SHORT COMMUNICATION

Cysteine Auxotrophs of *Salmonella typhimurium* which Grow without Cysteine in a Hydrogen/Carbon Dioxide Atmosphere

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Cysteine auxotrophs of *Salmonella typhimurium* mutated in *cysB*, *cysI* or *cysJ* grew with sulphate as a sulphur source when incubated under a hydrogen/carbon dioxide atmosphere. Yields obtained under these conditions were equivalent to those characteristic of wild-type *S. typhimurium*. The same mutants failed to grow with sulphate as a sulphur source when incubated aerobically. Auxotrophs mutated in *cysA*, *cysC*, *cysD*, *cysE*, *cysG* and *cysH* required cysteine for growth under both incubation conditions. The results suggest that mutations in *cysB* (regulation of the several *cys* operons) and also *cysI* and *cysJ* (sulphite reductase activity) can be circumvented during anaerobic growth under hydrogen.

INTRODUCTION

In *Salmonella typhimurium*, the synthesis of cysteine from sulphate requires the participation of 10 genes (Smith, 1971; Hulanicka *et al.*, 1974). *CysA* codes for a sulphate permease; *cysC*, *cysD* and *cysH* for the enzymes that reduce sulphate to sulphite; *cysG*, *cysI* and *cysJ* for a sulphite reductase enzyme system; and *cysE* for serine transacetylase. *CysK*, the most recently identified *cys* locus, produces the major *O*-acetylserine sulphhydrylase (Hulanicka *et al.*, 1974). *CysB* is the regulatory gene; it codes for a positive regulator protein which mediates the induction of the cysteine biosynthetic enzymes under conditions of cysteine deprivation (Kredich, 1971; Tully & Yudkin, 1975). Cysteine auxotrophs can be produced by single mutations in all except *cysK*. Apparently the *cysK* product can be functionally replaced by a different enzyme outside the cysteine regulon (Sledziewska & Hulanicka, 1978).

Because the gene-enzyme relationships in this biosynthetic pathway are well documented, we were surprised to note that certain cysteine auxotrophs (which we had obtained for genetic experiments involving anaerobic enzymes) behaved as prototrophs when grown on plates under an H₂/CO₂ atmosphere. To verify our initial observation, we decided to examine a larger collection of mapped cysteine auxotrophs for the ability to grow without cysteine under these anaerobic conditions. We report here yield studies of 17 such mutants representing all nine genes in which cysteine auxotrophs have been mapped. Our results suggest that mutational blocks in three of the *cys* genes can be circumvented during anaerobic growth under this atmosphere.

METHODS

Strains. Wild-type *Salmonella typhimurium* LT-2 was obtained from Dr B. N. Ames. Cysteine auxotrophs were all obtained from Dr K. E. Sanderson. Strain *cysH47* was derived from *S. typhimurium* LT-7; all other *cys* mutants were derived from *S. typhimurium* LT-2. Strains *cysH47* and *cysI51* require tryptophan for growth; the other *cys* mutants require only cysteine.

Media. Glucose minimal plates consisted of VBC medium (Vogel & Bonner, 1956) supplemented with glucose (1%, w/v) and tryptophan (0.1 mM), and solidified with 1.5% (w/v) agar. Liquid minimal medium consisted of VBC medium in which the MgSO_4 was replaced with equimolar MgCl_2 . It was supplemented with glucose (1%, w/v), tryptophan (0.1 mM), CaCl_2 (1.0 mM), FeSO_4 , Na_2MoO_4 and $\text{Na}_2\text{Se}_2\text{O}_3$ (each at 1.0 μM), and MnSO_4 , CuSO_4 and $\text{Co(NO}_3)_2$ (each at 0.1 μM). In each experiment, 0.1 mM sulphate or L-cysteine was added as the major sulphur source. Nutrient agar was obtained from Difco.

Yield determinations. Bacteria grown for 18 h on nutrient agar plates and then suspended in saline to a final concentration of 0.01 mg dry wt ml^{-1} were used for inocula; 0.1 ml inoculum was used for each 1.0 ml medium. All incubations were for 72 h at 37 °C. Aerobic cultures were grown on a shaker. Anaerobic incubations were carried out in GasPak anaerobic jars (BBL) which generate an atmosphere of 95% $\text{H}_2/5\%$ CO_2 . Absorbance was measured at 650 nm in a Bausch and Lomb Spectrometer 20 and was converted to mg dry wt ml^{-1} by means of a standard curve prepared for *S. typhimurium* LT-2 grown in the same medium and read in the same machine.

RESULTS AND DISCUSSION

Initially we examined a large number of cysteine auxotrophs for the ability to grow on glucose minimal plates incubated anaerobically under a H_2/CO_2 atmosphere. We tested two to four representatives from each *cys* gene in which auxotrophs have been mapped. We found anaerobic growth in four out of four *cysB* strains (*cysB14*, *cysB24*, *cysB87*, *cysB403*), two out of two *cysI* strains (*cysI51*, *cysI68*) and one out of two *cysJ* strains (*cysJ299* but not *cysJ538*). The same strains showed no detectable growth on glucose minimal plates incubated aerobically. None of the other *cys* mutants tested grew on this medium under either condition.

Our first attempts to repeat these results in liquid medium were not successful. The critical variables, however, were eventually identified: it was necessary to add a mineral supplement to the medium, and the inoculum could not be older than 24 h. Wild-type *S. typhimurium* did not exhibit the same requirements for anaerobic growth with sulphate, and the mutants did not exhibit the same requirements for aerobic growth with L-cysteine.

After determining the necessary growth conditions, we measured the cell yields of 17 *cys* mutants grown anaerobically and aerobically on sulphate and on L-cysteine (Table 1). The five mutant strains which grew anaerobically with sulphate were subcultured from the anaerobic sulphate tubes on to aerobic and anaerobic glucose minimal plates. In all cases, the aerobic/anaerobic difference was maintained. It appears that mutations in *cysB* (regulation of *cys* genes), and also in *cysI* and *cysJ* (sulphite reductase activity), can be circumvented during anaerobic growth under hydrogen. The difference in the results for the *cysJ* point mutant (*cysJ299*) and the *cysJ* deletion mutant (*cysJ538*) are probably due to polarity effects of the *cysJ* deletion on the neighbouring *cysH* gene. Loughlin (1975) showed that *cysJ538* and other *cysJ* deletion mutants lack the activity encoded by *cysH* as well as sulphite reductase activity.

Evidence has been presented which suggests that *cysJ* contains information for an iron-free flavoprotein responsible for the NADPH-diaphorase activity associated with sulphite reduction, while *cysI* and *cysG* are both involved in the production of a sirohaem-containing protein which can reduce sulphite with methyl viologen (Siegel *et al.*, 1974). Perhaps the *cysI* and *cysJ* functions in sulphite reduction can be assumed by other anaerobic reductases such as the anaerobic membrane-bound thiosulphate reductase, which is found in *S. typhimurium* and *Proteus mirabilis* but not in *E. coli* (Stouthamer, 1969). This enzyme catalyses the anaerobic production of H_2S from both thiosulphate and sulphite in complex media (i.e. media in which the cysteine biosynthetic enzymes would be expected to be repressed).

Table 1. Cell yields of *S. typhimurium* cysteine auxotrophs grown aerobically or anaerobically with 0.1 mM L-cysteine or sulphate

Strain	Nature of mutation	Cell yield (mg dry wt ml ⁻¹)*			
		L-Cysteine		Sulphate	
		Aerobic incubation	Anaerobic incubation	Aerobic incubation	Anaerobic incubation
LT-2	(Wild-type)	0.45	0.22	0.51	0.17
<i>cysA21</i>	Deletion in b cistron	0.40	0.16	< 0.005	< 0.005
<i>cysA381</i>	Point mutation	0.29	0.27	< 0.005	< 0.005
<i>cysB87</i>	Spontaneous mutation	0.49	0.26	< 0.005	0.17
<i>cysB403</i>	X-ray mutation	0.50	0.26	< 0.005	0.21
<i>cysC497</i>	Point mutation	0.44	0.25	< 0.005	< 0.005
<i>cysC520</i>	Deletion	0.45	0.24	< 0.005	< 0.005
<i>cysD220</i>	Point mutation	0.36	0.25	< 0.005	< 0.005
<i>cysD305</i>	Deletion	0.45	0.23	< 0.005	< 0.005
<i>cysE396</i>	X-ray mutation	0.45	0.23	< 0.005	< 0.005
<i>cysG384</i>	X-ray mutation	0.41	0.25	< 0.005	< 0.005
<i>cysG458</i>	Fast neutron mutation	0.37	0.18	< 0.005	< 0.005
<i>cysH47</i>	Point mutation	0.45	0.24	< 0.005	< 0.005
<i>cysH364</i>	Deletion	0.50	0.20	< 0.005	< 0.005
<i>cysI51</i>	Point mutation	0.34	0.18	< 0.005	0.16
<i>cysI68</i>	Deletion	0.37	0.21	< 0.005	0.16
<i>cysJ299</i>	Point mutation	0.45	0.25	< 0.005	0.19
<i>cysJ538</i>	Deletion	0.37	0.24	< 0.005	< 0.005

* Each value is an average of three to five experiments.

Studies of thiosulphate reductase in *P. mirabilis* have indicated that this enzyme, like the terminal reductases of the anaerobic sulphate-reducing bacteria, can be coupled to hydrogenase (deGroot & Stouthamer, 1970). The hydrogen present in the growth atmosphere during our experiments might be serving as a reducing agent for sulphite via a hydrogenase-thiosulphate reductase enzyme system.

Another possible candidate for an alternative sulphite-reducing activity is nitrite reductase, which is found in *E. coli* and presumably in *S. typhimurium* as well. Nitrite is not necessary for the induction of this enzyme, and sulphite has been shown to inhibit its activity with nitrite (Coleman *et al.*, 1978).

The anaerobic prototrophy of the *cysB* mutants has different implications. The *cysB* product has been shown to be a multisite regulator protein which interacts with at least three *cys* operons (Borum & Monty, 1976; Jagura & Hulanicka, 1978). It is possible that some *cysB* mutations are corrected by growth in a reducing atmosphere. On the other hand, the fact that all four *cysB* mutants tested on glucose minimal plates behaved as prototrophs under hydrogen, suggests that the *cysB* product may not be necessary for the induction of any of the *cys* operons in a reducing atmosphere. This would imply that the induction of each of the different *cys* operons (although involving separate *cysB* protein sites) may proceed by means of a common molecular mechanism.

Qureshi *et al.* (1975) reported the isolation of a number of *S. typhimurium* cysteine mutants, the growth of which could be supported by methionine as well as by cysteine. The mutants all mapped in known *cys* genes, usually at the end of the gene. Qureshi *et al.* (1975) showed that methionine was not being converted into cysteine, nor were the mutants leaky. Instead, it appeared that methionine was either inducing the specific *cys* gene, or was activating the associated enzyme. Since these '*cym*' mutations mapped in several *cys* genes, their existence might also be taken to suggest a common mechanism for the induction of the cysteine biosynthetic pathway operons.

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