihydroxyphenyl)alanine), a therapeutic drug for Parkinsonism and melphalan (4-[bis(2-chloroethyl)-amino]-L-phenylalanine), an antineoplastic L-phenylalanine mustard (Goldenberg *et al.*, 1979; Uchino *et al.*, 2002).

In general, compounds are transported into cells across the intestinal epithelium passively or by carrier-mediated routes (Palm *et al.*, 1997). Passive absorption can occur via either the transcellular or paracellular route. It is often generalized that rapidly transported hydrophobic compounds are absorbed across the membrane by the transcellular route, whereas slowly transported hydrophilic compounds are absorbed through the tight junctions via the paracellular pathway (Cereijido *et al.*, 1993).

The uptake of amino acids is mediated by energy-dependent (active) and independent (passive) transporters. Passive transporters mediate facilitated diffusion of substrate amino acids (Castagna *et al.*, 1997). A variety of amino acid transporters with overlapping substrate specificities have been found in epithelial cells (Ganapathy *et al.*, 1994; Castagna *et al.*, 1997). Molecular cloning approaches have revealed the molecular nature of amino acid transport systems (Segawa *et al.*, 1999; Kim *et al.*, 2001). Oligopeptide (Fei *et al.*, 1994; Ganapathy *et al.*, 1994), cation (Grundemann *et al.*, 1994), and anion transporters (Sekine *et al.*, 1997) are also well known.

Studies which are related to the phenomenon of multidrug resistance that accompanies cancer chemotherapy have focused on a membrane glycoprotein (termed P-glycoprotein) (Ueda *et al.*, 1986). P-glycoprotein is not only highly expressed in cancer cells but also expressed in normal intestinal and colonic epithelial cells (Peter *et al.*, 1992). This is a system that mediates drug transport in a secretory direction.

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Recently, studies on the substrate recognition mechanisms of amino acid transporters have been published. For instance, L-type amino acid transporter 1 (LAT1) subserving the amino acid transport system L is Na^+-independent and expressed in certain tissues such as brain, placenta, and testis (Kanai *et al.*, 1998; Uchino *et al.*, 2002). LAT1 was proposed to recognize the positively charged α-amino group, negatively charged α-carboxyl group, and large hydrophobic side chain (Uchino *et al.*, 2002). It was proposed that T-type amino acid transporter 1 (TAT1), which exhibited Na^+-independent transport of aromatic amino acids, recognized amino acid substrates as anions (Kim *et al.*, 2001). In addition, system L was reported to transport amino-acid related compounds such as L-DOPA and melphalan (Uchino *et al.*, 2002). However, the total mechanism of absorption and metabolism of structurally diverse amino acid derivatives across epithelial cells have not been fully understood.

To clarify structural factors of amino acids and their derivatives which influence their epithelial permeation and metabolism, we measured permeability of L-Trp and protected L-Trp derivatives: Trp-NH_2, acetyl (Ac)-Trp, Ac-Trp-NH_2, *tert*-butyloxycarbonyl (Boc)-Trp, carbobenzoxy (Cbz)-Trp, and 9-fluorenylmethoxycarbonyl (Fmoc)-Trp in an *in vitro* transport system. Trp is an essential amino acid for humans and plays an important role for the nervous system. We used Caco-2 cells as an *in vitro* model of human intestinal epithelial cells. Caco-2 cells, the cell line derived from human colon carcinoma, have been previously used in various transport experiments (Kimura *et al.*, 2001; Artursson and Karlsson, 1991). Good correlation between oral drug absorption in humans and apparent permeability coefficients in Caco-2 cells was reported (Artursson and Karlsson, 1991). The main absorption route of Trp and its derivatives mediated by Caco-2 cells is discussed in this report.

Materials and Methods

Materials

Trp and Trp derivatives were purchased from Nacalai Tesque (Kyoto, Japan), Kokusan Chemical Co Ltd. (Tokyo, Japan), or Wako Pure Chemical Industries (Osaka, Japan). Other amino acids and peptides were obtained from Wako Pure Chemical Industries or Sigma-Aldrich Japan (Tokyo, Japan). "Nissui 2" autoclavable Dulbecco's modified Eagle medium (DMEM) was obtained from Nissui Pharmaceutical (Tokyo, Japan). The potassium salt of penicillin G and streptomycin sulfate were purchased from JCN Biomedicals Inc. (OH, U.S.A.) and Wako Pure Chemical Industries, respectively. Transwell polycarbonate filters (12 mm and 6.5 mm in diameter, respectively, 0.4 μm-diameter pore size) were obtained from Corning Costar (MA, U.S.A.). All other reagents were of analytical grade and were purchased from Wako Pure Chemical Industries or Nacalai Tesque.

Cells

Caco-2 cells were obtained from the American Type Culture Collection (Rockville, MD, U.S.A.). The cells were cultured in flasks as described previously (Shima *et al.*, 1997). After trypsinization, the cells were seeded on Transwell polycarbonate filters for transport experiments at 4.5×10^5 cells/cm^2. The cells were cultivated for 20 to 22 days in DMEM with 10% fetal calf serum. The medium was changed three times a week. We used filters with electrical resistance of more than 300 $\Omega \cdot cm^2$. No mycoplasma was detectable upon DNA testing by Toray Research Center (Osaka, Japan) following DNA extraction from the cells with a DNA isolation kit (Wako Pure Chemical Industries).

Transport experiments

The transport experiments were done with Transwell filters (multiwell plates) as previously described (Kimura *et al.*, 2001). Filters of 12 mm in diameter were used for Ac-Trp because its apparent permeability coefficient was small and detectable only at the larger filter areas. Filters of 6.5 mm in diameter were used for the other compounds. Hank's balanced salt solution (HBSS) including 25 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid (HEPES), pH 7.3, was used as the apical (AP) and basolateral (BL) solutions. Trp and Trp derivatives of 0.02 ~ 2.7 mM were dissolved in the either AP or BL solution except for Boc-Trp, Cbz-Trp and Fmoc-Trp. Because of low solubility of Boc-Trp, Cbz-Trp and Fmoc-Trp in HBSS, 0.5 mM of Boc-Trp and Cbz-

Trp, and 0.1 mM Fmoc-Trp was dissolved in 1% dimethyl sulfoxide (DMSO) in HBSS.

In AP-to-BL absorption, after aspiration of the medium from the AP side, the filters were put in 0.5 ml (1.2 ml for Ac-Trp) of HBSS. One hundred microliters (0.5 ml for Ac-Trp) of the AP solution was added to the filters, followed by incubation for 10 ~ 15 min at 37 °C. The AP solutions were aspirated off after pre-incubation, and the filters were then put in 0.5 ml (1.2 ml for Ac-Trp) of the fresh BL solution. The transport experiments were started when 0.2 ml (0.5 ml for Ac-Trp) of the amino acid solution was added to the AP side. One hundred microliters of the AP solution was sampled soon after addition of the solution. The concentration of the solution was taken as the initial concentration. At 40-min intervals, 110 μl of the BL solution was sampled, and the same volume of fresh HBSS was added. In BL-to-AP absorption, after pre-incubation, the filters were put in 0.6 ml (1.3 ml for Ac-Trp) of the amino acid dissolved in the BL solution. One hundred microliters (0.4 ml for Ac-Trp) of the AP solution was added to the AP side and then 40 μl of the apical solution was sampled at 40 min intervals, and the same volume of fresh HBSS was added. In a similar way to AP-to-BL absorption, the initial concentration was that of the BL solution which was sampled (0.1 ml) soon after addition of the AP solution.

The amount of Trp and Trp derivatives in the sample was measured by their UV absorbance or fluorescence after HPLC (SHIMADZU LC-10AS, Japan) according to the following conditions.

HPLC conditions: C_{18} column (COSMOSIL ODS-A 4.6 × 150 mm); detectors: UV (220 nm) or fluorescence (Ex. 285 nm, Em. 345 nm); Trp: solvent: 40% MeOH in H_2O containing 0.05% TFA; flow rate: 0.8 ml/min; retention time: 3.3 min; Trp-NH_2: solvent: 40% MeOH in H_2O containing 0.05% TFA; flow rate: 0.8 ml/min; retention time: 2.5 min; Ac-Trp: solvent: 50% MeOH in H_2O containing 0.05% TFA; flow rate: 0.8 ml/min; retention time: 4.2 min; Ac-Trp-NH_2: solvent: 50% MeOH in H_2O containing 0.05% TFA; flow rate: 0.8 ml/min; retention time: 3.2 min.

The apparent permeability coefficient, P_{app}, of the compounds was calculated using the following equation:

$$P_{app} = 1/(AC_0) \cdot dQ/dt \quad (1)$$

where A is the area of the monolayer, which was 0.33 cm^2 (6.5 mm in diameter) or 1.13 cm^2 (12 mm in diameter), C_0 is the initial concentration of compounds in the donor side, Q is the amount of compounds transported to the receiver solution, and t is the time.

Inhibition of transport by amino acids and various compounds

To investigate interaction with transporters, the AP-to-BL permeability coefficient of the 0.2 mM Trp and Trp-NH_2 was determined in the presence of 2 mM amino acids, or various transporter substrates in the AP solutions. The effect of phenylalanine on the BL-to-AP absorption of Trp was also investigated.

Effect of pH on transport

The AP-to-BL permeability coefficient of the 2 mM Trp and Trp derivatives was measured at pH 6.3 as well as pH 7.3. HBSS including 25 mM 2-(*N*-morpholino) ethanesulfonic acid (MES), pH 6.3, was used as the AP solutions. The BL solutions were held at pH 7.3.

Measurement of partition coefficient (P) and distribution coefficient (D)

For Trp derivatives, the partition coefficient P (defined for the neutral or non-ionized form) in the 1-octanol/water system and the apparent distribution coefficient D under particular conditions (at pH 7 in this study) were measured at 25 ± 3 °C by the flask-shaking procedure. The log P value of the neutral Ac-Trp-NH_2 and Trp mostly taking a zwitterionized form at pH 7 as well as the log D value of ionizable Ac-Trp and Trp-NH_2 at pH 7 were obtained with 1-octanol and 0.1 M aqueous sodium phosphate : phosphoric acid (pH 7; ionic strength: 0.1) as the partitioning solvents under the same experimental conditions as reported before (Akamatsu *et al.*, 1989). The neutral log P value of acidic Ac-Trp and Cbz-Trp, and basic Trp-NH_2 were measured using 0.1 N HCl (pH 1) and 0.1 N NaOH (pH 13), respectively, as the aqueous phase. After the partitioning equilibrium was established, the concentration of Trp derivatives in the aqueous phase was measured by their UV

absorbance (SHIMADZU UV-1600PC). The concentration in the 1-octanol phase was taken as the difference. The measurement was repeated at least three times for each of the log *P* and log *D* values. The standard deviation of both values was mostly within ± 0.05. Because Boc-Trp was unstable in acidic solutions and Fmoc-Trp was too hydrophobic to measure its log *P*, their log *P* values were calculated with the use of the MacLogP software, ver. 4.0 (Hansch and Leo, 1995) and added −0.15, the difference between the log *P*(meas.) value (0.88) and the log *P*(calc.) value (1.03) for Ac-Trp.

Results and Discussion

We measured the dependence of the both AP-to-BL and BL-to-AP permeability coefficients on the concentration of Trp and its derivatives. If the compound is transported much faster from the AP to BL side than in the opposite direction that suggests the presence of transport systems with AP-to-BL polarity, whereas if the compound is transported more slowly from AP-to-BL this indicates involvement of an efflux mechanism by P-glycoprotein (Stenberg, *et al.*, 2001). The concentration-dependence of the permeability coefficients shows the existence of saturable transporters (Hidalgo and Borchardt, 1990).

Permeability coefficient of Trp

The influence of the Trp concentration on the AP-to-BL and BL-to-AP permeability coefficients of Trp is shown in Fig. 1A. The AP-to-BL permeability declined with increasing concentration from 0.01 mM to 1 mM and saturated at more than 1 mM. The saturated permeability coefficient was 4×10^{-6} cm/s. The BL-to-AP permeability coefficient was almost constant regardless of the Trp concentration, 1×10^{-6} cm/s, but slightly higher at concentrations below 0.8 mM. The results suggest involvement of active transport systems for Trp which were localized in the AP membrane of Caco-2 cells. Saturable transporters for Trp may also localize in the BL membrane. The saturated or constant permeability coefficients of Trp and its derivatives are listed in Table I.

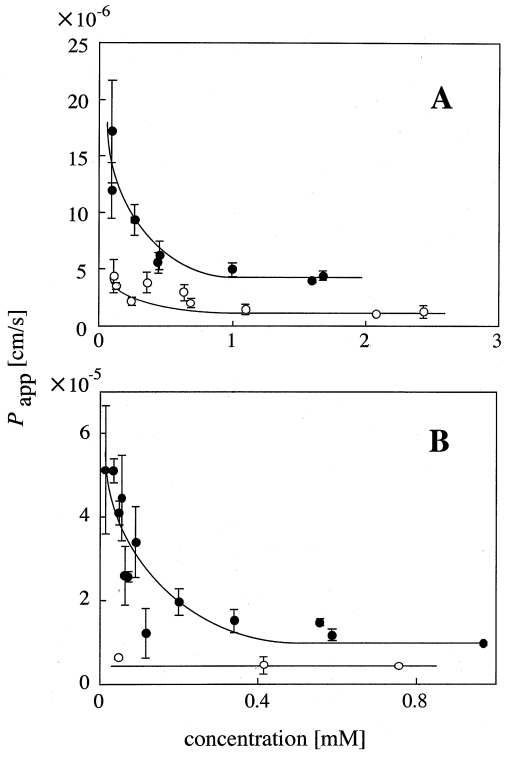

Fig. 1. A: The influence of the Trp concentration on the AP-to-BL (●) and BL-to-AP (○) permeability coefficients of Trp. B: The influence of the Trp-NH_2 concentration on the "combined" permeability coefficients of Trp-NH_2 [AP-to-BL (●) and BL-to-AP (○)].

Permeability coefficient of Trp-NH_2

Trp-NH_2 was hydrolysed by the cytosolic enzymes of Caco-2 cells and Trp was mainly detected after permeation of Trp-NH_2. Since Trp-NH_2 was only slightly degraded in the initial solution (10 ~ 20% degradation after 2 h) as well as in the BL solution, Trp was mainly regarded as a metabolite during permeation. Thus, the permeability coefficient of Trp-NH_2 was determined combining the amounts of Trp and Trp-NH_2 detected in the BL solution. The influence of the Trp-NH_2 concentration on the "combined" permeability coefficients of Trp-NH_2 is shown in Fig. 1B. The "combined" permeability coefficient decreased with an increase in Trp-NH_2 concentration up to 0.4 mM and saturated in the direction of AP-to-BL whereas it was constant in the direction of BL-to-AP. The saturated AP-to-BL and BL-to-AP permeability co-

Table I. Caco-2 cell permeability coefficients, partition coefficients in 1-octanol/water system and pK_a for Trp and its derivatives.

Compound[a]	P_{app}[b] (10^{-6} cm/s)		log P[c]	log D[d]	pK_a[e]	P_{app} (pH 6.3)/
	AP-to-BL	BL-to-AP		(pH 7)		P_{app} (pH 7.3)[f]
Trp	4.1	1.0	−1.06[g]	−1.06	2.43, 9.44[h]	1.3
Trp-NH_2	12.1	4.3	0.30	−0.32	7.55[i]	0.3
Ac-Trp	0.1	0.1	0.88	−2.01	3.69[j]	1.7
Ac-Trp-NH_2	2.4	2.5	0.42	0.42	–	1.3
Boc-Trp	2.2	2.7	2.65[f]	–	–	5.0
Cbz-Trp	1.6	1.6	3.20	–	–	8.5
Fmoc-Trp	17.4	18.6	4.93[f]	–	–	–

[a] Trp: tryptophan, Trp-NH_2: tryptophanamide, Ac-Trp: *N*-acetyl-tryptophan, Ac-Trp-NH_2: *N*-acetyl-tryptophanamide, Boc-Trp: *N*-butyloxycarbonyl-tryptophan, Cbz-Trp: *N*-carbobenzoxy-tryptophan, Fmoc-Trp: *N*-9-fluorenylmethoxycarbonyl-tryptophan.
[b] Apparent permeability coefficient.
[c] The partition coefficient defined for the neutral or non-ionized form in the 1-octanol/water system.
[d] The apparent distribution coefficient at pH 7.
[e] The acid dissociation constant of compounds or the conjugate acids.
[f] The ratio of P_{app}(pH 6.3) to P_{app} (pH 7.3).
[g] The log P value at the isoelectric point of Trp (5.94).
The log D value of Trp was same as the log P value in the range of pH 4.5 ~ 7.5.
[h] Calculated with the use of the MacLogP software, ver. 4.0 (BioByte Corp., Claremont, CA, USA) and added − 0.15, the difference between the log P(meas.) value and the log P(calc.) value for Ac-Trp.
[i] From literature (Perrin, 1965).
[j] pK_a of Ac-Gly (Perrin, 1965).

efficients were 1×10^{-5} and 0.4×10^{-5} cm/s, respectively, being the larger values compared to Trp, Ac-Trp, and Ac-Trp-NH_2.

Permeability coefficient of Ac-Trp and Ac-Trp-NH_2

Ac-Trp and Ac-Trp-NH_2 were relatively stable against cytosolic hydrolases and no metabolites were detected after permeation. The AP-to-BL and BL-to-AP permeability coefficients of each compound were independent on the concentration of the compound. The constant P_{app} values were 1×10^{-7} cm/s for Ac-Trp, one order less than that of Trp, and 2×10^{-6} cm/s for Ac-Trp-NH_2, in both directions.

Permeability coefficient of Boc-Trp, Cbz-Trp, and Fmoc-Trp

Permeability coefficients of Boc-Trp, Cbz-Trp and Fmoc-Trp were measured in the AP-to-BL and BL-to-AP directions (Table I). These compounds were also metabolically stable. That the permeability coefficients of each compound was similar in both directions suggests these Trp derivatives protected at the N-terminus as well as Ac-Trp are passively absorbed via either transcellular or paracellular route (Stenberg *et al.*, 2001).

Effect of transporter substrates on permeability coefficients

The existence of energy-dependent transporters for neutral amino acids such as Leu and Phe in AP membranes of Caco-2 cells has been shown (Hidalgo and Borchardt, 1990). In addition, competitive inhibition of Phe transport across Caco-2 cells by Trp was reported (Hidalgo and Borchardt, 1990). As shown in Table II, significant inhibition of the AP-to-BL permeation of Trp by Leu and Phe was observed, confirming the above hypothesis. Trp seems to be in part actively transported via the carrier-mediated route. Fig. 1A suggests the existence of transporters which work in the BL-to-AP direction with lower efficiency than AP transporters. The BL-to-AP permeation of Trp was significantly inhibited by Phe as expected (Table II).

A variety of amino acid transport systems, which are either Na^+-independent or -dependent, have been identified in both the AP membrane and the BL membrane of the small intestine (Ganapathy

Table II. Effect of amino acids on permeability coefficients of Trp.

Amino Acid	% of Control (SD) [a]
AP-to-BL	
Control	100 (15)
+Leu	78 (5)[b]
+Phe	71 (5)[b]
BL-to-AP	
Control	100 (38)
+Phe	63 (6)[b]

[a] The permeability coefficient of Trp in the presence of each amino acid was expressed as percent of that of Trp only.
[b] The significant inhibition was observed at the 95% level in t-test.

et al., 1994). It has been suggested that the Na^+-independent systems in the BL membrane are responsible for transport of amino acids from the intestinal cells into the blood whereas Na^+-dependent pathways participate in the transport from blood into the cells (Ganapathy *et al.*, 1994). Trp may be transported from the Caco-2 cells to the BL side by these systems or vice versa if all of them are expressed in the Caco-2 cells.

The high value and concentration dependence of Trp-NH_2 permeability coefficient in the AP-to-BL direction suggested the existence of a transporter for the derivative. The effect of various inhibitors on transport of Trp-NH_2 was investigated. Neither Phe nor the dipeptide Gly-Pro (a peptide transporter substrate) altered transport (data not shown). Tetraethylammonium bromide, a substrate of a cation transporter also did not show any effect (data not shown). This suggests Trp-NH_2 may be carried by different systems from Trp, oligopeptides and organic cations.

PH dependence of permeability coefficients

It is known that the neutral amino acid transporters are Na^+-dependent or Na^+-independent (Hidalgo and Borchardt, 1990) while the di-/tripeptide transporter, PepT1, expressed in Caco-2 cells, is H^+-dependent (Ganapathy *et al.*, 1998). Permeability coefficient of 2 mM Trp and its derivatives was measured at pH 6.3 and 7.3 in the AP-to-BL direction to investigate the effect of the H^+ concentration on permeation. Table I shows the P_{app}(pH 6.3)/P_{app}(pH 7.3) ratio of compounds. The P_{app} values of Ac-Trp-NH_2 having no charge and Trp mainly assuming a zwitterionized form at the neutral pH were independent of the pH variations. Ac-Trp, Boc-Trp, and Cbz-Trp had higher permeability coefficient at pH 6.3 than that at pH 7.3, whereas the permeability coefficient of Trp-NH_2 at pH 6.3 was lower than that at pH 7.3. This result also suggests that Trp-NH_2 does not interact with the H^+-dependent PepT1. Observed and estimated pK_a (K_a: the acid dissociation constant) values of compounds are listed in Table I. Since the ratio of the cationic form to the neutral form increases with decreasing pH from pK_a of the conjugate acid of Trp-NH_2, the lower P_{app} value at pH 6.3 may be caused by the increase of the more hydrophilic ionic form of Trp-NH_2. At lower pH, Ac-Trp, Boc-Trp, and Cbz-Trp are less charged, resulting in the higher P_{app} at pH 6.3.

Partition coefficients in the 1-octanol/water system

Hydrophobicity indices of Trp derivatives are shown in Table I in terms of the partition coefficient, log *P*, for the neutral or non-ionized forms and the distribution coefficient at pH 7, log *D*, in the 1-octanol/water system. Equations (2) and (3) represent the relationship between log *D* at a particular pH and log *P* for acids and bases, respectively, if the partition of ion-pair complexes can be neglected (Terada *et al.*, 1981).

$$\text{For acids:} \quad \log D = \log P - \text{pH} - \log (K_a + [H^+]) \qquad (2)$$
$$\text{For bases:} \quad \log D = \log P - pK_a - \log (K_a + [H^+]) \qquad (3)$$

$[H^+]$ is the hydrogen ion concentration of the aqueous phase. According to Eqs. (2) and (3), log *D* = log *P* for acids when pH << pK_a and for bases when pH>>pK_a (K_a: the acid dissociation constant of the conjugate acids). For acids, when pH >> pK_a, [log *D* = log *P* + pK_a – pH], whereas for bases, when pH << pK_a, [log *D* = log *P* – pK_a + pH].

pK_a values of Trp and Trp-NH_2 which have been previously reported (Perrin *et al.*, 1965) are listed in Table I. The pK_a of Ac-Trp is considered to be nearly equal to that of Ac-Gly (Table I). Trp showed the same log *D* values as the log *P* value at the isoelectric point (5.94) in the range of pH 4.5 ~ 7.5 (data not shown except that at pH 7). The log *D* values of Trp-NH_2 and Ac-Trp varied from log *P* according to Eqs. (3) and (2), respectively. The log *D* value at pH 7 (= log *P*) of Ac-Trp-NH_2 was highest among the compounds log *D* of which was

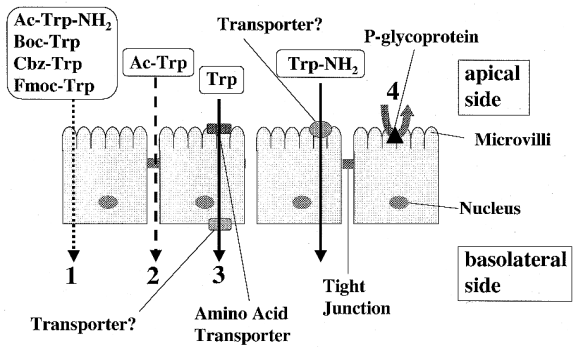

Fig. 2. The main absorption route of Trp and its derivatives across Caco-2 cells (AP-to-BL direction).
1. passive transcellular, 2. passive paracellular, 3. active carrier-mediated routes, and 4. P-glycoprotein efflux system.

measured. Although the log D values at pH 7 of Boc-, Cbz- and Fmoc-Trp were not measured, the value was predicted to be about [log P – 3] by Equ. (2), assuming their pK_a values are similar to that of Ac-Gly.

Main absorption route of Trp and Trp derivatives

Based on our results, a possible main absorption route of Trp and Trp derivatives across Caco-2 cells in the AP-to-BL direction, in which nutrients and drugs were ordinarily absorbed, is proposed and illustrated in the diagram shown in Fig. 2.

The most hydrophilic Trp was mainly absorbed by the amino-acid active transporter pathway. However, the result that uptake inhibition of Trp by Phe or Leu was not strong (29% or 22% inhibition) suggests the existence of another route for absorption of Trp. Since log P (log D) of Trp is too low to permeate across membranes, it is likely that the carrier-mediated facilitated diffusion (not shown in Fig. 2) contributes to the absorption of Trp.

Trp-NH_2 was carried by a kind of transporter probably different from those for amino acids, oligopeptides, and organic cations. Both Ac-Trp and Ac-Trp-NH_2 were passively transported. But, hydrophilic Ac-Trp at pH 7 (log D = –2.01) is presumably absorbed via the paracellular route and Ac-Trp-NH_2 seems to be hydrophobic enough to permeate mainly via the transcellular route (log D = 0.42). Boc-, Cbz- and Fmoc-Trp were also passively transported. Since predictable log D values of these compounds at pH 7 are higher than that of Ac-Trp, they are likely to permeate through the cell membrane, showing higher P_{app} values.

The present results for cell permeation of Trp derivatives should be useful for understanding absorption mechanism of amino-acid related therapeutic drugs. Currently work is in progress on evaluation of the relationship between structure and permeability coefficients of oligopeptides and their derivatives.

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Akamatsu M., Yoshida Y., Nakamura H., Asao M., Iwamura H., and Fujita T. (1989), Hydrophobicity of di- and tripeptides having unionizable side chains and correlation with substituent and structural parameters. Quant. Struct.-Act. Relat. **8**, 195–203.

Artursson P., and Karlsson J. (1991), Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial (Caco-2) cells. Biochem. Biophys. Res. Commun. **175**, 880–885.

Castagna M. A., Shayakul C., Trotti D., Sacci V. F., Harvey W. R., and Hediger M. A. (1997), Molecular characteristics of mammalian and insect amino acid transporters: implications for amino acid homeostasis. J. Exper. Biol. **200**, 269–286.

Cereijido M., Ruiz O., Gonzalez-Mariscal L., Contreras R.-G., Balda M. S., and Garcia-Villegas M.-R. (1993), The paracellular pathway. In: Biological Barriers to Protein Delivery (K. L. Audus and T. Raub, eds.). Plenum Press, New York, USA, 3–21.

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

Ganapathy, V., Brandsch, M., and Leibach, F. H. (1994), Intestinal transport of amino acids and peptides. In: Physiology of the Gastrointestinal Tract, Vol. 2 (L. R. Johnson, D. H. Alpers, J. Christensen, E. D. Jacobson, and J. H. Walsh, eds.). Raven Press, New York, USA, 1773–1794.

Ganapathy M. E., Huang W., Wang H., Ganapathy V., and Leibach F. H. (1998), Valacyclovir: a substrate for the intestinal and renal peptide transporters PEPT1 and PEPT2. Biochem. Biophys. Res. Commun. **246**, 470–475.

Goldenberg G. J., Lam H. Y., and Begleiter A. (1979), Active carrier-mediated transport of melphalan by two separate amino acid transport systems in LPC-1 plasmacytoma cells *in vitro*. J. Biol. Chem. **254**, 1057–1064.

Grundemann D., Gorboulev V., Gambaryan S., Veyhl M., and Koepsell H. (1994), Drug excretion mediated by a new prototype of polyspecific transporter. Nature **372**, 549–552.

Hansch C., and Leo A. (1995), Calculation of octanol-water partition coefficients by fragments. In: Exploring QSAR, Fundamentals and Applications in Chemistry and Biology (C. Hansch and A. Leo, eds.). American Chemical Society, Washington, DC, USA, 125–168.

Hidalgo I. J., and Borchardt T. (1990), Transport of a large neutral amino acid (phenylalanine) in a human intestinal epithelial cell line: Caco-2. Biochim. Biophys. Acta **1028**, 25–30.

Kanai Y., Segawa H., Miyamoto K. I., Uchino H., Takada E., and Endou H. (1998), Expression cloning and characterization of a transporter for large neutral amino acids activated by the heavy chain of 4F2 antigen (CD98). J. Biol. Chem. **273**, 23629–23632.

Kim D. K., Kanai Y., Chairoungdua A., Matsuo H., Cha S. H., and Endou H. (2001), Expression cloning of a Na^+-independent aromatic amino acid transporter with structural similarity to H^+/monocarboxylate transporters. J. Biol. Chem. **276**, 17221–17228.

Kimura Y., Hosoda Y., Yamaguchi M., Nagano H., Shima M., Adachi S., and Matsuno, R. (2001), Effect of medium-chain fatty acids on intracellular calcium levels and the cytoskeleton in human intestinal (Caco-2) cell monolayers. Biosci. Biotech. Biochem. **65**, 743–751.

Palm K., Artursson P., and Luthman K. (1997), Experimental and theoretical predictions of intestinal drug absorption. In: Computer-Assisted Lead Finding and Optimization: Current Tools for Medicinal Chemistry (H. van de Waterbeemd, B. Testa and G. Folkers, eds.). Wiley-VCH, Weinheim, Germany, 279–289.

Perrin D. D. (1965), Dissociation Constants of Organic Bases in Aqueous Solution, Butterworths, London, UK.

Peter W. H. M., Boon C. E. W., Roelofs H. M. J., Wobbes T., Nagengast F. M., and Kremers P. G. (1992), Expression of drug-metabolizing enzymes and P-170 glycoprotein in colorectal carcinoma and normal mucosa. Gastroenterology **103**, 448–455.

Segawa H., Fukasawa Y., Miyamoto K., Takeda E., Endou H., and Kanai Y. (1999), Identification and functional characterization of a Na^+-independent neutral amino acid transporter with broad substrate selectivity. J. Biol. Chem. **274**, 19745–19751.

Sekine T., Watanabe N., Hosoyamada M., Kanai Y., and Endou H. (1997), Expression cloning and characterization of a novel multispecific organic anion transporter. J. Biol. Chem. **272**, 18526–18529.

Shima M., Yohdoh K., Yamaguchi M., Kimura Y., Adachi S., and Matsuno R. (1997), Effect of medium-chain fatty acids and their acylglycerols on the transport of penicillin V across Caco-2 cell monolayers. Biosci. Biotech. Biochem. **61**, 1150–1155.

Stenberg P., Norinder U., Luthman K., and Artursson P. (2001), Experimental and computational screening models for the prediction of intestinal drug absorption. J. Med. Chem. **44**, 1927–1937.

Terada H., Kitagawa K., Yoshikawa Y., and Fujita T. (1981), Partition and ion-pair partition of 2,4-dinitrophenol, an uncoupler of oxidative phosphorylation. Chem. Pharm. Bull. **29**, 7–14.

Ueda K., Cornwell M. M., Gottesman M. M., Pastan I., Roninson I. B., Ling V., and Riordan J. R. (1986), The *mdr1* gene, responsible for multidrug-resistance, codes for P-glycoprotein. Biochem. Biophys. Res. Commun. **141**, 956–962.

Uchino H., Kanai Y., Kim D. K., Wempe M. F., Chairoungdua A., Morimoto E., Anders M. W., and Endou H. (2002), Transport of amino acid-related compounds mediated by L-type amino acid transporter 1 (LAT1): insights into the mechanisms of substrate recognition. Mol. Pharmacol. **61**, 729–737.