The *poc* Locus Is Required for 1,2-Propanediol-Dependent Transcription of the Cobalamin Biosynthetic (*cob*) and Propanediol Utilization (*pdu*) Genes of *Salmonella typhimurium*

MICHELLE R. RONDON AND JORGE C. ESCALANTE-SEMERENA*

Department of Bacteriology, University of Wisconsin—Madison,
1550 Linden Drive, Madison, Wisconsin 53706

Received 8 January 1992/Accepted 2 February 1992

In this communication we present evidence that indicates that 1,2-propanediol (1,2-PDL) is a positive effector of the transcription of the cobalamin (vitamin B₁₂) biosynthetic (*cob*) operon and of the 1,2-PDL utilization (*pdu*) genes. The stimulatory effects of 1,2-PDL were demonstrated using Mu d-*lac* transcriptional fusions to *cob* and *pdu*. Significantly increased levels of transcription of the *cob* and *pdu* operon fusions were measured in cultures grown under both anoxic and highly aerated conditions when 1,2-PDL was present in the culture medium. We have isolated mutants that carry lesions that render both *pdu* and *cob* transcription unresponsive to 1,2-PDL. These mutations were mapped to the region between *cob* and *pdu* (41 min), and they define the *poc* locus (PDL and cobalamin). The *poc* locus is required for the positive regulatory effects of 1,2-PDL to be exerted. Complementation of *Poc* function was achieved in *trans* by an F' plasmid carrying the *poc*⁺ locus, suggesting that at least one diffusible product regulates the expression of *cob* and *pdu* in *S. typhimurium*.

The enteric bacterium *Salmonella typhimurium* can synthesize cobalamin (vitamin B₁₂) de novo, but only under anaerobic growth conditions (14). The main cluster of cobalamin biosynthetic (*cob*) genes (at 41 min) has been divided into three regions, CobI, CobII, and CobIII. The *cobI* genes are required for the synthesis of adenosyl-cobinamide (Ado-Cbi); the *cobII* genes are required for the synthesis of the nucleotide dimethylbenzimidazole; and the *cobIII* genes are required to join Ado-Cbi and dimethylbenzimidazole to form adenosyl-cobalamin (10, 14). Previous work has shown that the Cob regions are contiguous in the order CobI, CobIII, and CobII; that they are transcribed counterclockwise; and that internal promoters allow basal, constitutive expression of *cobII* and *cobIII* genes under aerobic growth conditions (8, 14).

S. typhimurium does not make Ado-Cbi (the proposed end product of the CobI pathway) de novo in the presence of oxygen (14). Unlike CobI functions, CobII and CobIII functions are available for the synthesis of adenosyl-cobalamin under aerobic growth conditions provided that cobinamide is supplied in the culture medium. The reasons for the lack of synthesis of Ado-Cbi during aerobic growth of this bacterium have been investigated to a limited extent. The analysis of *cobI* transcription by using *lacZ* transcriptional fusions to the CobI promoter indicated that expression of the *cobI* genes was conditional to the absence of oxygen in the environment (9). It appeared from these results that the inability to synthesize Ado-Cbi under aerobic conditions was due to lack of transcription of the CobI region. However, Andersson and Roth (1) reported the isolation of mutants capable of transcribing the *cobI* genes to levels ≥80-fold higher than in the wild-type strain. These mutants were still unable to synthesize Ado-Cbi under aerobic growth conditions, which suggested that increasing the transcription of *cobI* was not sufficient to allow Ado-Cbi synthesis under aerobic conditions.

One metabolic pathway of *S. typhimurium* which requires cobalamin is the catabolism of 1,2-propanediol (1,2-PDL). 1,2-PDL can serve as the sole carbon and energy source for the cell under aerobic growth conditions, provided that the cells are supplemented with cobinamide or cobalamin. In this bacterium, the catabolism of 1,2-PDL proceeds via the cobalamin-dependent propanediol dehydratase, which converts it to propionaldehyde, which is then oxidized to propionate (13). The genes required for the catabolism of 1,2-PDL to propionate (PDL utilization or *pdu* genes) are located at 41 min, upstream of the *cob* operon. The physical distance between *pdu* and *cob* is unknown, but genetic markers in the two regions have been reported as being 12% cotransducible by bacteriophage P22 (13). It is also known that the *pdu* and *cob* genes are divergently transcribed and that *pdu* expression is regulated at the transcriptional level by 1,2-PDL (13).

In this paper, we present evidence that 1,2-PDL is a positive effector of *cob* transcription and report the isolation and initial characterization of mutants unresponsive to the stimulatory effects of 1,2-PDL. These mutants carry lesions that define a new locus referred to as *poc* (PDL and cobalamin). We propose that expression of the *cob* and *pdu* genes is coregulated at the transcriptional level by the *poc* gene product(s) in response to 1,2-PDL.

(Part of this work was presented at the 1991 American Society for Microbiology General Meeting in Dallas, Tex. [16a].)

MATERIALS AND METHODS

Bacterial strains, media, and growth conditions. All bacterial strains used in this study are derivatives of *S. typhimurium* LT2 and are listed in Table 1. As detailed in Table 1, all *cob*-24-*lac* strains are derivatives of JE1734 and all *pdu*-8-*lac* strains are derivatives of RT818. Nutrient broth (0.8% [wt/vol]; Difco, Detroit, Mich.) containing 85 mM NaCl was used as complex medium. No-carbon E medium supplemented with 1 mM MgSO₄ was used as the minimal medium

* Corresponding author.

TABLE 1. Strains used

Strain	Genotype	Source ^a
SA2779 (TR6583)	<i>metE205 ara-9</i>	K. Sanderson via J. R. Roth
RT818	<i>pdu-8::Mu d1-8</i>	R. M. Jeter
TR6583		
JE100	<i>cob-62::Mu d11734 cob-11::Tn10</i>	Lab collection
JE101	<i>cob-66::Mu d11734 cob-11::Tn10</i>	Lab collection
JE102	<i>cob-24::Mu d11734 cob-11::Tn10</i>	Lab collection
JE1734	<i>cob-24::Mu d11734</i>	Lab collection
JE1735	<i>cob-62::Mu d11734</i>	Lab collection
JE1736	<i>cob-66::Mu d11734</i>	Lab collection
JE1935	<i>poc-102::Tn10d(Tc)^b</i>	
JE1939	<i>poc-106::Tn10d(Tc)</i>	
JE1968 ^c	DEL299(<i>hisG-cob</i>) <i>proAB47 recA1</i> <i>strA1/F' 128 Pro⁺ lacZ950::Tn10</i> <i>zzf::Tn10DEL16DEL17(Cm)</i> <i>IS10::cobI⁺ poc⁺::IS10</i>	Lab collection
RT818		
JE1941	<i>poc-102::Tn10d(Tc)</i>	
JE1945	<i>poc-106::Tn10d(Tc)</i>	
JE1953	<i>recA1</i> derivative of JE1941	
JE1955	<i>recA1</i> derivative of JE1945	
JE1956	<i>recA1</i>	
JE1964	JE1953/F' <i>poc⁺</i>	
JE1966	JE1955/F' <i>poc⁺</i>	
JE1967	JE1956/F' <i>poc⁺</i>	
JE1734		
JE1929	<i>poc-102::Tn10d(Tc)</i>	
JE1933	<i>poc-106::Tn10d(Tc)</i>	
JE1947	<i>recA1</i> derivative of JE1929	
JE1951	<i>recA1</i> derivative of JE1933	
JE1952	<i>recA1</i>	
JE1958	JE1947/F' <i>poc⁺</i>	
JE1962	JE1951/F' <i>poc⁺</i>	
JE1963	JE1952/F' <i>poc⁺</i>	

^a Unless otherwise stated, the strains listed were obtained during the course of this study.

^b *Tn10d(Tc)*, *Tn10DEL16DEL17(Tc)*.

^c The F' plasmid strain JE1968 is referred to as F' *poc⁺* in all other strains.

(7). Unless otherwise stated, when provided in the medium, the final concentrations of compounds in the culture medium were as follows: glycerol, 22 mM; succinate, 35 mM; acetate, 10 mM; glucose, 11 mM; pyruvate, 50 mM; sodium nitrate, 50 mM; 1,2-PDL, 12 mM; 1,2-PDL analogs, 6 mM; methionine, 0.5 mM; 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-Gal; Bachem, Inc., Torrance, Calif.) 20 mg/liter; and lactose, 2.5 mM. In some cases (indicated below) acetate was added to the medium in addition to the main carbon and energy source to increase the rate of growth. Antibiotics in rich medium (per ml) were as follows: kanamycin, 50 μ g; ampicillin, 30 μ g; tetracycline, 20 μ g; and chloramphenicol, 20 μ g. In minimal medium, tetracycline was used at a concentration of 10 μ g/ml.

For cultures grown anaerobically on glucose, the glucose concentration was 22 mM. Regardless of the carbon and energy source used, all anoxic media also contained NaHCO_3 (40 mM) and trace minerals solution (10 ml/liter of medium) (3). Cell growth of anaerobic cultures was monitored with a Spectronic 20D spectrophotometer (Milton Roy Co., Rochester, N.Y.), with a light path of 1.5 cm. Cultures at an A_{650} of between 0.15 and 0.20 were in early log phase.

Aerobic cultures were routinely grown in 5 ml of medium

in a 125-ml Klett flask and vigorously shaken (400 rpm) to ensure high aeration. Cell growth for aerobic cultures was monitored with a Klett-Summerson colorimeter equipped with a red filter. An early-log-phase culture corresponded to a reading of 55 to 70 Klett units.

The typical inoculum size used for growing cells to measure transcription of the *cob* and *pdu* genes was about 2% (vol/vol; ca. 10^8 cells). Unless otherwise stated, inocula were started from isolated single colonies grown overnight in the same medium to be used for outgrowth.

Growth with 1,2-PDL as the sole carbon and energy source was determined by using NCE minimal medium containing 1,2-PDL (53 mM), CN-B_{12} (15 nM), and MgSO_4 and methionine as described above.

Cultures were routinely grown at 30°C.

Mutant isolation. A lysate of the high-transducing mutant bacteriophage P22 HT 105/1 *int-201* (18, 19) was prepared as described elsewhere (7) on a pool of approximately 50,000 strains, each carrying one insertion of the transposition-defective element *Tn10DEL16DEL17(Tc)* (21) [hereafter referred to as *Tn10d(Tc)*]. This lysate was used as donor to transduce JE1734 to tetracycline resistance. Transduction plates were replica printed onto minimal medium plates containing lactose with or without 1,2-PDL; alternatively, *Tc^r* colonies were directly selected on minimal medium with succinate, X-Gal, tetracycline, and 1,2-PDL. Methionine was added to satisfy the auxotrophic requirement of the strains used. Putative mutants were freed of contaminating phage on green indicator plates (7).

Conjugations. Equal volumes of donor and recipient full-density cultures (0.1 ml, ca. 10^8 cells) were mixed on a nutrient broth plate, incubated for 4 h at 37°C, and replica printed onto nutrient broth plates containing the appropriate antibiotic. Chloramphenicol was used to select for transfer of the F' plasmid, and either kanamycin or ampicillin was used to counterselect against the donor. Exconjugants were purified nonselectively on nutrient broth plates, and their phenotypes were tested.

β -Galactosidase enzyme activity assays. β -Galactosidase enzyme activity was determined by the method of Miller (15) in CHCl_3 -sodium dodecyl sulfate-permeabilized cells as described elsewhere (9). Routinely, cells were pelleted by centrifugation at $4,000 \times g$ for 10 min in a bench-top low-speed centrifuge (Sorvall; model GLC-2) and kept on ice until assayed. Cells were resuspended in 2 ml of sterile saline for the assay. Units of β -galactosidase activity are reported per A_{650} unit of the culture.

RESULTS

The experimental evidence presented in this communication is consistent with the existence of a 1,2-PDL-dependent regulatory system for the transcriptional control of both the *cob* and *pdu* genes. A highlight of this regulatory system is that it allows high levels of transcription of the *cobI* (Ado-Cbi biosynthesis) genes in the presence of oxygen.

1,2-PDL-dependent transcription of the *cob* genes. (i) **Effect of 1,2-PDL on *cob* transcription.** Under highly aerated growth conditions, the presence of 1,2-PDL in the culture medium resulted in a significant increase in the transcription of fusions to all three *Cob* regions (Table 2). *CobI* transcription increased dramatically (38-fold) when 1,2-PDL was present in the medium. *CobII* and *CobIII* transcription increased eight- and ninefold, respectively, in response to 1,2-PDL in the medium. This pattern of regulation, in which effectors of *cobI* transcription have a more pronounced effect on *CobI*

TABLE 2. Effect of 1,2-PDL on the transcription of *cob* fusions under aerobic growth conditions^a

<i>cob-lac</i> fusion (region)	β -Galactosidase activity (U/ <i>A</i> ₆₅₀ U) in cultures grown with:		
	No 1,2-PDL	1,2-PDL	Induction (fold)
<i>cob-24</i> (CobI)	15	565	38
<i>cob-62</i> (CobII)	30	240	8
<i>cob-66</i> (CobIII)	25	225	9

^a The basal medium used for all cultures was NCE-succinate containing methionine. Conditions for the preparation of the inoculum and its outgrowth are described under Materials and Methods. β -Galactosidase activity was assayed in early-log phase cultures.

than over CobII or CobIII, is consistent with previously reported studies on the transcriptional regulation of the *cob* genes (9). All of the experiments were performed with cultures grown at 30°C in light of our observations which indicated that an inverse relationship existed between the stimulatory effect of 1,2-PDL and temperature (data not shown).

Under anoxic conditions, the presence of 1,2-PDL in the medium caused a fourfold induction in the transcription of the *cobI-lac* fusion and sevenfold induction in the transcription of the *cobII-lac* and *cobIII-lac* fusions (data not shown). Expression of the *cobI-lac* fusion in the presence of 1,2-PDL was similar under aerobic and anaerobic conditions. The lower value for induction of the *cobI-lac* fusion (38-fold aerobically versus 4-fold anaerobically) was the result of a higher level of expression of the fusion under anaerobic conditions without added 1,2-PDL (110 U anaerobically versus 15 U aerobically). These data strongly suggest that 1,2-PDL is a positive regulator of transcription of all three regions of the *cob* operon under both aerobic and anaerobic growth conditions.

(ii) **Effect of 1,2-PDL on other genes.** 1,2-PDL had no stimulatory effect on the expression of operon fusions to *cobA*, a gene involved in cobalamin biosynthesis located outside the *cob* operon (10), or to *btuB*, a gene required for cobalamin transport across the outer membrane (4, 12) (data not shown). The only other genes whose transcription has been found to be regulated by 1,2-PDL were the PDL utilization (*pdu*) genes (13).

Specificity for 1,2-PDL. The specificity of the response to 1,2-PDL was examined by measuring the level of transcription of fusion *cob-24-lac* (CobI) in cultures grown aerobically in the presence of compounds structurally related to 1,2-PDL. We tested the following compounds: propan-1-ol, 1,3-PDL, ethylene glycol, glycerol, propionate, propionaldehyde, and ethanolamine. The average level of β -galactosidase activity measured in cultures grown in medium containing any one of these compounds was 14 ± 2 U per *A*₆₅₀ U. This average was the same as the level measured in the absence of any additions (15 U per *A*₆₅₀ U). It is possible that in some cases the failure to induce *cobI* transcription reflected a lack of transport of the compound in question. Even though this possibility was not addressed, it is known that *S. typhimurium* can grow on (and therefore transport) ethanolamine, propionate, and glycerol (11, 17).

We also compared the effects of *R*(-)- and *S*(+)-1,2-PDL on *cobI* transcription (Table 3). We measured a 30-fold increase in the transcription of fusion *cob-24-lac* when *S*(+)-1,2-PDL was present in the culture medium, a 18-fold increase in transcription of the fusion was measured when

TABLE 3. Enantiomeric specificity of the 1,2-PDL-dependent effect on *cobI* and *pdu* transcription in aerobically grown cells^a

Addition	β -Galactosidase activity				Growth ^b
	Of JE1734 (U/ <i>A</i> ₆₅₀ U)	Induction (fold)	Of RT818 (U/ <i>A</i> ₆₅₀ U)	Induction (fold)	
None	20	1	5	1	—
<i>R</i> (-)-1,2-PDL	355	18	60	12	+
<i>S</i> (+)-1,2-PDL	595	30	330	66	+
(<i>RS</i>)-1,2-PDL	470	24	110	22	+

^a Cultures of JE1734 (*cob-24-lac*) and RT818 (*pdu-8-lac*) were grown in NCE-succinate-acetate minimal medium containing magnesium ions, methionine, and the specified 1,2-PDL enantiomer under highly aerated conditions. The β -galactosidase enzyme activity level was measured in early-log-phase cultures.

^b Growth on a specific enantiomer of 1,2-PDL was assessed in NCE medium containing magnesium ions, cobalamin, and the given enantiomer at a final concentration of 12 mM.

R(-)-1,2-PDL was used, and a 24-fold increase in transcription was measured with the racemic mixture. These results show a slight preference for *S*(+)-1,2-PDL as an effector molecule. This apparent lack of specificity for either enantiomer could be explained by the presence of an isomerase in the cell which would convert *R*(-) to *S*(+)-1,2-PDL or, alternatively, by a lack of specificity for either one of the enantiomers. The latter is not unprecedented, since the 1,2-PDL dehydrase of *Klebsiella pneumoniae* catalyzes the dehydration of either enantiomer of 1,2-PDL, albeit at different rates (20). In our hands, *S. typhimurium* was able to grow aerobically on medium containing either of the enantiomers as a carbon and energy source and cobalamin (Table 3).

Similarly to *cobI* regulation, the level of transcription of an operon fusion to the PDL utilization (*pdu*) (13) region was affected by both 1,2-PDL enantiomers (Table 3). In this case, however, the effect of *S*(+)-1,2-PDL (66-fold increase) was more pronounced than the one measured with *R*(-)-1,2-PDL (12-fold increase); the racemate yielded an intermediate value of a 22-fold increase. The differences in the induction level between *cob* and *pdu* in response to 1,2-PDL may reflect the involvement of other factors that at this point remain undetermined.

Carbon and energy source effects on the 1,2-PDL-dependent transcription of *cobI*. Transcription of the *cob-24-lac* fusion in response to 1,2-PDL under aerobic growth conditions was clearly affected by the carbon and energy source present in the medium. Increases in β -galactosidase activity in response to 1,2-PDL were measured when the cells were grown in minimal medium containing a poor source of carbon. We found that the expression of the fusion in cells grown on succinate was greater than that in cells grown on pyruvate, which was greater than that in cells grown on acetate (data not shown). Some experiments (e.g., Table 3) were performed using succinate with acetate as a supplement to increase the rate of growth of the cultures. However, the cells grew very slowly anaerobically on this medium with nitrate as the alternative electron acceptor. Thus, for practical reasons subsequent culturing of strains under anoxic conditions was performed with a pyruvate-acetate medium. Cells grown under more energetically favorable growth conditions (e.g., minimal medium with glucose) also expressed *cobI* in response to 1,2-PDL; however, the levels of induction were much lower than in minimal medium with succinate.

TABLE 4. Effect of a *CobI* insertion mutation on the 1,2-PDL-dependent transcription of *cob-lac* fusions in aerobically grown cells^a

Strain	Relevant genotype	β -Galactosidase activity (U/A ₆₅₀ U) in cultures grown with:	
		No 1,2-PDL	1,2-PDL
JE1734	<i>cob-24-lac</i>	15	645
JE102	<i>cob-24-lac cob-11::Tn10</i>	1	10
JE1735	<i>cob-62-lac</i>	35	290
JE100	<i>cob-62-lac cob-11::Tn10</i>	35	40
JE1736	<i>cob-66-lac</i>	25	235
JE101	<i>cob-66-lac cob-11::Tn10</i>	15	20

^a Cultures were grown in NCE-succinate-methionine medium with or without added 1,2-PDL under highly aerated conditions.

1,2-PDL-stimulated transcription of *cobI* does not result in de novo synthesis of Ado-Cbi under aerobic growth conditions. An *S. typhimurium metE* mutant which depends on cobalamin for the synthesis of methionine from homocysteine (6) is prototrophic when grown anaerobically on cobalt-containing minimal medium. This correction of the auxotrophic requirement for methionine is due to de novo synthesis of cobalamin, the coenzyme of methionine synthase (MetH enzyme) (14). Aerobically, however, even when 1,2-PDL was included in the medium, the *metE* strain remained auxotrophic for methionine. In other words, despite the dramatic 1,2-PDL-dependent increase in *cobI* transcription under aerobic growth conditions, no net synthesis of the end product of the *CobI* pathway (Ado-Cbi) was observed. This observation is not unprecedented, as a previous report by Andersson and Roth (1) showed that mutants with significant (≥ 80 -fold increase) aerobic transcription of *cobI* genes were still unable to synthesize cobalamin aerobically.

The 1,2-PDL-dependent control region. The first indication of the location of the region that mediates the 1,2-PDL-dependent control of *cob* transcription was obtained from the following experiments. We measured β -galactosidase levels in strains carrying a *Tn10* insertion in *CobI* (*cob-11::Tn10*) and a fusion in *CobI* (*cob-24-lac*, JE102), *CobII* (*cob-62-lac*, JE100), or *CobIII* (*cob-66-lac*, JE101) (8). A strain carrying insertion *cob-11::Tn10* in an otherwise wild-type genetic background displayed a *CobI*⁻ phenotype. The *cob-11::Tn10* insertion has been previously shown to have strong polar effects on the transcription of all the above-mentioned *cob-lac* fusions, and the polar effects were not correctable by a *CobI*⁺ region provided in *trans* (8).

As shown in Table 4, insertion *cob-11::Tn10* had strong polar effects on the 1,2-PDL-dependent expression of all three *cob-lac* fusions tested. These data suggested that transcription from the *P_{cobII}* and *P_{cobIII}* promoters may not be regulated by the 1,2-PDL-dependent regulatory system, since one would expect no effect of the *cob-11::Tn10* insertion on the *cobII-lac* and *cobIII-lac* fusions if separate 1,2-PDL-dependent regulatory regions existed for each region of the operon. These data also suggested that the increase in *cobII* and *cobIII* expression previously observed in response to 1,2-PDL was probably due to transcription initiation at the *CobI* promoter.

Isolation of mutants unresponsive to 1,2-PDL. The isolation of mutants unable to regulate *cobI* transcription in response to 1,2-PDL was facilitated by two facts: (i) strain JE1734 (*cob-24-lac*) would grow with lactose as the sole carbon source only if 1,2-PDL was added to the medium, and (ii)

colonies of JE1734 growing on minimal medium containing X-Gal were white in the absence of 1,2-PDL and blue in its presence. We used these phenotypes to screen a pool of mutant strains each carrying one insertion of the *Tn10d(Tc)* element in their genome.

Using lactose-containing medium, we screened approximately 10,000 Tc^r transductants and found six mutants that were unable to grow on lactose (Lac⁻) even when 1,2-PDL was present in the medium. The isolation of Lac⁻ strains suggested the involvement of positive regulator mediating the effect of 1,2-PDL. In this screen no mutants with the ability to grow on lactose without added 1,2-PDL were found; i.e., constitutive expression of *cob* was not observed.

Using the color phenotype on X-Gal-containing plates as a screen, we isolated 17 Tc^r transductants (from approximately 10,000 Tc^r transductants analyzed) with a colony color lighter than that of the wild type. Again, the X-Gal⁻ phenotype of these mutants was consistent with mutations that prevented the synthesis of some component of the 1,2-PDL-dependent positive regulatory system.

We also isolated strains with the appropriate phenotype (Lac⁻ X-Gal⁻) but which were no longer kanamycin resistant. We presumed that these strains no longer contained the fusion and did not study these strains further.

Mapping the *Tn10d(Tc)* elements. Individual P22 phage lysates were grown on six of the strains (*Pdu*⁻ class; see below) containing the *Tn10d(Tc)* element in a TR6583 (*cob*⁺ *pdu*⁺) background and were used to transduce JE1734 (*cob-24-lac*) to Tc^r. This was done to reconstruct the mutant strains and to determine possible linkage of the insertions to *cob-24-lac*. The insertions showed 65 to 75% linkage to *cob-24-lac*. When the insertions were moved into RT818 (*pdu-8-lac*) and tested for linkage, they showed 10 to 20% linkage to the *pdu-8-lac* fusion. These results suggested that the insertions were probably located between the *cob* and *pdu* regions, as the two regions are about 12% linked by cotransduction (13).

Cob and Pdu phenotypes of the mutants: the *poc* locus. Several reasons prompted us to assess the *CobI* and *Pdu* phenotypes of the mutants carrying the *Tn10d(Tc)* elements. (i) The *cob* and *pdu* regions are closely linked, and they are divergently transcribed. This is one feature of genes sharing common regulatory systems (5). (ii) The expression of *cob* and *pdu* was positively regulated in response to 1,2-PDL. (iii) All of the *Tn10d(Tc)* elements affecting 1,2-PDL-dependent transcription of *cob* mapped in the region between *cob* and *pdu*.

The *Cob* and *Pdu* phenotypes of the isolated mutants were determined by mobilizing the *Tn10d(Tc)* elements into strain TR6583 (*cob*⁺ *pdu*⁺) by transduction. The *CobI* phenotype of these strains was assessed under anaerobic growth conditions that demanded synthesis of Ado-Cbi. The *Pdu* phenotype was assessed under aerobic growth conditions in medium containing 1,2-PDL as the sole carbon and energy source and cobalamin. Three classes of mutants were identified on the basis of their *Pdu* and *CobI* phenotypes: (i) *Pdu*⁺ *CobI*⁻ strains, (ii) *Pdu*⁻ *CobI*⁺ strains, and (iii) *Pdu*⁻ *CobI*⁻ strains. Representatives of the two classes of *Pdu*⁻ mutants were chosen for further study: JE1935 [*poc-102::Tn10d(Tc)*] represents the *Cob*⁺ *Pdu*⁻ class, and JE1939 [*poc-106::Tn10d(Tc)*] represents the *Cob*⁻ *Pdu*⁻ class. Transcription of *cobI-lac* and *pdu-lac* fusions was found to be uninducible or marginally inducible by 1,2-PDL in strains containing an insertion in the *poc* locus (Table 5). The effect of the *poc-102::Tn10d(Tc)* insertion on *cob* and *pdu* transcription

TABLE 5. Effect of *poc* mutations on the 1,2-PDL regulation of *cobI* and *pdu* transcription under anaerobic conditions^a

<i>poc</i> allele tested	<i>cob-24-lac</i> strain			<i>pdu-8-lac</i> strain		
	β-Galactosidase activity (U/A ₆₅₀ U) in cultures:		CobI phenotype	β-Galactosidase activity (U/A ₆₅₀ U) in cultures:		Pdu phenotype
	-PDL	+PDL		-PDL	+PDL	
<i>poc-102::Tn10d(Tc)</i>	25	80	+	5	100	—
<i>poc-106::Tn10d(Tc)</i>	10	15	—	5	5	—
<i>poc</i> ⁺ (JE1734)	30	310	+	NA	NA	NT
<i>poc</i> ⁺ (RT818)	NA	NA	NT	5	715	+

^a Cultures were grown on pyruvate-acetate minimal medium plus nitrate and bicarbonate, with or without added 1,2-PDL. Enzyme activity was determined in early-log phase cultures. CobI and Pdu phenotypes were determined in strains containing only the *poc* mutations. The CobI phenotype was determined by the ability to grow anaerobically on plates containing glucose as the carbon source, without added cobinamide. The Pdu phenotype was assessed in NCE medium containing magnesium ions, cobalamin, and 1,2-PDL. NA, not applicable; NT, not tested.

was reproducibly different from the effect of the *poc-106::Tn10d(Tc)* insertion.

In light of the fact that a single mutation affected the transcriptional regulation of both *pdu* and *cobI*, we referred to the locus affected by these mutations as the *poc* (PDL and cobalamin) locus.

Effect of the *poc::Tn10d(Tc)* insertions on the redox regulation of *cobI*. *cobI* gene expression was previously shown to be regulated by the redox level of the cell, such that expression is much higher anaerobically than aerobically (2, 9). We investigated the effect of *poc* insertions on the redox regulation of *cobI*. To do this we compared the levels of transcription of the previously studied *cob-24-lac* fusion in the presence or absence of a *poc* insertion in the chromosome.

The experiments were performed in cultures grown aerobically or anaerobically in medium containing glycerol as the carbon source and nitrate as the alternative electron acceptor. Glycerol was chosen as the carbon source, since growth on this medium best illustrates the redox control exerted on *cobI* expression (9). It is important to emphasize that in these experiments 1,2-PDL was not included in the culture medium. As shown in Table 6, *cobI* expression under anaerobic conditions was drastically reduced in *poc* mutants. One implication from these data is that the *poc* gene product(s) may be involved in the redox control of *cobI* expression. However, since the *poc* mutations tested were insertions, it is possible that their effect on redox control of

TABLE 6. Effect of *poc* mutations on the redox regulation of *cobI* transcription^a

<i>poc</i> allele tested	β-Galactosidase activity (U/A ₆₅₀ U) during:	
	Aerobic growth	Anoxic growth
<i>poc-102::Tn10d(Tc)</i>	5	60
<i>poc-106::Tn10d(Tc)</i>	5	15
<i>poc</i> ⁺	5	365

^a All strains are derivatives of JE1734 and carry the *cob-24-lac* operon fusion and the indicated *poc* allele. Cells were grown aerobically on glycerol minimal medium or anaerobically on the same medium plus nitrate and bicarbonate; 1,2-PDL was not present in the medium.

TABLE 7. Complementation of the *Poc* function(s)^a

<i>poc</i> allele tested	β-Galactosidase activity (U/A ₆₅₀ U) in strain:			
	Lacking F' <i>poc</i> ⁺ grown:		Carrying F' <i>poc</i> ⁺ grown:	
	-PDL	+PDL	-PDL	+PDL
<i>poc-102::Tn10d(Tc)</i>	3	35	3	310
<i>poc-106::Tn10d(Tc)</i>	3	3	3	205
<i>poc</i> ⁺	3	465	3	555

^a The strains tested are all derived from RT818 (*pdu-8-lac*). All strains carried a *recA1* allele. Cultures were grown aerobically on succinate minimal medium with or without 1,2-PDL. β-Galactosidase activity was determined in early-log-phase cultures.

cobI expression was due to polarity effects on genes whose products are required for the redox control to be exerted or on a gene required for the expression of the *poc* locus itself; or alternatively, it is possible that the insertion element somehow destabilizes the *cob* transcript.

Complementation of *poc* mutants. An F' plasmid carrying a wild-type copy of the region between *cobI* and *pdu* (1) was introduced into recombination-deficient (*recA1*) *cob-24-lac poc::Tn10d(Tc)* strains and *recA1 pdu-8::Mu d1-8 poc::Tn10d(Tc)* strains to test for complementation of *Poc* function(s). We compared the response to 1,2-PDL in *poc* mutants carrying or lacking the F' plasmid. Table 7 presents only the data obtained in experiments using the *pdu-8-lac* fusion, but similar results were obtained with strains carrying the *cob-24-lac* fusion (data not shown). The levels of β-galactosidase produced by *poc* mutant-F' *poc*⁺ merodiploids in response to 1,2-PDL increased 68- or 103-fold over those in strains lacking the plasmid [Table 7, *poc-106::Tn10d(Tc)* and *poc*⁺]. Complementation was also seen when the same strains were grown anaerobically (data not shown). These results suggest that the *poc* insertions disrupt the production of a diffusible factor and that the *Poc* phenotype is not due merely to disruption of the *cob* or *pdu* transcripts.

DISCUSSION

The data presented in this communication suggest that 1,2-PDL is a positive effector of transcription of the cobalamin biosynthetic (*cob*) and PDL utilization (*pdu*) genes of *S. typhimurium*. Prior to this report only the transcription of the *pdu* genes was known to be affected by this diol (13). The stimulatory effects of 1,2-PDL on *cob* and *pdu* transcription can be clearly observed both under aerobic and anaerobic growth conditions and appear to be mediated by the gene product(s) of the newly defined *poc* (PDL and cobalamin) locus. Mutations in *poc* render both *pdu* and *cob* unresponsive to the stimulatory effect of 1,2-PDL, suggesting that the *poc* gene product(s) alone is sufficient for the effect of 1,2-PDL to be exerted. The idea that *poc* synthesizes one or more diffusible gene products is supported by the fact that mutations in *poc* are recessive to the wild-type allele when the latter is provided in *trans*.

We think that it is unlikely that a metabolite of 1,2-PDL, rather than 1,2-PDL itself, is the effector molecule for several reasons: (i) the products of the *Pdu* pathway, propionaldehyde and propionate, did not cause an increase in transcription of fusion *cob-24::lac* (see above, "Specificity for 1,2-PDL"), even though at least propionate is known to serve as a carbon and energy source for *S. typhimurium* (11); and (ii) 1,2-PDL catabolism requires cobalamin, under aer-

obic conditions *de novo* synthesis of cobalamin does not occur in *S. typhimurium*, and cobalamin was never present in the culture medium in the experiments presented herein. Furthermore, a strain carrying fusion *cob-24-lac* (a strain unable to synthesize Ado-Cbi *de novo*) was able to regulate the transcription of the fusion in response to 1,2-PDL under anaerobic conditions in minimal medium lacking cobalamin (Table 5). Since cobalamin is required for 1,2-PDL catabolism, it is unlikely that a breakdown metabolite rather than 1,2-PDL is the effector.

The genetic organization of the *pdu* and *cob* genes relative to one another is common to other loci whose transcription is controlled by a single regulatory system (5). In *S. typhimurium* the *pdu* genes are located upstream and closely linked to the *cob* operon, and these regions are divergently transcribed. We have mapped the *poc* locus to the region located between *cob* and *pdu*.

The coregulation of the *cob* and *pdu* genes intuitively makes sense, since the utilization of 1,2-PDL as a carbon and energy source by *S. typhimurium* requires the availability of adenosyl-cobalamin, the end product of the Cob pathway. Even though our data clearly indicate that the *cobI* genes can be highly expressed under conditions of high oxygen levels, no net Ado-Cbi synthesis occurs under the conditions used in our experiments. Since our data suggest that under aerobic conditions in the presence of 1,2-PDL, the entire *cob* operon is transcribed (Table 5), it is unclear why no net synthesis of Ado-Cbi is achieved. One could suggest that failure to synthesize Ado-Cbi may be due to posttranscriptional regulation, to oxygen lability of Cob enzymes and/or intermediates of the CobI pathway, or to a combination of these or other unidentified factors.

It is interesting that under the laboratory conditions we have tested, utilization of 1,2-PDL as the carbon and energy source by *S. typhimurium* has been observed only under aerobic conditions, provided that cobalamin is present in the culture medium. In this bacterium synthesis of cobalamin has not been observed in aerobically grown cells. However, in the wild, *S. typhimurium* may encounter conditions of low oxygen levels where 1,2-PDL can be metabolized, and Ado-Cbi can be synthesized. Another possible scenario would be one in which under anoxic conditions compounds such as rhamnose are catabolized and yield 1,2-PDL as an end product (16). The availability of the latter could then enhance the 1,2-PDL-dependent transcription of *cob* and *pdu*, resulting in adenosyl-cobalamin biosynthesis and the ability to further degrade 1,2-PDL to propanol and propionate as previously reported (16).

ACKNOWLEDGMENTS

This work was supported in part by the College of Agricultural and Life Sciences of the University of Wisconsin-Madison, by Public Health Service grant GM40313 from the National Institute of General Medical Sciences to J.C.E.-S., and by National Science Foundation predoctoral fellowship RCD9154722 to M.R.R.

We thank R. Jeter and J. R. Roth for providing strains, D. C. Cameron for the gift of 1,2-PDL enantiomers, and D. M. Downs for critical reading of the manuscript.

ADDENDUM IN PROOF

Our finding on the positive effect of 1,2-PDL on the aerobic transcription of the *cob* genes has been confirmed by Bobik et al. in the accompanying report (T. A. Bobik, M. Ailion, and J. R. Roth, *J. Bacteriol.* 174:2253-2266, 1992), and both groups have isolated mutants that are no longer responsive to

1,2-PDL. In all instances, the lesions have been mapped to the region of the chromosome between *cob* and *pdu*.

REFERENCES

- Andersson, D. I., and J. R. Roth. 1989. Mutations affecting regulation of cobinamide biosynthesis in *Salmonella typhimurium*. *J. Bacteriol.* 171:6726-6733.
- Andersson, D. I., and J. R. Roth. 1989. Redox regulation of the genes for cobinamide biosynthesis in *Salmonella typhimurium*. *J. Bacteriol.* 171:6734-6739.
- Balch, W. E., and R. S. Wolfe. 1976. New approach to the cultivation of methanogenic bacteria: 2-mercaptoethanesulfonic acid (HS-CoM)-dependent growth of *Methanobacterium ruminantium* in a pressurized atmosphere. *Appl. Environ. Microbiol.* 32:781-791.
- Bassford, P. J., Jr., and R. J. Kadner. 1977. Genetic analysis of components involved in vitamin B₁₂ uptake in *Escherichia coli*. *J. Bacteriol.* 132:796-805.
- Beck, C., and R. Warren. 1988. Divergent promoters, a common form of gene organization. *Microbiol. Rev.* 52:318-326.
- Cohen, G. N., and I. Saint-Girons. 1987. Biosynthesis of threonine, lysine, and methionine, p. 429-444. In F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and E. Umberger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
- Davis, R. W., D. Botstein, and J. R. Roth. 1980. A manual for genetic engineering: advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Escalante-Semerena, J. C., M. G. Johnson, and J. R. Roth. 1992. The CobII and CobIII regions of the cobalamin (vitamin B₁₂) biosynthetic operon of *Salmonella typhimurium*. *J. Bacteriol.* 174:24-29.
- Escalante-Semerena, J. C., and J. R. Roth. 1987. Regulation of cobalamin biosynthetic operons in *Salmonella typhimurium*. *J. Bacteriol.* 169:2251-2258.
- Escalante-Semerena, J. C., S.-J. Suh, and J. R. Roth. 1990. *cobA* function is required for both *de novo* cobalamin biosynthesis and assimilation of exogenous corrinoids in *Salmonella typhimurium*. *J. Bacteriol.* 172:273-280.
- Gutnick, D., J. M. Calvo, T. Klopotoski, and B. N. Ames. 1969. Compounds which serve as the sole source of carbon or nitrogen for *Salmonella typhimurium*. *J. Bacteriol.* 100:215-219.
- Heller, K., and R. J. Kadner. 1985. Nucleotide sequence of the gene for the vitamin B₁₂ receptor protein in the outer membrane of *Escherichia coli*. *J. Bacteriol.* 161:904-908.
- Jeter, R. M. 1990. Cobalamin-dependent 1,2-propanediol utilization by *Salmonella typhimurium*. *J. Gen. Microbiol.* 136:887-896.
- Jeter, R. M., B. M. Olivera, and J. R. Roth. 1984. *Salmonella typhimurium* synthesizes cobalamin (vitamin B₁₂) *de novo* under anaerobic growth conditions. *J. Bacteriol.* 159:206-213.
- Miller, J. H. 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Obradors, N., J. Badia, L. Baldoma, and J. Aguilar. 1988. Anaerobic metabolism of the L-rhamnose fermentation product 1,2-propanediol in *Salmonella typhimurium*. *J. Bacteriol.* 170:2159-2162.
- Rondon, M. R., and J. Escalante-Semerena. 1991. Abstr. Gen. Meet. Am. Soc. Microbiol. 1991, K-112, p. 233.
- Roof, D. M., and J. R. Roth. 1988. Ethanolamine utilization in *Salmonella typhimurium*. *J. Bacteriol.* 170:3855-3863.
- Schmieger, H. 1971. A method for detection of phage mutants with altered transducing ability. *Mol. Gen. Genet.* 100:378-381.
- Schmieger, H., and H. Bakhaus. 1973. The origin of DNA in transducing particles of P22 mutants with increased transduction frequencies (HT-mutants). *Mol. Gen. Genet.* 120:181-190.
- Toraya, T., and S. Fukui. 1982. Diol dehydrase, p. 233-262. In D. Dolphin (ed.), B₁₂. John Wiley & Sons, Inc., New York.
- Way, J. C., M. A. Davis, D. Morisato, D. E. Roberts, and N. Kleckner. 1984. New Tn10 derivatives for transposon mutagenesis and for construction of *lacZ* operon fusions by transposition. *Gene* 32:369-379.