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

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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 [35S]methionine relative to the long-term [3H]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 35S in the methionine to cysteine or a contamination of the [35S]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 radioactive into RNA and protein in response to cysteine was measured in an otherwise isogenic relA/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 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. Mistranslating 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 [3H]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 [35S]cysteine. The incorporation of [35S]cysteine was monitored by using different specific activities of [35S]cysteine. The molar incorporation was almost proportional to the cysteine concentration, as expected if this were lower than the Km for uptake (data not shown). Dilution of the specific activity to, e.g., one half will therefore give the same incorporation because

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FIG. 1. Incorporation of $^{35}$Smethionine 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 minimal medium, similar to the other experiments described in this paper. Unlike the other inducers used in this paper, this strain was grown on a minimal medium with glycerol as the carbon source. The plasmid pMAS2 contains the wild-type lacZ gene inserted into the chromosome. With IPTG at 10$^{-3}$ M, the enzyme obtained from a bacterial cell culture was used for the measurement of the specific activity of the enzyme. The solid line is a plot of the time course of the specific activity of the enzyme measured in the experiment. The broken line represents the specific activity of the enzyme measured in the experiment. The time it takes before the plateau is reached is the translation time. A, no cysteine added; B, 2 μg of cysteine per ml added together with the [35S]methionine. Cysteine (purity >99%) was from Merck.

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 chloroform (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 (B). Strain SP536/pMAS2, grown in glycerol minimal medium at 37°C, was used. (A) Induction of β-galactosidase when 1 (●), 0.2 (□), 0.04 (○), or 0.0 (●) 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. 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. Cysteine should be carefully monitored in all short-term experiments exposing cells to even small concentrations of cysteine.

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

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