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SHORT COMMUNICATION

FORMATION OF *N*-CARBAMYLPUTRESCINE FROM CITRULLINE IN *SESAMUM*

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Abstract—The enzymic decarboxylation of citrulline has been demonstrated by production of ^{14}C-*N*-carbamylputrescine (NCP) from [carbamyl-^{14}C]-L-citrulline using extracts of leaves of K-deficient plants of *Sesamum indicum*. The levels of putrescine, agmatine and NCP were also determined in these leaves.

INTRODUCTION

IT WAS established by Smith[1] that agmatine and putrescine concentrations are elevated in several plant species growing under conditions of potassium stress. No activity was found when the arginine carboxy-lyase preparations of barley plants were incubated with other amino acids than arginine, such as citrulline. When agmatine was fed to barley seedlings *N*-carbamylputrescine (NCP) was identified as an intermediate of putrescine formation.[2] Yoshida and Mitake[3] showed that the pyrrolidine ring of nicotine in tobacco roots can be formed from agmatine, NCP or putrescine. In our laboratory we were able to detect putrescine,[4] agmatine and NCP[5] in the leaves of plants of *Sesamum indicum* L. growing in potassium-deficiency conditions. We also investigated the metabolism of [guanidine-^{14}C]-arginine injected in the stems of 2-month-old *S. indicum* plants maintained in K-deficient water nutrient solution showing symptoms of K-deficiency. Radioactivity was detected in agmatine, urea and NCP.[5] Recently, Maretski *et al.*[6] observed that sugar-cane cells supplied with ^{14}C-arginine convert an appreciable amount of this amino acid to NCP. Moreover they also found that this amine is formed more readily from citrulline than from arginine in these cell cultures.

RESULTS

We have investigated plants of *Sesamum indicum* for citrulline decarboxylase activity. Attempts to demonstrate this enzyme in very young seedlings (up to 10 days old) were unsuccessful, even in those seedlings allowed to germinate in K-deficient nutrient solution.

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[1] T. A. SMITH, *Phytochem.* **2**, 241 (1963).
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[3] D. YOSHIDA and T. MITAKE, *Plant Cell Physiol.* **7**, 301 (1966).
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However, this enzyme was found in concentrated extracts from old plants, using [carbamyl-^{14}C]-L-citrulline as substrate. Enzyme preparations were obtained by adding ammonium sulphate (to 72 per cent saturation) to cell-free extracts of 2-month-old normal and K-deficient plants. After the incubation periods of 24 and 48 hr, the enzyme reaction was stopped by adding ethanol and the soluble material was fractionated on Dowex 50 resin (H^+). The amines were cochromatographed with authentic NCP and subsequent radioautography demonstrated an exact coincidence of the radioactive and ninhydrin-positive areas of NCP in the extracts of K-deficient plants. Agmatine, which can be eluted with ammonium carbonate, was not detected on the radioautographs. No labelled NCP was detected on incubating the extract of normal plants with [carbamyl-^{14}C]-L-citrulline.

No NCP was found on incubating boiled enzyme preparation from leaves of normal and K-deficient plants with the labelled substrate.

In a parallel assay, leaves of 2-month-old *Sesamum* plants growing under the same conditions as above were harvested and the amines putrescine, agmatine and NCP were determined in the extracts. We were able to detect all these amines only in the extracts of K-deficient plants, putrescine being the amine having the highest concentration (45 μg/g fresh weight normal plants and 350 μg/g fresh weight K-deficient plants), and NCP having the lowest concentration (75 μg/g fresh weight K-deficient plants). This seems to be the first report of NCP in plants using no added precursor. Agmatine, the product of decarboxylation of arginine, was present in medium concentration (100 μg/g fresh weight K-deficient plants). Only putrescine was detected in the extracts of normal plants. The level of putrescine in K-deficient *Sesamum* leaves increased during the period from 1 to 8 weeks after germination (40 μg to 350 μg/g fresh weight), while in the normal plants the level of that amine remained constant and was very low (40 μg to 50 μg/g fresh weight).

EXPERIMENTAL

Chemicals

[Carbamyl-^{14}C]-L-citrulline (29·2 mc/mM) was obtained from the Radiochemical Centre, Amersham, and purified by paper chromatography; the citrulline zone on the paper was identified in the Actigraph II, model 1025, Nuclear-Chicago. *N*-Carbamylputrescine hydrochloride was prepared from putrescine dihydrochloride following the method of Smith and Garraway[2] and was identical with an authentic sample of *N*-carbamylputrescine hydrochloride. Putrescine dihydrochloride, from Nutritional Biochemicals Corporation, U.S.A., and agmatine sulphate, from Sigma Chemical Co., U.S.A., were purified by paper chromatography.

Plant Material

Sesamum indicum L. seeds were soaked overnight and planted in moist quartz at 28°. After 3 days in the dark the germinated seedlings were illuminated and irrigated with the complete nutrient solution of Hoagland and Arnon[7] diluted to 10%. After 7 days, when the seedlings were 5-cm long, they were placed in water culture, with full nutrient solution: some seedlings were maintained in complete nutrient solution and some were in K-deficient nutrient solution. After 2 months in the greenhouse (about 25°), when the necrotic areas in the leaves of the K-deficient plants were very large, the leaves from plants in both treatments were harvested and extracted. The experiments were made in June 1969 and repeated in August of the same year.

Amine Determination

A known weight of leaves was cut in small pieces and the cells were ruptured in an Omni-Mixer homogenizer, Sorvall, 60 sec, at 16,000 rpm, with 80% ethanol (about 10 ml/g fresh wt.). The extract was filtered through glass wool and the pigments were extracted into CCl_4. The alcohol-soluble material was then fractionated on a column (1 × 5 cm) of Dowex 50 × 8 resin (H^+), 200–400 mesh.

After elution of the amino acids (with 50 ml of 0·4 N NH_4OH), putrescine and NCP were eluted with 40 ml 5 N NH_4OH, and agmatine was subsequently eluted with 30 ml of a sat. aqu. $(NH_4)_2CO_3$. The first

[7] D. HOAGLAND and D. I. ARNON, *Calif. Agr. Expt. Sta., Berkeley, Calif., Circ.* **347**, 31 (1950).

amine fraction was evaporated in a stream of purified air, at 45°, and taken up in 1 ml of 0·05 N HCl. The fraction containing the agmatine was evaporated at 100°, and taken up in 1 ml of 0·05 N H_2SO_4. The three amines were quantitatively determined after separation by paper chromatography. The percentage recovery of known amounts of the three amines were: putrescine and NCP 85% and agmatine, 70%. Agmatine was determined by the method of Sakaguchi as modified by Smith[1] and the other amines by the ninhydrin reaction.[4] NCP was also identified by spraying the chromatograms with *p*-dimethylaminobenzaldehyde (0·1% in N HCl).[8]

Preparation of Enzyme Extracts

The procedure used by Crocomo and Fowden[9] was followed. Washed normal and K-deficient *Sesamum* leaves were ground at 0° in water (about 3 ml/g fresh wt.). The macerate was pressed through a fine cloth and the extract was centrifuged at 30,000 *g* for 15 min at 0°. $(NH_4)_2SO_4$ (5 g/10 ml) was added slowly to the supernatant at 0° and the solution was centrifuged at 16,000 *g* for 15 min at 0°. The precipitate was redissolved in cold distilled water. An aliquot was taken for the colorimetric determination of protein.[10] A portion of the suspension was boiled to give a control value.

Decarboxylase Assay

The ^{14}C-*N*-carbamylputrescine production from ^{14}C-citrulline was studied in reaction mixtures with 2 ml enzyme preparation (containing about 10 mg protein in the case of K-deficient plants, and 19 mg protein in the case of normal plants), 0·5 ml of 0·1 M citric acid and 0·2 M Na_2HPO_4 buffer, pH 6·5, and 0·5 ml [^{14}C carbamyl]-L-citrulline (2·5 μc). The reaction mixtures were placed in test tubes; toluene (1 ml) was added and the tubes were sealed with rubber stoppers. The tubes were incubated at 25° for periods of 24 and 48 hr. After the period of incubation, the reaction was stopped by the addition of ethanol to give a final concentration of 80%. The solution obtained on centrifuging was passed through a Dowex resin, as above. The NCP fraction was transferred, together with authentic carrier *N*-carbamylputrescine hydrochloride, to a 3MM Whatman paper chromatogram and run in *n*-BuOH–EtCOMe–NH_3–H_2O (5:3:1:1, v/v). The chromatograms were dried and the development process was twice repeated.[11] Subsequent radioautographs were prepared to ensure exact coincidence of radioactive and ninhydrin or *p*-dimethylaminobenzaldehyde positive areas.

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