タケノコ組織中へのL-フェニルアラニン-U-14C 及びL-チロシン-U-14C のトレーサー実験

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Tracer Experiment Administering L-Phenylalaine-U-^{14}C and L-Tyrosine-U-^{14}C to the Tissue Slices of Bamboo Shoots[1]

Etsuko KOZUKUE

Department of Home Economics, Kenmei Junior College, Honmachi, Himeji, Hyogo 670

Susumu MIZUNO

Faculty of Agriculture, Kobe University, Rokkodai, Nada, Kobe 657

Summary

Uniformly ^{14}C-labeled L-phenylalanine and L-tyrosine were administered to tissue slices of both top and base sections of bamboo shoots. Alcohol soluble substances were extracted and then separated into organic acid, sugar and amino acid fractions by ion exchange chromatography. The homogentisic acid fraction among the organic acids was collected by high-performance liquid chromatography (HPLC) and its radioactivity was measured, while the alcohol insoluble residue was used for the analysis of lignin aldehyde by the method of alkaline nitrobenzene oxidation.

1. The two labeled amino acids were steadily incorporated into the tissues during incubation and rapidly converted to organic acid, sugar and alcohol insoluble residue, especially the latter.
2. On determining the amount of phenylalanine converted to tyrosine, it was found that this was extremely small.
3. The incorporation of phenylalanine-U-^{14}C into alcohol insoluble residue was higher than that of tyrosine in both sections.
4. Although the conversion into lignin aldehyde from phenylalanine-U-^{14}C was higher than that from tyrosine-U-^{14}C, it was found that tyrosine incorporated into the shoots was converted to a remarkable extent for formation of lignin aldehyde.
5. The incorporation of phenylalanine and tyrosine into homogentisic acid was very low.

From these results, we assume that the conversion of phenylalanine to tyrosine or of tyrosine to homogentisic acid is very small, and that a part of the high amount of tyrosine in the shoots may be used for formation of lignin.

Introduction

In Japan, bamboo shoots are widely used as food. However there are only a few reports on the nutritional value of the shoots(2, 3, 11), and no report dealing with physiological or chemical studies on bamboo shoots has been published. In the previous paper(6, 7), we described the content of individual lipids, organic acids, sugars and amino acids in four different sections of bamboo shoots ; moreover, it has been conclusively demonstrated by gas chromatography-mass spectrometry that tyrosine, the main amino acid in the shoots, is the major component of the insoluble white clumpy substances produced rapidly on the nodal diaphragms and in the culm tissues after blanching of bamboo shoots.

In view of the high levels of tyrosine in the amino acids of the shoots, the present study was carried out to investigate the cause of this by tracer experiments involving administeration of phenylalanine-U-^{14}C and tyrosine-U-^{14}C to the tissues of bamboo shoots.

[1] Received for publication July 29, 1986.

Materials and Methods

Materials

Bamboo shoots (*Phyllostachys pubescens*) were obtained from a farmer in Oichi, Himeji City, Hyogo Prefecture. Plants were harvested at the proper time, and samples taken were about 32 cm in height and about 1,800 g in weight. Bamboo shoots were peeled and divided into four section from the top (A) to the base (D) as reported in the previous paper(6). For the present experiments both A and D sections were used.

Methods

1. *Phenylalanine-U-^{14}C and tyrosine-U-^{14}C feeding experiment*

^{14}C-feeding experiments were performed in tightly closed 50-ml glass tubes (3 cm I. D. × 15 cm) by incubating 2 g of sliced tissues (0.8 mm in thickness) from each section in 8 ml of 0.05 M phosphate buffer (pH=6.5) containing 4 μCi of uniformly labeled ^{14}C-phenylalanine and ^{14}C-tyrosine (New England Nuclear Corporation). In each test tube, a small vial containing 2 ml of a mixed solution of monoethanolamine and ethylcellosolve (3 : 1, v/v) was placed to trap the CO_2. During the incubation period, the test tubes were shaken gently in an incubator at 30°C. The incubation periods were 3, 6 and 9 hours. Each combination of treatments was tested in duplicate. At the end of each incubation period, the radioactivity of $^{14}CO_2$ was counted in 16 ml of toluene scintillator [6 g of PPO (2, 5-diphenyloxazole) and 0.5 g of POPOP (1, 4-bis-2-phenyloxazolebenzene) in 667 ml of toluene plus 333 ml of Triton X-100] (14). The radioactivity of $^{14}CO_2$ was measured using a Packard Tri-Carb scintillation spectrometer (Model-460). Each radioactivity value presented in the results is the mean of duplicate determinations. In addition to these procedures, the solution in each test tube was filtered through cheesecloth. The tissues were rinsed thoroughly with distilled water, blotted on filter paper and transferred to a 100-ml Erlenmeyer flask fitted with a reflux condenser to which 20 ml of 100% hot methyl alcohol was added and then boiled for 5 min to stop enzymatic activity.

2. *Extractions and fractionations*

The tissues were macerated in a glass mortar and centrifuged at 13,000 rpm for 10 min at 1°C. The pellet was re-extracted four times with 10 ml of 70% methyl alcohol and then centrifuged. The alcohol extracts were combined and evaporated to dryness at 35°C in a rotary evaporator, and then dissolved in 25 ml of 50% methyl alcohol. A 10 ml portion of the alcohol solutions was separated into organic acid, amino acid and carbohydrate fractions using ion exchange chromatography as reported in the previous paper(8). Individual fractions were evaporated to dryness and re-dissolved in 5 ml of 50% methyl alcohol. A 1 ml portion of this solution was used for the determination of the total radioactivity of individual fractions.

3. *Homogentisic acid, phenylalanine and tyrosine determinations*

For the determination of homogentisic acid administered in the organic acid fraction, a Hitachi high performance liquid chromatograph Model-635 equipped with a sampling valve with a 50 μl loop sample and an ultraviolet detector (210 nm) was employed. Homogentisic acid was separated with a 5 mm (I. D.) × 50 cm glass column packed with a strong cation resin (DIA ION, 10 μm, Mitsubishi Kasei Kogyo, Ltd. Tokyo, Japan) maintained at 50°C. The mobile phase was 0.2% H_3PO_4 solution at a flow rate of 0.8 ml/min. For the determination of administered tyrosine and phenylalanine, ion exchange chromatography using a high speed amino acid analyzer of our own making was used(9). Each component corresponding to homogentisic acid by HPLC and both tyrosine and phenylalanine by amino acid analyzer was collected, and then individual radioactivity levels were measured.

4. *Analysis of lignin aldehyde*

The alcohol insoluble residue was dried over silica gel in a desiccator. After being dried, it was weighed and the content of half the weight was used for the determination of the radioactivity of lignin aldehyde.

For the analysis of lignin aldehyde, alkaline nitrobenzene oxidation was carried out using the method of Stone and Blundell(13).

To 20 mg of dried alcohol insoluble residue, 0.1 ml of nitrobenzene and 3 ml of 2 N NaOH were added in a stainless steel bomb. After the mixture had been heated at 150–160°C for 2 hours, the bomb was cooled, and the mixture was centrifuged at 18,000 rpm for 10 min to separate the cell debris. Two ml of the supernatant was transferred to a test tube and repeatedly extracted with 300–350 ml of ethyl ether. The extracts were discarded and the aqueous layer was acidified to pH= 2.0 with 2 N HCl, and then repeatedly extracted with 100 ml of ethyl ether. This ether extract was dried and then dissolved in toluene scintillator for the determination of total lignin aldehyde.

Results

1. *Incorporation and distribution of phenylalanine-U-^{14}C and tyrosine-U-^{14}C into various fractions*

Figure 1 shows the determination of dpm of individual fractions incorporated into 2 g of both the top (A) and the base (D) sections of the shoots during incubations of 3, 6 and 9 hours after the application of L-phenylalanine-U-^{14}C and L-tyrosine-U-^{14}C. From the results of Fig. 1, it was found that the fed L-phenylalanine-U-^{14}C and L-tyrosine-U-^{14}C were rapidly translocated into the tissues of both A and D sections of the shoots and that the level of their radioactivity increased with the development of incubation time ; in phenylalanine administered into section A, the total radioactivity after 3 hours of incubation was 718×10^3 dpm/2 g fresh weight, and increased to 917×10^3 dpm and 1408×10^3 dpm after 6 and 9 hours, respectively, whereas in section D, the total activity showed the same tendency as that of section A, although the amount was somewhat lower than that of section A. While the total radioactivity of tyrosine administered into both A and D sections was approximately 36–72% that of phenylalanine, no significant difference between A and D sections was found. Two amino acids, once incorporated into the tissues, were steadily transferred to the alcohol-insoluble fraction during the incubation, but the incorporation of ^{14}C into the carbon dioxide, organic acid and carbohydrate fractions remained very low in both sections. From these results, it was found that both of the two amino acids were poorly metabolized to organic acid and carbohydrate.

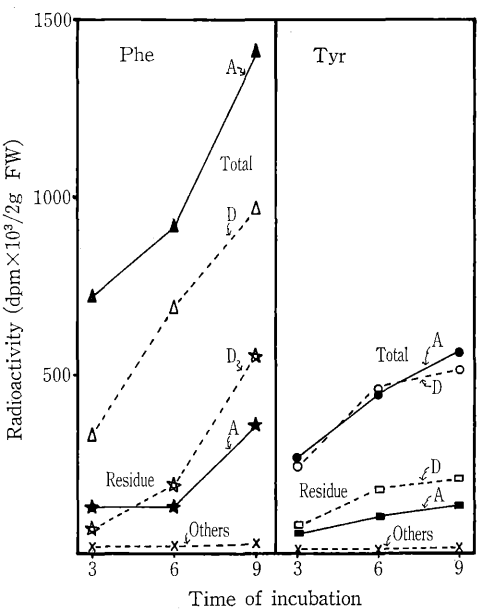

Fig. 1. Incorporation of phenylalanine-U-^{14}C and tyrosine-U-^{14}C into various fractions.
Residue : alcohol insoluble residue.
Others : the sums of organic acids, sugars and CO_2.
A : A sections. D : D sections.

2. *Conversion of phenylalanine to tyrosine and of tyrosine to phenylalanine*

In order to investigated the conversion of phenylalanine to tyrosine and of tyrosine to phenylalanine, L-phenylalanine-U-^{14}C and L-tyrosine-U-^{14}C were administered into the tissues of both A and D sections, and the radioactivity of tyrosine converted from phenylalanine and of phenylalanine to tyro-

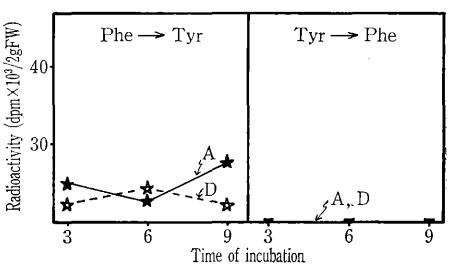

Fig. 2. Conversion of phenylalanine to tyrosine and of tyrosine to phenylalanine.
A : A sections. D : D sections.

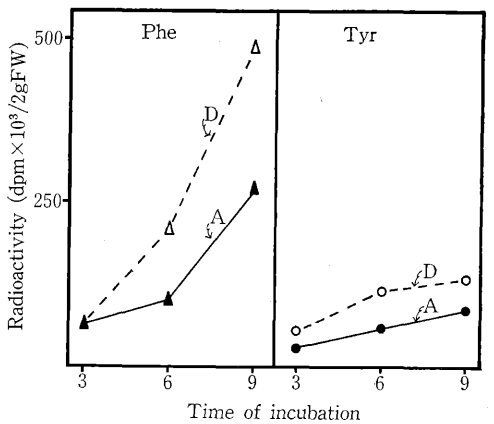

Fig. 3. ^{14}C-lignin aldehyde converted from phenylalanine-U-^{14}C and tyrosine-U-^{14}C.
A : A sections. D : D sections.

sine was measured (Fig. 2). As shown in Fig. 2, conversion of phenylalanine to tyrosine was slightly observed in both A and D sections, the activity in section A being a little higher than that in section D, but no conversion of tyrosine to phenylalanine was found.

3. *^{14}C-lignin aldehyde converted from phenylalanine-U-^{14}C and tyrosine-U-^{14}C*

Lignin aldehyde in the alcohol-insoluble residue was determined by the alkaline nitrobenzene oxidation method of Stone and Blundell(13). Figure 3 shows the total radioactivity count of lignin aldehyde contained in 2 g of both A and D sections. The radioactivity due to phenylalanine in both A and D sections increased during the incubation ; in addition, the activity in D section was appreciably higher than that in section A, while the activity due to tyrosine showed the same tendency as that for phenylalanine, although the activity for tyrosine was low in comparison with that for phenylalanine.

4. *^{14}C-homogentisic acid from phenylalanine-U-^{14}C and tyrosine-U-^{14}C*

HPLC chromatogram obtained from a standard homogentisic acid sample (8 μg) plus the organic acid fraction (50 μl) is presented in Fig. 4. By HPLC analysis, the peak corresponding to the standard homogentisic acid sample was collected, and then the radioactivity was measured. Figure 5 shows the radioactivity of ^{14}C-homogentisic acid con-

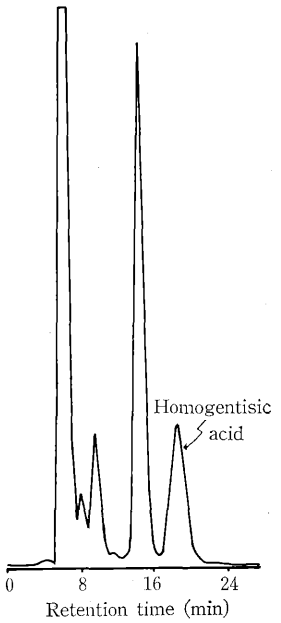

Fig. 4. HPLC chromatogram obtained from the organic acid of bamboo shoots containing a standard homogentisic acid.
Operating conditions : Column : strong cation resin. Detector : UV at 210 nm and 0.32 aufs. Flow rate : 0.8 ml/min. Column temperature : 50°C. Mobile phase : 0.2% H_3PO_4. Chart speed : 2.5 mm/min.

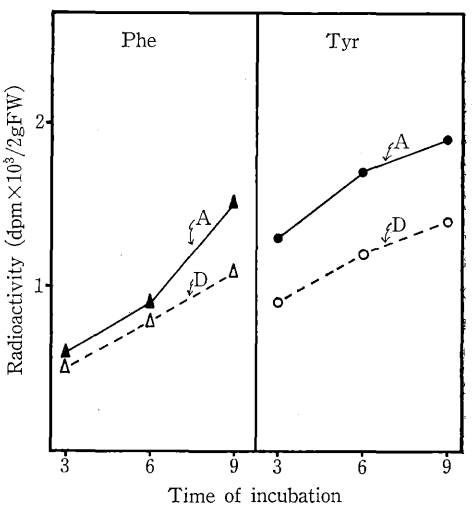

Fig. 5. ^{14}C-homogentisic acid converted from phenylalanine-U-^{14}C and tyrosine-U-^{14}C.
A : A sections. D : D sections.

verted from the two labeled amino acids in both A and D sections during the incubation. It was found that ^{14}C-homogentisic acid from phenylalanine increased in parallel in both A

and D sections during the incubation. On the other hand, the activity from tyrosine increased linearly during the incubation, but the activity in both A and D sections was slightly higher than that from phenylalanine.

Discussion

In this study, as a high tyrosine concentration had been noted in bamboo shoots, we applied labeled L-phenylalanine-U-^{14}C and L-tyrosine-U-^{14}C into the tissues of both the top (A) and the base (D) sections of the shoots, with the aim of investigation the pathway by which their ^{14}C was metabolized, in connection with organic acid, carbohydrate, amino acid fractions, carbon dioxide and lignin aldehyde, after incubations of 3, 6 and 9 hours at 30°C.

As shown in Fig. 1, the radioactivity of ^{14}C from the two labeled amino acids incorporated into both A and D sections increased with the progress of incubation time. The amount of ^{14}C converted from tyrosine was reduced from one third to three quarters of that from phenylalanine. Furthermore, the activiy incorporated into section A from phenylalanine was somewhat higher than that in D section, but for tyrosine no significant difference between A and D sections was found. Although the reason for the amount of total radioactivity (Fig. 1) incorporated into the tissues of bamboo shoots being considerably different from that of phenylalanine and tyrosine is not clear, we assume that, due to the high tyrosine level in the shoots, the penetration of tyrosine into the tissues may have been repressed.

^{14}C incorporated into the tissues of both A and D sections from the two labeled amino acids was converted rapidly to alcohol insoluble residue. Thus, after 3 hours of incubation nearly 20-30% of the total radioactivity of the two amino acids administered to the tissues was transferred to the residue, and the amount of ^{14}C incorporated increased during the incubation. After 9 hours the activity of the residue converted from phenylalanine reached 25% of the total activity in section A and 57% in D section, respectively, while 24% of the total activity in section A and 40% in section D from tyrosine was converted to the residue.

It is widely known that phenylalanine or tyrosine is a precursor of lignin formation (5). In this study, it was found that phenylalanine-U-^{14}C or tyrosine-U-^{14}C was incorporated efficiently into lignin aldehyde, an oxidation product of lignin (Fig. 3). The amount of ^{14}C incorporated into lignin aldehyde from phenylalanine was high with a range nearly 4 times greater in section A and 8 times greater in section D in comparison with those of tyrosine, indicating that lignification from phenylalanine is active. Although lignification from tyrosine is low in quantity, nearly 15% in section A and 26% in section D of the total radioactivity from tyrosine-U-^{14}C incorporated into the tissues after 9 hours of incubation proceeded to lignin aldehyde. It was therefore found that an appreciable proportion of tyrosin-U-^{14}C was also used for the production of lignin. Conversely, in A and D sections, it was found that ^{14}C incorporated into lignin aldehyde from both the two amino acids was much greater in section D than that in section A. This was coincident with the fact that a high lignin content was found in section D (unpublished data).

Bamboo shoots contain homogentisic acid which has a disagreeable pungent taste known as "egumi" in Japan(1), and the levels of homogentisic acid depend on the variety of cultivar, state of maturity, harvesting time, conditions of planting and the place of production (unpublished data). As shown in Fig. 5, a slight conversion of phenylalanine-U-^{14}C or tyrosine-U-^{14}C into homogentisic acid was found (i. e., the conversion ratio of each of the two labeled amino acids into homogentisic acid was below 0.5% of the total radioactivity). In fact, from the results of the determination of homogentisic acid by HPLC analysis (unpublished data), it was found that a small amount was contained in the shoots. Furthermore, we ate the shoots after blanching, and were unable to detect any pungent taste ; therefore, we supposed that the activity of metabolism of tyrosine to homogentisic acid was fairly low in

the shoots.

As an important part of the biosynthetic pathway of tyrosine, phenylalanine hydroxylase, which catalyzes the formation of tyrosine from phenylalanine, is known to exist in animals (4, 10) as well as in plants (12). From the conversion of phenylalanine-U-^{14}C to tyrosine (Fig. 2), it was found that the activity of this conversion was much lower in the shoots. Therefore, we assumed that a slight amount of tyrosine, via phenylalanine, was formed in bamboo shoots.

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タケノコ組織中への L-フェニルアラニン-U-^{14}C 及び L-チロシン-U-^{14}C のトレーサー実験

小 机 ゑつ子
賢明女子学院短期大学 670 姫路市本町

水 野 進
神戸大学農学部 657 神戸市灘区六甲台町

摘 要

本実験はタケノコ組織中に多量のチロシンが存在し，しかも湯煮後の白濁現象の主因となることから，この蓄積要因を追求すべく，L-フェニルアラニン-U-^{14}C 及び L-チロシン-U-^{14}C を組織にとりこませ，^{14}C の移行の追跡実験を行ない，蓄積機構についての新しい知見を見出したので報告する．

まず，剝皮したタケノコの先端部及び基底部の各組織切片に L-フェニルアラニン-U-^{14}C ならびに L-チロシン-U-^{14}C を 30℃ で，3，6，9 時間反応させたのち，アルコール可溶性抽出部とアルコール不溶性残渣部とに分けた．さらにアルコール可溶性抽出部は，糖，有機酸，アミノ酸に画分した．有機酸画分の中のホモゲンチジン酸は HPLC により，又アミノ酸画分中のフェニルアラニン及びチロシンはアミノ酸分析器により得た．

一方，リグニンアルデヒドはアルコール不溶性残渣部をアルカリニトロベンゼンで分解することにより得た．

① 両アミノ酸はすみやかに組織にとりこまれ，有機酸，糖画分及びアルコール不溶性残渣部へ移行したが，有機酸及び糖画分への活性は低かった．しかしアルコール不溶性残渣部へは 9 時間後に全活性の 24～57% と非常に高い値となった．

② リグニンアルデヒドについては，フェニルアラニン及びチロシンからきわめてすみやかにとりこまれることが確認された．

③ フェニルアラニンからチロシンへの転換はきわめて低く，チロシンからフェニルアラニンへの転換は全く認められなかった．

④ 両アミノ酸からホモゲンチジン酸への転換はほとんど行なわれていないものと推測した．