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ARTICLE *in* FEBS LETTERS · AUGUST 1970

Impact Factor: 3.17 · DOI: 10.1016/0014-5793(70)80323-4 · Source: PubMed

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THE INHIBITION OF RIBONUCLEIC ACID SYNTHESIS BY THE THIOL-OXIDIZING AGENT, DIAMIDE, IN *ESCHERICHIA COLI*

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Received 12 June 1970

The thiol-oxidizing agent, diamide, has been used to convert glutathione to glutathione disulfide within the cells of a stringent strain of *Escherichia coli* (CP 78), leading to a cessation of ^{14}C-leucine incorporation (protein synthesis) and ^{3}H-uracil incorporation (RNA synthesis). Parallel experiments with an isogenic relaxed strain (CP 79) gave similar results, providing evidence that glutathione is closely linked to RNA synthesis indepently of the link previously shown to protein synthesis.

A series of thiol-oxidizing agents has been utilized to convert intracellular glutathione into the disulfide, thereby revealing many heretofore unsuspected biochemical and biological functions for glutathione [1]. The reaction of one of these compounds, diamide, with glutathione (GSH) is illustrated in Eqn. 1 [2].

$$2GSH + (CH_3)_2NCON{=}NCON(CH_3)_2 \rightarrow GSSG + (CH_3)_2NCONHNHCON(CH_3)_2 \quad (1)$$

In the case of rabbit reticulocytes, diamide oxidation of GSH to GSSG leads to a lag in protein synthesis behind the regeneration of GSH. Substantial excesses of diamide produce not only a lag but a lowered rate of protein synthesis [3]. It seemed likely that inhibition of protein synthesis in the absence of GSH explained the inhibition of the growth of *E. coli* after diamide treatment [4]. However, we wished to probe a possible role for GSH in RNA synthesis, and therefore have carried out an investigation of the effect of diamide on a relaxed mutant of *E. coli,* in which RNA synthesis may proceed whether or not protein synthesis is under way [5].

We have examined the effect of diamide on ^{14}C-leucine incorporation (as a measure of protein synthesis) and ^{3}H-uracil incorporation (as a measure of RNA synthesis) in an isogenic mutant pair. One member of the pair is stringent (CP 78), in which RNA synthesis is seriously diminished in the absence of a required amino acid. The relaxed mutant (CP 79), like the stringent mutant, requires leucine, arginine, threonine, thiamine and histidine [6].

Cells were grown in a reciprocal shaker at 37° in M-9 medium [7]. After the cultures had reached an absorbancy of 0.1 at 625 nm, the cells were harvested by spinning at 10,000 rpm (Sorvall), washed with phosphate buffer, 0.01 M, pH 6.7, and spun down again. Washing and centrifugation were repeated twice more, after which the cells were suspended in fresh medium lacking threonine. Each culture was divided into four parts and additions were made as follows: (A) +threonine, –diamide (B) –threonine, +diamide (C) +threonine, +diamide (D) –threonine, –diamide. The final concentration of diamide was 10^{-3} M, a level sufficient to inhibit growth for more than six hours [4]. The present study was limited to the first 90 min after additions.

Uracil was present in the medium at a level of 10 μg/ml with 2.5 μCi/ml ^{3}H-uracil. Leucine was added

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North-Holland Publishing Company – Amsterdam

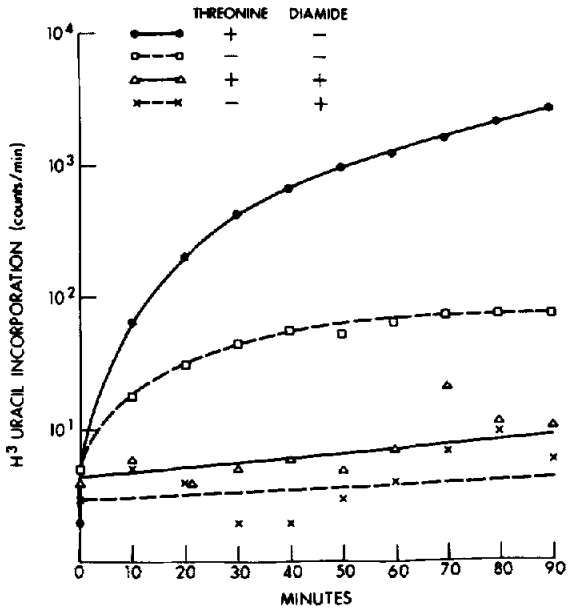

Fig. 1. ^{3}H-Uracil incorporation into a stringent strain of *E. coli* (CP 78) versus time. Additions to the medium are shown.

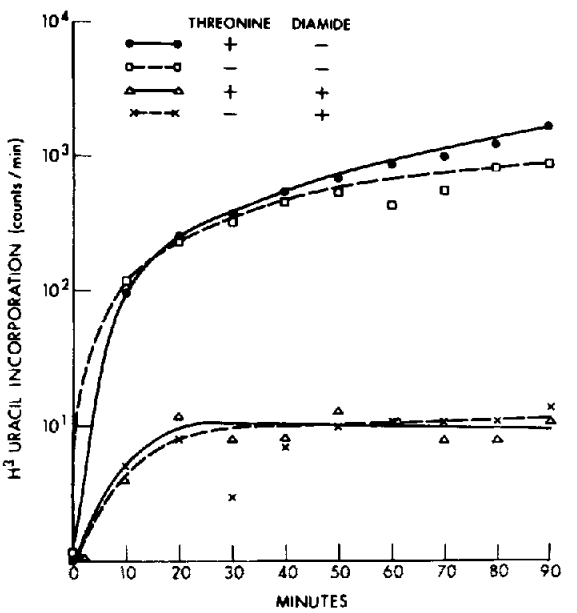

Fig. 2. ^{3}H-Uracil incorporation into a relaxed strain of *E. coli* (CP 79) versus time. Additions to the medium are shown.

to give a concentration of 10 μg/ml containing 0.1 μCi/ml ^{14}C-leucine. Cold 10% trichloracetic acid was added to 0.1 ml samples placed on Whatman 3 MM paper discs to precipitate protein and nucleic acid.

In the stringent strain (CP 78), as illustrated in fig. 1, restitution of threonine to the medium led to the incorporation of ^{3}H-uracil. Omission of the threonine diminished markedly the extent of the incorporation. Addition of diamide, thus converting the GSH to GSSG, led to complete loss of ^{3}H incorporation either in the presence or in the absence of threonine. Parallel results (not illustrated) were found for ^{14}C-leucine incorporation.

In the relaxed strain (CP 79), as expected, incorporation of ^{3}H-uracil was unaffected by threonine deprivation (fig. 2). The diamide effect on the incorporation of ^{3}H-uracil was just as dramatic for the relaxed strain as it had been for the stringent strain; almost no radioactivity was incorporated either in the presence or the absence of threonine. Diamide also abolished ^{14}C-leucine incorporation.

The simplest interpretation of these results is that GSH is closely linked to RNA synthesis. One possibility is that GSH serves as a cofactor in a key step. Alternatively, an increase in the intracellular pool of uracil or its derivatives through increased degradation of intracellular RNA might inhibit the transfer of external uracil into the cell [8]. Another plausible explanation could involve a requirement for GSH as an agent for conformational change through a thiol-disulfide interchange reaction, which might or might not involve an enzyme system directly concerned with RNA synthesis. The polymerization of triphosphates to RNA is not, apparently, the step which is directly affected since 10^{-3} M diamide does not affect the incorporation of label into RNA by purified RNA polymerase over 20 min in a mixture containing mixed triphosphates, DNA, Mg^{2+}, tris buffer and 10^{-4} M dithiothreitol [9].

Whatever the explanation on the molecular level, it seems clear that glutathione is a vital cellular component and is absolutely required for normal cellular function.

Acknowledgement

We thank Dr. M.Revel, Prof. U.Littauer, Prof. D. Elson and Prof. D.Danon for the facilities afforded us

in this study and Prof. N.S.Kosower for valuable advice.

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