0021-9193/78/0133-0409$02.00/0
JOURNAL OF BACTERIOLOGY, Jan. 1978, p. 409–410
Vol. 133, No. 1
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Printed in U.S.A.

Formation of *N*-Carbamyl Putrescine from Citrulline in *Escherichia coli*

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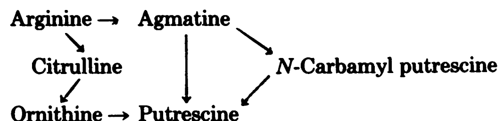
Received for publication 23 August 1977

Decarboxylation of citrulline by *Escherichia coli* enzyme was presented. The *N*-carbamyl putrescine produced showed the same properties as those of synthesized authentic samples in column chromatography, paper chromatography, and paper electrophoresis.

In bacteria arginine is decomposed to putrescine via citrulline and ornithine. On the other hand, arginine is decarboxylated to agmatine, which forms putrescine directly or via *N*-carbamyl putrescine. These pathways are summarized as follows (4, 6, 7):



Carbamylation of putrescine has been reported in *Streptococcus faecalis* (5, 7).

In our laboratory, decarboxylation of citrulline was observed in *Escherichia coli*, and the product was identified as *N*-carbamyl putrescine.

Authentic *N*-carbamyl putrescine hydrochloride was prepared by carbamylation of putrescine. Putrescine (9 g), dissolved in water (50 ml), was adjusted to pH 11 with concentrated HCl. Nitrourea (11 g) was added in small portions with stirring, and the reaction was kept at 50 to 60°C until bubbling ceased. After standing overnight at room temperature, the reaction mixture (pH 7) was adjusted to pH 4 with concentrated HCl and applied to an amberlite XE-64 Na^+ column (2.2 by 30 cm). The column was washed with water (450 ml, fraction I) and eluted with 0.2 M acetic acid (700 ml, fraction II) and 0.5 M acetic acid (500 ml, fraction III) successively. Fraction II was evaporated to dryness, dissolved in ethanol, and filtered. The filtrate was concentrated at 30°C, and white needles produced were recrystallized from water (yield, 2 g; mp, 186°C uncorrected). The melting point coincided with that reported by previous workers (5). The purity was checked by Kjeldahl N estimation. Ascending paper chromatography was carried out with solvent systems of *n*-butanol-acetic acid-water (4:1:5 by volume) and phenol saturated with water for 18 h. *N*-Carbamyl putrescine gave single spot (R_f of 0.36 and 0.78, respectively) both with ninhydrin reaction and *p*-dimethylaminobenzaldehyde reaction (1).

L-[*carbamyl*-^{14}C]citrulline (61 mCi/mmol; Radiochemical Centre, Amersham, England) was diluted with unlabeled L-citrulline to a specific activity of 1.25 μCi/μmol. Two milliliters of the labeled compound was applied to an amberlite XE-64 Na^+ column (0.8 by 8 cm). The column was washed with water (20 ml), and the washings were evaporated to dryness at 30°C. Water (2 ml) was added to the residue. The sample was spotted on Whatman no. 3 MM paper and subjected to paper electrophoresis in 8% formic acid at 25 V/cm for 1 h (8). A single peak of radioactivity, detected by Paper Radiochromatography Systems, Actigraph III (Nuclear-Chicago, Des Plaines, Ill.), was corresponded to L-citrulline.

E. coli IAM 1101 was inoculated on the agar medium containing 2% glucose, 3% Casitone, and 1% yeast extract, pH 6.0. After cultivation overnight, the cells were harvested by centrifugation, washed twice with 0.9% NaCl, and suspended in 6 volumes of 5 mM potassium phosphate buffer (pH 7.5) containing 0.5 mM dithiothreitol and 1 mM ethylenediaminetetraacetic acid. The bacteria were disrupted (20 kHz, 200 W) in a UR-200P ultrasonic vibrator (Tomy Seiko Co., Tokyo) for 10 min at 0°C. The sonic extract was dialyzed against the same buffer for 20 h at 4°C and centrifuged at 20,000 × *g* for 20 min. The supernatant was used as enzyme solution. Reaction mixture contained 100 μmol of acetate buffer (pH 5.0), 1 μmol of ethylenediaminetetraacetic acid, 1 μmol of dithiothreitol, 2 μmol of pyridoxal phosphate, 1.99 μmol (0.5 μCi) of L-[*carbamyl*-^{14}C]citrulline (purified as above) and 0.3 ml of enzyme (protein amount, determined by the method of Lowry et al. [3] with egg albumin as a standard, was 0.99 mg) in 1.0 ml. Boiled enzyme was used in a control experiment.

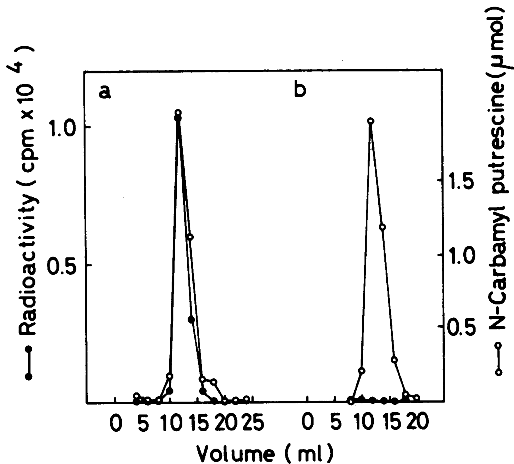


FIG. 1. *Column chromatography of enzymatic reaction product. (a) Complete system; (b) complete system except that boiled enzyme was used.*

This was incubated at 30°C for 2 h and stopped by boiling for 3 min. After adding 2 ml of water and 0.2 ml of 20 mM carbamyl putrescine, the denaturated protein was removed by centrifugation at 1,000 × *g* for 15 min. Resulting supernatant was applied to a column (0.8 by 8 cm) of amberlite XE-64 pyridine form (8). Citrulline was washed off the column with 30 ml of water. Elution was performed by 0.2 M acetic acid; fractions of 2 ml each were collected, and *N*-carbamyl putrescine was determined colorimetrically by the method of Koritz and Cohen (2). A portion was placed on a Toyo filter paper disk and assayed for radioactivity in 0.6% butyl-PBD in toluene with an LS-100 liquid scintillation system (Beckman Instruments, Fullerton, Calif.). The elution profile is shown in Fig. 1. Radioactive compound formed enzymatically coincided to authentic *N*-carbamyl putrescine. The radioactive fractions were combined, evaporated to dryness, and dissolved in a small volume of water. The sample was subjected to paper chromatography and paper electrophoresis. As was shown in Table 1, the radioactivity corresponded to *N*-carbamyl putrescine.

TABLE 1. *Paper chromatography and paper electrophoresis of N-carbamyl putrescine*

Compounds	R_f		Electrophoretic mobility[a] (cm)
	Solvent 1[b]	Solvent 2	
N-Carbamyl putrescine chemically synthesized	0.36	0.78	16.0
L-Citrulline	0.15	0.58	10.2
L-Ornithine hydrochloride	0.08	0.26	19.7
Putrescine dihydrochloride	0.11	0.30	30.5
L-Arginine	0.15	0.52	18.8
Agmatine sulfate	0.18	0.45	27.0
N-Carbamyl putrescine enzymatically synthesized (radioactive)	0.36	0.78	16.1

[a] Paper electrophoresis was carried out for 1 h at 25 V/cm on Whatman no. 3 MM paper with 8% formic acid (8). The compounds moved toward the cathode.

[b] The solvents were as follows: 1, *n*-butanol-acetic acid-water (4:1:5, by volume); 2, phenol saturated with water. R_f values were determined by ascending method with Toyo no. 51 paper, and spots were detected by ninhydrin reaction.

Studies on purification of the enzyme are in progress in this laboratory.

We wish to express our gratitude to the Institute of Applied Microbiology, Tokyo University, for supplying of *E. coli* strains.

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