Polyamine Limitation of Growth Slows the Rate of Polypeptide Chain Elongation in *Escherichia coli*

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The rate of polypeptide chain elongation during steady-state, polyamine-limited growth of a mutant of *Escherichia coli* was measured by two independent techniques. Analysis of polysome patterns gave values of 17.5 and 9.5 amino acids per s at 37 °C in unstarved and polyamine-limited cells, respectively. From the kinetics of entry of labeled amino acids into polyptides of defined molecular weights, values at 30 °C of 10.1 and 5.8 amino acids per s were obtained for unstarved and polyamine-limited cultures, respectively. Correction of these values to 37 °C resulted in rates of 15.0 and 8.7 amino acids per s. These results support the previous conclusion, based on the kinetics of β-galactosidase induction, that polyamine starvation decreases the rate of protein synthesis by limiting the velocity of polypeptide chain elongation.

As an approach to understanding the biological function of the polyamines, procedures have been developed for isolation of mutants of *Escherichia coli* defective in the synthesis of putrescine, and thereby spermidine (7, 11). The mutant that we have chosen for study has a partial block in polyamine synthesis and can be maintained indefinitely in steady-state, polyamine-limited growth (12). Under these conditions, the rates of ribonucleic acid (RNA) and protein accumulation were reduced to the same extent as the growth rate (12). To investigate the mechanism by which lowering the cellular content of polyamines limits the rates of RNA and protein synthesis, we investigated the rates of elongation of β-galactosidase and of its messenger RNA (10). We found that the rates of addition of monomers to these macromolecules were decreased proportionally to the reduction in growth rate produced by polyamine limitation. To investigate whether the reduction in polypeptide chain elongation rate is general, or characteristic of only a restricted class of proteins (e.g., induced enzymes), we measured the overall rate of polypeptide chain elongation in polyamine-limited cells by two independent techniques.

**MATERIALS AND METHODS**

**Polysome patterns.** *E. coli* strain DK6 (12) was grown at 37 °C in a phosphate minimal medium (4) supplemented with 0.2% glucose and all 20 amino acids (12). Polyamine starvation and growth of the cells in the presence (100 μg/ml) or absence of putrescine was carried out as previously described (12). The mass doubling rates under these conditions were 2.2 h⁻¹ and 1.3 h⁻¹ for unstarved and starved cultures, respectively. The cells were labeled for two doublings in mass with either [¹⁴C]uracil (5 μg/ml, 0.012 μCi/μg) in the absence of putrescine or [³H]uracil (5 μg/ml, 0.06 μCi/μg) with putrescine supplementation. At an absorbancy measured at 540 nm (A₅₄₀) of 0.1, the cultures were mixed, cells were harvested, and polysomes were isolated as described by Forchhammer and Lindahl (5). Polysomes were displayed in a 10-ml gradient of 15 to 30% sucrose in 5 mM tri(hydroxymethyl)aminomethane-hydrochloride (pH 7.5) containing 5 mM MgSO₄ and 60 mM KCl. Centrifugation was carried out at 40,000 rpm for 110 min in a Beckman SW41 rotor. Fractions were collected and counted in a Bio-Solv/toluene scintillation fluid (Beckman).

**Kinetics of entry of [³H]phenylalanine into polypeptides.** The cells were grown as described above except that the temperature was 30 °C and the aromatic amino acids were omitted from the culture medium. At this temperature the mass doubling rates of the unstarved and starved cultures were 1.4 h⁻¹ and 0.86 h⁻¹, respectively. Cultures were inoculated in the presence or absence of putrescine at an initial density of A₅₄₀ of 0.01 in the presence of a tracer amount of [¹⁴C]phenylalanine (0.1 μCi/ml, 375 mCi/mmoll). At a density of A₅₄₀ of 0.3 to 0.4, the experiment was initiated by exposing the cells to [³H]phenylalanine (20 μCi/ml, 7.8 Ci/mmoll). At 10-s intervals, 1-ml samples were taken into ice-cold 10% trichloroacetic acid containing unlabeled phenylalanine (100 μg/ml). The precipitates were centrifuged and washed once with 1 ml of 5% trichloroacetic acid containing phenylalanine as above. The precipitates were then dissolved at room temperature in 0.3 ml of 0.3 N NaOH and incubated for 10 min. The samples were reprecipitated by addition of 1 ml of cold 5% trichloroacetic acid, centrifuged, and washed once with 1 ml of...
The samples were stored frozen until electrophoresis. Prior to electrophoresis, the samples were heated at 100°C for 2 min and lysozyme that had been polymerized and diansylated (8) was added. The samples were subjected to electrophoresis in gels (0.5 by 8 cm) of 6% polyacrylamide, 0.24% N,N'-diallyltartardiamide (1). The current during electrophoresis was 8 mA per gel. The buffer was 0.1 M sodium phosphate (pH 7.2) containing 0.1% sodium dodecyl sulfate. Electrophoresis was halted when the bromphenol blue markers reached the bottoms of the gels. The fluorescent lysozyme bands corresponding to molecular weights 33,000, 47,000, 63,000, and 78,000, as well as the interband spaces corresponding to 54,000 and 72,000 molecular weights, were cut out with a razor blade; the slices were incubated overnight at room temperature in 0.5 ml of 2% periodic acid (1). The dissolved gel fractions were then combined with 10 ml of xylene-based scintillation fluid containing 25% Triton X114 and 3 g of 2,5-diphenyloxazole per liter (2). After sitting overnight in the dark at room temperature, the samples were counted in a Beckman LS200 scintillation counter. The ratio 14H/14C for a given molecular weight region was divided by 1H/14C of the total protein [F(M, t)] and plotted as a function of time. The synthesis times were calculated as described by Gausing (6).

Materials. [5-14H]Juracil (28 Ci/mmole) and [2-14C]Juracil (56.3 mCi/mmole) were obtained from Amersham/Searle; uniformly labeled [14C]phenylalanine (375 mCi/mmole) was from New England Nuclear. Acrylamide and N,N'-diallyltartardiamide were obtained from Eastman Organic Chemicals, putrescine was from Calbiochem, and lysozyme (twice crystallized) was obtained from Worthington Biochemicals Corp. Dansyl chloride was from Pierce Biochemicals, Triton X114 was from Rohn and Haas, and Bio-Solv was from Beckman.

RESULTS

Influence of polyamine limitation on polysome patterns. Forchhammer and Lindahl (5) estimated the rate of polypeptide chain growth in E. coli by calculating the rate of protein accumulation per active ribosome. The proportion of active ribosomes was determined from polysome profiles. We applied this approach to our polyamine-deficient mutant. Polysome patterns from cells grown in the presence and absence of putrescine are compared in Fig. 1. All of the polysomal material could be transferred to the mono- and disome regions of the sucrose gradients by treatment of the extracts with ribonuclease (7 μg/ml) or by exposure of the cells to puromycin (200 μg/ml) for 2 min prior to lysis. It is clear from inspection of the patterns and from the ratio 1H/14C across the gradient that no significant difference exists between the cells grown under the two conditions. Longer centrifugation times, designed to display more fully the monosome-subunit region, also failed to detect significant differences. In both cultures, 61% of the ribosomal material was in polysomes (fractions 1 to 41) and 18% was in monosomes (fractions 42 to 47). Assuming that half the monosome region represented active ribosomes (5), we arrived at a figure of 70% active ribosomes under both culture conditions. Using our previous values (12) of 87% of total RNA as ribosomal RNA and the cellular
ratio RNA/protein = 0.53 for unstarved and 0.59 for starved cultures, we calculated polypeptide chain elongation rates (5) of 17.5 amino acids per s in the unstarved cells and 9.5 amino acids per s in the polyamine-limited cells. (The elongation rate was calculated according to the following equation (5): \[ \frac{[(P/120) \times \mu \times 1n2]}{[(R/1.6 \times 10^8) \times f]} \], where \( P \) and \( R \) are protein and ribosomal RNA content of the cells in micrograms per milligrams dry weight, \( \mu \) is the specific growth rate in mass doublings per hour, and \( f \) is the fraction of ribosomes active in protein synthesis.)

**Kinetics of radioactive labeling.** The basic assumptions made in calculating polypeptide chain growth rates from polysome patterns were that all of the ribosomes in the polysome fraction were active and that the rate of protein synthesis was equal to the rate of accumulation under these two culture conditions (i.e., polyamine limitation did not lead to increased protein turnover). Thus, we sought to verify these results by a completely independent approach. Gausing (6) introduced a technique for estimating polypeptide chain growth rates by measuring the kinetics of entry of label into polypeptides of discrete molecular weight separated by sodium dodecyl sulfate gel electrophoresis. In our hands, the reproducibility of this technique was enhanced by including fluorescent molecular weight markers (8) during gel electrophoresis. As an example, the incorporation of \( ^{[\text{H}] \text{phenylalanine}} \) into polypeptides of 78,000 molecular weight is illustrated in Fig. 2A and B. The break points in these curves (72 and 108 s) represent the synthesis times for polypeptides of this molecular weight (3, 6) in unstarved and polyamine-limited cells. Our data from two separate experiments are summarized in Fig. 2C and D. The slopes of these lines yield average polypeptide chain elongation rates at 30 C of 10.1 ± 0.8 amino acids per s and 5.8 ± 0.4 amino acids per s for the unstarved and starved cultures, respectively. Correction of these values by the ratio of growth rates at 37 and 30 C leads to elongation rates at 37 C of 15.0 and 8.7 amino acids per s. These values agree with those calculated from polysome patterns. Given the assumptions inherent in obtaining elongation rates from polysome patterns, this agreement supports our previous conclusion that polyamine starvation does not induce extensive protein turnover (9).

**DISCUSSION**

The results reported here are summarized together with those obtained previously (10) in Table 1. The present data clearly substantiate our previous conclusions concerning the effect of polyamine starvation on the rate of polypeptide chain elongation and show that this is a general phenomenon and not associated only with the initial kinetics of enzyme induction. As was found with the rate of \( \beta \)-galactosidase elongation, polyamine limitation of growth produced a reduction in the average rate of polypeptide chain elongation identical to the reduction in growth rate. This suggests that polyamines are rather intimately involved in the processes of transcription, translation, or both. However, as pointed out previously (10), when one considers that the rate of messenger RNA elongation is reduced during polyamine limitation and that translation and transcription are coupled in
bacteria, it is difficult on the basis of these kinds of data to pinpoint the site of polyamine action in the transcription-translation process. Relevant to this question, Young and Srinivasan (13) recently presented evidence for a reduced rate of phage protein synthesis during infection of polyamine-starved cells by RNA phage f2. Since translation is not coupled to transcription in this phage system, and assuming that this result can be extrapolated to uninfected cells, their experiment suggests an influence of polyamines on protein synthesis independent of the reduction in the rate of messenger RNA elongation. However, this evidence does not exclude an influence of spermidine, either direct or indirect, on both translation and transcription.

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