

The Control of Sulphate Reduction in *Escherichia coli* by O-Acetyl-L-serine

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1. Extracts of *Escherichia coli* A.T.C.C. 9723 and K₁₂703 contain serine transacetylase and O-acetylserine sulphhydrylase. Synthesis of the latter enzyme is repressed by growth on L-cyst(e)ine and other sulphur compounds. 2. O-Acetyl-L-serine added to cells growing on glutathione or sulphate as source of sulphur induces the enzymes that catalyse (a) the activation of sulphate to adenosine 3'-phosphate 5'-sulphatophosphate (EC 2.7.7.4 and 2.7.1.25), (b) the reduction of adenosine 3'-phosphate 5'-sulphatophosphate to sulphite and (c) the reduction of sulphite to sulphide (EC 1.8.1.2). Hydrogen sulphide is liberated from cultures growing on sulphate as source of sulphur and in the presence of O-acetylserine. 3. The *cysE* mutants of *E. coli* K₁₂ lack serine transacetylase. Addition of O-acetylserine permits growth on sulphate as source of sulphur; at the same time the enzymes of sulphate reduction, previously absent, are synthesized. Such mutants have no detectable intracellular cyst(e)ine when starved of sulphur. 4. These results suggest that O-acetylserine is necessary for synthesizing the enzymes of sulphate reduction in *E. coli*. Its action does not appear to be by interference with the repressive control exerted over these enzymes by cyst(e)ine.

The discovery that O-acetyl-L-serine is an intermediate in cyst(e)ine biosynthesis (Kredich & Tomkins, 1966) prompted a reinvestigation of some of our results (Pasternak, Ellis, Jones-Mortimer & Crichton, 1965) concerning the control of this pathway. We have confirmed the presence of serine transacetylase and O-acetylserine sulphhydrylase in *Escherichia coli*; as in *Salmonella typhimurium* (Kredich & Tomkins, 1966), O-acetylserine sulphhydrylase is repressed by growth on L-cyst(e)ine.

We have also examined the effects of adding O-acetylserine to *E. coli* and to certain apparently pleiotropic mutants of *E. coli* growing on various sulphur sources. The results are compatible with sulphate reduction being controlled not only by the intracellular concentration of L-cyst(e)ine (Pasternak *et al.* 1965; Wheldrake, 1967) but also by that of O-acetylserine. A preliminary report (Spencer, Collins & Monty, 1967) suggests that the situation may be similar in *S. typhimurium*.

MATERIALS AND METHODS

Chemicals. O-Acetyl-L-serine was prepared by the method of Sheehan, Goodman & Hess (1956). Acetyl-CoA was prepared by the method of Stadtman (1957). Other

chemicals were as previously described (Pasternak, 1962; Ellis, Humphries & Pasternak, 1964; Pasternak *et al.* 1965).

Growth of organisms. *E. coli* A.T.C.C. 9723, *E. coli* K₁₂703 (given by Dr R. C. Clowes), *E. coli* K₁₂PA309 (a threonine-, leucine-, tryptophan-, histidine-, arginine- and thiamine-requiring strain given by Professor W. Hayes) and cyst(e)ine⁻ mutants, isolated after treatment of *E. coli* K₁₂703 or K₁₂PA309 with *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine or ultraviolet light respectively (Jones-Mortimer, 1968a) and classified (Mizobuchi, Demerec & Gillespie, 1962) as *cysB* mutants [mapping near tryptophan (Yanofsky & Lennox, 1959)] or *cysE* mutants [mapping near xylose (*cysA* mutants described by Taylor & Thoman, 1964)], were used. Cells were grown overnight on limiting glucose (0.1–0.15 mg. dry wt. of cells/ml.) as before (Ellis *et al.* 1964), except that the pH of the medium was 6.3 and that the necessary L-amino acids (each at 40 mg./l.) and thiamine hydrochloride (4 mg./l.) were added to cultures of *E. coli* K₁₂PA309 and its derivatives. Glucose (0.05% final concentration), solid O-acetyl-L-serine (where indicated) and additional solid S source of sulphur, with Na₂SO₃ (4.0 mM finally) or Na₂S (0.85 mM finally), were added and growth was continued for a further 1½–2½ hr. (0.15–0.25 mg. dry wt. of cells/ml.). The *cysE* mutants were grown overnight on L-cystine (0.85 mM), spun, resuspended in sulphur-free medium and grown for 2 hr. (approx. one cell division) with or without O-acetylserine as above. Cells were harvested and disrupted, and the supernatant fraction was prepared as described by Pasternak *et al.* (1965). Dry weight of cells and protein concentration were measured as described by Pasternak (1962). The growth response of mutants to various sulphur compounds in liquid medium at

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pH 6.3 was tested by the method of Ellis *et al.* (1964). Intracellular cyst(e)ine concentration was measured as described by Wheldrake (1967). Incorporation of [³⁵S]-sulphate by whole cells was determined by the method of Britten & McClure (1962).

Assay of enzymes. The following assays were used: the enzyme system catalysing the formation (Pasternak, 1962) and reduction (Pasternak *et al.* 1965) of adenosine 3'-phosphate 5'-sulphatophosphate; sulphite reductase (Ellis, 1964a); serine transacetylase (Kredich & Tomkins, 1966); *O*-acetylserine sulphhydrylase (Kredich & Tomkins, 1966). With the last enzyme cyst(e)ine formed was measured by the method of Grunert & Phillips (1951) after removal of hydrogen sulphide (Pasternak *et al.* 1965). The activity of an extract of *E. coli* 9723 grown on glutathione with serine as substrate was <0.1% of that with *O*-acetylserine. All results quoted are means of two or more experiments.

RESULTS AND DISCUSSION

Wild-type E. coli. Table 1 shows that extracts of *E. coli* 9723 contain serine transacetylase and *O*-acetylserine sulphhydrylase. The latter enzyme is repressed by growth on L-cystine. Essentially similar results were obtained with *E. coli* K₁₂703. The findings confirm those of Kredich & Tomkins (1966) with *S. typhimurium* and *E. coli* B. The physiological significance of serine sulphhydrylase (EC 4.2.1.22), which is insensitive to cyst(e)ine and of very much lower activity than *O*-acetylserine sulphhydrylase (Pasternak *et al.* 1965), is thus doubtful.

Addition of *O*-acetylserine to cells growing on sulphate or glutathione as source of sulphur stimulates the synthesis of the enzymes of sulphate activation and reduction (Table 1). [³⁵S]Sulphate

uptake by whole cells, which requires the presence of a permease in *E. coli* (Ellis, 1964b; R. J. Ellis & C. A. Pasternak, unpublished work) as well as in *S. typhimurium* (Dreyfuss, 1964), is also induced. Addition of L-serine is without significant effect on these enzymes, as noted previously (Ellis *et al.* 1964); this indicates that serine transacetylase is probably rate-limiting in the production of *O*-acetylserine *in vivo*. It was observed that cultures growing on sulphate liberate hydrogen sulphide (0.26 μmole of hydrogen sulphide from 23 mg. dry wt. of cells in 100 min.) when *O*-acetylserine (but not serine) is added to the medium; this is further evidence (Ellis *et al.* 1964) that sulphide is not the repressor of the sulphate-activating enzymes. The intracellular concentration of cyst(e)ine in sulphate-grown cells is increased rather than decreased by the addition of *O*-acetylserine, as might be expected, since *O*-acetylserine sulphhydrylase is clearly not rate-limiting (Table 1). Thus the induction of enzyme synthesis by *O*-acetylserine is not by restriction of cyst(e)ine biosynthesis. Results obtained with *cysE* mutants confirm this deduction.

Cyst(e)ine mutants of E. coli. Table 2 shows that *cysB* and *cysE* mutants differ in their response to sulphide and *O*-acetylserine; *cysB* mutants (which correspond to the *cysBb* mutants described by Mizobuchi *et al.* 1962) appear to be genuinely pleiotropic, whereas *cysE* mutants grow normally on sulphate when *O*-acetylserine is present. At the same time synthesis of the relevant enzymes occurs (Table 3). Hence *cysE* mutants appear to be deficient merely in the structural gene for serine transacetylase. This was confirmed by enzymic

Table 1. Control of sulphate-reducing enzymes in *E. coli* 9723

Cultures were grown, harvested and assayed as described in the Materials and Methods section. *O*-Acetylserine (1 mM) was added a few hours before harvesting, as described.

Source of sulphur for growth	<i>O</i> -Acetylserine added	Sp. activity (mμmoles/mg. of protein/min.)				
		Sulphate activation	Adenosine 3'-phosphate 5'-sulphatophosphate reductase	Sulphite reductase	Serine trans-acetylase	<i>O</i> -Acetylserine sulphhydrylase
Glutathione (0.2 mM)	—	3.3	0.32	9.1	4.8	18000
Rel. sp. activity						
Glutathione (0.2 mM)	—	(100)	(100)	(100)	(100)	(100)
Glutathione (0.2 mM)	+	200	210	150	140	64
Na ₂ SO ₄ (4.0 mM)	—	51	68	51	130	33
Na ₂ SO ₄ (4.0 mM)	+	230	160	87	150	64
Na ₂ SO ₃ (4.0 mM)	—	<1	25	22		7
Na ₂ SO ₃ (4.0 mM)	+	9	50	32		13
Na ₂ S (0.85 mM)	—	<1	45	6		2
Na ₂ S (0.85 mM)	+	<1	41	6		2
L-Cystine (0.2 mM)	—	<1	23	<1	180	4
L-Cystine (0.2 mM)	+	<1	9	<1	110	3

Table 2. *Growth characteristics of mutants of E. coli K₁₂*

Cultures were grown in sulphur-free medium as described in the Materials and Methods section. +, Good growth; —, no growth.

Strain	Parent	Locus	Additions to medium ...	Growth				
				Na ₂ SO ₄ (10mM)	Na ₂ SO ₄ (10mM) + O-acetylserine (1mM)	Na ₂ SO ₃ (0.42mM)	Na ₂ S (0.21mM)	L-Cystine (0.85mM)
PA 309	—	Wild-type		+	+	+	+	+
703	—	Wild-type		+	+	+	+	+
JM 13	703	<i>cysB</i>		—	—	—	+	+
JM 14	703	<i>cysB</i>		—	—	—	+	+
JM 63	PA 309	<i>cysB</i>		—	—	—	+	+
JM 15	703	<i>cysE</i>		—	+	—	—	+
JM 22	703	<i>cysE</i>		—	+	—	—	+
JM 70	PA 309	<i>cysE</i>		—	+	—	—	+

Table 3. *Sulphate-reducing enzymes in cysE mutants*

Cultures were grown on L-cystine (0.85 mM) followed by sulphur starvation with or without O-acetylserine (1 mM), as described in the Materials and Methods section.

Strain	O-Acetylserine added	Sp. activity (mμmoles/mg. of protein/min.)	
		Sulphate activation	Sulphite reduction
JM 15	—	< 0.01	< 0.02
JM 15	+	3.5	1.54
JM 70	—	< 0.02	< 0.01
JM 70	+	2.9	1.45

assay (<5% of the activity of wild-type trans-acetylase; normal O-acetylserine sulphhydrase). *S. typhimurium cysE* mutants have a similar defect (Kredich & Tomkins, 1966). The intracellular concentration of cyst(e)in e (< 0.02 μmole/mg. dry wt. of cells) in *cysE* mutants of *E. coli* grown in limiting sulphur was found to be below that required for substantial repression of the sulphate-activating enzymes (Wheldrake, 1967), which shows that lack of the enzymes in the absence of O-acetylserine is not due to repression by cyst(e)ine.

O-Acetylserine, then, is a necessary factor for the synthesis of the enzymes of sulphate reduction. Its action is not by interference with repression by cyst(e)ine (cf. Mandelstam & Jacoby, 1965). On the other hand cyst(e)ine may control induction by O-acetylserine since stimulation of wild-type enzymes is observed only when the amounts of enzyme are high (Table 1), that is when the concentration of cyst(e)ine is low. Competition between inducer and repressor for control of a pathway has been described in arginine biosynthesis (Gorini, 1963), which thus resembles cyst(e)ine biosynthesis in yet another (Pasternak *et al.* 1965) respect.

Genetic analysis of *cysB* and *cysE* mutants has shown (Jones-Mortimer, 1968*a,b*) that the type of regulation that governs the enzymes of sulphate reduction is 'positive' (e.g. Sheppard & Englesberg, 1966) rather than 'negative' (Jacob & Monod, 1961).

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