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Exolytic Hydrolysis of Toxic Plant Glucosides by Guinea Pig Liver Cytosolic β-Glucosidase*

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Venkatakrishnan Gopalan‡, Andrzej Pastuszyn‡, William R. Galey, Jr.§, and Robert H. Glew‡¶

From the Departments of ‡Biochemistry and §Physiology, School of Medicine, University of New Mexico, Albuquerque, New Mexico 87131

We demonstrate that although the guinea pig liver cytosolic β-glucosidase does not catalyze the hydrolysis of gentiobiose, it does hydrolyze, disaccharide-containing glycosides such as *p*-nitrophenyl-β-D-gentiobioside (Glcβ1→6Glcβ-pNP) and mandelonitrile-β-D-gentiobioside (amygdalin). Furthermore, we establish that the enzyme attacks disaccharide glycosides exolytically; specifically, we document the exolytic deglucosylation of amygdalin and the generation of the intermediate monosaccharide glycoside mandelonitrile-β-D-glucoside prior to the formation of the aglycone (mandelonitrile).

We also show that the cytosolic β-glucosidase catalyzes the hydrolysis of various phenolic (*e.g.* arbutin and salicin) and cyanogenic plant glucosides (*e.g.* prunasin). Using the everted gut-sack technique, we demonstrate that the plant glucosides, amygdalin, prunasin, and vicine, are transported across the small intestine of the guinea pig efficiently and without being hydrolyzed. Based on thess data we speculate that the cytosolic β-glucosidase may participate in biotransformation of toxic plant glucosides.

The cytosol of mammalian tissues such as liver and kidney is rich in β-glucosidase activity. Almost all of this β-glucosidase activity can be accounted for by a 60-kDa, neutral pH optimum glycohydrolase. This broad-specificity β-glucosidase catalyzes the hydrolysis of β-D-glucosides, β-D-galactosides, α-L-arabinosides, and β-D-xylosides of aryl aglycones such as *p*-nitrophenol and 4-methylumbelliferone (1–4). In the three decades since the enzyme was first described, the search for its physiologic role has eluded investigators.

Several reports have established that the broad-specificity mammalian cytosolic β-glucosidase is incapable of cleaving a variety of glucose- or galactose-terminated disaccharides, including gentiobiose, lactose, and cellobiose (2, 5). We recently conducted a study to gain insight into the chemical and physical nature of the active site of the cytosolic β-glucosidase and its lysosomal counterpart, glucocerebrosidase, and learned that a family of reversible, competitive inhibitors, namely alkyl-β-D-glucosides, displayed at least 100-fold lower K_I values for the cytosolic enzyme relative to the lysosomal glucocerebrosidase (6). This observation led us to hypothesize that the cytosolic β-glucosidase possesses a much more hydrophobic subsite in its catalytic center than glucocerebrosidase whose natural substrate is the highly lipophilic sphingolipid, glucocerebroside. On the basis of the results of that study, we suggested that in planning future studies aimed at identifying natural substrates of this β-glucosidase investigators should consider amphipathic glucosides as potential substrates.

LaMarco and Glew (7) demonstrated that L-picein, a plant phenolic glucoside, was an excellent substrate for the guinea pig liver β-glucosidase. In that same study, they also documented that a naturally occurring cyanogenic β-D-glucoside, dhurrin, was a potent inhibitor of the cytosolic β-glucosidase. On the basis of these results they speculated that the enzyme might play a role in the detoxification of toxic plant glucosides that find their way into the diets of man or animals.

In the present report we address three questions: (i) is the β-glucosidase capable of cleaving the sugar moieties of a cyanogenic disaccharide glycoside such as amygdalin and, if so, does the enzyme function as an exo- or endo-β-glucosidase? (ii) Do pathophysiologically relevant toxic plant glucosides serve as substrates for the guinea pig liver β-glucosidase? (iii) Are any of these toxic plant glucosides transported intact across the small intestine?

Herein, we document that a variety of toxic plant glucosides are hydrolyzed by the cytosolic β-glucosidase and also provide evidence for the exolytic nature of the cleavage of glycone moieties from amygdalin (Glcβ1→6Glcβ-mandelonitrile). Finally, we establish that these glycosides are transported efficiently across the gut, and we propose a biotransformation role for the hepatic β-glucosidase and discuss its pathophysiologic implications.

EXPERIMENTAL PROCEDURES

Materials

Amygdalin, *p*-nitrophenyl-β-D-glucoside (pNP-Glc),[1] 4-methylumbelliferyl-β-D-glucoside (4-MUGlc), *p*-nitrophenyl-β-D-gentiobioside (pNP-gentiobioside), *p*-nitrophenyl-β-D-cellobioside (pNP-cellobioside), and *p*-nitrophenyl-β-D-lactoside (pNP-lactoside) were purchased from Sigma. L-Picein (*p*-acetophenyl-β-D-glucoside) was obtained from Pfaltz and Bauer, Waterbury, CT. Arbutin was provided by Dr. Gordon Willick, National Research Council, Ottawa, Canada. Neolinustatin was a gift from Dr. David Seigler, University of Illinois, Urbana-Champaign, IL. Prunasin and linamarin were provided by Dr. Eric E. Conn, University of California, Davis, CA. [^{14}C]Inulin (specific activity 3 mCi/g) was purchased from ICN Biochemicals.

Cytosolic β-glucosidase was purified as described elsewhere from frozen guinea pig livers purchased from Pel Freez, Rogers, AR (7); the final preparation exhibited a specific activity of 506,000 units/mg protein in the standard β-glucosidase assay described below and which used 4-MUGlc as the substrate.

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¶ To whom correspondence should be addressed.

[1] The abbreviations used are: pNP-Glc, *p*-nitrophenyl-β-D-glucoside; 4-MUGlc, 4-methylumbelliferyl-β-↓-glucoside; pNP-gentiobioside, *p*-nitrophenyl-β-D-gentiobioside; pNP-cellobioside, *p*-nitrophenyl-β-D-cellobioside; pNP-lactoside, *p*-nitrophenyl-β-D-lactoside; HPLC, high performance liquid chromatography.

Methods

The Standard β-Glucosidase Assay—The standard assay contained substrate (at the specified concentration) in 0.2 M sodium citrate buffer, pH 6.0, and enzyme (1.0–5.0 ng) in a final volume of 0.1 ml. Assays were initiated by addition of enzyme and incubations were carried out at 37 °C for 15–30 min. When 4-MUGlc served as the substrate, the assay was terminated by the addition of 2.9 ml of 0.3 M ammonia-glycine buffer, pH 10.5, and release of the fluorescent product, 4-methylumbelliferone, was monitored using a Turner fluorometer as described elsewhere (8). One unit of enzyme activity corresponds to the release of 1 nmol of product in 1 h.

In the case of *p*-nitrophenyl substrates, incubations were terminated by addition of 0.9 ml of 0.02 M sodium borate, pH 9.5, solution. The release of *p*-nitrophenol from the various disaccharide glycosides was estimated based on its absorbance at 400 nm and molar absorptivity coefficient of 18,300 M^{-1} cm^{-1} (7).

Determination of D-Glucose—To measure the amount of D-glucose released in β-glucosidase assays, we utilized the hexokinase/glucose-6-phosphate dehydrogenase (Glu-6PD) coupled assay as described elsewhere (7). The amount of D-glucose release was calculated from a D-glucose calibration curve.

Studies on Uptake of Glucosides across Everted Gut Preparations of Guinea Pig Small Intestine—Guinea pigs were euthanized with sodium pentobarbital. After opening the abdomen, the proximal jejunum immediately distal to the duodenum was freed from mesenteric attachments and a gut segment (approximately 4-cm long) was removed from the animal. The section of jejunum was then everted, tied with a suture at one end, and filled with a 13.5 mM sodium phosphate buffer, pH 7.4, containing 145 mM sodium chloride, 5 mM potassium chloride, and 5 mM glucose (290–300 mOsm). The other end was then closed with a suture to form two filled, everted gut sacks. The sack was then immersed in buffer containing the glycoside solute (5 mM) whose permeability was to be determined. [^{14}C]Inulin was included in the external bathing medium to permit monitoring of the integrity of the intestinal sack. At the end of a 2-h incubation at 37 °C, the contents of each sack were removed and analyzed for both the test and ^{14}C tracer solutes. Aliquots from inside and outside the gut-sack were subjected to scintillation counting to measure [^{14}C] inulin leakage. Any sack exhibiting high permeability to the tracer (>25% of equilibrium) was considered defective and discarded; the mean [^{14}C]inulin leakage in our experiments was 16 ± 6%. The reversed-phase HPLC procedures described below were utilized to quantitate the concentration of test solutes inside the sack relative to the external bathing solution. Transport data are expressed as the percent of the concentration of solute inside the gut sack relative to the bathing solution; therefore, a value of 100% indicates complete equilibrium between the solute inside and outside the gut sack.

Reversed-phase High Pressure Liquid Chromatography—The β-glucosidase assay used to measure amygdalin hydrolyzing activity involved subjecting incubations to HPLC (Waters 600E Gradient controller, 484 Absorbance detector) on a 8 × 100-mm reversed-phase column (Delta Pak C_{18}, Waters, Milford, MA). The elution system consisted of 0.1% (v/v) trifluoroacetic acid in water (A) and 0.1% (v/v) trifluoroacetic acid in 95% (v/v) acetonitrile/water (B). The elution gradient was programed as follows: 5 min of solvent A, 20 min convex gradient (curve 3 on gradient programer) from 0 to 40% solvent B and 4 min concave gradient (curve 9 on gradient programer) from 40 to 100% solvent B. The flow rate was 1.0 ml/min. The eluent was monitored at 254 nm. Detector output was plotted on a Kipp and Zonen plotter, and simultaneously the data were acquired by a NEC 386/25 computer using Maxima software (Dynamic Solutions, Ventura, CA). The response of the detector was calibrated by chromatography of known amounts of authentic standards and integrating peak areas.

When either pNP-gentiobioside or Galβ1→6Galβ-pNP served as the substrate, the following elution gradient was employed: 5 min of solvent A, 20-min linear gradient from 0–20% solvent B, 8-min linear gradient from 20–80% solvent B, 2-min linear gradient from 80–100% solvent B, and a final 2-min wash with 100% solvent B.

In the case of pNP-lactoside and pNP-cellobioside, the separation of unused substrate and reaction products was achieved through the use of a 4.6 × 250-mm reversed-phase C_{18} ValuPak column (Regis, Morton Grove, IL). The solvent system consisted of water (A) and 100% methanol (B). The protocol for the separation of pNP-lactoside, pNP-Glc, and pNP involved use of a 50-min 0–100% linear gradient of solvent B, with a flow rate of 0.75 ml/min. The same gradient was also used to separate pNP-cellobioside, pNP-Glc, and pNP.

The permeability of the small intestine to vicine was estimated by subjecting samples from the serosal and the mucosal sides of the gut sack to a reversed-phase HPLC procedure which was similar to that described above for amygdalin, except that elution of vicine was monitored by measuring absorbance at 277 nm.

Protein Determination—The Coomassie dye-binding method of Bradford (9) was used to estimate the protein concentration and bovine serum albumin served as the standard.

RESULTS

Hydrolysis of Disaccharide Glycosides by Guinea Pig Liver Cytosolic β-Glucosidase—As stated in the Introduction, we demonstrated previously that the cytosolic β-glucosidase possesses a hyrophobic subsite in its catalytic center (6). It is reasonable therefore to attribute the inertness of disaccharides such as cellobiose, lactose, or gentiobiose in the β-glucosidase assay to their lack of a hydrophobic aglycone moiety. Reasoning that glycosides which contain a disaccharide moiety terminating in a β-linked glucose or galactose unit and possessing a hydrophobic aglycone such as *p*-nitrophenol might be substrates for the cytosolic β-glucosidase, we inquired if *p*-nitrophenyl disaccharides could be cleaved by the cytosolic β-glucosidase.

The *p*-nitrophenyl derivatives of cellobioside, gentiobioside, lactoside, and galactosyl-β(1→6)-galactopyranoside were therefore tested as substrates for the cytosolic β-glucosidase. In this experiment, the disappearance of the substrate, the appearance of likely intermediates (*e.g.* *p*-nitrophenyl-β-D-glucoside release from pNP-cellobioside or pNP-gentiobioside), and the formation of the common aglycone, namely *p*-nitrophenol, were monitored by means of reversed-phase HPLC. As shown in Table I, of the four structurally related disaccharide glycosides tested, only pNP-gentiobioside was cleaved at an appreciable rate by the enzyme; a specific activity of 94,900 units/mg was observed. Fig. 1, *panel A*, depicts the products and unused substrate present in an assay medium at the end of a 30-min incubation of pNP-gentiobioside, buffer, and cytosolic β-glucosidase. When the eluent was monitored at 310 nm, two peaks eluting at 27.4 and 38.3 min were observed; these times correspond precisely to those of pNP-gentiobioside and pNP standards. The extent of pNP formation corresponds to 15% the rate at which the enzyme catalyzes the hydrolysis of pNPGlc (Table I). No peak was observed at 29.5 min, the retention time for the pNP-Glc standard. No detectable hydrolysis of pNP-lactoside, pNP-cellobioside, or Galβ1→6Galβ-pNP was observed (Table I).

TABLE I

Relative rates of hydrolysis of various disaccharide glycosides by the guinea pig cytosolic β-glucosidase

Substrate[a]	Activity	Relative activity
	units/mg protein × 10^{-3}	%
pNP-β-D-Glc	632	100
Gentiobiose[b]	3.78	0.60
Cellobiose[b]	ND[c]	ND
Amygdalin[b]	167	26.3
pNP-β-D-gentiobioside[d]	94.9	15.1
pNP-β-D-lactoside	1.90	0.30
pNP-β-D-cellobioside	3.48	0.55
Galβ1→6Galβ-pNP	3.67	0.58

[a] The final substrate concentration in the assay medium was 10 mM.

[b] The extent of hydrolysis of these disaccharides were determined using the hexokinase/Glu-6PD-coupled assay that measures D-glucose release.

[c] Not detectable.

[d] The extent of hydrolysis of pNP-containing substrates was determined using the spectrophotometric procedure described under "Experimental Procedures."

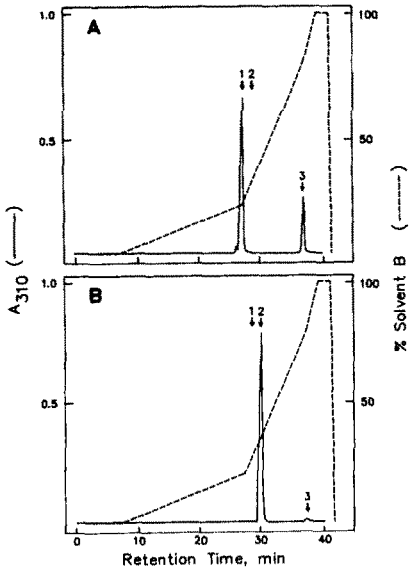


FIG. 1. **Hydrolysis of disaccharide glycosides by the cytosolic β-glucosidase: reversed-phase HPLC elution profile of the assay mixture obtained after incubation of the enzyme with 10 mM pNP-β-D-gentiobioside (*panel A*) and 10 mM pNP-β-D-cellobioside (*panel B*).** The absorbance at 310 nm recorded by the detector is plotted *versus* the retention time. In *panel A*, the *arrowheads labeled 1–3* correspond to the authentic standards of pNP-β-D-gentiobioside (27.4 min), pNP-Glc (29.5 min), and pNP (38.3 min), respectively. In *panel B*, the *arrowheads labeled 1–3* correspond to pNP-Glc (29.5 min), pNP-β-D-cellobioside (30.4 min), and pNP (38.3 min), respectively.

The rates of hydrolysis of all of these disaccharide glucosides relative to pNP-Glc are summarized in Table I. These findings indicate that the cytosolic β-glucosidase prefers substrates which contain a β(1→6) linkage between the two sugar moieties and that the enzyme will hydrolyze only a β-D-glucosyl residue located at the nonreducing end of disaccharide glucosides. It is noteworthy that human glucocerebrosidase did not catalyze the hydrolysis of pNP-gentiobioside or any of the other disaccharide glycosides listed in Table I (data not shown). There were no breakdown products when any of these substrates were incubated in the appropriate buffer in the absence of the cytosolic β-glucosidase.

One aspect of the chromatographic analysis of the products generated in the pNP-gentiobioside β-glucosidase incubation which warrants comment is that, despite the presence of a prominent *p*-nitrophenol peak indicating significant breakdown of pNP-gentiobioside, there was no evidence of the pNP-Glc intermediate that one would expect to see if the disaccharide pNP-gentiobioside had undergone step-wise exolytic attack by the enzyme (Fig. 1, *panel A*). However, we estimated pNP-gentiobioside-β-glucosidase activity in a parallel experiment using the hexokinase/Glu-6PD-coupled assay which measures D-glucose release. The results of this experiment revealed a 2:1 stoichiometry for the D-glucose: pNP generated from pNP-gentiobioside by the cytosolic β-glucosidase (data not shown). This observation provided the first indication that the guinea pig liver enzyme is an exoglucosidase. We tested this hypothesis further by carring out a time course analysis of the hydrolytic reaction using amygdalin, a plant gentiobioside, as the substrate.

The Cytosolic β-Glucosidase Is an Exo-β-glucosidase—We were interested in knowing if the plant disaccharide, amygdalin (Glcβ1→6Glcβ-mandelonitrile), could be cleaved by the cytosolic β-glucosidase and also in resolving the question of whether the cytosolic enzyme is an endo- or an exoglucosidase. As is illustrated in Fig. 2, the endolytic and exolytic pathways of amygdalin cleavage would generate distinct product patterns during the course of the enzymatic reaction. If the enzyme acts endolytically, the expected products of amygdalin hydrolysis would be gentiobiose and its aglycone, mandelonitrile. However, if the enzyme followed an exolytic pathway, generation of the monosaccharide glucoside, prunasin (mandelonitrile-β-D-glucoside), and the aglycone, mandelonitrile, would be expected. We therefore designed a reversed-phase HPLC procedure which would enable us to distinguish between these two pathways. Three peaks were observed when amygdalin was incubated with the guinea pig liver cytosolic β-glucosidase and the assay mixture was subjected to HPLC analysis (Fig. 3); the retention times of 18.7, 19.6, and 25.3 min corresponded precisely to those of authentic standards of amygdalin, prunasin, and mandelonitrile, respectively.

If the enzyme behaves as an exohydrolase, a time course analysis of the hydrolysis of amygdalin should exhibit a lag in the production of the aglycone D-mandelonitrile. The latter course is to be expected since an appreciable accumulation in the monosaccharide prunasin would be obligatory before enzymatic release of detectable amounts of mandelonitrile would be observed. Incubations of a fixed amount of both enzyme and amygdalin at pH 6.0 and at 37 °C were terminated at various time intervals from 0 to 60 min and analyzed by reversed-phase HPLC to quantitate the amounts of the intermediate, prunasin, and final product, mandelonitrile, generated. The concentration of amygdalin decreased with time while the concentration of prunasin increased concomitantly (Fig. 4). However, the aglycone, mandelonitrile, accumulated only after a lag of about 10 min. Thus, the kinetics of prunasin

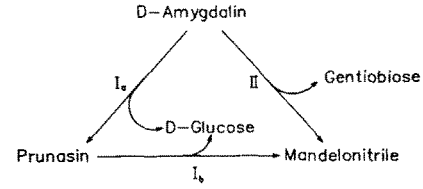


FIG. 2. **Two possible reaction pathways for the hydrolysis of amygdalin by the cytosolic β-glucosidase.**

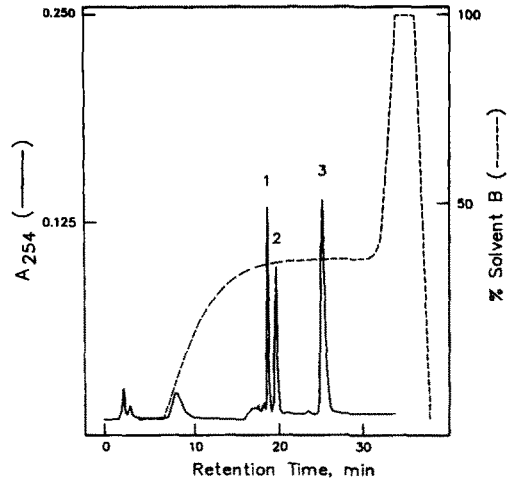


FIG. 3. **Hydrolysis of amygdalin by the cytosolic β-glucosidase: reversed-phase HPLC elution profile of the assay mixture obtained after incubation of the enzyme with amygdalin for 30 min under conditions described in the text.** The absorbance at 254 nm recorded by the detector is plotted *versus* the retention time. The *arrowheads labeled 1–3* correspond to amygdalin (18.7 min), prunasin (19.6 min), and mandelonitrile (25.3 min), respectively.

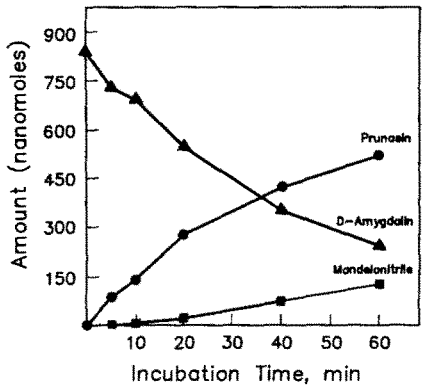
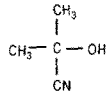
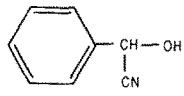
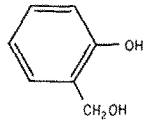
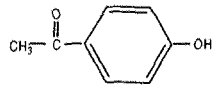
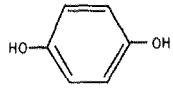
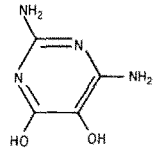


FIG. 4. **Time course of product release from the hydrolysis of amygdalin by the cytosolic β-glucosidase.** The amounts of prunasin and mandelonitrile generated and the amount of residual unused substrate present after subsequent incubation of 10 mM amygdalin with the cytosolic β-glucosidase for various periods of time were quantitated using the reversed-phase HPLC procedure described under "Experimental Procedures." The amounts of amygdalin (▲), prunasin (●), and mandelonitrile (■) in the assay medium after incubation of the enzyme with amygdalin is plotted *versus* the time of the incubation.

TABLE II

Aglycone structures of naturally occurring glucosides tested as substrates of the cytosolic β-glucosidase

NAME	AGLYCONE STRUCTURE	OCCURRENCE
Linamarin		*Linum usitatissimum*
Prunasin		*Prunus Sp*
Salicin		*Salix*
L-Picein		Willow bark, English mistletoe
Arbutin		*Pyrus communis*
Vicine		*Vicia faba*

and mandelonitrile appearance during the course of the enzyme incubation with amygdalin support the hypothesis that amygdalin is degraded exolytically by the guinea pig liver cytosolic β-glucosidase.

Plant β-D-Glucosides Are Substrates for Mammalian Cytosolic β-Glucosidase—LaMarco and Glew (7) reported that the plant phenolic glucoside, L-picein, was efficiently hydrolyzed by the guinea pig liver cytosolic β-glucosidase. In the present study we have examined the rates of hydrolysis of several other plant glucosides (Table II) which could conceivably find their way into the diets of humans or animals. It is well documented that cyanide poisoning can occur in mammals subsequent to hydrolysis of ingested plant cyanogenic glucosides (10–12). Furthermore, the presence of phenolic glucosides has been reported in nearly every higher plant species that has been examined (13). These glucosides are believed to be rapidly catabolized by mammals. We investigated the possibility that some of these phenolic glucosides or cyanogenic glucosides are hydrolyzed by the cytosolic β-glucosidase. We discovered that arbutin and salicin were hydrolyzed at 58 and 30%, respectively, the rate at which pNP-Glc was hydrolyzed (Table III). Of the cyanogenic glucosides tested, amygdalin was hydrolyzed most rapidly by the cytosolic β-glucosidase. The cyanogenic glucoside prunasin was hydrolyzed about one-tenth as rapidly as pNP-Glc. Linamarin and neolinustatin were also hydrolyzed, albeit at low rates. The pyrimidine glucoside, vicine, which is the causative agent in the form of hemolytic anemia known as favism (14, 15), was hydrolyzed at about 15% the rate at which 4-MUGlc was cleaved.

A comparison of the structures and rates of hydrolysis of these glucosides indicates that the guinea pig liver cytosolic β-glucosidase exhibits a preference for glucosides which possess an aryl, hydrophobic aglycone moiety. For instance, amygdalin, the β-gentiobioside of mandelonitrile, is hydrolyzed 3.3 times more rapidly than neolinustatin, the β-gentiobioside of the aliphatic aglycone, α-methylbutyronitrile (Table III). This observation indicates that future studies of this nature which are aimed at identifying natural substrates for the cytosolic β-glucosidase should emphasize amphipathic glucosides that possess a hydrophobic aryl, aglycone moiety.

Transport of Potentially Toxic Plant Glucosides across the Small Intestine of the Guinea Pig—If the naturally occurring glucosides mentioned above were to find their way into the diets of humans or animals, the question arises as to whether the liver or any other organ could play a role in their metabolism. An equally important question is whether these compounds are transported intact across the gut. To address these questions we used the everted gut sack technique (16) to determine if there is any significant transport or metabolism of prunasin, vicine, or amygdalin in the small intestine of the guinea pig. After incubating the everted gut sack for 2 h at 37 °C in a buffered solution supplemented with the respective glucoside, samples from the mucosal and the serosal sides of the gut sack were subjected to reversed-phase HPLC analysis.

TABLE III

Relative rates of hydrolysis of various toxic plant glucosides

The final substrate concentration in the assay medium was 10 mM.

Substrate	Activity	Relative activity
	units/mg protein × 10^{-3}	%
4-MUGlc	506	100
Phenolic glucosides		
L-Picein	425[a]	84
Salicin	152	30
Arbutin	294	58
Cyanogenic glucosides		
Amygdalin	167	33
Prunasin	65.8	13
Neolinustatin	50.6	10
Linamarin	25.3	5
Pyrimidine glucosides		
Vicine	75.9	15

[a] The activity was measured using the hexokinase/Glu-6PD-coupled assay that measures D-glucose release.

From this kind of analysis it was possible for us to determine not only the extent of glucoside transport but also whether or not significant hydrolysis of these glucosides had occurred. The results of this study indicated unambiguously that all three of these β-D-glucosides were indeed transported intact across the gut. As shown in Table IV, after a 2-h incubation, the concentrations of prunasin, amygdalin, and vicine inside the gut sack were 54.0% (± 6.0%), 42.5% (± 4.5%), and 89.6% (± 0.6%), of the theoretical equilibrium value, respectively. When 5 mM amygdalin and 5 mM prunasin were present together in the external solution bathing the everted gut, we found that the rates of uptake of the two glucosides were the same as those obtained when they were present individually (data not shown), indicating that they probably do not compete for a common carrier system. Current studies are aimed at characterizing the transporters involved in the uptake of these plant glucosides.

DISCUSSION

This study demonstrates that while gentiobiose itself is a very poor substrate of the cytosolic β-glucosidase, both amygdalin (β-D-gentiobiose linked to mandelonitrile) and pNP-gentiobioside are efficiently hydrolyzed by the guinea pig liver enzyme (Table I). Our results indicate that this β-glucosidase will catalyze the hydrolysis of glucose units in disaccharide glucosides only when the glucose units are linked to each other in a β(1→6) configuration, as in pNP-gentiobioside. The strict specificity of the enzyme with regard to the nature of the linkage between the two sugar units is borne out by the observation that both pNP-cellobioside and pNP-lactoside, both of which possess a β(1→4) linkage between their sugar units, are not hydrolyzed by the cytosolic β-glucosidase (Table I). It appears that these catalytic attributes of the mammalian cytosolic β-glucosidase are relatively unique since its lysosomal counterpart, namely glucocerebrosidase, does not catalyze the hydrolysis of any of the disaccharide glycosides we tested, including pNP-gentiobioside (data not shown).

It is well established that the mammalian cytosolic β-glucosidase is capable of hydrolyzing *p*-nitrophenyl-β-D-glucoside, -β-D-galactoside, -β-D-xyloside, and -α-L-arabinoside (1, 4, 7). Nevertheless, the inability of the cytosolic β-glucosidase to remove the terminal galactose unit of both pNP-lactoside and Galβ1→6Galβ-pNP suggests that the enzyme is not a β-galactosidase when one is addressing the nature of the glycone moiety at the nonreducing end of potential disaccharide glycoside substrates.

The sequential β-glucosidase-catalyzed release of first the monosaccharide, prunasin, and then the aglycone, mandelonitrile, from the disaccharide amygdalin established that the β-glucosidase follows an exolytic pathway when hydrolyzing disaccharide-containing glucoside substrates (Fig. 4). In light of the fact that gentiobiose is not a substrate for the enzyme, a likely explanation for our failure to detect *p*-nitrophenyl-β-D-glucoside during the hydrolysis of pNP-gentiobioside (Fig. 1, *panel A*) is that after exolytic attack of this substrate, the intermediate (pNP-Glc) undergoes immediate glycosidic cleavage before it dissociates from the active site of the β-glucosidase.

TABLE IV

Transport of plant glucosides across small intestine of guinea pig

Plant glucoside	[I]/[O], %[a]
Amygdalin	42.5 ± 4.5[b]
Prunasin	54.0 ± 6.0
Vicine	89.6 ± 0.6

[a] The [I]/[O] ratio represents the concentration of the solute inside the gut sack relative to the external solution.

[b] This ratio is the mean of two independent experiments.

Freese *et al.* (17) described a β(1→6)-glucosidase from feline kidney that is capable of removing the terminal glucose residue from amygdalin. Since they estimated the molecular mass of the feline enzyme to be 32 kDa, it is unclear if this activity is distinct from the 60-kDa guinea pig cytosolic β-glucosidase we have characterized in our study.

The catabolism of toxic plant glucosides is deserving of study since the diets of humans and animals in many parts of the world contain these compounds. For example, it is widely believed that chronic cyanide intoxication is the most important factor in the etiology of the tropical ataxic neuropathy which occurs in certain populations living in West Africa (10–12). We have demonstrated in this report that the cytosolic β-glucosidase catalyzes at appreciable rates the hydrolysis of the cyanogenic glucosides amygdalin and prunasin (Table III).

In order for the liver to be involved in biotransformation of any of these glycosides, they must first be transported across the gut whereupon they could be transported via the blood circulation to the liver for metabolism. Using everted gut sacks from guinea pigs, we have shown that two cyanogenic glucosides, prunasin and amygdalin, and another plant glucoside, vicine, are transported rapidly and without hydrolysis across the gut (Table IV). It remains to be seen if the liver or other organs extract these compounds from the systemic circulation.

We now attempt to integrate these findings on the hydrolysis of plant glucosides by the cytosolic β-glucosidase under a common pathophysiologic rubric. LaMarco and Glew (7) postulated a conventional biphasic detoxification pathway which would provide the cell with a means for disposing of potentially toxic glycosides. They suggested that the first phase of detoxification involves the cytosolic β-glucosidase-catalyzed release of sugar moieties from these compounds. The second phase would involve a UDP-glucuronyl transferase which conjugates a glucuronic acid residue from UDP-glucuronic acid to the nucleophilic acceptor site (*e.g.* a hydroxyl group) made available as a consequence of the first step of the pathway.

While the cytosolic β-glucosidase may play a protective role in the biotransformation pathway described above, the possibility that the enzyme contributes to the pathogenesis of toxic plant glucosides must also be considered. For example, in the case of vicine and other cyanogenic glucosides, the toxicity of these compounds becomes manifest only after the glucose residues have been removed. Specifically, the cleavage of vicine to its aglycone, divicine, results in the spontaneous generation of cytotoxic oxygen metabolites while the hydrolysis of cyanogenic glucosides to release highly unstable aglycones leads to the formation of HCN (10, 15). It is unclear if varying levels of the enzyme in humans could underlie variations in the susceptibility of different individuals to these toxic plant glucosides.

Circumstantial evidence exists which supports the hypothesis that the cytosolic β-glucosidase functions as a biotransformation enzyme. The cytosolic β-glycosidase satisfies at least three of the four characteristics of detoxification enzymes enumerated by Jakoby and Zeigler (18). First, the enzyme has an affinity for amphipathic xenobiotics owing to the presence of polar and hydrophobic domains in its catalytic center. Second, it displays a broad specificity with regard to the glycone and, to a limited extent, can tolerate variations in the aglycone moieties of its monosaccharide substrates.

Third, it is present in significant amounts in the liver and intestine.[2] Detoxification enzymes are usually concentrated in a specific organ at major points of entry of the xenobiotics to the body. The fourth property of most biotransformation enzymes, that of inducibility in the presence of the xenobiotic substrate, remains to be verified in the case of the guinea pig liver β-glucosidase.

The interesting question of the maximum length of the oligosaccharide in glycosides which are good substrates of the cytosolic β-glucosidase remains to be determined; for example, can the guinea pig cytosolic β-glucosidase remove terminal glucose units from a glucose-terminated tri-, tetra-, or pentasaccharide glycoside? Further studies performed using both naturally occurring and nonphysiologic glycosides as substrates for the cytosolic β-glucosidase are needed to delineate the mechanism of action and provide further insight into the physiologic role of the enzyme. It would be useful in this regard to determine if other naturally occurring cyanogenic disaccharides such as lucumin (xylosyl-β(1→6)-glucosyl-β-D-mandelonitrile) and vicianin (arabinosyl-β(1→6)-glucosyl-β-D-mandelonitrile) are substrates of the cytosolic β-glucosidase.

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[2] R. H. Glew and S. Macko, unpublished observations.

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