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Glutathione Is Required for Maximal Transcription of the Cobalamin Biosynthetic and 1,2-Propanediol Utilization (*cob*/*pdu*) Regulon and for the Catabolism of Ethanolamine, 1,2-Propanediol, and Propionate in *Salmonella typhimurium* LT2

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Transcription of the *cob*/*pdu* regulon of *Salmonella typhimurium* is activated by the PocR regulatory protein in response to 1,2-propanediol (1,2-PDL) in the environment. Nutritional analysis and DNA sequencing confirmed that a strain defective in expression of the *cob*/*pdu* regulon in response to 1,2-PDL lacked a functional *gshA* gene. *gshA* encodes γ-glutamylcysteine synthetase (L-glutamate:L-cysteine γ-ligase [ADP forming]; EC 6.3.2.2), the enzyme that catalyzes the first step in the synthesis of glutathione (GSH). The DNA sequence of *gshA* was partially determined, and the location of *gshA* in the chromosome was established by two-factor crosses. P22 cotransduction of *gshA* with nearby markers showed 21% linkage to *srl* and 1% linkage to *hyd*; *srl* was 9% cotransducible with *hyd*. In light of these data, the gene order *gshA srl hyd* is suggested. The level of reduced thiols in the *gshA* strain was 87% lower than the levels measured in the wild-type strain in both aerobically and anaerobically grown cells. 1,2-PDL-dependent transcription of *cob*/*pdu* was studied by using M. Casadaban's Mu-*lacZ* fusions. In aerobically grown cells, transcription of a *cbi-lacZ* fusion (the *cbi* genes are the subset of *cob* genes that encode functions needed for the synthesis of the corrin ring) was 4-fold lower and transcription of a *pdu-lacZ* fusion was 10-fold lower in a *gshA* mutant than in the wild-type strain. Expression of the *cob*/*pdu* regulon in response to 1,2-PDL was restored when GSH was included in the medium. In anaerobically grown cells, *cbi-lacZ* transcription was only 0.4-fold lower than in the *gshA*$^+$ strain; *pdu-lacZ* transcription was reduced only by 0.34-fold, despite the lower thiol levels in the mutant. *cobA-lacZ* transcription was used as negative control of a gene whose transcription is not controlled by the PocR/1,2-PDL system; under both conditions, *cobA* transcription remained unaffected. The *gshA* mutant strain was unable to utilize 1,2-PDL, ethanolamine, or propionate as a carbon and energy source. The defect in ethanolamine utilization appears to be at the level of ethanolamine ammonia-lyase activity, not at the transcriptional level. Possible roles for GSH in ethanolamine, 1,2-PDL, and propionate catabolism are proposed and discussed.

In *Salmonella typhimurium*, the cobalamin biosynthetic operon (*cob*) and the 1,2-propanediol (1,2-PDL) utilization (*pdu*) genes are coregulated at the transcriptional level in response to 1,2-PDL in the environment (9, 33). This induction requires the PocR protein (9, 33), a transcriptional activator with homology to the AraC protein (11, 36). Adenosylcobalamin serves as a cofactor for the first enzyme in the 1,2-PDL degradation pathway (21).

The *cob* and the *pdu* operons are located at min 41 on the chromosome and are transcribed divergently from each other (21). The intervening DNA between *cob* and *pdu* contains the *pocR* gene (9, 11, 33), which is transcribed in the same direction as the *cob* operon (9).

In this paper, we report the isolation and characterization of a mutant strain of *S. typhimurium* (JE2546) which is unable to utilize 1,2-PDL as the sole carbon and energy source under aerobic conditions. Interestingly, the mutation in this strain affects *cob* and *pdu* transcription in aerobically but not anaerobically grown cells. We document that the gene disrupted in strain JE2546 is *gshA*, whose product catalyzes the first step in glutathione (GSH) synthesis. In *E. coli*, the GshA protein, γ-glutamylcysteine synthetase (EC 6.3.2.2), has been shown to catalyze the synthesis of γ-glutamylcysteine from L-cysteine and L-glutamic acid (42). In *E. coli*, GSH is synthesized from γ-glutamylcysteine and glycine by the GshB protein (glutathione synthetase [EC 6.3.2.3]) (14).

GSH has been shown to play many roles in the physiology of eucaryotic and procaryotic cells, including protection against oxidative damage, toxic compounds, radiation, and heavy metals (28). Glutathione is also a cofactor or cosubstrate for many enzymes (28). Studies with *Escherichia coli* have documented the involvement of GSH in activation of the KefB potassium efflux system (16), in adaptation to osmotic stress (27), in the reactivation of aconitase (18), in arsenate resistance (31), and in the detoxification of the very reactive α-ketoaldehyde methylglyoxal (12).

We present data which document that GSH is required for maximal transcription of *cob* and *pdu* under aerobic growth conditions and that a GSH-deficient mutant of *S. typhimurium* is defective in a number of cellular processes. Our results suggest that the role of GSH in the detoxification of aldehydes may extend to the catabolism of poor carbon sources such as ethanolamine, 1,2-PDL, and probably propionate.

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MATERIALS AND METHODS

Bacterial strains, media, and growth conditions. The genotypes of strains and plasmids used in this work are listed in Table 1. Media and techniques used in

TABLE 1. Strains and plasmids used

Strain or plasmid	Genotype[a]	Source or reference
Strains		
S. typhimurium LT2		
TR6583 (formerly SA2979)	*metE205 ara-9*	K. Sanderson via J. Roth
JF1534	*hyd-1088*::MudJ	J. Foster via K. Sanderson
JR501	*hsdSA29 hsdSB121 hsdL6 trpC2 metA22 metE551 ilv452 leu3121 rpsL120 galE719 xyl-404 H1-b H2-e, n,x nml* (Fels2)$^-$ *fla-66*	41
TT15261	*proU1884*::MudP	8
Derivatives of TR6583		
JE2529	*srl-203*::Tn*10d*(Cm)	
JE2546	*gshA101*::Tn*10d*(Tc)	This study
JE2649	*cbi-24*::MudA	
JE3084	*cbi-24*::MudA *gshA101*::Tn*10d*(Tc)	
JE3562	*pdu-12*::MudA	
JE3085	*pdu-12*::MudA *gshA101*::Tn*10d*(Tc)	
JE1096	*cobA343*::MudJ	
JE3225	*cobA343*::MudJ *gshA101*::Tn*10d*(Tc)	
JE1684	*eutE18*::MudA	
JE2646	*eutE18*::MudA *gshA101*::Tn*10d*(Tc)	
JE2893	*proU1884*::MudP *gshA101*::Tn*10d*(Tc)	
E. coli DH5α/F′	F′/*endA1 hsdR17* (r_K^- m_K^+) *supE44 thi-1 recA1 gyrA* (Nal^r) *relA1* Δ(*lacZYA-argF*)*U169 deoR* [ϕ80d*lac*Δ(*lacZ*)*M15*]	6
Plasmids		
pSU19		Cm^r cloning vector (26)
pUC19		Ap^r cloning vector (30)

[a] Throughout this paper, Tn*10*DEL *16*DEL *17* (DEL = deletion) is abbreviated Tn*10d*(Tc), Mud1-8 is abbreviated MudA, and MudI 1734 is abbreviated MudJ. Mutation *cbi-24*::MudJ was formerly known as *cob-24*::MudJ.

this study have been described elsewhere (33). Cultures were grown at 37°C with shaking in 5-ml volumes in test tubes (20 by 150 mm); reduced-oxygen cultures were grown in filled screw-cap tubes without shaking. Incubation of agar plates under anoxic conditions was accomplished by using Gas-Pak jars (Bethesda Research Laboratories, Gaithersburg, Md.). Growth of cultures was monitored with a Spectronic 20D spectrophotometer (Milton Roy Co., Rochester, N.Y.). GSH was added to 0.1 mM (final concentration); 1,2-PDL was added to 12 mM (final concentration); carbon sources were present as noted in the text; auxotrophic requirements and antibiotics were added to concentrations reported earlier (33).

Chemicals. 2,4-Dinitrophenylhydrazine (DNPH), 3-methyl-2-benzothiazolinone hydrazone, 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), and glutathione reductase (GSSG reductase) were purchased from Sigma Chemical Co. (St. Louis, Mo.); all other chemicals were commercially available.

Assays. (i) β-Galactosidase. β-Galactosidase activity was measured in mid-log-phase cultures as previously described (33).

(ii) EAL. Ethanolamine ammonia-lyase (EAL) activity was assayed as previously described (32) except that higher amounts of protein (0.04 to 0.08 mg) were used. One unit of activity was defined as the amount of enzyme that produced 1 nmol of acetaldehyde per minute.

(iii) Determination of glutathione (GSH) levels. GSH levels in deproteinated cell extracts were measured by using the DTNB-GSSG reductase recycling assay (20) as described elsewhere (3). Extracts were prepared by precipitation of proteins with 1 volume of 100% methanol, followed by heating for 10 min at 65°C. The precipitate was removed by centrifugation, the supernatant was dried under vacuum, and the pellet was resuspended in GSH assay buffer (43 mM Na_2PO_4, 6.3 mM Na_4EDTA [pH 7.5]). The assay measured the difference in A_{412} of the reaction mixture after a 4-min incubation period at 30°C.

(iv) Determination of methylglyoxal levels. DNPH-reactible material in culture supernatants was assayed as described previously (22). The measurements were compared against a standard curve generated with commercially available methylglyoxal (Sigma). The culture medium itself contained no reactible material.

Genetic techniques. (i) Transductions. All crosses used bacteriophage P22 double mutant HT105/1 *int201* (37, 38). Transductions were performed as described previously (33).

(ii) Mapping. The Tn*10d*(Tc) element in JE2546 was mapped by using the Mud-P22 mapping kit described by Benson and Goldman (8), using transducing lysates prepared on the set of lysogens and selecting for the loss of tetracycline resistance (Tc^r) on Bochner plates (10, 24). Crude preparations of bacteriophage P22 tail spike protein were obtained as described previously (39, 44). Approximately 35 μg of protein of a crude extract containing P22 tail spike protein was routinely spread onto the plate prior to dispensing of Mud-P22 samples by means of a multiprong device.

Recombinant DNA techniques. (i) Cloning of *gshA101*::Tn*10d*(Tc). We used the Mud-P22 system (44) to clone the Tn*10d*(Tc) element from JE2546. The *proU1884*::MudP (clockwise packaging) element was introduced into strain JE2546 to yield strain JE2893. This strain was used to induce the P22 prophage by the addition of mitomycin C. Following an overnight incubation at 37°C with shaking, a phage lysate was prepared (13), and DNA was isolated from the phage heads (44). This DNA was digested to completion with *Pst*I (which does not cut within the Tn*10d*(Tc) element [43]) and ligated into *Pst*I-digested pSU19 (26). The ligation mix was electroporated into the restriction-deficient, modification-proficient *S. typhimurium* strain JR501 and plated on LB plates containing chloramphenicol to select for inheritance of plasmids. Plasmids carrying the Tn*10d*(Tc) element were identified by replica printing onto LB-chloramphenicol plates containing tetracycline at a concentration of 2 μg/ml (43). Plasmid DNA was prepared from these colonies via an alkaline lysis method (25) and used to transform strain JR501 to Cm^r. These plates were replica printed to test for coinheritance of the Tc^r and Cm^r markers. Plasmid DNA was prepared from double-drug-resistant colonies by using a Wizard MiniPrep kit (Promega, Madison, Wis.); DNA was digested with *Pst*I to determine the size of the insert and with *Eco*RI to cut at a unique site within the Tn*10d*(Tc) element (43). This analysis revealed that the cloned insert had no *Eco*RI site other than the one present in the Tn*10d*(Tc) element; thus, we were able to subclone the two *Eco*RI-*Pst*I fragments obtained from a double digest directly into pUC19 (30). These manipulations were performed in *E. coli* DH5α/F′ in order to use the blue/white screen for plasmids containing the cloned DNA (6). This subcloning step separated the two halves of the Tn*10d*(Tc) element, allowing us to use the primer 5′ATGTGTATCCACCTTAAC3′ (Genosys, The Woodlands, Tex.), which hybridized to the inverted repeat of the element, to sequence the junction site of the insertion and the flanking chromosomal DNA.

(ii) DNA sequencing. A Sequenase kit (version 2.0; U.S. Biochemical Co., Cleveland, Ohio) was used to sequence the DNA region flanking the Tn*10d*(Tc) element. Sequence data were compared with sequences in GenBank by using the BLAST algorithm developed by the National Center for Biotechnology Information at the National Library of Medicine (2).

RESULTS

We document that in *S. typhimurium* LT2, reduced GSH levels have a severe impact on the transcription of the *cob*/*pdu* regulon and on the utilization of ethanolamine, 1,2-PDL, and propionate as carbon and energy sources. Interestingly, these effects are seen only when cells are grown under aerobic conditions, and as expected, they are reversed by exogenous GSH

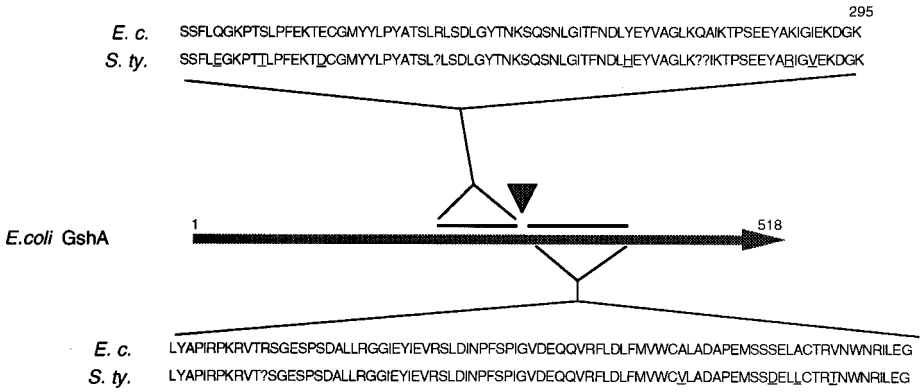

FIG. 1. Location of the Tn*10d*(Tc) element within *gshA*. The *E. coli* GshA protein is represented by the large arrow. The location of the Tn*10d*(Tc) element within the *S. typhimurium* coding region is indicated by the large inverted triangle. The small lines represent the locations of the sequences that were determined and are to scale. The partial amino acid sequence of the GshA protein of *E. coli* B, as well as the deduced sequence from *S. typhimurium*, is shown. The underlined amino acids indicate a difference between the *E. coli* (*E. c.*) and *S. typhimurium* (*S. ty.*) proteins. A question mark indicates that the residue was not determined at that location. Numbers indicate the size of the *E. coli* protein and the location of the insertion; 295 refers to the final lysine residue in the upper sequence.

or by its precursor γ-glutamylcysteine, the product of the reaction catalyzed by GshA.

We also report the partial determination of the nucleotide sequence of the *gshA* gene of *S. typhimurium* LT2. The chromosomal location of the *gshA* gene was established by genetic means, and the gene order relative to nearby markers was determined.

Identification and chromosomal location of *gshA* in *S. typhimurium*. Strain JE2546 [*gshA101*::Tn*10d*(Tc)] was isolated during a mutant search designed to identify genetic loci (other than those already known) required for aerobic growth with 1,2-PDL as the sole carbon source, using a phage P22 lysate prepared on a pool of approximately 80,000 strains, each carrying a Tn*10d*(Tc) element. The Tn*10d*(Tc) element in strain JE2546 did not map to any previously known loci involved in 1,2-PDL utilization (*pdu*, *pocR*, *btuB*, *tonB*, etc.). Mapping experiments using the Mud-P22 mapping kit of Benson and Goldman (8) determined that the general location of this element was in the 57- to 60-min region of the chromosome. Two-factor crosses with markers in this region showed that this element was 21% cotransducible by phage P22 with the *srl* locus and 1% cotransducible with *hyd*. We found that *srl* and *hyd* were 9% cotransducible, a finding that suggested the gene order *gshA srl hyd*. These results were consistent with the location of *gshA* in *E. coli* (7).

The DNA sequence flanking the Tn*10d*(Tc) element in strain JE2546 was determined and found to be 94% identical at the amino acid level (151 of 161 amino acid residues compared; 31% of the total protein length) to the GshA protein of *E. coli* B (Fig. 1) (42). *gshA* encodes L-glutamate:L-cysteine γ-ligase (ADP forming; EC 6.3.2.2), also known as γ-glutamylcysteine synthetase, which catalyzes the first step in the synthesis of GSH. The location of the insertion correlates with the location of *gshA* in *E. coli* (7). Hereafter, we refer to the insertion in JE2546 as *gshA101*::Tn*10d*(Tc).

Effect of mutation *gshA101*::Tn*10d*(Tc) on the intracellular level of reduced thiols. Deproteinated cell extracts of strain JE2546 contained only 13% of the level of DTNB-reactible material found in extracts of the wild-type strain. This was true for extracts prepared from aerobically grown cells and for extracts made from anaerobically grown cells, demonstrating that JE2546 is deficient in thiol synthesis under both growth conditions. Although the DTNB-GSSG reductase recycling assay is not specific for GSH, the reduction of DTNB-reactible material observed in the *gshA* mutant was significant, and it likely reflected the inability of the cell to synthesize GSH. These results were consistent with the insertion in strain JE2546 disrupting the *gshA* gene.

Phenotypes of the *gshA* mutant. (i) Growth phenotypes. The *gshA* mutant (JE2546) was unable to grow aerobically with 1,2-PDL as the sole carbon and energy source in culture medium supplemented with cobalamin. The *gshA*$^+$ strain grew on the same medium with a growth rate of 0.11 doublings per h. Strain JE2546 also failed to grow on ethanolamine (plus cobalamin) or propionate as the sole carbon and energy source. The growth rate of the *gshA*$^+$ strain on ethanolamine was 0.13 doublings per h; on propionate, the strain grew at a rate of 0.15 doublings per h. Strain JE2546 was able to use ethanolamine as the sole nitrogen source, suggesting that low but sufficient amounts of ethanolamine-degrading enzymes were synthesized in this strain. These levels were insufficient to support growth of the cell when ethanolamine served as the sole carbon and energy source. Only minor growth effects were observed when JE2546 was grown on other carbon sources such as succinate, fumarate, or pyruvate (data not shown).

In previous studies examining growth of *gsh* mutants in minimal medium, no or only minor differences in growth in comparison with a wild-type strain were observed (5, 17, 18, 29); we think that this reflects the carbon source provided (glucose or succinate in those cases).

Interestingly, no difference in growth between the *gshA* (JE2546) and *gshA*$^+$ (TR6583) strains was observed when cells were grown anaerobically on medium containing ethanolamine or propionate as the sole carbon and energy source and nitrate as the electron acceptor. 1,2-PDL was not tested since the wild-type strain does not grow on this compound anaerobically (33).

(ii) Transcription of the *cob* and *pdu* genes in aerobically grown cells. A dramatic reduction in *cob* and *pdu* transcription was observed in the GSH-deficient strain (Table 2). Transcription of the *cbi-24*::*lacZ* fusion was reduced 4-fold; transcription of the *pdu-8*::*lacZ* fusion was reduced 10-fold. This result helped explain the inability of the *gshA* mutant to grow on 1,2-PDL as a carbon and energy source.

For both *cbi* and *pdu*, wild-type levels of transcription were restored in cells grown in medium supplemented with GSH (Table 2), confirming that lack of GSH was responsible for the

TABLE 2. Effects of a *gshA* mutation on *cob* and *pdu* expression[a]

Strain	Relevant genotype	β-Galactosidase activity (U/A_{650}) in:				
		Aerobically grown cells			Anaerobically grown cells	
		No addition	1,2-PDL	1,2-PDL–GSH	No addition	1,2-PDL
JE2649	*cbi-24*::*lacZ gshA*$^+$	10	43	49	133	787
JE3084	*cbi-24*::*lacZ gshA101*::Tn*10d*(Tc)	8	11	50	142	474
JE3562	*pdu-12*::*lacZ gshA*$^+$	1	70	84	20	3,319
JE3085	*pdu-12*::*lacZ gshA101*::Tn*10d*(Tc)	2	7	103	13	2,176
JE1096	*cobA343*::*lacZ gshA*$^+$	58	57	65	45	50
JE3225	*cobA343*::*lacZ gshA101*::Tn*10d*(Tc)	45	43	56	50	52

[a] Cells were grown in NCE minimal medium containing $MgSO_4$, methionine, and D-(+)-xylose (30 mM) plus the stated additions (33). Aerobic growth was achieved by growing cultures in 5 ml of medium in test tubes (20 by 150 mm) with shaking (175 rpm); anaerobic growth was achieved by growing cultures in completely filled screw-cap tubes. 1,2-PDL was added to 12 mM; GSH was added to 0.1 mM. Cells were assayed during mid-log phase (A_{650} = 0.3 to 0.5). Values represent averages of three experiments; within each experiment, samples were assayed in duplicate.

altered transcription of the *cob*/*pdu* regulon seen in the *gshA* strain.

In contrast, transcription of a *cobA343*::*lacZ* fusion was reduced only about 25% in the mutant background. We chose the *cobA343*::*lacZ* fusion as a control for nonspecific effects. *cobA* encodes a protein required for the synthesis of cobalamin, yet it is not part of the *cob*/*pdu* regulon, as the *cobA* gene is located at a distance from the *cob* operon, and transcription of *cobA* is not regulated by 1,2-PDL (15, 34).

Together, these results indicated that decreased intracellular levels of GSH had a specific effect on *cob* and *pdu* expression and that our data did not reflect a general effect on gene transcription.

(iii) Transcription of *cob* and *pdu* in anaerobically grown cells. In anaerobically grown cells, the effect of the *gshA* mutation on *cob* and *pdu* transcription was not pronounced (Table 2). In the presence of 1,2-PDL, *cbi-24*::*lacZ* transcription was reduced only 0.4-fold; *pdu-8*::*lacZ* transcription was reduced only 0.34-fold; the control *cobA343*::*lacZ* fusion was unaffected. These results were consistent with our finding that growth of the *gshA* mutant under anaerobic conditions was not affected. Thus, it seems that for both transcription and growth, the *gshA* mutant has a phenotype only when the cells are grown aerobically.

(iv) Transcription of the ethanolamine utilization (*eut*) genes was not affected by reduced levels on GSH. Transcription of the *eut* operon is known to be induced by the presence of ethanolamine and cobalamin in the medium (35). Unlike transcription of *pdu* and *cob*, transcription of the *eut* operon was not significantly affected by the *gshA* mutation. Transcription of a *eut-lacZ* fusion in the *gshA* mutant was approximately 72% of the level measured in the *gshA*$^+$ strain. This reduction was similar to that of the control *cobA343*::*lacZ* fusion (77%), suggesting that the failure of the *gshA* strain to grow on ethanolamine was not due to a defect in activation of *eut* expression.

(v) EAL activity in the *gshA* mutant. We investigated the possibility that active EAL depends on the presence of GSH. The level of EAL activity was determined in cell extracts of *gshA*$^+$ (TR6583) and *gshA* (JE2546) strains grown under conditions known to induce transcription of the *eut* operon (35). A cell extract of JE2546 contained only 12% (13 U) of the EAL activity measured in the *gshA*$^+$ strain (110 U). EAL activity in a strain carrying an insertion within the *eut* operon, upstream of the *eutBC* genes encoding EAL, was only 6% (7 U) of the EAL activity found in the *gshA*$^+$ strain. Thus, the level of EAL activity in the *gshA* mutant was comparable to that in a strain that did not produce EAL. These results suggested that GSH was needed directly or indirectly to maintain EAL activity.

DISCUSSION

GSH has been identified as a key component for the utilization of ethanolamine, 1,2-PDL, and propionate in *S. typhimurium*. While the lack of GSH has a severe negative effect on transcription of the *cob*/*pdu* regulon, it appears to affect ethanolamine utilization by drastically reducing the level of active EAL. These effects may be due to the interaction of GSH directly with proteins or intermediates of these pathways.

Possible role of GSH in the catabolism of ethanolamine, 1,2-propanediol, and propionate. Analysis of the role of GSH in utilization of ethanolamine, 1,2-PDL, and propionate is complicated by the fact that the catabolic pathways for these compounds have not been completely elucidated. 1,2-PDL is catabolized to propionaldehyde by propanediol dehydratase, thought to be encoded by the *pdu* genes (21). The fate of propionaldehyde is not known. Ethanolamine is cleaved by EAL to produce acetaldehyde and ammonia (10a). Acetaldehyde is then catabolized to acetyl coenzyme A (40). The pathway for propionate utilization in *S. typhimurium* has not been studied; preliminary work in *E. coli* suggests that propionate may be catabolized via several pathways (15a).

Recent analyses of the *eut* and *pdu* genes in *S. typhimurium* have suggested that the Eut and Pdu catabolic enzymes may be confined within compartments where degradation of these compounds takes place (11, 40). A rationale for this hypothesis is that the first step in the catabolism of both ethanolamine and 1,2-PDL results in the formation of an aldehyde, which may be toxic to the cell. By localizing the enzymes and intermediates in a multiprotein compartment, the cell may have developed a mechanism to physically isolate these reactive aldehydes and thus protect itself from the negative effects of aldehyde reactivity.

GSH may play a key role in both the Eut and Pdu pathways, perhaps by protecting proteins in the complex from damage by aldehydes. GSH could function as an aldehyde scavenger under conditions in which these compounds are being locally produced. Alternatively, GSH might act as a cofactor for the aldehyde dehydrogenases which further metabolize acetaldehyde and propionaldehyde. GSH-dependent aldehyde dehydrogenases are widespread and have been documented in *E. coli* (19), although there is not evidence that the Eut and Pdu dehydrogenases are GSH dependent.

GSH-dependent detoxification of very reactive aldehydes has been demonstrated in the case of α-ketoaldehydes such as pyruvaldehyde (also known as methylglyoxal) (12, 23). Methylglyoxal synthesis in *E. coli* increases when the cell grows on carbon sources such as glycerol (12). GSH reacts with methylgyloxal to form a thiohemiacetal adduct which is converted to

lactoyl-S-glutathione by glyoxylase I and to D-lactate by glyoxylase II (12). In fact, we showed that when the *gshA*$^+$ (TR6583) and *gshA* (JE2546) strains were grown on glycerol, growth of the *gshA* strain was arrested before the culture reached mid-log phase (data not shown). At the onset of the arrest, sixfold more DNPH-reactible material was detected in the medium of the *gshA* mutant than in the medium of the *gshA*$^+$ strain at the same cell density. This level increased to 160-fold 18 h after growth of the *gshA* mutant ceased. These results indicated that the lack of GSH in JE2546 has a drastic effect on the ability of the cell to cope with aldehyde accumulation. A similar phenomenon may occur during growth on ethanolamine, propionate, and 1,2-PDL, although this hypothesis needs to be documented.

How does a lack of GSH affect *cob*/*pdu* transcription? Two proteins needed for the efficient transcription of the *cob*/*pdu* regulon in *S. typhimurium* are PduF (the proposed 1,2-PDL transport protein) and PocR (the transcription factor required for 1,2-PDL-dependent *cob* and *pdu* expression). We have previously shown that 1,2-PDL itself, and not a metabolite of 1,2-PDL, is the effector for PocR (33). Thus, it is unlikely that the effect of the *gshA* mutant is to prevent production of the true inducer of the *cob*/*pdu* regulon. To explain the effect of GSH on *cob*/*pdu* transcription, we propose that the lack of GSH may affect the function of PduF and/or of PocR. If this idea were correct, one possible explanation would be that GSH is needed to maintain some or all of the cysteinyl residues present in these proteins (eight Cys in PduF and nine Cys in PocR [11]) as reduced thiols in order to preserve biological activity. Effects on other proteins also known to affect *cob*/*pdu* transcription, i.e., catabolite gene activator protein and the ArcA/B system (1, 4), cannot be ruled out at this point.

Why is the effect of a lack of GSH evident only during aerobic conditions? As we have shown, *gshA* mutants have a phenotype only when grown aerobically, whereas the level of reduced thiols in the mutant is greatly decreased both during aerobic and anaerobic growth. This finding indicates that there is not an alternative pathway for GSH synthesis that functions anaerobically. There may be another molecule, synthesized only under anaerobic conditions, that is able to perform the functions of GSH when GSH is not available. Alternatively, the need for GSH may be greatly reduced under anaerobic growth conditions. If GSH is used by the cell to maintain redox-sensitive molecules in their active state, GSH may be dispensable in environments devoid of molecular oxygen. Aconitase may be one example of this, since GSH is needed aerobically to maintain sulfhydryl groups reduced for binding the iron-sulfur center required for enzyme activity (18). On the other hand, if GSH is needed aerobically to control aldehyde reactivity, either there must be an alternative mechanism for aldehyde detoxification anaerobically or utilization of these compounds may proceed differently under anaerobic conditions such that aldehydes do not accumulate. A better understanding of these pathways is needed to resolve these questions.

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