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## STUDIES ON THE POSSIBLE IDENTITY OF PARTICULATE $\beta$ -GLUCOSIDASE AND $\beta$ -XYLOSIDASE OF MOUSE LIVER

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### Summary

Mouse liver  $\beta$ -glucosidase ( $\beta$ -D-glucosidase glucohydrolase, EC 3.2.1.21) and  $\beta$ -xylosidase (1,4- $\beta$ -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  $\beta$ -glucosidase and  $\beta$ -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.  $\beta$ -Glucosidase activity is inhibited competitively by the two inhibitors, but  $\beta$ -xylosidase activity is inhibited non-competitively.

(c) Xylonolactone was a very poor inhibitor of both activities, but the inhibition of  $\beta$ -xylosidase activity was more pronounced than that of  $\beta$ -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  $\beta$ -D-glucoside and  $\beta$ -D-xyloside, respectively.

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### Introduction

The existence of different forms of mammalian  $\beta$ -glucosidases ( $\beta$ -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- $\beta$ -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  $\beta$ -glucosidase, in addition, apparently can hydrolyze other glycosides such as  $\beta$ -D-galactoside,  $\beta$ -D-fucoside,  $\alpha$ -L-arabinoside and  $\beta$ -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  $\beta$ -glucosidase from  $\beta$ -xylosidase (1,4- $\beta$ -D-xylan xylohydrolase, EC 3.2.1.37) and  $\alpha$ -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'  $\beta$ -glucosidase and  $\beta$ -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  $\beta$ -xylosidase activity in several tissues [14].

A particulate, lysosomal  $\beta$ -xylosidase has been reported present in rat liver and small intestine [15,16]. A single lysosomal enzyme seemed to be responsible for  $\beta$ -glucosidase and  $\beta$ -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  $\beta$ -glucosidase and  $\beta$ -xylosidase activities are due to one, or two different enzymes.

## Materials and Methods

4-Methylumbelliferyl- $\beta$ -D-glucoside and xylopyranosides, *p*-nitrophenyl- $\beta$ -D-glucoside 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-Elvehjem homogenizer, at 0–4°C. The homogenates were centrifuged at 100 000  $\times g$  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  $\times g$  for 15 min. The resultant supernatants were then centrifuged at 100 000  $\times g$  for 1 h and the pellet used. Protein was determined according to Itzhaki and Gill [19].

**Enzyme assay for  $\beta$ -glucosidase determination.** The incubation mixture

contained 1 M citrate/phosphate (or sodium acetate) buffer at the specified pH, 50  $\mu$ l; 10% sodium taurocholate, 10  $\mu$ l; 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  $\beta$ -glucosidase or for longer periods for  $\beta$ -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  $\beta$ -glucosidase and  $\beta$ -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  $\beta$ -glucosidase and  $\beta$ -xylosidase when sodium acetate was the buffer. When citrate/phosphate was used, the activities of both  $\beta$ -glucosidase and  $\beta$ -xylosidase were greater at the more acidic pH values (Fig. 1).

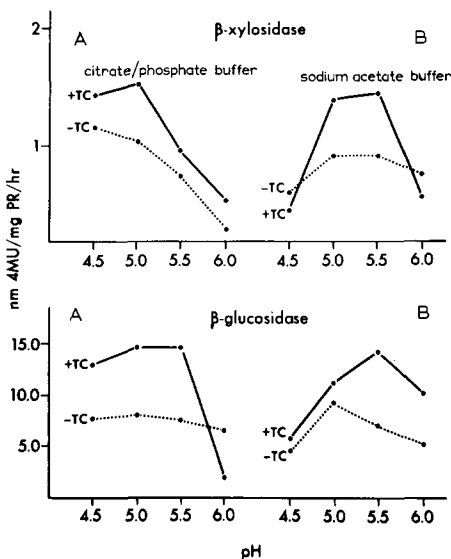


Fig. 1. Activities of  $\beta$ -glucosidase and  $\beta$ -xylosidase in the particulate (100 000  $\times$  g) fraction of mouse liver homogenates. Activities measured in the absence (●- - - -●) or presence (●- - - -●) of sodium taurocholate. (A) in citrate/phosphate buffer; (B) in sodium acetate buffer.

TABLE I

EFFECT OF DIFFERENT INHIBITORS ON PARTICULATE  $\beta$ -GLUCOSIDASE AND  $\beta$ -XYLOSIDASE ACTIVITIES OF MOUSE LIVER

Incubation mixture contained (final concentrations): 4-methylumbellyferyl- $\beta$ -D-glucoside (5 mM) or 4-methylumbellyferyl- $\beta$ -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)	$\beta$ -Glucosidase (nmol/mg protein per h)	$\beta$ -Xylosidase (nmol/mg protein per h)
<b>Preparation 1</b>		
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)
<b>Preparation 2</b>		
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  $\beta$ -glucosidase and  $\beta$ -xylosidase activities was further investigated. D-Gluconolactone inhibits  $\beta$ -glucosidase in a competitive manner (Fig. 2a), but inhibits  $\beta$ -xylosidase non-competitively (Fig. 2b). Similarly, conduritol B epoxide is a competitive inhibitor of  $\beta$ -glucosidase activity (Fig. 3a), and is a non-competitive inhibitor of  $\beta$ -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- $\beta$ -D-glucoside alone, *p*-nitrophenyl- $\beta$ -D-xyloside alone or with both substrates together and the activities

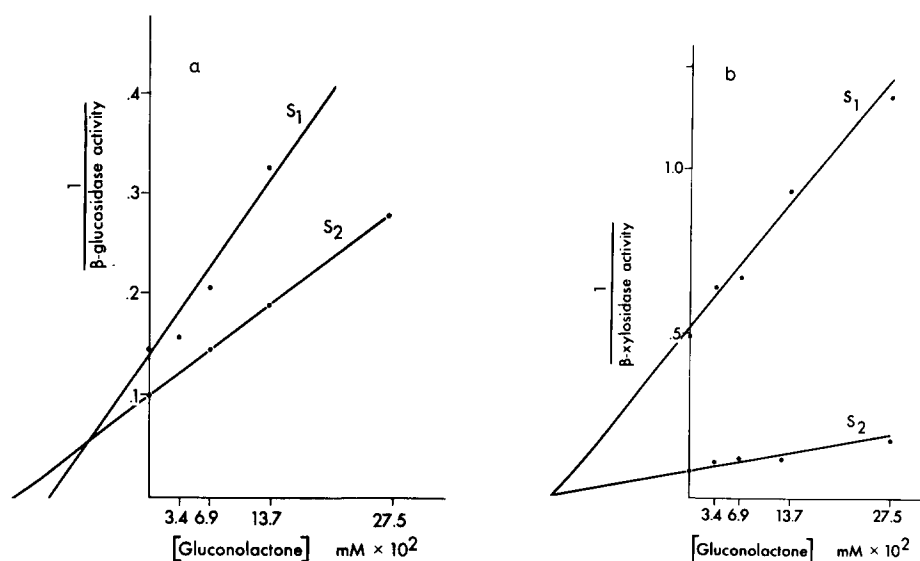


Fig. 2. (a) Inhibition of  $\beta$ -glucosidase activity in the particulate (100 000  $\times$  g) fraction of mouse liver homogenates by D-gluconolactone. Two different final substrate concentrations were used:  $S_1 = 1.25$  mM, and  $S_2 = 5$  mM. Activity was measured in citrate/phosphate buffer,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

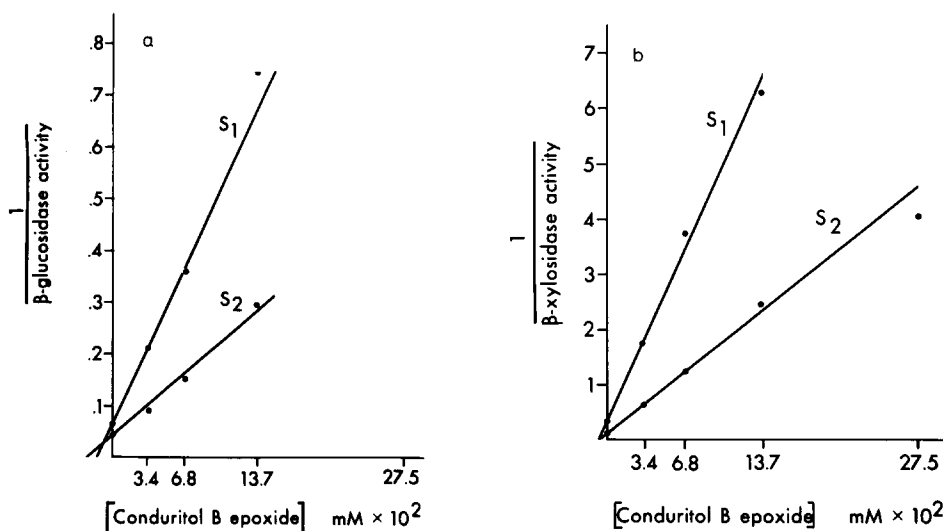


Fig. 3. (a) Inhibition of  $\beta$ -glucosidase activity in the particulate fraction of mouse liver homogenates by conduritol B epoxide.  $S_1 = 1.25$  mM and  $S_2 = 5$  mM of 4-methylumbelliferyl- $\beta$ -D-glucoside. Same incubation conditions as in Fig. 2a. (b) Inhibition of  $\beta$ -xylosidase 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  $\beta$ -GLUCOSIDASE AND  $\beta$ -XYLOSIDASE ACTIVITIES

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

Inhibitor	$K_i$ (M)	
	$\beta$ -Glucosidase	$\beta$ -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  $\beta$ -glucosidase and  $\beta$ -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-

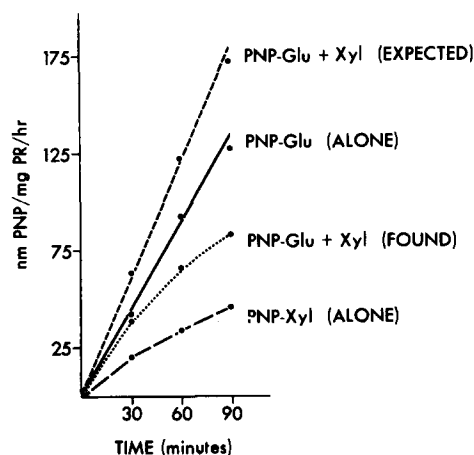


Fig. 4. Activities of  $\beta$ -glycosidase and  $\beta$ -xylosidase either present singularly or simultaneously as a function of time of incubation. Each substrate present at a final concentration of 5.0 mM.

TABLE III

EFFECT OF MIXED SUBSTRATES ON LIVER PARTICULATE  $\beta$ -GLUCOSIDASE AND  $\beta$ -XYLOSIDASE ACTIVITIES

Incubation conditions: liver homogenates centrifuged at  $600 \times g$ , 15 min, supernatant centrifuged at  $100\,000 \times g$ , 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,  $37^\circ\text{C}$ . 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- $\beta$ -D-glucoside (1 mM)	4-MU- $\beta$ -D-xyloside (1 mM)	26.1	29.3	32.0
4-MU- $\beta$ -D-glucoside (2 mM)	4-MU- $\beta$ -D-xyloside (2 mM)	39.6	41.2	50.7
4-MU- $\beta$ -D-glucoside (4 mM)	4-MU- $\beta$ -D-xyloside (4 mM)	61.5	58.4	77.1
PNP- $\beta$ -D-glucoside (0.5 mM)	PNP- $\beta$ -D-xyloside (1.0 mM)	20.4	22.7	27.4
PNP- $\beta$ -D-glucoside (1 mM)	PNP- $\beta$ -D-xyloside (2 mM)	21.1	30.7	41.2
PNP- $\beta$ -D-glucoside (2 mM)	PNP- $\beta$ -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  $\beta$ -D-glucoside and  $5.1 \cdot 10^{-3}$  M for the  $\beta$ -D-xyloside; for the *p*-nitrophenyl substrates they were  $0.85 \cdot 10^{-3}$  M for the  $\beta$ -D-glucoside and  $4.6 \cdot 10^{-3}$  M for the  $\beta$ -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  $\beta$ -glucosidase and  $\beta$ -xylosidase activities is particle bound, i.e. sediments in the  $100\,000 \times g$  pellet. This was also the case when the original homogenised fraction was first centrifuged at  $600 \times g$  for 15 min and the resultant supernatant was then centrifuged at  $100\,000 \times g$  for 1 h. Identical results were reported for rat liver [20]. The particulate  $\beta$ -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  $\beta$ -glucosidase activity, when measured with artificial substrates in the presence of detergent is believed to reflect glucocerebrosidase activity. The particulate  $\beta$ -xylosidase activity, which has been previously described in other animal species [9,20] was also present in mouse liver, and it has properties