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International Journal of Pharmaceutics 146 (1997) 205–212

international journal of pharmaceutics

β-Carboxylic acid esterified D-Asp-Ala retains a high affinity for the oligopeptide transporter in Caco-2 monolayers

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Received 20 August 1996; revised 15 October 1996; accepted 10 November 1996

Abstract

D-Asp-Ala, a metabolically stable dipeptide, possesses a relatively high affinity for the Caco-2 oligopeptide transporter ($IC_{50} = 5.75 \pm 0.09$ mM) as demonstrated by its ability to compete with [^{14}C]Gly-Sar in cellular uptake experiments. When the β-carboxylic acid of D-Asp-Ala is modified by esterification with a cyclohexyl group (D-Asp(OcHx)-Ala) or a benzyl group (D-Asp(OBzl)-Ala), the resulting compounds are still able to inhibit [^{14}C]Gly-Sar binding to the oligopeptide transporter, i.e., IC_{50} values for D-Asp(OcHx)-Ala and D-Asp(OBzl)-Ala were 2.80 ± 0.11 and 2.62 ± 0.35 mM, respectively. HPLC analysis shows that both D-Asp(OcHx)-Ala and D-Asp(OBzl)-Ala are fully resistant to degradation for up to 5 h when incubated in the apical media of confluent Caco-2 monolayers. These results demonstrate that it is possible to covalently modify the side chain of one amino acid in an enzymatically stabilized dipeptide with small, aromatic molecules while enabling them to retain their affinity for the oligopeptide transporter. © 1997 Elsevier Science B.V.

Keywords: Caco-2; Uptake; Oligopeptide transporter; Dipeptides; β-Carboxylic acid

1. Introduction

The oligopeptide transporter, which is expressed in mammalian small intestinal epithelia, is instrumental in the process of both essential and non-essential amino acid absorption via active uptake of digested di- and tripeptides from the gastrointestinal lumen (Matthews and Adibi, 1976; Ganapathy and Leibach, 1985). This transporter is energized by a Na^+-independent H^+-gradient, which is generated within the acid microclimate located adjacent to the apical surface of jejunal microvilli (Ganapathy and Leibach, 1985; Thwaites et al., 1993). As opposed to transport systems specific for single amino

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PII S0378-5173(96)04802-8

acids and sugars, the oligopeptide transporter has an immense number of potential di- and tripeptide substrates (Daniel et al., 1992). Various types of peptidomimetics have been shown to serve as efficient substrates for the oligopeptide transporter, including β-lactam antibiotics (Dantzig and Bergin, 1990; Kramer et al., 1990; Gochoco et al., 1994), angiotensin converting enzyme inhibitors (Hu and Amidon, 1988), and rennin inhibitors (Kramer et al., 1990). Free amino acids or intact tetrapeptides are not substrates for this transporter (Matthews and Adibi, 1976; Ganapathy and Leibach, 1985). Due to the fact that it displays such a broad affinity for a variety of structurally similar molecules, the oligopeptide transporter has attracted significant attention within the field of pharmaceutics for its potential use as a drug delivery vehicle (Smith et al., 1993; Swaan et al., 1993).

In both human (Liang et al., 1995) and rabbit (Fei et al., 1994) small intestine, a protein has been identified as one which is primarily responsible for di- and tripeptide absorption via a H^+-energized cotransporter. Interestingly, these human and rabbit H^+/peptide cotransporters exhibit an extremely high degree of cross-species homology (Liang et al., 1995). A functionally similar version of the oligopeptide transporter is expressed and utilized by the human adenocarcinoma cell line, Caco-2, once the monolayer is fully differentiated and polarized (Dantzig and Bergin, 1990; Saito and Inui, 1993; Thwaites et al., 1993; Gochoco et al., 1994). Although it is still not clear if there exists additional mechanisms capable of facilitating oligopeptide transport (Smith et al., 1993), several groups have described the valuable utility of conducting both uptake and transport experiments using confluent Caco-2 monolayers as a model system for screening potential oligopeptide transporter substrates (Dantzig and Bergin, 1990; Saito and Inui, 1993; Thwaites et al., 1993; Hidalgo et al., 1995). With respect to physicochemical features of substrates that interact with the oligopeptide transporter, several criteria have been established. It is possible that any dipeptide, regardless of the inclusion of acidic, basic, or hydrophobic amino acids, may serve as a suitable substrate for the oligopeptide transporter; yet, their inter- and intraspecies affinities can vary markedly (Smith et al., 1993). Greater than three amino acids in length, D-amino acids at both the amino and carboxy termini, or the lack of a free carboxyl group are all structural features that can significantly decrease a compound's affinity for the oligopeptide transporter (Bai and Amidon, 1992; Daniel et al., 1992; Eddy et al., 1995; Hidalgo et al., 1995); yet, the presence of a free N-terminal (α-amino group is not necessarily essential for efficient interaction (Hu and Amidon, 1988; Bai and Amidon, 1992; Daniel et al., 1992).

From the perspective of drug delivery, it may be possible to exploit substrates for the oligopeptide transporter to function as pro-moieties, thus enhancing the bioavailability of poorly absorbed drug compounds. This type of rational drug design has been suggested previously (Smith et al., 1993; Swaan et al., 1993); yet, to date, the use of a hydrolysis-resistant substrate in this manner has not been demonstrated. One conceivable tactic would be to introduce a D-configured amino acid within a dipeptide in efforts to circumvent the metabolic lability inherent to naturally occurring dipeptides (Bai and Amidon, 1992; Smith et al., 1993; Hidalgo et al., 1995). Several studies have demonstrated that the oligopeptide transporter displays a preferential affinity for L/L-configured dipeptides as opposed to L/D, D/L, or D/D combinations (Smith et al., 1993; Thwaites et al., 1994; Hidalgo et al., 1995). The structural requirements of peptides and peptidomimetics that influence their ability to interact with the oligopeptide transporter have been investigated, e.g. cyclization (Hidalgo et al., 1995) and assessment of hydrophobic potential (Daniel et al., 1992; Eddy et al., 1995); but, it has not yet been determined if covalent side-chain modification of one of the amino acids in a linear di- or tripeptide results in a loss of affinity for the oligopeptide transporter. In this report, we show that modification of the β-carboxyl of aspartic acid in D-Asp-Ala does not interfere with its ability to inhibit the uptake of [^{14}C]glycylsarcosine ([^{14}C]Gly-Sar), an enzymatically stable dipeptide having a high affinity for the oligopeptide transporter expressed in Caco-2 monolayers (Thwaites et al., 1993).

2. Materials and methods

2.1. Materials

[^{14}C]Gly-Sar (60 mCi/mmol) and [^{14}C]Mannitol (56 mCi/mmol) were purchased from Amersham International (Buckinghamshire, UK). Fetal bovine serum (FBS), Dulbecco's Modified Eagle Media (D-MEM), non-essential amino acids (NEAA, 100 ×), trypsin-EDTA (10 ×), L-glutamine (200 mM), penicillin (10 000 U/ml)/streptomycin (10 000 μg/ml) solution, and Hank's Balanced Salt Solution (HBSS) were purchased from Life Technologies (Roskilde, Denmark). 2-(*N*-morpholino)ethanesulfonic acid (Mes), *N*-2-hydroxyethylpiprazine-*N'*-2-ethanesulfonic acid (Hepes), and Bovine Serum Albumin (BSA) were purchased from Sigma (St. Louis, MO, USA). Various dipeptides, amino acids, Boc-D-Asp(OtBu)-OH, Boc-D-Asp(OcHx)-OH, Boc-D-Asp(OBzl)-OH, and H-Ala-OtBu were purchased from Bachem Feinchemikalien (Bubendorf, Switzerland). N-Hydroxybenzotriazole (HOBt) was purchased from Fluka (Buchs, Switzerland); 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU), diisopropylethylamine (DIPEA), dimethylforamide (DMF), trifluoroacetic acid (TFA), and dicyclohexylamine (DCHA) were purchased from NovaBiochem (Laufelfingen, Switzerland). All solvents used for dipeptide synthesis were obtained from Riedel-de Haën (Seelze, Germany), all analytical grade solvents used for HPLC analysis were obtained from Gerner and Jensen (Copenhagen, Denmark), and Ultima Gold scintillation fluid used was purchased from Packard (Groningen, The Netherlands).

2.2. Cell culture

Caco-2 epithelial cells were obtained from the ATCC (Rockville, MD), and were used between passages 21 and 40. They were seeded into tissue culture treated Transwells™ (4.7 cm^2, 0.4 μ pore size; Costar Corp., Cambridge, MA) at a density of 10^5 cells/cm^2. Cells were maintained in a humidified 5% CO_2 in air atmosphere at 37°C, and were cultured in D-MEM supplemented with 10% FBS, penicillin/streptomycin (100 U/ml and 100 μg/ml, respectively), 1% L-glutamine, and 1% NEAA. The growth media was replaced every other day, and apical and basal volumes were maintained at 1.5 and 2.5 ml, respectively. Confluent cell monolayers were obtained 17–21 days post-inoculation, with each well demonstrating a transepithelial electrical resistance (TEER) of between 500 and 600 Ωcm^2 as measured by an epithelial voltohmmeter (EVOM, World Precision Instruments, West Haven, CT). Cell monolayers could be maintained at a constant resistance for at least 7 days without suffering leakage between apical and basal compartments (as determined by [^{14}C]Mannitol flux) or a loss in TEER. The total amount of protein on each Transwell filter was calculated to be 0.42 mg/cm^2 using the Lowry method.

2.3. Uptake and transport experiments

Uptake experiments were performed in pH-adjusted HBSS supplemented with 0.05% BSA. Prior to conducting uptake experiments, Caco-2 monolayers were first rinsed and then incubated with HBSS (apical media = 0.05% BSA, 10 mM Mes, pH 6.0; basal media = 0.05% BSA, 10 mM Hepes, pH 7.4) for 15 min at 37°C under a 5% CO_2 atmosphere in order to equilibrate the cells to the change in pH gradient. Next, [^{14}C]Gly-Sar (0.5 μCi, 5.6 μM in the apical media), and in certain wells, inhibitors at various concentrations (20 mM for uptake inhibition studies; 20, 15, 10, 5, 1, 0.1, or 0.01 mM for IC_{50} determination experiments) were added concomitantly to the apical media of Caco-2 Transwells. Following either a 15 or 120 min incubation period, buffer was removed from both the apical and basal chambers and the cells were washed four times with ice-cold HBSS, pH 7. Following this washing step, the entire polycarbonate membrane was cut from the Transwell support and placed into a scintillation vial, scintillation fluid was added, and then the cell-associated radioactivity was counted via liquid scintillation spectrometry. Transport experiments were performed similarly, but following the incubation period the basolateral media was

removed and analyzed for the presence of either [^{14}C]Gly-Sar or [^{14}C]Mannitol.

2.4. Synthesis of D-Asp-Ala, D-Asp(OcHx)-Ala, and D-Asp(OBzl)-Ala

Boc-D-Asp(OtBu)-OH: Boc-D-Asp(OtBu)-OH was liberated from its DCHA salt by ethyl acetate extraction after dissolution in ice cold 2M H_2SO_4. The ethyl acetate was dried ($MgSO_4$) then removed by rotary evaporation ($< 40°C$) under reduced pressure to yield a colorless oil which was used without further purification.

Boc-D-Asp(OtBu)-Ala-OtBu: 1.23 g Boc-D-Asp(OtBu)-OH (4.25 mmol) was dissolved in 20 ml fresh distilled DMF at room temperature together with 0.574 g HOBt (4.25 mmol) and 1.365 g TBTU (4.25 mmol). The mixture was then stirred at room temperature for 5 min before adding 0.772 g H-Ala-OtBu (4.25 mmol) and 1.648 g DIPEA (12.75 mmol). The resulting mixture was stirred for 2 h at room temperature and then evaporated to dryness. The remainder was dissolved in 25 ml ethyl acetate and then extracted with 3×20 ml 10% $NaHCO_3$ in water, 3×20 ml 5% acetic acid in water v/v. The ethyl acetate phase was dried over Na_2SO_4 and then evaporated under reduced pressure, resulting in a colorless oil. The oil was used directly without further purification.

H-D-Asp-Ala-OH: The oil was dissolved in 20 ml DCM, then 20 ml concentrated TFA was added and the mixture stirred for 2 h at room temperature. The solvent was then evaporated under reduced pressure and the resulting oil was extracted with 3×10 ml ether, which turned the oil into 600 mg crystals, 70% in overall yield. H-D-Asp(OcHx)-Ala-OH and H-Asp(OBzl)-Ala-OH were prepared identically, resulting in 75% (910 mg) and 59% (735 mg) overall yields, respectively. The purity of all final products was $> 98\%$ as verified by TLC and HPLC, and identification of the products was done using ^{1}H-NMR. The structures of the final purified compounds are shown in Fig. 1.

2.5. HPLC determination of stability and partition coefficients

Analysis of the stability of D-Asp(OcHx)-Ala and D-Asp(OBzl)-Ala was performed using Caco-2 monolayers grown upon plastic 6-well plates under conditions identical to those used for the Transwells. Either D-Asp(OcHx)-Ala or D-Asp(OBzl)-Ala were added to certain wells to a final concentration of 5 mM, and media was sampled hourly over the course of 5 h. Following sampling, high performance liquid chromatography (HPLC) analysis was done in order to determine the extent of degradation of the modified compounds. HPLC equipment used were as follows: a Waters pump model 6000A, a Waters type 481 variable wavelength UV detector, a HiCHROM Spherisorb S5ODS2 column (5 μm, 4.6×250 mm^2), and a 20-μl loop injection valve. HPLC conditions used were as follows: the mobile phase was 20% v/v methanol, 0.001 M triethylamine, pH 7.0; the flow rate was 1.0 ml/min; and the column effluent was monitored at 220 nm. As controls, both cyclohexanol and benzyl alcohol were analyzed under identical conditions and the resulting elution profiles were compared with those for D-Asp(OcHx)-Ala and D-Asp(OBzl)-Ala. The apparent partition coefficient (P) of the D-Asp(OcHx)-Ala and D-Asp(OBzl)-Ala derivatives were determined in *n*-octanol/aqueous 0.02 M phosphate buffer at pH 7.4. The buffer solution and octanol were mutually saturated at 20–25°C prior to use; analysis was done using HPLC conditions as described above.

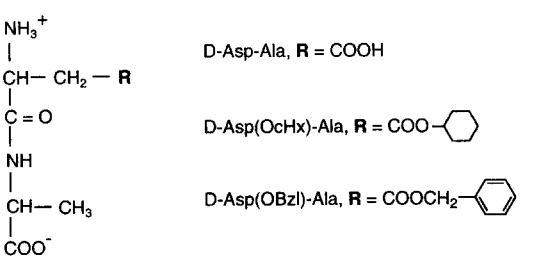

Fig. 1. The structure of D-Asp-Ala and its β-carboxyl esters.

3. Results

3.1. pH-dependence of [^{14}C]Gly-Sar uptake and transport

Previous studies using the Caco-2 model have shown that, although uptake of oligopeptide transporter substrates into Caco-2 monolayers occurs to a significant degree when both apical and basolateral pH are kept at 7.4, optimal conditions for substrate uptake via the apical oligopeptide transporter proceed at an apical pH of 6.0 and a basolateral pH of 7.4 (Dantzig and Bergin, 1990; Gochoco et al., 1994). Thus, these conditions were maintained for all controls in our experiments. The uptake and transport of [^{14}C]Gly-Sar following a 2 h incubation was 3.01 ± 0.11 and 0.98 ± 0.06 pmol/mg protein/min, respectively. When the apical pH was raised to 7.4, the degree of [^{14}C]Gly-Sar uptake and transport was significantly less, i.e. 1.72 ± 0.10 and 0.51 ± 0.13 pmol/mg protein/min, respectively. For transport experiments, the values have been corrected for [^{14}C]Mannitol flux across the monolayers, which was 0.06 ± 0.02 and 0.11 ± 0.02 pmol/mg protein/min for the pH 6 and 7.4 experiments, respectively.

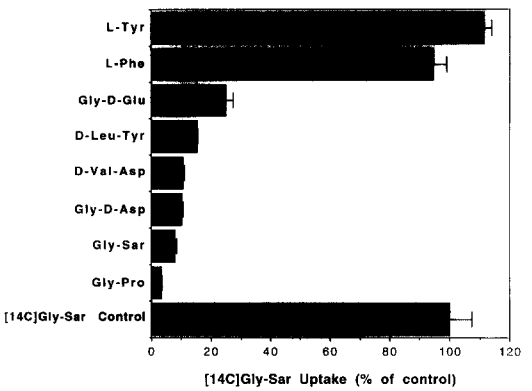

Fig. 2. Uptake of [^{14}C]Gly-Sar (0.5 μCi) in the presence or absence of either linear dipeptides (20 mM) or amino acids (20 mM). All Caco-2 cell monolayers had pH 6.0 buffer in the apical compartment and pH 7.4 buffer in the basal compartment. The degree of competitive [^{14}C]Gly-Sar uptake inhibition in monolayers treated with either free dipeptide or amino acid is depicted as a bar which represents the percentage of [^{14}C]Gly-Sar uptake vs. that of the control monolayers. Results shown represent the means $\pm$ S.D. for three separate filters under each condition.

3.2. [^{14}C]Gly-Sar uptake is inhibited by various dipeptides

The affinities of dipeptides of various configurations and hydrophobicities for the oligopeptide transporter were determined by their relative ability to inhibit the uptake of [^{14}C]Gly-Sar into Caco-2 monolayers at a concentration of 20 mM in the apical media (Fig. 2). The two tested L/L-configured dipeptides, Gly-Sar and Gly-Pro, inhibit the apical uptake of [^{14}C]Gly-Sar by $> 90\%$. Four dipeptides having a D-configured amino acid in either the first or second position, i.e. D-Leu-Tyr, D-Val-Asp, Gly-D-Glu, and Gly-D-Asp, demonstrate a reduced affinity for the oligopeptide transporter relative to the L/L-configured dipeptides; nonetheless, all were able to inhibit [^{14}C]Gly-Sar uptake by $> 75\%$. As an additional control, two L-amino acids, L-Tyr and L-Phe, were tested for their ability to inhibit [^{14}C]Gly-Sar uptake in an identical manner, and as is shown in Fig. 2, they were both unable to inhibit [^{14}C]Gly-Sar uptake to any significant degree.

3.3. D-Asp-Ala, D-Asp(OcHx)-Ala, and D-Asp(OBzl)-Ala inhibit [^{14}C]Gly-Sar uptake

As is shown in Fig. 3, the extent of inhibition of [^{14}C]Gly-Sar uptake is $> 95\%$ for all three compounds (each at 20 mM in the apical media) following 15 min of competitive inhibition, and $> 80\%$ following 120 min of competitive inhibition. After a series of several 15 min [^{14}C]Gly-Sar apical uptake inhibition experiments, IC_{50} values for D-Asp-Ala, D-Asp(OcHx)-Ala, and D-Asp(OBzl)-Ala were calculated and are listed in Table 1. Inhibitor concentrations used were those described in Section 2.3, and each concentration point used for the IC_{50} calculations represents results obtained from three separate Transwell-grown Caco-2 monolayers.

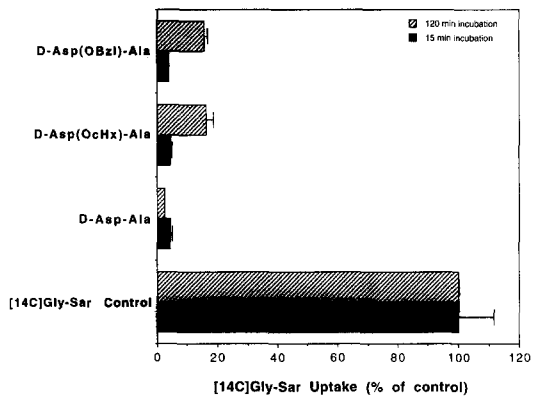

Fig. 3. Uptake of [^{14}C]Gly-Sar (0.5 μCi) in the presence or absence of D-Asp-Ala, D-Asp(OcHx)-Ala, or D-Asp(OBzl)-Ala (20 mM). The degree of competitive [^{14}C]Gly-Sar uptake inhibition in treated monolayers is depicted as a bar which represents the percentage of [^{14}C]Gly-Sar uptake vs. that of the control monolayers over either a 15 min (solid bars) or 120 min (hatched bars) incubation period. Results shown represent the means $\pm$ S.D. for three separate filters under each condition.

3.4. D-Asp(OcHx)-Ala and D-Asp(OBzl)-Ala stability and log P calculation

The stabilities of the β-carboxyl esterified dipeptides under experimental conditions were investigated. HPLC analysis of the apical media of Caco-2 cells loaded with the β-carboxyl modified D-Asp-Ala compounds shows no free cyclohexanol or benzyl alcohol after 5 h of incubation; thus, the β-carboxyl esters of D-Asp(OcHx)-Ala and D-Asp(OBzl)-Ala were completely stable for the duration of the uptake experiments. The apparent partition coefficients of D-Asp(OcHx)-Ala and D-Asp(OBzl)-Ala were also calculated. In order to insure that an equilibrious mixture was obtained, samples from the aqueous phase were analyzed at both 8 and 24 h. The log P values for D-Asp(OcHx)-Ala and D-Asp(OBzl)-Ala were -0.17 ± 0.01 and 0.15 ± 0.09, respectively; these values represent the means of $n = 3$ experiments, $\pm$ S.D. D-Asp-Ala could not be detected via HPLC due to its lack of retention on the column used; thus, its log P value could not be determined. There was no significant difference in the concentration of either compound in the aqueous phase 8 and 24 h after rotation was initiated, indicating that equilibrium was indeed reached after 8 h. There was no free cyclohexanol or benzyl alcohol seen in the HPLC chromatograms following 24 h of rotation.

Table 1
IC_{50} Values for D-Asp-Ala and β-carboxylic acid esterified D-Asp-Ala compounds

Compound	IC_{50} (mM $\pm$ S.D.)
D-Asp-Ala	5.75 ± 0.09
D-Asp(OcHx)-Ala	2.80 ± 0.11
D-Asp(OBzl)-Ala	2.62 ± 0.35

4. Discussion

In order to investigate the affinity of side chain modified dipeptides for the oligopeptide transporter, it was important to apply an assay system capable of evaluating the relative affinities of these compounds. The uptake and transepithelial transport of [^{14}C]Gly-Sar, a relatively small dipeptide that is not susceptible to enzymatic breakdown, across Caco-2 cells via a H^+-coupled oligopeptide transporter has been described previously (Thwaites et al., 1993, 1994). As opposed to using natural, L/L-configured dipeptides, which may present a problem with respect to hydrolysis at the apical membrane of the Caco-2 monolayers (Bai and Amidon, 1992; Smith et al., 1993), we chose to use a model dipeptide that would be enzymatically stable throughout the course of the experiment. Thus, in our experiments, [^{14}C]Gly-Sar served as an efficient marker for measuring competitive interaction with the oligopeptide transporter in the presence of D-Asp-Ala and its β-carboxyl esterified analogs. The pH dependency results are consistent with previously reported results (Ganapathy and Leibach, 1985; Dantzig and Bergin, 1990; Saito and Inui, 1993; Thwaites et al., 1993), i.e., uptake and transport of a substrate for the oligopeptide transporter is significantly more efficient when a H^+-gradient is established between the apical and basolateral membranes. The fact that [^{14}C]Gly-Sar uptake is not inhibited by either L-Phe or L-Tyr (Fig. 2) correlates with

results showing the lack of inhibition in the presence of L-Leu (Thwaites et al., 1994), and further indicates that [^{14}C]Gly-Sar uptake proceeds via an active, H^+-energized process utilizing the oligopeptide transporter.

As is shown in Fig. 2, a number of dipeptides are able to inhibit the uptake of [^{14}C]Gly-Sar in Caco-2 cells. These dipeptides were tested for their relative affinities for the oligopeptide transporter in order to provide information supplementing that which has already been published regarding possible combinations of D- and L-configured amino acids and their ability to be recognized by the oligopeptide transporter (Thwaites et al., 1994; Eddy et al., 1995; Hidalgo et al., 1995). As expected, L/L-configured dipeptides demonstrate a preferential affinity vs. D/L or L/D-configured dipeptides. It has been shown previously that D/D-configured dipeptides are poor substrates for the oligopeptide transporter (Bai and Amidon, 1992; Thwaites et al., 1994; Hidalgo et al., 1995). The inhibition studies using 20 mM of the dipeptides Gly-D-Glu, D-Leu-Tyr, D-Val-Asp, and Gly-D-Asp (Fig. 2) suggest that, whether the D-configured amino acid is in either the amino or carboxy position, there is no significant difference in inhibition of [^{14}C]Gly-Sar uptake into the Caco-2 monolayers. Thus, our rationale for choosing D-Asp-Ala from amongst the many possible combinations of dipeptides were as follows: Asp in either the amino or carboxy position of a dipeptide is able to retain an affinity for the oligopeptide transporter; only Asp and Glu have side-chain carboxyl-termini, thus providing two carboxyl groups per dipeptide in addition to a convenient arm for conjugation to other small molecules; and with respect to solubility, Asp may have a slight advantage over Glu due to its β-carboxyl as opposed to Glu's γ-carboxyl.

Following the synthesis of cyclohexyl- and benzyl-ester modified D-Asp-Ala, all three compounds were evaluated using Transwell-grown Caco-2 monolayers. During a 15 min uptake inhibition experiment each compound demonstrated a greater than 90% inhibition of [^{14}C]Gly-Sar uptake (Fig. 3). During a 2 h uptake experiment there was a slight difference in the degree of [^{14}C]Gly-Sar uptake inhibition noted for D-Asp(OcHx)-Ala and D-Asp(OBzl)-Ala ($> 80\%$) vs. that of D-Asp-Ala ($> 90\%$). The reason for this discrepancy is possibly due to the increase in hydrophobicities of D-Asp(OcHx)-Ala and D-Asp(OBzl)-Ala compared with that for D-Asp-Ala. Since D-Asp(OcHx)-Ala and D-Asp(OBzl)-Ala are more hydrophobic than D-Asp-Ala, their interaction with the Caco-2 monolayers could involve a passive component in addition to active interaction with the oligopeptide transporter. Nevertheless, the IC_{50} values listed in Table 1 show that D-Asp-Ala, D-Asp(OcHx)-Ala, and D-Asp(OBzl)-Ala all possess similar affinities for the oligopeptide transporter. Since stability studies done on D-Asp(OBzl)-Ala and D-Asp(OcHx)-Ala show that both β-carboxyl esterifications were fully stable in the apical media of Caco-2 monolayers for the duration of the uptake experiments, degradation of the β-carboxyl esterified compounds back to native D-Asp-Ala is not the mechanism by which [^{14}C]Gly-Sar uptake is inhibited.

To our knowledge, this is the first study showing that modification of the side-chain of an amino acid within a dipeptide results in retention of affinity for the oligopeptide transporter expressed in Caco-2 cells. Although D-Asp-Ala, D-Asp(cHex)-Ala, and D-Asp(OBzl)-Ala are all able to displace [^{14}C]Gly-Sar uptake into Caco-2 monolayers, it has been shown recently that interaction with the oligopeptide transporter does not necessarily imply that a compound enters the transepithelial transport pathway (Eddy et al., 1995; Hidalgo et al., 1995). Additionally, it has been proposed that perhaps the mechanism for basolateral efflux of a substrate for the oligopeptide transporter is not equivalent to that accessed for apical uptake (Tamura et al., 1995). Future experiments, using radiolabeled dipeptides and drug-dipeptide conjugates, will allow us to determine if D-Asp-Ala and similar molecules enter Caco-2 cells via the oligopeptide transporter and are able to access the transepithelial transport pathway. Such molecules may show potential for use as carriers to facilitate the absorption of poorly bioavailable drugs.

Acknowledgements

The authors would like to thank Dr Bernard A. Moss and Bettina Dinitzen for their excellent technical assistance. This work was supported by grants from the PharmaBiotec Research Center.

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