Evidence Concerning the Size of Amino Acid Incorporation Structures in Escherichia coli

Incorporation of exogenous amino acids into *Escherichia coli* has been shown to be at least a two-step process (*1, 2*) designated as incorporation into cold trichloroacetic acid (TCA) soluble fraction (metabolic pool) and incorporation into the trichloroacetic acid-insoluble amino acid fraction. In order to gain information on the functional units responsible for these processes, we have combined the techniques of uptake of radioactive tracers with radiation target analysis (*3*).

The basic protocol of the experiments is to grow *E. coli* on a minimal medium and to study the uptake of C^{14}- and S^{35}-labeled amino acids before and after irradiation with various doses of 1.3-Mev gamma radiation from a cobalt-60 source. The methods of determining incorporation into pool and trichloroacetic acid-insoluble fractions were those developed by the biophysics group at the department of terrestrial magnetism of the Carnegie Institution of Washington (*1, 4*). The results of the radiation inactivations were interpreted on the basis of previously developed target theory (*5, 6*).

Cultures of *E. coli* B were aerated overnight at 37°C in minimal (C) medium (*4*) with 5 g/lit of glucose. During this time, the culture grew to stationary phase; 50 ml of this suspension was then added to 150 ml of fresh medium and allowed to grow at 37°C with aeration for 2 hours. At this time the culture was in log phase, at a concentration of 0.5 to 1.0×10^9 cells per milliliter. Samples of 20 ml each were taken and frozen in Dry Ice. The cultures were kept at Dry-Ice temperatures during irradiation with a kilocurie cobalt-60 source. After irradiation, the samples were thawed and brought to 37°C. Freezing and thawing procedures took 5 to 10 minutes each.

The thawed cultures were added to equal volumes of minimal medium (with glucose) containing a labeled amino acid. Each 20-ml sample was supplied with 0.1 or 0.4 μc of C^{14}-L-leucine (Nuclear Instrument and Chemical Corp., specific activity 7.95 mc/mmole) or 0.2 μc of S^{35}-L-cystine (Abbot Laboratories, specific activity 3.48 mc/mmole).

The bacteria-amino acid incubation mixture was aerated at 37°C. Samples were taken during the first 15 minutes of culture with the labeled amino acid using the techniques of Britten, Roberts, and French (*1*); 2-ml samples were taken from the incubation mixture and drawn through a collodion membrane filter which retained the bacteria. The organisms were then washed with 2 ml of sterile, glucose-containing minimal medium. Other 2-ml samples of the incubation mixture were placed in 2 ml of 10-percent trichloroacetic acid. This was later filtered and then washed with 2 ml of 5-percent trichloroacetic acid. These two procedures produced the "whole-cell" and the "TCA-insoluble" samples. The difference between the whole-cell fraction and the TCA-insoluble fraction was called the "pool." The collodion membrane filters were dried in air and counted under a thin-window (1.4 mg/cm²) Geiger counter for either 4 or 5 minutes.

Control experiments with unfrozen, unirradiated bacteria indicated that the freezing process causes a 15 percent or less change in the rate of uptake of the amino acid, and the final value attained in 15 minutes is independent of the freezing.

The uptake of S^{35}-labeled cystine in the first 15 minutes in the whole-cell and TCA-insoluble fractions is shown in Fig. 1 for an unirradiated frozen sample and

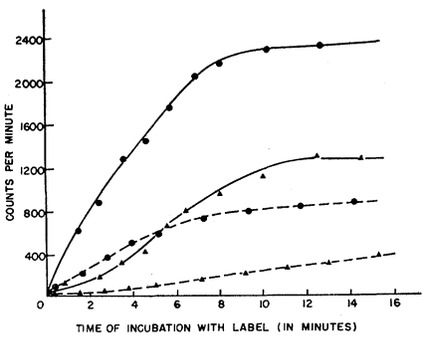

Fig. 1. Typical uptake of S^{35} cystine into *Escherichia coli*. Background was 15.2 ± 0.7 count/min and has been subtracted. Solid circles, whole cell; solid triangles, TCA-insoluble fraction; solid curve, no irradiation; broken curve, 443,000 r.

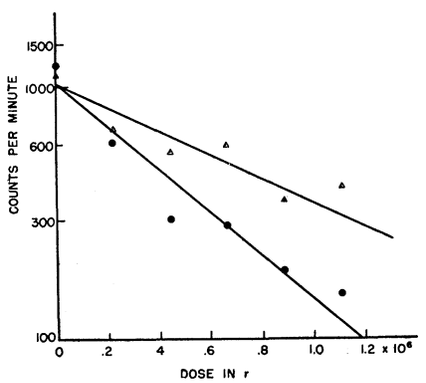

Fig. 2. Decrease in cystine incorporation with radiation dose. Bacteria were cultured with cystine for 14 minutes. Background was 15.2 ± 0.7 count/min and has been subtracted. Solid triangles, pool; solid circles, TCA-insoluble fraction.

for a sample that received 443,000 r. The TCA-insoluble and pool fractions at a given time decrease exponentially with dose, as is shown in Fig. 2 for the fractions after 14 minutes' incubation. If the decrease is characterized by the dose required to reduce the size of a fraction to 37 percent of the control, it is found that the 37-percent doses for the incorporation of a given amino acid are the same to within ± 10 percent for all times of incorporation.

Since 86 percent of the incorporated leucine and 63 percent of the incorporated cystine go into trichloroacetic acid-precipitable protein, while less than 0.3 percent of the leucine and only 2.8 percent of the cystine go into nucleic acids (*4*) (which may also be brought down by cold trichloroacetic acid), the use of the TCA-insoluble fraction as the measure of labeled protein is valid to a good approximation.

Considerations of target theory (*5*) indicate that the functional units involved with the pool and the protein formation are large. The average doses required to reduce incorporation to 37 percent of controls, and the apparent target volumes calculated from these, are as follows: cystine pool, 9.8×10^5 r and 2.0×10^{-18} cm³; cystine TCA insoluble, 4.5×10^5 r and 4.4×10^{-18} cm³; leucine pool, 2.2×10^5 r and 8.9×10^{-18} cm³; leucine TCA insoluble, 3.6×10^5 r and 5.4×10^{-18} cm³.

Any radicals formed can diffuse only very short (less than 30A) distances (*6*). Since, under the conditions of irradiation, freely diffusing radicals cannot play a dominating role in the inactivation process, the apparent inactivation volume is of the same order of magnitude as the actual physical structure involved in amino acid uptake. Although there are many complicating factors involved in obtaining physical dimensions from radiation inactivation volumes, these data strongly indicate that the physical structures involved have molecular weights between 1 and 10 million. If such a unit were spherical, the diameter would be 150 to 300 A.

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References and Notes

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3. This work was aided by a grant from the John A. Hartford Foundation.

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