In vitro biosynthesis of lactase in suckling and adult rabbits

Regulatory mechanisms involved in the decline of the lactase activity

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Steady state forms, levels and the in vitro biosynthesis of lactase-phlorizin hydrolase (LPH) proteins have been studied in proximal and middle intestine of suckling and adult rabbits. In most adult tissues the lactase activity and the LPH protein content were low and the synthesis rate of the 200 kDa lactase precursor was reduced in comparison to suckling tissues. In a few tissues with low enzymatic activity the LPH protein content was relatively high, and high lactase synthesis occurred. In addition, the ratio (labeled lactase)/lactase protein) was lower in the middle jejunum of the adult rabbit than in the proximal region. Both decreased synthesis of LPH precursor and increased turnover or inactivation of the enzyme may cause the decline of the lactase activity.

Lactase; Biosynthesis; Enzyme inactivation; Suckling and adult rabbit

1. INTRODUCTION

In previous studies on the biosynthesis of LPH in surgical biopsies from normal proximal jejunum of 37 Neapolitan adults [1], we demonstrated that there are three groups of hypolactasic human tissues: (i) with reduced [35S]methionine incorporation and normal processing of LPH proteins [2,3]; (ii) with reduced label incorporation and delayed processing [3]; (iii) with label incorporation into LPH proteins higher than expected on the basis of the low lactase activity. In rabbit [4] the rate of in vitro biosynthesis of LPH proteins and the strictly related levels of LPH mRNA were found to be high in the proximal and medial adult small intestine, whereas the lactase activity was lower in the proximal small intestine.

We have now measured in suckling and adult rabbits the in vitro biosynthesis of LPH proteins in proximal and middle intestine (in explants and isolated enterocytes): in most adults [35S]methionine incorporation into the LPH proteins was reduced, and in these samples the lactase activity and the LPH protein content were low as compared to the suckling. But there were also adults with low enzyme activity and relatively high steady state levels of LPH proteins; in some of these, high label incorporation into LPH proteins occurred.

2. MATERIALS AND METHODS

2.1. Animals and preparation of small intestine

New Zealand white rabbits 15 days or six months old were used. After sacrifice the jejunum plus ileum was excised and 3 cm sections from the proximal region of the adult intestine (3 cm after the ligament of Treitz) and from the middle region of the suckling and of the adult intestine were extensively rinsed with 0.9% NaCl and rapidly processed for organ culture, cell isolation, lactase and protein assays, or stored at −80°C.

2.2. Isolation of enterocytes

The cell isolation technique described by Smith et al. [5] was utilized. 3–4 mucosal specimens (150–200 mg wet weight) were incubated in a shaking water bath 10 min at 37°C in 20 ml of 1.5 mM KCl, 96 mM NaCl, 27 mM sodium citrate, 8 mM KH2PO4, 5.6 mM Na2HPO4, pH 7.3 and then 30 min in 1.5 mM EDTA, 0.5 mM diethiothreitol, 10 mM NaH2PO4, 154 mM NaCl, pH 7.3. The cell suspension was separated and centrifuged. The pellet was washed in 0.9% NaCl and utilized for DNA and lactase protein assays or stored at −80°C. Almost all of enterocytes were recovered, as assessed by measurement of residual lactase activity in the tissue specimens and by microscopic evaluation of the sample.

2.3. Determination of the LPH protein content

Lactase was immunosolated from small intestinal mucosa or isolated enterocytes and analyzed by SDS-PAGE as previously described [6]. After electrophoresis the gels were stained with Coomassie brilliant blue (R250) and dried. The lactase protein content was determined by densitometric scanning using the laser densitometer Ul-
troscan (LKB, Bromma, Sweden). Bovine serum albumin was utilized as standard [8].

2.4. Lactase and protein assays

The lactase activity and total protein were measured as previously described [9].

2.5. DNA quantitation

The quantitative determination of DNA in isolated cells was performed according to Labarca and Paigen [10]. Aliquots of homogenates before solubilization were resuspended in 3 ml of 50 mM sodium phosphate, 2 M NaCl, pH 7.4 containing 1 μg/ml fluorescent compound Hoechst 33258 (Sigma Chem. Corp., St. Louis, MO, USA). After 5 min the enhancement of fluorescence was measured (excitation: 356 nm; emission: 458 nm). DNA purified from the small intestine of adult rabbit was used as standard.

2.6. Organ culture and in vitro biosynthesis

Explants were cultivated as previously described [6]. After one hour incubation in methionine-free medium, 150 μCi [35S]methionine were added, for various labeling times. The samples were collected, rinsed in ice cold 0.9% NaCl, and processed as described in Section 2.3. Following SDS-PAGE the gels were stained with Coomassie brilliant blue, dried, and exposed to Hyperfilm β max (Amersham International plc, Buckinghamshire, England) or processed for fluorography as described by Bonner and Laskey [11]. Quantification of Coomassie stained and fluorographic bands was carried out by densitometric scanning.

2.7. Determination of labeled methionine/methionine ratio in total protein

Aliquots of labeled homogenates at 1 h were precipitated by 30% trichloroacetic acid (TCA) 30 min on ice. After centrifugation, the pellets were washed twice with 10% TCA, once with acetone and dried. The pellets were resuspended in 6 N HCl and incubated at 120°C 20 h under vacuum. The hydrolyzed samples were analyzed for methionine content by HPLC System Gold adapted amino acid analyzer (Beckman Instruments, Palo Alto, CA, USA), and for labeled methionine by liquid scintillation analyzer 1600 TR (Packard Instrument Co., CT, USA).

3. RESULTS

3.1. Lactase activity and proteins of small intestinal mucosa

LPH protein content and activity were determined in the small intestine of preweaning (15 days) and adult (6 months old) rabbits (Table I). The enzyme activity was higher in the suckling animals, ranging between 40 and 104 U/g protein, and lower in the adult animals, ranging between 3 and 16 U/g protein. Only two adult animals, n.8 and n.15, had more than 10 U/g protein in the middle region of the small intestine. In most adults the enzyme activity was similar in the proximal and middle intestine.

Suckling samples contained high levels of LPH proteins, as expected on the basis of the high enzyme activity, whereas most adult samples had low (or intermediate) levels of LPH proteins, corresponding to their lactase activity. The relation between the lactase specific activity and the level of lactase proteins showed in fact a quite good correlation (r = 0.84, P < 10^-6) for all the examined samples (Fig. 1A). When this type of relation was examined according to the developmental stage, correlation was found in the suckling population (r = 0.68, P = 0.09) but not in the adult samples (r = 0.32). When we plotted the lactase activity with the LPH protein content, we found that five samples from the adult population (from the proximal and middle region of No. 13 and from the middle region of No. 10, 12 and 15) had values above the 95% confidence limits of the regression line. In these 5 samples the ratio (units of lactase activity)/μg of lactase protein) was low (between 0.016 and 0.005) in comparison to the other adults (between 0.2 and 0.023) and to the suckling samples (between 0.121 and 0.042).

The LPH protein and the DNA contents were also measured in the enterocytes isolated from the small intestine of suckling and adult rabbits (Table I) and the
ratio LPH protein/mg DNA was plotted against the mucosal lactase activity in Fig. 1B. In preweanling animals the enterocytes contained large quantities of LPH proteins (>11 μg/mg DNA). The enterocytes of the adult mucosa had lower LPH protein content, whereas 3 out of the 4 examined adult samples with low enzyme activity and relatively high LPH protein content had, as expected, enterocytes with high protein content.

Fig. 2 shows the pattern of lactase proteins quantitatively immunoisolated from suckling and adult tissues. In two adults (lanes 1 and 2, No. 13 of Table I; lanes 7 and 8, No. 10) and two suckling rabbits (lanes 5 and 6, No. 2 and 1 of Table I) the lactase proteins were represented mainly by the 150 kDa mature form [6]. In one adult animal (No. 9 of Table I) accumulation of the 200 kDa high-mannose precursor was observed (lanes 3 and 4).

3.2. Initial synthesis rate of LPH proteins in suckling and adult rabbits

Small intestinal mucosal tissues were cultivated in vitro and labeled with [35S]methionine for 1 h. After 1 h only the high mannose 200 kDa precursor form was present [6]. It has been demonstrated that the label incorporation into this early precursor is closely related to the lactase mRNA level in the rabbit [4] and in the pig (Torp et al., unpublished results). Various labeled biopsies from each region were pooled and LPH proteins quantitatively immunoabsorbed as described in Section 2. The immunoisolated proteins were subjected to SDS-PAGE and revealed by Coomassie brilliant blue staining. Quantitation of radiolabeled and stained LPH proteins was performed by densitometric scanning.

In order to compare the synthesis rate in the suckling tissue with that in the adult animal tissue, the peak area of radiolabeled lactase (expressed in absolute absorb-

<table>
<thead>
<tr>
<th>Animal no.</th>
<th>Small intestinal region</th>
<th>Lactase specific activity (U/g protein)</th>
<th>LPH protein (μg/mg protein)</th>
<th>Lactase activity/LPH protein (U/μg LPH)</th>
<th>LPH protein (μg/mg DNA)</th>
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<tbody>
<tr>
<td>Sucklings (15 days)</td>
<td></td>
<td></td>
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<td>Adults (6 months)</td>
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<tr>
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<td>10</td>
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<td>0.067</td>
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<tr>
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<tr>
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<td>0.015</td>
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<tr>
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</table>
The label incorporation into the 200 kDa precursor was higher in the suckling tissues (second column of Table II) as compared to the adult with low or intermediate levels of lactase activity and LPH protein content. The label incorporation was as high as in the suckling tissues, in two (No. 13) of the three examined tissues with low lactase activity and high protein content. Similar results were obtained when the synthesis rate was measured in the enterocytes (last column of Table II).

Table II shows also that in adult rabbits the initial synthesis rate of LPH proteins in the proximal small intestine was as high or higher than in the middle intestine, whereas the opposite is true for the steady state level of LPH proteins. As a consequence, the ratio (labeled lactase)/(stained lactase) (Table II) was lower in the middle jejunum of the adult animal than in the proximal region.

4. DISCUSSION

In the present study we have demonstrated that in the adult rabbit there are various types of hypolactasic intestinal tissues. In most adults the lactase activity and the LPH protein content were low and the synthesis of the 200 kDa lactase precursor was reduced. In a few samples the enzyme activity was higher and the LPH protein content and the lactase synthesis were correspondingly increased. But there were also adults with low enzyme activity, and relatively high LPH protein content: in some of these samples high lactase synthesis occurred.

This and other studies [4,12,13] suggest that various mechanisms cause the decline of lactase activity in the small intestine of the adult mammal, as compared to the suckling:

(i) Decreased lactase mRNA levels and therefore decreased synthesis of LPH precursor. This may be due, at least in part, to the presence on the villus of enterocytes not committed to synthesis of lactase. Immuno-morphological and enzymohistochemical studies have in fact demonstrated that in the proximal intestine of the adult rabbit bands of lactase-negative enterocytes are found emanating from the crypts [14,15].

(ii) Increased turnover of the enzyme (in the brush border) and (partial) inactivation of the protein, as suggested in the present study, respectively: (a) by the relatively high synthesis rate in relation to the LPH protein content in the proximal as compared to the middle adult
intestine (Table II); (b) by the presence of adult hypolactasic tissues with relatively high LPH protein content and high lactase synthesis rate.

An increased luminal pancreatic (and bacterial) proteolysis of brush border enzymes may be one of the post-translational events producing in the adult intestine a decline of the lactase activity and, to a minor degree, also of other brush border enzymes such as sucrase [4] and aminopeptidase (Torp et al., unpublished results). The increased luminal proteolysis may explain the loss of the lactase from the surface of the enterocytes on the top of the villus: in fact, in proximal adult rabbit intestine, lactase-positive enterocytes are mainly localized in the lower part of the villus, suggesting that in these enterocytes lactase is produced and then lost as the cells move up the villus [14,15].

These different mechanisms causing adult-type hypolactasia apparently have variable importance probably not only in various species [16], but also in various individuals of the same species, in various parts of the small intestine, and probably also in the various enterocytes present on the villus. In proximal intestine of hypolactasic adult men, scattered cells in the upper part of the villus are positive, suggesting that the heterogeneity may also be determined at a late stage, when the cells move up the villus.

Many factors favour the presence of high lactase activity in the small intestine of the suckling mammals: a low enterocyte turnover, which permits that slowly (and in a complex way) processed proteins, such as lactase, reach the brush border in a fully active state after many metabolic changes; a commitment of all enterocytes emerging from the crypts to produce lactase; a low intraluminal pancreatic (and bacterial) proteolysis, etc. Changes of these favorable conditions and probably also decreased levels of lactase mRNA in the enterocyte itself in the adult intestine cause the hypolactasia of the adult mammal.

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