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NUTRITIONAL PROPERTIES AND SIGNIFICANCE OF VITAMIN GLYCOSIDES

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ABSTRACT

Glycosylated forms of pyridoxine, vitamin D, niacin, pantothenate, and riboflavin exist in nature, whereas glycosides of retinol and ascorbic acid are products of in vitro transglycosidation. β-Glucosides of pyridoxine (*a*) are prevalent in plant-derived foods, (*b*) contribute to human nutrition as partially available sources of vitamin B_6, (*c*) undergo partial hydrolysis by a novel mammalian cytosolic β-glucosidase, and (*d*) exert a weak antagonistic effect on the utilization of free pyridoxine. Niacin exists in grains as complexed forms with low bioavailability, whereas vitamin D glycosides are toxic components of certain calcinogenic plants of importance in animal health. Glycosides of pantothenate and riboflavin appear to be minor products of mammalian metabolism. Glycosylation of retinol or other hydrophobic alcohols may facilitate glycolipid turnover, whereas a stable ascorbyl glucoside may have nutritional applications. Glycosylation of vitamins exerts widely ranging chemical and biological effects, with great nutritional and metabolic significance.

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INTRODUCTION

As with many other biologically active compounds, glycoside derivatives of vitamins have been identified and their properties investigated. Examples include (*a*) naturally occurring glycosides of vitamin B_6, vitamin D, niacin, pantothenate, and riboflavin; (*b*) a retinyl glucoside identified as a product of in vitro enzymatic glycosylation; and (*c*) a synthetic ascorbyl glucoside that may have application as a stabilized form of vitamin C. The formation of glycosylated derivatives of vitamins—whether naturally in plants, animals, or microorganisms or by intentional chemical modification—represents a process that may cause dramatic changes in chemical, nutritional, and metabolic properties. This chapter examines glycosylated forms of vitamins and current understanding of the impact of glycosylation. Of the known glycosylated forms of vitamins, the pyridoxine glucosides arguably have the greatest nutritional significance and multiplicity of roles through their widespread natural occurrence in plants, their role as a partially available dietary form of vitamin B_6 in human nutrition, and the subtle antagonism of the metabolic utilization of nonglycosylated forms of the vitamin in mammals. Thus, primary attention is focused on the natural occurrence and nutritional properties of vitamin B_6 glycosides.

Current understanding of the functions of vitamin glycosides is incomplete, but several roles have been identified (Table 1). In essentially all examples of vitamin glycosides, in vivo hydrolysis is the key step governing biological activity (i.e. nutritional function). In the case of α-glucosides of vitamins, such hydrolysis may be catalyzed by intestinal brush border α-glucosidase (i.e. maltase) or by the ubiquitous cytosolic α-glucosidase activity expressed in many mammalian tissues (30), or possibly by pancreatic α-amylase. The limited hydrolysis of several dietary β-glucosides, such as certain cyanogenic glycosides, appears to be catalyzed by the mammalian cytosolic broad specificity β-glucosidase (14). However, the partial hydrolysis of pyridoxine-β-glucoside(s) appears to be catalyzed primarily by a distinct β-glucosidase, designated pyridoxine-β-D-glucoside hydrolase, found in the cytosolic fraction of intestinal mucosa (37, 42). The mechanism of formation of most naturally occurring vitamin glycosides is not well characterized, but it may occur via transglycosidation catalyzed by nonspecific glycosidases or by specific glycosylation with a UDP-sugar (e.g. UDP-glucose) as a donor. α-Glucoside formation

Table 1 Various functions of vitamin glycosides

Function	Example
Metabolically inert storage form of vitamin in plants	Pyridoxine glucosides; possibly niacin glycosides
Storage of a less active form of a toxic metabolite in calcinogenic plants	Vitamin D glycosides
Nutritional availability (wide range)	Pyridoxine glucosides Niacin glycosides
Possibly a detoxification or clearance process following large dose of the parent vitamin	Pantothenate partially excreted as glucoside
Potential antagonistic action against metabolic utilization of nonglycosylated forms	Pyridoxine glucosides
Increased chemical stability while retaining nutritional availability	Ascorbyl 2-α-glucoside
Unknown metabolic function, possibly a product of mammalian nonspecific transglycosidation	Riboflavin-α-glucosides

via transglycosidation reactions involving hepatic α-glucosidases occurs with certain xenobiotics (30) and may function in in vivo α-glycosylation of certain vitamins.

PYRIDOXINE GLUCOSIDES

Natural Occurrence and Identification of Pyridoxine Glucosides

Bound forms of vitamin B_6 were discovered during the initial development of quantitative assays for vitamin B_6 in rice bran concentrate and other materials of plant origin (52, 54, 55). Almost 30 years later, Yasumoto et al (80) reported that bound forms of pyridoxine comprised much of the vitamin B_6 in various grains and that the primary bound form was a β-glucoside of pyridoxine, although derivatives existed that were not hydrolyzed by β-glucosidase treatment. Nelson et al (46) detected and partially purified a low-molecular-weight bound form of pyridoxine in orange juice. Yasumoto and associates subsequently isolated the major vitamin B_6 glycoside from rice bran and conclusively identified it as pyridoxine-5′-β-D-glucoside (81) (Figure 1). Various other pyridoxine glucosides have been discovered (*a*) in sprouts of several types of plants germinated in the presence of exogenous pyridoxine and (*b*) occurring naturally in certain plants (Figure 1). These derivatives include both the 4′ and 5′ isomers of pyridoxine-β-D-glucoside, pyridoxine-5′-β-D-cellobioside

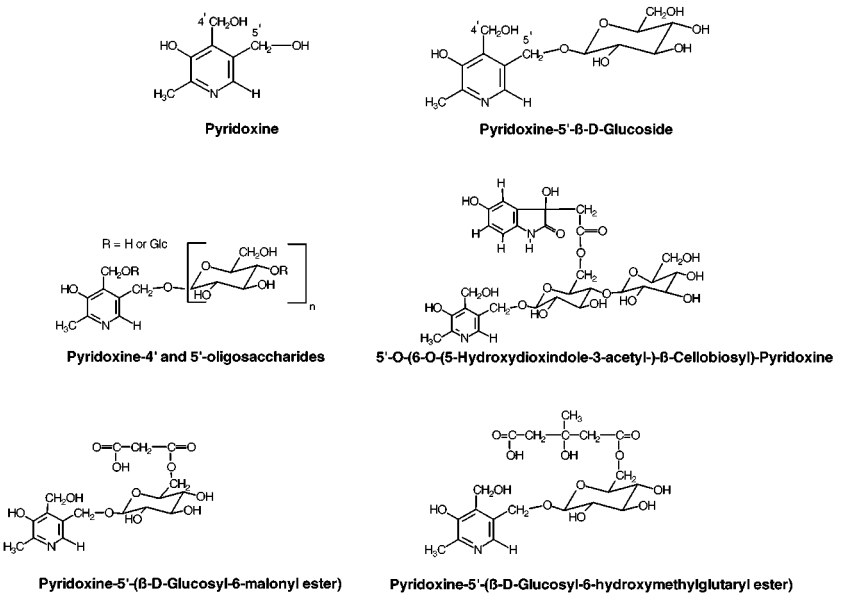

Figure 1 Structural formulas of pyridoxine and pyridoxine-β-D-glucoside derivatives.

and other oligosaccharides (58), pyridoxine-5′-β-D-glucoside esterified with malonic or 3-hydroxy-3-methylglutaric acid (59, 60), and a pyridoxine-5′-β-D-cellobiosyl-indoleacetyl ester (62). The identification of pyridoxine glucosides and the mechanism of their formation by plants and certain microbial species have been reviewed (17).

Several analytical methods have been devised for measurement of pyridoxine glucosides in foods. In a method reported by Kabir et al (28), total glycosylated vitamin B_6 is estimated as the incremental response in yeast growth assays before and after treatment of aqueous extracts with almond β-glucosidase. Gregory & Ink (19), followed by Tadera & Naka (61), developed liquid chromatographic procedures that enabled the measurement of individual forms of vitamin B_6, including pyridoxine-5′-β-D-glucoside. Selective hydrolytic steps were incorporated that allow indirect measurement of other forms of glycosylated vitamin B_6 in addition to pyridoxine-5′-β-D-glucoside (20).

Glycosylated forms of pyridoxine range approximately from 5% to 75% of the total vitamin B_6 in fruits, vegetables, and grains, with little or none in animal products (19, 20, 28, 33). Pyridoxine is the primary, possibly the only, glycosylated form of vitamin B_6, with no evidence of glycosylated pyridoxal or pyridoxamine. Pyridoxine-5′-β-D-glucoside is the major glycosylated form

of vitamin B_6 in most plant-derived foods (19, 20). Although the enzymatic synthesis of pyridoxine-α-D-glucoside has been reported (27, 47, 70), there is no evidence it occurs naturally in mammalian tissues or in plant-derived foods. The total quantity and percentage of glycosylated vitamin B_6 relative to total vitamin B_6 in any particular diet depends on food selection. For a typical mixed diet, glycosylated vitamin B_6 constitutes approximately 15% of the total vitamin B_6 ingested (1, 21). However, the range can vary from as high as 40–50% to as low as 5–10%.

Availability as a Source of Dietary Vitamin B_6

In view of the prevalent, natural occurrence of pyridoxine-5′-β-D-glucoside and other glycosylated forms of vitamin B_6, understanding their bioavailability and metabolic properties is essential to accurate interpretation of dietary vitamin B_6 intake data. Relevant questions include the following. What is the bioavailability of pyridoxine-5′-β-D-glucoside and other pyridoxine glucosides (i.e. to what extent do they undergo absorption and utilization in vitamin B_6 metabolism)? Where, how, and to what extent does hydrolysis of the glycosidic bond occur? Does glycosylated glucoside exert any inhibitory effect of vitamin B_6 metabolism? Both absorption and hydrolysis of the glycosidic bond must occur, though not necessarily in that order, before pyridoxine-5′-β-D-glucoside or other vitamin B_6 glucosides can function in vitamin B_6 metabolism.

The bioavailability of dietary vitamin B_6 is incomplete, and the influence of diet composition has not been fully determined (16, 18). For example, compared with the largely complete bioavailability of free pyridoxine in a formula diet, the bioavailability of the total vitamin B_6 in a mixed diet is approximately 75% (65). In controlled-feeding studies with humans, Kabir et al (29) found that the bioavailability of total vitamin B_6 in several foods was inversely related to the percentage glycosylated. Later studies with additional food items showed exceptions to this trend (4). Influences of food composition on intestinal transit time may have been responsible for the apparent variation in bioavailability. The extent to which the apparent incomplete bioavailability of glycosylated vitamin B_6 in mixed diets influences vitamin B_6 status is unclear.

Because vitamin B_6 glucosides exist in plant-derived foods, the question of whether the bioavailability of vitamin B_6 is lower in diets in which vitamin B_6 is mostly from plant sources must be considered. In comparisons between vegetarian and nonvegetarian populations, whose self-selected diets presumably differ in amounts of glycosylated vitamin B_6, little or no difference in vitamin B_6 status has been reported (53). Löwik et al (34) examined the vitamin B_6 nutritional status of a group of elderly Dutch. Diet type (vegetarian or nonvegetarian) did not result in a significantly different estimated mean daily vitamin B_6

intake. Vegetarians ($n = 29$) had slightly higher plasma pyridoxal 5′-phosphate concentrations than nonvegetarians had ($n = 426$, $P < 0.025$), but there was no significant difference in erythrocyte aspartate aminotransferase activity or activity coefficient. The intake of dietary fiber, a marker of plant-derived foods, did not adversely affect vitamin B_6 nutritional status. Although this study was not specifically designed to study bioavailability, these results suggest that differences in bioavailability between plant- and animal-derived foods were small relative to the statistical power of the study. In a study addressing the bioavailability of glycosylated vitamin B_6, Andon et al (1) examined the content of total and glycosylated vitamin B_6 in diet composites of 30 women. The intake of glycosylated vitamin B_6 (mean 15% of total) was not statistically related to the vitamin B_6 nutritional status of the women. This finding is consistent with partial availability of the glycosylated vitamin B_6. To investigate the nutritional significance of glycosylated vitamin B_6 more directly, Hansen et al (22) conducted a controlled study in which for 18 days young women were fed diets providing either 9% or 27% of the total vitamin B_6 intake in glycosylated form. These diets corresponded to low and moderately high percentages of glycosylated vitamin B_6, respectively. In spite of equivalent intake of total vitamin B_6, the higher intake of glycosylated vitamin B_6 caused a decrease in vitamin B_6 status, as reflected by plasma and erythrocyte pyridoxal phosphate, urinary vitamin B_6, urinary 4-pyridoxic acid, and the activity coefficient of erythrocyte aminotransferases. The reduction in vitamin B_6 status due to the higher intake of glycosylated vitamin B_6 corresponded to a 15–18% reduction in intake of available vitamin B_6. These findings indicate that the bioavailability of glycosylated vitamin B_6 (primarily pyridoxine-5′-β-D-glucoside) is incomplete and of sufficient magnitude to influence nutritional status when evaluated in a controlled study.

A number of studies of the bioavailability and metabolism of purified pyridoxine-5′-β-D-glucoside have yielded additional evidence of its importance in vitamin B_6 nutrition. Shortly after the identification of pyridoxine-5′-β-D-glucoside, Tsuji et al (71) reported that the vitamin B_6 availability of chemically synthesized glucoside was equivalent to that of pyridoxine when administered orally or injected intravenously in rats. Many other studies of pyridoxine-5′-β-D-glucoside utilization in rats have indicated that the glucoside is less available than free pyridoxine in rats (24, 40, 41, 67, 69), typically 20–30% relative to pyridoxine. One study of pregnant rats suggested that the metabolic utilization of orally administered pyridoxine-5′-β-D-glucoside was similar to that of free pyridoxine (9); however, nonpregnant control rats were not employed and these findings have not been confirmed. Variation exists among rodent species with respect to their extent of metabolic utilization of orally administered pyridoxine-5′-β-D-glucoside, with rats < hamsters = mice < guinea pigs (2).

Pyridoxine-5′-β-D-glucoside exhibits substantial but incomplete bioavailability in humans. Gregory et al (21) simultaneously administered [$^2H_2$]pyridoxine-5′-β-D-glucoside and [$^2H_5$]pyridoxine orally to five young adult men. On the basis of the molar ratio of the administered compounds and the ratio of $^2H_2$ and $^2H_5$ forms of the urinary metabolite 4-pyridoxic acid, the relative utilization of pyridoxine-5′-β-D-glucoside was 58 $\pm$ 13% (mean $\pm$ standard error of the mean). Urinary excretion of intact pyridoxine-5′-β-D-glucoside comprised approximately 35% of the intake (dietary plus purified dose), in contrast to the urinary excretion of 9% of ingested glycosylated vitamin B_6 reported by Hansen et al (22). In spite of the mean of 58% metabolic utilization, one subject actually showed equivalent utilization of the free and glycosylated forms administered. A second study was conducted to examine the bioavailability of pyridoxine-5′-β-D-glucoside in a protocol in which [$^2H_2$]pyridoxine-5′-β-D-glucoside and [$^2H_2$]pyridoxine were administered independently to all subjects (four men and four women) in consecutive trials (43). The metabolic utilization of [$^2H_2$]pyridoxine-5′-β-D-glucoside, as reflected by conversion to labeled 4-pyridoxic acid, was 50 $\pm$ 7% that of free pyridoxine, with no difference between men and women. These two studies indicate that in humans, dietary pyridoxine-5′-β-D-glucoside can contribute as a partially available source to vitamin B_6 nutrition.

Although pyridoxine-α-D-glucosides have not been detected in foods, the utilization of this derivative has been examined in rats and isolated rat hepatocytes. From the short-term response of plasma pyridoxal phosphate following oral administration of pyridoxine or the α-D- or β-D-glucosides in rats, Joseph et al (27) concluded that the α-glucoside was equivalent to free pyridoxine whereas the β-glucoside underwent far less utilization. In isolated rat hepatocytes, both pyridoxine-4′- and 5′-α-D-glucosides were effectively taken up and incorporated into vitamin B_6 metabolism (27). These observations regarding the facile metabolic utilization of pyridoxine-α-D-glucosides are similar to findings regarding the extensive hydrolysis and utilization of riboflavin-α-D-glucoside (26).

Intestinal Absorption and In Vivo Disposition

The intestinal absorption of nonglycosylated, nonphosphorylated forms of vitamin B_6 occurs by diffusion with metabolic trapping (5). Although the mechanism of intestinal absorption of pyridoxine-β-D-glucosides has not been determined, many lines of evidence indicate effective absorption.

In vitro studies with everted rat intestine showed that pyridoxine-5′-β-D-glucoside could be absorbed without prior hydrolysis, but the appearance of pyridoxine in serosal fluid indicated that partial hydrolysis could occur during absorption (70, 71). Similar studies also showed equivalent absorption of

pyridoxine-4′-β-D-glucoside and pyridoxine in rat intestine and that introduction of a β-glucosidase inhibitor, δ-gluconolactone, had no inhibitory effect on absorption (25).

In vivo studies involving orally administered tracer doses of pyridoxine-5′-β-D-glucoside and pyridoxine indicated extensive absorption of each form, as judged by radioactivity in excreted feces, which remained in the intestinal contents 24 h post-dose (<20% of dose for [^{3}H]pyridoxine-5′-β-D-glucoside and <10% of dose for [^{3}H]pyridoxine) (24, 66). Following an oral dose of [^{3}H]pyridoxine-5′-β-D-glucoside to rats, 39.5% of the radioactivity appeared in the urine in 24 h, and at least 85% of the urinary ^{3}H consisted of unchanged pyridoxine-5′-β-D-glucoside (24, 67). Thus, intestinal absorption of the intact glucoside occurs. The rat has a small but measurable ability to utilize pyridoxine-5′-β-D-glucoside as a source of available vitamin B_6, and the fraction not undergoing hydrolysis is excreted. Later kinetic studies of rats have shown that pyridoxine-5′-β-D-glucoside not hydrolyzed undergoes rapid urinary excretion largely within 6 h following ingestion (40). In human studies, urinary excretion of intact pyridoxine-5′-β-D-glucoside derived from dietary sources or administered doses of the glucoside also provides clear evidence of absorption, at least in part, of the intact glucoside (1, 21, 29, 43).

In vivo glycosylation of vitamin B_6 does not occur. Studies in which isotopically labeled pyridoxine was administered to humans or animals have shown no evidence of the excretion of correspondingly labeled pyridoxine-5′-β-D-glucoside in urine (21, 24, 43, 67). This suggests that in vivo glycosylation is not significant. In spite of reports that α-glycosylation can occur when exogenous pyridoxic acid is incubated with rat liver homogenates (63, 64), such derivatives have not been detected in analyses of tissues or urine from in vivo isotopic studies (24, 67, 68).

Whether pyridoxine-5′-β-D-glucoside consumed by women during lactation is secreted in milk is of potential relevance to infant nutrition. In a study by Andon et al (1), only trace amounts of a vitamin B_6 β-glucoside, corresponding to approximately 2% of the total vitamin B_6 secreted, were detected in the milk of women evaluated. Thus, the intact pyridoxine-5′-β-D-glucoside absorbed is preferentially excreted in urine rather than secreted into milk. A similar study involving radiolabeled pyridoxine-5′-β-D-glucoside administered to lactating rats confirmed these findings (68).

Enzymatic Hydrolysis of Pyridoxine-5′-β- -Glucoside

The β-glycosidic linkage of pyridoxine-5′-β-D-glucoside is stable, implying that in vivo nonenzymatic hydrolysis does not occur significantly, and the extent of in vivo utilization of glycosylated forms of pyridoxine is governed mainly by the extent of enzymatic hydrolysis (2, 21, 24, 43, 67). Initial investigation

of intestinal mucosa fractions indicated the presence of an enzymatic activity capable of slowly hydrolyzing pyridoxine-5′-β-D-glucoside (66). Differential centrifugation studies indicated that essentially all enzymatic activity capable of hydrolyzing pyridoxine-5′-β-D-glucoside is found in the high-speed supernatant fraction of jejunal mucosa (66). This activity, which exhibited a pH optimum of ∼6.0, was assumed to be due to the cytosolic broad specificity β-glucosidase found in many tissues (11, 14) that is believed to be responsible for the hydrolysis of certain other dietary glycosides from plant sources (14, 15, 32). To help identify the anatomic locus of the in vivo hydrolysis, studies compared the metabolic utilization of isotopically labeled pyridoxine-5′-β-D-glucoside administered orally or by injection (thus bypassing intestinal enzymatic processes). In rats, metabolic utilization of administered [^{3}H]pyridoxine-5′-β-D-glucoside is limited, but 5–10 times greater hydrolysis occurred when administration was oral (67). In humans, the utilization of [$^2H_2$]pyridoxine-5′-β-D-glucoside administered orally was ∼58% relative to concurrently administered [$^2H_5$]pyridoxine, whereas intravenous [$^2H_2$]pyridoxine-5′-β-D-glucoside underwent ∼28% utilization (21). Thus, in both species, both postabsorptive hydrolysis of pyridoxine-5′-β-D-glucoside and hydrolysis during the intestinal absorption process can occur, and the intestine is the primary site of hydrolysis. In analyses of rat tissues, enzymatic activity capable of hydrolyzing pyridoxine-5′-β-D-glucoside has been detected primarily in the intestinal mucosa and kidney, with little or no activity in the liver (41). Measurements of the specific activity of pyridoxine-5′-β-D-glucoside hydrolyzing enzyme activity showed that the cytosolic fraction of jejunal mucosal from humans has several times greater activity than in that from rats or guinea pigs (66). This difference may be responsible for the difference in utilization of orally administered pyridoxine-5′-β-D-glucoside between humans and rats. In contrast was the essentially complete metabolic utilization of pyridoxine-5′-β-D-glucoside observed in guinea pigs (2). This may be due to their large jejunal population of microorganisms with hydrolytic activity (3). Analyses of jejunal contents of rats also indicate hydrolytic activity, though its nutritional role is unclear (41). Because humans normally have less microbial colonization of the jejunal region of the small intestine than do rats, microorganisms in human small intestine should have little significance in the hydrolysis of pyridoxine-5′-β-D-glucoside. Analyses of jejunal cytosolic fractions from normal and germ-free mice indicated similar specific activity of the pyridoxine-5′-β-D-glucoside hydrolyzing enzyme in each species (LG McMahon, JF Gregory, unpublished data), which confirms that the detected cytosolic activity is of mammalian origin.

In an attempt to characterize more fully the biochemical aspects of pyridoxine-5′-β-D-glucoside hydrolysis, Nakano et al (42) purified cytosolic broad

specificity β-glucosidase from pig intestine. Although the crude fractions had abundant pyridoxine-5′-β-D-glucoside hydrolyzing activity, purified jejunal broad specificity β-glucosidase did not hydrolyze pyridoxine-5′-β-D-glucoside. A novel cytosolic enzyme, pyridoxine-β-D-glucoside hydrolase (42), is the actual catalyst. This enzyme has recently been purified and partially characterized (37). Pyridoxine-β-D-glucoside hydrolase effectively cleaves pyridoxine-5′-β-D-glucoside [K_m, 0.88 $\pm$ 0.12 mM ($\pm$ standard error); V_{max}, 13.2 $\pm$ 0.8 μmol^{-1} h^{-1} mg of protein], although it does not hydrolyze paranitrophenyl-β-D-glycosides that are substrates for the cytosolic broad specificity β-glucosidase (37). Pyridoxine-β-D-glucoside hydrolase exhibits an apparent molecular weight of ~130,000, in contrast to ~60,000 for broad specificity β-glucosidase (37). Another unexpected observation was that pyridoxine-β-D-glucoside hydrolase exhibits hydrolytic activity against lactose and cellobiose (37). Because this enzyme is found in the cytosolic fraction of the mucosal cells, it is unclear whether this disaccharidase activity has physiological relevance. Dietary lactose is hydrolyzed by lactase-phlorizin hydrolase associated with the jejunal brush border membrane prior to transport of the monosaccharides (39), whereas there is little or no hydrolysis or absorption of dietary cello-oligosaccharides or β-linked polysaccharides.

Of particular interest is the fact that the activity of pyridoxine-β-D-glucoside hydrolase in jejunal mucosa is influenced by vitamin B_6 nutritional status. In both rats and guinea pigs, activity of this mammalian enzyme is increased severalfold in vitamin B_6 deficiency (3, 41). The extent to which this regulation may occur in humans has not been determined, and its possible influence on the bioavailability of dietary pyridoxine-5′-β-D-glucoside will be investigated. A complicating factor in assessing the physiological significance of this nutritional regulation in rodents is the fact that microbial pyridoxine-5′-β-D-glucoside hydrolyzing activity in the jejunal contents increases with increased vitamin B_6 intake (3, 41). It is expected that nearly all of the hydrolysis of dietary pyridoxine-5′-β-D-glucoside in humans is catalyzed by the mammalian enzyme pyridoxine-β-D-glucoside hydrolase.

Antagonistic Effects of Pyridoxine-5′-β- -Glucoside

Because intact pyridoxine-5′-β-D-glucoside is a metabolically inactive analog of vitamin B_6, and because pyridoxine-5′-β-D-glucoside undergoes intestinal absorption but only partial hydrolysis, whether the intact glucoside might influence various aspects of vitamin B_6 metabolism was investigated. In vitro studies indicated that pyridoxine-5′-β-D-glucoside does not inhibit the key enzymes in the conversion of dietary forms of vitamin B_6 to pyridoxal phosphate (pyridoxal kinase and pyridoxine/pyridoxamine 5′-phosphate oxidase), and pyridoxine-5′-β-D-glucoside does not inhibit the activity of pyridoxal 5′-phosphate–dependent

glycogen phosphorylase or the reconstitution of the holoenzyme (12). However, an in vivo study with rats indicated that pyridoxine-5′-β-D-glucoside does partially inhibit the metabolic utilization of orally administered pyridoxine in a dose-dependent fashion (13). This study and a longer-term investigation of effects of dietary pyridoxine-5′-β-D-glucoside indicated that consumption of the glucoside in the presence of pyridoxine or alone caused in vivo alteration of vitamin B_6 metabolism, as evidenced by increased urinary excretion and a small accumulation of hepatic pyridoxine 5′-phosphate, a transient form usually not detected in tissues (13, 40). Short-term studies in rats evaluated the time course of the antagonistic effect following a single oral mixed dose of [^{3}H]pyridoxine and nonlabeled pyridoxine-5′-β-D-glucoside (40). The maximum inhibitory effect on [^{3}H]pyridoxine metabolism occurred $\sim$12 h after administration of the dose, consistent with the short in vivo retention of intact pyridoxine-5′-β-D-glucoside prior to urinary excretion. Although these studies demonstrate an antagonistic effect of pyridoxine-5′-β-D-glucoside in rats, they provide little information regarding the mechanism of this phenomenon. Studies with isolated rat hepatocytes indicated that pyridoxine-5′-β-D-glucoside is transported into liver cells at $\sim$20% the rate of pyridoxine, followed by limited hydrolysis (82). Pyridoxine-5′-β-D-glucoside also was found to inhibit competitively the cellular uptake of pyridoxine (K_i of 1.4 μM) (82), consistent with the short-term in vivo kinetics of the transient antagonistic effects observed. This study provided strong evidence that intact pyridoxine-5′-β-D-glucoside inhibits the transport of pyridoxine (and potentially pyridoxal and pyridoxamine) into cells by competing for the transport mechanism.

Pyridoxine-5′-β-D-glucoside also exerts this antagonistic effect on vitamin B_6 metabolism in humans, but the effect is less pronounced (43). Sequential trials were conducted in which [$^2H_2$]pyridoxine was administered with blends of nonlabeled pyridoxine and pyridoxine-5′-β-D-glucoside such that the glucoside comprised 0%, 15%, or 45% of the total vitamin B_6 dose. On the basis of rate of urinary excretion of [$^2H_2$]4-pyridoxic acid, this study showed that pyridoxine-5′-β-D-glucoside exerts a kinetically detectable delaying effect on pyridoxine metabolism. In spite of these changes in short-term kinetics, the area under the curve of 4-pyridoxic acid labeling, indicative of net metabolic utilization of the labeled pyridoxine dose, was not affected by the concurrently ingested pyridoxine-5′-β-D-glucoside. Thus, pyridoxine-5′-β-D-glucoside appears to have a weak, chronic effect in humans, with a slight modulating action but no pronounced inhibition. This weak antagonistic action of pyridoxine-5′-β-D-glucoside may have contributed to the worsening of vitamin B_6 status observed by Hansen et al (22) from diets high in the glucoside. This antagonistic effect is less notable in humans than in rats, possibly because of the greater in vivo hydrolysis of pyridoxine-5′-β-D-glucoside in humans than

rats and the consequently lower in vivo concentrations of pyridoxine-5′-β-D-glucoside.

In summary, pyridoxine-5′-β-D-glucoside is an interesting, naturally occurring derivative of vitamin B_6, with approximately 50% bioavailability as a source of vitamin B_6 in humans. Although partially available, pyridoxine-5′-β-D-glucoside bioavailability for humans is less than that of nonglycosylated forms of vitamin B_6. The weak antagonistic action of pyridoxine-5′-β-D-glucoside on the utilization of other forms of the vitamin complicates assessment of the overall bioavailability of total dietary vitamin B_6. In total, these data regarding the nutritional properties of pyridoxine-5′-β-D-glucoside make it the most thoroughly characterized of the vitamin glycosides.

GLYCOSIDES OF OTHER VITAMINS

Riboflavin

The existence of a glycosylated form of riboflavin was first reported by Whitby (77), who observed the formation of riboflavin-5′-α-D-glucoside (Figure 2) in vitro when rat liver fractions were incubated with riboflavin and either maltose or glycogen. Enzymes capable of catalyzing this transglycosylation reaction have been found in liver, certain microorganisms, and plants (56, 57, 77, 78), though conditions of transglycosylation reported are generally not relevant to intracellular concentrations of riboflavin or glycosyl donors.

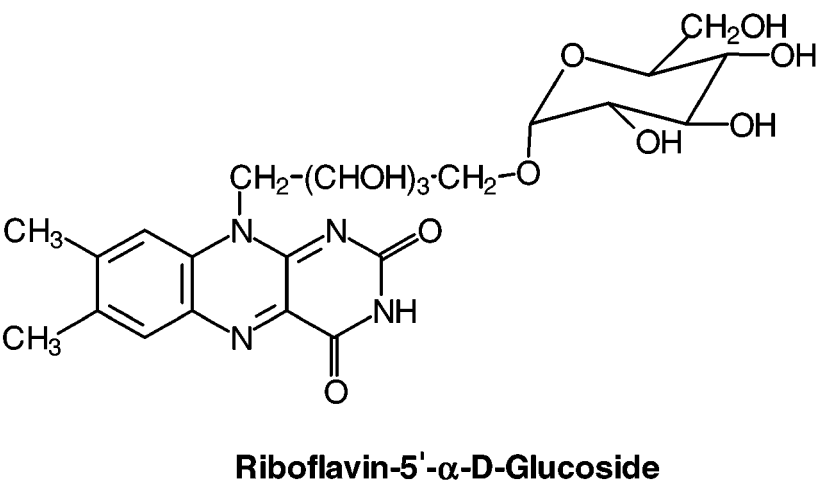

Figure 2 Structural formula of riboflavin-5′-α-D-glucoside.

Urinary riboflavin-5′-α-D-glucoside has been detected in urine of rats (5% of total excretory forms) following administration of a tracer dose of radiolabeled riboflavin (48), which suggests that glycosylation can occur to a small extent in vivo. Detailed analysis of the flavins in rat urine (8) and in human and bovine milk (50, 51) accounted for over 95% of flavins as nonglycosylated forms. This supports the conclusion that glycosylation of riboflavin is of minor significance in mammalian species. Riboflavin-5′-α-D-glucoside can be effectively taken up and fully metabolized by isolated liver cells, and when orally administered to rats, riboflavin-5′-α-D-glucoside and riboflavin exhibit comparable bioavailability (26). Although the in vitro glycosylation of riboflavin by a plant α-glucosidase has been reported (56), there is no evidence of dietary riboflavin glycosides in plant-derived foods. Thus, the significance of riboflavin-5′-α-D-glucoside as a dietary form of the vitamin and as a metabolite appears to be minor.

Niacin

Bound forms of niacin in grains such as corn and wheat have long been known to exist. In chemical methods of niacin determination, niacin remains in a bound state if extracted at neutral pH but is liberated if heated in alkaline medium. Bound forms of niacin exhibit little or no nutritional availability in animals (6, 35) and humans (7). Alkaline treatment, such as soaking corn in a lime solution, traditional in Central American production of tortillas, yields release of bound niacin and increased bioavailability (72).

The chemical structure of bound forms of niacin has not been determined fully (10, 31, 36, 74, 75). Elemental analysis, along with enzymatic and chemical hydrolysis, suggests that bound niacin from wheat bran (termed niacytin) has a single nicotinic acid moiety at least partially—in β-glycosidic linkages—ester-linked to an aromatic amine with glucose, xylose, and arabinose in a 6:3:1 molar ratio per molecule, with approximately three cinnamic acid esters (31). Later studies of this complexed niacin demonstrated far more heterogeneity in molecular mass (range, 1,500–17,000) and composition than was originally believed to exist, with variable quantities of hexoses, pentoses, phenolic acids, and amino-phenols (36). Similar composition was reported for the bound niacin in corn (10). The physiological function of these complexed niacin derivatives in plants has not been determined. Additional important findings involved the changes in the distribution of niacin compounds in corn during its development. Immature sweet corn is an effective source of available niacin, whereas niacin in mature field corn is largely unavailable (6). The availability of niacin in immature sweet corn is largely due to the predominance of NAD, NADP, and lesser quantities of other available niacin derivatives (74, 75).

Vitamin D

Glycosylated vitamin D is the toxic component of the calcinogenic plants *Solanum malacoxylon*, present in South America, and *Cestrum diurnum*, indigenous to Jamaica, Hawaii, and the southeastern United States. Both species induce a calcinosis and hypervitaminosis D in animals, including horses, cattle, and sheep. Aqueous extracts of *S. malacoxylon* contain a water-soluble vitamin D derivative that apparently is a glycoside derivative. Enzymatic hydrolysis with a mixed nonspecific glycosidase preparation yielded a lipophilic compound identified as 1,25-dihydroxyvitamin D_3 (Figure 3) (23, 45, 76). In *S. malacoxylon*, the specific site(s) of glycosylation of the 1,25-dihydroxyvitamin D_3 aglycone, the type of glycosidic bond involved, and the identity of the carbohydrate moiety have not been determined. The vitamin D derivative in *C. diurnum* is soluble in chloroform-methanol solution (23, 49, 76), in contrast to the water solubility of the vitamin D derivative in *S. malacoxylon*. This difference in solubility suggests greater polarity due to greater glycosylation of the derivative found in *S. malacoxylon*.

Rambeck et al (49) synthesized several vitamin D_3 glycosides, including vitamin D_3 β-D-glucoside, 1-hydroxyvitamin D_3 3-β-D-glucoside, and 1-hydroxyvitamin D_3 3-β-D-cellobioside, and examined them for vitamin D activity

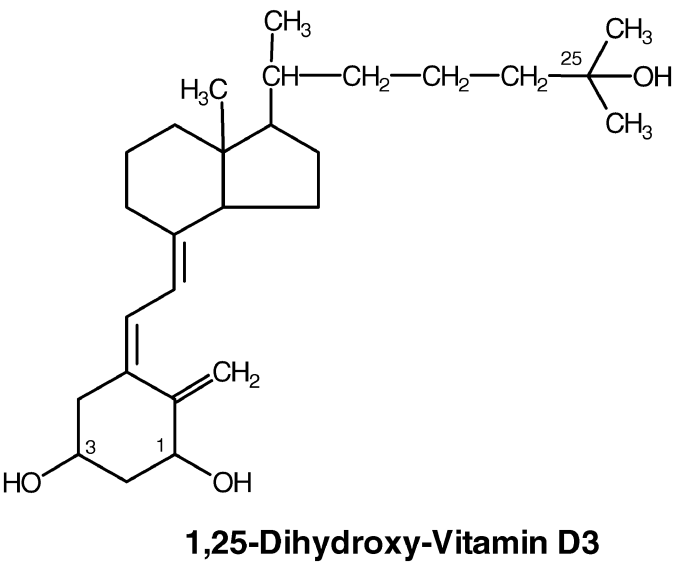

Figure 3 Structural formula of 1,25-dihydroxycholecalciferol (1,25-dihydroxy-vitamin D_3). The hydroxyl groups at the 1, 3, and 25 positions are potential sites of glycosylation.

in bioassays using rats, chicks, and quail. In rats and chicks, the aglycone (vitamin D_3) and the vitamin D_3 β-D-glucoside exhibited nearly equivalent activity, and the response of rats was equivalent when the compounds were administered orally or intravenously. In contrast, the β-D-glucoside of 1α-hydroxyvitamin D_3 exhibited only 10% activity relative to its aglycone (1α-hydroxyvitamin D_3) in rats, chicks, and quail, whereas the β-D-cellobioside (i.e. the disaccharide derivative) exhibited no vitamin D activity in a chick bioassay. A similar examination of structure-activity relationships has not been reported for 1,25-dihydroxyvitamin D_3 glycosides.

Retinol

There is no evidence glycosylated forms of dietary retinol or in vivo glycosylation of retinol in mammalian metabolism exist. However, in vitro findings suggest that limited in vivo glycosylation of retinol and other hydrophobic alcohols may occur through a glucocerebrosidase-mediated transglycosidation from a glycolipid substrate (73). This predominantly lysosomal enzyme normally catalyzes the hydrolysis of the β-glycosidic bond of glucocerebroside in glycolipid catabolism. Incubation of the enzyme with glucocerebroside and 0.5 mM retinol yielded the formation of a product identified as retinyl-β-D-glucoside (Figure 4). Of interest was the fact that the retinyl glucoside was not hydrolyzed by the glucocerebrosidase but could be hydrolyzed in vitro by two mammalian broad specificity β-glucosidases. This raises the interesting question of whether retinol or other lipophilic alcohols may participate in the catabolism of glucocerebrosides by serving as recipients in such a glucose transfer (73). The in vivo significance of these findings has not been determined.

Pantothenate

Studies of pantothenate metabolism in dogs have demonstrated in vivo glycosylation (44). Following administration of an oral dose of pantothenate (3 mg/kg), unchanged pantothenate comprised 60% of urinary excretion

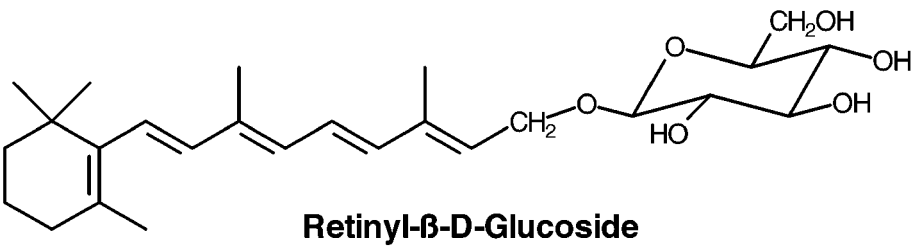

Figure 4 Structural formula of retinyl-β-D-glucoside.

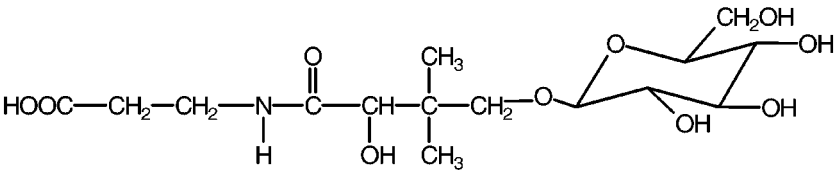

Figure 5 Structural formula of pantothenyl-4′-β-D-glucoside.

whereas a metabolite identified as pantothenate-4′-β-D-glucoside comprised 40% (Figure 5). The extent to which this compound is formed following more nutritionally relevant doses of pantothenate has not been determined. No evidence exists for the presence of dietary forms of glycosylated pantothenate.

Ascorbic Acid

Enzymatic glycosylation to form ascorbyl-2-α-D-glucoside (Figure 6) in vitro has been reported (79). Glycosylation of ascorbate at the 2-O position improves stability, as does formation of ascorbyl 2-O sulfates and phosphates. Ascorbyl-2-α-D-glucoside exhibits essentially full bioavailability as a source of vitamin C activity because of its extensive hydrolysis by intestinal brush border α-glucosidase (38). No applications of this derivative in either food fortification or as a nutritional supplement have been reported.

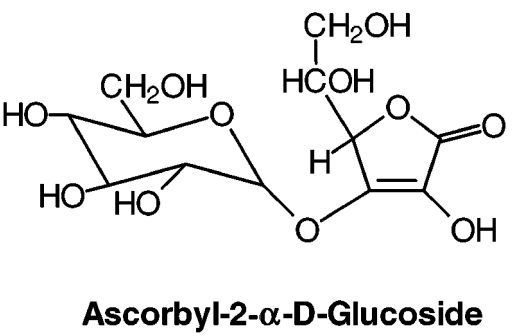

Figure 6 Structural formula of ascorbyl-2-α-D-glucoside.

SUMMARY

This review has illustrated the diversity of chemical properties and metabolic characteristics of the currently known vitamin glycosides. Glycosides of vitamin B_6 and niacin appear to have the greatest significance in human nutrition. Although important advances have been made in our understanding of their nutritional significance, more information is needed to permit a full assessment of vitamin glycosides in human nutrition. This is especially true of the pyridoxine glucosides and the nutritional regulation of the novel hydrolase responsible for their partial hydrolysis.

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