3 Absorption of Amino Acids and Peptides

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Introduction

Assimilation of dietary or microbial (ruminants) protein involves the interaction of a series of steps beginning in the stomach (non-ruminants), abomasum (ruminants), or proventriculus (poultry) and ending with the transport of amino acids and peptides from the basolateral membrane of the small intestine. In the glandular stomach, hydrochloric acid (HCl) denatures dietary protein and promotes proteolysis protein to large polypeptides via the action of pepsin. On entering the small intestine, pancreatic proteases principally hydrolyse large polypeptides and proteins into oligopeptides of six or less amino acid residues as well as free amino acids. Degradation of dietary protein continues by hydrolytic enzymes of the small intestine epithelia that are present in the luminal surface (apical membrane or brush border) of absorptive epithelial cells (enterocytes). Brush border peptidases split oligopeptides of six or less amino acids in length. Many of the resulting di- and tripeptides are transported into the enterocyte intact by a single H^+-coupled transporter and then hydrolysed to free amino acids by cytosolic peptidases (primary) or transported across the basolateral membrane. In contrast, free amino acids are absorbed by a variety of iron-dependent and -independent transporters. The fate of absorbed peptides is principally further hydrolysis to free amino acids by a variety of cytosolic peptidases, whether absorbed as free or peptide-bound amino acids, cytosolic amino acids are available as energy substrates, incorporation into constitutive protein, or transport across the basolateral membrane into blood. Ultimately, digested protein enters the hepatic portal circulation in the form of free amino acid and peptides.

The working hypothesis for assimilation of luminal proteins by enterocytes is illustrated in Fig. 3.1. The model identifies gastric, luminal, glycocalyx/apical membrane, and intracellular hydrolytic digestion events, in addition to apical and basolateral membrane-mediated absorption events of peptide-bound and free amino acids by specific transport proteins. Each component of the model is discussed in this chapter. Although poorly understood, and in contrast to the specificity of digestion and transport events, it is also important to note that the potential contribution to the absorption of amino acids by relatively non-specific transmembrane simple diffusion and paracellular flow events may be of nutritional significance.

Despite anatomical differences in the digestive tracts among farm animal species,

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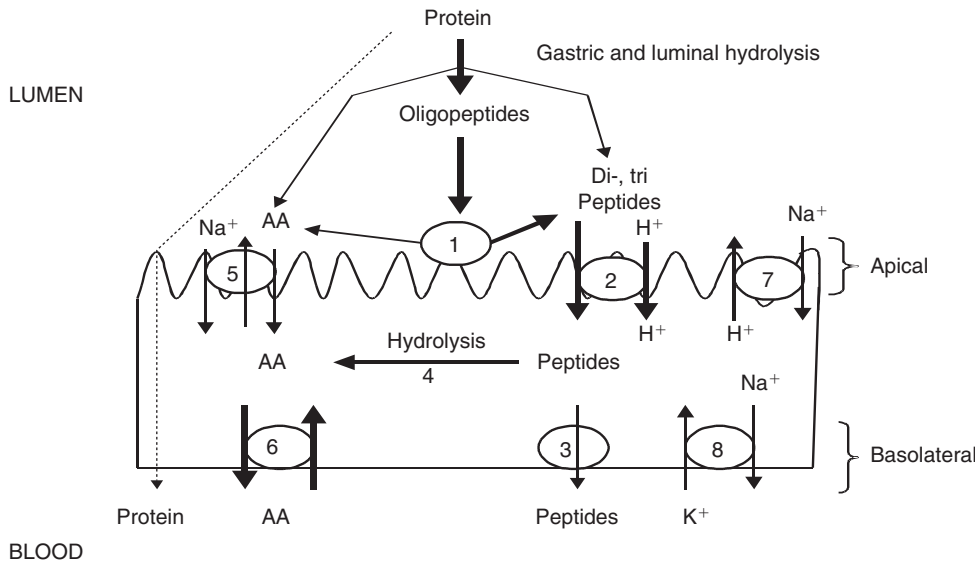

Fig. 3.1. The current model for the role of peptide uptake in protein assimilation, as adapted from Ganapathy *et al.* (1994). The relative contribution of peptide (di-, tri-) versus free amino acid (AA) to total protein assimilation through hydrolytic and transport events is indicated by the relative thickness of the lines. After hydrolysis by gastric proteases and luminal peptidases, oligopeptides are hydrolysed to small peptides and free amino acids by apical membrane-bound (1) peptidases. Peptides are absorbed across brush border membrane by PepT1 (2). Whereas a small proportion of absorbed peptides are then absorbed intact across the basolateral membrane by a H^+-independent transport activity (3), the majority are hydrolysed by intracellular peptidases (4). The resulting free AA, plus those absorbed across the apical membrane by a complement of Na^+-dependent and -independent amino acid transporters (5) are then transported across the basolateral membrane by a complement of Na^+-independent and amino acid exchanger transport proteins (6). The extracellular–intracellular H^+ gradient that drives PepT1 activity, is re-established by the combined function of the apical Na^+/H^+ exchanger (7) and the basolateral Na^+/K^+ ATPase (8), which re-establishes the extracellular–intracellular Na^+ gradients diminished by both Na^+/H^+ exchanger and Na^+-coupled free amino acid transport. The contribution to total protein assimilation by free AA uptake from the lumen is represented by a composite transporter model (7), representing AA transport by Na^+-coupled, AA counterexchange, and/or facilitated transport proteins. The transepithelial passage of intact proteins is also indicated (dashed line). The mechanisms responsible for this relatively minor, but immunologically important process, have been reviewed by Gardner (1994).

enzyme and transporter expression and activity of the associated tissues is fundamentally similar. Although differences do exist, enzymes and transport proteins responsible for digestion and absorption have probably adapted to the nature of the food more than the type of animal (Lassiter and Edwards, 1982). Therefore, processes involved in mammalian protein digestion and peptide and amino acid absorption are generally common to all species. We will attempt to point out differences where known. Our goal in this chapter is to review some of the more recent findings regarding peptide and amino acid net flux and transport processes. We begin with a brief review of processes involved in protein digestion in the glandular stomach. Pregastric digestion processes of ruminants and birds are topics of the other reviews.

Digestive Processes

Gastric digestion

Mammalian and avian protein digestion is initiated in the stomach (non-ruminants), abo-

masum (ruminants) or proventriculus (poultry) in the presence of HCl and pepsin. As discussed by Atasoglu and Wallace in Chapter 15, ruminant animals derive their amino acid supply from a mixture of feed protein that escapes ruminal degradation and microbial protein that is formed as a result of microbial fermentation in the reticulorumen. Microbial protein is readily digested by the host animal and constitutes a well-balanced array of essential amino acids for ruminants in many production systems. Gastric digestion involves the secretion of HCl by gastric parietal cells. Hydrochloric acid is required to initiate the conversion of pepsinogens into pepsins, and also to maintain pepsin activity. Pepsins are secreted as inactive precursors (i.e. pepsinogens) by chief cells in the stomach. In chickens, both gastric acid and pepsinogen are secreted by oxynticopeptic cells of the proventriculus (true stomach). Although HCI production is relatively high, little digestion occurs in the proventriculus as there is little storage capacity, and digesta transit rate is rapid. The synthesis and secretion of inactive precursors, known as zymogens or proenzymes, allow vertebrates to digest exogenous protein without destroying constituent protein in the stomach and pancreas. Once secreted and activated (pH<5.0), pepsin increases the susceptibility of native proteins to attack by pancreatic proteases by opening the tertiary and quaternary structure of the protein and exposing amino acid residues to the pancreatic endopeptidases (Guan and Green, 1996). For some dietary proteins, gastric predigestion is also important for stimulation of pancreatic exocrine secretion and cholecystokinin (CCK) release in the intestinal phase of digestion in the rat (Guan and Green, 1996) and dog (Meyer and Kelly, 1976). As mentioned, there are a number of pepsinogens secreted and these are converted into the analogous pepsins when the pH is less than 5.0. Once pepsin is present in the lumen of the stomach, the reaction becomes autocatalytic, which involves the splitting off of a peptide chain and peptide fragments. The pepsins are most active at pH less than 4.0 and become inactive at pH greater than 6.0, although the optimum pH for pepsin activity varies from species to species (Crevieu-Gabriel *et al.*, 1999).

Pepsins are most active at peptide bonds that include phenylalanine, tyrosine, leucine, valine and glutamic acid (Ulshen, 1987). Its most pronounced effect is between leucine and valine, tyrosine and leucine, or between the aromatic amino acids such as phenylalanine–phenylalanine or phenylalanine–tyrosine. The principal function of digestion in the stomach is the transformation of protein into large polypeptides, although peptic digestion in acid pH can produce free or peptide-bound amino acids. As suggested above, both amino acids and peptides are good stimuli for release of hormones that stimulate pancreatic enzyme secretion, e.g. CCK. Ultimately, the object of stomach proteolysis is to make peptide molecules available that are susceptible to further hydrolysis by proteolytic enzymes in the small intestine.

Digestion in the small intestinal lumen

The products of HCl and pepsin digestion enter the duodenum of the small intestine through the pyloric sphincter. In the duodenum, proteins and polypeptides serve as substrate for enzymes secreted from the pancreas and small intestine. Initially, protein and polypeptides entering the duodenum are broken down further in the intestine by pancreatic exopeptidases trypsin, chymotrypsin, elastase, and pancreatic endopeptidases carboxypeptidase A and B. The pancreas secretes proenzymes into the duodenum, which when activated hydrolyse peptide bonds. The conversion of inactive trypsinogen into active trypsin requires removal of an N-terminal peptide and is catalysed by the brush border enzyme enteropeptidase (formerly known as enterokinase). Enteropeptidase selectively cleaves a hexapeptide (H_2N-Val-Asp-Asp-Asp-Asp-Lys) from the amino terminus of trypsinogen resulting in trypsin (Kitamoto *et al.*, 1994). Enteropeptidase activity may be regulated by pancreatic secretion (Kwong *et al.*, 1978) and thus possibly by dietary protein content. Following its conversion from trypsinogen, trypsin activates the

other zymogens and to a lesser degree trypsinogen.

Trypsin, chymotrypsin, and elastase catalyse the breakdown of proteins, polypeptides, and peptides into smaller peptides and amino acids in the duodenum. Each pancreatic protease has a unique and complementary action. Trypsin catalyses the breakdown of bonds that involve lysine and/or arginine, whereas linkages involving aromatic amino acid residues are susceptible to chymotrypsin catalysis (Alpers, 1994). Elastases are less specific with regard to the type of peptide bonds, but in general catalyse the breakdown of peptide bonds containing aliphatic residues. The action of trypsin, chymotrypsin, and elastase releases numerous terminal peptide bonds, which in turn are further digested by aminopeptidases, carboxypeptidases, and other specific peptidases present in the lumen or mucosa of the small intestine. Pancreatic carboxypeptidase A and B are exopeptidases that catalyse the hydrolysis of the carboxy-terminal bonds in polypeptide chains, removing the amino acids in sequence. Proteolysis of an approximately 100-residue segment from the amino-terminal region results in the activation of procarboxypeptidases (Aviles *et al.*, 1985). Carboxy-terminal aromatic or non-polar amino acids exposed by the action of chymotrypsin and elastase are available to be cleaved by carboxypeptidase A, while carboxy-terminal basic amino acids exposed by trypsin can be cleaved by carboxypeptidase B. Both carboxypeptidase A and B are inhibited by proline. The products of pancreatic digestion are oligopeptides of up to six amino acid residues (approximately 60%) as well as free amino acids (approximately 40%; Alpers, 1994).

Mucosal phase of digestion

The final stages of non-fermentative protein digestion are carried out by a wide array of brush border and cytosolic peptidases. A review of the brush border and cytosolic peptidases of the small intestine has been conducted by Alpers (1994). The small intestinal peptidases are capable of splitting products of pancreatic digestion (i.e. oligopeptides of six or less amino acids). These enzymes are present in the enterocyte in two groups that are associated with different cell fractions, the apical membrane and the cytosol (Kim *et al.*, 1972, 1974). The apical-membrane enzymes are attached to the outer surface of the microvillus and extend out from the luminal surface of the enterocyte. In contrast, the cytosolic enzymes are found within the cell and do not make direct contact with the luminal contents. As such, these two groups of enzymes are distinct from one another, differing in location and physicochemical and immunochemical properties (Kim *et al.*, 1972; Nóren *et al.*, 1977; Tobey *et al.*, 1985). In addition, the apical enzymes seem to be unique to the small intestine, whereas similar cytosolic peptidases have been found in a number of tissues.

Many di- and tripeptides are absorbed intact and cleaved within the enterocyte by cytosolic peptidases. In mammals, as much as 90% of the total mucosal peptidase activity for dipeptides, 40% of activity for tripeptides, and 10% of the tetrapeptidase activity is associated with the cytosolic fraction (Sterchi and Woodley, 1980); therefore, the capability of cytosolic enzymes to hydrolyse oligopeptides with more than three amino acids appears to be limited. It should be noted, however, that the distribution of peptidase activity between the apical membrane and cytoplasm varies considerably with species and has been dependent on substrates used for the assay (Sterchi and Woodley, 1980). Oligopeptides of four to six amino acids in length are split to shorter peptides and free amino acids by apical microvillus membrane peptidases, whereas many di- and tripeptides are potential substrates for either apical membrane or cytosolic peptidases (Alpers, 1986). Therefore, membrane hydrolysis of peptides with subsequent absorption of amino acids, and transport of peptides followed by intracellular hydrolysis can occur. However, little is known about the importance of control of apical and cytosolic enzyme activity in relation to amino acid and peptide transport, or if location of specific peptidases along the longitudinal axis of the small intestine corresponds to specific amino acid and peptide transport proteins (Ugolev *et al.*, 1990).

Absorption of End Products of Protein Digestion

Theories about protein digestion and absorption, have evolved in the last 50 years. Initially it was thought that the vast majority of luminal proteins were completely hydrolysed to free amino acids before absorption from digesta (Cohnheim, 1901; Van Slyke and Meyer, 1912; Wiggans and Johnston, 1959). However, this classic hypothesis was challenged by an early report that at least dogs were capable of absorbing appreciable amounts of glycylglycine across the intestinal wall (Newey and Smyth, 1959), and subsequent reports that measured higher rates of absorption for amino acids when presented in the form of peptides than as free amino acids (Adibi and Morse, 1971; Matthews, 1983). From these and other studies (Matthews, 1991), it was understood that the absorption of protein in the form of small peptides was of tremendous nutritional importance. More specifically, most nutritional physiologists now accept the concept that about 70–85% of all luminal amino acids are absorbed from the digesta into enterocytes in the form of small peptides from the digesta, with the balance being absorbed as unbound (free) amino acids. After absorption into enterocytes, however, it is thought that about 85% of all absorbed amino acids appear in hepatic portal blood as free amino acids, as a result of intracellular hydrolysis (Matthews, 1991; Ganapathy *et al.*, 1994).

Net Portal-drained Visceral Flux of Peptides

Measurements of peptide and amino acid (and other nutrients) absorption and metabolism by tissues of the portal-drained viscera (PDV; gastrointestinal tract, pancreas, spleen and omental fat) can be obtained using chronic indwelling catheters in animals which allows for simultaneous sampling of arterial and venous blood draining the PDV, or sections of the PDV, and measuring blood flow through the same tissues (Reynolds, 2001). Net rates of peptide or amino acid release or removal by the PDV (or tissue of interest) can then be calculated as blood flow multiplied by venous–arterial concentration difference (Huntington *et al.*, 1989). Net portal appearance of peptide-bound or free amino acids is defined as the total amount of amino acids reaching portal blood minus the daily amounts entering the PDV via arterial blood. Peptides and amino acids liberated at the apical membrane or in the cytoplasm can be incorporated into proteins in the enterocyte, metabolized within the epithelium of the small intestine, or transported across the basolateral membrane, ultimately reaching the hepatic portal circulation. In addition, peptides and amino acids are mixed in the lumen and/or enterocyte with amino acids and peptides of endogenous origin during the digestion and absorption process. For these reasons the amino acids present in the hepatic portal vein do not completely reflect the amino acid pattern of the diet (Reynolds, 2001).

As discussed, it is now accepted that peptide absorption is an important physiological process in farm and other animals, and constitutes the primary source of absorbed amino acids. Peptide absorption across the PDV of ruminants has been reported by several researchers (McCormick and Webb, 1982; Seal and Parker, 1991; Koeln *et al.*, 1993; Han *et al.*, 2001), and has been shown to account for 63–92% of total amino acid flux by sheep and cattle. With net flux procedures, the origin of the peptides cannot be determined, but may be from absorption from the lumen, synthesis by tissues of the PDV, breakdown of endogenous protein by intracellular peptidases, or a combination of factors. At any rate, net flux of free amino acids appears to be low compared with peptide-bound amino acids in ruminants (Koeln *et al.*, 1993; Webb *et al.*, 1993; Han *et al.*, 2001). Based on evidence that chemical deproteinization overestimates the peptide amino acid concentration in plasma (Bernard and Rémond, 1996; Backwell *et al.*, 1997), it has been suggested that the high flux of peptides might be due to the procedure of sample deproteinization (Neutze *et al.*, 1996; Backwell *et al.*, 1997). Methods for measuring peptides have generally relied on the difference method, where amino acid analysis of deproteinized

samples before and after acid hydrolysis has occurred, with the difference being attributed to peptides. A modification of this procedure has included gel filtration after acid deproteinization of samples to ensure removal of residual protein before acid hydrolysis (Seal and Parker, 1996; Backwell *et al.*, 1997). However, Seal and Parker (1996) reported that, even after the treatment with both chemical deproteinization and physical filtration, peptide-bound amino acid flux still accounted for 63% of the net PDV appearance of total amino acids. The large appearance (438 g day^{-1}) of peptide-bound and free amino acids when calves were deprived of feed for 72 h (Koeln *et al.*, 1993) suggests that a large portion of small peptides might be the degradation products resulting from tissue protein in the gastrointestinal tract (GIT), spleen, pancreas or a combination of these organs, but also supports an increase in PepT1 mRNA and protein expression observed during dietary deprivation in rats (Thamotharan *et al.*, 1999a).

Interestingly, work of Webb *et al.* (1993) indicated that 86–90% of peptide-bound amino acids in the PDV of sheep and calves comes from the stomach tissues (rumen, reticulum, omasum, cranial abomasum, spleen and pancreas). Similar data from Seal and Parker (1996) suggested that 40% of portal peptide-bound amino acids were from the mesenteric-drained viscera (MDV; small intestine, caecum, colon, mesenteric fat and pancreas) suggesting that the remaining 60% were from the stomach tissues. Therefore, tissues of the stomach appear to account for the majority of peptide-bound amino acid release. Similarly, Han *et al.* (2001) found in yaks that 78% of the absorbed peptide-bound amino acids were from the stomach, whereas net flux of peptide-bound amino acids across the MDV accounted for 22% of the total peptide amino acids. In ruminants, the ability of the forestomach to absorb small peptides has been demonstrated *in vitro* (Matthews and Webb, 1995) and *in vivo* (Bernard and Rémond, 1999; see below). The lower absorption (10–40%) of peptide amino acids across tissues of the MDV supports the apparent high intracellular peptide hydrolysis observed in the cytoplasm of the small intestinal enterocyte (Matthews, 1991; Ganapathy *et al.*, 1994).

Han *et al.* (2001) found that the dominant amino acids appearing in the hepatic portal vein in the form of peptide were glutamate, aspartate, leucine, glycine, lysine, proline and serine. These peptide-bound amino acids accounted for 60% of the total peptide-bound amino acids appearing in hepatic portal blood. Han (1998) found similar results in Holstein steers and growing yaks, and similar results have been reported in calves (McCormick and Webb, 1982; Koeln *et al.*, 1993). Wallace *et al.* (1993) reported degradation-resistant peptides in rumen fluid of sheep 6 h after feeding. Interestingly, these peptides contained a significant proportion of aspartate, glycine and proline. In addition, several studies (Gardner and Wood, 1989; Daniel *et al.*, 1992; Pan *et al.*, 1996) have suggested that hydrophobic peptides and peptides resistant to mucosal hydrolysis are absorbed faster than hydrophilic and hydrolysis-susceptible peptides. As suggested by Han *et al.* (2001), resistance to mucosal hydrolysis might explain the larger fluxes of some peptide-bound amino acids. Also, the higher net absorption of glutamate, leucine, lysine, and serine in the form of peptides may imply that these amino acids exist more often in the form of peptides that are relatively hydrophobic and resistant to hydrolysis either by microbial peptidases in the rumen, or by apical-membrane and/or cytosolic peptidases of enterocytes.

Net Portal-drained Visceral Flux of Amino Acids

Early research in dogs (Elwyn *et al.*, 1968) showed an increase in free amino acids appearing in hepatic portal blood during protein digestion following a meal. During the absorptive period, amino acids appearing in portal blood were similar in composition to those ingested with the meal, except that glutamic and aspartic acids, which were removed by the PDV, and lysine and histidine appeared in greater amounts. Interestingly, net portal absorption of total amino acids was greater than the amount ingested, sug-

gesting complete hydrolysis of protein in the gut, and/or a contribution of amino acids from endogenous protein. In contrast, early comparisons of net PDV release of amino acids with amounts disappearing from the small intestine of ruminants showed that the amount of amino acids disappearing across the small intestine was 1.5–2 times greater than the amount appearing in hepatic portal plasma (Tagari and Bergman, 1978). These authors suggested that the gastrointestinal tract tissues selectively and preferentially used essential amino acids during absorption resulting in an imbalance in the profile of essential amino acids delivered to the liver.

More recent attempts in both sheep (MacRae *et al.*, 1997b) and cattle (Berthiaume *et al.*, 2001) have measured net flux of amino acids from the small intestine by placing a catheter at a point where the venous drainage from the small intestine first enters the mesenteric arcade, prior to the junction with the ileocaecal vein (Seal and Reynolds, 1993; MacRae *et al.*, 1997b). This approach has allowed researchers to measure net flux of amino acids across the small intestine, either from duodenum to ileum or from proximal jejunum to ileum (MacRae *et al.*, 1997b). Interestingly, in both sheep (MacRae *et al.*, 1997b) and cattle (Berthiaume *et al.*, 2001), net flux of essential amino acids across the PDV, when compared with the rates of disappearance of essential amino acids from the small intestine, confirmed the apparent loss of essential amino acids between the lumen of the small intestine and the hepatic portal vein. However, mean MDV net flux of essential amino acids averaged 106 and 103% of the small intestinal disappearance of essential amino acids in sheep and cattle, respectively. Berthiaume *et al.* (2001) reported that recovery of essential amino acids across the MDV of cattle was 102.8, 126.7, 102.2, 92.1, 76.5, 101.1, 100.0, 114.7 and 110.9% of intestinal disappearance for arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine, respectively. This indicates that across the MDV, a net balance of essential amino acids occurs such that inputs of essential amino acids for protein synthesis from both luminal and arterial sources are equivalent to outputs in mesenteric venous drainage from luminal amino acid transport and/or endogenous protein degradation (Reynolds, 2001). Interestingly, the ratio of PDV:MDV averaged 61% (range = 55–69%) in sheep and 62% (range = 38–76%) in cattle, indicating a substantial use of arterial amino acids by the stomach (reticulorumen, omasum, abomasum) and other tissues of the PDV (see Bequette, Chapter 5). MacRae *et al.* (1997b) hypothesized that low recovery of essential amino acids in PDV compared with MDV suggests that other tissues of the GIT not drained by the MDV (i.e. stomach and hindgut) do not exhibit a net balance between arterial sequestration and venous drainage from transport and/or protein turnover. In a study with the same sheep (MacRae *et al.*, 1997a), sequestration of arterial essential amino acids across the entire PDV accounted for 35–50% of the whole-body flux of essential amino acids. Therefore, the authors suggested the stomach and hindgut might sequester essential amino acids for tissue proteins from arterial sources at a greater rate than their degradation products are released into venous drainage as free amino acids. Alternatively, a greater portion of the products of stomach and hindgut protein degradation could be released as peptides as previously discussed.

The stomach (reticulorumen, omasum, abomasum) of the ruminant has the ability to absorb free amino acids. Calculations from Webb *et al.* (1993) indicated that 22 and 11% of total free amino acids appearing in portal blood of calves and sheep, respectively, were from stomach viscera. The potential for amino acid absorption across epithelial tissues of rumen and omasum have also been shown *in vitro* with radiolabelled and non-radiolabelled methionine (Matthews and Webb, 1995). Similarly, Rémond *et al.* (2000) demonstrated that free amino acids (glycine, serine, valine, methionine and lysine) can be absorbed across ruminal epithelium. However, the authors noted that absorption was low considering the small changes in net flux across the rumen wall relative to the large increases in ruminal concentrations.

In non-ruminants, experiments using radioisotopes have shown that digestion of most proteins in the small intestine is virtually

100% complete (Gaudichon *et al.*, 1996; Lien *et al.*, 1997). However, other studies suggest that less than 100% of ingested amino acids appear in hepatic portal blood following a meal (Rerat *et al.*, 1992; Stoll *et al.*, 1998; Wu, 1998; van Goudoever *et al.*, 2000). Results of portal recovery of essential amino acids ingested are similar to ruminants (56%); as a percentage of amino acid intake, 38, 57, 54, 60, 61, 48 and 69% of threonine, leucine, lysine, phenylalanine, valine, methionine and isoleucine were recovered in portal blood of young pigs (Stoll *et al.*, 1998). Similar results were reported by Rerat *et al.* (1992). Therefore, similar to the ruminant, there appears to be substantial utilization of dietary amino acids by tissues of the PDV during the absorptive process in the non-ruminant (Stoll *et al.*, 1998; Wu, 1998; van Goudoever *et al.*, 2000; and Bequette, Chapter 5). To our knowledge, rates of absorption across the MDV (i.e. small intestine) compared with the PDV have not been measured in pigs.

In dairy cattle (Berthiaume *et al.*, 2001), disappearance of total amino acids in the small intestine was 65.2%, which is in close agreement with values (57–76%) summarized by Reynolds *et al.* (1994) for ruminants in general. Essential amino acids disappearing in the small intestine were numerically higher than for non-essential amino acids (66.5 vs. 63.9%). Mesenteric-drained visceral flux accounted for 131% of intestinal disappearance of non-essential amino acids, and PDV flux represented 50% of MDV flux. In the case of non-essential amino acids, mean disappearance in the small intestine of aspartic acid, glutamic acid, cystine, proline and glycine was considerably higher than their respective MDV fluxes, while 116% of Ala and 123% of Ser that disappeared from the small intestine was recovered in the MDV. With the exception of serine, similar results were reported in piglets as a percentage of amino acid ingested (Stoll *et al.*, 1998). Reynolds (2001) suggested that the net loss of glutamate–glutamine and aspartate–asparagine pairs and other non-essential amino acids provide nitrogen for the synthesis of alanine using pyruvate arising from glycolysis (in part). In ruminants (Reynolds *et al.*, 1994; Reynolds, 2001) and non-ruminants (Rerat *et al.*, 1992; Prior and Gross, 1995), alanine is typically the amino acid released across the PDV in the largest amount of a net basis, transferring nitrogen and carbon to the liver for urea and glucose synthesis (Jungas *et al.*, 1992). The large release of alanine into the portal vein also reflects glutamate transamination and glutamine catabolism by the PDV, providing energy for gastrointestinal metabolism (Britton and Krehbiel, 1993; Reynolds *et al.*, 1994).

Peptide-bound and Free Amino Acid Transport Activities and Proteins

Paralleling tissue flux studies, which have described the relative flux and absorption potential of peptides and amino acids, has been research conducted to characterize specific biochemical activities (ion coupling, relative substrate affinities) and the molecular identity of specific gene products (proteins) capable of absorbing peptide-bound and free amino acids (transporters). The biochemical characterization of mammalian peptide-bound (peptide) or free (amino acid) amino acid transport systems has revealed the presence of at least two H^+-dependent and one H^+-independent peptide transport activities, one H^+-dependent amino acid transporter, and at least eight free amino acid systems in intestinal tissue (Fig. 3.2). The recent cloning and biochemical characterization of the cloned proteins responsible for these specific activities has revealed that, typically, several different proteins are capable of a biochemical 'system' activity, and that several Na^+-independent activities result from the function of two separate proteins. Although the data are limited compared with that for humans and laboratory animals, the intestinal epithelia of pigs, cattle and sheep appear to express a similar complement of transport activities and specific transport proteins as observed for other species. For all species, however, relatively little is known regarding how expression and function of individual transporters are coordinated with that of other transporters to account for the quantity and relative ratio of amino acids that are absorbed across the intestinal epithelium. Ultimately, to design

truly 'efficient' diets to support a desired level of animal production, knowledge regarding the intestinal luminal amino acid load must be matched with digestive and absorptive capacities for peptide-bound and free amino acids. The goal of this section is to identify and/or describe current information about peptide-bound and free amino acid transport.

Transport of Peptide-bound Amino Acids

Biochemical characterization of H^+-coupled peptide transport by enterocytes

As identified above, more moles of amino acids are absorbed as small di- and tripeptides (peptides) than as free amino acids. The importance of this process, along with the subsequent understanding that most β-lactam antibiotics appeared to compete for absorption with peptides, generated a tremendous amount of interest in identifying the process by which peptides were absorbed. Research using either hydrolysis-resistant peptides or poorly metabolized β-lactam antibiotics in whole-tissue, cell line and membrane vesicle experimental models demonstrated that many species are capable of mediated absorption of intact di- or tripeptides. An important understanding from early studies was that peptide transport activity is unique from free amino acid transport. Additionally, the transport of dipeptides is dependent on an extracellular–intracellular H^+ gradient and not a Na^+ gradient (Ganapathy *et al.*, 1984). Subsequent research showed that the pH dependence was specifically a H^+/peptide cotransport process (Daniel *et al.*, 1991, 1992). After coabsorption of the H^+ and peptide, the pH gradient is re-established by the Na^+/H^+ exchanger, which also resides in the brush border membrane. This counter transport is dependent on an extracellular–intracellular Na^+ gradient. The increased Na^+ concentration of the cell is re-established by the function of the basolateral membrane bound Na^+/K^+ ATPase. Because ATP is expended for two processes after absorption of the peptide, H^+/peptide-coupled transport is considered to be a 'tertiary' transport process. Research establishing the existence of an H^+ gradient across the 'microenvironment' (pH 5.5–6.3) of the apical membrane, and that H^+-coupled peptide transport is concentrative, has been well reviewed (Ganapathy *et al.*, 1994; Webb and Matthews, 1994). How these ion transporters function to support H^+-coupled peptide transport is illustrated in Fig. 3.1. The known biochemical parameters of endogenously and exogenously expressed PepT1 activities of farm animal species are summarized in Table 3.1. When compared to the functional activities of humans and laboratory animals (Table 3.2), it is clear that PepT1-like activity is well conserved among animals.

Table 3.1. Michaelis–Menten constants for low-affinity, H^+-dependent peptide transport activity of farm animal species.

Animal	Source tissue	Experimental model	Substrate	K_m (mM)	Source
Chicken	Intestine	PepT1 cDNA[a] expressed in oocytes	Gly-Sar[b]	0.47	Pan *et al.* (2001a)
Pig	Jejunum	Ussing-chamber	Gly-Gln	9.23 ± 0.14	
			Gly-Sar	11.62 ± 3.32	Winckler *et al.* (1999)
Sheep	Omasum	Poly(A)+ mRNA expressed in oocytes	Gly-Sar	0.4	Matthews *et al.* (1996)
		PepT1 cDNA expressed in oocytes	Gly-Sar	1.0 ± 0.01	Chen *et al.* (2002)
Cattle	Jejunum	BBMV[c]	Gly-Sar	1.28	Wolffram *et al.* (1998)
	Ileum	BBMV	Gly-Sar	0.93	

[a]Complementary DNA.
[b]Glycylsarcosine.
[c]BBMV, brush border membrane vesicles.

Table 3.2. Michaelis–Menten constants for low-affinity, H^+-dependent peptide transport activity of gastrointestinal tissues and cell lines.

Animal	Source tissue	Experimental model	Substrate	K_m (mM) K_i (mM)[a]	Source
Rat	Small intestine	BBMV[b]	Gly-Sar	2.2 ± 0.2	Tanaka *et al.* (1998)
Mouse	Intestine	Everted sleeves	Carnosine	12.6 ± 1.6	Ferraris *et al.* (1988)
Human	Small intestine	BBMV	Penicillin-G	13.9	Poschet *et al.* (1999)
		BBMV	Gly-Pro	4.1	Adibi (1997a)
			Gly-Gly-Pro	3.42	
	Jejunum	BBMV	Gly-Gln	0.64	
	Colon:	Apical membrane	Bestatin	0.34	Saito and Inui (1993)
	Caco-2 cells	Basolateral membrane	Bestatin	0.71	
		Apical membrane	Gly-Sar	1.1 ± 0.1	Brandsch *et al.* (1994)
		Apical membrane	Gly-Sar	3.9 ± 0.2	Walker *et al.* (1998)
		Apical membrane	Cefadroxil	0.98	Terada *et al.* (1999)
		Basolateral membrane	Cefadroxil	3.29	
		Apical membrane	Gly-Sar	0.65	
		Basolateral membrane	Gly-Sar	2.1	
Rabbit	Jejunum	Villus tip cells	Cephradine	3.6	Tomita *et al.* (1995)
		BLMV[c]	Gly-Pro	2 ± 0.2	Dyer *et al.* (1990)
	Small intestine	BBMV	Gly-Sar	17.3 ± 1.4	Ganapathy *et al.* (1984)
			Gly-Sar	19.5 ± 2	
		BBMV	Penicillin-G	21.7	Poschet *et al.* (1996)
Hamster	Jejunum	Everted rings	Val-Val	9.6	Matthews (1983)
			Gly-Sar	6.1	
			Leu-Leu	5.6	
			Gly-Gly	5.2	
			Ala-Ala	3.2	
Tilapia (fish)	Intestine	BBMV	Gly-Phe	9.8 ± 3.5	Reshkin and Ahearn (1991)
Eel	Intestine	BBMV	Gly-Gly	1.81 ± 0.49	Verri *et al.* (2000)
			Gly-Sar	1.75 ± 0.47	

[a] K_i for cefadroxil inhibition of Gly-Sar transport.
[b] BBMV, brush border membrane vesicles.
[c] BLMV, basolateral membrane vesicles.

In contrast to apical transport of peptides, convincing biochemical evidence for the mediated transfer of intact peptides across the basolateral membrane of these epithelia was not provided until recently. The transport of Gly-Pro across jejunal basolateral membranes isolated from rabbits was reported to be saturable, stimulated by an H^+ gradient, and competitively inhibited by other glycyl-containing peptides (Dyer *et al.*, 1996). These results led researchers to believe that the peptide transport systems expressed in the brush border membrane and in the basolateral membrane are very similar or identical. However, subsequent research using polarized Caco-2 cells and peptide and β-lactam antibiotic substrates (Table 3.2), demonstrated that the affinities of apical and basolateral membrane-localized peptide transport activities differ (Saito and Inui, 1993). Most recently, transport of Gly-Sar across the basolateral membrane of Caco-2 cells was shown to be less sensitive to changes in external pH, as compared to the apically located system (Terada *et al.*, 1999). In addition, the relative ability of cefadroxil to competitively inhibit Gly-Sar uptake was less for basolateral than apical transport, and basolateral transport was unable to concentrate intracellular Gly-Sar, in contrast to the apical transport. Therefore, it

is likely that basolateral peptide transport is mediated by a single low-affinity, H^+-independent, facilitated transport system. That peptide transport systems possess functional differences, depending on which membrane they are localized in (apical vs. basolateral) is analogous to differences in the location and function of many mammalian amino acid transport systems (Kilberg and Haussinger, 1992; see below). How the differential membrane localization of these transporters contributes to peptide-bound (and free) amino acid absorption across enterocytes is illustrated in Fig. 3.1.

In terms of the energetic cost associated with H^+-coupled peptide uptake, data from initial biochemical studies were mixed, indicating that either 1 or 2 H^+ were co-transported per peptide molecule (Ganapathy and Leibach, 1985; Hoshi, 1985). Initial studies that characterized the biochemical activity of a protein capable of H^+-coupled peptide uptake (PepT1; see below) after overexpression by *Xenopus laevis* oocytes (Fei *et al.*, 1994) determined that the H^+:peptide ratio for the neutral peptide Gly-Sar is 1:1. More extensive research with additional PepT1 substrates, however, has revealed that the number of H^+ required for peptide transport across the apical membrane of enterocytes depends on the charge of the peptide. For example, rabbit PepT1 displays H^+:peptide ratios of 1:1, 2:1 and 1:1 for neutral, acidic and basic dipeptides, respectively (Steel *et al.*, 1997). Whether acidic peptides are relatively less well recognized in the presence of a lower pH than are neutral or basic dipeptides, has not been definitively established, as evidenced by the contradictory data from whole tissue (Lister *et al.*, 1997) versus *in vitro* (Brandsch *et al.*, 1997) studies.

Molecular characteristics and tissue distribution of PepT1

As shown in Tables 3.1 and 3.2, expression of H^+/peptide co-transport activity by the gastrointestinal epithelia of a variety of animal species has been demonstrated, including that by the chicken, pig, sheep and cow. In addition, there is strong evidence that forestomach epithelia of ruminants are also capable of H^+-mediated peptide absorption (Matthews, 2000a). With the seminal cloning of PepT1 protein (Fei *et al.*, 1994), the mechanism responsible for H^+-coupled absorption of intact small peptides (predominantly di- and tripeptides) from the digesta of the intestinal lumen by enterocytes was identified. In mammals, PepT1 mRNA encodes an integral membrane protein that is predicted to possess 12 membrane-spanning domains, with a relatively large extracellular loop between transmembrane domains 9 and 10 (Leibach and Ganapathy, 1996). Chicken PepT1 mRNA, however, is significantly smaller than the mammalian PepT1 mRNA (1.9 kb versus about 3 kb; Chen *et al.*, 1999) and is predicted to encode a protein that lacks the large extracellular loop present in mammalian PepT1 (Pan *et al.*, 2001a).

The distribution of PepT1 mRNA along the small intestine also differs among species. The expression of PepT1 for a rabbit is most abundant in the duodenum and jejunum, lower in ileum, and very low in the colon (Freeman *et al.*, 1995). In contrast, PepT1 expression in rats is most abundant in the ileum as compared to the duodenum and jejunum (Miyamoto *et al.*, 1996). In ruminants, PepT1 is expressed by small intestinal epithelia, and by omasal and ruminal epithelium (Chen *et al.*, 1999; Pan *et al.*, 2001b). These differences in expression of PepT1 reflect the site of protein digestion and the availability of substrates for a given species and confirm previously identified biochemical activities. The molecular characteristics of known farm animal PepT1 mRNA orthologues, and their tissue-specific expression, are summarized in Table 3.3.

Regulation of PepT1 expression and activity

How PepT1 activity is regulated is of immense interest to nutritionists and pharmacologists, as this single protein activity accounts for much of total amino acid and β-lactam antibiotic absorption. Consequently, a variety of experimental regimens have been used to investigate how the expression of

Table 3.3. Molecular characteristics and tissue distribution of PepT1 mRNA expressed by farm animal species[a].

Animal	Genbank accession no.	Tissues screened[a]	Transcript size (kb)	Source
Chicken	AY029615	+ du – cr, pr, gi, ce, li, sm	1.9	Chen *et al.* (1999)
Pig		+ je	2.9	Winckler *et al.* (1999)
Sheep	AY027496	+ om, ru, du, je, il – li, ki, stm, lm, ce, co	2.8	Pan *et al.* (2001b)
Cattle		+ om, ru, du, je, il – ab, ce, co, li, sm, lm	2.8	Chen *et al.* (1999)

[a]ab, abomasum; br, brain; ce, caecum; co, colon; cr, crop; du, duodenum; gi, gizzard; il, ileum; je, jejunum; li, liver; lm, longissimus muscle; om, omasum; pr, proventriculus; ru, rumen; sm, skeletal muscle; stm, semitendinous muscle.

various species orthologues of PepT1 is regulated. As selectively collated in Table 3.4, the sensitivity of PepT1 mRNA and protein expression and/or functional activity to nutritional, ontogenic, disease and various metabolic affectors has been evaluated. In terms of dietary regulation of PepT1, increasing protein and casein content of the diet stimulates PepT1 expression and/or activity (Ferraris *et al.*, 1988; Erickson *et al.* 1995; Shiraga *et al.*, 1999). In terms of specific substrate regulation, either specific or mixtures of peptides appear capable of stimulating PepT1 activity (Ferraris *et al.*, 1988; Brandsch *et al.*, 1995; Adibi, 1997b) and expression (Shiraga *et al.*, 1999; Ogihara *et al.*, 1999), but not, generally, amino acids.

In an apparent paradox, dietary deprivation also stimulates PepT1 expression and activity. Specifically, the effect of a one day fast on the uptake of Gly-Gln by rat jejunal brush border membrane vesicles was to double H^+-dependent Gly-Gln uptake (Thamotharan *et al.*, 1999a). Concomitant with this increased activity was a threefold increase in the amount of PepT1 mRNA and protein content of the cells. The finding that starvation appears to stimulate was supported by the observation that starvation of rats for 4 days also results in increased levels of PepT1 mRNA by intestinal epithelia (Ogihara *et al.*, 1999; Ihara *et al.*, 2000). Consistent with the robust stimulation of PepT1 activity by nutritional surfeit or deficit, 5-fluorouracil-induced (a chemotherapeutic agent) injury to the intestinal epithelium of rats results in the preservation and production of PepT1 mRNA, relative to depressed mRNA levels observed for sugar (SGLT1) and amino acid (NBAT) transporters (Tanaka *et al.*, 1998). In keeping, PepT1 protein levels were unaffected, whereas sucrase and Na^+-dependent glucose SGLT1 activities were decreased. Collectively, the nutritional and disease status data suggest that the collective effect of nutritional challenge is to increase PepT1 uptake capacity.

With regard to identifying specific mechanism(s) responsible for PepT1 regulation, the culture of Caco-2 cells in the presence of pentazocine (a selective σ_1 receptor ligand) resulted in an increase in PepT1 mRNA and maximum velocity of Gly-Sar transport (Fujita *et al.*, 1999). In contrast, insulin seems to stimulate PepT1 activity in an acute, post-translational manner (Thamotharan *et al.*, 1999b). Specifically, culture of Caco-2 cells in physiological levels of insulin (5 nM) for more than 1 h apparently results from the insertion of pre-existing PepT1 from a cytoplasmic pool, and not from *de novo* synthesis. In keeping with these reports, PepT1 uptake capacity may be regulated by protein kinase C, as the blocking of calmodulin-regulated enzyme cascade depresses endogenously expressed canine (Brandsch *et al.*, 1995) and exogenously expressed ovine (Chen *et al.*, 2002) PepT1 activity. Whether this regulation occurs from a direct phosphorylation-dependent activation of PepT1, or through stimulation of trafficking pathways of cytoplasmic pools of PepT1 remains to be determined.

Table 3.4. Regulation of peptide transport activity in animal tissues and cell lines.

Animal	Tissue source	Model[a]	Experimental treatment	Activity			mRNA	Protein	Source
				Substrate	K_t (mM)	V_{max}			
Rat	Jejunum	NS	Control				100± 29[b]		Ihara *et al.* (2000)
			Starved (4 days)				161± 32		
			Semistarved (10 days)				164± 32		
			Parenteral nutrition (10 days)				179± 35		
	Small Intestine	NS	Low protein (7 days)				1		
			High protein (14 days)				1.5–2× ↑[c]		Erickson *et al.* (1995)
	Jejunum, BBMV	NS	Control	Gly-Gln	19.9± 1.6		1	1	Thamotharan *et al.* (1999a)
			Fasting (1 day)	Gly-Gln	41.4± 2.7		3× ↑[d]	3× ↑[e]	
	Small Intestine	NS	Fasting (4 days)					2× ↑	Ogihara *et al.* (1999)
			Oral AA mixture (4 days)					1.6× ↓	
	Jejunum	ON	4 days old				1		Miyamoto *et al.* (1996)
			28 days old				3.6× ↑		
	Ileum, BBMV	SR		Gly-Sar	2.1± 0.4	2.8± 0.3			Shiraga *et al.* (1999)
			Protein free (4 days)				1	1	
			5% casein (4 days)	Gly-Sa	2.0± 0.2	2.7± 0.2	0.9± 0.1	1.2± 0.3	
			20% casein (4 days)	Gly-Sar	3.6± 0.3*	2.7± 0.2	1.8± 0.3*	2.0± 0.3*	
			50% casein (4 days)	Gly-Sar	4.6± 0.2*	2.5± 0.2	2.0± 0.3*	2.2± 0.4*	
			10% Gly (4 days)	Gly-Sar	2.2± 0.3	2.6± 0.3	0.9± 0.1	0.9± 0.1	
			10% Phe (4 days)	Gly-Sar	4.7± 0.3*	2.6± 0.2	2.3± 0.2*	2.4± 0.5*	
			20% Gly-Phe (4 days)	Gly-Sar	5.5± 0.4*	2.4± 0.5	2.4± 0.3*	2.6± 0.3*	
	Jejunum, BBMV	DS	Control	Gly-Sar	3.6± 0.2	2.2± 0.2	2.3× ↑		Tanaka *et al.* (1998)
			5-Fluorouracil[f] (3 days)	Gly-Sar	3.2± 0.2	3.4± 0.3			
	Colon: Caco-2	SR	Control	Cefadroxil	0.19				Adibi *et al.* (1997b)
			GlySar 10 mM (1 day)	Cefadroxil	0.35				
	Colon: Caco-2	HS	Control	Gly-Sar	10.3± 0.7	0.51± 0.1			Fujita *et al.* (1999)
			(+) Pentazocine[g] 1 μM (1 day)	Gly-Sar	20.9± 1.1	0.69± 0.1			
	Colon: Caco-2	HS	Control	Gly-Gln	3.53± 0.61				Thamotharan *et al.* (1999b)
			Insulin 5 nM (1 h)	Gly-Gln	6.31± 0.5				
	Colon: Caco-2	PM	Control	Gly-Sar	13.8± 0.6	0.83± 0.1			Brandsch *et al.* (1997)
			DEP 0.4 mM (10 min)	Gly-Sar	6.4± 0.3	0.76± 0.1			

Continued

Table 3.4. *Continued.*

Animal	Tissue source	Model[a]	Experimental treatment	Activity			mRNA	Protein	Source
				Substrate	K_t (mM)	V_{max}			
Canine	Kidney: MDCK	SM	Control	Gly-Sar	132.9± 17.5				Brandsch *et al.* (1995)
			W-7[i] 50 μM (16 h)	Gly-Sar	67.6± 4.2				
			CGS-9343B[j] 30 μM (16 h)	Gly-Sar	79.8± 8				
			Calmidazolium[k] 10 μM (16 h)	Gly-Sar	88.5± 8				
		SR	DMEM[l] (4 days)	Gly-Sar	29.5± 2.98				
			LHM[m] (4 days)	Gly-Sar	114.5± 8.37				
Mouse	Small Intestine	SR	Low protein (18% casein)	Carnosine	5.5± 0.5				Ferraris *et al.* (1988)
			High protein (72% casein)	Carnosine	7.9± 0.6				
			54% casein	Carnosine	5.0± 0.6				
			54% casein hydrolysate[h]	Carnosine	6.6± 0.9				
			50% amino acids	Carnosine	4.9± 0.4				
Swine	Kidney: LLC-PK_1	SM	Control	D-Phe-L-Ala	92.8± 8.1				Wenzel *et al.* (1999)
			EGTA-AM°/Straurospine (3 h)	D-Phe-L-Ala	157.7± 6.3				
			Control	D-Phe-L-Ala	90.3± 1.4				

↑, ↓ denotes increase or decrease, respectively; × denotes multiples (or 'times'); *Differs ($P < 0.05$) from protein-free treatment.

[a]DS, disease status; HS, hormonal status; NS, nutritional status; ON, ontogenic effect; PM, protein modification; SM, second messenger effect; SR, substrate regulation.

[b]Percentages relative to control value.

[c]Middle and distal intestine.

[d]Intestinal mucosa.

[e]Brush border membrane.

[f]Diethylpyrocarbonate which modifies histidyl residues and blocks function (Brandsch *et al.*,1997).

[g]Anticancer drug that is toxic to cell growth.

[h]A selective σ_1 receptor ligand (Fujita *et al.*, 1999).

[i]W-7, *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulphonamide; a calmodulin inhibitor.

[j]CGS-9343B, zaldaride maleate; a calmodulin inhibitor with no effect on protein kinase C.

[k]Inhibitor of calmodulin-regulated enzymes.

[l]DMEM, Dulbecco's modified Eagle's medium.

[m]LHM, lactalbumin hydrolysate medium.

[n]Casein hydrolysate, consists of 50% free amino acids and 50% peptides.

[o]EGTA-AM, ethylene-glycol-bis-(β-aminoethyl)-*N, N, N′, N′*-tetraacetoxymethyl ester.

[p]dbcAMP, dibutyryl cyclic AMP.

Overall, despite the above-described understandings, it is still not known whether PepT1 expression is regulated by specific nutrient–gene interactions, intracellular signalling in response to cellular metabolic status, mechanical functioning of the transporter, and/or by hormones. What is clear, however, is that the need to maintain PepT1 functional capacity must be strong and of especial importance to absorptive epithelial function given that most metabolic challenges result in an increase in PepT1 activity. This conclusion is consistent with the understanding that the predominant form of intestinal protein assimilation is that of absorption as small peptides.

Mediated Absorption of Free Amino Acids

Molecular and functional properties of proteins capable of biochemically defined free amino acid transport 'system' activities

As indicated in Fig. 3.1, the mediated absorption of free amino acids across both apical and basolateral membranes is critical to the assimilation of luminal proteins. Remarkably, within the last 12 years, cDNA have been generated that encode proteins for six anionic, four cationic, 11 neutral, and five neutral and cationic free amino acid transporters. Except for exclusive proline and hydroxyproline transport by the IMINO system, at least one of these 26 proteins account for each of the major amino acid transport activities expressed by non-embryonic tissues, which have been biochemically defined over the previous 35 years. To facilitate a working knowledge base for understanding which proteins perform which transport activities, the biochemical and molecular properties of free amino acid transport systems and proteins have been collated in Table 3.5. An important understanding from this research is that the activities of seven biochemically defined transport systems are actually performed by more than one amino acid transporter. In addition, a subset of transporters actually function as heterodimer units, in conjunction with one of two glycoproteins (4F2hc, rBAT). These glycoprotein-associated amino acid transporter family members (Verrey *et al.*, 2000) account for all known Na^+-independent exchange transport activities, and system y^+L, which functions dually as a Na^+-independent exchanger of cationic amino acids and Na^+-dependent exchanger of neutral amino acids. A third concept is that about half of the cloned free amino acid transporters are not capable of net molar flux of amino acids across membranes. Instead, these 'exchangers' selectively import certain amino acids in exchange for export of selected other amino acids.

Free amino acid transporters expressed by intestinal epithelia

To facilitate an abbreviated discussion of which and how these transporters function to support absorption of amino acids across the gastrointestinal epithelium, the site of expression for the functional activities, mRNA, and protein (when reported) of free amino acid transporters expressed by gastrointestinal epithelia are collated in Table 3.6. Although few of the transporter proteins have actually been detected in apical or basolateral membranes, the matching of membrane-defined transport activity with detection of mRNA expression (Table 3.5) suggests that at least one molecularly defined transporter has been identified for all biochemically defined transport activities reported for apical and basolateral membranes. In terms of comparing specific substrates with the reported function of cloned transporters (Table 3.5) specific transporters are identified for apical membrane absorption of anionic L-glutamate, L-aspartate, D-aspartate by EAAT2 and/or EAAT3; cationic L-lysine, L-arginine, L-histidine and L-ornithine by CAT1 and L-arginine, L-lysine and L-histidine by $b^{0,+}$AT; and neutral amino acids by ASCT2 and $b^{0,+}$AT. Interestingly, 4F2-lc6 also encodes a protein capable of $b^{0,+}$ activity, but which associates with 4F2-hc, not RBAT. As with $b^{0,+}$AT, however, 4F2-lc6 transports cystine and neutral and cationic amino acids in a Na^+-independent manner. In terms of both cationic and neutral amino acid transport, system $B^{0,+}$ activity has long been identified with the apical membrane of intestinal epithelia. However, the

Table 3.5. Molecular and biochemical properties of free α-amino acid transport proteins[a].

CLONE	Alternate names	Genbank Acc. no.	Length	Transport system	Substrate specificity[bc]	Substrate affinity[d]	Co-substrate coupling	Source
Anionic								
EAAT1	GluT, GLAST	X63744	543	X^-_{AG}	D-,L-Asp, L-Glu	μM	Na^+_{in}, K^+_{out}, OH^-/HCO^-_{3out}	Storck *et al.* (1992)
EAAT2	GLT, GLAST2, GLTR	X67857	573	X^-_{AG}	D-,L-Asp, L-Glu	μM	Na^+_{in}, K^+_{out}, $OH^-/HCO_3^-{}_{out}$	Pines *et al.* (1992)
EAAT3	EAAC1	L12411	523–525	X^-_{AG}	D-,L-Asp, L-Glu	μM	Na^+_{in}, K^+_{out}, $OH^-/HCO_3^-{}_{out}$	Kanai and Hediger (1992)
EAAT4	——	U18244	564	X^-_{AG}	D-,L-Asp, L-Glu	μM	Na^+_{in}, K^+_{out}, $OH^-/HCO_3^-{}_{out}$	Fairman *et al.* (1995)
EAAT5	——	U76362	561	X^-_{AG}	D-,L-Asp, L-Glu	μM	Na^+_{in}, K^+_{out}, $OH^-/HCO_3^-{}_{out}$	Arriza *et al.* (1997)
xCT[ef]	4F2-lc4	AB022345 AB026891	502	X_c^-	CssC, L-Glu; L-Asp	μM	$AA1_{CssC}$;$AA2_{Glu}$	Sato *et al.* (1999)
		AF252872	501					Bridges *et al.* (2001)
Cationic								
CAT1	ecoR	M26687	622–629	y^+	Lys, Arg; Orn, His (when charged)	μM	none	Kim *et al.* (1991) Wang *et al.* (1991)
CAT2	CAT2β	NM003046	657–658	y^+	Lys, Arg;	μM	none	Closs *et al.* (1993a)
CAT2a	CAT2α	L03290	657–659	y^+	Lys, Arg; Orn	mM	none	Closs *et al.* (1993b)
CAT3	——	U70859	619	y^+	Arg	μM	none	Hosokawa *et al.* (1997)
Neutral								
GlnT (neuronal A)	ATA1	AF075704	485	A	Gln, Asn, His, Ala, Met, Ser, Gly; MeAIB, Pro	μM	Na^+_{in}	Varoqui *et al.* (2000)
ATA2		AF249673	504	A	MeAIB, Ala, Gly,	μM	Na^+_{in}	Sugawara *et al.* (2000a)
(classic A)	SA1	AF273024			Ser, Pro, Met, His,			Reimer *et al.* (2000)
	SAT2	AF173682			Asn, Gln			Yao *et al.* (2000)
ATA3 (hepatic A)		AF295535	547	A	Ala, Gly, Ser, Cys, Asn, Thr; Pro, Met, Gln, His; MeAIB, Lys	mM	Na^+_{in}	Hatanaka *et al.* (2000)
					Lys, Arg	μM	none	Sugawara *et al.* (2000b)

ASCT1	SATT	L14595	532	ASC	neutral, except for Gln at pH 7.5; plus anionic at pH 5.5	μM	$Na^+_{in}AA_{in,}$ $Na^+_{in}AA_{out}$	Arriza *et al.* (1993) Zerangue and Kavanaugh (1996)
ASCT2	ATB°	D-85044 U53347	553	B°	L-AA: Ala, Gln, Ser, Cys, Thr, Trp, Gln, Asn, Leu; Met, Val, Ile, Phe; Trp, Gly D-AA: Ser, Thr, Cys	μM	Na^+_{in}	Utsunomiya-Tate *et al.* (1996) Kekuda *et al.* (1996)
Asc-1[ef]		AB026688	530	asc	L-AA: Ala, Gly, Ser, Thr, Cys; Val, Met, Ile, Leu, His D-AA: Ala, Ser, β-Ala, AIB, Ala-methyl;Cys, Asn, Leu, Ile, Val, His; Gln, Met, Phe	μM	$AA1_{in}$;$AA2_{out}$	Fukasawa *et al.* (2000)
LAT1[ef]	4F2-lc1	AB015432 AF104032	512 507	L	L-AA: Leu, Ile, Phe, Met, Tyr His, Trp; Val D-AA: Leu, Phe, Met; Ile	low μM	$AA1_{in}$;$AA2_{out}$	Kanai *et al.* (1998) Prasad *et al.* (1999)
LAT2[ef]	4F2-lc5	AF171668	531	L	Phe; Leu; Ala; Gln, His	μM	$AA1_{in}$;$AA2_{out}$	Rossier *et al.* (1999)
		AF171669	535					
		AF170106	535		Thr, Phe, Trp; Ser, Gln, Leu, Ala, Cys, BCH	μM		Rajan *et al.* (2000)
SN1		NM006841	504	N	Gln, His	mM	$Na^+_{in}AA_{out}$,,	Chaudhry *et al.* (1999)
	NAT	AF159856	505		Gln, His; Ala	mM		Gu *et al.* (2000)
		AF244548	504		Gln, His; Asn, Ala	mM	$2Na^+_{in}AA_{out}$,, Li^+ or Na^+_{in}	Fei *et al.* (2000)
SN2		AF276870	472	N	His; Asn, Ser, Gln; Ala, Gly	mM	Li^+ or Na^+_{in}, H^+_{out},	Nakanishi *et al.* (2001)

Continued

Table 3.5. *Continued.*

CLONE	Alternate names	Genbank Acc. no.	Length	Transport system	Substrate specificity[bc]	Substrate affinity[d]	Co-substrate coupling	Source
TAT1		AB047324	514	T	L-AA: Tyr, Trp, Phe, L-Dopa, 3–0-methyl-Dopa D-AA: Trp, Phe	mM	none	Kim *et al.* (2001)
Neutral and cationic								
ATB^{0+}		AF151978	642	$B^{0,+}$	Ile, Leu, Trp, Met, Val, Ser; His, Tyr, Ala, Lys, Arg, Cys, Gly; Asn, Thr, Gln; Pro	NR	$2Na^+_{in}$, $2Cl^-_{in}$	Sloan and Mager (1999)
y^+LAT1[ef]	AmAT-L-lc	AF092032 AJ130718	511	y^+L	Leu; Arg, Lys, Gln, His	μM	$AA1_{in}$;$AA2_{out}$ Na^+_{in} or H^+	Torrents *et al.* (1998) Pfeiffer *et al.* (1999a)
	4F2-lc2	R82979			Lys, Arg, Orn; Met, Leu, His		(for neutral)	Kanai *et al.* (2000)
y^+LAT2[ef]	4F2-lc3	D87432	515	y^+L	Arg, Leu Arg, Lys, Gln, His, Met;	μM	$AA1_{in}$;Arg_{out} Na^+_{in} (for neutral)	Torrents *et al.* (1998) Broer *et al.* (2000)
BAT1[eg]	$b^{o,+}AT$	AB029559 AJ249198 Aj249199	487	$b^{o,+}$	Arg, Leu, Lys, Phe, Tyr; CssC, Ile, Val, Trp, His, Ala; Met, Gln, Asn, Thr, Cys, Ser	μM	neutral AA, dibasic AA exchange	Chairoungdua *et al.* (1999) Pfeiffer *et al.* (1999b)
4F2-lc6[ef]		AF155119	487	$b^{o,+}$	Leu, Trp, Phe, Met, Ala, Ser, Cys, Thr, Gln, Asn; Gly, CssC, BCH	μM	neutral AA, dibasic AA exchange	Rajan *et al.* (1999)

[a]Does not include members of the BGT, GAT, GLYT, TAUT or PRO neurotransmitter transporter families.
[b]';' denotes physiologically significant differences in degree of substrate affinity.
[c]CssC, L-cystine; Orn, ornithine; MeAIB, 2-methylaminoisobutyrate; AIB, α-aminoisobutyric acid; BCH, 2-aminobiyclo(2,2,1)heptane-2-carboxylic acid; Dopa, L-dihydroxyphenylalanine.
[d]When possible, values are data from overexpression of cDNA by mammalian cells, rather than by *Xenopus* oocytes.
[e]Member of the glycoprotein-associated amino acid transporter family.
[f]Associates with 4F2hc glycoprotein.
[g]Associates with rBAT glycoprotein.

Table 3.6. Expression of free α-amino acid transporter activities, mRNA and/or protein by mammalian gastrointestinal epithelia.

Transport system	Location of activity[a]	Specific transporter	Epithelia[bc] evaluated	mRNA	Protein[d]	Source
Anionic						
X^-_{AG}	Ap	EAAT2	R, O, D, J, I, Ce, Co	X	CMV	Howell *et al.* (2001)
		EAAT3	R, O, D, J, I, Ce, Co	X	CMV	Howell *et al.* (2001)
x_c^-	NR	xCT	intestine	X		Bassi *et al.* (2001)
Cationic						
y^+	Ap, Bl	CAT1	Small intestine	X		Kim *et al.* (1991); Wang *et al.* (1991)
Neutral						
A	Bl	ATA2	Small intestine	X		Sugawara *et al.* (2000a)
B°	Ap	ASCT2	Intestine	X		Kekuda *et al.* (1996, 1997)
asc	Bl	Asc-1	Small intestine	X		Fukasawa *et al.* (2000)
L	Bl	LAT2	Small intestine	X	Bl	Rossier *et al.* (1999)
N	Ap or Bl	SN2	Small intestine	X		Nakanishi *et al.* (2001)
T	Bl	TAT1	J, I, Co	X	Bl	Kim *et al.* (2001)
IMINO	Ap	Unknown				
Neutral and cationic						
$B^{o,+}$	Ap	$ATB^{o,+}$	Distal I, Ce, Co	X		Hatanaka *et al.* (2001)
y^+L	Bl	y^+LAT1	Small intestine	X		Torrents *et al.* (1998); Pfeiffer *et al.* (1999a)
	Bl	y^+LAT2	Small intestine	X		Broer *et al.* (2000)
$b^{o,+}$	Ap	BAT1	J, I	X		Chairoungdua *et al.* (1999)
	Ap	$b^{o,+}AT$	Intestine	X		Pfeiffer *et al.* (1999b)
	Ap	4F2-lc6	Small intestine	X		Rajan *et al.* (1999)

[a]As reviewed by Ganapathy *et al.* (1994), Mailliard *et al.* (1995), Palacin *et al.* (1998), Wagner *et al.* (2001), Bode (2001) and/or Matthews and Anderson (2002). Ap, apical; Bl, basolateral membrane; NR, not reported.
[b]R, rumen; O, omasum; D, duodenum; J, jejunum; I, ileum; Co, colon; Ce, caecum.
[c]When known, expression is reported for farm animal species.
[d]CMV, crude membrane vesicles isolated from homogenates of scraped epithelial tissues.

limited mRNA tissue distribution profiles for $ATB^{0,+}$, which encodes a protein capable of $B^{0,+}$ activity, shows no expression by the duodenum or jejunum and only weak expression by the distal ileum. In contrast, caecal and colonic expression is high. Therefore, it remains to be determined whether $ATB^{0,+}$ function contributes significantly to small intestinal absorption of amino acids, or whether another, as yet unidentified, $ATB^{0,+}$ isoform is responsible for system $B^{0,+}$ activity.

In terms of basolateral transport capacity, cationic amino acids are unilaterally transported by CAT1 uniport in counterexchange for neutral amino acids by y^+LAT1. In addition, given its intracellular binding preference for L-arginine and the high blood concentrations of L-glutamine, the predominant function of y^+LAT2 is to absorb L-glutamine into enterocytes in exchange for L-arginine. Accordingly, the function of y^+LAT2 may well be the mechanical coupling of the high intestinal L-glutamine uptake from, and L-arginine export into, splanchnic blood (Wu, 1998). In contrast, the presence of a basolateral anionic amino acid transporter has yet to be described (although xCT mRNA has been detected by RT-PCR, Bassi *et al.*, 2001), and may help explain the low arterial uptake of L-glutamate and L-aspartate by small intestinal epithelia. Neutral amino acid transport across the basolateral membrane of enterocytes appears to be achieved by a combination of activities by Na^+-dependent (ATA2) and Na^+-independent (TAT1) uniporters and ion-independent amino acid exchangers (Asc-1, LAT2, y^+LAT1, y^+LAT2). In addition, SN2 may contribute significantly to L-histidine, L-serine, L-asparagine and L-glutamine absorption by coupled Na^+/H^+ counter-exchange (Bode, 2001).

Asymmetrical expression, yet coordinated function, of amino acid transporters by polarized intestinal epithelia

In terms of how amino acid flux is mediated across enterocytes, all of the apical transporters are ion-dependent and capable of concentrative transport, except for the two system $b^{0,+}$ transporters. Consequently, the molar ratio of cationic and neutral amino acids initially absorbed from the lumen by concentrative transporters can be modulated by $ATB^{0,+}$ activity. Of the basolateral transporters, only ATA2 and TAT1 are uniporters. ATA2 (system A activity) activity is Na^+-dependent, capable of concentrative transport, and functions to transport amino acids into the cell, not into the blood. Conversely, TAT1 is a Na^+-independent system that selectively transports aromatic amino acids, down their concentration gradients. In contrast the other basolateral transporters are all exchangers. As a consequence of this differential expression of apical and basolateral transporters, it is likely that the bulk of amino acids that enter the blood through enterocytes is dependent on the concentration of amino acids in the cytosol of enterocytes.

A pertinent question that arises from the combined understandings gained from localization and functional studies with intestinal amino acid transporters is the degree to which the functions of apical (including PepT1) and basolateral amino acid transporters functions are coordinated. A working model that reflects current understanding of differential localization and identity of specific transporters responsible for mediated flux of amino acids across apical and basolateral membranes of enterocytes is presented in Fig. 3.2. How differential localization of transporters results in ion-dependent and substrate exchange-dependent vectoral transport of amino acids through enterocytes likely is similar to that proposed for renal epithelia presented by Palacin *et al.* (1998) and Verrey *et al.* (2000). As discussed above, it is generally accepted that the majority of amino acids are absorbed as small peptides, by PepT1 activity. After absorption, the peptide-bound amino acids are readily hydrolysed to free amino acids by intracellular peptidases (Fig. 3.1). As a consequence of these PepT1-dependent activities, and the activity of the Na^+-dependent X^-_{AG} and B^0 (and, perhaps, SN2 and $B^{0,+}$), an elevated supply of free amino acids exists to drive counterexchange across the apical membrane by BAT1 (and 4F2-lc6) and the counterexchange transport by basolateral transporters (Asc-1, LAT2) into the blood. Whereas the putative coordinated function of these differentially expressed transporters on transepithelial amino acid flux

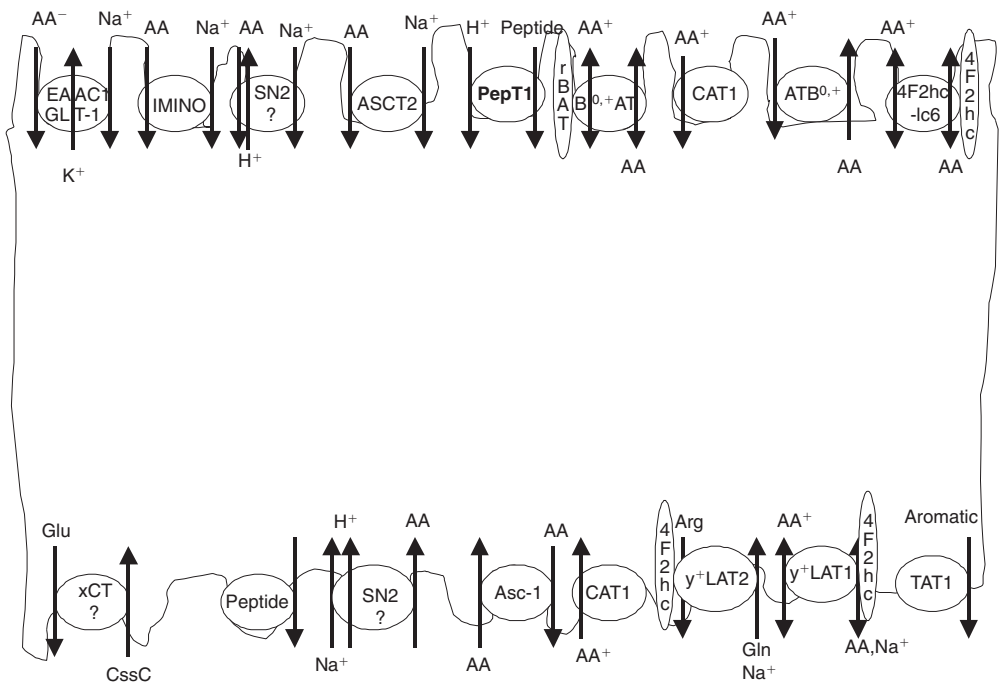

Fig. 3.2. Working model for the membrane-specific expression of peptide and free amino acid transporters by intestinal epithelial cells. The locations and predominant direction of substrate flow is derived from functional properties listed in Tables 3.4 and 3.5, and/or described in the text.

has not been evaluated, the influence of apically expressed PepT1 function on apical neutral and cationic amino acid uptake capacity by polarized Caco-2 cells has been (Wenzel *et al.*, 2001). PepT1 uptake of several neutral amino acid-containing dipeptides resulted in a 2.5- to 3.5-fold increased uptake of L-arginine by apical $b^{0,+}$AT activity. As this stimulation was dependent on intracellular hydrolysis of transported amino acids, it appears that PepT1 activity stimulated L-arginine by supplying requisite amino acids to drive $b^{0,+}$AT antiport uptake of arginine.

Emerging Concepts for the Role of Amino Acid Transporters

Potential for absorption of D-amino acids by intestinal epithelia

Given the fundamental differences in the amount of D-amino acids likely to be seen by animals that have a large pregastric fermentation capacity, and hence, a relatively greater production of bacterially derived D-amino acids, it is reasonable to suggest that the intestinal epithelia of ruminants may possess a larger capacity to absorb D-amino acids than postgastric fermenters. In this regard, several amino acid transport systems and proteins capable of D-amino acid absorption have been identified. Of particular importance is those systems capable of absorbing D-amino acids which are especially abundant in bacterial cell walls (D-glutamate, D-alanine and D-serine). Biochemically characterized system x^-_c activity has been defined as the Na^+-independent obligate exchange of L-glutamate and L-cysteine, which may be inhibited by D-glutamate (Dantzig *et al.*, 1978). Recently a cRNA has been generated (xCT) that apparently encodes for system x^-_c–like activity, when co-expressed with 4F2hc (Sato *et al.*, 1999). However, expression of x^-_c activity by enterocytes is unknown to these authors and investigation of

the ability of xCT to recognize and transport D-glutamate was not reported.

Although asc-like activity has been reported in the basolateral membranes of enterocytes (Mailliard *et al.*, 1995), the localization of Asc-1 has not been confirmed. In contrast, ASCT2 is localized to the apical membrane of intestinal epithelia. With regard to aromatic D-amino acids, both LAT1 and TAT1 are localized to the basolateral membrane and transport D-phenylalanine. In addition, the absorption of at least D-phenylalanine-containing peptides by PepT1 has been documented (Meredith and Boyd, 2000). The differential expression of ASCT2 and Asc-1 activity on both membranes of enterocytes indicates the capacity to absorb significant amounts of bacterial-derived D-amino acids from the intestinal lumen into the blood. These understandings, and that the flux of specific amino acids into the blood depends on their ability to compete for transport, suggest that the indiscriminant use of D-, L-isomer combinations as a supplemental source for one L-amino acid may perturb the flux of others into blood.

Other emerging concepts not explicitly covered in this review regarding the physiological consequences of transporter expression and function, include the potential pathological consequences that system y^+ (CAT1; Kim *et al.*, 1991; Wang *et al.*, 1991), ASC (ASCT1; Marin *et al.*, 2000), and B^0 (ASCT2; Rasko *et al.*, 1999; Tailor *et al.*, 2001) transporters serve as recognition molecules for various retroviruses and, in terms of ion fluxes, that system X^-_{AG} (EAAT5; Fairman *et al.*, 1995) and N (SN1, Chaudhry *et al.*, 1999) 'amino acid' transporters may be more accurately considered to function as amino acid-gated ion channels. Respectively, these understandings suggest that the ability to transport amino acids is associated with a health 'risk/cost' and indicate that amino acids can serve as 'signalling' molecules.

Conclusions

As reviewed previously in detail (Matthews, 2000a,b), and augmented with the current discussion, the evidence is strong that the gastrointestinal epithelia of pigs, chickens, sheep and cattle possess a large capacity to digest proteins and absorb their constituent amino acids by the same mechanisms as other animals, albeit with some important distinctions. Unanswered questions regarding the application of flux and transport data to the design of all animal diets include the following:

1. What is the capacity for peptide-bound versus free amino acid uptake by the gastrointestinal epithelia?
2. Can this capacity be regulated *in vivo* by diet and(or) feeding regimens?
3. Will increasing the amount of peptide-bound amino acids achieve greater amino acid absorption efficiencies?
4. Is the development and use of model substrates and(or) protein hydrolysates to potentiate peptide absorption capacity economically feasible?

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