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Growth Rate of *Escherichia coli* at Elevated Temperatures: Limitation by Methionine

ELIORA Z. RON AND BERNARD D. DAVIS

Department of Microbiology, Tel Aviv University, Israel, and Department of Microbiology and Molecular Genetics and Bacterial Physiology Unit, Harvard Medical School, Boston, Massachusetts 02115

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When *Escherichia coli* growing in minimal medium is shifted from 37 C to any temperature in the range 40 to 45 C, the growth rate immediately assumes a new, lower value, characteristic of that temperature. The decrease is shown to be due, in several strains, to decreased activity of the first enzyme of the methionine pathway, homoserine trans-succinylase, which thus appears to be more heat-sensitive than any other essential enzyme in the cell. This sensitivity does not involve progressive denaturation of the enzyme; rather, the response to a shift of temperature, in either direction, is immediate and reversible.

The effects of elevated temperatures on mesophilic bacteria have not been extensively analyzed at a molecular level. In early studies, the decreased rate of growth at temperatures above the optimum was generally regarded as the resultant of competition between killing and the continued growth of those cells that remained viable (4). However, the addition of various specific nutrients to the medium was later found to shift the temperature optimum upward in various organisms, and this finding suggested (3) that the temperature sensitivity of one or another specific enzyme may limit the growth of a wild-type organism at elevated temperatures, as it is known to do in temperature-sensitive mutants.

We have undertaken to define more precisely the mechanism by which elevated temperatures limit the growth of *Escherichia coli*. The results will show that a shift from 37 C to a higher temperature, up to 45 C, immediately establishes a characteristic, lower growth rate (measured in terms of protein synthesis), without killing. In all of the strains tested, this effect is due to decreased synthesis of methionine. The cause of this decrease is an immediate, reversible impairment of the activity of the first enzyme of that pathway, homoserine trans-succinylase (HTS; *O*-succinyl homoserine synthetase).

MATERIALS AND METHODS

Bacteria and growth conditions. *E. coli* strains used included B; W; W mutant M122-33, which requires methionine; $15T^{-}A^{-}$, which requires thymine and arginine; ML-35 (provided by J. Monod); K-12 strain 687-1, which requires arginine and has a "relaxed" (*rel*$^{-}$) control of ribonucleic acid (RNA) synthesis; and K-12 methionine auxotroph CW3747 (provided by S. Schlesinger), which lacks cystathionine γ-synthetase.

Growth conditions were as described previously (6). Minimal medium A (2) was used and was supplemented, when required, with specific amino acids (50 μg/ml each), uracil (20 μg/ml), and thymine (2 μg/ml).

Temperature was controlled with a precision of ±0.2 C. Culture volumes were small to assure quick equilibration upon shifts to new temperatures; equilibration time was shorter than 1 min.

Incorporation of radioactively labeled materials. Overnight cultures were diluted in fresh medium and grown to 3×10^8 to 6×10^8 cells/ml. The cultures were then diluted with 3 to 4 volumes of warmed medium containing either ^{14}C-leucine (0.01 μCi, 20 μg/ml), ^{14}C-uracil (0.01 μCi, 10 μg/ml), or ^{14}C-thymine (0.01 μCi, 2 μg/ml). Samples were transferred at intervals into an equal volume of 10% trichloroacetic acid. The precipitated macromolecules were collected on membrane filters (Millipore Corp.) and counted as previously described (6).

Synthesis of O-succinyl homoserine. The formation of *O*-succinyl homoserine by cells was measured by incubation with ^{14}C-homoserine, which was separated from ^{14}C-succinyl-homoserine on a Dowex-1-acetate column (7, 9). Samples of 1 to 2 ml at neutral *p*H were applied to a column (1 by 2 cm); homoserine was then eluted with 40 ml of water, and succinyl-homoserine was eluted with 10 ml of 0.2 M acetic acid. Samples (1 ml) were added to vials with Bray's solution (1) and counted in a Packard Tri-Carb liquid scintillation spectrometer.

Chemicals. ^{14}C-homoserine was purchased from CEA France and purified by elution from a Dowex-acetate column. All other radioactive compounds were purchased from New England Nuclear Corp. DL-Cystathionine, coenzyme A (CoA), and L-methionine were

purchased from Sigma Chemical Co. Dowex-1-Cl (× 2 100/200 mesh) was purchased from Fluka AG. Succinyl-CoA was synthesized as described by Schlesinger (9).

RESULTS

Effect of elevated temperature on growth and macromolecule synthesis. When a culture of *E. coli*, growing exponentially in minimal medium, was transferred from 37 to 45 C, growth stopped within a few minutes. However, there was no irreversible damage to the cell, since the viable number did not decline for several hours. The cessation of growth was evidently due to inhibition of synthesis either of protein or of RNA, since both these syntheses ceased rapidly, whereas that of deoxyribonucleic acid (DNA) ceased more slowly (Fig. 1).

Effect of elevated temperature on RNA synthesis in a "relaxed" strain. The strain used was *rel*$^+$, and RNA synthesis was stringently controlled; hence the inhibition of protein synthesis might be primary with a secondary inhibition of net RNA synthesis, or vice versa. To distinguish these alternatives, we examined a *rel*$^-$ mutant, in which deprival of a required amino acid stops protein but not net RNA synthesis (10). Figure 2 shows that with this strain a shift to 44 C (in the presence of the amino acid) had much the same effect as amino acid deprival; protein synthesis was inhibited immediately but RNA synthesis only gradually. The difference between the two strains suggests that elevated temperature blocks the formation or the activation of one or more amino acids.

Effect of methionine. The deficiency was further defined by showing that the addition of a complete amino acid mixture to a culture of the wild type at 44 C restored rapid growth and protein synthesis, and, after a brief lag, RNA synthesis also (Fig. 3). (The restoration to the values observed at 37 C is probably coincidental.) Moreover, methionine also restored protein synthesis, whereas a mixture of the other amino acids had no detectable effect (Fig. 4). On addition of methionine, the temperature required to effect a given degree of inhibition of protein synthesis was raised by about 1.5 C (Fig. 5).

Methionine antagonized the inhibitory effect of elevated temperatures on growth in all *E. coli*

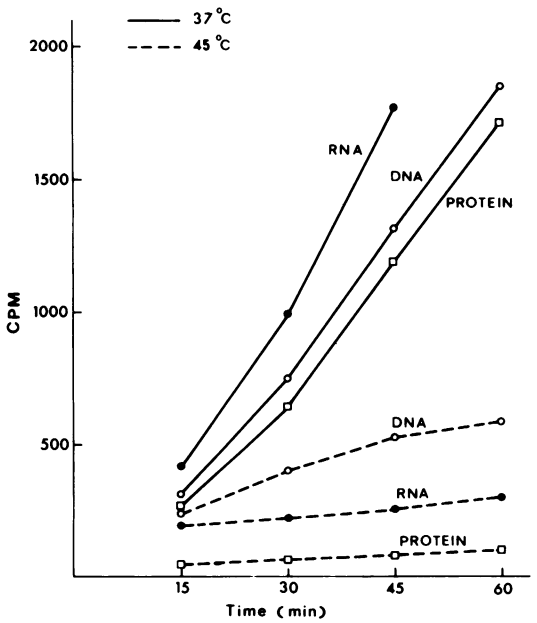

FIG. 1. *Synthesis of DNA, RNA, and protein in E. coli at 37 and 45 C. A culture of strain 15T*$^-$*A*$^-$*, growing exponentially at 37 C in minimal medium, plus thymine and arginine, was divided into three batches containing* ^{14}*C-thymine* (○), ^{14}*C-uracil* (●), *or* ^{14}*C-leucine* (□). *Part of each batch was further incubated at 37 C, and part was transferred to a bath at 45 C. Samples were analyzed as described in the text.*

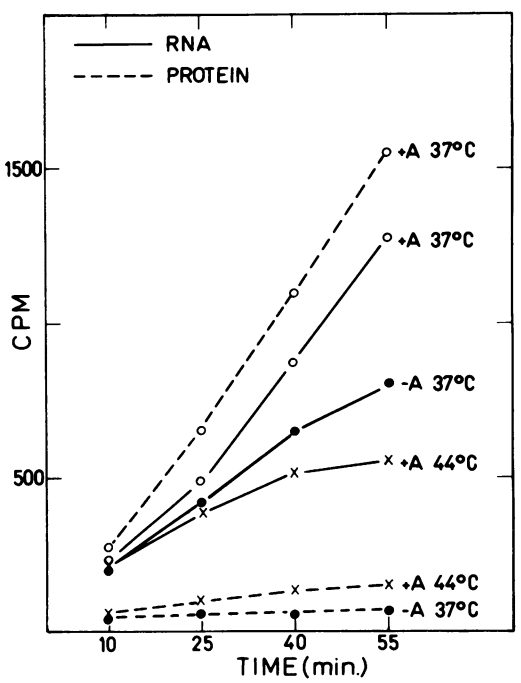

FIG. 2. *Synthesis of RNA and protein in a rel*$^-$ *arginine auxotroph. An exponentially growing culture of strain 687-1 was centrifuged, and the cells were washed twice and resuspended in minimal medium containing* ^{14}*C-uracil (solid line) or* ^{14}*C-leucine (broken line). Portions were incubated at 37 C, and other portions were supplemented with arginine (A) and incubated at 37 or 44 C.*

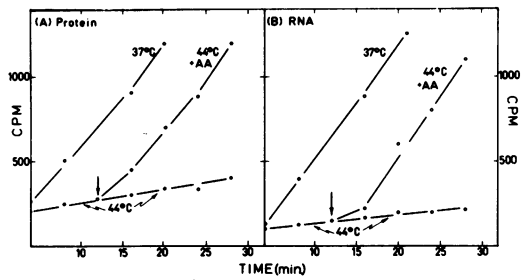

FIG. 3. *Effect of amino acids on growth at 44 C. Cultures of E. coli B growing in minimal medium were diluted with medium, with addition of ^{14}C-leucine (A) or ^{14}C-uracil (B), and were further incubated at 37 C or transferred to 44 C. After 12 min, a portion of the 44 C culture received a mixture of the 19 standard amino acids other than leucine (10^{-3} M final concentration of each). Samples were analyzed as described in the text.*

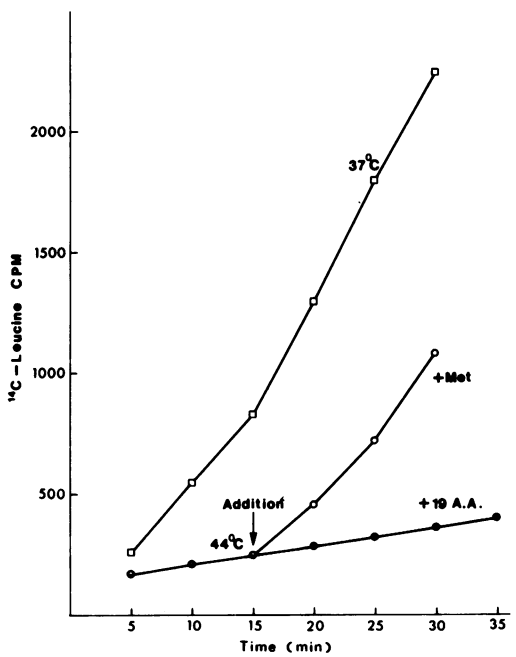

FIG. 4. *Effect of methionine on growth at 44 C. The experiment was done as described in Fig. 3, except that only the incorporation of ^{14}C-leucine was measured and additions, after 15 min, were either methionine or the amino acid mixture without methionine and leucine.*

strains tested, including B, W, K-12, 15T$^-$A$^-$, and ML. It is thus clear that growth of various *E. coli* strains at an elevated temperature is inhibited by a limitation in the endogenous supply of methionine relative to its requirement.

Effect of methionine and temperature on viability. In contrast to this effect on growth, methionine had no perceptible effect on killing. Thus, at 44 C the viable number remained constant for at least 18 min, with or without methionine, whereas at 47 C it decreased 90% in 20 min, with or without methionine (Fig. 6). Hence, the presence of methionine produced a more abrupt transition between the temperatures supporting growth and those causing killing.

Enzymatic site of the temperature effect. A limitation in the availability of methionine at elevated temperatures might be due to a decrease in the activity of any enzyme in this pathway. The locus of the inhibition was narrowed by tests with a methionine auxotroph (mutant M122-33) blocked in the synthesis of cystathionine (Fig. 7). Growth of this mutant is slower on cystathionine than on methionine, presumably because of relative impermeability; however, with either nutrient the growth rate was the same at 44 C as at 37 C (Fig. 8). It thus appears that the block in methionine synthesis at 44 C in the wild type does not impair the utilization of cystathionine, and hence must involve a step preceding this intermediate.

Cystathionine is synthesized by the first two enzymes of the methionine pathway (Fig. 7). The first of these has an allosteric response to methionine (8). To test whether it might also be especially sensitive to conformational changes induced by elevated temperature, we studied the

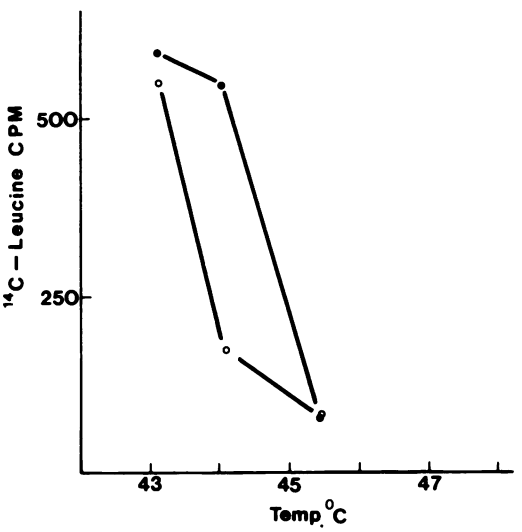

FIG. 5. *Protein synthesis as a function of temperature in the presence and the absence of methionine. From a culture of E. coli B, growing exponentially at 37 C, 2.5-ml samples were transferred to various temperatures as indicated. After 10 min of incubation, with (●) or without (○) methionine, 1 ml of prewarmed medium containing ^{14}C-leucine (0.1 μCi, 5 μg) was added; after 2 min, the incorporation was stopped by adding trichloroacetic acid.*

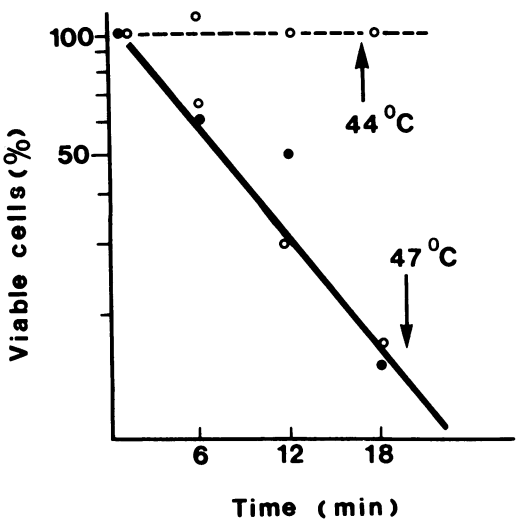

FIG. 6. *Effect of temperature on viability. A culture of E. coli B growing exponentially in minimal medium was transferred to 44 C or to 47 C and was incubated with (●) or without (○) added methionine. Samples were removed, diluted, and plated on nutrient agar. Colonies were counted after 18 hr at 37 C.*

accumulation of its product, *O*-succinyl homoserine (7), by a mutant of *E. coli* (CW3747) blocked in the next reaction (9). Methionine limitation, required for this accumulation, was achieved by growth on cystathionine; to prevent the precursors of the accumulated intermediate from being directed to other products, threonine and lysine were added. Under these conditions, a shift from 37 to 44 C immediately inhibited, by 90%, the formation of *O*-succinyl homoserine from ^{14}C-homoserine added to the growing culture (Fig. 9). Hence, the unusually temperature-sensitive enzyme in the pathway to methionine is evidently homoserine trans-succinylase.

Kinetics and reversibility of the temperature effect. The effect of temperature elevation on protein and RNA synthesis was similarly abrupt. There was a characteristic, new, lower rate of exponential synthesis for each temperature from appoximately 42 to 45 C (Fig. 10). Moreover, a reversal of the temperature shift, back to 37 C, immediately restored the rate of synthesis of both protein (Fig. 11) and *O*-succinyl homoserine (Fig. 9) to their initial values. It thus appears that, at each temperature in the range 42 to 45 C, the enzyme immediately assumes a particular conformation (or distribution of conformations) with a characteristic lower activity.

DISCUSSION

The results presented show that at temperatures above the optimum the rate of growth of *E. coli* in minimal medium is restricted by the endogenous supply of methionine. Thus, in the presence of added methionine growth is equally rapid at 44 and 37 C, whereas without methionine (even with 19 other amino acids) growth is only 20% as rapid at the higher temperature. The site of the inhibition was narrowed down by the demonstration that temperatures of 42 to 44 C also decrease the conversion of homoserine to *O*-succinyl homoserine by cells of a mutant blocked after the latter. It thus appears that these temperatures limit growth rate by decreasing the activity of the first enzyme of the methionine pathway, homoserine trans-succinylase.

This temperature sensitivity does not appear to depend either on conventional, cumulative denaturation of the enzyme or on an alteration in its rate of formation. Thus, a shift to 44 C results in the immediate establishment of a characteristic new rate of growth of the wild type and a parallel rate of accumulation by the mutant. Similarly, a shift back to 37 C immediately restores the rates to the old values. If these effects depended on an alteration in the rate of formation or the rate of irreversible inactivation of the sensitive enzyme, the changes in activity should have extended over at least several minutes, which would easily have been detected. The absence of such a lag strongly suggests that the decreased activity is due to a rapid, reversible alteration in the molecules of the sensitive enzyme already present in the cell. Since homoserine trans-succinylase is an allosteric enzyme, subject to feedback inhibition by methionine, its decreased activity in the cell at 42 to 44 C could be due either to impairment of its inherent activity or to increased sensitivity to methionine. Measurements on the extracted enzyme, presented in the following paper (5), establish a direct inhibition of its activity.

Methionine is required not only for incorpora-

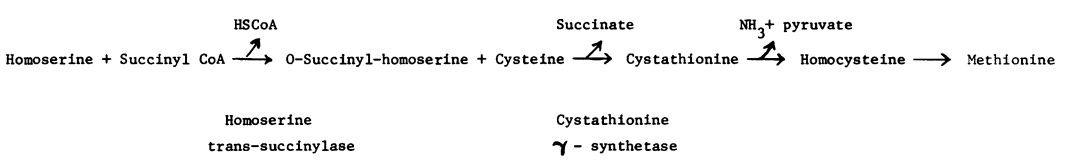

FIG. 7. *Pathway of biosynthesis of methionine.*

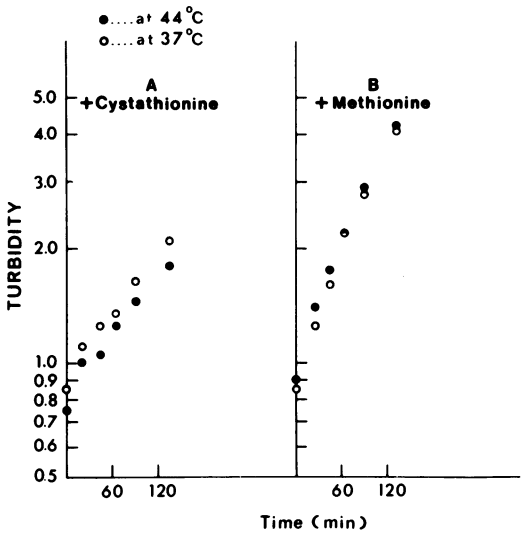

FIG. 8. *Growth of an E. coli methionine auxotroph on cystathionine at 44 and 37 C. Cells of mutant M122-33 were washed and resuspended in medium containing either methionine or cystathionine. Part of each culture was incubated at 37 C and part at 44 C, and the turbidity was measured at intervals in a photoelectric colorimeter. A value of 1.0 corresponds to 10^8 cells/ml.*

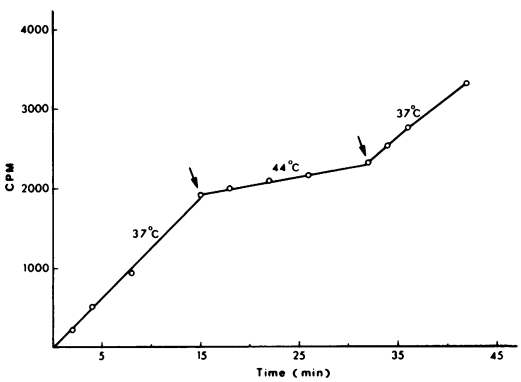

FIG. 9. *Accumulation of O-succinyl homoserine by a methionine auxotroph. To a culture of E. coli mutant CW3747 growing with cystathionine as source of methionine, ^{14}C-homoserine (0.1 μCi, 20 μg/ml) was added, together with lysine and threonine (20 μg/ml of each), at zero time. Temperature was shifted as indicated. Samples were removed, chilled, and centrifuged in the cold, and the supernatant fluid was analyzed for O-succinyl homoserine.*

tion into proteins but also for initiation of protein synthesis, for various methylation reactions, and for synthesis of polyamines; hence control of its synthesis would directly control many aspects of growth. However, the teleonomic value of an especially sensitive response of this pathway to elevated temperatures is not obvious, especially

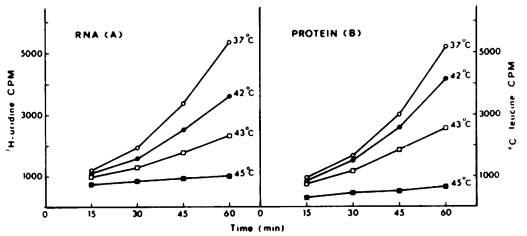

FIG. 10. *Rates of protein and RNA synthesis at various temperatures. Exponentially growing cultures of E. coli B were transferred to various temperatures. ^{3}H-uridine (0.1 μCi, 20 μg/ml) or ^{14}C-leucine was added; at intervals, samples were taken for analysis.*

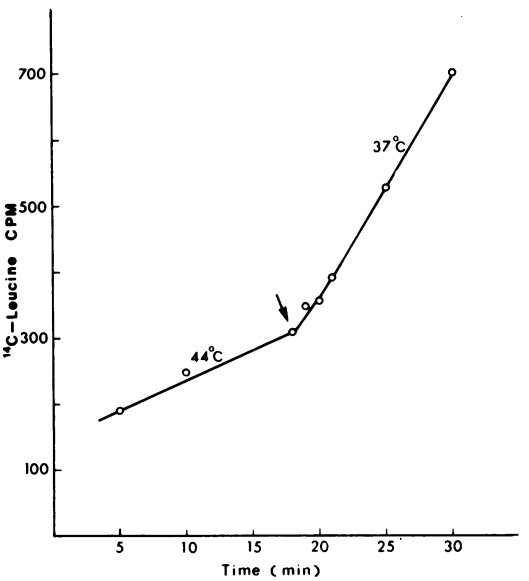

FIG. 11. *Immediate reversal of the temperature effect on protein synthesis. A culture of E. coli B growing at 37 C was warmed to 44 C, and ^{14}C-leucine was added; after 15 min, the temperature was rapidly shifted back to 37 C. Samples were taken at intervals.*

since this response has no effect on growth in media containing methionine. Moreover, the presence or absence of methionine does not influence the rate of killing by heat (Fig. 6), which evidently involves quite a different mechanism from that responsible for the inhibition of growth. Nevertheless, it is noteworthy that the pathway to methionine appeared to be the most temperature-sensitive pathway in all five wild-type strains of *E. coli* tested. Although this uniformity might simply reflect genetic stability in the structure of homoserine trans-succinylase, it also suggests the possibility of some undisclosed selective advantage.

ACKNOWLEDGMENTS

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