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NOTES

Amino Acid Antagonist Death in *Escherichia coli*

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Six analogues of amino acids killed corresponding auxotrophs of *Escherichia coli*. With all but one analogue, protein synthesis was required for lethality.

It has long been known that incorporation of structural analogues of amino acids into cellular proteins of microorganisms can contribute to the formation of partially active or inactive enzymes and inhibit growth (4, 8, 12, 15, 19). Only recently, however, incorporation of the arginine analogue, canavanine, has been shown to produce irreversible damage and death of the affected organism (14). We investigated this with several other amino acid antagonists and found that death following their incorporation may be a general phenomenon.

One analogue each of tryptophan and isoleucine and two of leucine and proline were found to kill corresponding auxotrophs of *Escherichia coli* in the absence of the corresponding metabolite. The structures of these analogues beside their corresponding natural metabolites are shown in Fig. 1.

Figure 2 shows the surviving fraction after 5 hr of incubation with various concentrations of amino acid antagonists, and Fig. 3 shows the time course of killing at one concentration of antagonist.

Several of the amino acid antagonists studied have been reported to be incorporated into protein of *E. coli*: azatryptophan (10), *O*-methylthreonine (17), azetidine carboxylic acid (5), and thiazolidine carboxylic acid (18). The leucine antagonists, methallylglycine (3) and azaleucine (16), have been shown to inhibit growth, but their incorporation into protein has not been demonstrated. To determine whether the incorporation of the antagonist into protein was a requisite for lethality, protein synthesis was inhibited with chloramphenicol (1). At a concentration of 50 μg/ml, this antibiotic completely protected cells

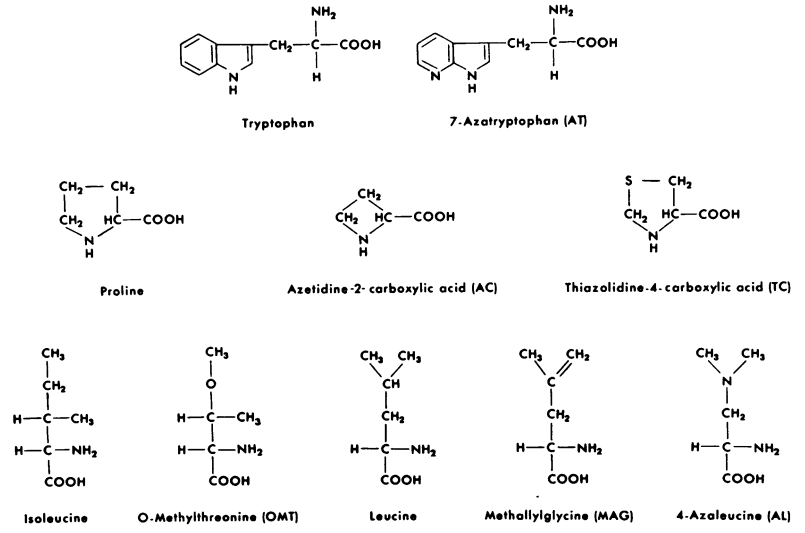

FIG. 1. *Amino acids and their analogues. Abbreviations in parentheses are used to designate individual analogues in Fig. 2-4. AC, TC, and OMT were of the* L*-configuration; AT, MAG, and AL were present as the racemates.*

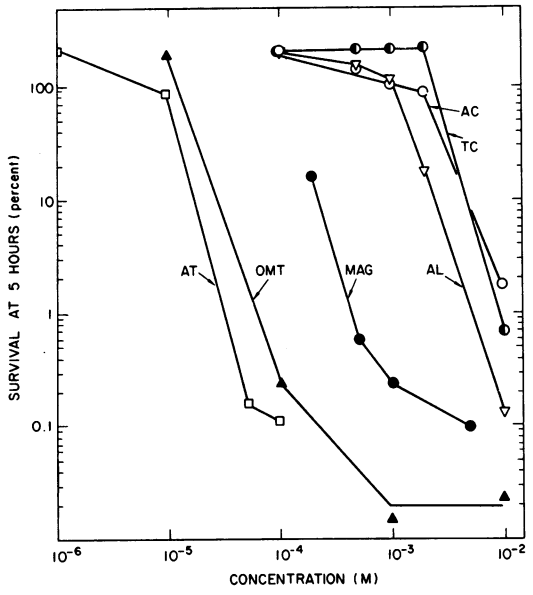

FIG. 2. *Viability of E. coli cultures after 5 hr of incubation in the presence of amino acid analogues. E. coli strains were grown overnight at 37 C in a New Brunswick gyratory shaker at maximal speed in fortified medium [medium A of Davis and Mingioli (2) supplemented with a mixture of amino acids, purines, pyrimidines, and vitamins (9) and containing 100 μM thymine and 5.74 μM L-arginine]. Cells from the overnight culture were suspended at a concentration of 2 × 10^8 cells per ml in fresh fortified medium and grown to a concentration of 6 × 10^8 cells per ml. These exponential-phase cells were harvested by centrifugation, washed twice with medium A minus glucose, resuspended in medium A, and transferred to Coleman round cuvettes (19 by 105 mm), containing fortified medium and the analogue in place of the natural amino acid, to give a final concentration of approximately 6 × 10^7 cells per ml and a final volume of 10 ml. Abbreviations for individual analogues are shown in Fig. 1. Viable bacteria were determined by diluting culture samples in medium A minus glucose and plating appropriate dilutions on Difco Nutrient Agar plates. E. coli WWU (obtained from R. C. Bockrath) requiring proline, tryptophan, arginine, methionine, thymine, and uracil was used for studies with the proline, tryptophan, and arginine antagonists. E. coli 70V3 isoleucine⁻, requiring isoleucine and thymine, was employed to study the action of O-methylthreonine; E. coli 70V3 leucine⁻, requiring leucine and thymine, was used to evaluate the action of the leucine antagonists.*

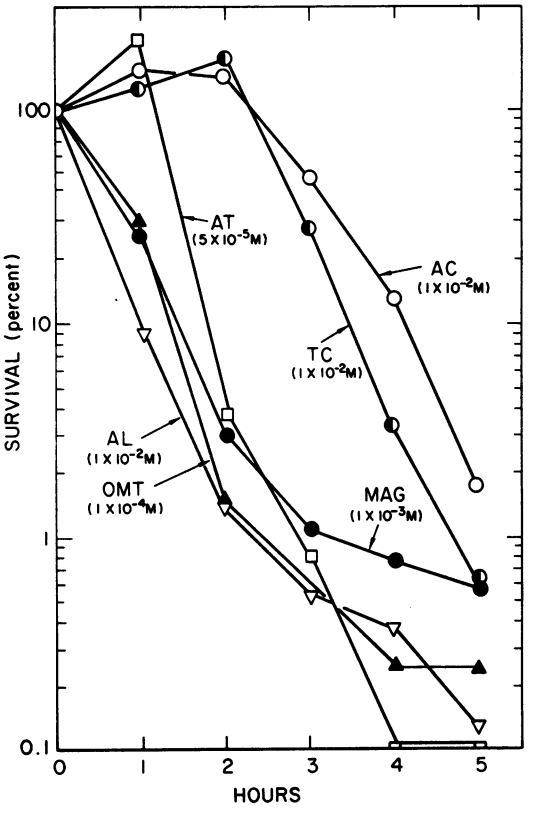

FIG. 3. *Loss of viability as a function of time. Experimental conditions are as described in Fig. 2.*

against the lethal effects of a 4-hr incubation with all of the antagonists except thiazolidine carboxylic acid. In the multiple amino acid auxotroph of *E. coli*, strain WWU, inhibition of protein synthesis by omission of proline and arginine prevented the lethal effect of azatryptophan, and omission of tryptophan and arginine prevented the lethal effect of azetidine carboxylic acid but not that of thiazolidine carboxylic acid.

Examination of thin sections of cells killed by exposure to analogues indicated some marked changes in morphology compared to the control (Fig. 4, −TRP). There was a general disarray of the nuclear region as had been shown for canavanine-induced death by Schachtele et al. (13). These authors also reported the presence of dense canavanine bodies which were attached to the cell membrane. Our observations confirm their results (Fig. 4, CANAV.). Cells killed by incorporation of several of the other analogues exhibited similar morphology. Symmetrical and asymmetrical plasmolysis were characteristics of cells treated with thiazolidine carboxylic acid and *O*-methylthreonine, respectively. The former analogue also induced the production of giant cells and the latter appeared to promote vacuole formation. An explanation of the similarities and differences in the lethal action of the various analogues and the striking concentration dependence seen with some (Fig. 2) must await a more detailed comparison of their effects on macromolecular synthesis.

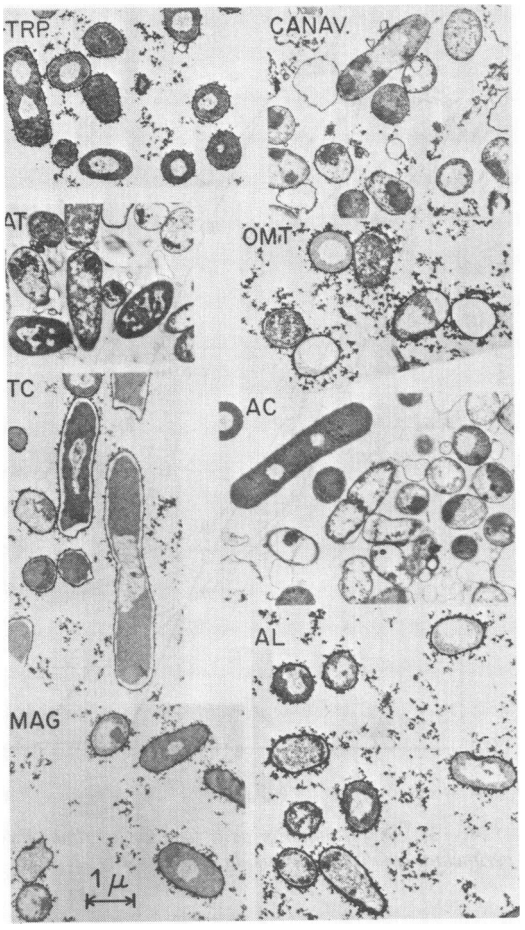

FIG. 4. *Thin sections of cells killed by amino acid analogues. After 5 hr of incubation as described in Fig. 2, 30 ml of culture was fixed by the "standard fixation" procedure of Kellenberger et al. (7), except that embedding in agar was omitted. The pellet was treated for 1 hr with a solution of 0.5% uranyl acetate and 10% Formalin, dehydrated in ethyl alcohol (70, 80, 90, 95, and 100%) for 15 min each, and treated twice with propylene oxide for 15 min. The preparation was held overnight at 4 C in a mixture of 50% Epon-Araldite and 50% propylene oxide and then embedded in Epon-Araldite. Thin sections were cut with diamond knives on a LKB ultramicrotome, placed on collodion-carbon coated grids, and stained with uranyl acetate (6) and lead citrate (11) for 15 min each. Sections were examined in an RCA EMU-3G electron microscope at an accelerating voltage of 50 kv. Objective aperture size was 25 μm. Incubation in the absence of tryptophan, −TRP; incubation without arginine and with canavanine at a final concentration of 10^{-3} M, CANAV.*

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