Cysteine and Growth Inhibition of *Escherichia coli*: Threonine Deaminase as the Target Enzyme

CHARLES L. HARRIS

Department of Biochemistry, West Virginia University School of Medicine, Morgantown, West Virginia 26506

Cysteine has been shown to inhibit growth in *Escherichia coli* strains C6 and HfrH 72, but not M108A. Growth inhibition was overcome by inclusion of isoleucine, leucine, and valine in the medium. Isoleucine biosynthesis was apparently affected, since addition of this amino acid alone could alter the inhibitory effects of cysteine. Homocysteine, mercaptoethylamine, and mercaptoethanol inhibited growth to varying degrees in some strains, these effects also being prevented by addition of branched-chain amino acids. Cysteine, mercaptoethylamine, and homocysteine were inhibitors of threonine deaminase but not transaminase B, two enzymes of the ilvEDA operon. Cysteine inhibition of threonine deaminase was reversed by threonine, although the pattern of inhibition was mixed. These results suggest a relationship between the growth-inhibitory effects of cysteine and other sulfur compounds and the inhibition of isoleucine synthesis at the level of threonine deaminase.

The growth of microorganisms in minimal medium is inhibited by the presence of certain amino acids, a subject recently reviewed by De Felice et al. (2). The best studied example is growth inhibition of *Escherichia coli* K-12 by valine (13), which is due to the inhibition of acetohydroxy acid synthase. This inhibition, in turn, leads to isoleucine starvation (7, 16). Strains that are resistant to valine have been shown to contain an acetohydroxy acid synthase isozyme that is not inhibited by valine. Although this system is fairly well understood, the metabolic reasons for growth inhibition by other amino acids remain obscure.

Over 20 years ago, Rowley observed that cysteine inhibited the growth of 40 strains of *E. coli*, and that this effect was prevented by methionine (12). Roberts et al., in their extensive studies of sulfur metabolism in *E. coli*, observed that cysteine was a potent inhibitor of growth, but that cystine or reduced glutathione was not (11). They further observed that cysteine prevented the conversion of methionine to isoleucine in *Torulopsis utilis*, but did not determine how this inhibition was mediated. More recently, Kanzaki and Anraku observed that the inhibition of growth of *E. coli* K-12 by cysteine was related to the valine sensitivity of the cell (5). This effect was overcome by the addition of a mixture of threonine, isoleucine, and leucine. These results suggest that cysteine or one of its metabolites has some direct effect on an enzyme involved in the biosynthesis of the branched-chain amino acids, although no evidence has yet been reported to support this. We provide evidence here that the growth-inhibitory effects of cysteine are overcome by isoleucine, leucine, and valine. These growth effects are shown to be related to the inhibition of threonine deaminase by cysteine.

**MATERIALS AND METHODS**

**Bacterial strains and growth.** The strains used in this study and their properties are as follows: *E. coli* HfrC relA Met+ Cys+ (λ) (hereafter called C6) was isolated in this laboratory (4). *E. coli* strains HfrH 72 lacZ (Am) Thi- φ80 φ90dyrT and M108A lacZ (Oc) ups-1 Tyr- φ80 φ80dupC were originally obtained from H. Echols of the University of California at Berkeley and have been maintained in this laboratory for a number of years. All strains were grown in the M9 medium of Anderson (1) and, except where noted, were supplemented with 20 μg of each required amino acid or vitamin per ml. Cultures were maintained at 37°C in a shaking water bath, and growth was monitored at 650 nm.

**Extract preparation and enzyme assays.** At the determined stage of growth, cultures were harvested by centrifugation at 12,000 × g for 10 min and washed once with M9 salts. Cell pellets were stored at −90°C until used, with no loss of enzyme activity being observed. Cells were thawed and suspended in 0.05 M Tris-hydrochloride buffer (pH 7.6) containing 0.05 M MgCl₂, 0.025 M KCl, 1 mM 2-mercaptoethanol, and 5% glycerol (1 to 2 ml/g of cells). The cell suspension was disrupted at 75 W with a Branson sonifier for five pulses of 20 s with a 20-s rest between pulses. Microscopic examination showed complete cell breakage under these conditions. The cell extract was clarified by centrifugation at 17,000 × g for 20 min, and the
supernatant solution was assayed for threonine deaminase and transaminase B as soon as possible, as described below.

Threonine deaminase assays were carried out by the method of Ratzkin et al. (10) under the following conditions. Assays were carried out in a total volume of 1.0 ml and contained 0.1 M Tris-hydrochloride (pH 8.0), 0.1 M NH₄Cl, 0.1 mM pyridoxal phosphate, and 0.04 M threonine. After the addition of 0.1 ml of cell extract, the tubes were incubated for 20 min at 37°C, after which 0.1 ml of 50% trichloroacetic acid was added. From 0.2 to 0.8 ml of this reaction mixture was diluted to 1 ml, and 0.5 ml of 0.025% 2,4-dinitrophenylhydrazine in 0.5 M HCl was added. After 15 min at 25°C, 1.0 ml of 40% NaOH was added and the resultant color was determined at 540 nm.

Transaminase B assays were carried out according to the method of Duggan and Wechler (3) and contained, per ml: 0.1 M Tris-hydrochloride (pH 8.0), 0.08 mM pyridoxal phosphate, 15 mM α-ketoglutarate, 25 mM L-valine, and 0.1 ml of cell extract. The reaction was stopped as above, and after color development, the amount of product formed was determined by comparison with a standard curve using α-ketoisovaleric acid. All enzymatic assays were carried out at enzyme levels at which the initial velocity of the reaction was proportional to protein concentration. The amount of protein in cell extracts was determined by the Bio-Rad method (Bio-Rad Laboratories, Richmond, Calif.), using bovine serum albumin as a standard.

Chemicals. Methionine was purchased from Schwarz-Mann, Orangeburg, N. Y. Other amino and α-keto acids were products of Sigma Chemical Co., St. Louis, Mo., as were pyridoxal phosphate and 2,4-dinitrophenylhydrazine. All other chemicals were of the highest quality commercially available.

RESULTS

Studies with growing cultures. The effect of cysteine on the growth of E. coli strains C6, HfrH 72, and M108A is shown in Fig. 1. Strain C6 is auxotrophic for cysteine, but still shows extensive growth inhibition in the presence of 0.48 mM cysteine. This inhibition is transient, as growth eventually reaches the same rate as the control culture. The addition of branched-chain amino acids completely eliminated growth inhibition by cysteine. High concentrations of isoleucine, leucine, and valine were necessary, possibly due to a transport defect for these amino acids in strain C6. Since methionine is also present in the growth medium for C6, the former amino acid did not prevent growth inhibition by cysteine (12). Strain HfrH 72 appeared to be more sensitive to cysteine than strain C6 (Fig. 1). Whereas growth inhibition was nearly complete, other experiments showed that longer incubation resulted in the eventual growth of strain HfrH 72 at the same rate as the control culture. The length of the growth delay with cysteine was shortened, but not eliminated, by the inclusion of methionine in the growth medium (data not shown). The reason for this effect is not known at present. Inhibition by cysteine was completely prevented by the addition of isoleucine, leucine, and valine to cultures of HfrH 72. Finally, the growth of strain M108A was not sensitive to growth inhibition by cysteine.

The specificity of this phenomenon was examined by determining the effect of various sulfur compounds on the growth of E. coli C6 and HfrH 72. Table 1 shows that the growth of strain C6 was inhibited by cysteine, homocysteine, and mercaptoethanolamine, but not by mercaptopropanol or cysteic acid. In other experiments, strain HfrH 72 showed a more complicated sensitivity: cysteine was the most inhibitory, mercaptopropanol and mercaptoethanolamine were slightly inhibitory, and homocysteine and cysteic acid were without effect. Cysteine was found to be less inhibitory than cysteine for both C6 and HfrH 72, whereas mercaptopropionate (0.33 mM) and glutathione (0.32 mM) were shown to have little effect on the growth of either strain (data not shown).

Since inhibition of growth by cysteine was overcome by the branched-chain amino acids, we determined their effect on growth inhibition by the sulfur compounds used above. In each instance where growth inhibition occurred, the addition of isoleucine, leucine, and valine (as in

![Graph](https://via.placeholder.com/150)

**Fig. 1. Effect of cysteine on growth of three E. coli strains.** Cultures were inoculated to give approximately comparable cell densities of strains C6, HfrH 72, and M108A and incubated at 37°C with vibratory shaking. The concentrations of branched-chain amino acids added were 0.5 mM for isoleucine and leucine and 1.0 mM for valine, except with strain C6, where the levels were 12.5 mM for isoleucine and leucine and 25 mM for valine. Symbols for C6: ○, 0.16 mM cysteine; ●, 0.48 mM cysteine; □, 0.48 mM cysteine plus isoleucine, leucine, and valine. Symbols for HfrH 72 and M108A: ○, control; ●, 0.15 mM cysteine; □, 0.16 mM cysteine plus isoleucine, leucine and valine.
the legend to Fig. 1) prevented the effect (data not shown). Therefore, the sulfur compounds tested appear to inhibit growth by interfering with some step in the isoleucine, leucine, and valine biosynthetic pathway. To learn which step is involved, each amino acid was added individually, or in combination, and the effect on growth inhibition of strain HfrH 72 by cysteine was determined. We found that isoleucine alone fosters the resumption of growth in the presence of cysteine, albeit after a long lag period. Leucine alone overcame the effects of cysteine also, but was less effective than isoleucine. Valine alone prolonged growth inhibition as expected, since strain HfrH 72 contains a valine-sensitive acetohydroxy acid synthase (2). The addition of isoleucine plus valine completely eliminated growth inhibition by cysteine, as did isoleucine plus leucine (data not shown). These results suggest that the primary effect of cysteine is on isoleucine synthesis, and that the sulfur amino acid might also inhibit some step on the branch of the pathway leading to leucine. The addition of valine plus isoleucine success- fully eliminated the effects of cysteine, presumably because of the reversibility of the valine transamination step. Thus, inclusion of valine would supply carbon units for leucine production by its conversion to α-ketoisovalerate (3, 11).

The results obtained here with cysteine are similar to those reported by Uzan and Danchin (17), who found that 10 mM serine caused growth inhibition in E. coli due to isoleucine limitation. The step in the metabolic pathway that was inhibited was not determined, although it was not at the level of threonine deaminase. Because serine supplies the carbon units for cysteine synthesis in E. coli (6), the effects of serine seen by Uzan and Danchin (17) could be due to its conversion to cysteine. If so, serine should inhibit the growth of strain HfrH 72 but not C6, since the latter mutant is unable to convert serine to cysteine (4). With strain C6, 1.0 mM serine inhibited growth after one cell doubling, whereas 10.0 mM serine was needed to attain the same inhibitory effect with strain HfrH 72 (data not shown). This is in agreement with Uzan and Danchin (17), who found that relA strains were more sensitive to serine than relA+ strains. Furthermore, the effect of serine and the lag usually caused by cysteine were both prevented in strain C6 by the addition of isoleucine, leucine, and valine. These results indicate that serine and cysteine both inhibit some step in the isoleucine and valine biosynthetic pathway, but their exact sites of action must differ.

Enzyme studies. The following experiments were carried out to determine which enzyme of the isoleucine and valine biosynthetic pathway was being inhibited by cysteine and the other sulfur compounds that affected growth of E. coli. Figure 2 shows that cysteine is an inhibitor of threonine deaminase, the first enzyme in the pathway leading to isoleucine. At 3.3 mM, cysteine caused a 68% inhibition of threonine deaminase activity, but had only a very slight effect on transaminase B activity (the last enzyme in the pathway). Cysteine caused equal inhibition of threonine deaminase activity in extracts from strains C6, HfrH 72, and M108A. However, the specific activity of this enzyme was 266 nmol/min per mg of protein in the latter.

**Table 1. Effect of sulfur compounds on the growth and threonine deaminase activity of E. coli**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Growth effect</th>
<th>Threonine deaminase activity (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C6</td>
</tr>
<tr>
<td>None</td>
<td>None</td>
<td>100.0</td>
</tr>
<tr>
<td>Cysteine</td>
<td>Inhibition</td>
<td>61.0</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>Inhibition</td>
<td>60.0</td>
</tr>
<tr>
<td>Mercaptoethylamine</td>
<td>Inhibition</td>
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<td>Mercaptoethanol</td>
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<tr>
<td>Cysteic acid</td>
<td>None</td>
<td>93.0</td>
</tr>
<tr>
<td>Cystine</td>
<td>Slight inhibition</td>
<td>231.0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Growth studies given above refer to strain C6 grown in M9 medium supplemented with 0.16 mM cysteine and 0.13 mM methionine. Results with strain HfrH 72 are discussed in the text. The concentrations of each sulfur compound were: cysteine, 0.33 mM; homocysteine, 0.3 mM; mercaptoethylamine, 0.35 mM; mercaptoethanol, 0.51 mM; cysteic acid, 0.24 mM; and cystine, 0.17 mM.

<sup>b</sup> Threonine deaminase assays were carried out in duplicate as described in the text, and the data are averages of two separate experiments. The activities of threonine deaminase in extracts from strains C6 and HfrH 72 (untreated controls) were 61.2 (n = 7) and 94.5 (n = 4) nmol/min per mg of protein. Each sulfur compound was added at 0.4 mg/ml, giving the following concentrations: cysteine, 3.3 mM; homocysteine, 3.0 mM; mercaptoethylamine, 3.5 mM; mercaptoethanol, 5.1 mM; cysteic acid, 2.4 mM; and cystine, 1.7 mM.

**Fig. 2. Effect of cysteine on threonine deaminase (○) and transaminase B (●) activities from E. coli C6.** Enzyme assays were carried out as described in the text, except that various concentrations of cysteine were added as indicated. The specific activities of threonine deaminase and transaminase B in the absence of cysteine were 72.0 and 79.9 nmol/min per mg of protein, respectively.
strain, in contrast to values of 61 and 75 nmol/min per mg of protein observed with strains C6 and HfrH 72, respectively. The presence of higher threonine deaminase levels in M108A may be responsible for the lack of growth inhibition by cysteine seen in Fig. 1.

Threonine deaminase activity was measured as a function of the concentration of threonine, both in the absence and presence of cysteine. Figure 3 shows the result of this kinetic study, in which the $K_m$ for threonine was estimated to be 5.55 mM and the $K_i$ for cysteine was 4.1 mM. The values for the apparent $K_m$ for threonine in the presence of 2 and 7.5 mM cysteine were 8.0 and 16.7 mM, respectively. It was thought that cysteine could conceivably be complexing with pyridoxal phosphate and hence depleting the coenzyme (14). However, addition of higher amounts of pyridoxal phosphate to reaction mixtures did not overcome the inhibition. Thus, cysteine may be inhibiting threonine deaminase in some other manner (a sulfhydryl effect) as its concentration is increased. Whatever the mode of inhibition, it is clear that cysteine did not alter the hyperbolic shape of the velocity-versus-substrate curve, suggesting that the inhibition is not the same as that observed with isoleucine (14).

The growth experiments summarized in Table 1 suggested that other sulfur compounds might also inhibit some step in the isoleucine and valine biosynthetic pathway, perhaps threonine deaminase. Table 1 shows that in addition to cysteine, homocysteine and mercaptoethalamine are both effective inhibitors of this enzyme. Both compounds inhibited growth in strain C6, but only mercaptoethalamine was inhibitory to strain HfrH 72. The reason for this discrepancy is not clear, but it may be due to an inability of the latter strain to take up homocysteine from the medium. Mercaptoethanol had no effect on growth or threonine deaminase activity. Cysteic acid was also without effect on growth or enzyme activity, demonstrating the importance of the reduced sulfhydryl group for both effects. Cysteine had a slight effect on growth, but was stimulatory to threonine deaminase. The stimulation was not due to the presence of mercaptoeryurate as a contaminant of cysteine (8), because no color was observed with reaction mixtures with cysteine but not enzyme. Similar experiments with mercaptoeryurate gave intense colors with enzyme, due to a direct reaction of the $\alpha$-keto compound with dinitrophenylhydrazine. The effect of cysteine on growth may be due to conversion to cysteine, although an inhibition of some other enzyme related to isoleucine synthesis cannot be ruled out. Hence, with the exceptions noted, there appears to be a correlation between growth inhibition by these sulfur compounds and inhibition of threonine deaminase.

**DISCUSSION**

The inhibition of growth of *E. coli* by cysteine is shown here to be overcome by branched-chain amino acids. Isoleucine alone is effective in this regard, although the inclusion of leucine further influences the release of growth inhibition. The growth effects have been correlated with an inhibition of threonine deaminase by cysteine. Transaminase B, the last enzyme in the pathway leading to isoleucine, was unaffected by cysteine. Because of the leucine effect, and the fact that several other enzymes are a part of the isoleucine and valine biosynthetic pathway (15), it is difficult to be certain that the growth effects are solely caused by cysteine inhibition of threonine deaminase. In support of this contention, we observed that *E. coli* M108A had a fourfold higher level of threonine deaminase than other strains tested, and was insensitive to cysteine in the medium. In this case, it is possible that insufficient cysteine was added to completely inhibit this enzyme. Other experiments showed that 1.0 mM $\alpha$-aminobutyrate eliminated the growth effect of cysteine on *E. coli* C6 (C. L. Harris, unpublished data). The former compound is converted to $\alpha$-ketobutyrate by *E. coli* (N. Charon, personal communication), thus supplying sufficient isoleucine to overcome the effect of cysteine on threonine deaminase. Finally,
preliminary results show that derepression of the ilvEDΔ operon occurs in E. coli C6 in the presence of growth-limiting concentrations of cysteine (Harris, unpublished data). Hence, cysteine treatment apparently lowers the isoleucine pool sufficiently both to inhibit growth and to exert an effect on the regulation of the ilvEDΔ operon (15).

The nature of the inhibition of threonine deaminase by cysteine is somewhat complex. At 2 mM cysteine, the inhibition was overcome by threonine, indicating a competitive inhibition (Fig. 3). However, at higher cysteine concentrations, the pattern of inhibition appears to be of the mixed type. Under these conditions, increasing the concentration of threonine and pyridoxal phosphate together was without effect. Hence, cysteine appears to have more than one inhibitory effect on this enzyme, but at present we are unable to make any definite conclusions as to their nature.

Previous reports indicated that methionine prevents the inhibition of growth by cysteine in E. coli (12). We failed to observe this effect in E. coli C6, a methionine auxotroph. Methionine did shorten the growth lag caused by cysteine with E. coli HfrH 72, suggesting that in this case methionine might eventually be able to supply isoleucine to allow growth to begin. We did not test this possibility directly, but others observed a conversion of methionine to ammonia, methyl mercaptan, and α-ketobutyrate in E. coli (9). This may be the mechanism whereby methionine overcomes cysteine inhibition in strain HfrH 72 and perhaps accounts for the lesser effects of cysteine on the growth of strain C6. That is, the latter strain may have the enzymes that degrade methionine already induced because it is present in the growth medium, whereas strain HfrH 72 requires some time to allow their synthesis.

Finally, the effects of structurally related amino acids on the growth and enzymatic activities in E. coli have been described (2). However, our observations suggest that amino acids synthesized from markedly different precursors may have effects on enzymes of other amino acid biosynthetic pathways. Since nutrients are important to growth regulation, it is possible that the inhibitions seen here may be of physiological significance. The amino acids in question are in different families, but are not totally unrelated (15). Serine is used in E. coli to produce cysteine, the sulfur of which is used in methionine synthesis. Homoserine is a precursor to both methionine and threonine synthesis, the latter amino acid being the first compound in the pathway leading to isoleucine. Whether the effects of cysteine and other related compounds are involved in a metabolic control of growth remains conjectural at present.

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LITERATURE CITED