Dietary vitamin B_6 effects on the distribution of intestinal mucosal and microbial β-glucosidase activities toward pyridoxine-5′-β-D-glucoside in the guinea pig

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Guinea pigs utilize dietary pyridoxine-5′-β-D-glucoside (PN-glucoside) as a source of vitamin B_6 more extensively than other species studied to date, including rats, mice, hamsters, and humans. In this study, we evaluated the sources of β-glucosidase activity involved in the hydrolysis and utilization of dietary PN-glucoside and the potential influence of vitamin B_6 nutritional status. Male guinea pigs (~400 g, 3 to 6/diet group) were fed a sucrose/casein based diet containing 0, 1, or 3 mg/kg pyridoxine (as pyridoxine · HCl) for 4 wk. Animals fed diets containing the two lower concentrations of pyridoxine exhibited loss of body weight, although plasma pyridoxal 5′-phosphate did not vary as a function of dietary pyridoxine concentration. However, significant differences in plasma and muscle taurine and erythrocyte aspartate aminotransferase activity among diets indicated functional differences in vitamin B_6 status. β-glucosidase activity (pmol/hr/mg protein), as measured using [^{3}H] pyridoxine-glucoside as the substrate, was higher in lumenal contents than mucosal cytosol. Specific activity in mucosal cytosol was significantly higher in animals fed diets containing 0 mg/kg pyridoxine than either 1 or 3 mg/kg. In contrast, the specific β-glucosidase activity of lumenal contents was significantly lower in guinea pigs fed 0 mg/kg pyridoxine versus 1 or 3 mg/kg pyridoxine diets. Although the absolute quantity of the mammalian and microbial enzyme available for in vivo small intestinal hydrolysis of dietary pyridoxine-glucoside cannot be directly determined, these data indicate that both mucosal and microbial β-glucosidase activities may contribute to the intestinal hydrolysis of pyridoxine-glucoside, and that the distribution of these activities is influenced by the concentration of dietary vitamin B_6. (J. Nutr. Biochem. 5:238–242, 1994.)

Keywords: vitamin B_6; pyridoxine β-D-glucoside; guinea pig; β-glucosidase

Introduction

Pyridoxine-5′-β-D-glucoside (PN-glucoside) is a vitamin B_6 derivative that contributes to human nutrition due to its wide occurrence in plant products and partial bioavailability.[1–3] For in vivo utilization of PN-glucoside to occur, enzymatic hydrolysis of the β-glycosidic bond is necessary either before or after intestinal absorption.[4,5]

Intensive study of the metabolism of PN-glucoside has followed its isolation and identification in 1977.[2] The bioavailability of this B_6 glycoconjugate has been estimated in humans[4] and in several laboratory animal species, including the rat,[5–7] mouse, hamster, and guinea pig.[8] While rats display

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a limited capability (10 to 34%) to hydrolyze PN-glucoside to pyridoxine (PN), mice and hamsters metabolize PN-glucoside much more extensively (69% and 70%, respectively); thus, the responses of these species more closely resemble that of the human (58% bioavailability). The intestinal absorption of PN-glucoside is rapid and extensive, presumably in the small intestine, as with other forms of vitamin B_6.[2,7] Studies in humans and rats have indicated that the utilization of PN-glucoside in vitamin B_6 metabolism when injected intraperitoneally or intravenously is only half as effective as when administered orally,[4,7] which indicates a substantial contribution of the intestine to the (albeit incomplete) release of metabolically active PN from PN-glucoside. Guinea pigs display more extensive utilization (92%) of PN-glucoside than any other species studied thus far,[8] possibly due to a greater extent of microbial action in the small intestine, as well as hind-gut fermentation and associated hydrolytic ability.[9]

The present study was conducted to estimate the relative contributions of intestinal mucosal and microbial β-glucosidase activities to the utilization of dietary PN-glucoside, as well as to assess the possible influence of the concentration of dietary vitamin B_6 on the distribution of these enzyme activities in this animal species.

Methods and materials

Animals, diets and protocols

Male Hartley guinea pigs weighing 250 to 300 g were obtained from Harlan-Sprague Dawley, Indianapolis, IN USA. They were housed in pairs in polycarbonate cages and fed a non-purified, stock diet (Mini-Friends Guinea Pig Chow, Purina Mills, St. Louis, MO USA) for 1 week before beginning the experiment for acclimatization to the animal facility. For the following 4-week period, they were given free access to a purified casein-sucrose (40%:60%) based diet[10] that was either: (a) deficient (0 mg/kg PN added), (b) marginally adequate (1 mg/kg PN added), or (c) fully adequate (3 mg/kg PN added) in vitamin B_6 (PN added as pyridoxine · HCl; obtained from Sigma Chemical Co., St. Louis, MO USA). All diet ingredients except PN were obtained from ICN Nutritional Biochemicals (Cleveland, OH USA), and the diets were pelleted by the supplier. Except for the variable concentration of vitamin B_6, these diets provided adequate amounts of all known essential nutrients.[9,11] This protocol was conducted according to National Institutes of Health guidelines and was approved by the University of Florida Institutional Animal Care and Use Committee.

At the end of the 4-week feeding period, the animals were anesthetized using 2.0 mL of 65 mg/mL sodium pentobarbital. Blood samples were obtained by venipuncture of the inferior vena cava through a midline abdominal incision, transferred to heparinized collection tubes, and centrifuged at 3000 *g* for 20 min to obtain plasma. Erythrocytes were washed three times by resuspension in an isotonic saline solution followed by centrifugation. The small intestine was removed, placed on ice, and gently flushed three times with cold isotonic saline solution to obtain the lumenal contents. Small intestinal mucosa were obtained by scraping the longitudinally sectioned intestine with a glass microscope slide in the presence of ice-cold isotonic saline solution.

Analytical procedures

Protein concentrations were estimated by the method of Bradford et al.[12] Erythrocyte aspartate transaminase (AST) activity was determined by a spectrophotometric method[13] using a commercially obtained kit (Sigma Chemical Co.). Hemoglobin concentrations were measured as the cyanomethemoglobin derivative.[14]

Free taurine concentration in deproteinated plasma was measured using a high performance liquid chromatography (HPLC) technique, as previously described.[15] For measurement of total taurine in muscle samples with this procedure, samples were acid-digested in 6 mol/L HCl at 80° C, then adjusted with NaOH to pH 7.2 prior to analysis. Plasma pyridoxal 5′-phosphate (PLP) concentration was determined by HPLC using a post-column fluorogenic derivatization technique.[10]

Assay of β-glucosidase activity

Intestinal mucosal and intralumenal β-glucosidase activities were measured using 5 μmol/L [^{3}H]PN-glucoside as the substrate.[16] This assay involves measurement of the [^{3}H]PN released from [^{3}H]PN-glucoside using reverse-phase HPLC with liquid scintillation counting of the collected fractions. Samples of mucosa and lumenal contents were prepared for this assay by homogenization (Polytron with PT 10/35 probe; Brinkmann Instruments, Inc.; Westbury, NY USA) for 60 sec in 5 volumes of ice-cold sodium acetate buffer (0.2 mol/L, pH 5.5) containing 1 mmol/L phenylmethylsulfonyl fluoride and 10 mmol/L 2-mercaptoethanol, followed by centrifugation at 20,000*g* for 20 min at 4° C, as modified from Daniels et al.[17] Assays were conducted at 37° C under conditions that provided linearity as a function of enzyme concentration and reaction time. The reactions were terminated by the addition of trichloroacetic acid to a final concentration of 5% (wt/vol).

Statistical methods

Statistical differences between dietary treatments were evaluated by analysis of variance with multiple comparisons by the Tukey procedure.[18] Results were considered to be significant at $P < 0.05$.

Results

PLP concentration was not influenced by the concentration of dietary PN in these animals *(Table 1)*. This result was consistent with the findings of a previous study comparing the metabolism of PN-glucoside in various laboratory animals in which plasma PLP did not respond to the concentration of dietary PN between 0 and 1 mg/kg over a 4-wk study.[8] The body weight of the guinea pigs fed the 3 mg PN/kg diet did not change significantly over the 4-week period ($P > 0.05$), although the animals fed the 0 and 1 mg PN/kg diets lost weight ($P < 0.05$). Mean body weights

Table 1 Plasma pyridoxal-5′-phosphate concentration and erythrocyte aspartate aminotransferase activity in guinea pigs fed graded levels of pyridoxine for 4 weeks

Diet	Plasma [PLP] (nmol/L)	AST activity (nmol * min^{-1} * (mg Hb)$^{-1}$)
0 mg/kg PN	24.4 ± 2.1[a]	0.276 ± 0.069[a]
1 mg/kg PN	23.0 ± 2.1[a]	0.776 ± 0.183[b]
3 mg/kg PN	25.1 ± 1.7[a]	1.32 ± 0.26[c]

Data are means ± SEM, n = 3 animals per group.
Within each column, values followed by the same superscript letter are not significantly different ($P > 0.05$).
PLP, plasma pyridoxal-5′-phosphate; AST, erythrocyte aspartate aminotransferase.

(initial / final) were: 427 g / 340 g (0 mg PN/kg); 421 g / 324 g (1 mg PN/kg); and 424 g / 395 g (3 mg PN/kg).

In contrast to the lack of dietary response observed with plasma PLP, the two functional indices of vitamin B_6 status were related to the dietary intake of pyridoxine by guinea pigs in this study. AST, in which PLP functions as a coenzyme, exhibited significant differences in specific activity between the three dietary groups, with increasing activity as a function of dietary vitamin B_6 ($P < 0.05$; *Table 1*). The concentration of taurine in tissues is related to vitamin B_6 status because taurine synthesis is PLP-dependent.[19,20] In this regard, the animals fed the 0 mg PN/kg diet exhibited significantly lower total taurine concentration in muscle, relative to the 1 and 3 mg PN/kg diets *(Table 2)*. Plasma taurine concentration declined with increasing dietary PN ($P < 0.05$), which apparently reflects a redistribution of this compound into plasma during vitamin B_6 deficiency with a transfer into organs of high metabolic demand.[21] These results indicate that functional differences existed in the vitamin B_6 status of the guinea pigs in this study.

Concurrent with the above metabolic changes, the specific activity of β-glucosidase in the small intestinal mucosa was significantly decreased and lumenal β-glucosidase significantly increased *(Table 3)* in guinea pigs fed the highest level (3 mg/kg) of pyridoxine ($P < 0.05$). Furthermore, the magnitude of the microbial enzyme activity was increased by nearly 10 times in guinea pigs fed the marginally (1 mg/kg PN) or fully (3 mg/kg PN) adequate quantity of dietary pyridoxine. These results suggest that, under conditions in which guinea pigs consume an adequate amount of vitamin B_6, the specific activity of intralumenal β-glucosidase activity would ordinarily predominate over that of the intestinal mucosal source of this enzyme. The actual activity of each enzyme and its contribution to the in vivo hydrolysis of PN-glucoside cannot, however, be directly determined.

Table 2 Plasma and muscle taurine levels in guinea pigs fed graded levels of pyridoxine (PN) for 4 weeks

Diet	Taurine concentration	
	Plasma (μmol/L)	Muscle (μmol/g)
0 mg/kg PN	38.1 ± 2.4[a]	14.45 ± 3.93[a]
1 mg/kg PN	31.5 ± 2.8[a,b]	25.26 ± 1.10[b]
3 mg/kg PN	24.5 ± 4.7[b]	23.54 ± 1.83[b]

Values represent means ± SEM, n = 3 to 6 animals per group. Values with different superscripts differ significantly at the $P < 0.05$ level.

Table 3 Mucosal cytosolic and intralumenal β-glucosidase activity of small intestine in guinea pigs fed graded levels of pyridoxine for 4 weeks

Diet	β-Glucosidase activity (pmol PN * h^{-1} * [mg protein]$^{-1}$)	
	Mucosal	Lumenal
0 mg/kg PN	65.8 ± 10.7[a]	84.8 ± 37.3[a]
1 mg/kg PN	39.7 ± 27.1[b]	348 ± 27[b]
3 mg/kg PN	34.8 ± 5.2[b]	323 ± 59[b]

Values represent means ± SEM for three animals. Within each column, values followed by the same superscript letter are not significantly different ($P > 0.05$).

Discussion

Taken together, the data on body weight, AST activity, and taurine concentration in the guinea pigs indicate that different metabolic responses occurred in animals fed different amounts of pyridoxine in the diet, and that these responses reflected the functional vitamin B_6 status of the animals. The results of this study are consistent with the reported requirement for vitamin B_6 in guinea pigs of 3 mg/kg of diet.[9,11] Vitamin B_6 metabolism has been examined previously in guinea pigs,[22,23] but the relationships between vitamin B_6 intake, plasma PLP concentration, and other indicators of nutritional status have not been reported.

Although the existence in mammalian tissues of a broad specificity cytosolic β-glucosidase activity has long been recognized, the function of this enzyme is not well understood. The cytosolic β-glucosidase has been shown to be capable of hydrolyzing several steroid β-glucosides[24,25]; the physiological significance of this may be minimal, however.[26] Disaccharides are poor substrates[26,27] for the cytosolic β-glucosidase, which indicates that this enzyme has little or no digestive function. Glew et al. reported that the cytosolic β-glucosidase from guinea pig liver could hydrolyze picein, a naturally occurring aryl glucoside, and suggested a possible role of the enzyme in detoxification of such compounds.[26,27] Conversely, this enzyme may also participate in the hydrolysis and pathogenic effects of certain cyanogenic glycosides that undergo intestinal absorption in intact form.[26,28]

The in vivo hydrolysis of dietary pyridoxine-5′-β-D-glucoside, a naturally occurring substrate that is present in essentially all human diets, is a nutritional function of the mammalian broad specificity β-glucosidase. Dietary PN-glucoside contributes to the vitamin B_6 nutrition of a human or animal to the extent that it undergoes hydrolysis of the glycosidic bond (e.g., mean 58% in humans, 25 to 30% rats, and ca 90% in guinea pigs).[4–8] When injected intravenously or intraperitoneally, PN-glucoside is utilized in vitamin B_6 metabolism, although about 50% less efficiently than observed with oral administration. These observations indicate that β-glucosidase activity of internal organs (e.g., liver and kidney) participates in the hydrolysis of PN-glucoside following parenteral administration. We have shown that the cytosolic β-glucosidase of mammalian small intestinal mucosa is capable of releasing PN from PN-glucoside,[16] although the possible role of β-glucosidases from intestinal microorganisms in the hydrolysis of dietary PN-glucoside has not been previously examined. The results of the present study *(Table 3)* indicate that in guinea pigs there is significant β-glucosidase activity, presumably of bacterial origin, in the small intestinal lumen. Thus, in the guinea pig, bacterial β-glucosidase in the small intestine appears to play a role, along with the cytosolic mammalian enzyme, in the extensive hydrolysis of dietary PN-glucoside. Further studies are needed to assess the relative contribution of microbial β-glucosidase activity in animal species, including humans, that have less bacterial colonization of the small intestine

than occurs in guinea pigs. Developmental changes in the activity of small intestinal cytosolic β-glucosidase[29–31] may also influence the extent of PN-glucoside hydrolysis by this enzyme.

The methods used in this study do not differentiate between β-glucosidase activity from microbial and mammalian sources; therefore, intralumenal β-glucosidase activity cannot be unequivocally identified as being of microbial origin. Additional studies involving chromatographic or electrophoretic separation will address this issue. In view of the lower β-glucosidase activity associated with the intestinal mucosal cells[16] relative to lumenal contents, it is unlikely that the enzyme from sloughed cells or intestinal secretions would be a major source of intralumenal β-glucosidase activity. Pancreatic secretions have not been examined as a source of β-glucosidase activity; however, the minimal digestion of dietary β-glucosides in most mammalian species suggests that secretion of this enzyme is minimal. The use of gnotobiotic guinea pigs[23] may provide useful information regarding the specific roles of mammalian β-glucosidase activity.

The influence of dietary vitamin B_6 intake on the activity of various enzymes of the intestinal microflora, as well as the distributions and numbers of microbial species, has not been previously reported. The observation of increased β-glucosidase activity in the intestinal lumen in this study with increasing dietary PN suggests an influence of PN on the bacterial synthesis of this enzyme because there is no vitamin B_6 coenzyme requirement by β-glucosidases. Although apparent induction of enzyme synthesis by dietary vitamin B_6 has been reported in the case of an intestinal aromatic amino acid aminotransferase,[31] to our knowledge such an enhancement has not been seen previously in intestinal microflora. We recently reported that the utilization of PN-glucoside in vitamin B_6 metabolism in guinea pigs is nearly as extensive as that of free PN, and that the vitamin B_6 status of the animals (i.e., at two suboptimal levels) did not influence the utilization of PN-glucoside.[8] The results of the present study indicate that the relative contribution of intralumenal (i.e., bacterial) and mammalian β-glucosidases in this hydrolysis would likely differ as a function of dietary vitamin B_6 intake. With respect to the mucosal cytosolic β-glucosidase, the results of this study contrast with our previous findings that there is no significant influence of the long-term intake of either PN or PN-glucoside in the diet on the activity of this enzyme in rat small intestine.[32]

We have previously shown that the intestinal β-glucosidase activity of guinea pigs is higher than that of rats, yet less than that of humans.[16] However, this difference alone is not enough to account for the much higher bioavailability of PN-glucoside in guinea pigs than in either rats or humans. It is uncertain to what degree hind-gut fermentation contributes to human nutriture in general, much less with regard to the specific nutrient vitamin B_6. Guinea pigs, however, are known to display a high degree of hind-gut fermentation, in comparison with other laboratory rodents.[9,33] The apparently high degree of β-glucosidase activity in the small intestinal lumen observed in this study may be complimented further by the hydrolytic activity associated with the extensive cecal and colonic microflora in guinea pigs.[34]

In this regard, guinea pigs may not represent an adequate animal model of human PN-glucoside utilization. Nevertheless, studies of the metabolism of PN-glucoside in this species would yield informative comparative results concerning the relative roles of microbial and mammalian β-glucosidases as a function of dietary vitamin B_6.

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