Peptide Digestion and Absorption in the Small Intestinal Mucosa During Acute and Chronic Diarrhea

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Before dietary protein or endogenous protein of the alimentary tract can be assimilated, it must be hydrolyzed by intraluminal digestive processes into small peptides and free amino acids. Borgström et al. (1) found that in normal subjects, 60 to 80% of 1 g of radioiodinated human serum albumin disappeared at the level of the proximal jejunum. Subsequently, Nixon and Mawer (2,3) measured the protein concentration and amino acid composition of gut content at the various levels of the intestine after a 15-g protein meal. They found that the major part of the meal protein was digested and absorbed in the duodenum and upper jejunum.

More recently Adibi and Mercer (4) reported an experiment in which human subjects were fed 50 g of bovine serum albumin in a mixed meal, after which samples were taken from points 110 and 220 cm from the teeth, and the ingested protein was identified by SDS-polyacrylamide gel electrophoresis. They found that undigested protein could be detected from the jejunum and upper ileum for as long as 4 hr after ingestion and that exogenous protein was the principal contributor to increases above fasting levels of intraluminal amino acids and peptides in the upper gut during this period. They further reported that greater amounts of amino acids in the intestinal aspirates were present as small peptides than in the free form.

Chung and co-workers (5) studied the extent of digestion and absorption of 59 g of bovine serum albumin contained in a mixed meal. Sixty percent of the meal protein was digested and absorbed at the level of the proximal jejunum, and 99% by the distal ileum. These data indicate that almost complete digestion and absorption of protein occurs by the time it reaches the terminal ileum. However, bovine serum albumin was still present in the proximal ileal content, thus suggesting that the ileum is important for normal protein digestion.

All of these studies have also demonstrated that peptides account for the largest amount of nitrogen in the intestinal lumen during protein digestion.

Chung et al. (5) found that, in general, over 50% of each amino acid is present in peptide form in the intestinal aspirates (Table 1). I should like to stress that almost all acidic amino acids and proline are present as peptides; it is, in fact,
well known that peptide bonds containing glutamic acid and proline are resistant to pancreatic proteolysis (6,7).

All of the studies cited were performed on human adults. In a study on infants, digestion of protein from breast milk was compared to that of protein from an adapted cow’s milk formula in two infants aged 4 months with an artificial anus applied in the ascending colon region. In both infants, when breast milk was given, stools contained a considerably lower α-amino nitrogen and protein concentration: the concentration was nearly twice as high during the cow’s milk period than during the breast milk period, although the amount of protein given daily in the cow’s milk was only 1.25 times higher than that contained in the breast milk diet (8). Similar differences in nitrogen concentration have been shown between ordinary stools obtained by breast milk feeding and feeding on a cow’s milk preparation (8). These studies do not give information on the percentage of dietary protein absorption, but they suggest that different dietary proteins have different digestibilities.

The luminal peptides are digested and absorbed in the small intestinal mucosa through two mechanisms. One is transport into the cell and intracellular hydrolysis, and the other is brush border hydrolysis followed by transport into the cell of the released smaller peptides or amino acids.

In a recent review, Ganapathy and Leibach (10) discussed the possible mechanisms of peptide transport and hydrolysis in the small intestine on the basis of studies on peptide transport in purified brush border vesicles. They claim that peptide transport in the intestine is Na+ independent, carrier mediated,
and, perhaps, a passive process occurring down a concentration gradient. However, the actual molecular mechanisms involved in peptide transport at the membrane level are far from clear.

The carrier-mediated uptake process consists mainly or exclusively of one transport system of broad specificity for dipeptides and tripeptides. Tetrapeptides, with some possible exceptions, are apparently not transported intact, an observation consistent with the generally very low or absent hydrolytic activity of cytoplasmic enzymes on tetrapeptides. The mechanisms of transport and intracellular hydrolysis are therefore mainly specialized for the digestion of tripeptides and dipeptides. A critical review of this problem was published by Silk in 1981 (11).

The soluble peptidases responsible for the intracellular hydrolysis of transported peptides are a complex mixture of various enzymes. Some of these have been purified in animals, and their substrate specificity has been studied.

1. A dipeptidase ("master" dipeptidase) (12-16). This enzyme is able to hydrolyze many dipeptides, with the exception of those with C-terminal proline and some with N-terminal proline or basic amino acids. This enzyme has neither tripeptidase or aminoacyl-β-naphthylamide hydrolase activity. A suitable substrate for the assay of this enzyme is glycyl-L-leucine.

2. An aminoacyl-proline hydrolase (prolidase) (16,17). This enzyme can hydrolyze dipeptides with C-terminal proline or hydroxyproline. Glycyl-L-proline is a substrate for the assay of the enzyme.

3. An aminotripeptidase (18). This enzyme is responsible for almost all of the aminotripeptidase activity of cytosol, and it cannot hydrolyze dipeptides or peptides larger than tripeptides. The enzyme is able to hydrolyze a large series of tripeptides but not those with either a charged N-terminal residue (e.g., lysine or glutamic acid) or a proline residue in the second position. The most specific tripeptide that could be used to monitor the aminotripeptidase in a subcellular fractionation is L-prolylglycylglycine.

Other cytosol enzymes have been identified in the small intestine (for a review see ref. 19).

Very little information is available on the substrate specificity of soluble peptidases in human intestine. It has been demonstrated (20,21) that, in human intestinal mucosa, the enzymatic hydrolysis of glycyl-L-leucine, L-leucylglycylglycine, and glycyl-L-proline is mainly carried out by soluble enzymes. The soluble enzymes responsible for the hydrolysis of these three substrates are probably the "master" dipeptidase, the tripeptidase, and the prolidase, respectively. These peptides are therefore probably suitable substrates for the assay of cytosol peptidases in total homogenates of human intestinal mucosa.

The second mechanism for the hydrolysis of dipeptides and tripeptides is brush border hydrolysis followed by the absorption of the released amino acids or dipeptides. The amount of di- or tripeptide that follows the first or the second mechanism for intestinal absorption and digestion probably depends on the
relative affinity of the peptide for the brush border peptidases or the carrier system.

Tetra- and higher peptides are, in contrast, hydrolyzed *in vivo* in the brush border membrane. The peptidases recently identified in the brush border are in fact able to hydrolyze large peptides using different mechanisms: aminopeptidase, dipeptidylaminopeptidase, carboxypeptidase, and endopeptidase hydrolysis. Furthermore, some are able to hydrolyze peptide bonds containing glutamic acid or proline, which are resistant to pancreatic peptidases (6,7) and cytosol peptidases (12,13,22,23).

The following peptidases are brush border enzymes:

1. Oligoaminopeptidase (24–33). This enzyme is able to hydrolyze di- and oligopeptides up to at least octapeptides (29). The best substrates for the human enzymes are peptides, especially tri- and tetrapeptides, with a large neutral or aromatic amino acid at the N-terminal (25,30a). The L-leucyl-β-naphthylamide is a suitable substrate for the assay of this enzyme in total homogenate of human intestinal mucosa (20).

2. Dipeptidylaminopeptidase IV. The enzyme purified from hog intestine (34) is a serine protease able to free N-terminal dipeptides from peptides having penultimate proline, alanine, or leucine residues. This enzyme probably plays a role in the terminal digestion of proteins, releasing from oligopeptides (or proteins) (35,36) the aminoacylproline types of dipeptide, which are poorly hydrolyzed in the brush border membrane (37–39) but which are transported into the cells by the peptide carrier (40). Glycyl-L-prolyl-β-naphthylamide is a suitable substrate for the assay of this enzyme in total homogenate of human intestinal mucosa (20).

3. Aminopeptidase A (41–43). This enzyme is probably involved in the digestion of oligopeptides containing N-terminal glutamic (and aspartic) acid (44). The brush border of hog (45) and rat (46) intestine is, in fact, able to digest peptic peptides of gluten, which are rich in glutamic acid. α-L-Glutamyl-β-naphthylamide in the presence of Ca²⁺ is a suitable substrate for the assay of this enzyme in total homogenate of human intestinal mucosa (20).

4. A carboxypeptidase (20,47–50). A similar enzyme has been purified from kidney and is able to hydrolyze the C-terminal residue from a large series of N-blocked peptides, most of them having proline as C-penultimate amino acid (51); this enzyme was recently identified as a brush border peptidase in pig kidney (52). N-CBZ-L-Prolyl-L-alanine (or L-leucine) in the presence of Co³⁺ may be used as a substrate for the assay of this enzyme in total homogenate of human intestinal mucosa (20).

5. γ-Glutamyltranspeptidase (41,53,54). It has been suggested that this enzymatic activity is involved in the transport of amino acids and dipeptides across the brush border (55,55a).

In addition to these enzymes, one or two other peptidases are probably responsible for the hydrolysis of some di- and tripeptides in the brush border (41,48,56–61).
An endopeptidase activity has also been identified in the brush border that is able to hydrolyze the B-chain of insulin (62-65).

Enterokinase is another mucosal peptidase; it reaches its highest activity in the duodenum (66) and is probably predominantly localized in the brush border (67). This enzyme, which triggers the activation of proteolytic pancreatic zymogens in the small intestinal lumen, is a key enzyme for protein digestion and absorption.

In conclusion, brush border peptidases are able to hydrolyze, with different mechanisms, not only di- and tripeptides but also larger peptides (or proteins). This probably explains the results of a study demonstrating digestion and absorption of exogenous whole protein from rat small intestine in the absence of demonstrable luminal pancreatic proteolytic enzyme activity; partial hydrolysis and denaturation of dietary protein by gastric pepsin and HCl increase the efficiency of this intestinal process (68).

**PEPTIDE DIGESTION AND ABSORPTION IN THE SMALL INTESTINAL MUCOSA DURING ACUTE AND CHRONIC DIARRHEA**

Very few reports are available on the two routes of peptide absorption and digestion in diarrhea.

Regarding the intestinal transport of peptides, the information we have is very scanty: the study of this problem is complicated by the difficulty in selecting a sufficient range of representative peptides. Even if attention is limited to dipeptides and tripeptides, the range of possible peptides is still wide, and results obtained on a few peptides cannot be extrapolated to draw general conclusions.

With this limitation in mind, we recall that Hellier and co-workers (69) used the intestinal perfusion technique to demonstrate impaired absorption of glycylglycine in adult subjects with tropical sprue.

Guandalini and colleagues (70) have demonstrated that cyclic guanosine monophosphate has no effect on peptide transport in rabbit ileum. They studied the absorption of two dipeptides: one, glycylsarcosine, that can be translocated into the cell via the carrier system, and the other, glycylphenylalanine, which can be split at the brush border membrane by peptidases, with the subsequent transport of the released amino acids. These results appear to suggest that peptide absorption is normal in acute diarrhea caused by heat-stable enterotoxins, such as that from *E. coli* or *Y. enterocolitica*, which cause fluid secretion in the small intestine by stimulating guanylate cyclase.

In the atrophic small intestinal mucosa of coeliac children, we (20) have recently measured the activities of a series of brush border and soluble peptidases by using substrates that are specific or almost specific for these enzymes. In this way we were able to measure in total homogenate of single biopsies all enzyme activities together (Table 2). We found that in atrophic intestinal mucosa of children with active celiac disease, all of the enzyme activities studied were
significantly reduced compared to normal controls. However, the peptidase activities were not equally reduced. Among the brush border peptidases, aminopeptidase A and carboxypeptidase were as low as was sucrase (31, 46, and 35% of the control values, respectively); dipeptidylaminopeptidase IV and oligoaminopeptidase were less markedly reduced (64 and 70% of control values, respectively). Of the soluble peptidase activities, glycyl-L-leucine hydrolase activity showed a greater reduction than did L-leucylglycylglycine hydrolase activity (46 and 81% of the control values, respectively).

The fact that not all peptidase activities are equally decreased in the atrophic mucosa may result from the fact that some enzymes of the enterocyte are more affected than others by the disease: it is well known that lactase shows a greater reduction than does sucrase in the atrophic mucosa of celiac patients. Another possibility is that peptidases, besides being located in epithelial cells, are found also in other cells, for example, in immunocytes, which are numerous in the atrophic mucosa (20). Therefore, the overall enzyme activity of the biopsy may not reflect the true digestive capacity of the enterocytes.

In a study performed in our laboratory by Giunta and co-workers (71), the activity of three brush border peptidases, oligoaminopeptidase, aminopeptidase A, and dipeptidylaminopeptidase, were measured in small intestinal biopsies of seven infants aged between 2 and 6 months affected by chronic intractable diarrhea, severely malnourished (with a body weight ranging between 2.2 kg and 4.6 kg), before total parenteral alimenation (Table 3). Oligoaminopeptidase and aminopeptidase A were severely depressed; dipeptidylaminopeptidase was only slightly reduced. More markedly affected were the enzyme activities in the biopsy samples with the more severe mucosal damage. In four subjects in whom the enzyme activities were measured again after 20 to 40 days of total parenteral alimenation, the mucosa was morphologically normal, and the brush border peptidases had also returned to normal, although the sucrase was still reduced, thus suggesting that the peptidase activities are less affected by deprivation of oral nutrition than is the sucrase.
TABLE 3. Brush border peptidase and sucrase activities in infants (aged between 2 and 6 months) with intractable diarrhea before total parenteral alimentation (units/g protein, mean ± 1 SD and, in parentheses, percentage of the control values)

<table>
<thead>
<tr>
<th></th>
<th>Sucrase (n = 9)</th>
<th>Oligoaminopeptidase (n = 9)</th>
<th>Aminopeptidase A (n = 9)</th>
<th>Dipeptidyl-aminopeptidase (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>92 ± 30</td>
<td>42 ± 13</td>
<td>13.9 ± 3.5</td>
<td>10.6 ± 4.3</td>
</tr>
<tr>
<td>Intractable diarrhea</td>
<td>57 ± 7</td>
<td>25 ± 5</td>
<td>7.6 ± 0.8</td>
<td>8 ± 1.1</td>
</tr>
<tr>
<td>(62%)</td>
<td>(59%)</td>
<td>(55%)</td>
<td>(75%)</td>
<td></td>
</tr>
</tbody>
</table>

From Giunta et al., (71) with permission.

In a study performed in 1971, Kumar and co-workers (72) demonstrated that in eight undernourished children with diarrhea, mean intestinal mucosal glycylproline hydrolase activity was reduced to 40% of the control values. In the adult with malnutrition and normal, or almost normal, intestinal mucosa, Hazuria et al. (73) demonstrated that the glycyl-L-leucine dipeptidase and glycyl-L-valine dipeptidase activities are markedly reduced.

The conclusion of these few studies is that the enzymatic activities of mucosal brush border peptidases are reduced in patients with acute and chronic diarrhea, particularly when malnutrition and/or small intestinal lesions are also present; it is, in fact, well known from autopsy studies (74) and biopsy studies in children (75) that acute diarrhea may also cause severe small intestinal mucosa damage. However, it should be mentioned that Branski and co-workers found that in suckling mice infected with reovirus type 3, the oligoaminopeptidase activity is, unlike the activities reported above, increased (76).

CONSEQUENCES OF IMPAIRMENT OF MUCOSAL DIGESTION AND ABSORPTION OF PEPTIDES IN ACUTE AND CHRONIC DIARRHEA

One possible consequence of impaired mucosal digestion and absorption of peptides in acute and chronic diarrhea, especially when accompanied by malnutrition, could be a generalized protein malabsorption. It is well known that infectious diarrhea may produce a transient but significant malabsorption syndrome. Most investigations on this subject have been devoted to malabsorption of sugars and fats, but some evidence also exists for nitrogen, amino acid, and protein malabsorption (77,78).

Molla and Molla (79) studied the effect of diarrheal illness on the absorption coefficient of protein in 29 children, all aged below 5 years, with acute watery diarrhea; these were caused in 13 cases by rotavirus, in 10 by enterotoxin-producing E. coli (half of them heat-stable toxin and half heat-labile toxin), and in six by Shigella. The children were studied both in the acute stage of diarrhea and at 2 and 8 weeks after recovery.
In rotavirus diarrhea, protein absorption was significantly lower than in _E. coli_ diarrhea and it did not improve even 8 weeks after recovery from the disease. In rotavirus infection the villous epithelial cells are known to be specifically affected (80), and the inflammation may be prolonged, resulting in generalized malabsorption.

In _Shigella_ patients, nitrogen absorption in the acute stage of the disease was minimum (only 41%). _Shigella_ is a disease of the lower bowel, and the nitrogen malabsorption probably represented a direct loss of protein from the gut rather than failure of intestinal absorption. Absorption of all nutrients improved considerably 2 weeks after recovery from the disease.

In children with _E. coli_ diarrhea, although absorption of all nutrients during the acute stage was affected less than in the other two forms of infectious diarrhea studied, absorption still failed to improve 2 weeks after recovery. At 8 weeks, there was an increase in the absorption of nutrients.

During the recovery period from choleric secretory diarrhea, the nitrogen balance in children improves rapidly with milk feeding, even before the diarrhea is fully controlled and irrespective of the clinical severity of the disease. There is little nitrogen loss in the stool, and the apparent absorption of protein is substantial (81).

Protein absorption is also severely depressed in infants with prolonged diarrhea: large stool losses of nitrogen have been measured by Mann and co-workers (82) in 22 infants who had severe diarrhea for 7 days without known pathogens in the stool. The apparent nitrogen absorption was very poor, only about 50%, on three diets (full-cream milk, soy milk, or low-lactose milk). An appreciable proportion of this nitrogen loss appeared to be endogenous. Likely sources are gastrointestinal mucosa exfoliation, secretions, and plasma proteins.

The mechanisms by which acute and chronic diarrheal disease produce protein malabsorption have yet to be clarified. There are some data implicating the loss of excessive protein from the g.i. tract through protein and exfoliative enteropathy, as has been demonstrated in acute measles enteritis (83) and in children suffering from acute rotavirus diarrhea or from acute diarrhea without any apparent microbial pathogen (84).

Bacterial overgrowth, similar to that seen in the contaminated small bowel syndrome, may contribute to protein malabsorption in diarrheal disease. Possible action mechanisms are competition with the host for the uptake of ingested nutrients, metabolism of proteins and amino acids, and inhibition of mucosal amino acid uptake. Recently, protein-losing enteropathy has also been demonstrated in human blind loop syndrome (85).

In contrast, it is most improbable that faulty mucosal digestion and absorption of peptides is an important mechanism of protein malabsorption in acute and chronic diarrhea, as we know that in severe active celiac disease, in which there is extensive mucosal damage, protein malabsorption is either absent or of only minor importance (86). This suggests that peptide digestion and absorption in
the small intestinal mucosa have a large reserve capacity and are generally not limiting factors in dietary protein absorption in g.i. disease.

A further possible consequence of impaired mucosal digestion and absorption of peptides in acute and chronic diarrhea could be malabsorption of peculiar amino acid sequences present in some proteins. As mentioned previously, brush border peptidases are probably specialized in the hydrolysis of peptides that contain glutamic acid and proline. Brush border peptidases are therefore most probably of great importance in the digestion of proteins such as gliadins, which are very rich in glutamic acid and proline residues. As these peptidase activities are decreased in the atrophic mucosa, digestibility of glutamyl and prolyl peptides is expected to be particularly reduced in celiac disease and in other forms of severe diarrhea with atrophic small intestinal mucosa (20). Also, impaired intestinal proteolysis may be involved in increased macromolecular absorption, as suggested by Udall and Walker (87).

Finally, it may be speculated that alterations of the structure of brush border peptidases may occur as a consequence of variations of the enterocyte cell cycle, as in viral infections (88,89), or as a consequence of bacterial degradation of brush border glycoproteins, as in luminal bacterial overgrowth (90,91). Structural modifications of brush border peptidases, as well as of other brush border glycoproteins, may alter their capacity to interact with the intraluminal content.

CONCLUSION

Excessive gastrointestinal protein loss in diarrhea may pass unnoticed in well-nourished children, but it may trigger the development of malnutrition in already undernourished children. It is therefore generally recommended that food intake continue whenever possible during the diarrheal episode. The problem arises as to which is, from a nutritional point of view, the best form of amino nitrogen intake for a child with acute or chronic diarrhea. As mentioned previously, the diminished total capacity of the small intestinal mucosa to digest and absorb peptides is probably still sufficient to cover the nutritional needs of the infant or, at least, is not an important limiting factor in protein absorption in diarrhea. Hence, oligopeptide-based nitrogen sources could be recommended in cases of acute and chronic diarrhea because they are probably relatively well digested and absorbed; nevertheless, the question remains whether oligopeptides, amino acids, or protein itself is the best nitrogen source in these diets.

REFERENCES


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