Intracellular Concentration of Cysteine in *Escherichia coli* and its Relation to Repression of the Sulphate-Activating Enzymes

By J. F. WHELDRAKE*

Department of Biochemistry, University of Oxford

(Received 26 April 1967)

1. The intracellular cysteine and glutathione concentrations were measured in *Escherichia coli* under a variety of growth conditions. 2. An inverse relation between intracellular cysteine concentration and the specific activity of the sulphate-activating enzymes was found. 3. This is compatible with the view that the intracellular cysteine concentration controls the rate of synthesis of these enzymes.

In *Escherichia coli* the synthesis of the enzymes catalysing the reduction of sulphate to sulphide is repressed by cysteine (Mager, 1960; Pasternak, 1961, 1962; Kemp, Atkinson, Ehret & Lazzarini, 1963; Ellis, Humphries & Pasternak, 1964; Pasternak, Ellis, Jones-Mortimer & Crichton, 1965; Wheldrake & Pasternak, 1965). Pasternak *et al.* (1965), who showed that the sulphate-reducing enzymes are not all repressed in a co-ordinate manner, suggested that a mechanism of 'differential repression' might operate. They envisaged a situation whereby intermediates of the pathway, such as sulphite and sulphide, give rise to higher intracellular concentrations of cysteine than does sulphate, the starting material, and suggested that the synthesis of the early enzymes on the pathway is sensitive to lower concentrations of cysteine than is the synthesis of the later enzymes. This mechanism explains how cells can grow on intermediates of the biosynthetic pathway without synthesizing the unnecessary early enzymes. One prediction of this model is that the amounts of the sulphate-reducing enzymes will vary inversely with the intracellular cysteine concentration, which itself will vary with growth conditions. This prediction has now been tested and the results are reported below.

Two different approaches were used to measure the intracellular cysteine concentration. In the first, cells were treated with ^{14}C -labelled NEM† and the adducts formed with cysteine and other reduced sulphur compounds separated by two-dimensional paper chromatography. In the second, cells were grown in the presence of $^{35}\text{SO}_4^{2-}$ of known specific activity until the radioactivity in the intracellular

cysteine had reached a constant value. Non-radioactive NEM was then added and the ^{35}S -labelled NEM adducts were separated by two-dimensional paper chromatography as before. Both methods gave essentially similar results.

MATERIALS AND METHODS

Chemicals. [^{14}C]NEM (4.7 $\mu\text{C}/\mu\text{mole}$) was obtained from Schwarz Bio-Research Inc., Orangeberg, N.Y., U.S.A. It contained some radioactive impurities, but these do not move in or near the region corresponding to the cysteine or GSH adducts of NEM in the chromatography system used. NEM was obtained from Koch-Light Laboratories Ltd., Colnbrook, Bucks.

$\text{Na}_2^{35}\text{SO}_4$ (carrier-free) was obtained from The Radiochemical Centre, Amersham, Bucks.

Growth of organisms. *E. coli* A.T.C.C. 9723 was grown and maintained as described by Ellis *et al.* (1964). Cultures (600 ml. in 21. flasks) were incubated overnight at 37° on a Gyrotary shaker (New Brunswick Scientific Co., New Brunswick, N.J., U.S.A.) and harvested towards the end of exponential phase (0.1–0.4 mg. dry wt./ml.). The cells from 200 ml. were resuspended in 1–2 ml. of minimal medium (Pasternak, 1962) and subsequently used for determination of the intracellular cysteine concentration. The cells from 400 ml. were disrupted and used to assay the enzymes catalysing the formation of PAPS from sulphate and ATP.

Assay of intracellular concentrations. Resuspended cells (0.25 ml.) were diluted to 5 ml. with minimal medium containing glucose (0.2%) and except for GSH-grown cells the sulphur compound used for growth. The concentration was that used for growth, except that 0.2 mM- Na_2SO_3 and 0.2 mM- Na_2S were used. Na_2SO_4 (1 mM) was added for GSH-grown cells. [^{35}S]Sulphate (500–1000 μC) was added where indicated. This suspension was incubated in a shaking water bath at 37°, and samples were removed at intervals and added to 1.2 ml. of ethanol containing either 20 μmoles of NEM in the presence of $^{35}\text{SO}_4^{2-}$ or 0.21 μmole of [^{14}C]NEM in its absence. After 2–3 hr. at room temperature the

* Present address: Department of Biochemistry, University of Adelaide, Adelaide, South Australia.

† Abbreviations: NEM, *N*-ethylmaleimide; PAPS, adenosine 3'-phosphate 5'-sulphatophosphate.

mixture was centrifuged and 1 ml. of the supernatant transferred to Whatman no. 3 paper, together with an L-methionine marker (10 μ g.). Chromatography and location of the radioactive areas were carried out as described below. The quantity of cysteine present was calculated from the radioactivity of the area corresponding to NEM-cysteine determined with an end-window probe (efficiency 1.3%) and, if the counts were less than 10 times background, by cutting out the area, eluting with 5 ml. of water and plating out samples (0.2–0.5 ml.) which were then dried and counted (low-background automatic sample changer; Isotope Developments Ltd.) (efficiency 4.7%). Standards were performed by treating 0.21 μ mole of [14 C]NEM with 0.1 μ mole of cysteine and carrying out the above procedure. This gave 103 500 counts/min./ μ mole on paper and 388 000 counts/min. after elution, compared with expected values of 134 000 counts/min. and 484 000 counts/min. respectively, based on a 14 C standard solution (The Radiochemical Centre) and the specific activity of the [14 C]NEM. A blank for [14 C]NEM + medium without cells was also performed. This gave a value of only 20% greater than background, corresponding to 0.02 n-mole of cysteine.

Chromatography and location of radioactive compounds. Two-dimensional paper chromatography as described by Margolis & Mandl (1958) was used. The solvents were: (1) ethanol–2-methylpropan-2-ol–aq. ammonia (sp.gr. 0.88)–water (300:100:4:75, by vol.) followed by (2) 2-methylpropan-2-ol–formic acid (90%, w/v)–water (14:3:3, by vol.). The chromatograms were sprayed with ninhydrin to locate the methionine that was used as a marker, since NEM-cysteine does not give a colour under these conditions (Ellis, 1966). The chromatograms were then subjected to radioautography for 3–6 weeks. The radioactive area that moves the same distance as methionine in solvent (1) and just ahead of it in solvent (2) was taken to be NEM-cysteine (Ellis, 1966).

Enzyme assays. The enzyme system catalysing the synthesis of PAPS from sulphate and ATP was assayed as described by Pasternak (1962).

Protein was estimated as described by Wheldrake & Pasternak (1965).

Cell density determination. Cell density was determined nephelometrically by means of an EEL Unigalvo type 20 instrument calibrated against a Perspex standard set at 25.

RESULTS

Comparison of [14 C]NEM and [35 S]sulphate methods. The intracellular cysteine concentration was found to be so small under certain conditions that it could not be accurately measured with [14 C]NEM at the specific activity available, and it was therefore necessary to use the $^{35}\text{SO}_4^{2-}$ method, for which material of higher specific activity can be used.

To demonstrate that the two methods gave comparable results the intracellular GSH concentration was determined in mid-exponential-phase *E. coli* grown from a small inoculum in one culture on 1 mM- $\text{Na}_2^{35}\text{SO}_4$ (10 $\mu\text{C}/\mu\text{mole}$) and in a second culture on 1 mM- Na_2SO_4 . Samples of the cultures (0.4 ml.) were transferred directly into

Table 1. *Time-course for equilibration of cysteine concentration in E. coli*

Cultures of cells grown overnight as indicated were harvested, resuspended and incubated with 1 mM- $\text{Na}_2^{35}\text{SO}_4$ or 0.42 mM-cystine for the times shown. Intracellular cysteine concentration was determined by treating with unlabelled NEM or [14 C]NEM respectively.

Time (min.)	Concn. of cysteine (n-moles/mg. dry wt.)	
	Grown on 1 mM- Na_2SO_4	Grown on 0.42 mM-cystine
5	0.018	27.2
10	0.031	31.0
15	0.112	
30	0.232	
45	0.277	
60	0.255	

1.2 ml. of ethanol containing 20 μ moles of NEM or 0.21 μ mole of [14 C]NEM respectively. The values obtained for the GSH concentration were 49.2 and 50.2 n-moles/mg. dry wt. respectively.

Intracellular cysteine concentration. The time required to achieve steady-state conditions was normally less than 5 min. although it was 30 min. for sulphate (Table 1).

Table 2 shows the steady-state concentrations of cysteine and GSH in the cell during growth on various sulphur sources. The cysteine concentration of GSH-grown cells was measured in cells grown on GSH and transferred to $^{35}\text{SO}_4^{2-}$. This represents a maximum value for that concentration, since the sulphate-reducing enzymes are depressed under these growth conditions and the rate of sulphur incorporation from GSH is lower than that from sulphate.

The methionine concentrations in cells growing on sulphate was also determined and found to be 0.65 n-mole/mg. dry wt.

Repression of PAPS-synthesizing enzymes. The results are shown in Table 2. The values are similar to those obtained by Ellis *et al.* (1964).

DISCUSSION

Although it has been assumed that the intracellular concentration of the end product of a biosynthetic pathway is the factor controlling the synthesis of its (biosynthetic) enzymes (Gorini & Maas, 1957; Novick & Maas, 1961) there do not seem to have been many attempts to correlate the actual concentration with the specific activity of these enzymes. Intracellular amino acid concentrations have been measured in many organisms under one specified set of conditions (Holden, 1962); Britten & McClure (1962) showed that the intracellular proline concentration is higher in cells

Table 2. *Comparison of intracellular concentrations of cysteine and GSH with PAPS-synthesizing ability of E. coli*

Cultures of cells were grown overnight as indicated, and intracellular cysteine and GSH concentrations and PAPS-synthesizing ability were determined as described in the Materials and Methods section. The results for intracellular concentrations are expressed as means $\pm$ s.e.m. with the numbers of determinations in parentheses. N.D., Not determined.

Sulphur source for growth	Concn. of cysteine (n-moles/mg. dry wt.)	Concn. of GSH (n-moles/mg. dry wt.)	PAPS synthesized (n-moles/mg. of protein/min.)
1 mM-Na ₂ SO ₄	0.22 $\pm$ 0.017* (6), < 0.4 (2)	86.5 $\pm$ 12.3 (4)	1.54
0.67 mM-Methionine + 1 mM-Na ₂ SO ₄	0.644 $\pm$ 0.064* (7)	N.D.	0.22
0.8 mM-Na ₂ SO ₃	1.53 $\pm$ 0.17 (4)	14.8 $\pm$ 1.4 (4)	0.07
4.0 mM-Na ₂ S	1.55 $\pm$ 0.30 (4)	17.8 $\pm$ 2.2 (4)	0.04
0.42 mM-Cystine	21.2 $\pm$ 3.7 (5)	54.7 $\pm$ 12.6 (5)	< 0.01
0.2 mM-GSH	0.037 $\pm$ 0.0025* (6), < 0.2 (1)	N.D.	5.23

* Determined with ³⁵SO₄²⁻; all other values were determined with [¹⁴C]NEM.

grown in the presence of proline than in those grown in its absence.

The results in Table 2 show that, in the cysteine-biosynthetic pathway, there is an inverse correlation between the specific activity of the sulphate-activating enzymes and intracellular cysteine concentration in *E. coli*. This is evidence in favour of the mechanism of repression proposed by Pasternak *et al.* (1965), although it does not rule out the possibility that sulphide, which was not measured, might be the repressor.

This technique does not attempt to distinguish between cysteine and cysteinyl-transfer-RNA as repressors (cf. Neidhardt, 1966). However, it seems likely that, below some saturating level, the concentration of cysteinyl-transfer-RNA will be closely related to the cysteine concentration.

The reason for the marked difference in GSH concentration in sulphide-grown and sulphite-grown cells as opposed to those grown on sulphate or cystine is not clear.

I thank Dr C. A. Pasternak and Dr R. J. Ellis for their invaluable advice and comment during this work. I was a

graduate scholar of St Cross College, Oxford, during part of this work, which was supported by the Medical Research Council.

REFERENCES

- Britten, R. J. & McClure, F. T., (1962). *Bact. Rev.* **26**, 292.
 Ellis, R. J. (1966). *Nature, Lond.*, **211**, 1266.
 Ellis, R. J., Humphries, S. K. & Pasternak, C. A. (1964). *Biochem. J.* **92**, 167.
 Gorini, L. & Maas, W. K. (1957). *Biochim. biophys. Acta*, **25**, 208.
 Holden, J. T. (1962). In *Amino Acid Pools*, p. 73. Ed. by Holden, J. T. Amsterdam: Elsevier Publishing Co.
 Kemp, J. D., Atkinson, D. E., Ehret, A. & Lazzarini, R. A. (1963). *J. biol. Chem.* **238**, 3466.
 Mager, J. (1960). *Biochim. biophys. Acta*, **41**, 553.
 Margolis, D. & Mandl, R. H. (1958). *Contrib. Boyce Thompson Inst.* **19**, 509.
 Neidhardt, F. C. (1966). *Bact. Rev.* **30**, 701.
 Novick, R. P. & Maas, W. K. (1961). *J. Bact.* **81**, 236.
 Pasternak, C. A. (1961). *Biochem. J.* **81**, 2p.
 Pasternak, C. A. (1962). *Biochem. J.* **85**, 44.
 Pasternak, C. A., Ellis, R. J., Jones-Mortimer, M. C. & Crichton, C. E. (1965). *Biochem. J.* **96**, 270.
 Wheldrake, J. F. & Pasternak, C. A. (1965). *Biochem. J.* **96**, 276.