Delayed Inducibility of Sulphite Reductase in *cysM* Mutants of *Salmonella typhimurium* Under Anaerobic Conditions

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(Received 23 July 1981; revised 2 October 1981)

Salmonella typhimurium cysM mutants grow at a normal rate under aerobic conditions, but only after a lag period under anaerobic conditions. No difference in the induction of two cysteine biosynthetic enzymes – sulphite reductase and *O*-acetyl-L-serine sulphydrylase – in wild-type, *cysK* and *cysM* strains was observed under aerobic conditions. Under anaerobic conditions, however, the *cysM* strain differed from the others in showing a long delay in the induction of sulphite reductase. These observations are consistent with the assumption that the observed growth delay of the *cysM* mutant under anaerobic conditions is the result of abnormalities in the regulation of sulphite reductase.

INTRODUCTION

The final step of L-cysteine biosynthesis in *Salmonella typhimurium* and *Escherichia coli* is the sulphydrylation of *O*-acetyl-L-serine by either free sulphide or protein-bound hydro-disulphide (Tsang & Schiff, 1976). In *S. typhimurium* two enzymes have been described which are capable of this reaction using free sulphide as substrate, *O*-acetylserine sulphydrylase-A and -B coded by the *cysK* and *cysM* genes, respectively (Hulanicka *et al.*, 1979). Strains lacking either *O*-acetylserine sulphydrylase-A or -B are cysteine prototrophs, but *cysM cysK* double mutants are cysteine auxotrophs. The expression of the *cysM* gene is regulated by the nutritional and genetic factors that control the cysteine regulon.

This report describes the observation that although *cysM* strains grow well aerobically on sulphate, they are cysteine bradytrophs under anaerobic conditions. This observation may eventually provide an answer to the question of why *S. typhimurium* has two distinct enzymes catalysing the same reaction.

METHODS

Organisms. All bacteria used were derivatives of *S. typhimurium* LT2 (Table 1).

Media and culture conditions. The minimal medium (BS) was medium C of Vogel & Bonner (1956) except that MgSO_4 was replaced by an equimolar amount of MgCl_2 , and the sulphur source was provided by adding 0.5 mM-L-cystine as described by Kredich (1971) or 0.1 mM- Na_2SO_4 . Supplements consisted of 0.5% (w/v) glucose and, when appropriate for the growth of auxotrophs, 0.2 mM-uracil and 0.2 mM of the required amino acid. Solid media were prepared by the addition of agar (Oxoid).

Liquid cultures under aerobic conditions were grown with rotary shaking. Anaerobic conditions for the liquid cultures were obtained by bubbling highly purified nitrogen through the medium for at least 20 min. Commercial nitrogen was purified by passage through a system of four oxygen column traps (two gas washing bottles filled with an alkaline solution of pyrogallol and two columns containing copper chips heated to 200–250 °C). The bottles were closed with mercury valves.

Bacteria from overnight cultures grown in the presence of 0.5 mM-L-cystine were harvested carefully, washed and resuspended in fresh medium at an A_{650} of 0.2–0.3. These cultures were grown under aerobic or anaerobic conditions as described above. They were harvested at a suitable time and *O*-acetylserine sulphydrylase (EC

Table 1. *Designation and derivation of S. typhimurium LT2 strains*

Strain	Genotype	Source or derivation
TK1000	Wild-type	N. D. Zinder
TK1411	<i>trpC109</i>	K. E. Sanderson
TK181	<i>trpC109 cysK1772</i>	Spontaneous mutation to 1,2,4-triazole resistance in TK1411
SB3751	<i>trpB223 Δ(cysK-ptsHI182)</i>	J. C. Cordaro
TK2058	<i>trpB223 Δ(cysK-ptsHI182) cysM2328</i>	Spontaneous mutation to azaserine resistance in SB3751
TK2072	<i>trpB223 cysM2328</i>	Transductant from TK1000 lysate × TK2058

4.2.99.8) and sulphite reductase (EC 1.8.1.2) were assayed in cell-free extracts. Cell densities were estimated by measuring turbidity at 650 nm. Bacteria for enzyme studies were harvested from exponentially growing cultures by centrifugation.

Enzyme studies. *O*-Acetyl-L-serine sulphydrylase was determined as described previously (Kredich *et al.*, 1969). One unit (U) of *O*-acetylserine sulphydrylase is defined as the amount of enzyme catalysing the formation of 1 μmol cysteine min⁻¹. NADPH-sulphite reductase was assayed by the method of Vito & Dreyfuss (1964). Protein was determined by the biuret method (Gornall *et al.*, 1949) with bovine albumin as the standard.

Chemicals. *O*-Acetyl-L-serine was prepared by the method of Sakami & Toennies (1942). Other chemicals were commercial products of reagent grade.

RESULTS

Cysteine auxotrophy of cysM mutants under anaerobic conditions

The observation that growth of *cysM* strains on minimal medium without shaking was impaired suggested that growth of these mutants depends on aeration. We therefore studied the properties of *cysM* mutants grown under aerobic and anaerobic conditions.

In the first experiments the division times (Table 2) of the wild-type (TK1411), *cysK* (TK181) and *cysM* (TK2072) strains growing aerobically and anaerobically were measured. The division times of the wild-type and the *cysK* mutant were practically the same under both conditions of growth. However, the generation time of the *cysM* mutant growing aerobically on sulphate was prolonged compared with the wild-type and *cysK* mutant, and in a nitrogen atmosphere the *cysM* strain did not start to grow for 24 h. The addition of cystine restored the normal growth rate (Table 2), but neither L-serine nor L-methionine had this effect. After the lag of up to 24 h, the *cysM* strain resumed growth with the same generation time as observed under aerobic conditions. The duration of the lag depended on the sulphur source in the medium in which the inoculum was grown. It was longest when L-cystine was used as the sole source of sulphur, which suggests that anaerobiosis causes some abnormalities in the induction of the cysteine biosynthetic enzymes in the *cysM* strain.

Delayed inducibility of sulphite reductase in cysM mutants under anaerobic conditions

The kinetics of induction of two enzymes of cysteine biosynthesis – *O*-acetylserine sulphydrylase and sulphite reductase – in the wild-type (TK1411), *cysK* (TK181) and *cysM* (TK2072) strains were determined under aerobic and anaerobic conditions (Table 3, Fig. 1). The repressed levels of sulphite reductase and *O*-acetylserine sulphydrylase observed at time 0 (the start of incubation) resulted from the presence of L-cystine in the inoculum. The increase in enzyme activities during incubation of the three strains tested was similar under aerobic conditions. However, a significant difference was noted in the induction of sulphite reductase in the *cysM* mutant growing anaerobically. No increase in sulphite reductase activity in the *cysM* mutant was detected during 90 min incubation, whereas induction of this enzyme in the wild-type (TK1411) and *cysK* (TK181) strains was similar to that observed under aerobic conditions.

Table 2. Division times under aerobic and anaerobic conditions

Bacteria from overnight cultures grown aerobically in minimal medium supplemented with 0.5 mM-L-cystine were harvested, washed three times with 0.9% (w/v) NaCl and resuspended in fresh medium with the sulphur source indicated. The inocula for anaerobic experiments were saturated with N₂ and inoculated into medium protected from O₂ by a few millilitres of paraffin layered on the top of the bacterial culture.

Strain	Relevant genotype	Division time (min)			
		Aerobic		Anaerobic	
		Sulphate	L-Cystine	Sulphate	L-Cystine
TK1411	<i>cys</i> ⁺	55	50	65	55
TK181	<i>cysK</i> 1772	60	55	60	50
TK2072	<i>cysM</i> 2328	108	60	NG	60

NG, No growth for 24 h.

Table 3. Activities of *O*-acetylserine sulphydrylase under aerobic and anaerobic conditions

Bacteria from overnight cultures grown anaerobically in the presence of L-cystine were harvested, washed twice at room temperature with 0.9% (w/v) NaCl and resuspended in minimal medium (BS) supplemented with 0.1 mM-Na₂SO₄. Each suspension was divided into two: one part was incubated with aeration and the other was incubated in a nitrogen atmosphere. Samples were taken at the times indicated and enzyme activities in crude extracts were assayed.

Time (min)	<i>O</i> -Acetylserine sulphydrylase [U (mg protein) ⁻¹]					
	TK1411 (<i>cys</i> ⁺)		TK181 (<i>cysK</i>)		TK2072 (<i>cysM</i>)	
	Aerobic	Anaerobic	Aerobic	Anaerobic	Aerobic	Anaerobic
0	0.41	0.41	0.01	0.01	0.30	0.84
30	1.32	1.14	0.01	0.01	0.83	0.61
60	2.62	ND	0.04	0.03	1.32	0.54
90	6.96	3.75	0.05	0.05	2.65	1.53

ND, Not determined.

Fig. 1. Induction of sulphite reductase under aerobic (○) and anaerobic (●) conditions. For experimental details, see legend to Table 3.

Thus, it seems likely that the observed growth delay of the *cysM* mutant under anaerobic conditions is caused by a slower rate of induction of sulphite reductase and is the consequence of the lack of sulphide for sulphydrylation of *O*-acetyl-L-serine.

DISCUSSION

The final step in cysteine biosynthesis, sulphydrylation of *O*-acetyl-L-serine, is catalysed by two enzymes: *O*-acetylserine sulphydrylase-A and *O*-acetylserine sulphydrylase-B coded by the *cysK* and *cysM* genes, respectively. Mutation in one of these two genes does not lead to cysteine auxotrophy. In general, it is very unusual for organisms such as *S. typhimurium* to carry and express genes for two different enzymes which catalyse the same reaction. The *cysM* and *cysK* strains grow aerobically on minimal medium with the same division time (Table 2). However, under anaerobic conditions, the *cysM* strain is a cysteine bradytroph. The lag in the growth of the *cysM* strain depended on the sulphur source in the medium in which the inoculum was grown: the lag was longest when L-cystine was used as the sulphur source. It is well known that this compound causes a repressed level of cysteine biosynthetic enzymes (Kredich, 1971).

The determination of the kinetics of induction of *O*-acetylserine sulphydrylase and sulphite reductase showed a significant delay in induction of the latter enzyme in the *cysM* mutants under anaerobic conditions (Fig. 1). Thus, it seems that *O*-acetylserine sulphydrylase-B is somehow required or at least preferred for the induction of sulphite reductase under anaerobic conditions.

This work was supported by the Polish Academy of Sciences within Project 09.7.

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