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β-GLUCOSYLPYRIDOXINES IN GERMINATING SEEDS CULTURED IN THE PRESENCE OF PYRIDOXINE*

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Key Word Index—*Hordeum vulgare*; *Triticum aestivum*; *Oryza sativa*; Gramineae; *Glycine max*; Leguminosae; 4′-*O*-(β-glucosyl)pyridoxine; 5′-*O*-(β-glucosyl)pyridoxine.

Abstract—5′-*O*-(β-Glucosyl)pyridoxine and 4′-*O*-(β-glucosyl)pyridoxine were formed in germinating seeds of wheat, barley and rice cultured on a pyridoxine solution; the ratio was 1:1 in the case of wheat and barley. On similar germination of soybean seeds, only 5′-*O*-(β-glucosyl)pyridoxine was formed. Plant seedlings also conjugated exogenously supplied pyridoxine in the same way.

INTRODUCTION

Vitamin B_6 occurs naturally in plants largely in bound form [1, 2]. Bound forms of vitamin B_6 of unknown structure were reported in rice bran and urine [3, 4] and later a glucose conjugate was described [5]. A vitamin B_6 conjugate(s) in orange juice was reported to be a non-protein compound with a molecular weight less than 3500, but its exact structure was not determined [6]. In previous papers [7, 8], we reported that two conjugated forms of vitamin B_6 (pyridoxine 4′- and 5′-β-glucosides) were formed when cellobiose and pyridoxine were incubated with wheat bran β-glucosidase (cellobiase).

The present paper describes the formation of these two glucosylpyridoxines in several germinating cereal seeds cultured in a pyridoxine solution, the characterization of one of them in germinating soybean seeds similarly cultured, and the distribution of these two conjugates in various plant seedlings grown in the presence of pyridoxine.

RESULTS AND DISCUSSION

A bound form of vitamin B_6, β-glucosylated pyridoxine, was formed in germinating cereal seeds, legumes and other plants cultured in the presence of pyridoxine. During germination of seeds of wheat and barley, two β-glucosylpyridoxines [5′-*O*-(β-glucosyl)pyridoxine and 4′-*O*-(β-glucosyl)pyridoxine] as the main metabolites of pyridoxine were formed in a 1:1 molar ratio, whereas similar experiments with rice seeds showed that the 5′-glucoside was the major conjugate but that the 4′-glucoside was also formed at the later stages of germination (Fig. 1). In germinating soybean seeds, the accumulation of only one pyridoxine derivative was observed (Fig. 1). The amount of the derivative accumulated was much more than that of pyridoxine at all stages of germination. The derivative was purified by prep. PC, ion-exchange column chromatography on Dowex 50W × 8, gel filtration on Sephadex G-10, DEAE-cellulose column chromatography and lyophilization, and isolated as the crystalline hexaacetate (mp 85–87°). The derivative was identical to 5′-*O*-(β-glucosyl)pyridoxine, synthesized enzymatically from cellobiose and pyridoxine by wheat bran β-glucosidase [8], in R_f, mp, elemental analyses, UV, IR and ^{1}H NMR spectra, electrophoretic mobilities, colour reaction with 2,6-dichloroquinone 4-chlorimide in the presence of boric acid, and liberation of glucose and pyridoxine (molar ratio 1:1) by hydrolysis with almond β-glucosidase. Thus, the derivative is 5′-*O*-(β-glucosyl)pyridoxine. Its microbiological activity for *Saccharomyces carlsbergensis* was found to be not more than 10% of that of equivalent mole of pyridoxine after a 16 hr incubation.

5′-*O*-(α-Glucosyl)pyridoxine and 4′-*O*-(α-glucosyl)-pyridoxine, new derivatives of vitamin B_6, were first found by Ogata *et al.* [9, 10] in the culture broth of *Sarcina lutea* which was grown in the medium consisting of sucrose and pyridoxine. Subsequently, Suzuki *et al.* reported that glycosylpyridoxine-forming activities were found in partially purified preparations of α-glucosidases from *Aspergillus niger* and *Mucor javanicus*, and in crystalline β-galactosidase from *Escherichia coli* [11]. Two new derivatives of pyridoxine [4′-*O*-(β-galactosyl)pyridoxine and 5′-*O*-(β-galactosyl)pyridoxine] were formed by incubation of *o*-nitrophenyl-β-D-galactopyranoside and pyridoxine with crystalline β-galactosidase from *E. coli*, and isolated and characterized [12]. Also, a highly purified α-glucosylpyridoxine-forming enzyme from *Micrococcus* sp. was purified to homogeneity in polyacrylamide disc gel electrophoresis and ultracentrifugation. This purified enzyme was shown to have α-glucosidase activity as well as α-glucosylpyridoxine-forming activity [13]. These microbial glycosidases [9–13] and wheat bran β-glucosidase [8] catalysed the transfer of a glycosyl residue from disaccharides to both the 5′- and 4′-hydroxymethyl groups of pyridoxine. On the other hand, uridine 5′-diphosphoglucuronyltransferase from rabbit liver catalysed the formation of a β-glucuronylpyridoxine from

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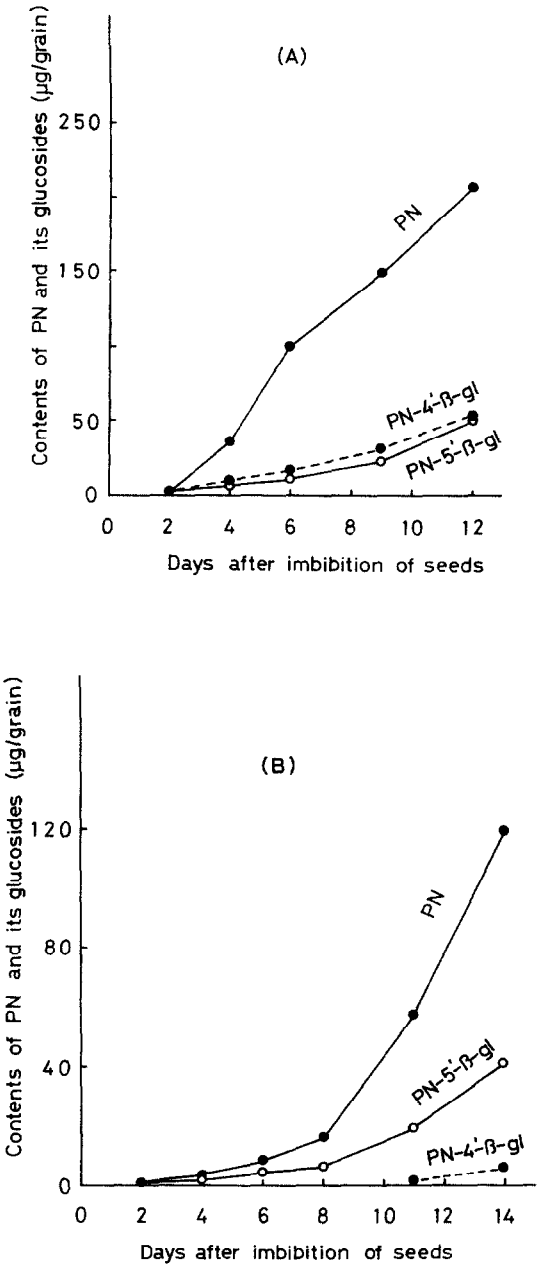
uridine 5'-diphosphoglucuronate and pyridoxine [14]. Also, a particulate enzyme in seedlings of *Pisum sativum* L. cv. Kinusaya was reported to catalyze the transfer of glucose from uridine diphosphoglucose to the 5'-, but not 4'-hydroxymethyl group of pyridoxine [15].

These data on the enzymes catalysing the formation of glycosylpyridoxines suggest that the two β-glucosyl pyridoxines in germinating seeds of wheat and barley may be formed by β-glucosidase, and one β-glucosyl pyridoxine[5'-*O*-(β-glucosyl)pyridoxine] in germinating soybean seeds by uridine diphosphoglucose-pyridoxine glucosyltransferase. The distribution of the β-glucosyl pyridoxine-forming activity in various plant seedlings cultured in a pyridoxine solution (Table 1) shows that β-glucosylpyridoxine occurs widely in various plants.



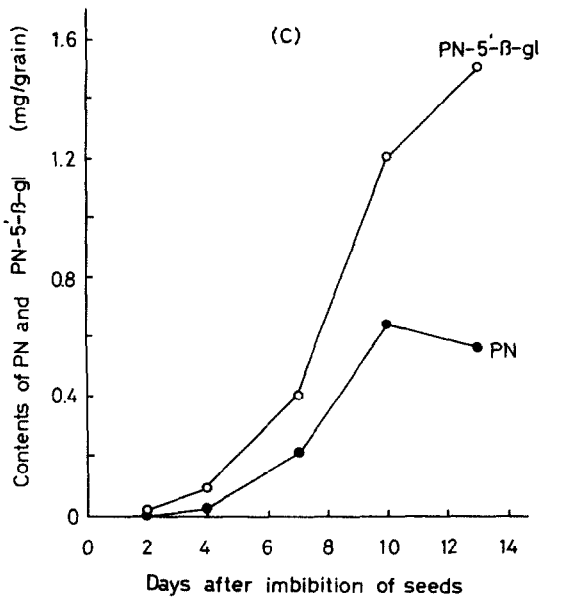


Fig. 1. Changes in content of pyridoxine and its glucosides in germinating seeds cultured on 10 mM pyridoxine during germination. (A) Wheat; (B) rice; (C) soybean. PN, Pyridoxine; PN-4'-β-gl, 4'-*O*-(β-glucosyl)pyridoxine; PN-5'-β-gl, 5'-*O*-(β-glucosyl)pyridoxine.

EXPERIMENTAL

Materials. Barley seeds (*Hordeum vulgare* L. var. nudum cv. Kikaihadaka), wheat seed (*Triticum aestivum* L. cv. Shirasagi), rice seeds (*Oryza sativa* L. cv. Taichung 65, nonwaxy and *Oryza sativa* L. cv. Norin 18, nonwaxy) and soybean seeds (*Glycine max* L. Merr. cv. Tamanishiki) were harvested in the experimental field of our Institute. Other plant seeds were obtained from Yamato Plantation Co. Ltd., Tenri, Japan. Pyridoxine · HCl was obtained from Nakarai Chemicals Co. Ltd., Kyoto, Japan.

Assays of pyridoxine and its derivative in germinating seeds. Seeds were soaked in a disinfectant soln (0.25% Homai WP, Nippon Soda Co. Ltd.,) for 15 min at 40° and then for 5 hr at room temp. After the seeds were thoroughly rinsed in running water, they were sowed in vermiculite placed on plastic plates. The seeds were germinated at 20–27° in the dark. The sterilized and deionized water or a 10 mM pyridoxine · HCl soln (neutralized to pH 4.8 by NaOH) was sprinkled on each plastic plate every other day. The germination rate of seeds was over 90%. At intervals of 2 or 3 days, germination seeds on each of two plastic plates were harvested, washed and heated for 15 min in a boiling water bath, and then homogenized in a mortar with 2 vols of 0.02 M acetate buffer, pH 4.8. The homogenate was again heated for 15 min in a boiling water bath. One vol. of EtOH was added to the homogenate and centrifuged. The supernatant was concd to 30° under red. pres. The concentrate was applied as bands on a Toyo No. 50 filter paper (40 × 40 cm), and developed twice by ascent in *n*-BuOH–pyridine–H_2O (6:4:3). After drying, purplish fluorescent bands of pyridoxine and its derivatives were detected on the chromatogram under a UV lamp, cut out, and eluted with 0.1 M Pi buffer, pH 7.0, for 3 hr at 37°. The amounts of pyridoxine compounds in the effluent were determined fluorometrically and colorimetrically as follows. The values obtained by both methods resembled each other. The fluorescent intensity of the buffered eluate was measured by a spectrofluorometer attached to a Hitachi model EPS-3T automatic recording

Table 1. Formation of β-glucosylpyridoxines in various plant seedlings cultured on a pyridoxine solution

	Days after imbibition	PN	PN-5′-β-gl	PN-4′-β-gl
		(μg/10 grains)		
Beta vulgaris L. var. *cicla* L.	10	224	216	15
Beta vulgaris L. var. *rapa*	10	81	58	+
Brassica rapa L.	10	15	49	+
Brassica oleracea L.	10	50	208	
Raphanus sativus L.	10	387	1003	83
Vicia faba L.	12	3063	3338	
Pisum sativum L. cv. Arasuka	10	227	1137	
Pisum sativum L. cv. Kinusaya	10	386	1514	
Vigna unguiculata	10	2154	762	
Hibiscus esculentus L.	10	1914	2486	
Cucumis sativus L.	10	614	1166	34
Cucurbita maxima	12	1541	1371	
Cryptotaenia japonica	23	245	1116	
Daucus carota L.	15	68	189	+
Solanum melongena L.	15	22	65	
Lycopersicon esculentum	12	623	1892	
Chrysanthemum coronarium L.	10	31	22	
Arctium lappa L.	10	231	78	
Lactuca sativa L.	12	56	94	11
Allium tuberosum L.	15	116	246	
Allium fistulosum L.	10	65	47	
Allium cepa L.	12	96	123	+
Oryza sativa L. cv. Norin 18	14	1380	425	70
Oryza sativa L. cv. Taichung 65	14	1200	410	60

PN, Pyridoxine; PN-5′-β-gl, 5′-*O*-(β-glucosyl)pyridoxine; PN-4′-β-gl, 4′-*O*-(β-glucosyl)pyridoxine.

spectrophotometer (excitation at 325 nm; emission at 400 nm). The pyridoxine compounds were also measured by diazotized *p*-aminoacetophenone method with slight modification [10]. Since the concentrate of the extracts from germinating seeds cultured on water gave no band of pyridoxine and its derivatives on paper chromatograms, the areas corresponding to the compounds were cut and eluted. The eluates were used as the blank in both fluorometry and colorimetry. The content of the pyridoxine derivatives was expressed as pyridoxine equivalent.

Microbial assay of pyridoxine and its derivative. Microbiological activity of pyridoxine and its 5′-glucoside was assayed by the method of Atkin *et al.* [16], using *Saccharomyces carlsbergensis* strain 4228 ATCC 9080.

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