Hypolactasia as a molecular basis of lactose intolerance

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Received 23 May 2006; revised 21 July 2006

Lactase-phlorizin hydrolase (LPH), a membrane-bound glycoprotein present in the luminal surface of enterocytes in the intestine is responsible for lactose intolerance, a phenomenon prevalent in humans worldwide. In the rodent intestine, the post-natal development of the LPH follows a specific pattern, such that the enzyme levels are high in the peri-natal period, but declines considerably upon maturation. The observed maturational decline in the LPH activity is very similar to adult-type hypolactasia observed in humans. Majority of the studies have been carried out using animal models or cell lines and a number of hypotheses have been put forward to explain the maturational decline of lactase activity such as: (a) decreased amount of lactase protein, (b) defect in post-translational modification of precursor lactase to the mature enzyme, and (c) synthesis of an inactive, high molecular weight lactase with altered glycosylation, however, the precise underlying mechanism of adult-type hypolactasia remains undefined. The present review describes the recent developments in understanding the regulation of lactase expression and the possible mechanism of adult-type hypolactasia, as a cause of lactose intolerance.

Keywords: Lactase-phlorizin hydrolase, Lactase activity, Adult-type hypolactasia, Lactase glycosylation and regulation, Lactose intolerance, Lactose gene expression

Introduction

Lactose, the major carbohydrate of milk is a disaccharide consisting of glucose and galactose linked through β-1,4-glycosidic linkage. It is synthesized in the mammary glands, during late pregnancy and lactation. Lactase-phlorizin hydrolase (LPH), a bifunctional enzyme having lactase (β-galactosidase; EC 3.2.1.23) and glucosidase (EC 3.2.1.62) activities1 is present in the brush border membrane of small intestinal enterocytes2. Lactase hydrolyzes lactose, whereas glucosidase is involved in the breakdown of substances such as phlorizin, flavonoid glucosides3 and pyridoxine-5′-β-D-glucoside4. LPH is the only enzyme that cleaves lactose into glucose and galactose and is essential for the survival of mammals early in the life. Post-natal development of LPH follows a specific pattern, such that the enzyme activity is quite high at birth, but decreases considerably upon weaning in most of the mammalian species, including the majority of human population, who are described as lactase non-persistent5,6. The deficiency of lactase activity leads to milk intolerance, a phenomenon prevalent in humans worldwide and is associated with symptoms such as abdominal pain, flatulence, nausea and diarrhoea7.

Lactose malabsorption

Lactose malabsorption is a normal physiological pattern8. It occurs as a result of the inability of some persons to digest significant amounts of lactose, because of inadequate amounts of lactase activity. Deficiency of lactase causes accumulation of undigested lactose in small bowel, leading to increased influx of fluids inside the intestinal lumen. The unabsorbed lactose is passed into the large intestine, which in addition to increasing fluid volume of gastro-intestinal content, is acted upon by the colonic bacteria, resulting in the production of short-chain fatty acids and hydrogen gas. The gas produced can cause abdominal pain, cramps, nausea and diarrhoea. In patients with common adult-type hypolactasia, the amount of ingested lactose required
to produce symptoms varies, but is reported to be 12-18 g lactose or 8-12 oz of milk. The severity of lactose malabsorption and the extent of symptoms are not only a function of lactase levels in the small intestine, but also of several other factors such as: (a) consumption of large quantities of lactose, exceeding the lactose digestion capacity (LDC), (b) rate of gastric emptying time, (c) intestinal transit time, (d) colonic microflora, and (e) age of subject

Biosynthesis of lactase

Lactase is encoded by a single lactase gene (LCT) of approx. 50 kb, located on chromosome 2q.21 in humans. The gene has 17 exons (Fig. 2) and encodes an mRNA transcript of 6274 nucleotides (Genbank X07994). However, in case of rodents, lactase gene (Lct) has a location on chromosome 13q12.

Lactase protein consists of 2027 amino acids in humans, 1926 in rabbits and 1928 in rats. It is a single polypeptide composed of a putative signal peptide of 19 amino acids, a large pro-portion of 849 amino acids and a mature protein that contains two catalytic sites and at the C-terminal end, a membrane spanning domain and short cytoplasmic domain (Fig. 3). Between the signal sequence and the membrane anchor, four homologous regions (I to IV) have been recognized. Only regions III (lactase) and IV (phlorizin hydrolase), the membrane spanning segment and the cytosolic C-terminus makes the “mature” LPH, which can be isolated from the brush border membrane.

Initially, lactase is produced as a 200-220 kDa precursor (prolactase) containing high mannose N-glycosylation. The prolactase is synthesized and folded into a 3-D configuration within rough endoplasmic reticulum (RER) and subsequently transported to Golgi apparatus, where N-linked glycans are converted into complex forms. Commonly, this complex N-glycosylated prolactase becomes O-glycosylated to some extent, leading to an increase in molecular mass from 225-240 kDa of prolactase. During or shortly after the transport of the prolactase to the microvillus membrane, the precursor is proteolytically cleaved into its mature form of 130-160 kDa in humans and 120-130 kDa in rats. Proteolytic processing of lactase by trypsin occurs after its passage through the Golgi apparatus, but before insertion into the plasma membrane (Fig. 4). A prodomain, comprising the N-terminally, located 734 amino acids of pro-LPH (LPHα) is found to be an intramolecular chaperone that is critically essential in facilitating the folding of the intermediate form LPHβinitial.

Developmental pattern of intestinal lactase activity

During post-natal development, intestinal maturation is associated with morphological changes, proliferation and differentiation of cells and digestive
adaptation to nutritional changes such as decrease in lactase activity and a rise in sucrase, maltase and aminopeptidase activities. Lactase is present predominantly along the brush border membrane of differentiated enterocytes and exhibits a well-characterized development pattern in all mammals. This restricted expression makes it an ideal marker for fully differentiated enterocytes.

mRNA encoding LPH in the mature human intestinal epithelium has a complex expression along the crypt-villus axis. The expression of mRNA and protein is very low in crypt cells. At crypt-villus junction high levels of LPH mRNA appear, which are maintained at similar levels in villus tip cells. Another interesting observation is that like lactase protein, mRNA encoding lactase is not uniformly distributed in mature enterocytes and is concentrated in the cytoplasm, located below the apical membrane. Thus, there seems to be co-localization between the lactase mRNA and lactase protein in the apical part of the enterocytes. Recently, we demonstrated that in vitro translation of lactase is impaired in adult rat intestine, which suggested that translational efficiency of mRNA to lactase is greatly curtailed during post-natal development. However, the mechanism and expression of the restricted localization of the lactase mRNA are not known.

Lactase activity exhibits a characteristic proximal to distal gradient of expression along the length of small intestine. In adult rat and rabbit, it is highest in the middle jejunum and decreases both proximally and distally, resulting in minimal activity in proximal duodenum and terminal ileum. The lactase expression around birth differs between rodents and humans. In rats, the lactase activity is detectable on 18th day of gestation and is maximal during 1st week after birth and then begins to decline, reaching the adult values by the end of 4th post-natal week. However, in humans, the lactase expression is highest at the time of birth, probably reflecting a difference in the intestinal maturation between rodents and humans. In contrast to humans, the rodent intestine is not fully mature at birth, as fully developed crypts are not detected until 2-3 days after birth. Lactase expression after weaning is patchy in the distal part of the small intestine in rabbits. The same phenomenon has been found in persons with adult-type hypolactasia, both at the protein and mRNA levels as well as in distal parts of the small intestine in weaned rats. These findings imply that the low lactase activity observed after weaning is not a consequence of general down-regulation of lactase expression in all enterocytes, but a complete turn-off of lactase expression in majority of the enterocytes.

**Etiology**

Lactose malabsorption is prevalent worldwide and occurs due to primary lactase deficiency or adult type hypolactasia, secondary lactase deficiency or acquired hypolactasia, and congenital lactase deficiency (CLD) or alactasia. Adult-type hypolactasia is the most common form of lactose malabsorption, which occurs due to deficiency of lactase. Acquired hypolactasia is present in a variety of gastro-intestinal diseases with histological evidence of mucosal damage and increased transit time in the jejunal mucosa. CLD is very rare and occurs as a result of complete loss of lactase activity. It manifests itself as severe diarrhoea during breast-feeding in first few days of life, which leads to poor weight gain, as long as lactose-containing milk is given to the patient. It is more common in the isolated Finnish population and the underlying gene defect has been assigned to chromosome 2, having location 2q21-22.

Different methods have been used for determining the lactase deficiency. Although, an intestinal biopsy is the only direct method for determining lactase activity, it cannot be regarded as a standard method, because mucosal lactase activity varies along the length of small intestine. The genetic test of C/T (-13910) polymorphism could be used as first-stage screening test for adult-type hypolactasia in children. In this case, intestinal biopsies after assaying lactase activity could be genotyped for the C/T (-13910) variant using PCR mini-sequencing.

![Fig. 4—Schematic representation of lactase-phlorizin hydrolase biosynthesis](image-url)
Measuring blood glucose concentration, after an oral dose of 50 g lactose is the most commonly used method for testing tolerance to lactose\textsuperscript{42}; rise in blood glucose levels less than 20 mg/dL (1.1 mmol/L) is considered as an indication of lactose malabsorption. However, because of inter-individual variations in gastric emptying and glucose metabolism, this test cannot be considered sensitive to establish lactase deficiency. Another test involves the determination of urinary galactose after inclusion of ethanol with the lactose load by decreasing galactose metabolism to glucose.

The measurement of breath hydrogen response after oral dose of lactose is considered to be fairly reliable test in detecting lactose malabsorption\textsuperscript{43}. This test is based on the principle that unhydrolyzed lactose is fermented by the colonic microflora, producing hydrogen and methane, which get diffused into the blood circulation and excreted in expired air. The test is positive in 90% of patients with lactose malabsorption\textsuperscript{43,44}. Recently, an automated rapid genotyping assay for LPH C→T\textsubscript{13910} has been developed and a relationship was found between positive lactose breath hydrogen test (LBHT) and symptoms of lactose intolerance\textsuperscript{45}.

**Prevalence of adult-type hypolactasia**

Lactase non-persistence, also known as adult-type hypolactasia is an autosomal recessive condition that affects about half of the world’s population\textsuperscript{38}. Its prevalence varies considerably between different races and populations. It is the predominant phenotype in the native populations of Australia and America and in the Pacific, East and Southeast Asia and Tropical Africa\textsuperscript{46}. With the exception of the population of northern and central Europe, 70-100% of adults worldwide are lactose malabsorbers. Lactose tolerance or breath hydrogen tests have provided accurate mapping of the prevalence of lactose malabsorption worldwide. The prevalence of primary lactose malabsorption is 3-5% in Scandinavia, 17% in Finland, 2-15% in northern Europeans and 6-23% in American Whites and central Europeans\textsuperscript{47}. The maximum frequency occurs in case of American Indians (80-100%) and Asians (95-100%)\textsuperscript{10}. The frequency increases as one moves south and west. A similar trend is seen from north to south of India\textsuperscript{48}. The prevalence of lactose malabsorption is found to be 30% in Northern India and 70% in Southern India respectively\textsuperscript{49,50}.

**Polymorphisms associated with adult-type hypolactasia**

Several nucleotide polymorphisms have been described in the lactase gene (LCT) and the surrounding regions\textsuperscript{51,52}. Two single nucleotide polymorphisms (SNPs) C/T\textsubscript{−13910} and G/A\textsubscript{−22018} are found to be associated with adult-type hypolactasia\textsuperscript{52}. C\textsubscript{−13910} at position -13910 upstream of lactase gene is 100% associated and a G\textsubscript{−22018} at position -22018 is more than 95% associated with lactase non-persistence, in Finnish population. Troelsen\textit{et al}\textsuperscript{53} investigated the role of these nucleotide polymorphisms for LPH gene expression. They reported that T\textsubscript{−13910} variant enhances the LPH promoter approximately 4 times more than the C\textsubscript{−13910} variant in differentiated Caco-2 cells, showing that the molecular difference between lactase persistence and non-persistence is caused by mutation at position -13910. The T\textsubscript{−13910} and A\textsubscript{−22018} variants are associated with lactase persistence\textsuperscript{54}. Individuals with the persistent T\textsubscript{−13910} allele showed 11-times higher LCT mRNA content in their intestinal mucosa, compared to those with the non-persistent C\textsubscript{−13910} allele, supporting regulation of LCT gene at the transcriptional level. Identification of the C/T\textsubscript{−13910} variant has enabled haplotype analysis of the close polymorphisms and microsatellite markers in the genomic region\textsuperscript{54}.

**Lactase and intestinal glycosylation**

The apical membrane of intestinal cell is exposed to a rapidly changing environment in suckling animals, which culminates at weaning and requires adaptive alteration in membrane composition. Chemical analysis of microvillus surface during suckling period shows that membrane is rich in sialic acid\textsuperscript{35}, but has low level of fucose. Upon weaning, sialic acid content decreases sharply, while fucose content begins to rise to attain adult levels. After weaning, there occurs a complete reversal of molar ratio of fucose/sialic acid from suckling animals\textsuperscript{56}.

The age-related changes from sialylation to fucosylation of BBM-bound glycoproteins are due to a shift in sialyl and fucosyl transferase levels in small intestine\textsuperscript{57}. During post-natal development, the active form of lactase, before weaning is sialylated and inactive form after weaning is fucosylated\textsuperscript{58}. This shift from sialylation to fucosylation may also be associated with susceptibility of lactase to degradation by luminal proteases, thus leading to hypolactasia. Kaur\textit{et al}\textsuperscript{59} have shown the decrease in lactase
activity in 8-day-old pups on incubating the membranes with luminal wash isolated from the adult rat intestine. Some investigators have suggested that the decrease in lactase-specific activity in adult rats is due to the synthesis of an inactive high molecular weight protein with altered glycosylation, rather than active form found in young animals. The structure of glycan chains may also be important for the transport of lactase and for the integration into the apical membrane of the enterocyte. Administration of thyroxine to rats is reported to decrease lactase activity and a simultaneous increase of a fucosytated inactive form of this enzyme, which is abundant in adult membranes, but only present in trace amounts in suckling rat intestine.

**Regulation of lactase gene expression**

The lactase gene has a complicated pattern of regulation during post-natal development. It is maximally expressed in apical villus cells and the dietary sucrose elicits the enhancement of its expression in villus cells, where respective mRNA transcripts are accumulated in large quantities. Its expression is spatially restricted along the longitudinal axis of the gut. Although the mechanism of regulation of the spatial and temporal restriction of its expression is not fully understood, however, it is widely accepted that regulation of its expression occurs mainly at the post-transcriptional level in the jejunum, whereas it is controlled at the pre-translational level in the colon. A close correlation exists between lactase activity and its mRNA levels in rats and humans. The lactase activity is also reported to be regulated at the level of gene transcription. It has been demonstrated that a distinct 5'-region of the lactase promoter directs the intestine-specific expression in small intestine of transgenic mice and the regulatory sequences are localized to a 1.2 kb region upstream of the lactase transcription start site.

*In vivo* studies have identified the lactase promoter and the transcription factors regulating its expression. A lactase promoter of 150 bps, found just upstream of the transcriptional initiation site is conserved in rodents, rabbits and humans. The presence of these conserved sequences indicates the localization of regulatory cis-elements (transcription factor-binding sites) in this region. Three cis-elements, i.e. CE1α, CE2c and a GATA site have been identified using deleted and mutated versions of receptor/promoter gene constructs. The cis-elements bind to the transcription factors for activating a particular gene expression. The importance of binding of transcription factors (Cdx-1, HNF1α and GATA-4) to these sites has been investigated in cell cultures using CaCo-2 cells, isolated from a primary colonic tumour from a 72-year-old Caucasian male. The expression of the transcription factors plays a key role in demonstrating the regulation of LCT gene expression.

Cdx-2 (caudal-related homeodomain) is an intestine-specific homeodomain-containing transcription factor that activates the promoters of intestinal genes through specific interactions with cis element CE1α. It plays an important role as a regulator of intestinal specific expression and differentiation of the lactase and sucrase-isomaltase promoters. LPH gene expression is repressed in the non-Cdx-2 producing cells, because their binding sequence is different from that of Cdx-2 binding sites. The Cdx-2 is highly expressed in colon and its expression decreases from distal to proximal portion of the intestine, but its expression is not the sole criterion for demonstrating lactase gene expression. Other transcription factors such as HNF-1α and GATA also play a key role in regulating the lactase expression.

HNF1 (hepatocyte nuclear factor – 1α and 1-β) specifically binds to CE2-site, which has been shown to be important for lactase promoter activity. HNF-1α is expressed along the entire length of the small intestine. It is the main activator through CE2c site, as it activates the lactase promoter approximately 10-times more than HNF-1β. Interaction (*in vivo* and *in vitro*) between GATA-5 and HNF-1α is required for the synergistic activation of the human LPH promoter. It occurs through C-terminal zinc finger and basic regions of GATA-5 and the homeodomain of HNF-1α. GATA factors (-4, -5 and -6) also play a role in binding and activating lactase promoter genes. GATA-4 is the main GATA factor that binds the lactase promoter. GATA-4 and 5 are mainly expressed in the differentiated intestinal epithelial cells, whereas GATA-6 is highly expressed in the proliferating crypt cells. GATA-4 exhibits a gradual expression is repressed in the non-Cdx-2 producing cells, because their binding sequence is different from that of Cdx-2 binding sites. The Cdx-2 is highly expressed in colon and its expression decreases from distal to proximal portion of the intestine, but its expression is not the sole criterion for demonstrating lactase gene expression. Other transcription factors such as HNF-1α and GATA also play a key role in regulating the lactase expression.

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The expression of HNF-1α increases after weaning, whereas the expression of GATA-4 and Cdx-2 remained relatively unchanged during post-natal development of rat intestine. However, the influence of these changes on the post-natal decline of lactase activity remains unknown. Studies by Wang *et al.*
have shown that rat lactase promoter contains a pdx-1 binding site, which is expressed in duodenum and proximal jejunum and its expression is inversely related with lactase expression.

**Dietary and hormonal regulation of lactase**

Both nutritional and hormonal factors are involved in the decline of specific activity of lactase. The starvation, however, induces lactase levels in rats. A high carbohydrate diet for 1 week, with corn starch forming 70% of the total energy and with no added lactose, increased disaccharidase activities (lactase, sucrase and maltase) in the rat jejunum. Elevated mRNA levels for lactase have been reported in rats fed sucrose-enriched diet, within 12 h of intake, suggesting that dietary sucrose enhances the transcription of lactase gene.

Kaur et al. reported the effect of saturated dietary fats on the activity of intestinal disaccharidases. Results showed a decrease in lactase activity in rats fed saturated diets (coconut oil) compared to control. However, polyunsaturated fat (corn oil) or fish oil diets did not alter lactase activity. The high protein diet (30% protein) reduced lactase activity and resulted in low levels of total hexoses and sialic acid content in brush borders of mice intestine. The imposition of undernutrition in weaning rats, modulate the activities of various brush border enzymes. It is well established that lactase activity is high and sucrase activity is almost absent during the peri-natal period. The nutritional restrictions imposed in suckling rats augmented lactase and diminished sucrase activities, suggesting that maturational development of these enzymes was delayed under these conditions.

Conflicting results of the effects of alcohol intake on lactase activity have been reported. The lactase activity is reported to decline in adult rats exposed to 30% ethanol [in drinking water (v/v)] for 3 months.

Lactose intolerance is also manifested by poor tolerance of dairy products, leading to low calcium intake and poor calcium absorption from dairy products. Increased bone turnover and decreased bone mass have been reported, especially in men and menopausal women, suffering from lactose intolerance. This has been attributed to elevated levels of bone turnover markers (urinary deoxy pyridinoline cross-links) that were negatively correlated with total daily calcium intake and positively correlated with parathyroid hormone.

A number of hormones influence lactase activity, especially during the post-natal development. Generally, pituitary and adrenal cortical hormones enhance intestinal maturation during post-natal development. Administration of thyroxine decreases lactase activity in dose-dependent manner. In thyroxine-treated animals, lactase activity returns to age-specific normal levels, before the low lactase activity of adult rats was attained. Administration of thyroxine to adult rats leads to the development of villus hyperplasia and down-regulation of lactase mRNA. In adult rats, thyroxine specifically decreases the amount of lactase, whereas the structure of crypt-villus remains unaffected. However, Castillo et al. demonstrated that in the absence of thyroxine and growth hormone, the intestinal maturation gets impaired, whereas lactase activity remained abnormally high in 6 days-old pups.

The thyroxine injection soon after birth led to decline in lactase activity in the colon than in the jejunum in rats. These findings have been confirmed by comparing the lactase activity both in control (injected with NaCl) and hormone-injected rats. Administration of thyroxine soon after birth showed a 20% and 70% decrease in the lactase activity in jejunum and colon respectively, in rats. In the jejunum, thyroxine caused a 25% and 15% decline in LPH-type proteins and LPH mRNA, respectively, whereas in the colon, the LPH-type proteins as well as the 6.3 kb mRNA were 3-fold less compared to controls. However, exogenous administration of epidermal growth factor (EGF) caused a slight increase of specific activity of lactase, and the amounts of LPH-type proteins and mRNA. In the colon, in contrast, EGF injections caused simultaneous decrease (70%) in 6.3 kb mRNA transcript, LPH-type proteins and the specific activity of lactase. Similarly, cortisone administration increased both mRNA levels and lactase activities in 6 days-old pups, whereas the administration of cortisone along with thyroxine antagonized the enhancing effect of cortisone on lactase gene expression. In the absence of thyroxine, lactase activity remained elevated in neo-natal rats and failed to decline at weaning, while half-life of the enzyme was doubled.

**Conclusions**

It is apparent that multiple factors, such as (a) decreased amount of lactase protein, (b) synthesis of an inactive, high molecular weight lactase or defect in post-translational modification of precursor lactase to the mature enzyme, and (c) mRNA levels encoding lactase are differentially altered and translated during...
post-natal development⁴⁹, are involved in observed decline and regulation of lactase activity in intestine. The phenomenon of lactase expression is apparently complex and multifactorial in nature. This needs further studies to establish the precise mechanism of hypolactasia in human populations.

Acknowledgement

We thank Indian Council of Medical Research, New Delhi for the award of Senior Research Fellowship to Ms. Kamaljit Kaur.

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