JOURNAL OF BACTERIOLOGY, Jan. 1979, p. 694-696
0021-9193/79-01-0694/03$02.00/0

Vol. 137, No. 1

Accumulation of Peptidyl tRNA Is Lethal to *Escherichia coli*†

JOHN R. MENNINGER

Zoology Department, University of Iowa, Iowa City, Iowa 52242

Received for publication 2 June 1978

A mutant strain of *Escherichia coli* with temperature-sensitive peptidyl-tRNA hydrolase grows at 30°C but, when shifted to 40°C, dies at rates affected by physiological, pharmacological, and genetical perturbations. The rate of killing correlates with the relative accumulation of peptidyl-tRNA, suggesting that it is responsible for the death of the cells.

The enzyme peptidyl-tRNA hydrolase, referred to below simply as hydrolase, catalyzes the hydrolysis of *N*-acylated amino acids from *N*-acyl-aminoacyl-tRNA's that are not bound to ribosomes. We have previously reported the isolation and characterization of a strain of *Escherichia coli* that has a mutation in the structural gene (*pth*) for hydrolase. This mutation allows the enzyme to be rapidly and irreversibly inactivated at elevated temperatures (1, 4). *E. coli* cells with the temperature-sensitive hydrolase can grow exponentially at 30°C, but when cultures are shifted to 43°C there is an accumulation of peptidyl-tRNA and an inhibition of protein synthesis (1, 2, 4). Also a fraction of the cells die, unable to form a colony when replaced at 30°C (J. R. Menninger, Fed. Proc. **33**:1335, 1974). Data presented below suggest that the peptidyl-tRNA that accumulates after the inactivation of hydrolase is responsible in some way for the death of cells.

The fundamental observation is shown in Fig. 1, which depicts the fractional survival of a culture of *E. coli* strain *ts*8 at various times after shifting the temperature to 43°C. There was usually evidence of a lag before killing was observed, and the final fractional survival was in the range of 1 to 10%. The density of cells at the time of temperature shift appeared to have little influence on the killing, provided the cells were growing exponentially.

Figure 2 shows the effects of chloramphenicol and erythromycin on cell survival. Doses were chosen as the minimum necessary to inhibit growth of the wild-type CA244 cells at the elevated temperature (data not shown). Antibiotic was added 15 min before and was present during the incubation at 40°C to guarantee that an effective dose would be present at the time of the temperature shift. Chloramphenicol at 4 μg/ml completely prevented the death of the *ts*8 cells at 40°C. This is consistent with this drug's effect on the accumulation of peptidyl-tRNA, which is significantly slowed in cultures treated the same as those depicted in Fig. 2 (2). Other antibiotics known to block elongation of peptides also protected *ts*8 from being killed at high temperatures (data not shown).

In contrast, the presence of 80 μg of erythromycin per ml enhanced both the rate and extent of killing of strain *ts*8 at 40°C. This, too, is consistent with the effects of erythromycin on the rate of dissociation of peptidyl-tRNA from ribosomes and its accumulation in the cells with inactivated hydrolase (2). This is another manifestation of the mechanism of antibiotic action of erythromycin, namely the destabilization of the peptidyl-tRNA/ribosome binding interaction (2, 5). Other macrolide antibiotics have similar effects on the survival of *pth* strains (J. R. Menninger, manuscript in preparation).

The unperturbed fractional survival of 1 to 10% was stable, neither increasing nor decreasing for at least 24 h (data not shown), and did not increase if the colonies were incubated at room temperature, rather than 30°C (data not shown). The surviving cells were essentially identical genetically to the starting culture, since the frequency of phenotypic revertants, cells able to grow in liquid culture at nonpermissive temperatures, was less than 10^{-5}. Furthermore, when the survivors were replaced at 30°C and grown for a few generations, they died with a time course similar to Fig. 1 after being shifted to a nonpermissive temperature (data not shown). The growth of the survivors at 30°C did not seem to be synchronized (data not shown). The rate and extent of killing of *ts*8 cells placed at 40°C were not greatly different if the cells were first grown in minimal medium lacking amino

† This is paper no. 5 in the series "Studies on the Metabolic Role of Peptidyl-tRNA Hydrolase." The preceding paper is reference 3.

acids and with glycerol as a carbon source (data not shown).

The data of Fig. 1 and 2 show that shifting a culture with a temperature-sensitive peptidyl-tRNA hydrolase to nonpermissive temperatures leads to death of most of the cells. This cytotoxic effect is positively correlated with the immediate accumulation in the cells of peptidyl-tRNA that dissociates from ribosomes during protein biosynthesis (2, 3).

The correlation is maintained during physiological, pharmacological, and genetic perturbations. Starving the cells for an amino acid enhances both the accumulation of peptidyl-tRNA's and the rate and extent of killing (A. C. Caplan, P. E. Gingrich, and J. R. Menninger, unpublished data). Drugs, such as chloramphenicol, that block the accumulation of peptidyl-tRNA (2) also blocked the cytotoxicity (Fig. 2). Drugs like erythromycin, which enhance the accumulation of peptidyl-tRNA (2), enhanced both the rate and extent of killing (Fig. 2). Mutations have been found that reduce both the rate of accumulation of peptidyl-tRNA and the rate of killing (e.g., *relA*; J. R. Menninger and A. G. Atherly, Fed. Proc. **34**:707, 1975) or enhance both phenomena (A. C. Caplan and J. R. Menninger, unpublished data). We have isolated pseudo-revertants of the *pth* cells (by selection in liquid media at 43°C) whose hydrolase is still temperature sensitive but whose levels of peptidyl-tRNA at high temperature are less than the mutant parent. These cells can grow at the high temperature (J. R. Menninger and P. E. Gingrich, Fed. Proc. **37**:1625, 1978).

These data, taken together, strongly imply that the accumulation of peptidyl-tRNA is the cause of cell death. We already know that this accumulation precedes and, therefore, probably causes an abrupt reduction in the rate of protein synthesis which occurs about 5 min after shifting to nonpermissive temperatures (2). It seems likely that this rapid loss of protein synthetic capability plays a role in the observed killing.

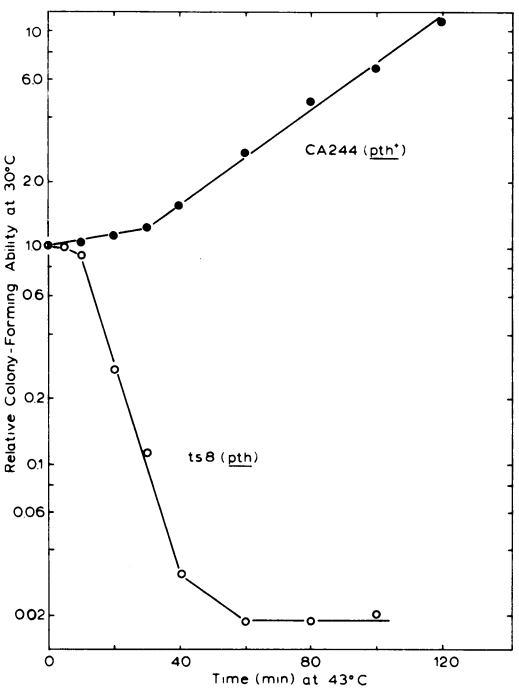

FIG. 1. *Time course of survival of mutant pth (ts8) and wild-type (CA244) cells after shift to 43°C. Cells growing exponentially in broth at 30°C at a density of 2.1 × 10⁸/ml (CA244, solid circles) or 0.75 × 10⁸/ml (ts8, open circles) were diluted into prewarmed aerating broth at 43°C. At various times afterward, samples were diluted and plated (broth agar) for colony-forming ability at room temperature. The number of colonies formed after overnight incubation at 30°C was divided by the number found for a sample taken at the time of the temperature shift to give the fractional survival.*

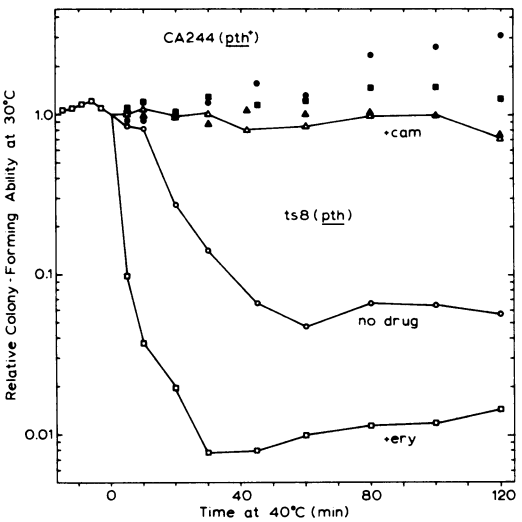

FIG. 2. *Time course of survival of mutant pth (ts8) and wild-type (CA244) cells after shift to 40°C in the presence of chloramphenicol or erythromycin. Cells were growing exponentially at 30°C in M9 minimal medium supplemented with 19 of the 20 common amino acids (leucine omitted) (2). At 15 min prior to attaining a density of 2 × 10⁸ to 9 × 10⁸/ml, chloramphenicol (4 μg/ml, triangles) or erythromycin (80 μg/ml, squares) was added. Cultures of ts8 (open symbols) and CA244 (closed symbols) were also tested without drugs in this medium as a control (circles). At zero time the cultures were diluted into prewarmed, aerating medium at 40°C containing the drug or diluent as required. Further sampling was performed as described in Fig. 1.*

It is well known that merely interrupting protein synthesis does not cause death to bacterial cells (e.g. Fig. 2, chloramphenicol data). There must be an irreversible element in the cell's response that makes it unable to escape the accumulation of peptidyl-tRNA. Further, one needs to explain the apparent immunity from being killed of a significant fraction of unperturbed cells at high temperature (1 to 10%; Fig. 1 and 2).

A plausible but unproved model can be built on the assumptions that (i) all hydrolase molecules that are in the cytoplasm at the time of temperature shift are immediately and irreversibly inactivated (see ref. 4); (ii) no very active alternate scavenging system exists for peptidyl-tRNA; and (iii) doomed cells are those that were not translating an mRNA that specified hydrolase at the time of severe inhibition of protein synthesis. The surviving cells are presumed to escape being killed by completing, at a very low rate, the synthesis of at least one nascent hydrolase chain on return to the low temperature.

This work was supported in part by Public Health Service grant GM-20626 from the National Institute of General Medical Sciences.

I thank Don Otto and Lesley Menninger for research assistance and Pat Gingrich and Allen Caplan for permission to quote unpublished data.

LITERATURE CITED

1. **Atherly, A. G., and J. R. Menninger.** 1972. Mutant *E. coli* strain with temperature sensitive peptidyl-transfer RNA hydrolase. Nature (London) New Biol. **240:** 245–246.
2. **Menninger, J. R.** 1976. Peptidyl-transfer RNA dissociates during protein synthesis from ribosomes of *Escherichia coli*. J. Biol. Chem. **251:**3392–3398.
3. **Menninger, J. R.** 1978. The accumulation as peptidyl-transfer RNA of isoaccepting families in *Escherichia coli* with temperature-sensitive peptidyl-transfer RNA hydrolase. J. Biol. Chem. **253:**6808–6813.
4. **Menninger, J. R., C. Walker, P. F. Tan, and A. G. Atherly.** 1973. Studies of the metabolic role of peptidyl-tRNA hydrolase. 1. Properties of a mutant *E. coli* with temperature-sensitive peptidyl-tRNA hydrolase. Mol. Gen. Genet. **121:**307–324.
5. **Otaka, T., and A. Kaji.** 1975. Release of (oligo) peptidyl-tRNA from ribosomes by erythromycin A. Proc. Natl. Acad. Sci. U.S.A. **72:**2649–2652.