Effect of the Thiol-Oxidizing Agent Diamide on the Growth of Escherichia coli

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Oxidation of glutathione within Escherichia coli cells by diamide, (CH$_3$)$_2$NCON$\equiv$NCON(CH$_3$)$_2$, stops growth but does not cause cell death. Normal growth rates are resumed after periods which vary in length according to the diamide concentration. Consumption of excess reagent with added glutathione quickly reverses the inhibition. Another thiol-oxidizing agent, azoester, C$_6$H$_5$N$\equiv$NCOOCH$_3$, causes lysis.

There is almost a complete lack of information concerning the role of intracellular low molecular weight thiols, of which the major component is glutathione (GSH), in the functioning of the cell, except for suggestions that such thiols may play some part in cell division (9). One difficulty in elucidating the significance of high intracellular GSH concentrations [ca. $10^{-4}$ M in Escherichia coli (10); ca. $10^{-4}$ M in human red blood cells (3)] is due to the lack of a reagent suitable for the removal of intracellular GSH without damage to cellular systems. Kosower and Kosower recently developed a series of reagents which oxidize intracellular GSH to glutathione disulfide (GSSG) (2–8). The stoichiometry found for the reaction of one of these reagents, diamide, with GSH within the mature human red blood cell corresponds almost exactly to that expected from equation 1 (Kosower et al., Biochem. Biophys. Res. Commun., in press):

$$2 \text{GSH} + (\text{CH}_3)_2\text{NCON} \quad \text{NCON(\text{CH}_3)_2} \rightarrow$$
$$\text{GSSG} + (\text{CH}_3)_2\text{NCONHNCON(\text{CH}_3)_2}$$

(1)

The indirect evidence of the stoichiometry of the reaction with intracellular GSH and the direct evidence of the failure of hemoglobin SH groups to react with azoester (see below) demonstrate the specificity of this series of thiol-oxidizing agents to GSH and equivalently reactive low molecular weight thiols. It may be noted that cysteine reacts at about one-half the rate of GSH with azoester (6).

A concentrated solution of diamide was added to a culture of E. coli B growing exponentially in minimal medium M-9 (1) at a density of $10^8$ cells/ml. Suspensions were incubated at 37 C, and growth was followed by the optical density at 625 nm with a Zeiss spectrophotometer. The effects produced by diamide concentrations between $10^{-4}$ and $6 \times 10^{-4}$ M on the growth of E. coli B are shown in the optical density versus time curves illustrated in Fig. 1.

Concentrations of diamide as high as $3 \times 10^{-3}$ M have been tested against E. coli B. If the diamide was removed from the cells by washing on a membrane filter (Millipore Corp., Bedford, Mass.) or by adding excess GSH (to consume the reagent according to equation 1), growth resumed. Filtration gave cells which lagged slightly in attaining normal growth; cell suspensions from which the diamide had been removed by the addition of GSH resumed normal growth rates within 10 min. If, in addition to GSH, the culture was treated with a mixture of sulfur amino acids (cysteine and methionine, each at 20 µg/ml), no increase in subsequent growth rate was observed. Colony counts made after 15-min treatments of E. coli B suspensions with high concentrations of diamide ($3 \times 10^{-3}$ M) showed that no death had occurred. Microscopic observation indicated that cells recovering from a diamide-induced lag looked normal and no increase in frequency of filaments was evident.

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Another thiol-oxidizing agent, azoester, \( \text{C}_6\text{H}_5\text{N}==\text{NCOOCH}_3 \) inhibited growth at concentrations above \( 10^{-4} \text{ M} \) (7, 8). With this reagent, inhibition of growth was accompanied by a fairly rapid lysis (ca. 50% in 1.8 hr). Azoester, in excess of that required for complete oxidation of the intracellular GSH, produces lysis in many oxygen-deficient cells, such as carbon monoxide-loaded red blood cells suspended in oxygenated buffer (5).

It is not now possible to specify the molecular mechanisms through which inhibition of growth occurs. It is conceivable, for example, that diamide reacts with the small intracellular pool of cysteine and homocysteine and thereby inhibits protein synthesis and growth. We believe that a more important factor in growth inhibition is GSH, which is intimately involved in maintaining certain parts of the growth system and, in particular, protein synthesis (Zehavi, Kosower, and Kosower, unpublished data). The length of the lag during which no growth occurs almost certainly reflects the rate at which the culture regenerates its low molecular weight thiols and thereby the rate at which excess diamide may be consumed.

Diamide appears to be a useful reagent for microbiological investigations.

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**LITERATURE CITED**


**Fig. 1.** Growth after diamide addition. A culture of *E. coli B* growing exponentially in M9 medium at 37 C was divided into separate flasks on a reciprocal shaker. Sufficient diamide was added to achieve the following final concentrations: ○, no diamide; △, \( 10^{-4} \text{ M} \); ●, \( 2 \times 10^{-4} \text{ M} \); ○, \( 3 \times 10^{-4} \text{ M} \); △, \( 4 \times 10^{-4} \text{ M} \); X, \( 5 \times 10^{-4} \text{ M} \); □, \( 6 \times 10^{-4} \text{ M} \).