Biochem. J. (1965) **96**, 492

Activity of β-Galactosidase in Homogenates and Isolated Microvilli Fraction of Jejunal Mucosa from Suckling Rats

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(*Received* 16 *October* 1964)

1. β-Galactosidase activity was studied in homogenates and isolated microvilli fraction of jejunal mucosa from 14-day-old suckling rats. *o*-Nitrophenyl β-D-galactoside served as the substrate. 2. The microvilli fraction contains about one-third of the total activity of the original homogenate. 3. The pH optimum of the β-galactosidase was 3·5 in the total homogenate and supernatant fraction, whereas in the microvilli fraction the maximum activity was at pH 5·5. 4. This work gives further support to the view that two β-galactosidases exist in the jejunal mucosa.

Activity of β-galactosidase (β-D-galactoside galactohydrolase, EC 3.2.1.23) is high in the small intestine of infant mammals (see Herzenberg & Herzenberg, 1959; Koldovský, 1963). Doell & Kretchmer (1962, 1963) found two β-galactosidase activities in homogenates of the whole jejunoileum from 3–6-day-old rats by ultracentrifugal separation. They differed in localization (microsomal and soluble fractions) and in substrate affinity. Recently it has become possible to isolate the 'brush border' (microvilli) from homogenates of the small-intestinal mucosa of hamsters (Miller & Crane, 1961) and adult rats (Gallo & Treadwell, 1963; Rutloff, Noack, Frise & Schenk, 1965). This microvilli fraction has been shown to contain a considerable amount of different disaccharidases. By using a modification for the isolation of the brush border (Noack & Schenk, 1965) β-galactosidase activity was determined in this fraction. Only the jejunal mucosa was used since in previous work there was some indication that predominantly this part of the small intestine contains two β-galactosidases differing in their pH optima (Koldovský & Chytil, 1965).

EXPERIMENTAL

Animals. Rats of the Wistar strain removed from the mother immediately before being killed were decapitated on the fourteenth postnatal day. The small intestine was removed and rinsed with cold 5mM-EDTA solution, pH 7·4, and the mucosa was scraped off the proximal third (jejunum). All procedures were performed at 1–4°.

Isolation of brush border. For the separation of brush border from the rest of the cell, 2g. of mucosa was first homogenized in 10ml. of 5mM-EDTA solution, pH 7·4, with a Dounce-type homogenizer (Dounce, 1955) with 15 strokes. Then 8ml. of the homogenate was mixed with 40ml. of 5mM-EDTA solution, pH 7·4, and centrifuged at 300*g* for 15min. in the cold to give supernatant S_1 and precipitate P_1. The supernatant was collected and precipitate P_1 resuspended in 5mM-EDTA to give a volume of 10ml. with the Dounce homogenizer. Fraction P_1, in agreement with Miller & Crane (1961), contains no nuclei since these had been broken down by the hypo-osmotic medium. Fraction P_1 contains about 5% of fraction S_1 and perhaps also some mitochondria and microsomes enclosed in mucin. In addition, remnants of villi, muscle fibres and vessels are present.

Counting of brush-border particles. A 1ml. portion of fraction P_1 was placed on a density gradient consisting of 2ml. of 0·35M-sucrose, 2ml. of 0·95M-sucrose, 1·5ml. of 1·25M-sucrose and 1·0ml. of 1·45M-sucrose (all in 5mM-EDTA, pH 7·4). After centrifugation at 300*g* for 15min. the sucrose phases (see Fig. 1) were collected separately. The border layer was always added to the subsequent phase and again centrifuged (at 800*g* for 20min.). The supernatant was discarded and 0·1ml. of 5mM-EDTA, pH 7·4, was added. Brush-border particles were then counted in a blood-cell count chamber. Brush-border particles could also be counted directly in fraction P_1. No losses occurred during the preparation.

Preparative separation of fraction P_1. In some experiments a purer fraction than P_1 was prepared. A 5g. sample of mucosa was homogenized with 25ml. of 5mM-EDTA, pH 7·4, as described above, and then 160ml. of EDTA was added and the suspension centrifuged at 300*g* for 15min. Fraction

P_1 was recentrifuged at 8000g for 10min. (centrifuge supplied by Janetzki, Leipzig, Germany) to separate off the remainder of the supernatant, thus giving fraction P_2. This was again placed on the density gradient. Phases AB, D, R_1 and R_2 (see Fig. 1) were collected and centrifuged at 12500g (centrifuge supplied by Janetzki) for 10min. to remove sucrose, and the precipitate was again suspended in 5mM-EDTA, pH7·4. Phase AB contains many microscopically unidentified particles, phase D a few brush-border particles, phase R_1, in addition to brush-border particles, a very few remnants of villi, and phase R_2 many villi and vessel remnants (see Fig. 1).

Enzymic assay. This was performed according to the method of Lederberg (1950) (see also Koldovský, Chytil & Muzyčenkova, 1964; Koldovský & Chytil, 1965): 0·5ml. of buffer plus 0·5ml. of the fraction assayed were diluted with 5mM-EDTA so as to give a linear relationship between activity and concentration and time, and 0·5ml. of 3mM-*o*-nitrophenyl β-D-galactoside was added. After 20min. incubation at 37° the reaction was stopped with 2ml. of M-K_2CO_3. The *o*-nitrophenol liberated was determined photometrically at 420mμ, and activity is expressed as μmoles liberated. In some experiments $MgCl_2$ (20mM) was added to the buffer (see Paigen, 1963).

Buffers. All buffers were 0·3M solutions. For pH1–2·5 sodium citrate was used; sodium acetate served as buffer for pH3·0–5·5, and potassium phosphate for pH6·0–8·0. A Pye (Cambridge) pH-meter was used together with standard solutions supplied by Burroughs Wellcome (buffer-solution tablets).

RESULTS

Table 1 shows that fraction P_1 contains about one-third of the total activity of the original homogenate (H). The total recovery is good regardless of whether magnesium chloride was added or not. At pH3·5 less β-galactosidase activity is found in fraction P_1 than at pH5·5. The ratio of activities at pH5·5 and 3·5 illustrates this (Table 1). Thus β-galactosidase activity is higher at pH3·5 in fractions H and S, but this does not hold for fraction P_1.

β-Galactosidase activity in the different fractions of the density gradient (Fig. 1) follows the curve of brush-border particles much better at pH5·5 than at pH3·5. This is considered to demonstrate that part of the β-galactosidase activity is localized in the microvilli. In addition, it appears that the β-galactosidase activity in the brush-border particles differs from that in the rest of the cell. To throw further light on this, β-galactosidase activity was determined in fractions S_1 (containing most of the mucosal cells) and R_1 (containing most brush-border particles) at different pH values. Fig. 2 demonstrates that the pH optimum for β-galactosidase activity is different in fractions S_1 and R_2.

DISCUSSION

This work gives further support to the view (Koldovský & Chytil, 1965) that two β-galactosidases exist in the jejunal mucosa. One appears to be localized in the brush-border particles and its pH optimum is at 5·5; the other is contained in the rest of the cell and has a pH optimum at 3·5. In whole homogenates of the jejunum both enzymes are present, giving a badly defined pH optimum of activity (Koldovský & Chytil, 1965). Doell & Kretchmer (1962, 1963) have also suggested the presence of two β-galactosidase activities in the

Table 1. *Distribution of β-galactosidase activity in homogenate of jejunal mucosa of suckling rats*

Experimental details are given in the text. Homogenates were prepared from 1g. of mucosa from 24 rats in each experiment. Activities were determined both with no added $MgCl_2$ and with $MgCl_2$ (20mM) added to the buffer (see the Experimental section). The results are given as means ± S.E.M. of three experiments.

	β-Galactosidase activity (μmoles/hr./fraction)				Ratio of activities at pH5·5/pH3·5	
	At pH3·5		At pH5·5			
Fraction	No $MgCl_2$	With $MgCl_2$	No $MgCl_2$	With $MgCl_2$	No $MgCl_2$	With $MgCl_2$
Whole homogenate	94±4·9	101±13·0	56±7·8	76±4·3	0·58±0·03	0·79±0·07
S_1 (supernatant)	86±2·6	90± 5·7	44±3·3	58±3·7	0·52±0·03	0·65±0·01
P_1 (precipitate)	13±4·2	18± 3·3	23±4·8	23±5·2	1·46±0·24	1·23±0·04

	β-Galactosidase activity (% of activity of whole homogenate)			
	At pH3·5		At pH5·5	
S_1	92±2·2	94±8·9	81±2·8	77±4·1
P_1	18±3·1	19±2·8	41±7·4	31±6·5
S_1+P_1 (recovery)	110	113	123	104

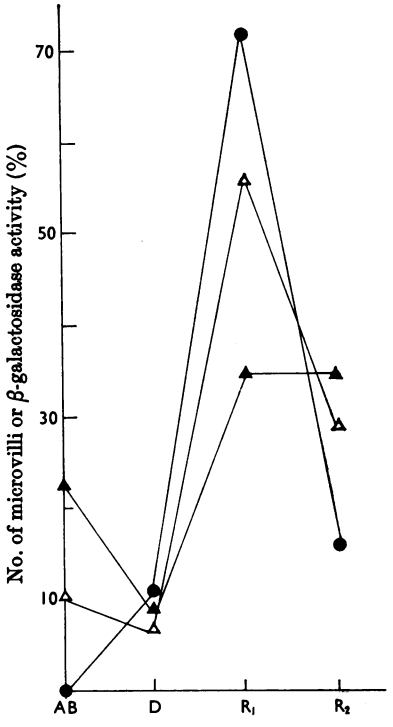

Fig. 1. Distribution of β-galactosidase activity and microvilli in fractions of the density gradient. Ordinate: the numbers of microvilli (●) or β-galactosidase activities at pH 3·5 (▲) and at pH 5·5 (△) in each fraction are expressed as percentages of the total numbers of microvilli or activity applied to the density gradient. Experimental details are given in the text. Homogenates were prepared from mucosa from 40 rats. Two other similar experiments gave the same results. The density-gradient phases were as follows: AB, 5 mM-EDTA; D, 0·35 M-sucrose in 5 mM-EDTA; R_1, 0·95 M-sucrose in 5 mM-EDTA; R_2, 1·29 M-sucrose in 5 mM-EDTA.

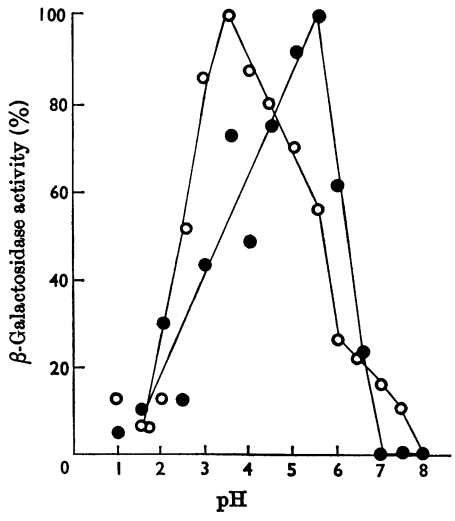

Fig. 2. pH–activity curves of β-galactosidase activity in fractions S_1 (○) and R_1 (●). Ordinate: β-galactosidase activities are expressed as percentages of the maximum value for the fraction at optimum pH. Experimental details are given in the text. Mucosa from 40 animals was used, and the buffer contained $MgCl_2$ (20 mM). Two other similar experiments gave the same results.

jejunoileum of 3–6-day-old rats. They fractionated mucosal cells and found a more alkaline pH optimum for β-galactosidase in the nuclear fraction. This fraction might well contain brush-border particles. In other respects their results cannot be directly compared with ours since (*a*) they used the whole small intestine, (*b*) they fractionated the cells in a different manner and (*c*) they used younger animals.

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