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Hydrolysis of a naturally occurring β-glucoside by a broad-specificity β-glucosidase from liver

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We have isolated from guinea-pig liver a broad-specificity β-glucosidase of unknown function that utilizes as its substrate non-physiological aryl glycosides (e.g. 4-methylumbelliferyl β-D-glucopyranoside, *p*-nitrophenyl β-D-glucopyranoside). The present paper documents that this enzyme can be inhibited by various naturally occurring glycosides, including L-picein, dhurrin and glucocheirolin. In addition, L-picein, which acts as a competitive inhibitor of the broad-specificity β-glucosidase (K_i 0.65 mM), is also a substrate for this enzyme (K_m 0.63 mM; $V_{max.}$ 277000 units/mg). Heat-denaturation, kinetic competition studies, chromatographic properties and pH optima all argue strongly that the broad-specificity β-glucosidase is responsible for the hydrolysis of both the non-physiological aryl glycosides and L-picein. This paper demonstrates that β-glucosidase can catalyse the hydrolysis of a natural glycoside, and may provide a key to understanding the function of this enigmatic enzyme. A possible role in the metabolism of xenobiotic compounds is discussed.

INTRODUCTION

Mammalian tissues contain two β-glucosidase isoenzymes that hydrolyse aryl β-D-glucosides (e.g. MUGlc, *p*NPG) (Dance *et al.*, 1969; Glew *et al.*, 1976; Peters *et al.*, 1976). The lysosomal β-glucosidase, glucocerebrosidase, is the deficient enzyme in Gaucher's disease, and its role in the degradation of glucocerebroside has been firmly established (Patrick, 1965; Brady *et al.*, 1965). The second, non-lysosomal, β-glucosidase is actually a broad-specificity β-glycosidase with no known function (Daniels *et al.*, 1981). The broad-specificity β-glucosidase hydrolyses various low-M_r non-physiological aryl glycosides, including 4-methylumbelliferyl derivatives of β-D-glucosides, galactosides, xylosides and α-L-arabinosides (Daniels *et al.*, 1981). However, previous efforts to identify physiological substrates that support high levels of hydrolysis by this enzyme have been unsuccessful; consequently, no concrete evidence exists that defines a possible function for the broad-specificity enzyme.

The observation that the broad-specificity β-glucosidase displays a preference for low-M_r aryl glycosides prompted us to seek compounds in Nature that have similar aryl structures. Since plants that serve as food sources (e.g. almonds, cassava, sorghum) contain xenobiotic compounds, many of which are glucosides, we entertained the hypothesis that the function of the broad-specificity β-glucosidase is to hydrolyse low-M_r glycosides that, by virtue of their existence in plants, may find their way into the diet.

In the present paper we demonstrate that (1) a variety of naturally occurring glycosides act as inhibitors of the broad-specificity β-glucosidase isolated from guinea-pig liver and (2) L-picein, an aryl glucoside present in willow bark, effectively serves as a substrate for the broad-specificity enzyme. This is the first report of a naturally occurring glycoside that supports high rates of hydrolysis by the broad-specificity β-glucosidase.

MATERIALS AND METHODS

Materials

English short-hair guinea pigs were purchased from Hilltop Lab Animals (Scottdale, PA, U.S.A.). Hexokinase, glucose-6-phosphate dehydrogenase, amygdalin, laminarin, $NADP^+$, ATP, MUGlc, MUGal, MUXyl and MUAra were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). DE-52 DEAE-cellulose (Whatman) resin was obtained from Reeve Angel (Clifton, NJ, U.S.A.), and octyl-Sepharose resin was purchased from Pharmacia Fine Chemicals (Piscataway, NJ, U.S.A.). *p*NPG was obtained from Pierce Chemical Co. (Rockford, IL, U.S.A.). L-Picein, loganin, dhurrin, glucocheirolin and lactobionic acid were purchased from Atomergic Chemetals Corp. (Plainview, NY, U.S.A.). Castanospermine was kindly provided by Dr. A. D. Elbein (University of Texas Health Science Center, San Antonio, TX, U.S.A.).

β-Glucosidase assay with MUGlc as the substrate

All incubations were carried out at 37 °C. The standard assay was conducted at pH 6.0 and the reaction mixture contained 0.2 M-sodium citrate, 5.0 mM of the respective 4-methylumbelliferyl glycoside substrate and enzyme protein in a final volume of 0.1 ml. Release of 4-methylumbelliferone was measured fluorimetrically as described previously (Peters *et al.*, 1975). Where specified, various natural glycosides were included in the assay as inhibitors in concentrations indicated in the Results section. For the kinetic analyses, the substrate concentration was varied over a 20–30-fold range; kinetic constants were determined by fitting the data to a

Abbreviations used: MUGlc, 4-methylumbelliferyl β-D-glucopyranoside; MUGal, 4-methylumbelliferyl β-D-galactopyranoside; MUXyl, 4-methylumbelliferyl β-D-xylopyranoside; MUAra, 4-methylumbelliferyl α-L-arabinopyranoside; *p*NPG, *p*-nitrophenyl β-D-glucopyranoside.

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rectangular hyperbola by using a computer analysis method described by Cleland (1979). One unit of enzyme activity is defined as that amount by enzyme required to hydrolyse 1 nmol of substrate/h.

Hexokinase/glucose-6-phosphate dehydrogenase coupled assay of β-glucosidase

This assay measures release of glucose from the substrate, and was performed essentially as described previously (Lowry *et al.*, 1964). Briefly, in the standard reaction, 3 mM-L-picein was incubated at 37 °C and at pH 6.0, with 0.2 M-sodium citrate and various amounts of enzyme protein in a final volume of 0.1 ml. After boiling for 2 min, 0.02 ml of the reaction mixture was subjected to the hexokinase/glucose-6-phosphate dehydrogenase coupled reactions along with a 0–5.0 nmol glucose standard curve. Master mix I (0.1 ml) containing 0.33 mM-ATP, 5.0 mM-$MgCl_2$ and 100 mM-Tris/HCl buffer, pH 8.0, was added to 0.02 ml of the reaction mixture, followed by 0.010 ml of hexokinase (0.30 μmol/min per ml) stock prepared as described by Lowry *et al.* (1964), and the resultant mixture was incubated for 20 min at 37 °C. After the reaction had been stopped by boiling for 2 min, 1.1 ml of master mix II, containing 0.03 mM-$NADP^+$, 5.0 mM-$MgCl_2$ and 100 mM-Tris/HCl buffer, pH 8.0, and 1.7 ml of water was added, followed by 0.010 ml of a glucose-6-phosphate dehydrogenase (0.30 μmol/min per ml stock) preparation (Lowry *et al.*, 1964). The mixture was subsequently incubated for 40 min at 37 °C. Formation of NADPH was measured fluorimetrically by using a Turner fluorimeter with a 365 nm primary filter and a > 485 nm sharp-cut secondary filter; NADPH concentration was then defined in terms of glucose released by calibrating the fluorimeter with the glucose standard curve. The reaction was linear for 2 h, and with up to 0.02 ml of enzyme. One unit of enzyme activity is defined as that amount of enzyme required to catalyse the release of 1.0 nmol of glucose from the substrate/h.

Protein determination

Protein concentration was measured by using the Coomassie dye-binding method of Bradford (1976), with bovine serum albumin as the standard.

Purification of the broad-specificity β-glucosidase from guinea-pig liver

The broad-specificity β-glucosidase was purified essentially as described previously (LaMarco & Glew, 1985) to a specific activity of 475000 units/mg of protein. Chromatography on octyl-Sepharose was performed exactly as described previously (LaMarco & Glew, 1985).

DE-52 DEAE-cellulose column chromatography

The 100000 *g* supernatant fraction of the β-glucosidase preparation was applied to a 5 cm × 22 cm column of DE-52 DEAE-cellulose equilibrated with 10 mM-sodium phosphate buffer, pH 6.0. After the column had been washed with 10 column volumes of the same buffer, a 2-litre 0–0.3 M-NaCl linear gradient was applied to the column, and 20 ml fractions were collected. Conductivity was monitored with a Radiometer conductivity-meter.

Kinetic competition experiment

To calculate the velocity of the reaction in mixtures containing *p*NPG and L-picein, the two substrates were added to the standard reaction mixture in various concentrations, and glucose release from both substrates was measured by using the hexokinase/glucose-6-phosphate dehydrogenase coupled assay as described above.

RESULTS

Isolation of the broad-specificity β-glucosidase from guinea-pig liver

Purification of the broad-specificity β-glucosidase from guinea-pig liver was performed essentially as described previously (LaMarco & Glew, 1985), to a specific activity of 475000 units/mg, a value comparable with those for β-glucosidases from other sources that have been purified to homogeneity (Daniels *et al.*, 1981; LaMarco & Glew, 1985). Hydrophobic affinity chromatography on octyl-Sepharose was utilized in the purification, indicating that the enzyme has a significant degree of hydrophobic character. This step provided greater than 50-fold purification, and a typical profile is shown in Fig. 1. A large portion of the contaminating protein passed through the column, while essentially all the β-glucosidase activity remained bound. The enzyme was eluted from the column as a sharp peak with 80% (v/v) ethylene glycol; all four glycosidase activities were co-eluted, indicating that guinea-pig β-glucosidase is indeed a broad-specificity β-glycosidase comparable with the broad-specificity β-glucosidase isolated from human liver (Daniels *et al.*, 1981; LaMarco & Glew, 1985).

Properties of the purified broad-specificity β-glucosidase

Substrate saturation curves for β-glucosidase were constructed with as substrates MUGlc, MUGal, MUAra and MUXyl. Hydrolysis of all four substrates obeyed Michaelis–Menten kinetics, and the K_m and $V_{max.}$ values are summarized in Table 1. The catalytic efficiency ($V_{max.}/K_m$ ratio) was highest for MUGlc; thus, since MUGlc is the preferred substrate, the broad-specificity enzyme is commonly referred to as β-glucosidase.

Sedimentation of the purified β-glucosidase in a 5–20% (w/v) sucrose gradient yielded an $S_{20,w}$ value of 5.1 S; the apparent M_r calculated with respect to an alkaline phosphatase marker was 70000, a value similar to that estimated by SDS/polyacrylamide-gel electrophoresis (Laemmli, 1970) for human liver β-glucosidase.

Naturally occurring glycosides as inhibitors of the broad-specificity β-glucosidase

β-Glucosides can be found in Nature in a variety of plants. Some of these naturally occurring glycosides are low-M_r water-soluble aryl glycosides with structures comparable with those of MUGlc or *p*NPG, two non-physiological substrates that support high rates of hydrolysis by the broad-specificity β-glucosidase. Since plants constitute a considerable fraction of the foods consumed by omnivores, it is conceivable that, after consumption, these low-M_r plant glycosides could serve as inhibitors of or substrates for the broad-specificity β-glucosidase *in vivo*. A variety of these glycosides were tested as inhibitors of the purified β-glucosidase utilizing MUGlc as the substrate, and the following compounds were without effect: loganin, castanospermine, amygdalin and laminarin (see Fig. 2). Given the historical relationship between the broad-specificity β-glucosidase

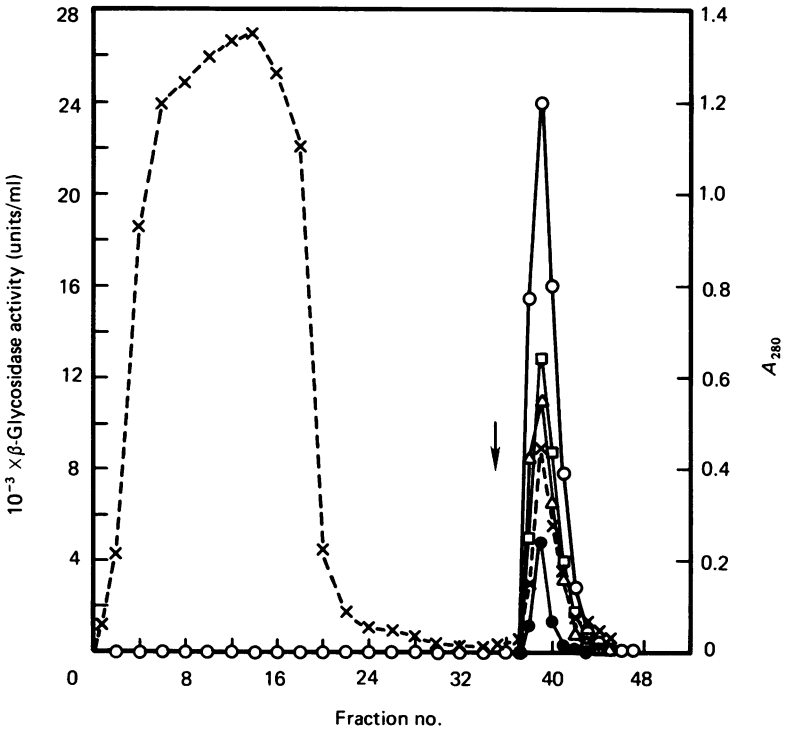

Fig. 1 Chromatography of the broad-specificity β-glucosidase on octyl-Sepharose

Glycosidase activity was measured with one of the following 4-methylumbelliferyl derivatives as the substrate: ○, MUGlc; □, MUGal; △, MUAra; ●, MUXyl. Protein (×) was monitored by absorbance at 280 nm. The column was loaded up to fraction 24, then washed with 0.5 litre of equilibrating buffer. Elution with 80% ethylene glycol was initiated at the arrow.

Table 1. Kinetic parameters of the broad-specificity β-glucosidase from guinea-pig liver

Substrate	K_m (mM)	$V_{max.}$ (units/mg)	$10^{-4} \times V_{max.}/K_m$
MUGlc	0.13	442000	325
MUGal	0.51	325000	63.7
MUAra	0.07	90000	129
MUXyl	0.12	19500	16.3

and glucocerebroside β-glucosidase (Daniels *et al.*, 1981; LaMarco & Glew, 1985), and the observation that castanospermine is a potent inhibitor of the latter enzyme (Saul *et al.*, 1983), it is curious that this product of the Australian legume does not inhibit the broad-specificity β-glucosidase of guinea-pig liver.

Four of the compounds tested acted as inhibitors of the broad-specificity β-glucosidase: glucocheirolin, dhurrin, lactobionic acid and L-picein. To define the nature of the inhibition caused by the natural glycosides, substrate saturation curves were composed with MUGlc as the substrate, in the presence of increasing concentrations of each of the four glycosides. Slopes and intercepts were calculated by computer analysis that fits the data to a rectangular hyperbola as described by Cleland (1979). In each case a replot of slope versus inhibitor concentration was constructed and the abscissa intercept was calculated by linear-regression analysis; the equilibrium constant for each inhibitor (K_i) was estimated from the abscissa intercepts of the replots. The structures of these glycosides are shown in Fig. 3.

Glucocheirolin, a thioglucoside present in mustard oil and wallflower seeds, yielded straight lines that intersected to the left of the ordinate axis (results not shown), a pattern characteristic of mixed-type inhibition. A K_i of 3.33 mM for glucocheirolin revealed that this glycoside was the poorest inhibitor of the four tested. Dhurrin (see Fig. 3), a cyanogenic aryl glucoside isolated from *Sorghum vulgare*, also exhibited mixed-type inhibition (Fig. 4), but was a considerably more effective inhibitor, possessing a K_i value of 0.16 mM (Fig. 4 inset).

The remaining two natural glycosides proved to be competitive inhibitors, as the inhibitor curves intersected at a point on the ordinate axis; intersection on the ordinate axis indicates that, even at infinite substrate concentrations, the $V_{max.}$ remains unchanged, a feature of competitive inhibition. Lactobionic acid, which can be obtained by oxidation of lactose at position 1 of the glucose moiety, was a good inhibitor, exhibiting a K_i of 0.18 mM (results not shown). L-Picein, which can be isolated from English mistletoe and willow bark, was also an effective competitive inhibitor (Fig. 5), with a K_i value of 0.65 mM (Fig. 5 inset).

L-Picein as a substrate for the broad-specificity β-glucosidase

The β-glucoside L-picein, as shown in Fig. 3, has a structure very similar to that of *p*NPG; in addition, the fact that it is a competitive inhibitor (Fig. 5), and therefore interacts with the enzyme at the substrate-binding site, led us to investigate the possibility that

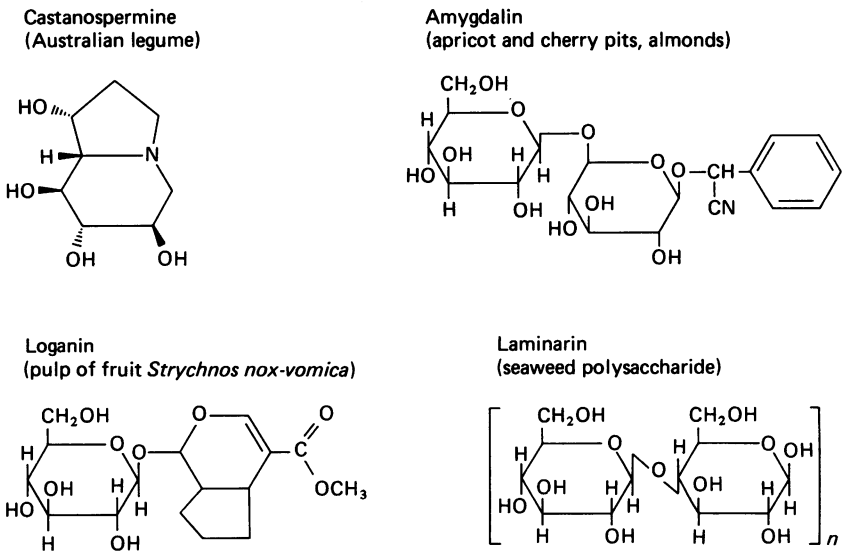

Fig. 2. Structures of four naturally occurring compounds that do not inhibit β-glucosidase

The concentrations for preliminary inhibition studies were as follows: castanospermine (0–0.2 mg/ml); amygdalin (0–5.0 mM); loganin (0–5.0 mM); laminarin (0–0.3 mg/ml).

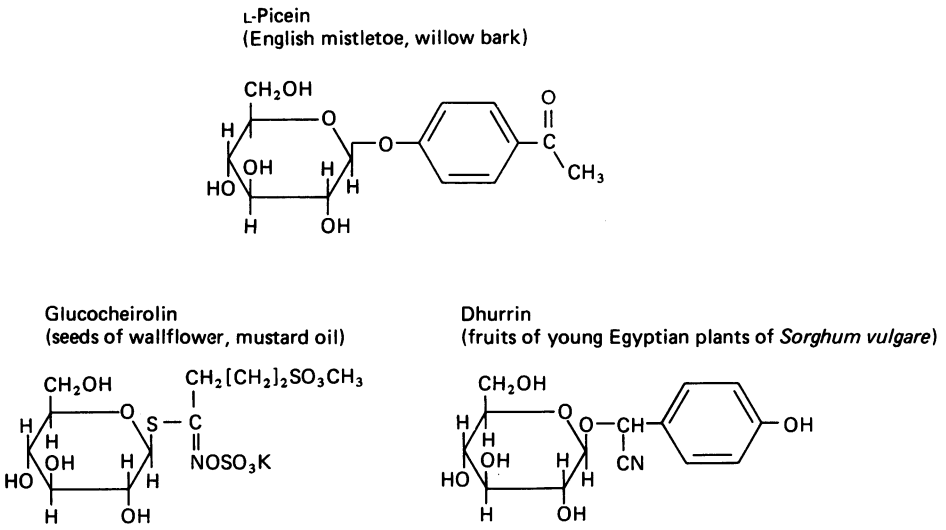

Fig. 3. Structures of three naturally occurring glycosides that inhibit the broad-specificity β-glucosidase

L-picein may be a substrate for the broad-specificity β-glucosidase. Glucose release was measured by a hexokinase/glucose-6-phosphate dehydrogenase coupled assay, as described in the Materials and methods section. A substrate saturation curve, constructed with L-picein in the concentration range 0.06–2 mM, revealed that L-picein could indeed serve as a substrate for the broad-specificity β-glucosidase. Kinetic constants were calculated from a double-reciprocal plot, as shown in Fig. 6; the K_m for L-picein was 0.63 mM, a value in close agreement with the apparent K_i (0.65 mM; Fig. 5 inset), and the $V_{max.}$ was 277000 units/mg, about 66% of the $V_{max.}$ for MUGlc hydrolysis. None of the other compounds mentioned has been tested as substrate.

A variety of experiments were then performed to provide evidence that L-picein hydrolysis was accomplished by the identical broad-specificity β-glucosidase that hydrolyses well-characterized non-physiological substrates (e.g. MUGlc, *p*NPG), and not by some minor contaminating glucosidase activity that might have been present in the extensively purified enzyme preparation.

The variation of β-glucosidase activity over the pH range 4.0–8.0 was investigated with MUGlc and L-picein as the substrates (Fig. 7). The general bell-shape of the curve was the same for the two substrates, and hydrolysis of both substrates was maximal at pH 6.0. A shoulder was evident on the alkaline side of the pH optimum, and, although it was present with both substrates, and may represent microheterogeneity in the enzyme preparation, we performed subsequent experiments to support the hypothesis that the two activities resided in the same enzyme molecule.

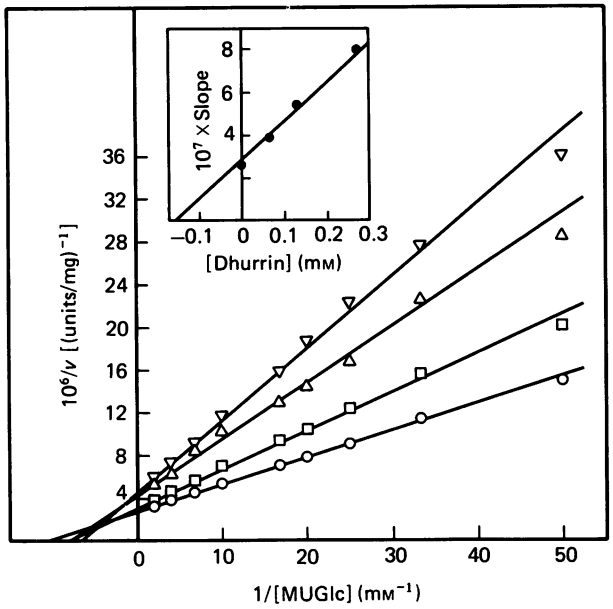

Fig. 4. Lineweaver–Burk analysis of the broad-specificity β-glucosidase inhibited by increasing concentrations of dhurrin

β-Glucosidase activity was measured with MUGlc as the substrate, in the absence (○) or presence of the following concentrations of dhurrin: □, 0.07 mM; △, 0.13 mM; ▽, 0.26 mM.

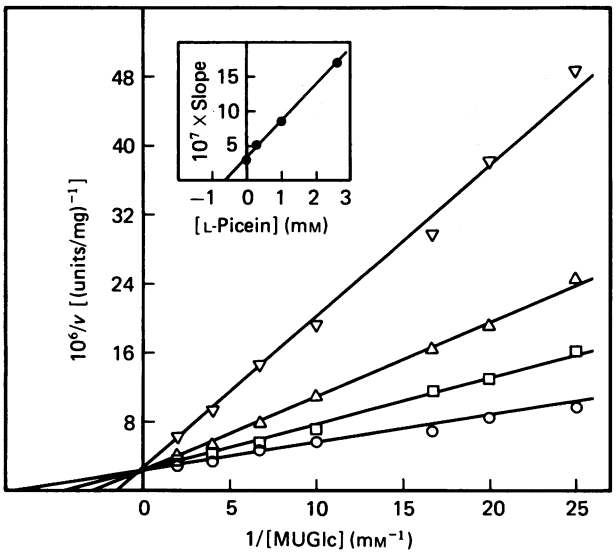

Fig. 5. Lineweaver–Burk analysis of the broad-specificity β-glucosidase inhibited by increasing concentrations of L-picein

β-Glucosidase activity was measured with MUGlc as the substrate, in the absence (○) or presence of the following concentrations of L-picein: □, 0.25 mM; △, 1.00 mM; ▽, 2.60 mM.

If hydrolysis of MUGlc and L-picein is catalysed by the same enzyme, glucose release from both substrates should be equally heat-sensitive. We therefore subjected the β-glucosidase preparation to heat inactivation and then measured its ability to hydrolyse both substrates. As

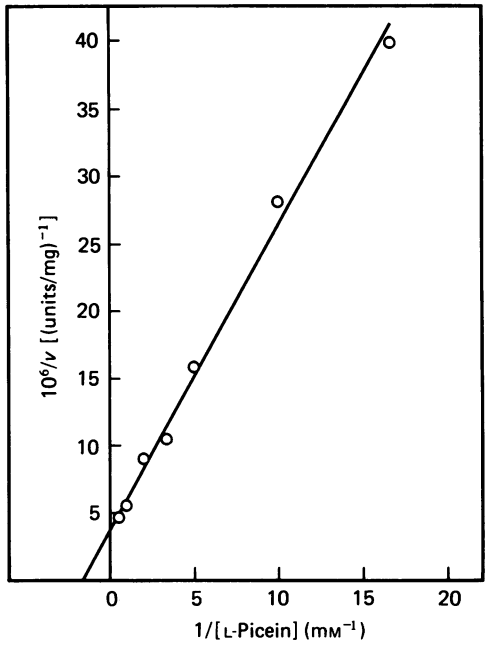

Fig. 6. Lineweaver–Burk analysis of the broad-specificity β-glucosidase with L-picein as the substrate

Glucose release from L-picein (○) was determined under standard conditions by the hexokinase/glucose-6-phosphate dehydrogenase coupled assay described in the Materials and methods section.

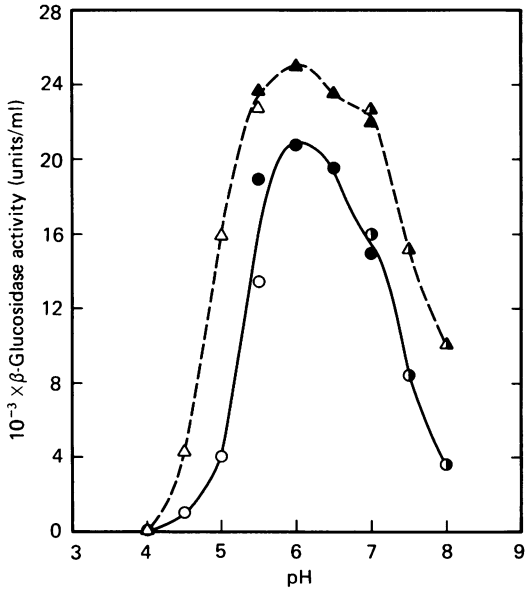

Fig. 7. Effect of pH on β-glucosidase activity measured with either MUGlc or L-picein as the substrate

β-Glucosidase activity was measured with (1) MUGlc as the substrate as described in the Materials and methods section (△, ▲ and ◮) or (2) L-picein as the substrate by the coupled assay described in the Materials and methods section (○, ● and ◑). The assays were conducted exactly as described except for the substitution of the following buffers at a final concentration of 0.2 M: △ and ○, sodium acetate; ▲ and ●, sodium citrate; ◮ and ◑, sodium phosphate.

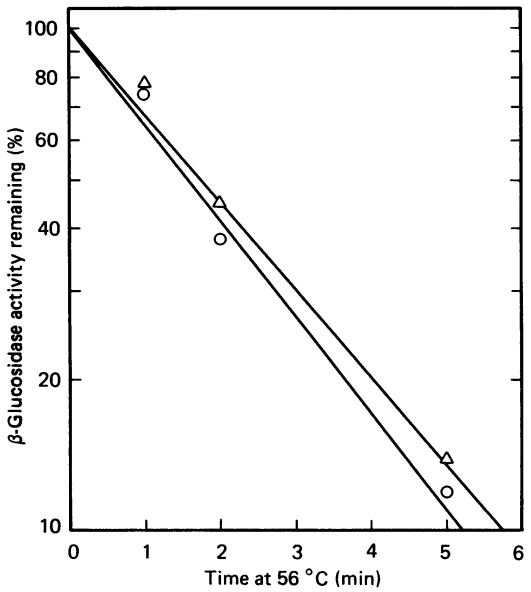

Fig. 8. Heat denaturation of the broad-specificity β-glucosidase

A portion of the purified β-glucosidase preparation was heated at 56 °C for a period of time varying between 0 and 5 min. β-Glucosidase activity was measured under standard conditions with either MUGlc (○) or L-picein (△) as the substrate, as described in the Materials and methods section.

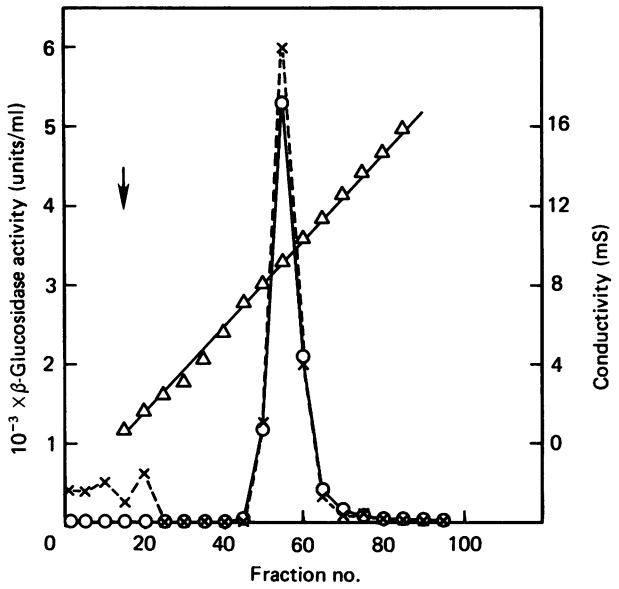

Fig. 9. Chromatography of the broad-specificity β-glucosidase on a DE-52 DEAE-cellulose column

β-Glucosidase activity was detected with either MUGlc (○) or L-picein (×) as the substrate exactly as described in the Materials and methods section. A 30 ml 0–0.3 M-NaCl gradient was begun at the position of the arrow, and conductivity was monitored (△).

witnessed in Fig. 8, hydrolysis of both substrates decreased at an identical rate ($slope_{MUGlc} = -0.20$; $slope_{L\text{-picein}} = -0.19$), supporting the hypothesis that both substrates are hydrolysed by the broad-specificity β-glucosidase.

Since the broad-specificity β-glucosidase binds to the anion-exchanger DE-52 DEAE-cellulose, and can be subsequently eluted with a 0–0.3 M-NaCl gradient, we next attempted to demonstrate coelution, from a DE-52 DEAE-cellulose column, of β-glucosidase activity measured with MUGlc and L-picein as substrates. A portion of the 100000 *g* supernatant fraction, which contains over 98% of the broad-specificity enzyme activity, was chromatographed on a DE-52 DEAE-cellulose column, and the results of this experiment are presented in Fig. 9. More than 96% of the picein-hydrolysing activity co-chromatographed with the MUGlc-hydrolysing peak; the small amount of apparent activity in the early column fractions is not real, but is the result of contaminating pigments that interfere with the coupled assay.

In a final attempt to verify that the same active site catalysed the hydrolysis of both non-physiological aryl glycosides and L-picein, we performed kinetic competition studies with two competitive substrates. When enzymic activity is measured in the presence of two competitive substrates, the hydrolysis of each substrate in the mixture will be less than the hydrolysis of that same substrate tested alone (Thorn, 1949; Dixon & Webb, 1964). The following equation can be used to calculate the velocity, v, of the reaction when one enzyme hydrolyses two substrates in a mixture:

$$v = \frac{\frac{V_a \cdot a}{K_a} + \frac{V_b \cdot b}{K_b}}{1 + \frac{a}{K_a} + \frac{b}{K_b}}$$

where V_a and V_b are the maximum velocities, K_a and K_b are the Michaelis constants and a and b are the substrate concentrations of the two substrates. If two separate enzymes are each hydrolysing their respective substrate, then the reaction velocity will be equal to the sum of the velocities measured when only one substrate is present. The results of the competition studies performed at two *p*NPG concentrations are presented in Table 2; *p*NPG was utilized as the alternative substrate, since the highly fluorescent MUGlc interfered with the coupled assay. On measuring glucose release at both *p*NPG concentrations, the observed velocity, as measured by the coupled assay, was remarkably similar to the velocity calculated for one enzyme that hydrolyses two substrates, indicating that one enzyme in the preparation was responsible for the hydrolysis of both *p*NPG and L-picein.

Taken together, the results in this section argue strongly that the β-glucosidase activities that hydrolyse non-physiological aryl glycosides and L-picein reside in the same enzyme molecule, and therefore that L-picein is a *bona fide* substrate for the broad-specificity β-glucosidase.

DISCUSSION

The physiological function of the broad-specificity β-glucosidase has eluded investigators for decades. Our observation that selected naturally occurring glycosides serve as inhibitors of, and in one case as a substrate for, the broad-specificity β-glucosidase may aid in defining a physiological role for this enzyme. Inhibitor studies showing L-picein to be a competitive inhibitor of the broad-specificity β-glucosidase, as well as the experiments

Table 2. Competition studies: calculated and observed reaction rates

K_m (*p*NPG) = 0.76 mM; $V_{max.}$ (*p*NPG) = 175000 units/mg.

Substrates	10^{-3} Calculated velocity (units/mg)		10^{-3} Observed velocity (units/mg)	Number of enzymes
	One enzyme	Two enzymes		
2 mM-L-Picein + 0.2 mM-*p*NPG	126	159	132	1
2 mM-L-Picein + 0.6 mM-*p*NPG	131	200	143	1

with L-picein as a substrate (e.g. heat denaturation, co-elution from a DE-52 DEAE-cellulose column, pH–activity curves, kinetic competition experiments) strongly imply that hydrolysis of L-picein is indeed catalysed by the broad-specificity enzyme; L-picein is therefore the first natural substrate shown to be rapidly hydrolysed *in vitro* by β-glucosidase.

The fact that L-picein is hydrolysed by β-glucosidase 66% as efficiently as MUGlc, and that this natural glycoside is structurally similar to the non-physiological aryl glycoside substrates, suggests that the enzyme prefers substrates containing a hydrophobic domain; this is supported by previous studies that revealed that hexyl and heptyl thioxylosides acted as competitive inhibitors of the broad-specificity β-glucosidase of mammalian liver, whereas the short-chain, less hydrophobic alkyl thioxylosides did not (Daniels, 1983).

Although dhurrin is structurally similar to L-picein (Fig. 3), it acts as a mixed-type inhibitor of the broad-specificity β-glucosidase, indicating that it binds to some site other than the substrate-binding site. The bulky cyanide group found in dhurrin may prevent binding to the substrate-binding site; however, a second glycoside-binding site on β-glucosidase must exist, which binds dhurrin and allows it to inhibit catalysis in a mixed-type fashion. Other evidence exists that implies the existence of a second glycoside-binding domain; for example, both glucosyl- and galactosyl-sphingosine act as mixed-type inhibitors of the human broad-specificity β-glucosidase (Daniels, 1983; LaMarco & Glew, 1985). Glucocheirolin acts also as a mixed-type inhibitor of β-glucosidase, albeit a poor one. Perhaps the negatively charged sulphate group of the aglycone (Fig. 3) retards binding of this compound to the putative glycoside-binding site. Amygdalin and loganin possess aromatic groups in the aglycone (Fig. 2), but do not inhibit β-glucosidase activity; the additional glucose residue of amygdalin and the substituted heterocyclic ring of loganin may sterically hinder binding of these glycosides to either the enzyme's substrate-binding site or the second glycoside-binding site that influences turnover. The fact that lactobionic acid is a competitive inhibitor of β-glucosidase reveals that the substrate-binding site can accommodate small charged disaccharides. The enzyme did not, however, recognize laminarin; perhaps interaction between the non-reducing end of the polysaccharide and the enzyme's active site was a rare event, and the repeat unit would be a more effective inhibitor.

The realization that mammals ingest food stuffs that contain these glycosides, and the recognition that these xenobiotic compounds can have toxic effects, compel us to speculate about the role of β-glucosidase in detoxication. If these naturally occurring glycosides are absorbed intact in the gut and subsequently transported to the liver, where they can come in contact with the broad-specificity β-glucosidase, then perhaps the function of this enzyme is to remove the sugar moiety from a variety of glycosides and create a site for conjugation to some charged substance (e.g. glucuronic acid) that renders the compound more water-soluble.

Detoxication has been defined as 'those chemical changes which foreign organic compounds undergo in the mammalian body' (Williams, 1947), and consists of one or both of two phases of metabolism (Caldwell, 1980). Phase I metabolism includes oxidation, reduction and hydrolysis, and phase II metabolism involves conjugation of the compound to glucuronic acid, sulphate or a variety of other charged molecules. One belief is that the enzymes involved in detoxication serve to render lipophilic substrates more water-soluble, and therefore more easily excretable (Caldwell, 1980). To interact with lipophilic compounds, these enzymes must possess a hydrophobic substrate-binding pocket (Jakoby, 1980); the broad-specificity β-glucosidase has been shown to contain such a binding pocket by the enzyme's ability to adhere to octyl-Sepharose (see Fig. 1) and to be inhibited by hydrophobic glycolipids (Daniels, 1983; LaMarco & Glew, 1985). Another property shared by β-glucosidase and the detoxication enzymes is acceptance of a wide range of substrates (Jakoby 1980; Daniels *et al.*, 1981). A broad specificity allows these enzymes to oblige a variety of substrates and catalyse a range of reactions, perhaps at the expense of lower rates of catalysis (Jakoby, 1980). Since plant glycosides may contain a variety of sugars, including D-glucose, D-galactose, D-xylose and L-arabinose (Scheline, 1978), having one broad-specificity enzyme capable of hydrolysing all four sugars from glycosides spares the cell from synthesizing four separate highly selective glycosidases.

It is conceivable that before conjugation can occur the sugar residue must be cleaved from the parent glycoside, producing an aglycone with an acceptor site for conjugation. Although this aglycone may be a highly reactive intermediate, detoxication enzymes are believed to form an interacting group of catalysts that may function in a fashion analogous to other enzyme systems that constitute metabolic pathways (e.g., glycolytic enzymes) (Jakoby, 1980); thus the aglycone intermediate should be channelled into phase II of its metabolism with relative ease.

It is generally believed that glycosidases in mammalian tissues do not contribute significantly to the hydrolysis of

ingested plant glycosides, owing to the subcellular localization of these enzymes (Scheline, 1978). Presumably, polar glycosides cannot make contact with glycosidases located in microsomes; however, if these compounds, like L-picein, contain aromatic hydrophobic domains, the possibility exists that they can be metabolized by microsomal glycosidases, especially since other detoxication enzymes are housed in the endoplasmic reticulum (Scheline, 1978). This hypothesis is not without precedence, as sugar residues of highly lipophilic cardiac glycosides are believed to be hydrolysed by microsomal enzymes (Scheline, 1978).

Hydrolysis of glucuronide-conjugated xenobiotics has been predominantly attributed to the microflora of the gastrointestinal tract; these compounds are thought to undergo conjugation in the liver and then excretion into the bile, so that the conjugated forms eventually interact with the gut bacteria (Scheline, 1978; Goldman, 1980). Some of the conjugates are then hydrolysed by bacterial enzymes, the aglycones being re-absorbed and excreted in the urine. An obvious question that remains is: if the naturally occurring xenobiotic compound is a glycoside, where does hydrolysis occur so that subsequent conjugation can take place? Experiments have been conducted with germ-free rats to address this question. For example, if metabolites found in the urine and faeces of normal rats after administration of a particular compound are not detected in germ-free rats under the same conditions, involvement of the intestinal microflora is assumed (Goldman, 1980). Experiments of this kind have assigned the role of glycoside hydrolysis to the intestinal microflora; however, metabolism of a xenobiotic compound may differ in a germ-free animal. If absorption of the compound is inhibited in germ-free animals, then interference with its access to the liver will alter the contribution of mammalian enzymes to its metabolism (Goldman, 1980). Often an animal is rendered germ-free by treatment with antibiotics that exhibit a multiplicity of effects on the animal's metabolism. Aminoglycosides bind to and inhibit absorption of certain test compounds; additionally, antibiotics often cause diarrhoea, thus decreasing the intestinal transit time and ultimately inhibiting absorption (Goldman, 1980).

Whether or not a xenobiotic is metabolized by intestinal bacteria also depends on the spatial distribution of the microflora, as well as the absorptive properties of the compound. Intestinal flora normally reside beyond the ileocaecal valve; thus compounds such as L-picein with a hydrophobic domain, or drugs designed to facilitate absorption, will probably be absorbed before they meet the intestinal microflora (Goldman, 1980). This would necessitate insertion of a glycoside hydrolysis step before the conjugation reaction; we propose that the broad-specificity β-glucosidase may function in this manner *in vivo*.

In areas of the world where large quantities of cassava root or sorghum grain are consumed, a significant percentage of the population is afflicted with neurological disease. Since these food sources contain cyanogenic glycosides, it is believed that the causative agent is the free cyanide produced *in vivo* from breakdown of these compounds (Westly, 1980; Umoh *et al.*, 1985). The present paper documents that dhurrin (Fig. 2) is a mixed-type inhibitor of the broad-specificity β-glucosidase (Fig. 4); therefore, if dhurrin is absorbed in the gut as the glucoside, and comes in contact with β-glucosidase *in vivo*, the possibility exists that some of the pathophysiology associated with ingestion of cyanogenic glucosides can result from inhibition of the broad-specificity β-glucosidase. Inhibition of enzymes *in vivo* by ingested plant compounds has previously been shown to cause physiological dysfunction; cattle grazing on a legume containing swainsonine, a potent inhibitor of lysosomal α-mannosidase, develop a syndrome that mimics genetic mannosidosis (Ockerman, 1969; Dorling *et al.*, 1978).

In conclusion, we have identified, for the first time, a natural substrate rapidly hydrolysed by the broad-specificity β-glucosidase, which may indicate that this enzyme is involved in hepatic detoxication of xenobiotic compounds.

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