Incorporation of 5-Methyltryptophan into the Protein of Escherichia coli 15T⁻ (555–7)

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Previous studies have indicated that essential amino acids must be supplied for the initiation of deoxyribonucleic acid (DNA) synthesis by amino acid auxotrophs (5, 7, 11). Analogues of required amino acids can be substituted in a number of instances (3). Thus, the methionine analogues, norleucine and ethionine, and the tryptophan analogue, 7-azatryptophan, allow initiation of DNA replication in E. coli 15T⁻ after starvation for methionine or tryptophan. These analogues, as well as 4-methyltryptophan, are incorporated into protein in place of their respective amino acid counterparts (1, 2, 12, 14). It is surprising, however, that 5-methyltryptophan (5-MT) also permits DNA replication after starvation for tryptophan (3). This suggests that 5-MT may be incorporated into protein despite previous reports to the contrary (9, 10). An indication that it might be incorporated was reported in studies on polynucleotide phosphorylase (6, 15).

In the experiments below, I shall describe the incorporation of radioactive 5-MT into a hot trichloroacetic acid-insoluble fraction of the cell, presumably protein. A similar observation has been reported by D. H. Ezekiel and N. Carlson (Bacteriol. Proc., p. 116, 1967).

All of the experiments were carried out with the quadruple auxotroph E. coli 15T⁻, met⁻, trp⁻, arg⁻. The growth of this organism in thymine and in amino acid-supplemented M9 medium has been described in detail (5, 13). Estimation of radioactivity by scintillation spectrometry has also been described (13).

DL-5-MT was titrated by Schwartz Bio Research Inc., Orangeburg, N.J., by use of catalytic exchange. This material was purified in our laboratory by two passages through Sephadex G-25 with 0.01 M NH₄OH as the solvent. Four additional passages through columns of amberlite IRC50 eluting with 0.3 M ammonium acetate (pH 5.28) at 50 C (4, 8) resulted in a product which appeared to be pure upon two-dimensional chromatography in tert-butyl alcohol-methyl ethyl ketone-water (4:4:2) and tert-butylalcohol-methanol-water (4:5:1). The specific activity of the product was 1.2 c/mmmole.

When cells of 15T⁻ were incubated with this material, it was incorporated into the hot trichloroacetic acid-insoluble fraction (Table 1). The incorporation was eliminated by the addition of minute amounts of tryptophan, but was only moderately reduced by addition of nonradioactive 5-MT. Incorporation of L-tryptophan was not concentration-dependent above 0.5 μg/ml, whereas incorporation of DL-5-MT decreased with concentrations below 6 μg/ml.

The incorporated material was not soluble in alcohol or ether. Pronase digestion followed by column chromatography was used to identify the incorporated radioactivity. Two preparations were examined: (i) a culture which had incorporated ^14C-5-MT, and (ii) a culture which had been allowed to incorporate ^14C-tryptophan for 40 min and then had been transferred, by collection and washing on a membrane filter (13), to medium in which ^14C-5-MT was substituted for tryptophan.

The labeled cells were harvested and washed by membrane filtration; then they were precipitated and washed twice with 10% trichloroacetic acid, washed twice with alcohol, and washed twice with ether. The dried material was dissolved in 1 ml of 0.05 M NH₄HCO₃ (pH 7.45), and Pronase was added to a concentration of 0.5 mg/ml. After 15 hr of incubation at 37 C, the material was chromatographed on a column (20 by 2 cm) of Sephadex G-25 eluting with 0.01 M NH₄OH. Fractions (1.5 ml) were collected, and radioactivity could be measured in fractions 32 to 46. Pronase emerged with the solvent front in fractions 13 to 18. The radioactive fractions were pooled, dried, and chromatographed at 50 C on columns (24 by 1.2 cm) of amberlite IRC50 (4) eluting with 0.3 M ammonium acetate, pH 5.28. (The samples were dissolved in a volume of 2 ml of 0.01 M acetic acid and were immediately added to the amberlite columns; ammonium acetate buffer was used to wash the sample into the column.) Suitable nonradioactive standards of tryptophan or 5-MT were added to the samples for identification purposes. Figures 1A, 1B, and 2 present the results.

Some channeling of the columns occurred; this may have been responsible for the observation that the peaks are asymmetric and emerge at somewhat different effluent volumes in different

980
runs. Nevertheless, it is clear that Pronase digests the acid-insoluble radioactive material to yield a product which is not tryptophan. Between 66 and 75% of this material is indistinguishable from 5-MT and elutes with the added 5-MT. The remaining material does not bind to the column and may represent degradation products of 5-MT or undigested peptides containing 5-MT. A similar unidentified fraction was noted by Ezekiel and Carlson (Bacteriol. Proc., p. 116, 1967).

These data demonstrate that 5-MT is incorpo-

TABLE 1. Incorporation of radioactive 5-methyl-tryptophan (5-MT) or tryptophan by E. coli 15T- 55S-7a

<table>
<thead>
<tr>
<th>Amt of 5-MT or tryptophanb</th>
<th>Amt of radioactivity</th>
<th>Amt incorporated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1 µg of 4H-DL-5-MT (3 µc)</td>
<td>2,040</td>
<td>3.0 x 10^-3</td>
</tr>
<tr>
<td>1.1 µg of 4H-DL-5-MT + 2.2 µg of DL-5-MT</td>
<td>1,434</td>
<td>6.3 x 10^-3</td>
</tr>
<tr>
<td>1.1 µg of 4H-DL-5-MT + 4.4 µg of DL-5-MT</td>
<td>1,170</td>
<td>9.4 x 10^-3</td>
</tr>
<tr>
<td>1.1 µg of 4H-DL-5-MT + 1.0 µg of DL-tryptophan</td>
<td>744</td>
<td>10.9 x 10^-3</td>
</tr>
<tr>
<td>1.1 µg of 4H-DL-5-MT + 2.0 µg of DL-tryptophan</td>
<td>101</td>
<td>1.1 x 10^-3</td>
</tr>
<tr>
<td>0.5 µg of 14C-L-tryptophan (0.11 µc)</td>
<td>10,825</td>
<td>8.5 x 10^-3</td>
</tr>
<tr>
<td>0.5 µg of 14C-L-tryptophan + 55 µg of DL-5-MT</td>
<td>4,084</td>
<td>9.5 x 10^-3</td>
</tr>
<tr>
<td>0.5 µg of 14C-L-tryptophan + 2 µg of DL-tryptophan</td>
<td>2,744</td>
<td>10.7 x 10^-3</td>
</tr>
</tbody>
</table>

* An exponential culture of 15T- was washed and suspended in M9 medium lacking tryptophan, but containing all other supplements necessary for growth. After 10 min of tryptophan starvation, 1-ml samples of the culture (3 x 10^8 cells/ml) were pipetted onto the amino acids listed and were incubated for 5 min at 37 C. Trichloroacetic acid was added to a concentration of 10%, and the samples were heated to 90 C for 20 min and then washed five times with 10% trichloroacetic acid by reprecipitation from 0.01 M NH4OH. The washed samples were resuspended in 0.01 M NH4OH and counted in an aqueous scintillation fluid (13). The amount (µg) of material incorporated was calculated from the specific activities of the 4H-DL-5-MT (3 µc/0.005 µmole) and the 14C-L-tryptophan (1 µc/0.025 µmole) and from the counting efficiency of the scintillation spectrometer.

b In 1-ml incubation mixture.

Fig. 1. Chromatographic separation of a Pronase digest of cells labeled with 4H-DL-5-MT. A 50-ml culture was grown to 4 x 10^9 cells/ml and then was transferred to supplemental M9 medium in which tryptophan was replaced by 4H-DL-5-MT (1 µc/ml; 0.16 µg/ml). After 80 min of incubation at 37 C, the cells were harvested and washed on a membrane filter. Pronase digestion was carried out as described in the text. After a preliminary purification on Sephadex G-25, the digest was chromatographed on amberlite IRC50. (A) To one sample (one-half of the digest), 500 µg of 5-MT was added for an optical density reference; (B) to another sample (one-fourth of the digest), 1 mg of tryptophan was added for an optical density reference. Symbols: ●, radioactivity; ○, optical density at 280 nm.
Fig. 2. Chromatographic separation of a Pronase digest of cells labeled with $^{14}$C-L-tryptophan and subsequently with $^3$H-L-5-MT. A 25-ml culture of 15 T
557 (2 x $10^8$ cells/ml) was grown for 40 min at 37°C in a supplemented M9 medium containing $^{14}$C-L-tryptophan (0.4 $\mu$g/ml; 0.16 $\mu$g/ml); then it was transferred to a medium in which the tryptophan was replaced by L-
$^3$H-5-MT (1 $\mu$g/ml; 0.16 $\mu$g/ml) and was incubated for 80 min at 37°C. The cells were harvested and digested as described in the text. After a preliminary purification of the digest on Sephadex G-25, the sample was co-chro-
matographed with 500 $\mu$g of 5-MT on amberlite IRC50 as described in the text. Symbols: $\oplus$, $^{14}$C; $\ominus$, $^3$H; solid line, optical density of 5-MT reference at 280 nm.

rated as such into a hot acid-insoluble fraction of the cell which can be digested by Pronase. In view of the findings of Ezekiel and Carlson (Bacteriol. Proc., pp. 116, 1967), it seems safe to conclude that it is incorporated into protein. It is not known whether 5-MT is incorporated into all cell pro-
teins or only into some.

NOTES

J. BACTERIOL.

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