Intestinal Transport of Amino Acid Residues of Dipeptides

I. INFLUX OF THE GLYCINE RESIDUE OF GLYCYL-L-PROLINE ACROSS MUCOSAL BORDER*

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SUMMARY

The flux of the glycine residue of glycyl-L-proline (Gly-Pro) from intestinal lumen into mucosal cell was investigated in rabbit ileum. Measurements of tissue 14C, after 60 s exposure of the mucosal surface to [Gly-14C]Gly-Pro at concentrations from 0.05 to 32 mM, suggest two saturable influx processes, one with a V max of 0.59 μmole per cm² hr and an apparent affinity constant (K) of 0.93 mM, and the other with a V max of 4.03 μmole per cm² hr and a K of 56.8 mM. The low K process was investigated further. After brief exposure to 0.5 mM [Gly-14C]Gly-Pro, all 14C in the tissue was in the form of free glycine. The influx of [Gly-14C]Gly-Pro (Gly(Gly-Pro) influx) at 0.5 mM Gly-Pro concentration was not inhibited by 20 mM glycine and inhibited only 8% by 20 mM L-proline. Conversely, Gly-Pro (20 mM) did not inhibit the influx of either [3H]glycine (0.5 mM) or [3H]proline (0.5 mM), L-Methionine, L-phenylalanine, and L-leucine all markedly inhibited glycine influx but had little or no effect on Gly(Gly-Pro) influx. In contrast, Met-Pro and Phe-Pro strongly inhibited Gly(Gly-Pro) influx but had little effect on glycine influx. All peptides tested inhibited Gly(Gly-Pro) influx except those which lacked a free NH₂-group in the α position. In the case of Leu-Leu, this inhibition was shown to be competitive.

Gly(Gly-Pro) influx was dependent on the Na⁺ concentration in the mucosal medium, although, at a Na⁺ concentration close to zero, influx was far greater than could be accounted for by passive diffusion. Removal of Na⁺ inhibited Gly(Gly-Pro) influx by reducing V max, whereas it inhibited glycine influx by increasing K.

Loading tissues first with 50 mM glycine or Gly-Pro reduced Gly(Gly-Pro) influx, whereas prior loading with 50 mM proline, leucine, or d-proline did not inhibit.

It is concluded that most Gly(Gly-Pro) influx at low Gly-Pro concentrations occurs by a process selective for dipeptides with a free NH₂ group in the α position. The process is partially inhibited by a low Na⁺ concentration in the mucosal medium and also by a high glycine concentration in the mucosal cell.

Low molecular weight peptides account for most of the nitrogenous material in the intestinal lumen during the digestion of a protein meal (1-3). With very few exceptions, peptides do not cross the intestinal epithelium intact, but they reach the serosal side in the form of free amino acids (4-6). Enzymes hydrolyzing small peptides are not found in significant amounts in the intestinal lumen but are associated with mucosal cells (7-13). The hydrolysis of peptides could occur on the luminal surface of the brush border with release of the constituent amino acids back into the lumen before they are absorbed. Several observations suggest, however, that the relationship between peptide hydrolysis and transepithelial transport of their amino acid residues may be more complex. Asatoor et al. (14, 15) studied the absorption of L-histidine, L-tryptophan, and L-phenylalanine in patients with Hartnup disease, a condition in which there is defective intestinal absorption of neutral amino acids. When these amino acids were ingested in the form of dipeptides, they were normally absorbed, whereas, when ingested as free amino acids, their absorption was less than normal. In the rat, glycine and L-methionine were found to be more rapidly absorbed from di- and tripeptides containing these two amino acids than from equivalent mixtures of the free amino acids (16, 17). Also in the rat, glycine and L-proline were found to accumulate inside everted gut sacs more rapidly when presented to the luminal surface as glycyl-L-proline than when presented as equivalent amounts of the free amino acids.1 The above observations suggest that the amino acid residues of small peptides are translocated across the intestinal brush border by a mechanism different from that for free amino acids.

In the present study, we have investigated the influx of the glycine residue of [glycyl-14C]glycyl-L-proline across the mucosal border of rabbit ileum. The study demonstrates a selective influx process which is shared by other peptides but not by free amino acids.

1 A. Rubino and S. Auricchio, unpublished observations.
METHODS

New Zealand white male rabbits weighing from 2.5 to 4 kg and fed a standard rabbit chow ad libitum were used. The animals were killed by a blow to the neck and 20- to 30-cm long segments of ileum were removed, beginning distally at the last Peyer's patch.

Measurements of Lumen to Cell Influx—The procedure and apparatus used are essentially those described by Schultz et al. (18). Briefly, an 8-cm segment of ileum is opened and then pinned, with the mucosal side up, over the bottom portion of a two-piece chamber. The chamber creates four separate mucosal ports, each with a tissue opening of 1.12-cm² area. Enough ileum is taken from one animal to fill two of these chambers (eight ports). Each port has a separate gas inlet and holds a separate bathing solution. The entire experiment is conducted in a constant temperature environment (37°). Unless specified otherwise, the Ringer solution used contained: NaCl, 140 mM; KCl, 10 mM; KH₂PO₄, 1.2 mM; CaCl₂, 1.2 mM; and MgCl₂, 1.2 mM (pH 7.2). After a 30- to 45-min preliminary incubation period, the initial solution is replaced with a test solution containing the radioactively labeled compound under study and ³H- or ¹⁴C-labeled inulin, added as an extracellular marker. After 10 to 60 s, the test solution is quickly replaced with cold 0.3 M mannitol and the tissue is punched out, rinsed quickly again in mannitol solution, and placed in 2 ml of 0.1 N HNO₃. Radioactivity is then extracted from the tissue by shaking for 2 hours. The extract is centrifuged at 1500 × g for 10 min. Equal volumes of the supernatant and the test solution diluted in 0.1 N HNO₃ are then added to Bray’s solution and assayed for radioactivity in a liquid scintillation counter. Under these circumstances, quenching variations among samples were shown to be negligible by Schultz et al. (18) and this was reconfirmed in the present study. The unidirectional flux of a compound, X, from lumen to mucosal cells is then calculated by Equation 1

\[
\text{Influx (X)} = \left( \frac{X^* - I^{*\text{m}}}{[X]^* - [I^{*\text{m}}]} \right) \frac{[X]_m - 3600}{T \cdot A}
\]

where \([X]\) is the concentration of X in micromoles per ml; the subscripts \(t\) and \(m\) refer to tissue and medium, respectively; and \(X^*\) and \([X]^*\) are, respectively, counts per min and counts per min per ml due to Compound X labeled with one radioisotope. \(I^{*\text{m}}\) and \([I^{*\text{m}}]\) are, respectively, counts per min and counts per min per ml due to inulin labeled with another radioisotope. \(T\) is the number of seconds of mucosal exposure to the test solution, and \(A\) is the area of exposed mucosa (1.12 cm²).

In Fig. 1, tissue uptakes of radioactivity from media containing \([\text{glycyl-}^{14}\text{C}]\text{glycyl-L-proline, [3H}]=\text{glycine, or [3H}]=\text{L-proline have been plotted as functions of time after first correcting for the inulin “space.” The uptakes are linear for at least 60 s and extrapolate to the origin. It can be concluded, therefore, that they accurately reflect unidirectional influx. It was also shown, for all of the radioactively labeled compounds used in the study, that more than 97% of the tissue radioactivity was in the supernatant after the extraction procedure. This was determined by quickly washing the remaining tissue with 0.9% NaCl, dissolving the tissue with a “solubilizer” (Nuclear-Chicago, No. 190610), and then assaying the dissolved material for the remaining radioactivity.

![Graph showing tracer uptake over time](http://www.jbc.org)
Materials—[glycyl-14C]glycyl-L-proline (10 mCi per mmole) was obtained from Schwarz BioResearch. Radiochemical purity was rechecked at 3-month intervals. [2-3H]Glycine (4.67 Ci per mmole), [3,4-3H]L-proline (5.1 Ci per mmole), [carboxyl-14C]inulin (2.05 mCi per g), and [methoxy-3H]inulin (19.5 mCi per g) were obtained from New England Nuclear.

Unlabeled products were obtained as follows: glycyl-L-proline, L-leucyl-L-leucine, and glycylglycine were from Nutritional Biochemicals; L-prolylglycine hydrate, L-phenylalanlyglycine, and glycylglycylglycine were from Mann; L-methionyl-L-proline, L-phenylalanly-L-proline, N-acetylglucylglycine, and L-glutarylserosine were from Fox (Los Angeles, California); and L-phenylalanine, L-leucine, L-methionine, and L-tryptophan were from Calbiochem. All other chemicals were reagent grade.

RESULTS

Effect of Concentration of [glycyl-14C]Glycyl-L-Proline in Mucosal Medium on Initial Rate of Accumulation of Radioactivity in Mucosal Cells—The influx of 14C was determined at [glycyl-14C]-glycyl-L-proline (14C-Gly-Pro) concentrations in the mucosal medium that ranged from 0.05 to 32 mM. In Fig. 2, influx, expressed as micromoles of Gly-Pro per cm² hr, has been plotted as a function of the ratio of influx over Gly-Pro concentration. If the influx process had displayed simple Michaelis-Menten kinetics, then a nonperpendicular linear relationship would have been found (19). The actual relationship is curvilinear with each end of the curve approximating linearity. Lines drawn through the last several points at each end of the curve both intersect the ordinate, suggesting that the influx process consists of two saturable components, one with a high apparent affinity for Gly-Pro and a low maximal velocity and the other with a low apparent affinity for Gly-Pro and a higher maximal velocity. In order to establish separate kinetic constants for the high affinity system, we have assumed that 14C influx from [14C]Gly-Pro can be described by Equation 2

\[
\text{Influx} = \frac{v_{\text{max}}(1) C}{K_t(1) + C} + \frac{v_{\text{max}}(2) C}{K_t(2) + C}
\]

where C is the concentration of 14C-Gly-Pro in the medium, \(v_{\text{max}}(1)\) and \(v_{\text{max}}(2)\) are the maximal velocities of two independent translocation processes, and \(K_t(1)\) and \(K_t(2)\) are the apparent affinity constants for the two processes. The kinetic constants which provide the best possible fit of the data to the above equation are given in the legend of Fig. 2. The theoretical curve based on these constants is shown in Fig. 2. It fits closely the actual points over the full range of the curve. In the present study the characteristics of the high affinity system have been examined. Therefore, influx measurements have been made at low Gly-Pro concentrations. At 0.5 mM Gly-Pro, for example, 83.4% of the [14C]Gly-Pro influx should occur via the high affinity process.

Chemical Form of Tissue Radioactivity following Brief Exposure to 0.0 mM [14C]Gly-Pro—The chemical forms of 14C present in the mucosa following a 60-s exposure to 0.5 mM [14C]Gly-Pro were determined by descending paper chromatography. At the end of 60 s, the only detectable form of 14C present in the 1 ml of incubation medium to which the mucosa had been exposed was [14C]Gly-Pro. However, the amount of radioactivity found in the tissue in the form of [14C]Gly-Pro was only 15 to 22% of the total tissue radioactivity when analyzed with the phenol solvent system (two experiments) and 18 to 21% when analyzed with the 2-propanol, formic acid solvent system (two experiments). The remainder of the tissue radioactivity was in the form of [14C]glycine. The amount of incubation medium which was present in the extracellular space of the tissue (the [H]inulin space), and which could be assumed, therefore, to contain [14C]Gly-Pro at its medium concentration, was not determined in these particular experiments. In 29 other experiments in which the incubation conditions were identical with those used in the present experiments, the per cent of tissue 14C which was extracellular, based on the [H]inulin counts, was 19.9 ± 1.2 (1 S.E.). In the present experiments, therefore, most or all of the [14C]Gly-Pro extracted from the tissue must have been from the extracellular space and essentially all of the intracellular 14C must have been present as free glycine. At low [14C]Gly-Pro concentration, therefore, the accumulation of 14C in the tissue during the 1st min of exposure can be equated
Table I
Interaction between glycyl-L-proline and its constituent amino acids

Substrate and inhibitor concentrations in the incubation media are given in parentheses. Influx in presence of inhibitors is expressed as per cent of control influx. Control and inhibited tissues were alternated in the influx chamber and the data were treated as pairs by determining per cent of control influx for adjacent ports. Number of pairs was eight for all groups.

<table>
<thead>
<tr>
<th>Influx</th>
<th>Inhibitor (20 mM)</th>
<th>Per cent of control influx (mean ± 1 S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycyl-(Gly-Pro) (0.5 mM)</td>
<td>Glycine</td>
<td>100.1 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>L-Proline</td>
<td>92.3 ± 2.0a</td>
</tr>
<tr>
<td>Glycine (1 mM)</td>
<td>Glycyl-L-proline</td>
<td>96.4 ± 1.7</td>
</tr>
<tr>
<td>Proline (1 mM)</td>
<td>Glycyl-L-proline</td>
<td>102.3 ± 4.0</td>
</tr>
</tbody>
</table>

* p < 0.05.

Table II
Effect of amino acids and peptides on [14C]Gly-Pro influx and [3H]glycine influx

Substrate and inhibitor concentrations in the incubation media are indicated in parentheses. Control and inhibited tissues were alternated in the influx chamber and treated as pairs. Number of pairs was eight for each inhibitor tested.

<table>
<thead>
<tr>
<th>Inhibitor (20 mM)</th>
<th>[14C]Gly-Pro influx (0.5 mM)</th>
<th>[3H]Glycine influx (2 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% control influx (± 1 S.E.)</td>
<td></td>
</tr>
<tr>
<td>L-Phenylalanine</td>
<td>97.9 ± 5.8</td>
<td>17.2 ± 3.3a</td>
</tr>
<tr>
<td>L-Leucine</td>
<td>79.2 ± 4.5a</td>
<td>8.2 ± 0.9</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>90.5 ± 2.7a</td>
<td>6.3 ± 1.3a</td>
</tr>
<tr>
<td>L-Methionyl-L-proline</td>
<td>7.9 ± 1.0a</td>
<td>72.3 ± 3.8a</td>
</tr>
<tr>
<td>L-Phenylalanyl-L-proline</td>
<td>11.9 ± 1.3a</td>
<td>81.8 ± 4.0a</td>
</tr>
<tr>
<td>L-Leucyl-L-leucine</td>
<td>11.6 ± 1.8a</td>
<td>13.6 ± 1.5</td>
</tr>
<tr>
<td>L-Phenylalanylglucose</td>
<td>18.5 ± 2.4a</td>
<td>17.5 ± 1.2a</td>
</tr>
<tr>
<td>Glycylglycine</td>
<td>46.3 ± 2.2a</td>
<td>52.3 ± 4.2a</td>
</tr>
<tr>
<td>Glycylasparagine</td>
<td>62.4 ± 8.4a</td>
<td></td>
</tr>
<tr>
<td>L-Prolylglycine</td>
<td>104.3 ± 8.4</td>
<td></td>
</tr>
<tr>
<td>N-Acetylglycylglycine</td>
<td>99.2 ± 4.2</td>
<td></td>
</tr>
<tr>
<td>Glycylglycineglycine</td>
<td>42.8 ± 3.1a</td>
<td></td>
</tr>
</tbody>
</table>

* p < 0.05.

with the accumulation of [14C]glycine. For convenience we refer to this process as "Gly(Gly-Pro) influx."

Effect of Glycine, Proline, and Other a-Amino acids on Gly(Gly-Pro) Influx and Effects of Gly-Pro on Glycine and Proline Influxes—If a significant portion of Gly(Gly-Pro) influx at low Gly-Pro concentrations occurred by pathways also shared by glycine or proline free in the lumen, then it should have been possible both to inhibit Gly(Gly-Pro) influx with glycine or proline and to inhibit glycine or proline influx with Gly-Pro. As indicated in Table I, Gly(Gly-Pro) influx at 0.5 mM Gly-Pro concentration was not inhibited at all by a 40-fold excess of glycine in the mucosal medium and was only minimally inhibited by a 40-fold excess of proline. Furthermore, neither the influx of glycine nor that of proline (1 mM concentrations) was inhibited by a 20-fold excess of Gly-Pro. These results strongly suggest that the influx of the dipeptide occurs by a pathway not shared by the amino acids and vice versa. Three other neutral amino acids were tested at a 20 mM concentration against both 0.5 mM Gly-Pro and 2 mM glycine (Table II). They all markedly inhibited glycine influx but produced either no inhibition or, at the most 20% inhibition of Gly(Gly-Pro) influx. The reason for the small amount of inhibition of Gly(Gly-Pro) influx that was observed in the presence of a large excess of leucine, methionine, or proline is not known. It is possible that these amino acids inhibited the low affinity Gly(Gly-Pro) transport system which accounts for about 15% of the influx at 0.5 mM Gly-Pro concentration.

Fig. 3. Effect of L-leucyl-L-leucine on Gly(Gly-Pro) influx. In control studies (O—O) glycryl-L-proline concentrations in the incubation media ranged between 0.05 and 2 mM. Each control point is the average of four to six determinations in two to three animals. The effect of 5 mM L-leucyl-L-leucine (•—•) was determined over a Gly-Pro concentration range of 0.15 to 2 mM. Each point is the average of eight determinations in four animals.
inhibition of glycine influx by L-leucyl-L-leucine, L-phenylalanylglycine, and glycylglycine resulted from a competitive interaction between glycine and the amino acids released by these dipeptides. On the other hand, there is no evidence to date that the hydrolase known to split dipeptides with L-proline in the COOH-terminal position (glycyl-L-proline dipeptido hydrolase, EC 3.4.3.7) is present in the brush border. In the rat small intestinal mucosa, this enzyme appears to be located, for the most part, in the cytoplasmic fraction of the cell.

Sodium Dependence of Gly(Gly-Pro) Influx—Replacement of NaCl in the mucosal medium with choline chloride (approximately 1 mM Na+ remained) reduced Gly(Gly-Pro) influx by 60% of 0.5 mM Gly(Gly-Pro) concentration. In order to determine whether this inhibition resulted from an alteration of the $K_t$ or of the $V_{\text{max}}$ of the Gly(Gly-Pro) high affinity translocation process, Gly(Gly-Pro) influx was determined in choline Ringer solution at varying Gly-Pro concentrations. Results are shown in Fig. 4 and Table III. $V_{\text{max}}$ was significantly reduced in choline-Ringer. This reduction was significant even after control values were corrected for the low affinity translocation process. $K_t$, in choline-Ringer, was significantly less than the uncorrected but not the corrected $K_t$ in sodium-Ringer. Fig. 4 and Table III also indicate the effect of a low Na+ (40 mM) choline-Ringer solution on glycine influx at low glycine concentrations. The $V_{\text{max}}$ remained the same, but the $K_t$ was increased.

Effects of Prior Tissue Loading on Gly(Gly-Pro) or Glycine on Gly(Gly-Pro) Influx—Following a 30-min preliminary incubation of the mucosa with either 50 mM glycine or 50 mM Gly-Pro, Gly(Gly-Pro) influx was reduced by about 25% (Table IV). No inhibition of Gly(Gly-Pro) influx occurred when the mucosa was first incubated with 2 mM glycine or 50 mM proline, leucine, or glucose. The inhibition caused by prior incubation with high concentrations of glycine or Gly-Pro appears, therefore

### Table III

**Kinetic constants of high affinity Gly(Gly-Pro) and glycine influx processes**

<table>
<thead>
<tr>
<th>Incubation conditions</th>
<th>$n$</th>
<th>$V_{\text{max}}$ (μoles/cm² hr)</th>
<th>$K_t$ (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gly(Gly-Pro) influx</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>140 mM Na⁺</td>
<td>5</td>
<td>0.91 ± 0.08</td>
<td>1.43 ± 0.15</td>
</tr>
<tr>
<td>5 mM Leu-Leu, 140 mM Na⁺</td>
<td>4</td>
<td>0.92 ± 0.18</td>
<td>3.90 ± 0.62a</td>
</tr>
<tr>
<td>Na⁺ free</td>
<td>5</td>
<td>0.42 ± 0.09</td>
<td>0.16 ± 0.05a</td>
</tr>
<tr>
<td>Glycine influx</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>140 mM Na⁺</td>
<td>3</td>
<td>0.80 ± 0.03</td>
<td>1.26 ± 0.06</td>
</tr>
<tr>
<td>40 mM Na⁺</td>
<td>3</td>
<td>1.10 ± 0.23</td>
<td>3.47 ± 0.75a</td>
</tr>
</tbody>
</table>

*p < 0.05 compared to control.

### Table IV

**Effect of prior tissue loading on Gly(Gly-Pro) influx**

The concentration of Gly-Pro in the incubation medium was 0.5 mM. Tissues were first incubated for 30 min with preliminary loading compounds at the concentrations given in parentheses. Mannitol at the same concentrations was included in the preliminary incubation media of control tissues. Previously loaded and control tissues were alternated and the data were treated as pairs by determining per cent of control influx in adjacent ports. Number of pairs was eight for each previously loaded compound. Values given are means ± 1 S.E.

<table>
<thead>
<tr>
<th>Previously loaded compound</th>
<th>Per cent of control influx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycyl-L-proline (50 mM)</td>
<td>78.5 ± 2.5a</td>
</tr>
<tr>
<td>Glycine (50 mM)</td>
<td>73.8 ± 2.7a</td>
</tr>
<tr>
<td>Glycine (2 mM)</td>
<td>102.9 ± 8.7</td>
</tr>
<tr>
<td>L-Proline (50 mM)</td>
<td>99.9 ± 3.6</td>
</tr>
<tr>
<td>L-Leucine (50 mM)</td>
<td>101.2 ± 3.8</td>
</tr>
<tr>
<td>Glucose (50 mM)</td>
<td>102.2 ± 6.1</td>
</tr>
</tbody>
</table>

*p < 0.05.
to be highly specific. As most or all of the Gly-Pro in the mucosal cells was probably hydrolyzed, the inhibition of Gly-Gly-Pro influx was probably due to the high intracellular concentration of glycine. Proline, leucine, and glucose are all accumulated in small intestinal mucosa to at least as great an extent as glycine (21-24), indicating that osmotic swelling of the mucosal cells could not have been responsible for the reduced Gly-Gly-Pro influx. Furthermore, as an excess of glycine in the mucosal medium does not inhibit Gly-Gly-Pro influx (Table I), the inhibition observed in the presence of a high intracellular concentration of glycine could not have been due to back diffusion of glycine into the mucosal medium and subsequent interaction, at the outer surface of the brush border, with the Gly-Gly-Pro translocation process. Instead, either glycine itself, or one of its metabolic products, appears to interact in an inhibitory fashion with the Gly-Gly-Pro translocation process at some location beyond the luminal surface of the brush border membrane.

**DISCUSSION**

The mucosal influx of the glycine residue of glycyl proline appears to occur by two saturable processes, one with a high apparent affinity for Gly-Pro and a low maximal velocity, and the other with a low apparent affinity and a high maximal velocity. The former of these two processes was investigated further in the present report.

Following brief exposure of the luminal surface to 0.5 mM [14C]Gly-Pro, all of the radioactivity in the mucosal cells was found to be in the form of free glycine. This glycine clearly did not enter the mucosal cells by a pathway open to glycine free in the lumen. Studies of the interactions of Gly-Pro and glycine with each other and with other amino acids and peptides indicate that Gly-Gly-Pro influx and glycine influx have widely different molecular specificities: (a) a large excess of glycine in the mucosal medium did not inhibit Gly-Gly-Pro influx; (b) a large excess of Gly-Pro in the mucosal medium did not inhibit glycine influx; (c) large excesses of phenylalanine, leucine, and methionine all markedly inhibited glycine influx but had little or no effect on Gly-Gly-Pro influx; and (d) large excesses of L-methionyl-L-proline and L-phenylalaninyl-L-proline markedly inhibited Gly-Gly-Pro influx but had very little effect on glycine influx.

Further evidence that glycine influx and Gly-Gly-Pro influx occur by different pathways was obtained from experiments done at reduced luminal Na+ concentrations. Although both processes are inhibited by a reduction in luminal Na+, the characteristics of their interaction with Na+ differ fundamentally. A reduction in luminal Na+ leads to an increase in the $K_r$ for glycine influx but does not alter the $V_{max}$. This is the typical effect of luminal Na+ concentration on the kinetics of amino acid influx, as has been shown in rabbit ileum with glycine (21), with alanine, leucine, and valine (25), and with lysine (26). In contrast, measurements of Gly-Gly-Pro influx in the nearly complete absence of luminal Na+ indicate a reduction in $V_{max}$ and no increase in $K_r$.

With respect to both molecular specificity and interaction with Na+, therefore, the influx processes for free glycine and for the glycyl residue of Gly-Pro are entirely different. Gly-Gly-Pro influx also does not occur over a pathway shared by proline. A large excess of proline caused only 8% inhibition of Gly-Gly-Pro influx and a large excess of Gly-Pro had no effect on proline influx. Instead, the translocation process for Gly-Gly-Pro appears to be unique for peptides. Varying degrees of inhibition of Gly-Gly-Pro influx were observed with a number of dipeptides and also the tripeptide glycyglycylglycine. In the case of L-leucyl-L-leucine, this inhibition was shown to be competitive. One essential structural requirement for this inhibition is the presence of a free amino group in the $\alpha$ position: with an imino group or an acetylated amino group in this position, there was no inhibition of Gly-Gly-Pro influx.

As only free [14C]glycine was found in the mucosal cells following brief exposure to [14C]Gly-Pro, it is uncertain whether the dipeptide is translocated intact or whether it is hydrolyzed within the brush border membrane. Three possible mechanisms of Gly-Gly-Pro influx can be postulated. (a) Gly-Pro may be translocated intact and then hydrolyzed inside the cell. (b) Gly-Pro may be hydrolyzed at the outer surface of the brush border, and the liberated glycine may then enter the cell by a translocation process which is not open to glycine free in the lumen; a similar mechanism has recently been proposed by Crane et al. (27) to explain the transport of the glucose subunit of disaccharides in hamster small intestine. (c) A membrane hydrolase may function also as a transport protein by binding Gly-Pro at the luminal side of the membrene and releasing free glycine and proline at the intracellular side. The latter two possibilities require the presence of Gly-Pro peptidase activity in the brush border. While peptidase activities in general have been found both in the cytoplasmic fraction of the mucosal cell and in isolated brush borders (11-13, 20), the dipeptidase specific for peptide bonds lacking a peptidase hydrogen (glycyl-L-proline dipeptidase, EC 3.4.3.7) has been found almost entirely in the cytoplasmic fraction of the cell. The possibility remains, nonetheless, that a much smaller amount of enzyme activity located in the brush border may play a significant role in the hydrolysis and transport of Gly-Pro. Also, measurements of Gly-Pro dipeptidase activity were carried out in the rat and may not apply to the rabbit.

Two characteristics of Gly-Gly-Pro influx deserve additional comment: the interaction with Na+ and the transconcentration effect. Replacement of Na+ in the incubation medium with choline reduced Gly-Gly-Pro influx, but an appreciable influx still remained that could not be attributed to simple diffusion: influx in the nearly complete absence of Na+ still obeyed saturation kinetics and, furthermore, was considerably larger (0.095 pmole per cm2 hr at 0.5 mm Gly-Pro) than influx in the presence of 140 mm Na+ + 20 mm L-leucyl-L-leucine (0.030 pmole per cm2 hr). It is of interest to consider these characteristics of the Na+-Gly-Gly-Pro interaction within the context of the known effects of Na+ on the mucosal influx of amino acids and disaccharides. Curran and Schultz and their co-workers have analyzed these effects in terms of a general model which consists of: (a) association-dissociation constants for the binary and the ternary complexes of carrier, nonelectrolyte, and Na+ at both outer and inner boundaries of the brush border membrane, and (b) rates of translocation of these different complexes across the brush border membrane (25, 26). They have assumed that the translocation rates are slow relative to the association-dissociation reactions. In the case of the amino acids, a reduction in luminal Na+ concentration increases the $K_r$ of influx without altering the $V_{max}$. This can be explained by postulating that the translocation rates of the carrier-amino acid complex and the carrier-amino acid-Na+ complex are approximately equal, and that the function of Na+ is to stabilize the complex at the outside boundary of the membrane, thereby causing a larger fraction of total carrier to be present in a complexed form. Re-
moval of Na⁺, therefore, is equivalent to adding a competitive inhibitor. In the case of the monosaccharides, a reduction in luminal Na⁺ concentration does not alter the Kᵢ but reduces the Vₘₐₓ of influx, and total replacement of Na⁺ nearly abolishes influx. This can be explained by assuming that the effect of Na⁺ is not to stabilize the carrier-monosaccharide complex (in fact, the opposite proved to be the case) but to render that complex suitable for translocation. Only the tertiary carrier-monosaccharide-Na⁺ complex is translocated, there being no detectable transfer of the binary carrier-monosaccharide complex. In the present study, we have shown that a reduction in luminal Na⁺ concentration decreased the Vₘₐₓ and had little effect on the Kᵢ of Gly(Gly-Pro) influx. To this extent the effect of Na⁺ on Gly(Gly-Pro) influx resembles its effect on monosaccharide influx and differs from its effect on amino acid influx. However, Gly(Gly-Pro) influx was still considerable in the nearly complete absence of Na⁺ differing in this respect from monosaccharide influx. These characteristics of the Na⁺-Gly(Gly-Pro) interaction can be explained in terms of the general model outlined above, if one assumes that: (a) the role of sodium with respect to Gly(Gly-Pro) influx is not to stabilize the carrier-substrate complex but to facilitate the translocation of this complex, and (b) the rate of translocation of the binary carrier-substrate complex is appreciable, although slower than that of the tertiary carrier-substrate-Na⁺ complex. It is uncertain, however, that the general model proposed by Curran et al. (25) is applicable to the present case. If Gly(Gly-Pro) is first hydrolyzed and the released amino acids are then transferred into the mucosal cell, then a more complex model will need to be formulated. Na⁺ could interact separately with the hydrolytic reaction and with the transfer process. Goldner, Schultz, and Curran (28) point out that an inhibitory transconcentration effect will be found if the rate of translocation of the binary complex is less than that of the ternary complex. This prediction should also be valid for a more complex model, which places an additional set of reactions in front of those already postulated. It is of interest, therefore, that prior loading with either glycine or Gly-Pro caused a 25% inhibition of Gly(Gly-Pro) influx. As, in both cases, the inhibiting species is probably glycine, the transconcentration effect suggests that glycine and not Gly-Pro is the translocated species. This suggestion is further supported by the fact that prior loading with proline, leucine, or glucose did not inhibit Gly(Gly-Pro) influx. In a number of bacterial and fungal systems, however, feedback control of nonelectrolyte transport appears to be mediated by metabolic derivatives of the transported substrates (29–39). The transconcentration effect observed in the present study, therefore, may not be as straightforward as that implied in the model of Curran et al. (25, 28). Until the ambiguity concerning the relationship between hydrolysis and translocation is resolved, it will be premature to attempt to formulate and test a precise model for Gly(Gly-Pro) influx.

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REFERENCES


Intestinal Transport of Amino Acid Residues of Dipeptides: 1. INFLUX OF THE GLYCINE RESIDUE OF GLYCYL-L-PROLINE ACROSS MUCOSAL BORDER

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