CONVERSION OF 3-AMINO-1, 2, 4-TRIAZOLE INTO 3-AMINO-1, 2, 4-TRIAZOLYL ALANINE AND ITS INCORPORATION INTO PROTEIN BY ESCHERICHIA COLI

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Metabolites of the herbicide, 3-amino-1, 2, 4-triazole (3AT), have been detected in tissues of higher plants by several investigators. Most of these compounds have not been sufficiently characterized for identification. The accumulation of a 3AT metabolite in bean and tomato plants, however, was described by Massini (1963) and identified as β (3-amino-1, 2, 4-triazolyl-1-) α-alanine (3ATA). It was presumed by Massini that 3ATA was formed by coupling 3AT with serine. Since 3ATA was shown to be non-phytotoxic, the suggestion was made that formation of this metabolite represents the mechanism by which 3AT-poisoned plants recover.

Yeast inhibited by 3AT has been reported to accumulate imidazoleglycerol phosphate and to excrete its derivative, imidazoleglycerol, into the medium (Klopotowski and Hulanicka, 1963). The appearance of these compounds was simultaneously accompanied by

an extended lag phase during the growth of the microorganism. The inhibition was non-competitively reversed by L-histidine. The suggestion was made that 3AT inhibited imidazoleglycerol phosphate dehydrase and that this inhibition was reversed by histidine. The possibility that inhibition was due to a metabolite of 3AT rather than the parent compound was not explored.

When Escherichia coli was cultured in the presence of 3AT, an unidentified compound was observed to accumulate in the medium. This compound was found to react with ninhydrin and to contain a diazotizable amine as evidenced by the diazo-coupling assay for aryl amine determination according to the procedure of Bratton and Marshall (1939). These properties suggested that this compound might be 3ATA. Thus, experiments were carried out to isolate and to identify this compound as follows:

After culturing for 24 hr in a glucose-mineral salts medium containing 0.1% 3AT, cells of E. coli were removed by centrifugation. The supernatant was concentrated under reduced pressure and low temperature to one tenth of the original volume and filtered through Whatman No. 40 filter paper. Approximately 50 ml were adsorbed on a column (3.5 x 30 cm) of Amberlite IR-120 in the H^+-form and eluted with 0.5 N NH_4OH. All fractions containing diazotizable amines were pooled and concentrated to about 10 ml. Using the procedure employed by Massini (1963) to precipitate and purify 3ATA, a material was obtained from this eluate which possessed properties of 3ATA as follows: (1) Paper chromotography of this compound revealed a R_f identical to that reported for 3ATA by Massini (1963); and, (2) After hydrolysis for 4 hr at 120 C in 2 N KOH, paper chromotography

of the hydrolysate revealed a ninhydrin-reactive spot with a R_f identical to L-alanine. This compound could not be detected in cultures grown in the absence of 3AT.

Since 3ATA shows a structural similarity to histidine, the possibility that this substance functions as an analogue of histidine and subsequently is incorporated into protein was investigated. Preliminary experiments indicated that a compound with the characteristics of 3ATA listed above but not 3AT was detected by paper chromotography of the hydrolysate of a protein fraction isolated from E. coli grown in the presence of 3AT. The protein fraction, isolated by the method of Siekevitz (1952), was hydrolyzed for 18 hr at 120 C in 6 N HCl.

These results suggested that 3ATA was incorporated into cellular protein. This possibility was tested by allowing E. coli to incubate in the presence of 3-amino-1, 2, 4-triazole-5-C^{14} (^{14}C-3AT). After incubation for 60 min, the reactions were stopped by addition of trichloracetic acid and protein fractions were isolated according to the technique of Siekevitz (1952). The protein fractions were dissolved in 80% formic acid and aliquots were spotted on filter paper discs. After drying, radioactivity was determined by means of a Packard Tri-Carb liquid scintillation spectrometer.

Table 1 shows the results of these experiments. Reactions in which the ^{14}C-3AT was diluted with unlabelled 3AT (^{12}C-3AT) showed an increase in the amount of total 3AT (or 3ATA) incorporated into protein. Addition of amounts greater than 5 μmoles ^{12}C-3AT per 1 ml of reaction mixture, however, did not result in further increases of incorporation. The increase observed in the incorporation was presumably due to increased inhibition of histidine biosynthesis at higher 3AT con-

TABLE I

Incorporation of 3AT into cellular protein of Escherichia coli

System	mμmoles 3AT/mg protein
Complete	0.11
+CAP	0
+2.5 μmoles ^{12}C-3AT	1.13
+2.5 μmoles ^{12}C-3AT + CAP	0
+12.5 μmoles ^{12}C-3AT	6.04
+12.5 μmoles ^{12}C-3AT + CAP	0
+25.0 μmoles ^{12}C-3AT	6.85
+25.0 μmoles ^{12}C-3AT + CAP	0

Complete system: 1 ml cells suspended in 0.1 M tris(hydroxymethyl)aminomethane, pH 7.5 to give 5% light transmission at 550 mμ; 1 μC ^{14}C-3AT (specific activity = 3.8 μC/μmole); total volume, 2.5 ml. This system was incubated for 1 hr at 35 C and 200 μg CAP was used in each of those experiments indicated. Values were calculated from the specific activity of the isotope after addition of ^{12}C-3AT. Protein was estimated with the Folin reagent described by Lowry et al. (1951). See text for further details.

centrations. The latter has been suggested by Klopotowski and Hulanicka (1963) for yeasts. The addition of chloramphenicol (CAP) to the reactions resulted in a complete inhibition of incorporation. Repetition of these experiments provided data with closely parallel results between the experiments.

Using techniques previously described, paper chromatography of hydrolysates of the protein fractions showed that radioactivity was found only in spots having the characteristics of 3ATA.

Although not conclusive, these data suggest that 3AT (or 3ATA) competes with histidine for incorporation into protein. Experiments designed to further test this hypothesis are in progress.

In summary, E. coli was shown to convert the herbicide, 3-amino-

1, 2, 4-triazole, into a metabolite, 3-amino-1, 2, 4-triazolyl alanine. This latter compound, presumably an analogue of histidine, was in turn incorporated into cellular protein.

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