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Inhibition of Respiration of *Escherichia coli* by Thioglycerol

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Anaerobic growth on glucose significantly protected *Escherichia coli* from growth inhibition by thioglycerol. Methionine and anaerobiosis completely overcame growth inhibition by 2 to 90 mM thioglycerol. The respiration of aerobically growing cells was partially inhibited by 20 to 90 mM thioglycerol.

Thioglycerol inhibits bacterial growth at 2 to 90 mM concentrations (4). Although thiols interact with numerous proteins, inactivation of proteins is not the cause of growth inhibition by 2 to 40 mM thioglycerol. Rather, it was found that thioglycerol lowered the intracellular concentrations of *S*-adenosylmethionine (SAM), thioglycerol itself becoming a major methyl acceptor molecule (3). Exogenous methionine reversed the growth inhibition by more than doubling the intracellular level of SAM, so that besides methylating thioglycerol, an ample amount of SAM remained to satisfy all demands for it.

Nevertheless, methionine supplementation could not significantly reverse the growth inhibition by 40 to 90 mM thioglycerol. This meant that at these concentrations, there was another mode of interaction between the cells and thioglycerol that resulted in growth inhibition. My results suggest that this second growth inhibitory site is the aerobic respiration system of the cell.

MATERIALS AND METHODS

Organisms and reagents. Organisms and reagents used were those described in the accompanying article (3).

Growth. Anaerobic growth was achieved by bubbling a mixture of 95% N_2 and 5% CO_2 gas through the cultures for 15 min and placing these cultures in test tubes (12.5 by 1.5 cm) capped with rubber septa. Whenever additions were made or samples were removed from the tubes, they were flushed with N_2-CO_2 gas before the septa were replaced. The tubes were gently agitated at 37°C in an upright position. The growth was monitored at 520 nm, and the percent inhibition of mean growth rate was calculated as described in the accompanying article (3).

Respiration. Oxygen uptake was measured by a Clark oxygen electrode attached to a YSI model 53 biological oxygen monitor (Yellow Springs Instrument Co.). Constant temperature (30°C) was maintained throughout the experiments with a Lauda K-2/R circulator. The dissolved oxygen levels were constantly monitored during measurements by a Curken model 250 recorder. For purposes of calculating the amount of oxygen uptake by cells, it was assumed that an optical density of 520 nm (OD_{520}) of 1 equaled 10^9 cells per ml.

RESULTS

The extent of growth inhibition by thioglycerol was influenced by the nature of the carbon-energy source used (Fig. 1). Cells proliferating on succinate were most inhibited by thioglycerol, and cells using glucose were the least sensitive. Succinate-grown cells depend on aerobic metabolism for biosynthesis and energy generation, whereas aerobically growing *Escherichia coli* cells on glucose still use glycolysis to meet the bulk of their energy needs (5) until they reach late-log and stationary phase (2). It was conceivable that the growth inhibition by thioglycerol of succinate-grown cells was enhanced by their exclusively aerobic metabolism. Therefore, we compared the inhibitory effect of thioglycerol on aerobically and anaerobically growing cells that were using glucose as their carbon-energy source. Anaerobiosis conferred substantial protection from thioglycerol (Fig. 2). Low concentrations (2 to 10 mM) were still somewhat inhibitory, but any further increase in thiol concentration was of no consequence to the cells.

To test whether the inhibition by 2 to 10 mM thioglycerol could be counteracted by methionine, anaerobically growing cells were exposed to thioglycerol in the presence of methionine. Exogenous methionine completely prevented inhibition of anaerobically growing cultures by 2 to 90 mM thioglycerol (Fig. 2).

Since aerobic growth apparently sensitized cells to thioglycerol, we measured the rate of oxygen uptake by cells growing on glucose, exposed to this thiol at 2 to 90 mM. Thioglycerol above 10 mM immediately hindered oxygen uptake (Fig. 3). Sixty minutes of exposure of cells to thioglycerol caused a decline in respiration at all concentrations used (Fig. 3).

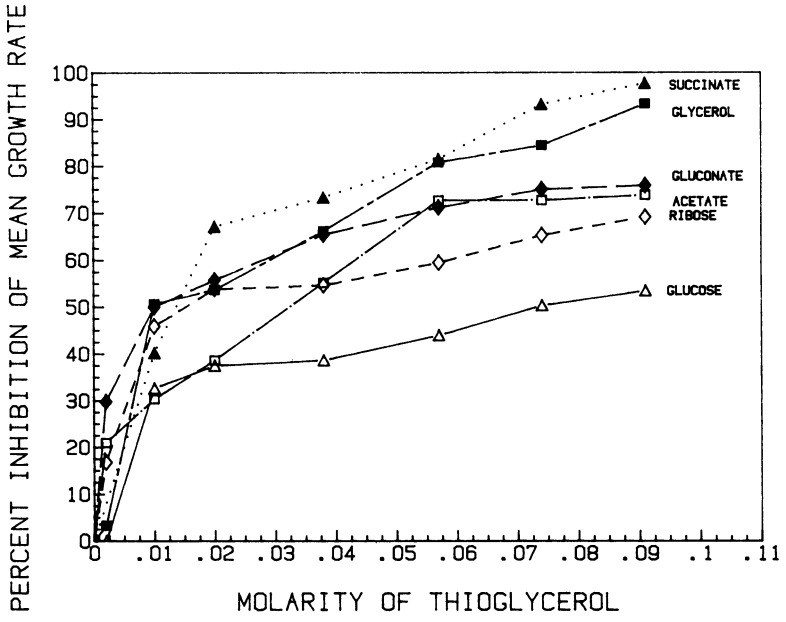

FIG. 1. The relationship between the carbon-energy source and the growth inhibitory effect of thioglycerol. Cultures (45 ml each) growing logarithmically in the presence of air and on the carbon energy source indicated (0.3% [wt/vol]) to OD = 0.21 were divided into 5-ml portions. Each aliquot received thioglycerol to the final concentration indicated, and the turbidity of each culture was monitored.

We measured the uptake of radioactive mercaptoethanol (a close analog of thioglycerol) into aerobically or anaerobically growing *E. coli* cells. There were no substantial differences in the amounts retained by either culture (data not shown).

DISCUSSION

There are at least two possible explanations for the resistance of *E. coli* to thioglycerol during anaerobic growth. If the permeability of *E. coli* to thioglycerol decreased during anaero-

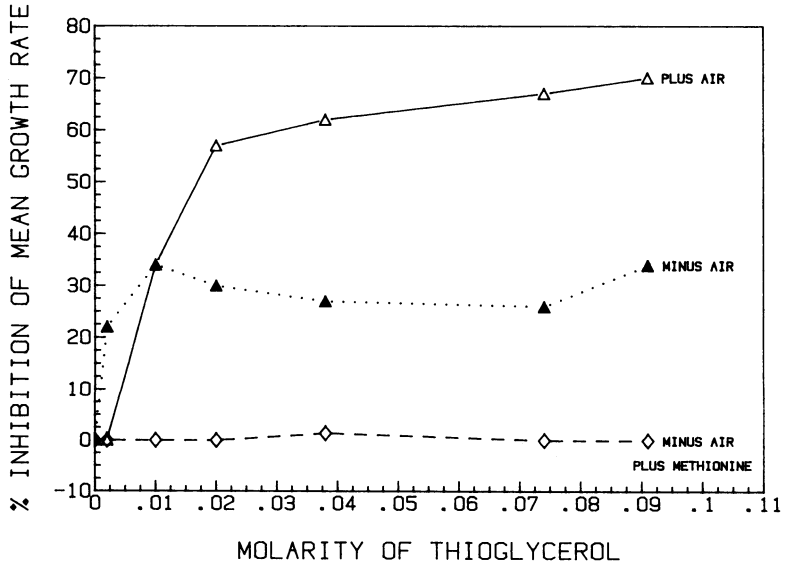

FIG. 2. Protection of *E. coli* THU from thioglycerol by anaerobic growth and by methionine. The experiment described in the legend to Fig. 1 was repeated on three cultures, all growing on glucose, one in the presence of air and two in the absence of air. One of the anaerobic cultures was supplemented with 2.7 mM methionine.

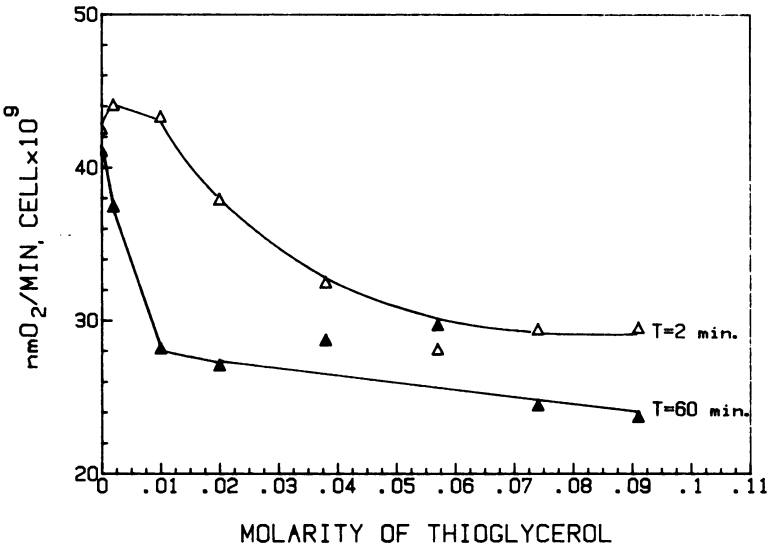

FIG. 3. Inhibition of respiration by thioglycerol. From 45-ml cultures of *E. coli* THU growing logarithmically at 30°C in the presence of air, using glucose as carbon energy source, 5-ml portions were removed and mixed with increasing amounts of thioglycerol. The rates of oxygen uptake were determined either immediately (within 2 min of mixing) or after 60 min of growth in the presence of thioglycerol.

bic growth, the smaller amounts of intracellular thiol would inhibit the growth to a lesser degree. The second explanation is that thioglycerol may hinder oxygen utilization, and during anaerobic growth, the target of thioglycerol is nonfunctional or not present in the cell. The two explanations are not mutually exclusive, and in theory, both may contribute to the observed phenomenon. Since anaerobic growth did not appear to hinder the uptake of radioactive mercaptoethanol (a close analog of thioglycerol), it seemed likely that thioglycerol acted primarily by blocking oxygen utilization.

By determining the rate of oxygen intake, I found that respiration was rapidly inhibited by thioglycerol at concentrations of 10 mM and higher. The extent of inhibition increased during prolonged exposure to thioglycerol, and even 2 mM thioglycerol (the lowest concentration tried) inhibited respiration somewhat.

Within the respiratory apparatus, the target of thioglycerol is likely to be the cytochrome oxidase since glutathione, cysteine, 2,3-dimercaptoethanol, thioglycolate (1), and various alkane thiols (6) are known inhibitors of this enzyme. In vitro studies will be necessary to confirm this notion.

Thioglycerol (2 to 10 mM) possibly lowered the intracellular SAM concentration in anaerobically growing cells, because exogenous methionine successfully counteracted the growth inhibition. There were no other growth inhibitory targets for thioglycerol in anaerobically growing cells. The finding that methionine abolishes inhibition by thioglycerol during anaerobic growth may be helpful in studies in which this thiol is added to cells to maintain a reducing environment and in which growth inhibition is undesirable.

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