Susceptibility of lactase to luminal proteases in developing rat intestine

Kamaljit Kaur, Safrun Mahmood,* Akhtar Mahmood

Department of Biochemistry, Panjab University, Chandigarh 160 014 and Department of Experimental Medicine and Biotechnology, Postgraduate Institute of Medical Education and Research, Chandigarh 160 012

**Background:** Postnatal development of rat intestine is associated with a decline in brush-border lactase activity. This phenomenon is similar to the adulthood hypolactasia in humans. However, the mechanism underlying this process is not understood. **Methods:** The effect of luminal proteases from adult rat intestine on the intestinal lactase activity in animals aged 7, 14, 21 and 30 days was studied in in vitro experiments. Lactase levels were estimated using enzyme assays and Western blot analysis. **Results:** Incubation of purified brush borders with increasing concentrations of luminal proteases reduced the lactase activity in intestine of 7-day-old rats, but not in that of adult animals. Western blot analysis revealed low signal of the 220-kDa lactase protein in 7-day-old animals, but not that of older weaned animals. **Conclusions:** Our findings suggest that luminal proteases may be responsible for the maturational decline in intestinal lactase activity.

*Indian J Gastroenterol 2006;25:179-181*

Deficiency of lactase phlorizin hydrolase (LPH; EC 3.2.1.23.62) is responsible for milk intolerance among humans, a disorder prevalent worldwide. Postnatal development of this enzyme follows a specific pattern in rodents, such that the enzyme levels are high in the perinatal period but decline considerably upon maturation. This maturational decline in lactase activity is similar to the adult-type hypolactasia in humans, but its underlying mechanism is unknown.

The present study was done to examine the effect of luminal proteases on brush-border lactase during postnatal development of rat intestine.

**Methods**

Inbred albino rats (Wistar strain; 10-15 in each group) of different ages (7, 14, 21 and 30 days) and male adult rats free of any infection were used. The animals were maintained as per standard guidelines. The experimental protocol was approved by the ethics committee of our institution. Rats were sacrificed under light ether anesthesia, and intestinal tissues were taken out and cleaned by flushing with chilled normal saline.

**Preparation of brush border membranes (BBM)**

BBM were prepared from 10-15 pooled intestines by the method of Kessler *et al.*, and suspended in 50 mM sodium-maleate buffer pH 6.8; this led to 10-12-fold enrichment of lactase enzyme.

**Biochemical determinations**

To determine the susceptibility of lactase activity to degradation by luminal proteases, BBM (400 mg protein) were incubated in vitro with different concentrations of luminal fluid isolated from adult rat intestine (40-240 mg protein/mL) at 37°C for 1 hour. The reaction was stopped by adding 10 mM phenylmethanesulphonyl fluoride (PMSF) and 2 mM EDTA. Lactase activity was then assayed in the BBM, using the method of Dahlqvist. Protein was determined by the method of Lowry *et al.*, using bovine serum albumin as the standard.

**Western blotting**

BBM proteins were resolved on SDS-PAGE using the method of Lammeli. In brief, 10% separating gel (lower gel) and 3% stacking gel (upper gel), both freshly prepared, were used. Western blot analysis was performed as per the method described earlier, using a rabbit anti-rat intestinal lactase (a gift from Dr. D H Alpers, Washington University Medical School, St. Louis, MO, USA) as the primary antibody and goat anti-rabbit IgG (Bangalore Geneli, India) as the secondary antibody.

Statistical analysis was done by using the Student’s *t* test.

**Results**

Lactase activity in the purified rat intestinal BBM on day 30 was nearly 88% lower than that on day 7 of life (Fig. 1). Western blot analysis for lactase in BBM revealed two bands of 220-kDa and 130-kDa proteins at all ages of postnatal development. The intensity of the 220-kDa band was high in 7- and 14-day-old rat intestine and decreased with age thereafter (Fig. 2). The 220-kDa lactase isoforms protein showed a progressive decline in amount with age.

Incubation with increasing concentration of
Fig 1: Postnatal development of lactase activity in rat intestine. Values are mean (SD) of 4 observations each.

Luminal contents induced a progressive decline (46%) in lactase activity of BBM from 7-day-old rat intestine (Table 1), but not of that from adult animals. On Western blot analysis, incubation of BBM with increasing concentrations of luminal wash (from 0.04 to 0.24 mg protein/mL) led to a progressive decline in the intensity of the 220-kDa lactase band (Table 2). In comparison, the intensity of this band did not change in BBM from adult animals after incubation with luminal wash.

Discussion

Our data show that intestinal lactase activity decreases significantly after day 14, as has been shown previously by Lee et al.3 Treatment with increasing concentrations of luminal wash led to a decline in the lactase activity of BBM from the intestine of sucking mice; however, no such effect was seen in BBM from adult animals. These results are in accordance with previous findings of an increase in the activity of luminal proteases in rat intestine after weaning.9

Several mechanisms have been proposed to explain the occurrence of adult-type hypolactasia, including (a) decreased production of lactase protein,10 (b) synthesis of an inactive, high-molecular-weight lactase,11 and (c) defective post-translational modification of a lactase precursor to the mature enzyme;12 however, the exact mechanism for decline in lactase activity remains unknown.

In the perinatal period, the BBM is rich in sialic acid and contains low amounts of fucose. During weaning, there is a shift from sialylation to fucosylation,13 due to a change in levels of sialyl and fucosyl transferases in the small intestine.14 We propose that this shift makes the intestinal lactase more susceptible to proteolytic degradation by luminal proteases. In young ones, the enzyme is sialylated with little luminal protease activity; decreasing sialylation with age leads to increased protease activity.15,16

Studies by Buller et al17 suggested that lower levels of lactase mRNA in adult rat intestinal tissue than those in the suckling animals are responsible for reduction in lactase enzyme activity among adult animals. However, Freund et al18 described significant amounts of lactase-encoding RNA in 3- and 24-month-old adult rats with low lactase activity, and failed to find a correlation between the lactase RNA transcript levels and the lactase activity. We recently showed that in vitro translation of RNA to lactase is impaired in adult rat intestine, which suggests that

Table 1: Effect of luminal contents on intestinal brush border lactase activity in suckling and adult rat intestine

<table>
<thead>
<tr>
<th>Concentration of luminal contents (mg/mL)</th>
<th>Suckling</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.097 (0.018)</td>
<td>0.0027 (0.001)</td>
</tr>
<tr>
<td>0.04</td>
<td>0.079 (0.024)</td>
<td>0.0027 (0.005)</td>
</tr>
<tr>
<td>0.08</td>
<td>0.076 (0.021)</td>
<td>0.0031 (0.001)</td>
</tr>
<tr>
<td>0.16</td>
<td>0.063 (0.013)*</td>
<td>0.0026 (0.0002)</td>
</tr>
<tr>
<td>0.24</td>
<td>0.052 (0.006)**</td>
<td>0.0025 (0.001)</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01 as compared to adult

Values are mean (SD) of 3 observations.

Table 2: Densitometric scan of Western blot for brush border lactase in 7-day-old and adult rat intestine incubated with increasing concentrations of luminal wash

<table>
<thead>
<tr>
<th>Concentration of luminal contents (mg/mL)</th>
<th>Arbitrary units</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7-day-old</td>
</tr>
<tr>
<td>0</td>
<td>5.4</td>
</tr>
<tr>
<td>0.04</td>
<td>5.1</td>
</tr>
<tr>
<td>0.08</td>
<td>4.5</td>
</tr>
<tr>
<td>0.16</td>
<td>3.7</td>
</tr>
<tr>
<td>0.24</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 2: Densitometric scan of Western blot for brush border lactase in 7-day-old and adult rat intestine incubated with increasing concentrations of luminal wash.
translational efficiency of mRNA to lactase decreases during postnatal development.\textsuperscript{19}

In conclusion, the observed decline in rat intestinal lactase activity on maturation is multi-factorial, and is mediated at least in part by an increase in the protease activity of the intestinal luminal contents.

\textbf{References}


Correspondence to: Professor Akhtar Mahmood. E-mail: akhtarmah@yahoo.com

Acknowledgement: Ms Kamaljit Kaur was supported by a Senior Research Fellowship of the Indian Council of Medical Research, New Delhi

Received August 25, 2005. Received in final revised form June 2, 2006. Accepted June 9, 2006