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Biophysical Aspects of Enzyme Regulation with Particular Reference to Defects in Sugar Digestion

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Important observations about the genetic defects of intestinal disaccharidases have come through the study of their intracellular pathways and processing of enzyme molecules in normal and altered human as well as animal mucosa using various techniques of molecular biology. The knowledge of these investigations has increased considerably our understanding of how primary and secondary deficiencies of brush-border-membrane (BBM) hydrolases are defined on a molecular level. In order to understand the pathology of intracellular misarrangements of BBM-hydrolases, the normal biosynthesis and processing of these microvillar proteins are discussed briefly.

METHODS

The study of biosynthesis and intracellular transport was performed using previously published techniques (1–4). Briefly, intestinal biopsies taken from patients undergoing investigations for malabsorption syndrome were studied *in vitro* using the organ culture technique (5), as modified by us (3). Newly synthesized proteins were immunoprecipitated with a panel of monoclonal antibodies after biolabeling of the mucosal explants with [^{35}S]-methionine. These were directed against the following BBM hydrolases: sucrase-isomaltase (SI), lactase-phlorizin-hydrolase (LPH), maltase-glucoamylase (MGA), aminopeptidase N (APN), dipeptidyldipeptidase IV (DPP IV), angiotensin-converting enzyme (ACE), and PABA-peptide hydrolase (PPH), a metalloendopeptidase capable of hydrolyzing biologically active peptides (6,7). Immunoprecipitates were subjected to SDS-PAGE under reducing or nonreducing conditions, followed by fluorography. Further subcellular localization of BBM molecules in Lowicryl-fixed tissue was performed by immunoelectron microscopy using the protein-A gold labeling technique (8).

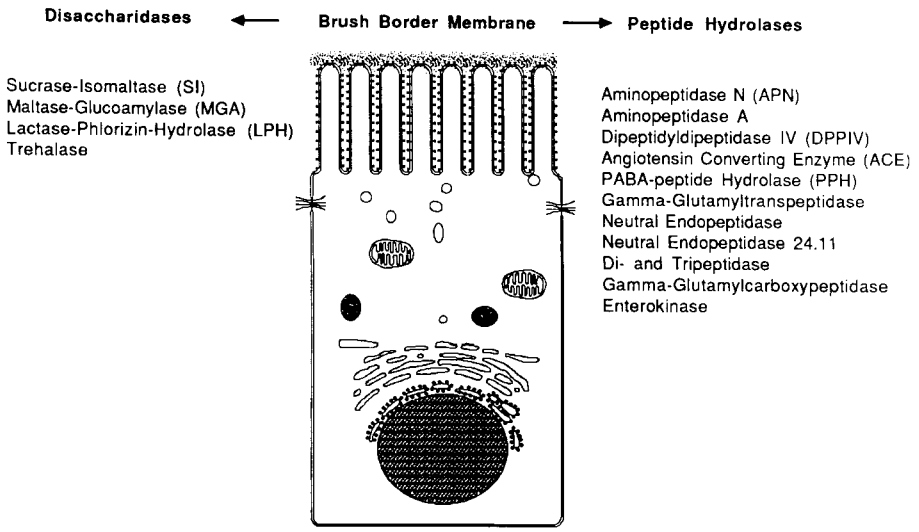

FIG. 1. Disaccharidases and peptide hydrolases involved in the digestion of disaccharides and oligosaccharides as well as oligopeptides from nutrients, pancreatic proenzymes, and biologically active peptides.

BIOSYNTHESIS OF INTESTINAL BRUSH-BORDER-MEMBRANE HYDROLASES IN STRUCTURALLY NORMAL MUCOSA

The mature intestinal epithelial cells are highly polarized and are composed of two main membranous regions: the apical cell membrane with its unique features of a brush border and the basolateral membrane. The microvillar membrane is characterized by a network of microvilli that contain the important glycoproteins responsible for the hydrolysis and absorption of micronutrients and minerals. For the degradation of various sugar and peptide molecules of different composition and chain length, the intestinal disaccharidases sucrase-isomaltase, maltase-glucoamlylase, lactase-phlorizin-hydrolase, trehalase, and a variety of peptide hydrolases (Fig. 1) are present within the microvillar region of the columnar epithelia in order to digest carbohydrate molecules and oligopeptides from nutritional intakes. The disaccharidases are the best studied brush-border hydrolases. Their enzymatic activities and their distribution throughout the gastrointestinal tract, as well as their age dependency, have been investigated by many groups of researchers (9–13). The biogenesis of the disaccharidases produced and processed by the mature enterocyte has been elucidated in mammals as well as in humans, demonstrating common pathways within the translational and posttranslational routes.

Sucrase-isomaltase is the best studied BBM hydrolase in all species, including humans. The data accumulated from these studies have led to a general understanding of how these hydrolases are synthesized and processed within the small intestinal enterocyte. After transcription (Fig. 2), a single-chain precursor (pro-SI_h) is produced

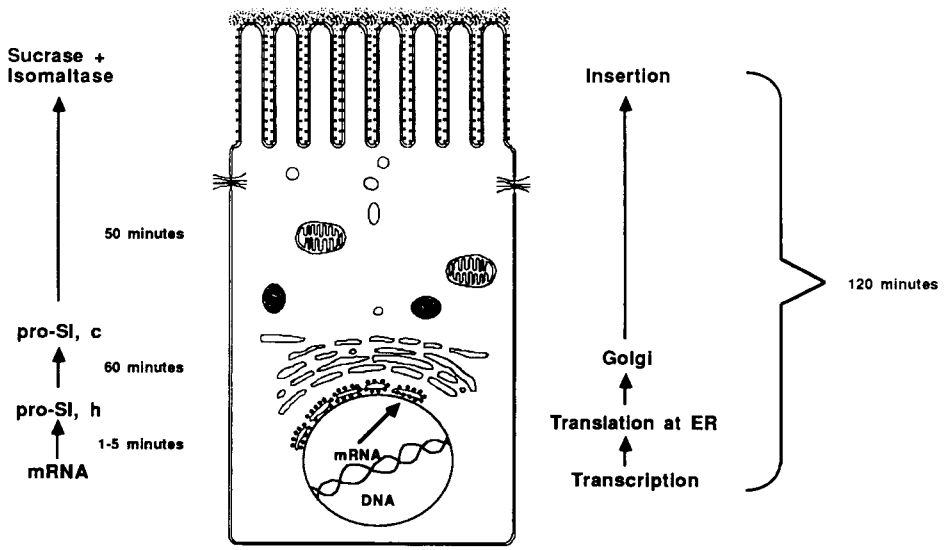

FIG. 2. Schematic outline of the biosynthesis and sorting of sucrase-isomaltase. ER, endoplasmic reticulum.

in the rough endoplasmic reticulum rich in mannose (i.e., high mannose precursor) containing carbohydrate residues that are N-glycosylated and with an apparrent molecular weight of 210 kDa in humans (14–18). From the rough endoplasmic reticulum, the pro-SI_h is transported to the Golgi apparatus, where trimming of the mannose residues and addition of complex carbohydrates occur to yield pro-SI_c (Mr = 245 kDa) (19). The complete primary structure of the pro-SI from rabbit is composed of 1,827 amino acid residues containing the two active catalytic subunits, isomaltase (140 kDa) and sucrase (120 kDa), which are associated by noncovalent, ionic interactions (20). After complex glycosylation in the Golgi, the pro-SI_c is translocated and inserted into the microvillous membrane by vesicular transport directly into the apical microvillous membrane. The exact route of how glycoproteins are transported from the Golgi to the microvillar membrane remains to be established. The time course of transport of the pro-SI_c from the Golgi into the brush-border membrane in a human colon carcinoma cell line (CaCo-2 cells) is rather slow (21). Similar transport kinetics were obtained when the biosynthesis of SI was investigated in the organ culture of human intestinal explants (4). Insertion of pro-SI_c into the microvillous membranes was obtained by anchoring a single hydrophobic segment of the molecule, which is located at the N-terminus of isomaltase (22). After insertion into the microvillar membrane, pro-SI_c is cleaved into sucrase and isomaltase by pancreatic proteases (4,15). The mature catalytic enzymes sucrase and isomaltase cleave various substrates, including sucrose, isomaltose, maltose, maltotriose, and amylose, as well as limit α-dextrins, which are derived from hydrolysis of amylopectins (23). Sucrase-isomaltase and maltase-glucoamylase play a major role in starch digestion during the

first month of life, since in human infants α-amylase is not developed during the first 6 months of life (24,25).

Lactase-phlorizin-hydrolase as the only β-glycosidase of the brush-border membrane has been reported in earlier work to be synthesized also as a single-chain precursor with a molecular weight of 150 kDa (26). However, conflicting results were obtained on the structure and identification of the precursor molecules. Sjöström *et al.* demonstrated in the pig that a precursor protein of 200 kDa was revealed (27). The same group reported that the precursor molecule of LPH in the pig small intestine is a membrane-bound polypeptide of 225 kDa that is intracellularly cleaved after complex glycosylation (28). Similar data were obtained in CaCo-2 cells (21). In human intestinal epithelial cells, a high-mannose precursor of 215 kDa was demonstrated in intestinal explants maintained in organ culture (3). Here, the intracellular cleavage of the high mannose precursor occurs during translocation of the molecule across the Golgi *before* complex glycosylation takes place. The mature form of LPH is then inserted into the membrane with an Mr of 160 kDa. The primary structure of the human lactase molecule is known and comprised of 1,927 amino acids in human and 1,926 amino acids in the rabbit (29). The mature form of lactase is cleaved from its precursor on position 866 of the whole molecule. In contrast to most other BBM hydrolases, the mature lactase is anchored within the lipid bilayer from its carboxyl end of the protein chain (29).

Maltase-glucoamylase (MGA) hydrolyzes 1 to 4 α-glycosidic linked glucose polymers, including maltose and maltotriose (30). The enzyme is developed early in gestation and contributes to the digestion of starch after birth (24). The biosynthesis of MGA is similar to that of sucrase-isomaltase as a precursor molecule of 225 and 245 kDa. The former represents the high mannose and the latter the complex glycosylated precursor of maltase-glucoamylase in the pig small intestine (27). Recently, we studied the biosynthesis and processing of MGA in human intestinal biopsy specimens and demonstrated that the molecule in contrast to SI and LPH does not undergo intracellular or extracellular proteolytic modifications (31).

BIOSYNTHESIS AND INTRACELLULAR PROCESSING OF BRUSH-BORDER HYDROLASES IN CONGENITAL DISORDERS

The study of genetic defects of disaccharidases in humans involves interesting models for understanding the molecular basis of selective protein transport from one cell organelle to the other because at present these steps of selective protein transport are poorly understood (32). The autosomal recessive disorder of congenital sucrase-isomaltase deficiency (33) has been suggested as an excellent model for the investigation of membrane traffic of proteins (1). Using the protein-A gold labeling technique (8) in combination with electron microscopy in an intestinal biopsy specimen of a girl with congenital sucrase-isomaltase deficiency, a posttranslational block of pro-SI could be detected where the immunoreactive precursor molecules became

stuck within the Golgi apparatus and were not further translocated into the brush-border membrane (34). Immunoprecipitation of this molecule revealed that it was indistinguishable from pro-SI_h. The existence of a defective pro-SI of the high-mannose type was later confirmed in another patient with the same disorder (35). Early studies on this enzyme deficiency suggested that there exist at least two types of sucrase-isomaltase deficiency because, in some of the patients described, a relatively high amount of remaining isomaltase activity was found. Whether this reflected also different translational or posttranslational defects within the enterocyte could only be speculated about (36). Studies on eight patients with congenital sucrase-isomaltase deficiency from different parts of Europe, however, using the same techniques demonstrated the existence of at least three phenotypes of this disorder: one in which sucrase-isomaltase accumulated intracellularly probably in the endoplasmic reticulum, as a membrane associated high-mannose precursor, one in which the intracellular transport was blocked in the Golgi apparatus, and one in which catalytically altered enzyme was transported to the cell surface (2).

The molecular defect in lactase deficiency is still a matter of debate. The congenital form of lactase deficiency, a very rare disorder in infancy, seems to be similar to adult-type hypolactasia in that the remaining lactase molecule is present (37). Both enzymatic activity and the amount of immunoreactive lactase are reduced in adult hypolactasia, suggesting a reduced synthesis of the molecule rather than a defective enzyme (38). Gel electrophoretic comparison of an infant with congenital lactase deficiency and an adult with lactase deficiency showed similarly reduced lactase protein bands (39). In a recent study performed by us in adult volunteers with lactose intolerance (40), it could be demonstrated that the rate of synthesis in individuals with LPH restriction was 6% of LPH-persistent controls, whereas biosynthesis of SI was increased to 230%. From data in organ culture experiments, lactose-intolerant adults could be divided into two groups. In the first group, the expression of LPH was very low over the culture period, and the rate of conversion from LPH precursor to the mature enzyme was similar to that of high LPH controls. In the second group, however, the initial expression of LPH during the first 4 hours of culture was greater but declined after 20 hours. This was accompanied by an accumulation of immunoreactive LPH in the Golgi region of the enterocyte. This finding was similar to that in earlier studies in patients with congenital SI deficiency (2).

In animals, two forms of lactase have been demonstrated, a proximal and a distal form of lactase within the small intestine, different in their carbohydrate composition (41).

Trehalase also has been shown to be deficient in a son and his father suffering from intolerance of young mushrooms (42). The physiological role of this particular brush-border enzyme as well as its deficiency state are poorly understood, and it remains unclear why evolution persisted in expressing an enzyme that does not seem to play a major role in the digestion of micronutrients, although it is very sensitive and can be induced precociously by prostaglandins in suckling animals (43).

BRUSH-BORDER HYDROLASES AND MUCOSAL INJURY

Whereas in normal intestinal biopsy specimens, precursor and mature forms of the majority of the BBM hydrolases are detected within 30 to 90 minutes, the biosynthetic situation in damaged mucosa is quite different. In acute untreated celiac disease, SI and APN are clearly detected in biosynthetic labeling experiments (44). The labeling intensity is, however, reduced in comparison to that in normal mucosa. DPP IV revealed very low labeling, and LPH, ACE, MGA, and PPH are absent within 6 hours of organ culture. A specific deficiency of a brush-border membrane hydrolase was not found. Small intestinal mucosa from patients with CD in remission synthesizes BBM hydrolases as in normal mucosa. When challenged with gluten at a standard dose of 0.5 g/kg/day for 1 month, the tissue showed only a slight mucosal damage, and the biosynthesis of BBM hydrolases became similar to that in the mucosa of untreated celiac disease. This suggests that the biosynthesis of BBM hydrolases is partly turned off by partial damage to the mucosal surface. Of particular interest is the fact that the biosynthesis of ACE is abolished completely in partially damaged mucosa. This enzyme has been shown to be responsible for the degradation of luminal gliadins up to 30% (45). In this regard, the undigested gliadins could exert a toxic effect on the mucosal surface and enhance the damage to the intestinal epithelia. The role of ACE in celiac disease needs to be studied in detail. In order to simulate the profound influence of gliadin on the biosynthesis of BBM hydrolases, we investigated this effect *in vitro* using mucosal biopsies of patients with CD before oral gluten challenge. The mucosal biopsies were cultured *in vitro* in the presence or absence of gliadin (Frazer fraction III, 1 mg/ml of medium) under the same culture conditions as described earlier. It could be demonstrated that within 24 hours of culture the biosynthesis of LPH, DDP IV, ACE, and PPH was completely abolished in the presence of gliadin, whereas that of SI was reduced by 72% and that of APN and MGA by 87%. Casein used as a control protein also inhibited the biosynthesis of LPH, MGA, DPP IV, ACE, and PPH to a certain degree, about 30%, much less than gliadin.

CONCLUSION

The study of BBM hydrolases on a molecular level has considerable potential in detecting transcriptional, posttranscriptional, and posttranslational defects in functionally and structurally damaged human intestinal mucosa. The techniques of cell biology contribute to our understanding of how cell organelles are involved in maintaining and repairing the function of the microvillous membrane. This will give us insights into the cellular events on the intestinal mucosa in acute and chronic malnutrition. In the near future, studies must be devised to determine for the turnover and degradation of these microvillar proteins in order to understand the pathomechanism of mucosal destruction on a molecular level. It remains speculative whether

transporters of minerals and micronutrients within the microvillous membrane undergo similar changes as are seen with the BBM hydrolases.

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DISCUSSION

Dr. Diamond: Here is a functional and evolutionary question about lactase. If you were devising a person from scratch and were told to make a person in whom lactase was expressed in childhood and not in adulthood, you probably would not waste any biosynthetic energy on lactase in adulthood. You would surely not put the energy into lactase and then fail to express it. Why do you think nature acts in such a nonsensical way?

Dr. Lentze: We know that the messenger is there and that the amount of messenger produced in lactase-deficient persons is less than it is in normal subjects. This suggests that there is a fine balance between the amount of protein being synthesized and the processing within the cell. There may be a requirement for a certain amount of protein to escape degradation while the enzyme is processed in the cell. If there is a lower mass of lactase in the cell, processing may be so slow that this particular part of the protein is rapidly degraded. That would explain our results of a posttranslational defect in lactase deficiency, but I have no idea why the messenger is still there. It would be much easier for nature to turn it off and not have it any more. So why waste all this energy still producing and degrading it? I can't answer the question fully.

Dr. Kretchmer: Isn't it rather strange that the message disappears during development? In the rabbit, the RNA disappears and then reappears. That result is very odd and begs the question: Is the second messenger, in fact, the same as the first messenger?

Dr. Lentze: We do not know at present. It is much more complicated than previously thought.

Dr. Rossi: Can you explain why in sucrase deficiency there is an increase of lactase activity? The second question is whether the enteropeptidases are involved? And finally, how do you think the signal for downregulation of biosynthesis of brush-border-membrane enzymes is achieved?

Dr. Lentze: In answer to your first question, to our surprise we found a marked oversynthesis of sucrase in a lactase-deficient individual compared to a normal. This represents a compensation for lack of biosynthesis of lactase because there is more space in the microvillous membrane. So nature probably compensates for lack of protein molecules of lactase, for example, by sucrase-isomaltase. As to the second question, I think enteropeptidases are not involved in this process. However, some other peptidases are involved in regulating these enzymes. They are not involved in the sugar hydrolases, but PABA-peptide hydrolase (PPH) is thought to regulate other proteins. We don't know which one, but there are many examples, such as secretory IgA. The secretory IgA molecule needs a final cleavage on the membranous part of the intestinal cell to be released into the lumen. It is not known which of the peptidases does this, but it may be PPH. In answer to the last question, it is very difficult to identify what signal actually is involved. There are several candidates, for example, second messengers, which would favor receptor binding or some other mediators, such as lymphokines.

Dr. Gracey: Michael, what is the situation in that very rare experiment of nature, congenital alactasia?

Dr. Lentze: We have not been able to study this because of the scarcity of patients.

Dr. Guesry: We have heard about the appearance of lactase at birth and its decline on

weaning. It has been suggested that lactase could be induced by feeding lactose (1). Could you please comment?

Dr. Lentze: It is known from various data that lactase in animals responds to substrate feeding, but lactase in humans is not inducible. There is no way of manipulating lactase in humans. There must be species differences, even in milk-intolerant people. If you force them to drink more milk, lactase activity will not be increased.

Dr. Kretchmer: The literature on the so-called induction of lactase goes back to Pfeiffer in 1909. What Dr. Lentze pointed out is quite correct. There is no evidence of induction of lactase in humans or, in fact, in any other animal. I believe the data suggesting that an increase in activity in the presence of substrate is protection of the enzyme from degradation, and the result is an increase in activity but it is not new synthesis.

Dr. Diamond: To go back to the question with which we began this discussion, Dr. Lentze's response illustrates the difference between what are called proximate and ultimate relationships. On this question of the disappearance of adult lactase and the continued energy expenditure for lactase synthesis, your response was in terms of the underlying cellular mechanism. That is a valid response, but there is also a separate question: Why does natural selection result in cellular mechanisms when natural selection could easily have disposed of them and saved biosynthetic energy?

Dr. Lentze: There may be another gene controlling the synthesis or turn-on and turn-off of lactase, but the so-called regulatory gene might not be working effectively. Evolutionally, one could argue that there is still some milk consumed in adulthood, but this is actually not the case. Perhaps Dr. Kretchmer could explain why we don't get rid of lactase.

Dr. Kretchmer: It is known that genetic systems leak. Take the example of phenylketonuria. There is still phenylalanine hydroxylase in the liver of individuals with that disease, but they cannot metabolize phenylalanine.

Dr. Truswell: How is sucrase-isomaltase degraded so quickly? Is it degraded by cells from the microvilli sloughing off? Is it degraded when it has a big load of substrate to metabolize? How does the messenger RNA get that information? Is it, for example, from the fructose passing through the cell?

Dr. Lentze: As far as degradation is concerned, we have no way of exploring this question in detail because it is so difficult technically. The only thing we know is the turnover time, which is measured in hours. As to your second question, yes, sucrase is inducible, but we have no idea how the signal reaches the nucleus.

Dr. Shafrir: I would like to extend the question about the induction of sucrase-isomaltase. It may be possible that it is not a message delivered by fructose or its metabolites to the protein synthesis system but a proliferation of the sucrase-producing cells. Do you have any evidence that there is such a proliferation on a sucrose-rich diet?

Dr. Lentze: As far as I know, at least from animal work, this has not been the case. The experiments reported on yesterday by Dr. Diamond showed that transporter binding sites are coming out of immature cells, but once they are on the villus you are not able to manipulate them. It might be similar for these enzyme. It is not the one cell already being there—it might just induce the ones in the lower part of the villi. Although by blocking it, the reverse is true. We are just at the beginning of understanding how all these intracellular events lead to clinical applications.

Dr. Gaskin: Do you mean in your description of cleavage of sucrase-isomaltase by trypsin that the enzyme is inactive in its combined state?

Dr. Lentze: No, it is active. The mature precursor of sucrase-isomaltase is as active as it is when it is hydrolyzed by trypsin. We know that from experiments in cells from a colon

cancer cell line that can be grown in culture dishes and exhibit a polar structure, even a brush border. From these experiments, it is known that the mature precursor is enzymatically active, but it is not known whether the high mannose precursor is enzymatically active or if only the complex glucosylated one is active.

Dr. Truswell: What is the angiotensin-converting enzyme doing in this position?

Dr. Lentze: We have underestimated angiotensin-converting enzyme in gastroenterology as a whole. Angiotensin-converting enzyme is thought to be responsible for the breakdown of gliadin peptides, and this has relevance to celiac disease.

Dr. Diamond: One detail from your report: a case of trehalase deficiency discovered in a son and father intolerant of young mushrooms. Can you tell me the natural distribution of trehalase, which I thought was confined to insects. Does this mean that mushrooms and social insects were important in early human diets?

Dr. Lentze: We have not been able to raise monoclonal antibodies against trehalase. As for the food content of trehalase, I should refer this question to Dr. Kretchmer.

Dr. Kretchmer: As you said, trehalase is the major sugar in insects, and it is also concentrated in the spores of mushrooms and other fungi. We have had a great deal of trouble raising an antibody, although there is no problem with lactase, sucrase, and maltase. The amazing thing about trehalase, in contrast to lactase and sucrase, is that it is present in other tissues, but its distribution is spotty throughout nature. It does not occur in the carnivores, for example. In the rat and in the rabbit, trehalase is present in the intestine and the kidney, and under certain conditions, renal trehalase serves as a digestive enzyme. Could you tell us if there is a homology among the sequences of lactase and sucrase.

Dr. Lentze: No, there is not.

Dr. Kretchmer: That disappoints me a great deal because I thought lactase was the mother enzyme, since it is in milk.

REFERENCES

1. McNeish AS, et al. Development of intestinal disaccharidases in preterm infants. *Lancet* 1983;2: 622–3.