JOURNAL OF BACTERIOLOGY, Aug. 1991, p. 5244–5246
0021-9193/91/165244-03$02.00/0

Vol. 173, No. 16

Cysteine, Even in Low Concentrations, Induces Transient Amino Acid Starvation in *Escherichia coli*

MICHAEL A. SØRENSEN AND STEEN PEDERSEN*

Institute of Microbiology, University of Copenhagen, Øster Farimagsgade 2A, DK-1353 Copenhagen, Denmark

Received 30 August 1990/Accepted 24 May 1991

Cysteine, in concentrations down to 0.04 µg/ml, induces transient amino acid starvation in *Escherichia coli* growing in minimal medium. The duration depends on the concentration and is 5 min at 2 µg of cysteine per ml. At low cysteine concentrations, threonine and isoleucine almost completely abolish the starvation.

Previously, we observed a larger incorporation of [^{35}S]methionine relative to the long-term [^{3}H]lysine incorporation for one β-galactosidase construct compared with another. This was somewhat surprising because the labelling conditions were made identical by mixing strains (10). The result was obtained with an inserted sequence with high cysteine content, and transfer of ^{35}S in the methionine to cysteine or a contamination of the [^{35}S]methionine might explain the result. We tried to eliminate this problem by adding cysteine but found the cell physiology to be disturbed, even with concentrations of cysteine several orders of magnitude lower than 60 µg/ml, previously reported to inhibit threonine deaminase (3). Figure 1 shows that addition of 2 µg of cysteine per ml increases the translation time for the *lacZ* mRNA significantly, from 82 to 95 s. This indicated a starvation condition, because particularly a *relA* strain, as used here, has a reduced peptide elongation rate during starvation (4). The accumulation of radioactivity into RNA and protein in response to cysteine was measured in an otherwise isogenic *rel*$^{+}$/*relA* pair of *E. coli* B, JF858 and JF859 (7). The response was as expected from their *rel* genotype for amino acid starvation (data not shown). Cells undergo the same starvation period when exposed to cysteine, whether they grow in glucose or in glycerol minimal medium (data not shown).

The expression of β-galactosidase is very sensitive to amino acid starvation (1), and we used this to assay the effect of cysteine. Figure 2 shows that 0.2 and even 0.04 µg of cysteine per ml prolong the β-galactosidase induction lag and that the induction kinetics is unaffected by cysteine when added 20 or 30 min before induction. Growth is transiently inhibited for approximately 20 min with 10 µg of cysteine per ml (Fig. 3, closed circles after time A) and for approximately 5 min with 2 µg of cysteine per ml (Fig. 4). These amounts of cysteine should not be this rapidly consumed in protein synthesis. After the starvation, the same growth rate as before is gradually achieved. Isoleucine and threonine diminish the effect of 10 µg of cysteine per ml (Fig. 3) and seem to abolish starvation at 2 µg/ml for the first few minutes, although the rate of protein synthesis seems slightly reduced at later times (Fig. 4). We also found that 50 µg of cysteine per ml (Fig. 3, time D) inhibited growth for a long time and that addition of threonine late in this severe cysteine response did not reverse starvation. Figure 3 also shows that cells adapted to 10 µg of cysteine per ml in the presence of 10 µg of threonine per ml could tolerate 50 µg of cysteine per ml without a severe starvation response, in contrast to when the cells adapted to 10 µg of cysteine per ml in the absence of threonine. We note from Fig. 3 that, even in the presence of threonine, a high cysteine concentration reduces the final growth rate by about 20% (Fig. 3, compare the broken line after time B with the normal growth curve represented by the solid line). This inhibition was seen even after exponential growth for 16 h (data not shown).

Cysteine was previously reported to be bacteriostatic, possibly because the threonine deaminase is inhibited by cysteine in high concentrations by a sulfhydryl effect (3). Harris (3) also found that a mutant which overproduces the enzyme is less sensitive to cysteine and that mixtures of threonine and isoleucine relieve the cysteine effect. We found this reversal of the starvation to be only partial and the effect of high cysteine concentrations complicated, possibly because of an effect on other enzymes or enzyme complexes (9). It is likely that the reason that homoserine shortens the duration of starvation (Fig. 2 and 4) is that it increases the pools of threonine and isoleucine after a lag period.

In Fig. 2, the time lag before the appearance of active β-galactosidase exceeded 3 min at 2 µg of cysteine per ml. This is twice the translation time measured in Fig. 1 with the same strain and amount of cysteine. The reason for this twofold difference is probably that other amino acids are being incorporated instead of threonine or isoleucine when cysteine is added. Mistranslation during starvation is a known *relA* phenotype (2, 6). No enzymatically active β-galactosidase molecule will therefore appear until one translation time after the starvation is terminated, i.e., after 3 to 4 min, if misincorporation in either end of β-galactosidase inactivates the enzyme. One conclusion from our work is, therefore, that the time of appearance of active β-galactosidase is not necessarily a measure of the translation rate. Threonine or homoserine also reduced the cysteine effect on the induction lag, although the rate of active β-galactosidase synthesis was reduced (Fig. 2C). This suggests that mistranslation still takes place to some degree at 1 µg of cysteine per ml in the presence of threonine, even if Fig. 4 showed that [^{3}H]lysine incorporation was close to normal.

Finally, we investigated whether cysteine in concentrations 10- and 100-fold lower than 0.04 µg/ml would be effective in a pulse-chase experiment with [^{35}S]cysteine. The incorporation of [^{35}S]cysteine was monitored by using different specific activities of [^{35}S]cysteine. The molar incorporation was almost proportional to the cysteine concentration, as expected if this were lower than the K_m for uptake (data not shown). Dilution of the specific activity to, e.g., one half will therefore give the same incorporation because

---

* Corresponding author.

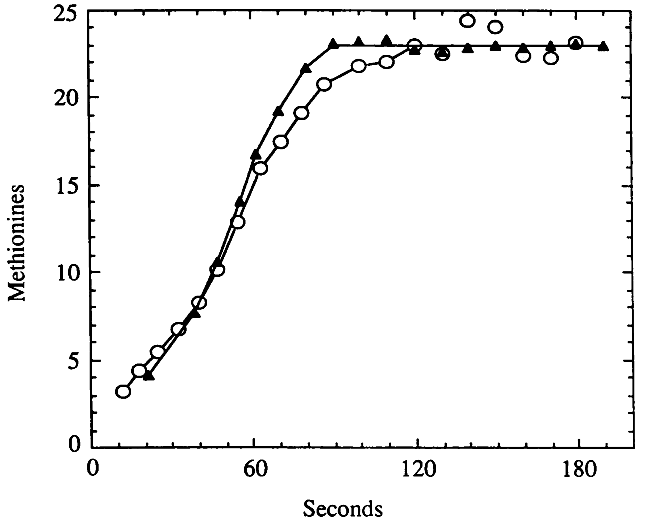

FIG. 1. Incorporation of [^{35}S]methionine into finished β-galactosidase protein after a 5-s pulse followed by a chase. Strain SP536/pMAS2 was used; it was grown at 37°C in glycerol medium and induced with 10^{-3} M isopropyl-β-D-thiogalactopyranoside (IPTG) 15 min before the experiment. The plasmid pMAS2 has the wild-type *lacZ* gene inserted into pBR322 (10). Isotopes were obtained from Dupont NEN, Inc. (Boston, Mass.). The incorporated radioactivity is converted to represent methionine residues (10), and these are plotted versus the time after the start of the pulse. The time it takes before the plateau is reached is the translation time. ▲, no cysteine added. ○, 2 μg of cysteine per ml added together with the [^{35}S]methionine. Cysteine (purity >99%) was from Merck.

twice as much cysteine is taken up. It is therefore conceivable that no concentration of cysteine is effective in a chase without having an adverse effect on growth. Addition of isoleucine or threonine in such experiments might diminish the otherwise dramatic effect on growth, but cell physiology should be carefully monitored in all short-term experiments exposing cells to even small concentrations of cysteine.

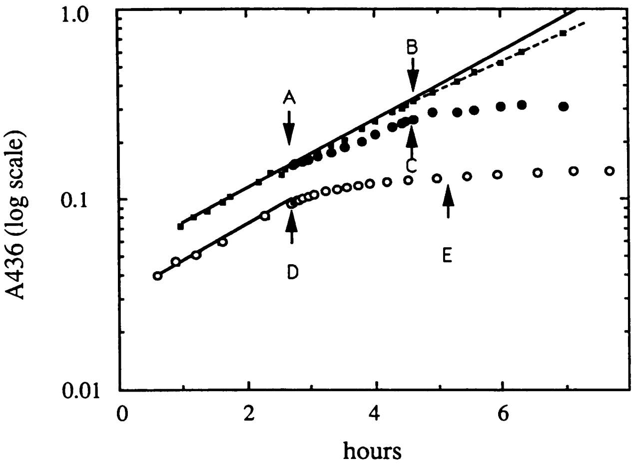

FIG. 3. Growth curve of SP536/pMAS2 growing in glycerol minimal medium at 37°C without addition of isopropyl-β-D-thiogalactopyranoside (IPTG). The results for two separate experiments are presented. At time A, half of the culture received 10 μg of cysteine per ml plus 10 μg of threonine per ml (■), whereas the other half received only 10 μg of cysteine per ml (●). At times B and C, cysteine was added to a final concentration of 50 μg/ml to both cultures. The solid line is an extension of the growth curve before time A; the broken line represents the growth curve after time B. In the other experiment (○), 50 μg of cysteine per ml was added at time D, and at time E, 50 μg of threonine per ml was added.

We have not determined the mechanism by which cysteine gives amino acid starvation, and at least two mechanisms are possible. One is that threonine deaminase is the target (3) for a sulfhydryl effect or, alternatively, that the reaction in which cysteine reacts with *O*-succinylhomoserine to give cystathione in the methionine pathway, in vivo is rate limited by cysteine, and that therefore cysteine addition transiently diverts all homoserine into this pathway, thereby inducing

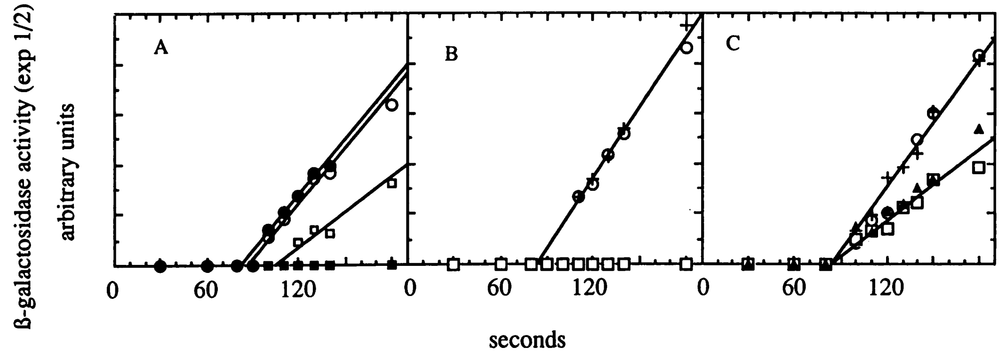

FIG. 2. Induction kinetics for β-galactosidase. To induce the *lac* operon we used 10^{-3} M isopropyl-β-D-thiogalactopyranoside (IPTG). Samples of aliquots were taken after induction into chloramphenicol (final concentration, 2.5 mg/ml) at 0°C. Cells were opened by sonication, and the β-galactosidase activity was assayed (5). The enzyme activity per ml at a normalized cell density was calculated and corrected for the basal level of β-galactosidase, and the square root versus the time of sampling was plotted. This gives a linearly rising curve permitting estimation of the induction lag (8). Strain SP536/pMAS2, grown in glycerol medium at 37°C, was used. (A) Induction of β-galactosidase when 1 (■), 0.2 (□), 0.04 (○), or 0 (●) μg of cysteine per ml is added together with the IPTG. (B) Induction of β-galactosidase when cysteine is added at various times relative to the inducer. Symbols: ○, no cysteine added; +, cysteine (2 μg/ml) added 20 min before IPTG; □, cysteine (2 μg/ml) added together with the inducer. (C) Simultaneous addition of IPTG and homoserine plus cysteine (▲) or IPTG and threonine plus cysteine (□) or addition of IPTG only (○) or of cysteine (+) 30 min before the inducer. All amino acids were added at final concentrations of 1 μg/ml.

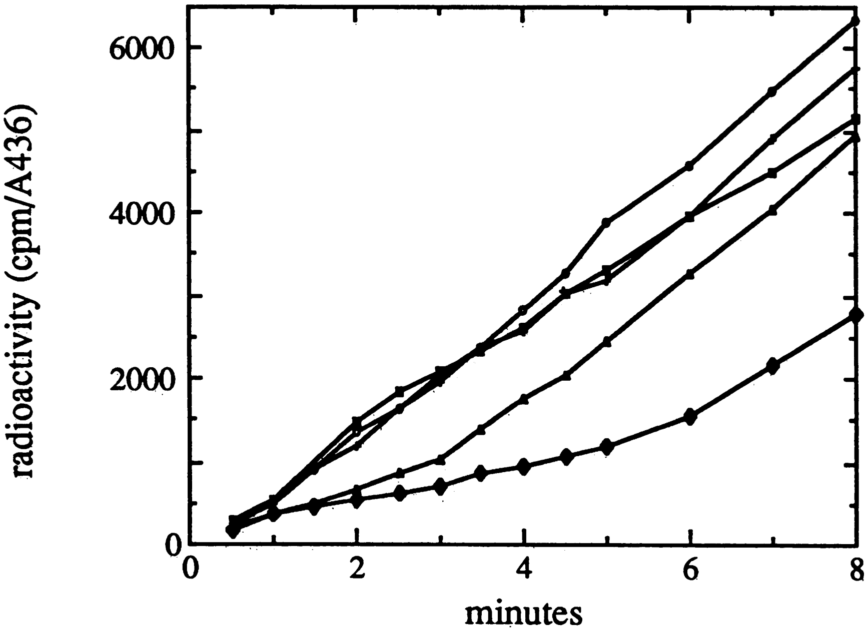

FIG. 4. Incorporation of $[^3H]$lysine (0.14 μCi/ml and 0.7 μg/ml) into trichloroacetic acid precipitable material in SP536/pMAS2 growing in glycerol medium at 37°C (normalized to the same cell density). At time 0, the following, together with the $[^3H]$lysine, was added: 2 μg of cysteine per ml (◆), 2 μg of cysteine plus 1 μg of threonine per ml (■), 2 μg of cysteine plus 1 μg of isoleucine per ml (+), 2 μg of cysteine plus 1 μg of homoserine per ml (▲), or nothing (○).

starvation for threonine or isoleucine. In either case, we fail to understand fully why threonine and homoserine do not reverse the effect of cysteine.

We thank M. Warrer for technical assistance and D. Weilguny and K. V. Rasmussen for helpful discussions.

The work was supported by the Danish Center of Microbiology.

REFERENCES

1. **Fiil, N.** 1972. A functional analysis of the *rel* gene in *Escherichia coli*. J. Mol. Biol. **45**:195–203.
2. **Hall, B., and J. Gallant.** 1972. Defective translation in RC-cells. Nature (London) New Biol. **237**:131–135.
3. **Harris, C. L.** 1981. Cysteine and growth inhibition of *Escherichia coli*: threonine deaminase as the target enzyme. J. Bacteriol. **145**:1031–1035.
4. **Johnsen, K., S. Molin, O. Karlström, and O. Maaløe.** 1977. Control of protein synthesis in *Escherichia coli*: analysis of an energy source shift-down. J. Bacteriol. **131**:18–29.
5. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
6. **O'Farrell, P. H.** 1978. The suppression of defective translation by ppGpp and its role in the stringent response. Cell **14**:545–557.
7. **Reeh, S., S. Pedersen, and J. D. Friesen.** 1976. Biosynthetic regulation of individual proteins in *relA*+ and *relA* strains of *Escherichia coli* during amino acid starvation. Mol. Gen. Genet. **149**:279–289.
8. **Schleif, R., W. Hess, S. Finkelstein, and D. Ellis.** 1973. Induction kinetics of the L-arabinose operon of *Escherichia coli*. J. Bacteriol. **115**:9–14.
9. **Singer, P. A., M. Levinthal, and L. S. Williams.** 1984. Synthesis of the isoleucyl- and valyl-tRNA synthetases and the isoleucine-valine biosynthetic enzymes in a threonine deaminase regulatory mutant of Escherichia coli K-12. J. Mol. Biol. **175**:39–55.
10. **Sørensen, M. A., C. G. Kurland, and S. Pedersen.** 1989. Codon usage determines the translation rate in *Escherichia coli*. J. Mol. Biol. **207**:365–377.