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STUDIES ON THE POSSIBLE IDENTITY OF PARTICULATE β-GLUCOSIDASE AND β-XYLOSIDASE OF MOUSE LIVER

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Summary

Mouse liver β-glucosidase (β-D-glucosidase glucohydrolase, EC 3.2.1.21) and β-xylosidase (1,4-β-D-xylan xylohydrolase, EC 3.2.1.37) activities were studied under different conditions of incubation in an attempt to determine whether these two activities are due to a single enzyme or two separate enzymes. The results showed that:

(a) Particle-bound β-glucosidase and β-xylosidase activities exhibit similar characteristics with different buffers and at various pH values, in the presence or absence of taurocholate.

(b) Both activities are inhibited by gluconolactone and conduritol B epoxide. β-Glucosidase activity is inhibited competitively by the two inhibitors, but β-xylosidase activity is inhibited non-competitively.

(c) Xylonolactone was a very poor inhibitor of both activities, but the inhibition of β-xylosidase activity was more pronounced than that of β-glucosidase.

(d) The presence of glucosides or xylosides simultaneously in the incubation medium suggested the presence of one enzyme with both activities.

These results, together with the mode of inhibition produced by gluconolactone and conduritol B epoxide also suggest the presence of two different binding sites for the β-D-glucoside and β-D-xyloside, respectively.

Introduction

The existence of different forms of mammalian β-glucosidases (β-D-glucoside glucohydrolases, EC 3.2.1.21) has been reported. A lysosomal form is capable of hydrolyzing glucocerebroside and glucosylsphingosine, in addition to

steroid-β-D-glucosides and artificial substrates [1,2]. This particulate enzymic activity is greatly diminished in Gaucher's disease and believed responsible for cerebroside accumulation in patients [3]. In addition, a cytosolic or soluble form has been described which is incapable of hydrolyzing the cerebroside, but will hydrolyze the other substrates [4—7]. The soluble β-glucosidase, in addition, apparently can hydrolyze other glycosides such as β-D-galactoside, β-D-fucoside, α-L-arabinoside and β-D-xyloside [6,8—10], and may be identical with the general 'aryl' glycosidase [11]. All these hydrolytic activities are thought to reside in a single 'soluble' enzyme protein. Partial dissociation of β-glucosidase from β-xylosidase (1,4-β-D-xylan xylohydrolase, EC 3.2.1.37) and α-L-arabinosidase activities of pig kidney suggests that more than one enzyme may be involved [7]. Thus, the assignment of these hydrolytic activities to the same enzyme is not unequivocal. Using a different approach, Öckerman [8] and Chiao et al. [12,13] demonstrated that patients with Gaucher's disease lacked both 'soluble' β-glucosidase and β-xylosidase activities in spleen, leukocytes, liver, kidney and in fibroblasts, suggesting that a common enzyme might be responsible for these two activities. Also, the Gaucher mouse exhibited decreased β-xylosidase activity in several tissues [14].

A particulate, lysosomal β-xylosidase has been reported present in rat liver and small intestine [15,16]. A single lysosomal enzyme seemed to be responsible for β-glucosidase and β-xylosidase activities in rat liver, but two enzymes were implicated for intestine.

In the present report we have compared some properties of these two enzymic activities in the particulate fraction of mouse liver. Through the use of various inhibitors and artificial substrates, an investigation was carried out to determine whether the particulate β-glucosidase and β-xylosidase activities are due to one, or two different enzymes.

Materials and Methods

4-Methylumbellyferyl-β-D-gluco- and xylopyranosides, *p*-nitrophenyl-β-D-gluco- and xylopyranosides were purchased from Koch-Light Laboratories Ltd. Conduritol B epoxide was prepared by Dr. G. Legler [17]. D-Glucono-1,5-lactone was purchased from General Biochemicals; D-xylonolactone and xylosylamine were synthesized according to published procedures [18,19]. D-Xylose was purchased from Fisher Scientific and sodium taurocholate from Pfanstiehl Inc.

Enzyme preparation. Adult BALB/c mice were killed and 25% tissue homogenates were prepared in 0.25 M sucrose in all-glass Potter-Elvejhem homogenizer, at 0—4°C. The homogenates were centrifuged at 100 000 × *g* for 1 h in a Beckman model L3-40 centrifuge, and the resulting supernatant and pellet were collected separately. The pellet fraction was rehomogenized in double-distilled water (25% homogenate) and aliquots were used directly as the enzyme source. For the study of the effect of mixed substrates, the 25% sucrose homogenates were first centrifuged at 600 × *g* for 15 min. The resultant supernatants were then centrifuged at 100 000 × *g* for 1 h and the pellet used. Protein was determined according to Itzhaki and Gill [19].

Enzyme assay for β-glucosidase determination. The incubation mixture

contained 1 M citrate/phosphate (or sodium acetate) buffer at the specified pH, 50 μl; 10% sodium taurocholate, 10 μl; aliquots of enzyme preparations, substrates and other additions, in a total volume of 0.2 ml. Tubes were incubated at 37°C for 30 min for β-glucosidase or for longer periods for β-xylosidase. The reaction was terminated by the addition of 2 ml of 0.2 M glycine buffer, pH 10.3, and all samples were centrifuged at 2000 rev./min for 15 min. The supernatant was taken either for measurement of 4-methylumbelliferone released, in an Aminco-Bowman spectrofluorometer (366 nm excitation and 446 nm emission), or for measurement of *p*-nitrophenol liberated in a Gilford 250 spectrophotometer (405 nm). The pH, protein and substrates concentrations, as well as any additions, are indicated in the figures and tables. All values were corrected for boiled enzyme samples.

Results

Soluble vs. particulate activities

In mouse liver, 94—97% of both β-glucosidase and β-xylosidase activities is particle bound. The former is approximately ten fold more active than the latter.

Effect of pH and taurocholate

Both hydrolytic activities are enhanced by the presence of taurocholate, and the pH profiles obtained in the presence and absence of detergent were quite similar for both β-glucosidase and β-xylosidase when sodium acetate was the buffer. When citrate/phosphate was used, the activities of both β-glucosidase and β-xylosidase were greater at the more acidic pH values (Fig. 1).

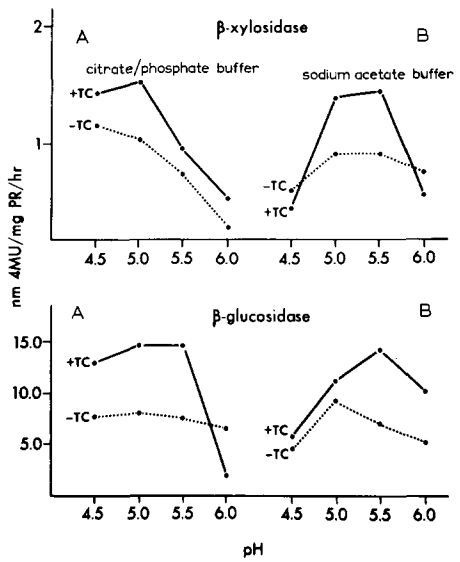

Fig. 1. Activities of β-glucosidase and β-xylosidase in the particulate (100 000 × *g*) fraction of mouse liver homogenates. Activities measured in the absence (●- - - - -●) or presence (●——●) of sodium taurocholate. (A) in citrate/phosphate buffer; (B) in sodium acetate buffer.

TABLE I

EFFECT OF DIFFERENT INHIBITORS ON PARTICULATE β-GLUCOSIDASE AND β-XYLOSIDASE ACTIVITIES OF MOUSE LIVER

Incubation mixture contained (final concentrations): 4-methylumbellyferyl-β-D-glucoside (5 mM) or 4-methylumbellyferyl-β-xyloside (2.5 mM); citrate/phosphate buffer (0.25 M), pH 4.5; sodium taurocholate (0.5%). Preparation 1, from 1.3 to 2.1 mg protein/0.2 ml. Preparation 2, from 0.8 to 6.4 mg protein/0.2 ml. n.d., not determined. Data in parentheses are percentage of residual activity.

Inhibitor (mM)	β-Glucosidase (nmol/mg protein per h)	β-Xylosidase (nmol/mg protein per h)
Preparation 1		
None	17.0	0.91
Glucono-(1-5)-lactone		
0.55	4.4 (26)	0.12 (13)
1.1	3.2 (19)	0.09 (10)
1.65	2.1 (12)	0.07 (8)
Xylono-1,4-lactone		
8.2	17.0	0.78
16.4	17.2	0.72
24.6	15.7 (92)	0.76 (82)
Conduritol B epoxide		
0.55	1.2 (7)	0.01 (1)
1.1	1.0 (6)	0.004 (0.4)
1.65	0.8 (5)	0.002 (0.2)
Preparation 2		
None	7.8	1.2
D-Xylosylamine		
4	n.d.	0.9
8	8.1	0.74
D-Xylose		
8	7.8	0.96

Inhibitors

Both enzyme activities respond similarly to the different inhibitors employed and extensive inhibition of both activities was observed with gluconolactone and conduritol B epoxide while there was little if any in the presence of xylonolactone, and xylosylamine and xylose, respectively (Table I).

Kinetics of inhibition by gluconolactone and conduritol B epoxide

The inhibitory action of gluconolactone and conduritol B epoxide on the particulate β-glucosidase and β-xylosidase activities was further investigated. D-Gluconolactone inhibits β-glucosidase in a competitive manner (Fig. 2a), but inhibits β-xylosidase non-competitively (Fig. 2b). Similarly, conduritol B epoxide is a competitive inhibitor of β-glucosidase activity (Fig. 3a), and is a non-competitive inhibitor of β-xylosidase activity (Fig. 3b). The apparent K_i values obtained from these data are summarized in Table II.

Mixed substrates incubations

The possibility of competition between the substrates, D-glucosides and D-xylosides was investigated.

Particles were incubated with *p*-nitrophenyl-β-D-glucoside alone, *p*-nitrophenyl-β-D-xyloside alone or with both substrates together and the activities

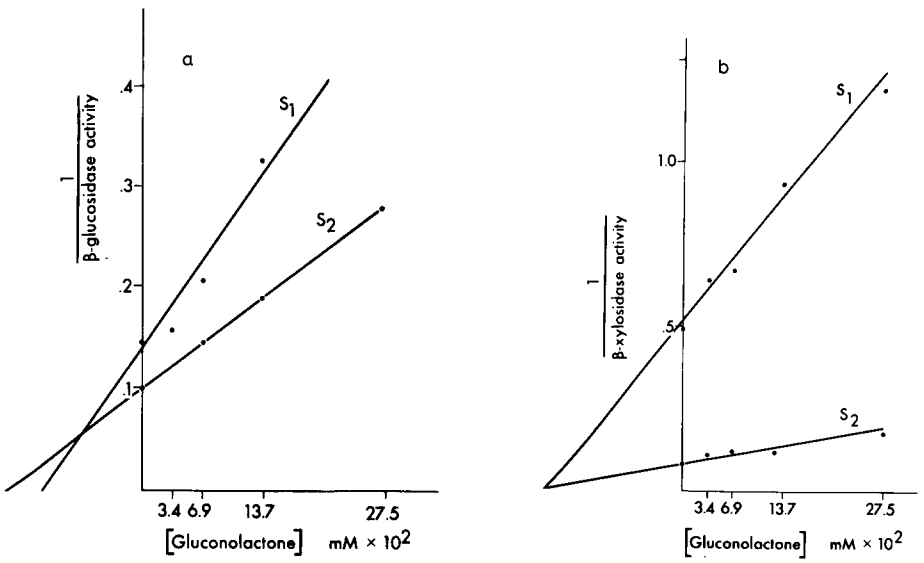

Fig. 2. (a) Inhibition of β-glucosidase activity in the particulate (100 000 × *g*) fraction of mouse liver homogenates by D-gluconolactone. Two different final substrate concentrations were used: S_1 = 1.25 mM, and S_2 = 5 mM. Activity was measured in citrate/phosphate buffer, pH 4.5, in the presence of sodium taurocholate. Substrate used was 4-methylumbellyferyl-β-D-glucoside. (b) Inhibition of β-xylosidase activity in the particulate fraction of mouse liver homogenates by D-gluconolactone. S_1 = 1.25 mM, and S_2 = 3.75 mM of 4-methylumbellyferyl-β-D-xyloside. Conditions of assay as in (a).

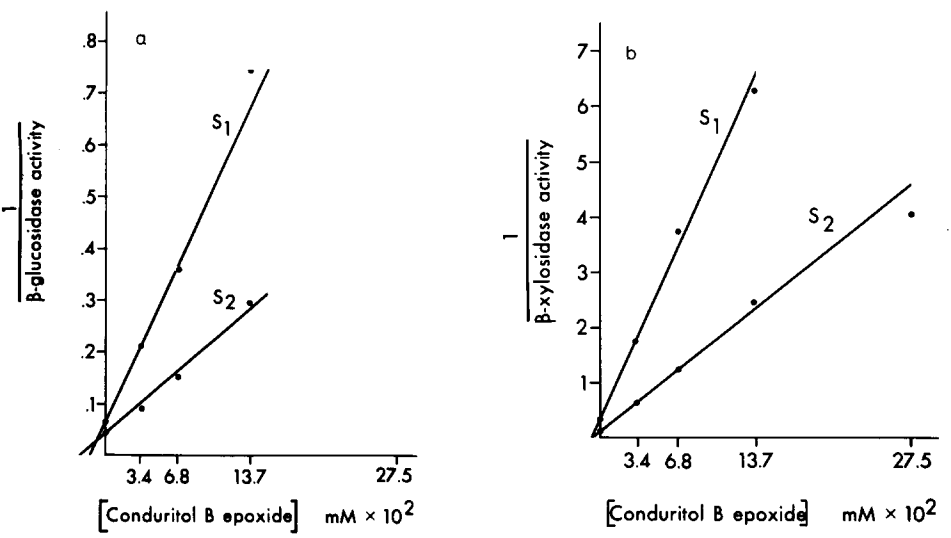

Fig. 3. (a) Inhibition of β-glucosidase activity in the particulate fraction of mouse liver homogenates by conduritol B epoxide. S_1 = 1.25 mM and S_2 = 5 mM of 4-methylumbellyferyl-β-D-glucoside. Same incubation conditions as in Fig. 2a. (b) Inhibition of β-xylosidase activity of mouse liver particulate fraction by conduritol B epoxide. Conditions as described in Fig. 2b.

TABLE II

APPARENT K_i VALUES OF D-GLUCONOLACTONE AND CONDURITOL B EPOXIDE ON MOUSE LIVER PARTICULATE β-GLUCOSIDASE AND β-XYLOSIDASE ACTIVITIES

Assays performed with the 4-methylumbellyferyl-glycosides as substrates. Incubation conditions as described in Table I.

Inhibitor	K_i (M)	
	β-Glucosidase	β-Xylosidase
Glucono-1,5-lactone	$3.8 \cdot 10^{-5}$	$1.3 \cdot 10^{-4}$
Conduritol B epoxide	$1.2 \cdot 10^{-5}$	$9.2 \cdot 10^{-6}$

analyzed as a function of time of incubation. If β-glucosidase and β-xylosidase are two separate enzymes than the rate of the reaction in the presence of both glycosides would be the sum of each activity determined separately. However, if one enzyme catalyzes both reactions, the rate obtained would be less than the sum of each activity determined separately and this is what was observed (Fig. 4).

Finally, a comparison was made of the enzymic activities found by mixing two substrates, and the calculated theoretical activities expected for the case of one, or two enzymes. In the latter case the expected velocity (v) would be the sum of the velocities with each substrate determined separately ($v_A + v_B$); in the former case v should fit the following equation:

$$v = \frac{v_A\left(1 + \frac{A}{K_{m(A)}}\right) + v_B\left(1 + \frac{B}{K_{m(B)}}\right)}{1 + \frac{A}{K_{m(A)}} + \frac{B}{K_{m(B)}}}$$

where v_A and v_B are velocities with substrates A and B determined indepen-

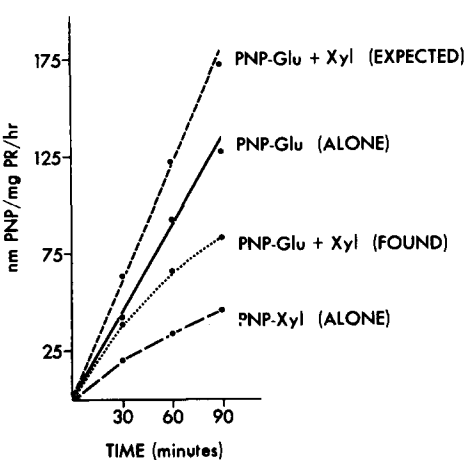

Fig. 4. Activities of β-glycosidase and β-xylosidase either present singularly or simultaneously as a function of time of incubation. Each substrate present at a final concentration of 5.0 mM.

TABLE III

EFFECT OF MIXED SUBSTRATES ON LIVER PARTICULATE β-GLUCOSIDASE AND β-XYLOSIDASE ACTIVITIES

Incubation conditions: liver homogenates centrifuged at 600 × *g*, 15 min, supernatant centrifuged at 100 000 × *g*, 60 min, and pellets used. Citrate/phosphate buffer, 0.25 M, pH 5.0 (final concentration): sodium taurocholate, 0.5% (final concentration); enzyme preparation, 0.33—1 mg protein/tube; substrates in final concentration as indicated. Incubations for 30 or 60 min, 37°C. MU, methylumbellyferyl, PNP, *p*-nitrophenyl.

Substrate A	Substrate B	Observed activity (nmol/mg protein per h)	Calculated activity (nmol/mg protein per h)	
			One enzyme	Two enzymes
4-MU-β-D-glucoside (1 mM)	4-MU-β-D-xyloside (1 mM)	26.1	29.3	32.0
4-MU-β-D-glucoside (2 mM)	4-MU-β-D-xyloside (2 mM)	39.6	41.2	50.7
4-MU-β-D-glucoside (4 mM)	4-MU-β-D-xyloside (4 mM)	61.5	58.4	77.1
PNP-β-D-glucoside (0.5 mM)	PNP-β-D-xyloside (1.0 mM)	20.4	22.7	27.4
PNP-β-D-glucoside (1 mM)	PNP-β-D-xyloside (2 mM)	21.1	30.7	41.2
PNP-β-D-glucoside (2 mM)	PNP-β-D-xyloside (4 mM)	29.0	40.8	60.0

dently; A and B are concentrations of the two respective substrates and K_m (A or B) have the usual meaning. The K_m values of the 4-methylumbellyferyl-glycosides were $2.5 \cdot 10^{-3}$ M for the β-D-glucoside and $5.1 \cdot 10^{-3}$ M for the β-D-xyloside; for the *p*-nitrophenyl substrates they were $0.85 \cdot 10^{-3}$ M for the β-D-glucoside and $4.6 \cdot 10^{-3}$ M for the β-D-xyloside.

The results (Table III) show that the velocities found at any substrate concentration, were always closer to those calculated for one enzyme catalyzing the two reactions.

Discussion

Centrifugation of homogenised mouse liver in 0.25 M sucrose indicated that the bulk of β-glucosidase and β-xylosidase activities is particle bound, i.e. sediments in the 100 000 × *g* pellet. This was also the case when the original homogenised fraction was first centrifuged at 600 × *g* for 15 min and the resultant supernatant was then centrifuged at 100 000 × *g* for 1 h. Identical results were reported for rat liver [20]. The particulate β-glucosidase of mouse liver had similar properties to those reported in the literature for other animal species [1,21] in that it has an acidic pH optimum and is activated by taurocholate. This β-glucosidase activity, when measured with artificial substrates in the presence of detergent is believed to reflect glucocerebrosidase activity. The particulate β-xylosidase activity, which has been previously described in other animal species [9,20] was also present in mouse liver, and it has properties

similar to the particulate β-glucosidase, when the effects of buffer, pH and taurocholate are considered.

The soluble β-glucosidase of mouse liver had properties similar to those reported in the literature for other animal species and tissues [7]; it had a more alkaline pH optimum and was inhibited by detergents. Again, the pH profiles and behavior in the presence of detergent were similar for mouse liver soluble β-xylosidase and β-glucosidase activities (results not shown).

Gluconolactone and conduritol B epoxide were very effective inhibitors of both β-glucosidase and β-xylosidase (Table I). The differential inhibition of two enzymic activities by gluconolactone was observed with a β-glucosidase/β-galactosidase of intestinal rat mucosa and the possibility of more than a single binding site for the two substrates was suggested [22]. Xylono-1,4-lactone produced only slight inhibition of β-glucosidase activity at very high concentrations. Nevertheless, activity of β-xylosidase was reduced to about 82% by these high concentrations of inhibitor. A somewhat similar finding was reported for limpet β-glucosidase and β-xylosidase activities, where glucono-1,4- or -1,5-lactones were better inhibitors of β-glucosidase and β-xylosidase activities than xylono-1,4-lactone, although the latter produced a higher inhibition of β-xylosidase than β-glucosidase [23]. This differential action of the two types of aldones might reflect lower affinity of the enzyme for xyloside; this is supported by the higher K_m values we found for 4-methylumbellyferyl and *p*-nitrophenyl-xylosides in comparison to the respective glucosides. In other non-mammalian tissues, however, the enzyme β-glucosidase is inhibited by gluconolactone but not by xylonolactone or xylosylamine [24], whereas the enzyme β-xylosidase is inhibited by the xylosyl derivatives but not by gluconolactone [25].

When the kinetics of inhibition by gluconolactone and conduritol B epoxide were examined differences were seen between β-glucosidase and β-xylosidase activities (Figs. 2a and b, and 3a and b). A similar phenomenon was noted by Grover and Aushley [26], where competitive inhibition by gluconolactone on β-glucosidase activity, and non-competitive inhibition on β-galactosidase were seen with a purified almond emulsin β-glucosidase/β-galactosidase. The reverse was noted for the β-galactosidase activity of the same enzyme preparation. These authors suggested that two catalytic sites exist in the same enzyme preparation.

We employed an additional method for determining whether the two enzymic activities are due to one or two enzymes by incubating the enzyme preparation with both substrates and the results suggest that only one enzyme is responsible for both activities (Fig. 4). In the case of a purified soluble human liver β-glucosidase, others arrived at the same conclusion [10]. Interestingly though, by this method Walker and Axelrod [27] concluded that only one active site was present in the purified almond emulsin β-glucosidase/β-galactosidase [26]. When we studied quantitatively the effect of mixed substrates, at several different substrate concentrations (Table III), the results favor the hypothesis for the existence of one, rather than two enzymes for the two activities. However, a lower activity than that calculated for one enzyme was found (in this calculation it is assumed that the inhibition is of a fully competitive type [28]), especially for the case of *p*-nitrophenyl-glycosides. This

cannot be explained at the moment. Kraml et al. [22] obtained the same type of results with the β-glucosidase/β-galactosidase of rat intestinal mucosa. In their studies the kinetics with the *p*-nitrophenyl substrates were of a mixed type, and the existence of more than a single binding site for the two substrates could not therefore be excluded. This might be a possible explanation for our results as well; kinetics of inhibition by gluconolactone and conduritol B epoxide seem to support this hypothesis.

In conclusion, our results with mouse liver support the hypothesis for the existence of a particulate enzyme possessing both β-glucosidase and β-xylosidase activities. The data obtained with the inhibitors, and two substrates simultaneously present suggest the existence of separate binding sites for the two substrates as seen for instance with carboxypeptidase A [29].

The existence of a single β-glucosidase/β-xylosidase activity could explain the finding of decreased xylosidase activity in tissues of patients with Gaucher's disease [13].

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