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FORMATION OF β-GLUCOSYLPYRIDOXINES IN SOYBEAN AND RICE CALLUS

YUKIO SUZUKI, HIROYUKI ISHII, KEIKO SUGA and KEI UCHIDA

Institute for Agricultural and Biological Sciences, Okayama University, Kurashiki, 710, Japan

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Key Word Index—*Oryza Sativa*; Gramineae; rice; *Glycine max*; Leguminosae; soybean; callus tissues; pyridoxine metabolism; 4'-glucoside; 5'-glucoside.

Abstract—5'-*O*-(β-Glucosyl)pyridoxine accumulated in soybean calluses and cultured cells grown on a sucrose medium in the presence of pyridoxine. In rice calluses, 5'-*O*-(β-glucosyl)pyridoxine as the main metabolite was accompanied by small amounts of 4'-*O*-(β-glucosyl)pyridoxine.

INTRODUCTION

Conjugates of pyridoxine and glucose (pyridoxine β-glucosides) can be synthesized by incubation of cellobiose and pyridoxine in the presence of the wheat bran β-glucosidase (cellobiase) [1, 2]. The resultant derivatives, 4'-*O*-(β-glucosyl)pyridoxine and 5'-*O*-(β-glucosyl)-pyridoxine, are formed in germinating seeds of wheat, barley and rice cultured in a pyridoxine solution, whereas only 5'-*O*-(β-glucosyl)pyridoxine accumulates in germinating soybean seeds. Also, β-glucosylpyridoxine formation has been shown to be widely distributed in various plant seedlings cultured in a pyridoxine solution [3]. The present paper describes the formation of β-glucosylpyridoxines in plant calluses and cells grown on the sucrose media with pyridoxine. There is no earlier report on the formation of vitamin glycosides in plant tissue cultures.

RESULTS AND DISCUSSION

Formation of a β-glucosylpyridoxine by soybean calluses and cultured cells

A purplish fluorescent spot corresponding to β-glucosylpyridoxine was detected, in addition to the spot of pyridoxine, on a paper chromatogram of the extracts of soybean calluses which were grown in the Murashige and Skoog's agar medium containing sucrose and 10 mM pyridoxine. Its fluorescent intensity was much greater than that of pyridoxine at all stages of cultivation. The spot was not detected in calluses grown on the sucrose medium in the absence of 10 mM pyridoxine. The formation of the derivative [5'-*O*-(β-glucosyl)pyridoxine] was observed in the 3-day-old calluses, followed by the gradual increase. After this lag period from 3 to 10 days, the content increased rapidly with an increase of callus growth. The amount of the derivative accumulated was seven times that of pyridoxine after 21 days cultivation. The pyridoxine content was very low throughout the course of cultivation. Pyridoxine incorporated was rapidly transformed to the derivative in soybean calluses.

Furthermore, the formation of the derivative was examined in soybean cultured cells, when grown on 50 ml of Murashige and Skoog's liquid medium containing sucrose and 10 mM pyridoxine in 500 ml flasks at 25° in the dark with shaking. A small amount of the derivative was detectable in soybean cells after 3 days of cultivation, and thereafter the concentration of conjugate increased abruptly. After 21 days cultivation, the content of the derivative in cells was about 50% of the pyridoxine added to the liquid medium. On prolonged cultivation (30 days), about 80% of pyridoxine added was converted into the derivative in cells. Very little of the derivative was present in the culture fluid at all stages of cultivation.

The derivative, after 21 days cultivation, was partially purified from the extracts of soybean calluses by gel filtration on Sephadex G-10 column and by band prep. PC with solvent A. The partially purified derivative was confirmed to be 5'-*O*-(β-glucosyl)pyridoxine as follows. Its R_f values were identical with those of an authentic specimen on paper in the solvent systems: *n*-BuOH satd with 0.2 M acetate buffer, pH 5.0, *n*-BuOH–HOAc–H_2O (4:1:5, v/v, upper layer), *iso*-PrOH–EtOH–H_2O (7:1:2, v/v) and solvent A. β-Glucosidase hydrolysed the derivative to give pyridoxine and glucose, while α-glucosidases had no effect. The UV absorption spectra of the derivative in 0.1 N HCl, 0.1 M Pi buffer, pH 7.0, and 0.1 N NaOH closely resembled those of pyridoxine.

Formation of two β-glucosylpyridoxines by rice calluses

Two β-glucosylpyridoxine derivatives were observed in rice calluses grown on a sucrose agar media with 10 mM pyridoxine at 25° in the dark. The fluctuation in their amounts is presented in Fig. 1. The calluses grown on the K medium with 10 mM pyridoxine contained the larger amounts of conjugates. The main metabolite was the 5'-glucoside, the minor one being the 4'-glucoside.

EXPERIMENTAL

The sterilized soybean seeds (*Glycine max* L. cv. Tamanishiki) and hulled rice seeds (*Oryza sativa* L. cv. Taichung 65, non-waxy) were put on the basal agar medium of Murashige and Skoog [4] containing 3% sucrose as carbon source and 2,4-dichlorophenoxy acetic acid (2,4-D) at a concn of 4.52 μM (for soybean

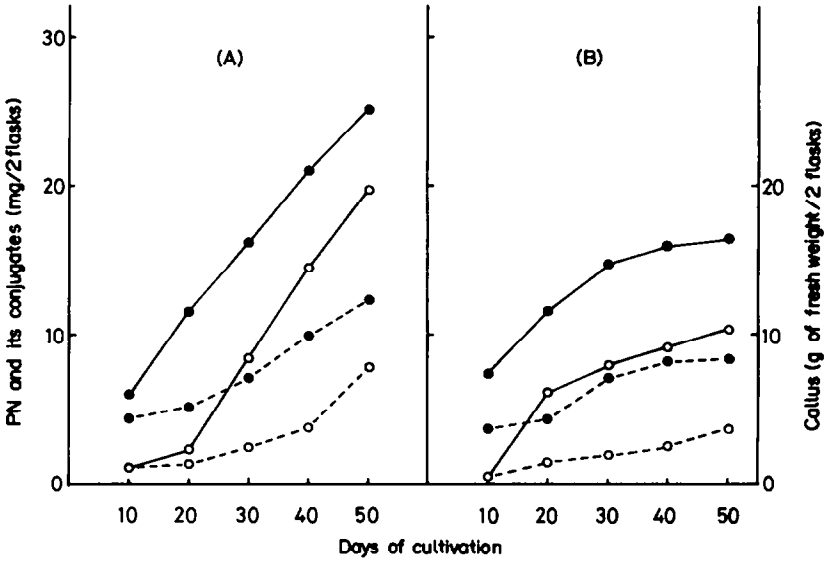

Fig. 1. Changes in contents of two β-glucosylpyridoxines in rice calluses grown on sucrose agar media with 10 mM pyridoxine. ●——● PN (pyridoxine); ○——○ 5′-*O*-(β-glucosyl)pyridoxine; ○ - - - ○ 4′-*O*-(β-glucosyl)pyridoxine; ● - - - ● callus. (A) K medium; (B) R-2 medium.

seeds) or 45.2 μM (for rice seeds). After about 3 weeks at 25° in the dark, the calluses induced from the germinating seeds of soybean and rice were transferred on the agar medium containing 3% sucrose and 2,4-D (4.52 μM for soybean callus; 45.2 μM for rice callus), and successively subcultured more than 20 times at intervals of 3 weeks, and then used for the present studies. A piece of the soybean callus was grown on 50 ml the agar medium containing 3% sucrose and 4.52 μM 2,4-D with or without 10 mM pyridoxine·HCl (neutralized to pH 5.7 by NaOH) in 100 ml flasks at 25° in the dark. After subculture twice on each of the slightly modified K and R-2 media [5, 6], the rice callus was grown on each 50 ml of the media with or without 10 mM pyridoxine at 25° in the dark. The compositions of these media were as follows. The modified K medium contained 30 g sucrose, 5 g casamino acid, 1.65 g NH_4NO_3, 1.9 g KNO_3, 0.44 g $CaCl_2 \cdot 2H_2O$, 0.37 g $MgSO_4 \cdot 7H_2O$, 0.17 g KH_2PO_4, 6.2 mg H_3BO_3, 22.3 mg $MnSO_4 \cdot H_2O$, 10.6 mg $ZnSO_4 \cdot 7H_2O$, 0.83 mg KI, 0.25 mg $NaMoO_4 \cdot 2H_2O$, 55.7 mg $FeSO_4 \cdot 7H_2O$, 74.5 mg Na_2-EDTA, 25 μg $CuSO_4 \cdot 5H_2O$, 25 μg $CoCl_2 \cdot 6H_2O$, 0.2 g inositol, 1 mg thiamine·HCl, 0.5 mg pyridoxine·HCl, 0.5 mg nicotinic acid, 22.1 μg 2,4-D, 10.75 mg kinetin, and 8 g agar in 1 l. of distilled water, being adjusted to pH 5.7. The modified R-2 medium contained 20 g sucrose, 0.33 g $(NH_4)_2SO_4$, 4 g KNO_3, 0.312 g $NaH_2PO_4 \cdot 2H_2O$, 0.247 g $MgSO_4 \cdot 7H_2O$, 0.147 g $CaCl_2 \cdot 2H_2O$, 12.5 mg $FeSO_4 \cdot 7H_2O$, 16.7 mg Na_2-EDTA, 1.54 mg $MnSO_4 \cdot H_2O$, 2.2 mg $ZnSO_4 \cdot 7H_2O$, 2.86 mg H_3BO_3, 0.13 mg $NaMoO_4 \cdot 2H_2O$, 0.2 mg $CuSO_4 \cdot 5H_2O$, 1 mg thiamine·HCl, 0.1 mg pyridoxine·HCl, 0.1 mg nicotinic acid, 2 mg 2,4-D, and 8 g agar in 1 l. of distilled water, being adjusted to pH 5.7. At intervals of 3–4 days or 10 days, calluses on two or three flasks of each plant were harvested, heated for 15 min at 100° and homogenized with 2 vols 0.02 M acetate buffer, pH 4.8. The homogenate was again heated for 15 min at 100°. One vol. of EtOH was added to the homogenate and centrifuged. The supernatant was concd at 30° under red. pres. The concentrate was applied to PPC, using *n*-BuOH–pyridine–H_2O (6:4:3) (solvent A). The pyridoxine glucosides on PC were measured with diazotized *p*-aminoacetophenone [7]. Yeast α-glucosidase and almond β-glucosidase were commercial samples. The crystalline α-glucosidase from the mycelia of *Mucor javanicus* was prepared, as described earlier [8]. 4′-*O*-(β-Glucosyl)pyridoxine and 5′-*O*-(β-glucosyl)pyridoxine were obtained as faint yellow powders from the incubation mixture containing cellobiose and pyridoxine with wheat bran β-glucosidase [2], and as white powders from the extracts of the germinating soybean seeds cultured on a pyridoxine soln [3], respectively.

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