Regulation of carbon utilization by sulfur availability in *Escherichia coli* and *Salmonella typhimurium*

John A. Quan,¹ Barbara L. Schneider,¹ Ian T. Paulsen,^{1†}
Mamoru Yamada,² Nicholas M. Kredich³ and Milton H. Saier, Jr¹

Author for correspondence: Milton H. Saier, Jr. Tel: +1 858 534 4084. Fax: +1 858 534 7108.
e-mail: msaier@ucsd.edu

¹ Department of Biology,
University of California at
San Diego, La Jolla,
CA 92093-0116, USA

² Department of
Biochemistry, Yamaguchi
University School of
Medicine, Ube,
Yamaguchi, Japan 755

³ Departments of Medicine
and Biochemistry, Duke
University Medical Center,
Durham, NC 27710, USA

Different pleiotropic transcriptional regulators are known to function in the coordination of regulons concerned with carbon, nitrogen, sulfur, phosphorus and iron metabolism, but how expression profiles of these different regulons are coordinated with each other is not known. The basis for the effects of *cysB* mutations on carbon utilization in *Escherichia coli* and *Salmonella typhimurium* was examined. *cysB* mutations affected the utilization of some carbon sources more than others and these effects could be partially, but not completely, reversed by the inclusion of cysteine or djenkolate in the growth medium. Assays of transport systems and enzymes concerned with glucitol and alanine utilization showed that these activities were depressed in *cysB* mutants relative to isogenic wild-type strains, and cysteine or djenkolate present in the growth media partially restored these activities. Using transcriptional fusions to the *fdo* (formate dehydrogenase) and *gut* (glucitol) operons, it was shown that decreased expression resulted from defects at the transcriptional level. Furthermore, the effects of loss of CysB were much less pronounced under conditions of catabolite repression than in the absence of a catabolite-repressing carbon source, and cAMP largely reversed the effect of the loss of CysB. Comparable effects were seen for *E. coli lacZ* gene expression under the control of its own native promoter, and sulfur limitation in a *cysB* mutant depressed net cAMP production in a cAMP phosphodiesterase mutant. Adenylate cyclase thus appears to be responsive to sulfur deprivation. These observations may have physiological significance allowing carbon and sulfur regulon coordination during the growth of enteric bacteria in response to nutrient availability.

Keywords: regulon, transcription, pleiotropic regulators, adenylate cyclase, CysB

INTRODUCTION

Bacteria require an ample supply of a variety of nutrients to maintain continuous growth. Among these nutrients are sources of carbon, nitrogen, phosphorus, sulfur and iron. The use of each such nutrient is in general controlled by one or a few pleiotropic transcriptional regulatory proteins. Thus, in *Escherichia coli* and the closely related *Salmonella typhimurium*, carbon utilization is regulated by the cAMP-receptor protein (CRP)

and the catabolite repressor/activator (Cra) protein (Saier & Ramseier, 1996; Saier *et al.*, 1996), nitrogen utilization is controlled by the NtrBC sensor kinase/response regulator pair in conjunction with the GlnB and GlnD proteins (Magasanik, 1996; Reitzer, 1996a, b), phosphorus utilization is controlled by the PhoR/PhoB sensor kinase/response regulator pair (Wanner, 1996), iron utilization is regulated by the Fur transcription factor (Earhart, 1996; Gralla & Collado-Vides, 1996) and sulfur metabolism is controlled by the CysB transcriptional activator (Kredich, 1992).

The cysteine regulon includes most of the genes required for synthesis of cysteine and genes for uptake of sulfur sources such as L-cystine, sulfate, thiosulfate and taur-

[†] **Present address:** The Institute for Genomic Research, 9712 Medical Center Drive, Manassas, VA 20850, USA.

Abbreviations: CRP, cAMP-receptor protein; EMB, eosin-methylene blue.

ine. Transcriptional activation of these genes requires CysB, the inducer *N*-acetyl-L-serine and conditions of sulfur limitation (Kredich, 1992, 1996). CysB is a tetrameric LysR-type regulator with an N-terminal DNA-binding domain, a central inducer-binding domain and a C-terminal oligomerization domain that is essential for stability (Lochowska *et al.*, 2001). Its activity is regulated by an efflux pump specific for cysteine metabolites (Dassler *et al.*, 2000). CysB is also an autorepressor, preventing expression of its own structural gene, *cysB*.

E. coli and *S. typhimurium* can utilize a number of sulfur-containing compounds as sole sulfur source, including sulfate, sulfite, thiosulfate, sulfide, glutathione, lanthionine, taurine and L-djenkolate [*S,S'*-methylene bis(cysteine)] as well as cysteine and cystine (Kredich, 1996; van der Ploeg *et al.*, 1997). Although these organisms cannot utilize methionine as a sole sulfur source, *Klebsiella* strains can (Seiflein & Lawrence, 2001). Sulfide and thiosulfate are anti-inducers, probably exerting their effects by competing with *N*-acetyl-L-serine for binding to the CysB activator (Hryniewicz & Kredich, 1991; Ostrowski & Kredich, 1990). Cysteine inhibits inducer synthesis, resulting in maximal repression of the sulfur regulon. Growth with poor sulfur sources such as L-djenkolate or glutathione results in maximal derepression of the sulfur regulon. Rapid growth on rich media results in moderate induction of the regulon (Antón, 2000).

Hartmann & Boos (1993) demonstrated that mutations in *phoB*, the gene encoding the pleiotropic activator of the *pho* regulon, affect the carbohydrate fermentation phenotype in *E. coli* under certain physiological conditions, particularly under conditions of phosphate starvation. CysB, normally considered to be the central pleiotropic regulator of sulfur metabolism in enteric bacteria, has been shown to be required for normal acid induction of arginine decarboxylase (Shi & Bennett, 1994) as well as for sensitivity to novobiocin (Rakonjac *et al.*, 1991) and mecillinam (Oppezzo & Antón, 1995). Thus, pleiotropic effects of *phoB* and *cysB* mutations other than those expected on the basis of their involvement in phosphorus and sulfur metabolic regulation, respectively, have been reported. In this report we present the results of our preliminary studies with representative examples of *cysB* mutant strains and provide analyses of the basis for specific carbohydrate utilization defects observed for these mutants.

METHODS

Strains and plasmids. The strains used in this study are listed in Table 1. The *cysB* allele in MDA7K was transferred by P1 transduction to three different genetic backgrounds (bottom of Table 1), selecting for tetracycline resistance and screening for the *cysB* phenotype (non-growth on minimal medium and growth upon the addition of cysteine). Plasmids were introduced into the various *E. coli* strains by electroporation as described by O'Callaghan & Charbit (1990).

Growth medium. The minimal medium contained W salts (100 mM potassium phosphate, pH 7, plus 1 mM MgSO₄)

(Smith *et al.*, 1971) to which was added 0.01% L-tryptophan when required (i.e. for strains LJ4508, LJ4510, LJ4511 and LJ4512) plus 0.2% (NH₄)₂SO₄ or another nitrogen source (when indicated) and the specified carbon source. For sulfur-free medium, MgSO₄ was replaced by MgCl₂ and 0.2% (NH₄)₂SO₄ was replaced by 0.4% NH₄Cl. The rich medium used was Luria-Bertani (LB) broth or nutrient broth (NB). These media are limiting for cysteine, and growth of *cysB* mutants in these media requires addition of cysteine (an efficient sulfur source) or djenkolate (a limiting sulfur source).

Cell growth was always at 37 °C. Fermentation was tested on eosin-methylene blue (EMB) plates lacking lactose, but containing the indicated carbon source at 1%, except for rhamnose which was present at 0.5%. These plates, like the rich media described above, are sulfur-limited. Carbon oxidation was assayed using 96-well Biolog plates (Bochner, 1993). Cysteine, when present, was added to a final concentration of 0.2 mM. Although cysteine can be growth-inhibiting at high concentrations (Harris & Lui, 1981), it is not inhibitory at this concentration. For all enzyme assays, cells were harvested during exponential growth.

Enzyme assays. β -Galactosidase was assayed by the method described by Miller (1972). For the data presented in Table 5, which used minimal media, IPTG (1 mM) was present during growth to eliminate any effect of the *lac* operon repressor. β -Galactosidase activities are either expressed in Miller units ($\mu\text{mol min}^{-1}$ (mg protein)⁻¹ (see Table 5) or in relative activities (see Figs 2 and 3). For reporter gene transcriptional fusion assays, cells were grown overnight in 5 ml NB plus 1% glucose, glucitol or formate at 37 °C with agitation. Fresh NB (100 ml) of the same composition was inoculated from the overnight culture and cells were harvested during exponential growth at 37 °C (OD₆₀₀ approx. 0.5). The cell density was adjusted and two drops of toluene were added to permeabilize the cells during vortexing for 30 s. The samples were incubated at 30 °C and aliquots were transferred to the appropriate buffers for reporter gene product assay. The β -galactosidase buffer consisted of 2 mM EDTA, 70 mM Na₂HPO₄, 35 mM NaH₂PO₄ (pH 7.0), 1 mM MgSO₄, 10 mM KCl and 10 mM β -mercaptoethanol. The same conditions were used to assay native β -galactosidase as reported in Table 5. The β -glucuronidase buffer consisted of 50 mM sodium phosphate, pH 7.0, 0.1% Triton X-100 and 10 mM β -mercaptoethanol. The assay mixtures were allowed to equilibrate to room temperature for 10 min before addition of 200 μl 0.4% *o*-nitrophenyl galactopyranoside (*gut* operon expression) or 100 μl 10 mM *p*-nitrophenyl- β -D-glucuronide (*fdo* operon expression) to give a final volume of 1.0 ml. The nitrophenol released was measured after addition of 400 μl 1 M Na₂CO₃ (*gut* operon expression) or 400 μl 2.5 M 2-amino-2-methylpropanediol (*fdo* operon expression) to stop the reaction. The samples were then read at 420 or 415 nm, respectively (Jefferson *et al.*, 1986; Miller, 1972). Protein concentrations were measured by the Lowry method.

Cell extracts for the assay of glucitol-6-phosphate dehydrogenase, Enzyme II^{Gut} and D-alanine dehydrogenase activities were prepared as follows. Cells were grown with shaking in LB broth at 37 °C, harvested by centrifugation, washed three times with minimal medium 63 and resuspended in the same medium. PMSF (0.1 mM) and β -mercaptoethanol (0.5 mg ml⁻¹) were added to the samples and the cells were lysed using a French press at 10000 p.s.i. (69 MPa). After centrifugation to remove cell debris, the supernatant was used for the assays. For the assay of glucitol-6-phosphate dehydrogenase activity, aliquots of the cell extracts were added to a solution containing 0.1 M Tris/HCl (pH 8.5), 1.5 mM

Table 1. Bacterial strains and plasmids used in this study

Strain/plasmid	Genotype/description	Reference or source
<i>S. typhimurium</i>		
LT2	Wild-type	Ostrowski & Kredich (1989)
LT2 (<i>cysB403</i>)	<i>cysB403</i>	Ostrowski & Kredich (1989)
<i>E. coli</i>		
GNB7145K	MC4100 <i>adi::MudI1734(Kan^R lac) relA1</i>	Shi & Bennett (1994)
MDA7K	MC4100 <i>adi::MudI1734(Kan^R lac) cysB trpB::Tn10 relA1</i>	Shi & Bennett (1994)
SB2249*	<i>cpd</i> (Crooke's strain)	Castro <i>et al.</i> (1976)
LJ2703*	<i>cpd ptsI^{ts} crr</i>	Castro <i>et al.</i> (1976)
LJ4508*	<i>cpd cysB trpB::Tn10</i>	This study
LJ4510*	<i>cpd ptsI^{ts} crr cysB trpB::Tn10</i>	This study
LJ4511*	<i>cpd trpB::Tn10</i>	This study
LJ4512*	<i>cpd ptsI^{ts} crr trpB::Tn10</i>	This study
Plasmids		
pCB182	<i>lacZ</i> promoter probe vector (Amp ^R)	Schneider & Beck (1986)
pHA6	Amp ^R <i>fdo::uidA</i> (Kan ^R)	Abaibou <i>et al.</i> (1995)
pGUTALAC1	Amp ^R <i>gutB::lacZ</i> †	This study

* All these strains are isogenic, being in the genetic background of Crooke's strain (*cpd*).

† The *gutB* gene is also designated *srIB* (Berlyn, 1998).

NAD⁺, 1 mM dithiothreitol and 2 mM glucitol 6-phosphate. The optical density at 340 nm was followed over a period of 2 min. For the assay of Enzyme II^{Gut}, 0, 1, 2, 5, 10 and 25 µl samples of extract were added to 100 µl twofold concentrated assay mix containing 100 mM potassium phosphate buffer, pH 6.0, 20 µM [¹⁴C]D-glucitol, 20 mM glucitol 6-phosphate, 50 mM KF, 25 mM MgCl₂ and 5 mM dithiothreitol. The samples were brought to 200 µl, final volume, vortexed and incubated at 37 °C for 40 min. One millilitre ice-cold water was then added to each assay tube to stop the reaction. The samples were applied to NaCl pre-washed ion-exchange columns (Saier *et al.*, 1977), the columns were washed twice with 12 ml water and the samples were eluted with 12 ml 1 M LiCl into scintillation vials containing Biosafe II scintillation cocktail in preparation for scintillation counting. For the D-alanine dehydrogenase assay, aliquots of cell extracts were added to a solution containing 2 mM D-alanine, 10 mM potassium phosphate, pH 7.5, and 0.33 mg dinitrophenyl hydrazine ml⁻¹. At time intervals of 10 min, the reaction was stopped by addition of 1.67 ml 10% NaOH and the absorbance was read at 445 nm as described by Wild *et al.* (1974; see also Saier *et al.*, 1980).

Transport assays. Cells were grown with shaking at 37 °C in LB medium, harvested during exponential growth by centrifugation, washed three times with minimal medium 63 and resuspended in the same medium. The cell suspensions were pre-warmed at 37 °C for 10 min before ¹⁴C-labelled glucitol or D-alanine was added to a final concentration of 0.1 mM. At intervals, samples were transferred to pre-washed 0.2 µm (pore diameter) millipore filters. Cells were washed three times with medium 63 before the filters were dried, transferred to scintillation vials containing Biosafe-NA scintillation cocktail and counted.

cAMP assays. Cultures were grown for net cAMP production in minimal salts medium containing 1% lactate or 1% lactate plus 0.5% glucose for 5 h before samples were removed for extraction of cAMP (Castro *et al.*, 1976). The optical density of the cultures (600 nm) was approximately 0.6. Samples were

placed in a boiling water bath for 5 min, chilled to 0 °C and centrifuged to remove cell debris. Total cAMP was determined using partially purified cAMP-binding protein from beef muscle (Gilman, 1970), essentially as described previously (Castro *et al.*, 1976; Feucht & Saier, 1980). The amount of cAMP produced (Table 6) is expressed in nmol (mg dry wt)⁻¹. No background activity was subtracted from the values presented.

RESULTS

Effects of *cysB* mutations on carbon utilization in *E. coli* and *S. typhimurium*

Table 2 records the Biolog results observed for *cysB* mutants of both *E. coli* and *S. typhimurium* relative to their isogenic wild-type (*cysB*⁺) strains. The results observed for the *S. typhimurium* *cysB* mutant generally (but not always) paralleled those obtained with the *E. coli* mutant. Table 2 records the most pronounced effects observed. Clear-cut parallel effects were observed for D-alanine, L-fucose, D-glucitol, glucuronamide, α-hydroxyglutaric acid γ-lactone and L-rhamnose. In two cases (D-glucuronate and D-gluconate), the *S. typhimurium* *cysB* mutant exhibited decreased utilization relative to its parental strain, but the *E. coli* mutant did not. In two cases (glycolate and glyoxylate), the *E. coli* *cysB* mutant exhibited decreased utilization relative to its wild-type strain, but the wild-type *S. typhimurium* strain did not exhibit a positive response towards either compound. In one case (formate), opposite responses were observed for *S. typhimurium* and *E. coli* *cysB* mutants (increased utilization in the former strain; decreased utilization for the latter strain). Although the catalytic repertoires for using carbon sources are similar in *E. coli* and *Salmonella* species, significant differences

Table 2. Carbon oxidation by wild-type and *cysB* mutant strains of *S. typhimurium* and *E. coli*

Carbon oxidation was assayed using Biolog plates (Bochner, 1993). Relative responses were recorded as follows: + + +, very strong; + +, strong; +, weak; —, no response.

Substrate	<i>S. typhimurium</i>		<i>E. coli</i>	
	WT	<i>cysB</i>	WT	<i>cysB</i>
D-Alanine	+	+	+	+
Cellobiose	—	—	+	—
Formate	+	+	+	+
L-Fucose	+	—	+	—
D-Glucitol	+	—	+	—
Glucuronamide	+	—	+	—
D-Glucuronate	+	+	+	+
D-Gluconate	+	+	+	+
Glycolate	—	—	+	—
Glyoxylate	—	—	+	—
α -Hydroxyglutaric acid γ -lactone	+	—	+	—
L-Rhamnose	+	+	+	—

Table 3. Fermentation properties of *cysB* mutants of *S. typhimurium* and *E. coli* on solid medium

Sugars were present at 1%, with the exception of rhamnose (0.5%). Djenkolate and cysteine when present were included in the fermentation medium at 1 mg ml⁻¹. + +, Strong fermentation; —, no fermentation; + or $\pm$, intermediate fermentation responses. Relative colour on EMB plates was evaluated for individual colonies. Triplicate determinations gave comparable results.

Fermentation (EMB)	<i>S. typhimurium</i>		<i>E. coli</i>	
	WT	<i>cysB</i>	WT	<i>cysB</i>
L-Fucose	+	$\pm$	+	$\pm$
Glucose	+	+	+	+
Galactose	+	+	+	+
Mannitol	+	+	+	+
L-Rhamnose	+	$\pm$	+	—
Glucitol	+	$\pm$	+	$\pm$
Glucitol + djenkolate	+	+	+	+
Glucitol + cysteine	+	+	+	+

are found even among the strains and substrains of each species (Lin, 1996).

Fermentation properties of *cysB* mutants of *S. typhimurium* and *E. coli*

Table 3 summarizes the results of carbohydrate fermentation responses of wild-type and *cysB* mutant

Fig. 1. Uptake of [¹⁴C]glucitol by wild-type cells (◆) and by *cysB* mutant cells grown in LB broth plus 0.5% glucitol with (▲) or without (■) djenkolate at a concentration of 1 mg ml⁻¹. Cells were harvested 3 h after inoculation during exponential growth as described under Methods.

strains to select compounds. The *cysB* mutants of *S. typhimurium* and *E. coli* generally did not ferment carbohydrates as well as did the isogenic parents, and for both bacterial species, responses towards L-rhamnose and D-glucitol appeared to be most pronounced. On minimal medium, both *cysB* mutants exhibited negligible growth regardless of the carbon source present, as expected, since the *cysB* mutation gives rise to a requirement for exogenous cysteine or another sulfur source (data not shown). In all cases examined, inclusion of cysteine or djenkolate in the media partially, but not completely, reversed the growth phenotype. Even high concentrations of these compounds did not restore wild-type growth responses (data not shown).

Glucitol uptake in *S. typhimurium*

Fig. 1 shows time courses for the uptake of [¹⁴C]D-glucitol by *S. typhimurium* strain LT-2 and by the isogenic *cysB* mutant following growth in LB broth (moderately sulfur-limiting conditions comparable to our fermentation conditions). Uptake was reduced nearly tenfold by loss of CysB. When the *cysB* mutant was grown in the presence of glucitol plus djenkolate, uptake was partially restored. These results are in full agreement with the fermentation results recorded in Table 3.

Table 4. Effects of a *cysB* mutation on the specific activities of transport systems and enzymes concerned with glucitol and alanine utilization in *S. typhimurium*

Cells were grown in LB broth (moderately sulfur-limiting conditions ± 1 mg L-djenkolate ml⁻¹), harvested in the exponential growth phase and prepared for transport or enzyme assay as described in Methods. One hundred percent corresponds to the following values: glucitol transport, 5 nmol (mg dry wt)⁻¹ min⁻¹; Enzyme II^{Gut} (measured *in vitro*), 750 nmol (mg protein)⁻¹ min⁻¹; glucitol-6-phosphate dehydrogenase, 630 nmol (mg protein)⁻¹ min⁻¹; D-alanine transport, 1.2 nmol (mg dry wt)⁻¹ min⁻¹; D-alanine dehydrogenase, 70 nmol (mg protein)⁻¹ min⁻¹. ND, Not determined.

Activity measured	Relative activity (%)		
	WT	<i>cysB</i>	<i>cysB</i> + djenkolate
Glucitol transport	100 $\pm$ 2	13 $\pm$ 3	48 $\pm$ 5
Enzyme II ^{Gut}	100 $\pm$ 22	52 $\pm$ 24	73 $\pm$ 18
Glucitol-6-phosphate dehydrogenase	100 $\pm$ 38	54 $\pm$ 23	63 $\pm$ 23
D-Alanine transport	100 $\pm$ 15	71 $\pm$ 11	ND
D-Alanine dehydrogenase	100 $\pm$ 30	9 $\pm$ 6	ND

Effects of *cysB* mutations on the specific activities of enzymes and transport systems concerned with glucitol and D-alanine utilization

Table 4 summarizes the results of studies aimed at quantifying the specific activities of transport systems and catabolic enzymes concerned with glucitol and D-alanine utilization in *S. typhimurium*. Glucitol is utilized via a pathway that involves concomitant transport and phosphorylation of the sugar, yielding cytoplasmic glucitol 6-phosphate, followed by oxidation of glucitol 6-phosphate to fructose 6-phosphate (Lengeler, 1975a, b). The Enzyme II^{Gut} (glucitol PTS permease) and glucitol-6-phosphate dehydrogenase were therefore assayed in crude extracts derived from wild-type and *cysB* mutant cells of *S. typhimurium*. As summarized in Table 4, both activities were depressed to about 50 % of the wild-type level by the *cysB* mutation, and the inclusion of djenkolate in the growth medium partially restored these activities. Inclusion of cysteine had an effect similar to that of djenkolate (data not shown). These results are in agreement with the fermentation and transport results cited above.

When D-alanine uptake and D-alanine dehydrogenase were similarly assayed, qualitatively similar results were obtained. Thus, D-alanine transport activity was reduced to 70 % whilst D-alanine dehydrogenase activity decreased to 9 %. The dramatic effect of the *cysB* mutation on D-alanine dehydrogenase activity can account for the poor response to alanine as measured with Biolog plates.

Use of reporter gene transcriptional fusions to study the effects of a *cysB* mutation on gene expression

A plasmid bearing a *gutB-lacZ* transcriptional fusion was transformed into wild-type and *cysB* mutant strains

of *E. coli*. Cells bearing this multicopy plasmid were then grown in nutrient medium under non-inducing, repressing and inducing conditions as described in Methods. The lack of induction by glucitol presumably reflects the multiple copies of the *gut* operon control region since the chromosomally encoded regulatory proteins, GutM and GutR (Yamada & Saier, 1988), may not be present in sufficient amounts to regulate all plasmid operators. The results depicted in Fig. 2(a) clearly show that when cells were grown under either non-inducing conditions (NB alone) or inducing conditions (NB plus 1 % glucitol), the presence of the *cysB* mutation drastically reduced glucitol operon expression (Yamada & Saier, 1987, 1988; Yamada *et al.*, 1990). CRP is present in *E. coli* cells in large amounts. Consequently, catabolite repression would not be expected to be altered by expression of a target gene on a multicopy plasmid. As can be seen, strong catabolite repression is observed. Unexpectedly, however, there was little effect of the *cysB* mutation under glucose-repressing conditions.

Comparable experiments were conducted with a transcriptional reporter gene fusion to the *fdoGHI* (aerobic formate dehydrogenase) operon. In this *fdoG-uidA* fusion, the reporter gene encodes β -glucuronidase (Abaibou *et al.*, 1995) (Fig. 2b). Results paralleled those observed for the glucitol operon gene fusion. Thus, induced and uninduced activities were comparable, and unrepressed activity (i.e. in NB-grown cells) was reduced about twofold by the *cysB* mutation. This activity was largely restored by inclusion of cysteine in the growth medium. By contrast, glucose-repressed activity was only weakly affected by the *cysB* mutation. These results led to the possibility that the *cysB* mutation prevented the normal cAMP-CRP-mediated activation of catabolite-sensitive operons under non-repressing conditions. Activity under non-repressing conditions

Fig. 2. Transcription of the glucitol (*gut*, a) and formate dehydrogenase (*fdo*, b) operons in wild-type (WT) and *cysB* mutant strains of *E. coli*. (a) A plasmid-encoded *gutB-lacZ* fusion was introduced into the two strains by electroporation as described in Methods. Cells were grown in NB with or without glucose (Glc; 1%) or glucitol (Gut; 1%) at 37 °C for 3 h before harvesting, cell rupture and assay of β -galactosidase. (b) A plasmid-encoded *fdoG-uidA* fusion was similarly introduced into the two strains by electroporation. The transformed cells were grown in nutrient broth with or without formate (Form) or glucose (Glc) (1% each) for 3 h before harvesting, cell rupture and assay of β -glucuronidase as described in Methods. White boxes at the top of the dark bars denote the error expressed in standard deviations with the measured mean values denoted by the dark bars. The lack of induction reflects the use of high-copy-number plasmids.

was thus affected by the *cysB* mutation much more than that under repressing conditions.

Effect of exogenous cAMP on gene expression

The results reported in Fig. 2 suggested that the *cysB* defect might interfere with the function of the cAMP-CRP complex that normally activates catabolite-sensitive genes. To test this possibility, expression of the two reporter gene fusions (*gutB-lacZ* and *fdoG-uidA*) was studied as a function of the concentration of exogenous cAMP. The results are summarized in Fig. 3. As can be seen, cAMP increased expression of both fusions in the *cysB* genetic background, but decreased expression of both fusions in the wild-type background. The mild repressive effect in the wild-type background suggests that, in the absence of exogenous cAMP, these cells synthesize sufficient cAMP for maximal transcription. The results suggest that a primary deficiency, giving rise to defective expression of carbon catabolic enzyme-encoding genes, is related to depressed levels of cytoplasmic cAMP in the *cysB* mutant relative to the wild-type strain.

Fig. 3. Effects of cAMP on expression of the genes encoding Enzyme IIA of the glucitol PTS (a) and formate dehydrogenase (b) of *E. coli*. Cells (WT, $\blacklozenge$; *cysB*, $\blacksquare$) were grown at 37 °C in nutrient broth for 3 h in the presence of the concentration of exogenous cAMP indicated. β -Galactosidase (a) or β -glucuronidase (b) was assayed as described in Methods. Five millimolar cAMP depressed growth rates of the wild-type strain, but not of the *cysB* mutant.

Table 5. β -Galactosidase levels in Crooke's strain and the isogenic *cysB* mutant strain

Growth conditions were as described in Methods using the minimal sulfate-free medium with 1% DL-lactate, 0.5% D-glucose, 0.01% L-tryptophan, 0.2 mM cysteine or 1 mM djenkolate as indicated above. IPTG (1 mM) was present under all conditions to neutralize the effect of the *lac* operon repressor.

Medium	β -Galactosidase activity (Miller units)	
	Wild-type	<i>cysB</i>
Lactate djenkolate	3030 $\pm$ 240	2500 $\pm$ 290
Lactate cysteine	3610 $\pm$ 290	3200 $\pm$ 220
Glucose djenkolate	1900 $\pm$ 100	450 $\pm$ 110
Glucose cysteine	1700 $\pm$ 110	1600 $\pm$ 120

Control of *lac* operon expression in response to sulfur limitation

Table 5 presents the results of experiments designed to test the effects of sulfur limitation on native β -galactosidase synthesis when *lacZ* is expressed under the control of the *lac* operon promoter and control region. These experiments were conducted in the genetic background of Crooke's strain (SB2249; *cpd*; Castro *et al.*, 1976). Since this strain lacks cAMP phospho-

Table 6. cAMP levels in strains grown on lactate and djenkolate

The concentrations were as follows: DL-lactate, 1%; L-djenkolate, 1 mM; L-tryptophan, 0.01% (present in all cultures); D-glucose, 0.5% (present + glucose only).

Strain	Relevant genotype	cAMP [nmol (mg dry wt) ⁻¹]	
			+ Glucose
LJ4511	<i>cpd</i>	1.10 ± 0.11	0.39 ± 0.04
LJ4508	<i>cpd cysB</i>	1.02 ± 0.14	0.29 ± 0.03
LJ4512	<i>cpd ptsI^{ts} crr</i>	0.45 ± 0.08	0.61 ± 0.12
LJ4510	<i>cpd ptsI^{ts} crr cysB</i>	0.34 ± 0.04	0.56 ± 0.07

diesterase, cAMP degradation cannot occur. This leads to higher cytoplasmic levels of this nucleotide. In the wild-type strain, β -galactosidase activity was high when cells were grown in 1% lactate-containing minimal medium and substantially lower when grown in the presence of 0.5% glucose. Under these conditions, the presence of djenkolate or cysteine had little effect on *lacZ* expression, although expression was slightly higher when cysteine (0.2 mM) was present as compared with djenkolate (1 mM). These results are consistent with the conclusion that in *cysB*⁺ cells, cAMP is present at high cytoplasmic concentrations and glucose only moderately reduces these concentrations. However, in the isogenic *cysB* mutant, lactate-grown cells showed substantially less β -galactosidase activity than the wild-type strain and the difference between cysteine- and djenkolate-grown cells was somewhat greater. Growth in the presence of djenkolate is known to promote sulfur limitation in a *cysB* mutant to a greater extent than growth in the presence of cysteine (Kredich, 1996). Most striking, however, was the effect of sulfur limitation on the *cysB* mutant when grown in the presence of glucose plus djenkolate. β -Galactosidase activity dropped to about 20% of the value obtained when the wild-type strain was grown with glucose with or without either cysteine or djenkolate. Activity was also high when the *cysB* mutant was grown with glucose plus cysteine. These results show that *lacZ* expression is greatly reduced only in the *cysB* mutant under conditions of sulfur starvation and substantiate the suggestion that sulfur limitation lowers cellular cAMP levels.

Direct measurement of cAMP production

Total cAMP production (cells plus medium) was quantified in the same isogenic *cpd* and *cpd cysB* strains used in the experiments described in the previous section (Table 6). In this experiment, cells were grown in the same minimal medium supplemented with lactate or glucose plus djenkolate. Because Crooke's strain (SB2249) lacks the cAMP-degradative enzyme, cAMP phosphodiesterase, it is clear that the effects are due to the regulation of cAMP synthesis, not cAMP degradation.

The *cysB* mutant showed slightly lower cAMP production than the isogenic *cysB*⁺ strain when lactate was the sole carbon source present. In the presence of glucose, however, cAMP production was greatly reduced and the *cysB* mutation reduced production even further. The presence of the *ptsI crr* double mutations reduced cAMP production during growth on lactate, but increased production in the presence of glucose. In this case, glucose may have increased cAMP production by increasing energy availability and thereby increasing the level of ATP in the cell. *crr* mutants are insensitive to PTS-mediated regulation of adenylate cyclase (Castro *et al.*, 1976; Feucht & Saier, 1980). In the *crr* genetic background, in the presence of glucose, the *cysB* mutation did not reduce cAMP production levels to those observed for glucose-grown LJ4508 (*cysB*). The results are therefore in qualitative agreement with the results presented in Table 5, assuming that cellular cAMP levels explain the results reported therein. The data suggest, but are insufficient to establish, that CysB exerts its effect on adenylate cyclase by influencing the PTS-regulatory apparatus rather than directly affecting adenylate cyclase itself.

DISCUSSION

Mutations affecting pleiotropic transcription factors controlling nitrogen, sulfur, phosphorus or iron also have a negative effect on carbon utilization (Hantke, 1987; Hartmann & Boos, 1993; Shi & Bennett, 1994; and our unpublished results). Thus, for example, in *E. coli*, using Biolog plates, a *phoB* mutant showed decreased glucose, maltose, melibiose and trehalose utilization, a *glnB* mutant exhibited overall weaker responses than did the parental strain and a *fur* mutant showed poor utilization of proline, L-fucose, D,L- α -glycerol phosphate, formate, L-rhamnose, fumarate and glycyl-L-aspartate. In some cases, these effects might be indirect, due to a deficiency for one or more specific compound(s) synthesized or secured by the cell. Such a compound might be required for carbon metabolic processes to proceed normally. Such indirect effects may be of great physiological significance, allowing the bacterium to coordinate the utilization of one nutrient class of compounds with another. We anticipate that such regulatory effects have been selected for during evolution. However, very few studies have addressed the mechanisms by which these regulatory interactions occur.

Recently, evidence for a connection between the cAMP-CRP-regulated modulon and the stringent response in *E. coli* has been reported (Johansson *et al.*, 2000). Moreover, the homologous nucleoid proteins, H-NS and StpA, were shown to play a positive role in expression of stringently regulated genes as well as in cell growth. The results reported suggest that catabolite repression, the stringent response and control of gene expression by structural nucleoid proteins are all interconnected (Johansson *et al.*, 2000). The relationship between these observations and those reported here, if any, have yet to

be investigated, but the results of Johansson *et al.* (2000) corroborate our postulate that the different regulons in the bacterial cell are interrelated.

In this communication, we not only document the effects of *cysB* mutations in *E. coli* and *S. typhimurium* on carbon oxidation and carbohydrate fermentation, but we also pursue the effect of the *cysB* mutation on carbon utilization to the mechanistic level. The results reported revealed that the effects were at the transcriptional level for both the *gut* and the *fdo* operons. Both effects appear to be partially reversed by exogenous cAMP or a sulfur source such as cysteine or djenkolate. Furthermore, we provide evidence that the effect is on regulation of the cAMP biosynthetic enzyme, adenylate cyclase, by the IIA^{Glc} protein of the PTS, and not on the inherent activity of adenylate cyclase itself. Our current postulate is that these effects are indirect, that they affect the regulation of adenylate cyclase as a primary target and that the level and/or activity of this enzyme will prove to be hypersensitive to the metabolic state of the cell. Such sensitivity would allow the bacterium to coordinate carbon/energy metabolism with the availability of other macro- and micronutrients. The mechanistic details of these interactions are currently under study in our laboratory.

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