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# 16 Production and gene expression of brush border disaccharidases and peptidases during development in pigs and calves

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This review presents recent information on the expression of intestinal brush-border disaccharidases (maltase-glucoamylase, sucrase-isomaltase, lactase and trehalase) and peptidases (aminopeptidases A and N and dipeptidyl peptidase IV) during development in growing animals. The biosynthesis, structure and function of these intestinal enzymes are reported. Analysis of their localisation along the crypt-villus axis shows that levels of intestinal enzyme mRNA and specific activity are at their highest in the lower half of the villi. The distribution of intestinal enzymes along the proximal-distal axis of the intestine changes after birth. This results in the establishment of specific distribution patterns in sucking or weaned animals, according to the enzyme examined. The expression of brush-border enzymes is developmentally imprinted. Nevertheless, there is a role for substrates in the regulation of enzyme expression. Weaning induces dramatic changes in the specific activities of the disaccharidases, and to a lesser extent in peptidase specific activities. Finally, it is concluded that studies of the regulation of intestinal gene expression are relatively scarce in porcine and bovine species. Development of new molecular tools, in particular high-density microarrays, is expected to provide new and relevant information for improved understanding of the physiology of the small intestine.

## 1. INTRODUCTION

This review examines the current status of knowledge of the intestinal brush border disaccharidases and peptidases, which are essential for the digestion of carbohydrates and protein in livestock. Focusing on pigs and calves, various literature data concerning biochemical and molecular biological characteristics of enzymes are discussed in a physiological context. The roles of intestinal enzymes are described, taking into account complementarity with salivary, gastric and pancreatic digestive enzymes and their crucial hydrolytic function in the process of absorption. Gene expression of the enzymes and nutritional regulation of their expression appear during postnatal development up to maturity. Abundant information is available on intestinal enzyme structure and function and therefore in this chapter this subject is covered only briefly, and the reader is referred rather to excellent reviews in this domain (Semenza, 1986; Galand, 1989; van Beers et al., 1995). The information and references outlined in this review should serve for readers as an initial source of information and to stimulate deeper studies.

## 2. DISACCHARIDASES

In monogastrics, the first starch-degrading enzyme encountered by the ingested feed is  $\alpha$ -amylase secreted by the submaxillary and sublingual salivary glands (but in low levels). This salivary enzyme is however absent in ruminant species. Hydrolysis of internal  $\alpha$  (1–4) glucosidic linkages of amylose and amylopectin in starch results in a mixture of dextrans, maltose and maltotriose. Starch, hemicellulose and hexose breakdown may also occur in the upper regions of the stomach, due to fermentation by gastric microflora in monogastrics and more significantly by ruminal flora in ruminants. Thereafter, pancreatic  $\alpha$ -amylase hydrolysis produces large amounts of reducing sugars, especially maltose, in the intestinal lumen where brush border disaccharidases play a part in carbohydrate hydrolysis.

The small intestine is known to possess at least four separate maltase activities. Two of these maltase activities can be assigned to the sucrase-isomaltase enzyme complex and the two others can be attributed to the maltase-glucoamylase complex. Therefore, the starch-derived products of  $\alpha$ -amylase digestion are further degraded by the combined actions of maltase-glucoamylase and sucrase-isomaltase complexes whereas lactase and trehalase hydrolyse lactose and trehalose, respectively. The significance of trehalose is rather minor, as this disaccharide is confined to yeast, mushrooms and insects (van Beers et al., 1995). The monosaccharides produced as end products of digestion, are absorbed along the small intestine by

transcellular transport through specific transporters expressed in the plasma membrane of the epithelial cells and/or by paracellular transport via the tight junctions between these epithelial cells.

Any starch or sugars which escape digestion by the host's enzymes may be partly degraded by the autochthonic/allochthonic flora of the small intestine, although such degradation mainly takes place in the large intestine.

## **2.1. Biosynthesis and structure**

### **2.1.1. *Maltase-glucoamylase complex***

After translation of the specific mRNA, a single precursor of maltase-glucoamylase (pro-MG), rich in mannose, is produced in the rough endoplasmic reticulum (RER); in pigs it has an apparent molecular weight of 225 kDa. Following transportation from the RER to the Golgi apparatus, mannose residues are trimmed and carbohydrate residues are added, yielding a pro-MG of 245 kDa. It has been shown that the complexly N-glycosylated maltase-glucoamylase precursor is sulphated on the tyrosine residues (Danielsen, 1987). Then, the anchorage of a single hydrophobic segment, located at the N-terminus of the glucoamylase subunit, permits the direct translocation of the pro-MG into the apical membrane of the microvilli. This transmembrane sequence constitutes an uncleaved RER targeting signal sequence which, in addition, serves as a brush border anchor conferring a transmembrane protein type. Finally, pro-MG is cleaved into maltase and glucoamylase by pancreatic proteases, but the subunits remain associated by noncovalent, ionic interactions. Therefore, the complete primary structure of the pig pro-MG contains the two associated active catalytic subunits, sucrase (125 kDa) and isomaltase (135 kDa) (Danielsen, 1994). In addition, Noren et al. (1986) demonstrated by electron microscopy that pig maltase-glucoamylase heterodimers may dimerize during biosynthesis in the RER to form homohetero-tetramers. In contrast, in the human neither proteolytic cleavage of the intestinal maltase-glucoamylase complex of 335 kDa nor dimerization of the complex have been demonstrated (Naim et al., 1988).

### **2.1.2. *Sucrase-isomaltase complex***

The biosynthesis of sucrase-isomaltase is similar to that of maltase-glucoamylase, as a mannose-rich, N-glycosylated precursor of 270 kDa (in rabbits) and a N- and O-glycosylated precursor with an approximately 20 kDa higher molecular mass than the high mannose form. Sucrase-isomaltase is

also tyrosine-sulphated as a result of a trans-Golgi event. The conversion to mature sucrase-isomaltase was prevented when the pancreatic duct was disconnected, confirming the need for proteolytic cleavage of the mature complex into non-covalently associated sucrase (120 to 140 kDa and isomaltase (140 to 151 kDa in human, pig and rat species (Cowell et al., 1986). The N-terminal sequence of the pig isomaltase that serves as an anchor in the apical membrane shares 45% homology with that of the pig maltase-glucoamylase. The deduced peptidic sequences of human (1827 amino acids), rabbit (1827 amino acids) and rat (1841 amino acids) sucrase-isomaltase show a twofold internal homology in their sucrase and isomaltase regions. The sucrase and isomaltase subunits share 38 to 41% identity. The sucrase-isomaltase may form dimers of  $\alpha_2\beta_2$ -type in the RER, except in humans (Cowell et al., 1986). The mean turnover time of rat sucrase-isomaltase *in vivo* is around 6 h. Therefore, in order to maintain a steady state activity level, enterocytes need to synthesize daily 4 times the amount of mature enzyme expressed in the intestinal membrane (Dudley et al., 1993).

### 2.1.3. *Lactase*

Pro-lactase occurs as a 200 to 220 kDa precursor containing high mannose N-glycosylation. After transportation to the Golgi apparatus, pro-lactase becomes the complexly O-glycosylated form with a slightly greater molecular mass of 225 to 240 kDa. During the transport to the brush border membrane, but primarily before insertion in the membrane, the precursor is proteolytically cleaved into the mature form (130 to 160 kDa and a propeptide corresponding to the N-terminal domain. This process evidently results from the action of an intrinsic proteinase of the enterocyte since it occurs *in vivo* in the complete absence of luminal proteases, when the pancreatic duct is ligated (Hauri et al., 1985). However, as for maltase-glucoamylase and sucrase-isomaltase, proteolytic processing is not essential for the specific activity of lactase. The pro-peptide of approximately 100 kDa functions as an intramolecular chaperone during the initial folding of lactase in the RER (Naim et al., 1994). It is not associated with the mature lactase, nor transported to the cell surface or the luminal medium, but rapidly intracellularly degraded. In contrast to the two other disaccharidases described above, the mature lactase is anchored in the brush border membrane at the carboxyl end of its protein chain. The primary sequence of lactase from human (1927 amino acids), rabbit (1926 amino acids) and rat (1928 amino acids) revealed a fourfold internal homology; domains I and II form the pro-peptide and domains III and IV, corresponding to the mature part of the polypeptide, contain the two active sites. Lactase probably forms

non-covalent dimers at a late step in biosynthesis (Danielsen, 1990; Naim, 2001). In the adult rat, the mean residence time of lactase in enterocytes is around 8 h (Dudley et al., 1993).

#### 2.1.4. *Trehalase*

Trehalase is rather unique among the disaccharidase family, from several different points of view. The primary translation product of small intestinal trehalase consists of a relatively small enzyme (65.5 kDa compared to other intestinal disaccharidases). Trehalase contains four sites of N-glycosylation, but there is no evidence for O-glycosylation. The N- and C-terminal extremities possess hydrophobic sequences. The N-terminal sequence is a cleavable signal-sequence for translocation into the RER, while the C-terminal sequence is a transient transmembrane sequence. After the translation of the polypeptide is complete, the C-terminal sequence is replaced by a glycosylphosphatidylinositol (GPI)-anchor, as shown by its selective solubilization by phosphatidylinositol-specific phospholipase C (Takesue et al., 1986). In contrast, the other intestinal disaccharidases are anchored by a permanent transmembrane sequence that is released upon papain digestion. The GPI-anchor functions as a very specific targeting signal for apical transport (Lisanti and Rodriguez-Boulan, 1989). Finally, there is no internal homology within trehalase, indicating that there is most likely only one active site (Ishihara et al., 1997), in contrast to the several internal homology areas described for lactase and sucrase-isomaltase.

## 2.2. Functions

In most mammalian species, the small intestine expresses four major brush border disaccharidases: maltase-glucoamylase, sucrase-isomaltase, lactase and trehalase. Each of the subunits in maltase-glucoamylase and sucrase-isomaltase complexes has maltase and maltotriase activity, hydrolysing the  $\alpha(1-4)$  glucosidic bonds of non-reducing glucose units (table 1). Thus the name "maltase" is in a way deceptive since there are four maltases. By using maltose as substrate, maltase activity results from the action of one to four enzymes without a clear distinction between the contribution of sucrase-isomaltase and maltase-glucoamylase to this activity. In contrast, the glucoamylase activity unique to the complex maltase-glucoamylase can be assayed by measuring glucose production from amylose and/or amylopectin. The  $\alpha(1-6)$  glucosidic bonds in maltose and maltotriose are hydrolysed by the isomaltase enzyme expressed in both bovine and porcine species. Lactase is the only enzyme in the small intestine possessing

Table 1  
Nomenclature and substrate specificities of intestinal brush border disaccharidases and peptidases

Enzyme	EC number	Subunits	Activities	Substrates
Disaccharidase Maltase-glucoamylase	3.2.1.20	Maltase Glucoamylase	$\alpha$ (1-4) glucosidase <sup>1</sup>	Maltose, maltodextrose, starch, glycogen, $\alpha$ -limit dextrins, amylose, amylopectins, isomaltose (minor activity)
Sucrase-isomaltase	3.2.1.10, -48	Sucrase Isomaltase	$\alpha$ (1-4) glucosidase $\alpha$ (1-6) glucosidase	Sucrose, maltose, maltotriose Isomaltose, maltose, maltotriose, $\alpha$ -limit dextrins (minor activity)
Lactase	3.2.1.23, -45, -46, -62, -108		$\beta$ (1-4) galactosidase $\beta$ (1-4) glucosidase $\alpha$ - $\alpha'$ -Trehalase	Lactose Cellobiose, cellulose $\alpha$ - $\alpha'$ -Trehalose
Peptidase Aminopeptidase N	3.4.11.2	Aminopeptidase N	exopeptidase	Release of a N-terminal amino acid, that is preferentially Ala, Leu or Met
Aminopeptidase A	3.4.11.7	Aminopeptidase A	exopeptidase	Release of a N-terminal amino acid, that is preferentially Glu or Asp
Dipeptidyl peptidase IV	3.4.14.5	Dipeptidyl peptidase IV	exopeptidase	Release of a N-terminal dipeptide, X-Y, preferentially when Y is Pro

<sup>1</sup>The two subunits of this enzyme complex display nearly identical enzyme activities.

$\beta(1-4)$  galactosidase activity, and is thus a crucial enzyme during early postnatal mammalian development. In addition to lactose, lactase can also hydrolyse cellobiose, conferring on lactase both  $\beta(1-4)$  galactosidase and  $\beta(1-4)$  glucosidase activities. Sucrose that becomes part of the diet mainly after weaning, is exclusively hydrolysed by the sucrase subunit of the sucrase-isomaltase heterodimer. In the calf, sucrase activity is completely absent. Intestinal trehalase is the sole enzyme responsible for hydrolysis of trehalose. These enzyme complexes are true exoglucosidases, producing free monomeric glucose from their di- and oligomeric substrates.

Galand (1989) indicated that almost half of 18 mammalian species examined, did not express sucrase as newborns and/or adults, but in contrast maltase activity has been detected in all mammals studied. Therefore the sucrase-isomaltase complex is absent in cattle and sheep, as well as in rodents, rabbits and horses at birth. In contrast, humans, dogs and cats possess high sucrase, isomaltase and maltase activities throughout life. In rats and rabbits maltase-glucoamylase accounts for about 70% of the maltase activity, while in humans sucrase-isomaltase activity represents 80% of the maltase activity (van Beers et al., 1995). In the sucrase-isomaltase complex, maltase activity is restricted to the isomaltase subunit in monkeys and to the sucrase subunit in pigs (Hertel et al., 2000).

The expression of maltase-glucoamylase and trehalase in the small intestine and the kidney suggests that their tissue-specific gene regulation is different from that of sucrase-isomaltase and lactase, which are confined to the intestine. Moreover, enzymes may possess properties other than hydrolytic activity per se. Recently, Finn et al. (2001) demonstrated for the first time, that a digestive enzyme can function as an ion channel. Thus, sucrase-isomaltase is also a cAMP-dependent epithelial chloride channel.

### 3. PEPTIDASES

In contrast to the relatively small number of carbohydrases, the number of peptidases found in enterocytes in the small intestine is large. This is necessary because of the large number of different peptide bonds in oligopeptides produced by the action of pancreatic proteases. Enterokinase, an endopeptidase responsible for the activation of trypsinogen, has been isolated in the brush border membrane. Most of the other peptidases identified in the enterocytes are aminopeptidases and dipeptidases located in the brush border and the cytoplasm, respectively. The major brush border hydrolases include aminopeptidase N, aminopeptidase A and dipeptidyl peptidase IV. They participate in the final stages of protein digestion. Peptides consisting of four or more amino acids are hydrolysed extracellularly by brush border



enzymes whereas tri- and dipeptides are hydrolysed either at the brush border level or intracellularly, or are absorbed and transported intact into the blood circulation. Several cytoplasmic hydrolases have been described in the literature and are important in the hydrolysis of absorbed peptides. For example, the glycyllucine-dipeptide hydrolase hydrolyses dipeptides containing neutral amino acids, the aminoacylproline hydrolase (prolinase) hydrolyses dipeptides with C-terminal proline or hydroxyproline and aminotripeptidases hydrolyse only tripeptides. In this review, the structure, function and expression of brush border peptidases will be presented.

### 3.1. Structure

Peptidases of brush border membranes are glycoproteins. The sugar can represent quite a sizeable percentage of the total molecular weight and both N- and O-glycosidic linkages are found. During the process of maturation, the earliest products detected are N-glycosylated forms to high mannose forms. Pulse chase experiments demonstrate that high mannose forms prevail for 30–60 min, a time that presumably reflects their movement from the RER to the Golgi complex (Danielsen et al., 1984). Thereafter, the trimming steps, complex glycosylation and O-glycosylation take place and lead to the final forms of intestinal peptidases. Thus the molecular weight of different forms change during the maturation process. For example, the molecular weight of the primary translation product of aminopeptidase N is 115 kDa that of the high mannose form is 140 kDa and that of the final complexly glycosylated form is 160 kDa (Danielsen et al., 1982).

Aminopeptidases N and A and dipeptidyl peptidase IV have similar subunit structures and modes of anchoring in the membrane. Aminopeptidase N is composed of a single type of subunit of approximately 160 kDa which occurs as a monomer in the rabbit and as a homodimer in the human, pig and rat. Aminopeptidase A also occurs as a homodimer, with a subunit of approximately 170 kDa in pig intestine. Dipeptidyl peptidase IV from pig intestine is a homodimer with a subunit of approximately 137 kDa. These proteins are transmembrane proteins anchored via a hydrophobic segment located in the N-terminal region of the polypeptide chain. The hydrophobic segment has a dual role, that of a signal during biosynthesis and insertion and that of anchor in the final “mature” enzyme.

### 3.2. Functions

Aminopeptidase N plays a key role in the hydrolysis of peptides in the lumen, shortening oligopeptides by stepwise hydrolysis (table 1). It is most

active against neutral and basic amino acids and hydrolysis ceases when proline or 5-hydroxyproline is the final or penultimate N-amino acid. Dipeptidyl peptidase IV has a complementary activity to that of aminopeptidase N. It hydrolyses the N-terminal dipeptides most efficiently when the penultimate amino acid is proline, but efficiency is much lower when a neutral amino acid occupies the penultimate position. Aminopeptidase A preferentially hydrolyses peptides with aspartic acid and glutamic acid in the N-terminal position.

Besides the property of digestive function, brush border peptidases may have other roles. Aminopeptidase N acts also as a receptor. The study of Delmas et al. (1992) provides strong evidence that pig aminopeptidase N serves as a receptor for an envelope RNA virus, the transmissible gastroenteritis virus, and thus facilitates virus entry into cells. Determinants essential for the virus-receptor interaction resides within a domain of aminopeptidase N that is distinct from the enzymatic site (Delmas et al., 1994). On the other hand, dipeptidyl peptidase IV present on the surface of most mammalian cells is an activation marker for various types of immune cells. It plays a role in the regulation of differentiation and growth of lymphocytes, and the inhibition of dipeptidyl peptidase IV activity by specific inhibitors leads to a reduction of cytokine production in stimulated T-cells (Arndt et al., 2000).

#### **4. REGULATION OF ENZYME EXPRESSION**

The digestive function (disaccharidase and peptidase activities) of the enterocytes and their microvilli only begins when structural differentiation is complete, which is usually during the period of migration over the crypt-villus junction (Smith et al., 1989). However, brush border enzymes are present in a mature form from the bottom of the crypt to the top of the villus. The development of absorptive function for both sugar and amino acid transport appears in enterocytes at the upper to mid-level of the villus and continues to increase until they are shed at the villus tip (Cranwell, 1995).

##### **4.1. Developmental gene and protein expressions**

It is widely accepted that the regulation of brush border enzyme expression is primarily transcriptional. In several species expression of disaccharidase and peptidase correlate well with the amount of corresponding mRNA. Additional levels of regulation may however exist and involve differential efficiency of mRNA translation, stability of mRNA and protein or different rates of protein maturation.

#### 4.1.1. *Crypt-villus distribution*

The epithelium of the small intestine mainly consists of absorptive cells which are constantly renewed. Mitotic active cells located in the low region of the crypts give rise to nondividing cells which gradually migrate along the crypt-villus axis, finally being extruded into the intestinal lumen from the top of the villi. Sucrase-isomaltase, lactase, aminopeptidase N and dipeptidyl peptidase IV are present in pig enterocytes from the crypts to the villi (Hansen et al., 1994; Fan et al., 2001). In 14d-old formula-fed piglets, the specific activities of aminopeptidase N, sucrase and lactase increase several fold from the bottom of the crypt to the middle part of the villus before reaching an asymptotic value from the middle to the tip of the villus (Fan et al., 2001). In 3 week-old suckling piglets, lactase activity increases linearly along the villus axis, attaining maximal values at approximately 400  $\mu\text{m}$  from the crypt-villus junction (Kelly et al., 1991a). These biochemical data have been confirmed by histochemical and immunocytochemical analysis.

According to Hansen et al. (1994), the biosynthesis of rat sucrase-isomaltase and dipeptidyl peptidase IV starts shortly after the generation of the enterocytes, whereas biosynthesis of dipeptidyl peptidase IV begins a few hours later. Dipeptidyl peptidase IV mRNA is present in crypt cells, but at a 7-fold lower level than in villus cells in rat jejunum (Darmoul et al., 1991). The preferential expression of dipeptidyl peptidase IV in villus cells may thus result from an increased accumulation of corresponding mRNA which may be explained by changes in the transcription rate of the gene observed as the intestinal cells enter a differentiation process. Aminopeptidase N (Noren et al., 1989) and sucrase-isomaltase (Leeper and Henning, 1990; Traber, 1990) genes are probably controlled in a similar differentiation-dependent way. Sucrase-isomaltase mRNA is however poorly expressed in crypt enterocytes of rat small intestine (Traber et al., 1992). Interestingly, lactase and sucrase-isomaltase mRNA expression are maximal in the lower half of the villus, and the level gradually declines towards the villus tip, as demonstrated in rat, rabbit and human small intestine (Traber et al., 1992; Freeman et al., 1993). It seems that in fully functional villi, the transcription of the genes encoding brush border disaccharidases are turned on 1 to 2 days before the cells are extruded from the epithelium at the villus tip.

#### 4.1.2. *Distribution along the intestine*

The specific activity of lactase is found to be highest in the proximal region and lowest in the distal region of the small intestine in neonate piglets (fig. 1)

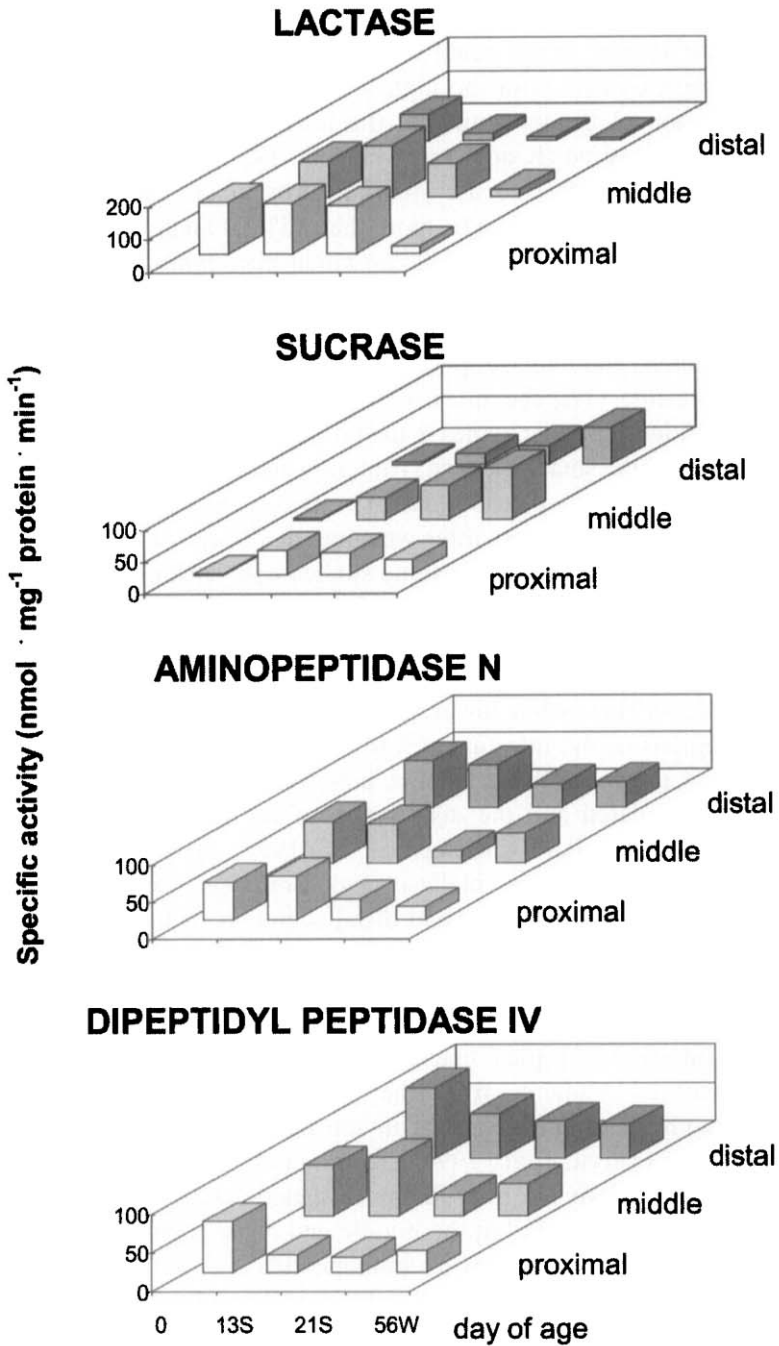


Fig. 1. The specific activities of four brush-border enzymes in the proximal, middle and distal segments of the small intestine of piglets from 0 to 56 days of age. S, sucking piglets; W, piglets weaned at 21 days of age. (Le Huërou-Luron et al., 2001).

and calves (Le Huërou et al., 1992). A definite pattern of distribution of lactase specific activity in the small intestine is evident in sucking pigs from about 14 days of age, with the highest activities being found in the first 30–50% region of the intestine and the lowest activities in the last 30% region of the intestine (Kidder and Manners, 1980; Hampson and Kidder, 1986; Kelly et al., 1991b,c; Le Huërou-Luron et al., 2001). In milk-fed calves, from about 7 days of age, more than 95% of lactase specific activity is observed in the duodenum and the proximal (the highest values) and middle segments of the jejunum (Le Huërou et al., 1992). These site-specific expressions are similarly observed in weaned pigs (fig. 1) and calves. At birth, the sucrase and maltase specific activities are evenly distributed along the small intestine. Over the time course of lactation sucking piglets develop greater activities of both enzymes in the proximal to mid-jejunum than in the distal regions of the small intestine (Aumaitre and Corring, 1978). It is interesting that the opposite distributions of activities are observed in the post-weaning period, i.e. 5 weeks after weaning, at 21 days of age (Le Huërou-Luron et al., 2001). However, in some studies with sucking piglets, sucrase activity is greater in the distal half of the small intestine after 2 weeks (Aumaitre and Corring, 1978) or 5 weeks (Kidder and Manners, 1980); maltase activity is equally distributed along the intestine at 2–3 weeks of age (Kelly et al., 1991a) or has lower activities in the 10% proximal and 10% terminal regions of the intestine at 5 to 8 weeks of age (Kidder and Manners, 1980; Cranwell, 1995). In pigs, activities of sucrase and isomaltase are highly correlated and the distribution of the two enzymes along the intestine is very similar (van Beers et al., 1995). In preruminant (milk-fed) and ruminant calves maltase and isomaltase exhibit maximum specific activities in the middle and distal segments of the small intestine, whereas in the duodenum these activities are decisively lower (Le Huërou et al., 1992).

Distribution of aminopeptidase N and dipeptidyl peptidase IV activities along the intestine of piglets changes after birth (fig. 1). It results in the establishment of gradients from the duodenum to the ileum of both enzymes, with maximum activities in the distal segment in sucking and weaned piglets (Tarvid et al., 1994; Lizardo et al., 1995; Le Huërou-Luron et al., 2001). Similarly, in milk-fed and weaned calves aminopeptidase N activity is mainly expressed in the middle and distal segments of the small intestine, whereas no variation in aminopeptidase A activity is observed along the intestine (Le Huërou et al., 1992). At 4 months of age the specific activity of aminopeptidase N is 60 to 110% greater in the ileum than in the distal jejunum.

It is worth stressing that in 14 days-old piglets weaned at 7 days of age, the expression profiles of the specific activity and mRNA of lactase

and aminopeptidase N are superimposable (Le Huërou-Luron, Marion and Le Dividich, unpublished results) (fig. 2). The level of maltase mRNA is evenly distributed, whereas that of dipeptidyl peptidase IV mRNA is 2-fold higher in the proximal segment than in the middle and distal segments of the intestine. This implies that the site-specific expression of enzymes along the intestine results from the major transcriptional level of regulation for lactase and aminopeptidase N and also the additional levels of regulation for both maltase and dipeptidyl peptidase IV. In the adult rat, lactase mRNA is restricted to the jejunum and proximal ileum, whereas sucrase-isomaltase mRNA is detected throughout the small intestine, a pattern regulated by transcriptional rate.

#### **4.1.3. Age**

Lactase activity is present at high levels in newborn pigs and calves. In the time course following birth, lactase specific activity is slightly decreased in the proximal and middle segments whereas a sharper decrease is observed in the distal segment of the small intestine of sucking piglets (fig. 1). Expressed per g mucosa, there is a fall in the amount of the relative activity between birth and 8 weeks of age (Manners and Stevens, 1972). In calves, a drop of lactase specific activity is observed between birth and two days of age whereas no significant change is measured thereafter (Le Huërou et al., 1992). Conversely, the other disaccharidases have only low activities at birth in both species. Thereafter, in piglets specific activities of both maltase and sucrase increase rapidly to reach a peak around 2 weeks of age and to plateau at 2 (fig. 1) or 3 weeks of age (Aumaitre and Corring, 1978; Sangild et al. 1991; Cranwell, 1995). These activities remain at similar plateau levels in the period from 3–4 weeks of age up to 6–8 weeks in suckling pigs (Aumaitre and Corring, 1978) or continue to increase up to 8 weeks of age as isomaltase and trehalase activities (Kidder and Manners, 1980; Miller et al., 1986). However, total activities of maltase and sucrase in the small intestine of sucking pigs increase 26- and 14-fold, respectively, from 1 to 8 weeks of age (Aumaitre and Corring, 1978) whereas total lactase activity is more or less constant over this period. The decrease in the specific activity of lactase with age is compensated for by the increased weight of the small intestine. In calves from 1 week of age, maltase and isomaltase specific activities increase regularly with age. Between 1 and 17 weeks of age, these activities were enhanced 10- and 6-fold for maltase and isomaltase, respectively (Le Huërou et al., 1992).

We found that the specific activities of aminopeptidases A and N and dipeptidyl peptidase IV are generally high at birth but decrease gradually

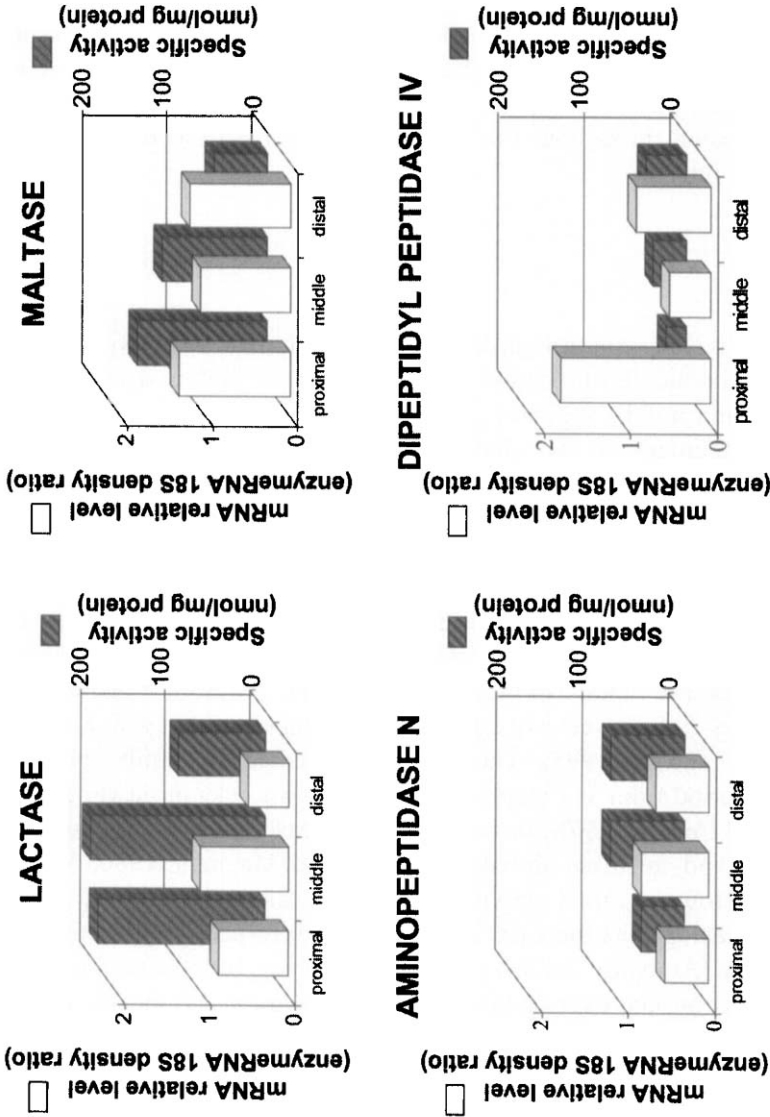


Fig. 2. The mRNA relative levels and the specific activities of four brush-border enzymes in the proximal, middle and distal segments of the small intestine of 14 d-old piglets weaned at 7 days of age. Relative levels of mRNA were measured by reverse transcription-polymerase chain reaction using 18S RNA internal standard as described by Petersen et al. (2001). (Le Huërou-Luron, I., Marion, J. and Le Dividich, J., unpublished results).

during suckling (fig. 1); i.e. aminopeptidase N and dipeptidyl peptidase IV specific activities were 42 and 59% lower in 3-week-old sucking piglets than at birth. A similar observation was reported by Sangild et al. (1991). In contrast Tivey and Smith (1989) found that intact villi from 28-d-old pigs contain 30% more aminopeptidase N and 400% more dipeptidyl peptidase IV activity than villi from newborn pigs.

To our knowledge, no data are available on the changes occurring in the mRNA coding brush border digestive enzymes during early development in piglets and calves. During development of rodents, maltase-glucoamylase expression is upregulated concomitantly with the development of the intestinal microvilli and is detected earlier than lactase and sucrase-isomaltase, clearly indicating different regulation of these genes with respect to timing of expression (van Beers et al., 1995). In rats, a coordinated relationship between lactase specific activity, lactase protein and lactase mRNA abundance is observed during development of the small intestine, suggesting that there is primarily a transcriptional level of expression regulation (Rings et al., 1992).

#### **4.1. Nutritional regulation**

Factors other than age which influence the development of brush border enzymes in the small intestine are numerous and may include: creep feeding during the suckling period (Hampson and Kidder, 1986), weaning on to dry diets or liquid diets (Deprez et al., 1987), composition of feeds, growth factors and hormones. In addition to endogenous sources, growth factors and hormones may be provided by colostrum and milk before weaning or via supplements after weaning. However the information concerning their effects on postnatal intestinal development is beyond the scope of our review.

##### **4.2.1. Weaning**

Weaning exerts significant effects on the development of the brush border enzymes and on intestinal morphology. Interestingly, structural and functional alterations of the small intestine observed in response to weaning appear firstly in the proximal segments, and with varying time-delays over the following days in the more distal regions (Marion et al., unpublished results). In piglets weaned at 7 days of age, maltase specific activity in the proximal segment is increased 7-fold during the first postweaning week and remains unchanged during the second postweaning week, whereas



this activity is 4- and 3-fold increased in the distal region, respectively. Following weaning at 2 or 3 weeks of age, lactase and sucrase specific activities start to decrease during the first four days (Hampson and Kidder, 1986; Tang et al., 1999). Miller et al. (1986) reported that in piglets weaned at 3 or 5 weeks of age, the specific activities of lactase, sucrase and isomaltase on d 5 after weaning were significantly lower (2 to 5-fold) than those in sucking pigs of similar ages. Maltase activity increases significantly by 2.4-fold on d 5 post-weaning in piglets weaned at 5 weeks of age but not in those weaned at 3 weeks of age. By 11 days after weaning sucrase activity shows a partial recovery which is enhanced when piglets receive creep feed before weaning (Hampson and Kidder, 1986). With the exception of lactase, the specific activities of the disaccharidases increase progressively from 2 weeks after weaning; maltase and trehalase activities continue to increase up to 200 d of age, while sucrase and isomaltase activities continue to increase even beyond that age. (Manners and Stevens, 1972; Kidder and Manners, 1980). In contrast, lactase activity continues to decline in both unweaned and weaned piglets until a plateau is reached after 8-weeks of age (Kidder and Manners, 1980).

Peptidase activities are less influenced by weaning than disaccharidase activities. After weaning at 7 days of age the specific activity of aminopeptidase A does not change and that of aminopeptidase N increases by 31% during the 3 days after weaning and remains constant up to 2 weeks after weaning (Marion et al., 2001). That of dipeptidyl peptidase IV increases transiently during the first 3 postweaning days, whereas no difference with sucking pigs is observed at 21 days of age. A tendency for a postweaning recovery in the activity of aminopeptidase N and dipeptidyl peptidase IV is only observed in the jejunum of piglets 5 weeks after weaning. However, variations in specific activity underestimate post-weaning reductions in total intestinal enzyme activity since the marked decline in villus height (30–70%) after weaning results in a reduced mucosa weight. In addition, the length of the microvilli is reduced in the 3–7 d period following weaning at 3 weeks of age (Cera et al., 1988). The experiment of Kelly et al. (1991c) with pigs weaned at 14 days of age and force-fed a weaner diet by gastric intubation clearly documented that low post-weaning feed intakes caused alterations in the small intestinal function and structure immediately after weaning. In weaned calves, the specific activities of aminopeptidase N, maltase and isomaltase increase after weaning. The activities of aminopeptidase N, maltase and isomaltase are 2.1-, 3.2- and 2.0-fold greater than those recorded in unweaned calves at similar ages (2–3 months of age), while the activity of lactase is 1.7-fold lower in 2 month-old weaned calves as compared to milk-fed calves (Le Huërou et al., 1992).

Data on expression of intestinal digestive enzymes at mRNA levels are scarce in pigs and calves. In rats, lactase mRNA transcripts are abundant before weaning, despite the progressive decline of enzyme activity, and then decrease 2- to 4-fold during weaning (Duluc et al., 1993). In contrast, sucrase-isomaltase mRNA is first detected 14 days after birth and increases rapidly to abundant levels by 28 days of age. Lactase transcriptional rate declines whereas that of sucrase-isomaltase increases after weaning (Krasinski et al., 1994). In early-weaned pigs the increase of maltase specific activity observed during the 7 days after weaning parallels the increase in the corresponding mRNA (Le Huërou-Luron, Marion, and Le Dividich, unpublished results).

Apart from the primary regulation of lactase and sucrase-isomaltase at the transcriptional level, there are indications for some post-translational (protein stability) regulatory mechanisms. The half-life of lactase and sucrase-isomaltase activities in aging rodents is generally found to decrease, probably due to enhanced degradation or inactivation of the enzymes (van Beers et al., 1995). The brush border disaccharidases would appear to have different sensitivities towards luminal hydrolysis in the intestine. Pancreatic elastase is the most potent in releasing the enzymes from the plasma membrane. The kinetics of release of lactase, maltase and sucrase activities are similar, but released sucrase-isomaltase and maltase-glucoamylase still contribute to the luminal digestion of disaccharides, whereas lactase activity is lost after release from the membrane (Young and Das, 1990).

In pigs, just after weaning, either low or no feed intake occurs for a few days, but the severity and the length of the underfed period greatly depends on the animal-dependent adaptation ability and stress susceptibility. Malnutrition, induced by restriction of the diet to 40% of the daily control intake, for a 30-day period in nursing piglets, leads to an increase in specific activities of all disaccharidases (maltase, sucrase, isomaltase and lactase), whereas segmental activities are reduced (Nunez et al., 1996). This implies that enzymatic proteins involved in nutrient digestion are preferentially maintained in comparison with other intestinal proteins. In agreement with these observations, lactase and sucrase-isomaltase mRNA in rat small intestine increase during a severe short-lasting feed restriction (for 2 to 4 days) and return to baseline after refeeding (Nsi-Emvo et al., 1994). When rats are refed after a 2-day starvation, the migrating enterocytes at the crypt-villus junction stop expressing sucrase-isomaltase, whereas enterocytes passing the crypt-villus junction are apparently not able to down-regulate gene expression, once committed to sucrase-isomaltase expression. This suggests a "point-of-no-return" principle for the up-regulation of brush border glycohydrolases at the

crypt-villus junction (van Beers et al., 1995). In contrast, a long-lasting period (for 8 weeks) of protein deficiency by piglets weaned at 3 weeks of age decreases both lactase mRNA abundance and post-translational processing, resulting in a lower absolute synthesis rate of lactase (Dudley et al., 1997).

#### 4.2.2. *Dietary regulation*

The dietary regulation of brush-border enzymes is still poorly understood and data are very scarce, particularly in piglets and calves. Indeed, dietary effects should consider all the changes induced by modifications in feed composition and intake on the luminal environment of enterocytes (Sanderson, 1998). In the intestinal lumen are bacteria, their byproducts and endogenous substances. Moreover, dietary changes may affect crypt cell proliferation, alter the level of luminal nutrition or change the rate of pancreatobiliary secretions into the intestine (Smith, 1992). A long-chain triglyceride-rich diet leads to enhanced degradation of sucrase-isomaltase in the jejunum of rats, presumably caused by an increased bile acid secretion (Shinohara et al., 1993). A high protein, low carbohydrate diet leads to a similar effect by increased pancreatic protease accumulation in the lumen (Goda et al., 1988).

The expression of brush border disaccharidases is developmentally imprinted. Nevertheless, there is still a role for carbohydrate substrates in the regulation of the level of the individual disaccharidases. Thus, all evidence suggests that the level of lactase activity is genetically determined; only minor control may be exerted by glucose, but no regulation is exerted by lactose (Goda et al., 1995; Le Huërou-Luron et al., 2001). Sucrase-isomaltase is regulated in a more substrate-dependent manner than lactase (Henning 1985; Bustamente et al., 1986). A decreased intake of starch leads to a specific decrease in all brush border disaccharidases, while brush border peptidases are not affected (Goda et al., 1983). The upregulation of sucrase-isomaltase by dietary sucrose is a specific adaptation that increases the amount of active enzymes per cell, as no change in the cell migration rate, the number of enterocytes, or crypt-villus ratio have been reported (van Beers et al., 1995). In cultured pig small intestinal explants, fructose prevents the expression of aminopeptidase N, and induces a very rapid degradation of newly synthesized enzymes, probably due to aberrant N-glycosylation and perturbed intracellular membrane traffic in the enterocyte (Danielsen, 1989). In piglets four weeks after weaning at 3 weeks of age, dietary composition (pelleted milk replacer vs. commercial starter feeds) did not affect peptidase and disaccharidase activities (Le Huërou-Luron et al.,

2001). Inclusions of dietary fibres in diets may induce modifications of rat disaccharidase activities (Khokhar, 1994), but results from different studies have not always been consistent.

## **5. FUTURE PERSPECTIVES**

A fairly extensive knowledge base has been generated on various aspects of the structure, biosynthesis and function of brush border enzymes in several mammalian species. Studies on regulation of intestinal gene and enzyme activity expression have mainly been conducted with rodents and relevant information is scarce for porcine and bovine species. However, this basic information is of particular importance for elucidating the mechanisms that control the expression of genes for intestinal enzymes, in particular when digestive disorders occur. Development of molecular tools would bring new insights to the mechanisms of adaptation of genes to the environment. For several years, molecular biologists have been developing a variety of techniques to analyse gene expression, including Northern-blot, differential display assays, ribonuclease protection assays, qualitative and semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR). More recently, real time RT-PCR has allowed quantitative analysis of specific mRNAs. However, these methods are limited by the number of samples that can be simultaneously analysed. The widespread application of high-density microarrays should completely change our approach to answer scientific questions. The high-density microarrays provide the important advantage of allowing parallel quantification of the expression of a great number (almost unlimited) of genes from a given genome (Freeman et al., 2000; Deyholos et al., 2001). The cDNA microarrays can be used to answer almost any gene-dependent biological question in any organism. For instance, the human epithelial cell responses to infection with an invasive pathogen has been recently evaluated by using microarrays, in order to characterize and understand the host-pathogen interaction (Eckmann et al., 2000). Besides genomics, the use of proteomics would also bring additional and complementary knowledge. Finally, discovery of new roles and implications of digestive proteins would also help to expand our understanding of fundamental physiology in animals and humans. Such modern techniques and investigations would be expected to yield relevant data for elaborating feeding strategies that take into account the complex interactions between the diet, the microflora, the luminal milieu and the physiology of the small intestine, including the optimal functioning of the immunological and endocrine systems.

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