LACTASE ENZYMES IN THE INTESTINAL BRUSH BORDER MEMBRANE OF THE SUCKLING RAT

A second enzyme restricted to ileum

J. R. GREEN and H.-P. HAURI
Department of Gastroenterology, University Children's Hospital, 3010 Bern, Switzerland

Received 11 October 1977
Revised version received 18 October 1977

1. Introduction

The lactose hydrolysing enzyme (β-D-galactoside galactohydrolase, EC 3.2.1.23) of the intestinal brush border membrane has been studied extensively because of its role in the nutrition of young mammals and also because of the problems associated with its absence. In rats lactase activity is detectable before birth, it reaches high levels between days 12 and 20 and then declines to the low adult levels [1] but during this development the proximal and distal regions of the small intestine behave differently [2]. In those initial studies total intestinal lactase activity was measured, including the lysosomal β-galactosidase and the cytoplasmic hetero-β-galactosidase as well as the true brush border membrane lactase. However, the advent of a specific assay for brush border membrane lactase [3] together with improvements in the fractionation of brush border membrane components have facilitated this present study which describes a second lactase enzyme restricted to the distal small intestine of the suckling rat. This enzyme differs from that of the proximal small intestine in its electrophoretic mobility and developmental pattern. The change-over point between the two lactases occurs three quarters of the way along the small intestine.

2. Materials and methods

Pairs of rats between 1 and 28 days of age were anaesthetized with ether, the entire small intestine was removed, cut into quarters (or eighths in one series of experiments) and rinsed out with saline. The pieces were split open and the mucosa was scraped off. With the intestines from 1–4 day old rats this was not possible so the entire piece of split intestine had to be used. Homogenisation and the isolation of a purified brush border fraction were carried out according [4] as modified [5]. Protein was measured by the procedure [6], brush border lactase was assayed, at pH 5.8, in the presence of *p*-chloro-mercuri-benzoate [3], the brush border membrane marker enzyme sucrase was measured by the method [7] and phlorizin hydrolase activity by that of [8]. Gel electrophoresis was performed on 6% or 7.5% polyacrylamide gels using the system [9] except that the sample (50 μg protein) was solubilized in the sample buffer for 15 min at room temperature in the absence of mercaptoethanol. After electrophoresis the gels were split longitudinally, one half was stained for protein with Coomassie blue, the other half was sliced into 0.5 mm sections and eluted in 1 ml water. Lactase activity was measured in these eluates and the relative mobility (R_F) of the region of enzymatic activity was compared with the R_F values of the stained protein bands on a scan of the other half of the gel.

3. Results and discussion

The values in table 1 for the development of lactase activity in proximal and distal small intestinal mucosa show a similar pattern to that obtained previously

Table 1
Development of lactase in proximal (P) and distal (D) quarters of rat small intestine

Age (days)	Lactase (total IU) P	Lactase (total IU) D	Lactase (IU/mg BBM) P	Lactase (IU/mg BBM) D
1	2.56	0.73	0.74	0.31
4	3.69	1.72	0.64	0.18
8	3.73	4.46	0.96	0.74
12	3.91	4.26	0.72	0.48
16	4.47	5.20	0.69	0.54
20	3.01	2.55	0.50	0.25
24	3.12	0.43	0.15	0.09
28	0.37	0	0.07	0

Expressed as total international units (IU) in tissue homogenates and as specific activity per mg purified brush border membrane protein (IU/mg BBM). Values are means of triplicate assays on pooled tissue from 8 animals of each age group

with the non-specific assay [2]. The activity of the true brush border lactase, expressed either as total units or as specific activity, showed little change in the proximal small intestine until day 16 when it began to fall to approach adult values by day 28. The distal activity rose from its initial low level to reach a peak between days 8 and 16, after which it decreased rapidly to be totally absent by day 28. In animals older than 16 days intestinal sucrase was detectable and its specific activity (IU/mg protein) was used to calculate the purification factors of the brush border membrane fractions over the original tissue homogenates; these ranged from 12.7–29.8 (mean 21.1) for sucrase compared with 10.8–28.7 (mean 19.0) for lactase.

Given these developmental differences, the possibility that the proximal and distal lactases were not identical molecules was investigated by gel electrophoresis. Figure 1 shows the combined protein and lactase scans of gels loaded with proximal or distal brush border membranes from a 13 day old rat. Comparison of the protein and enzyme patterns shows that the lactase activity comigrates with a major protein band of R_F 0.227 for the proximal enzyme and R_F 0.242 for the distal one. This small difference in R_F was consistently observed in all the suckling rats of age 1–24 days, in older animals the disappearance of the distal lactase activity was concommitant with the loss of the major stained protein band. The absolute designation of lactase enzymatic activity to the comigrating stained protein band was discussed

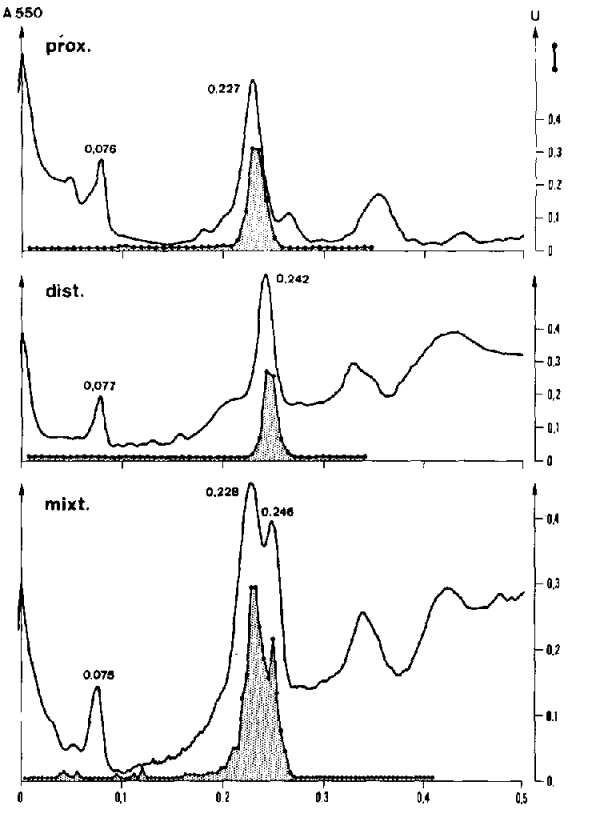

Fig.1. Polyacrylamide gel electrophoresis of brush border membrane protein from the small intestine of the suckling rat. Protein stained with Coomassie blue and scanned at 550 nm (solid line), lactase enzymatic activity in arbitrary units in eluates of sliced gel (shaded area). Top scan shows the gel pattern obtained with brush border membranes from the proximal quarter of the small intestine, middle scan shows the same from the distal quarter, and bottom scan is from a mixture of proximal and distal brush border membranes. Peak numbers indicate mobility relative to the electrophoretic front.

[10]. In order to confirm the small difference in the relative mobilities of the lactase enzymes gels were loaded with a mixture of proximal and distal brush border membrane protein and run under identical conditions. The lower scan in fig.1 shows that the gel system resolved this mixture into a double peak of both enzymatic activity and stained lactase protein. Another notable feature of the protein scans given in fig.1 is that the band of R_F 0.076 had a constant R_F (± 0.001 SD) on all gels.

To locate more precisely the change-over point between the proximal and distal lactases the small intestines from rats aged 12, 14, 19 and 23 days were divided into 8 sections of equal length and the brush border membrane proteins analysed as before. From the R_F values of the lactases, it was possible to classify the lactase enzyme present in sections 1–6 as the proximal type and that in sections 7 and 8 as the distal type. In no section was the simultaneous presence of the two forms of the enzyme observed, and there was no evidence for a gradual change in mobility of any of the bands as would be expected if the observed differences were due to intraluminal proteolysis of a proximal protein to produce an electrophoretically faster distal form. Further efforts to identify additional differences between the lactase enzymes, apart from their different electrophoretic mobilities and developmental patterns, have been restricted by the availability of sufficient quantities of purified brush border membranes from suckling rats. However, it has been established that both enzymes have an identical pH curve with an optimum at pH 5.2, they follow similar heat inactivation profiles at 50°C and they both possess phlorizin hydrolase activity.

Acknowledgement

This work was supported by grants numbers 3.547-0.75 and 3.676-0.75 from the Swiss National Science Foundation.

References

[1] Alvarez, A. and Sas, J. (1961) Nature 190, 826–827.
[2] Koldovsky, O. and Chytil, F. (1965) Biochem. J. 94, 266–270.
[3] Asp, N. G. and Dahlqvist, A. (1972) Anal. Biochem. 47, 527–538.
[4] Schmitz, J., Preiser, H., Maestracci, D., Gosh, B. K., Cerda, J. J. and Crane, R. K. (1973) Biochim. Biophys. Acta 323, 98–112.
[5] Hauri, H. P., Kedinger, M., Haffen, K., Freiburghaus, A., Grenier, J. F. and Hadorn, B. (1977) Biochim. Biophys. Acta 467, 327–339.
[6] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[7] Messer, M. and Dahlqvist, A. (1966) Anal. Biochem. 14, 376–392.
[8] Leese, H. J. and Semenza, G. (1973) J. Biol. Chem. 248, 8170–8173.
[9] Laemmli, U. K. (1970) Nature 227, 680–685.
[10] Green, J. R. and Hadorn, B. (1977) Biochim. Biophys. Acta 467, 86–90.