Cobalamin-dependent 1,2-propanediol utilization by *Salmonella typhimurium*

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(Received 23 October 1989; revised 5 January 1990; accepted 18 January 1990)

The enteric bacterium *Salmonella typhimurium* utilizes 1,2-propanediol as a sole carbon and energy source during aerobic growth, but only when the cells are also provided with cobalamin as a nutritional supplement. This metabolism is mediated by the cobalamin-dependent propanediol dehydratase enzyme pathway. Thirty-three insertion mutants were isolated that lacked the ability to utilize propanediol, but retained the ability to degrade propionate. This phenotype is consistent with specific blocks in one or more steps of the propanediol dehydratase pathway. Enzyme assays confirmed that propanediol dehydratase activity was absent in some of the mutants. Thus, the affected genes were designated *pdu* (for defects in propanediol utilization). Seventeen mutants carried *pdu::lac* operon fusions, and these fusions were induced by propanediol in the culture medium. All of the *pdu* mutations were located in a single region (41 map units) on the *S. typhimurium* chromosome between the *his* (histidine biosynthesis) and branch I *cob* (cobalamin biosynthesis) operons. They were shown to be P22-cotransducible with a branch I *cob* marker at a mean frequency of 12%. Mutants that carried deletions of the genetic material between *his* and *cob* also failed to utilize propanediol as a sole carbon source. Based upon the formation of duplications and deletions between different pairs of *his::MudA* and *pdu::MudA* insertions, the *pdu* genes were transcribed in a clockwise direction relative to the *S. typhimurium* genetic map.

Introduction

Salmonella typhimurium synthesizes cobalamin (vitamin B₁₂) *de novo*, but only during anaerobic growth (Jeter *et al.*, 1984). This enteric bacterium makes a substantial genetic commitment to produce cobalamin: an estimated 20 to 30 enzymes are uniquely employed for its synthesis (Jeter & Roth, 1987). The genes that encode the cobalamin biosynthetic enzymes are clustered at 41 map units on the *S. typhimurium* chromosome. These genes (designated *cob*) are organized into three operons that generally correspond to the biochemical functions of their gene products. The branch I *cob* operon is responsible for the synthesis of the corrinoid intermediate cobinamide, the branch II *cob* operon for the synthesis of 5,6-dimethylbenzimidazole, and the branch III *cob* operon for the incorporation of these components into the complete cobalamin molecule (Jeter *et al.*, 1987; Jeter & Roth, 1987).

The importance of cobalamin to the general metabolism of *S. typhimurium* remains poorly understood. Until recently, only two cobalamin-dependent enzymes were known to occur in *S. typhimurium*. Tetrahydropteroylglutamate–homocysteine methyltransferase

(EC 2.1.1.13) catalyses the transfer of a methyl group from *N*⁵-methyltetrahydrofolate to L-homocysteine, yielding L-methionine as a product (Cauthen *et al.*, 1966). This enzyme is encoded by the *metH* gene (Childs & Smith, 1969). However, *S. typhimurium* possesses a second enzyme (encoded by the *metE* gene) that catalyses the same reaction but does not require cobalamin for activity (Smith, 1961). Thus, cobalamin normally does not need to be available in order for the cells to carry out this last step in methionine biosynthesis. Ethanolamine ammonia-lyase (EC 4.3.1.7) catalyses the cleavage of ethanolamine to acetaldehyde and ammonia. In the presence of exogenously supplied cobalamin, this enzyme allows *S. typhimurium* to use ethanolamine as a sole source of carbon and energy for aerobic growth (Chang & Chang, 1975; Roof & Roth, 1988).

A third enzymic function that requires cobalamin has been identified recently in *S. typhimurium*: the reduction of epoxyqueuosine to queuosine (Frey *et al.*, 1988). Queuosine is a modified nucleoside that replaces guanosine in the anticodons of certain transfer RNA (tRNA) molecules (Björk, 1987; Björk *et al.*, 1987). In the absence of exogenously supplied cobalamin, anaerobically grown *S. typhimurium* produces tRNAs that contain

primarily queuosine, whereas aerobically grown *S. typhimurium* produces tRNAs that contain only the precursor epoxyqueuosine (Frey *et al.*, 1988).

Propanediol dehydratase (EC 4.2.1.28) is another cobalamin-dependent enzyme that is found in certain genera of the enteric bacteria (e.g. *Klebsiella*) but not in others (e.g. *Escherichia*). This enzyme, which catalyses the removal of water from 1,2-propanediol to yield propionaldehyde, has been purified from *K. pneumoniae* and its reaction mechanism has been characterized extensively (Abeles & Lee, 1961; Lee & Abeles, 1963; Toraya *et al.*, 1977). Propanediol dehydratase catalyses the first step in an anaerobic pathway that converts 1,2-propanediol into equimolar amounts of 1-propanol and propionate (Toraya *et al.*, 1979). Both products are excreted into the culture medium. Thus, propanediol does not serve as a sole carbon source for anaerobic growth (Forage & Foster, 1982). Instead, the enzyme pathway appears to operate for the generation of additional ATP by substrate-level phosphorylation (Toraya *et al.*, 1979). This pathway has been detected recently in anaerobically grown cultures of *S. typhimurium* (Obradors *et al.*, 1988). Thus, propanediol dehydratase represents a fourth cobalamin-dependent function that occurs in *S. typhimurium*.

In this paper, I report that *S. typhimurium* is also able to grow aerobically by utilizing 1,2-propanediol as a sole source of carbon and energy. However, this growth occurs only when cobalamin is present in the culture medium as a nutritional supplement. I describe the isolation and genetic characterization of mutants that are defective in the utilization of 1,2-propanediol. All of these mutations are located in a single region (41 map units) on the *S. typhimurium* chromosome and they lie close to the *cob* genes.

Methods

Bacterial and bacteriophage strains. The genotypes of the bacterial strains that were used in this study are given in Table 1. All of the strains were derived from *S. typhimurium* LT2. The bacteriophage that was used in all transductional crosses was P22 HT 105/1 *int-201*. This derivative of bacteriophage P22 performs generalized transduction at higher frequencies (75-fold and above) than wild-type P22 and is defective for integration into the bacterial chromosome (Anderson & Roth, 1978; Schmieger, 1971, 1972). MudA is a conditionally transposition-deficient derivative of the Mud1 (Amp, Lac) bacteriophage of Casadaban & Cohen (1979). MudA does not transpose except in host cells that also carry an amber (nonsense) suppressor mutation (Hughes & Roth, 1984). MudJ is the MudIII1734 derivative that was originally isolated by Castilho *et al.* (1984). Since MudJ carries a deletion that includes the A and B genes of Mu, a separate genetic element must provide the missing transposition functions by complementation (Hughes & Roth, 1988). MudA and MudJ carry resistance determinants to the antibiotics ampicillin and kanamycin, respectively.

Culture media and growth conditions. Nutrient broth (8 g l⁻¹, Difco) with the addition of NaCl (5 g l⁻¹) was used as the complex medium for the routine cultivation of bacterial strains. The E medium of Vogel & Bonner (1956) with the addition of D-glucose (14 mM) as a carbon source was generally used as the minimal medium. NCE (no carbon E) minimal medium base (Davis *et al.*, 1980) was substituted when the cells were utilizing carbon sources other than glucose. Alternative carbon sources included DL-1,2-propanediol, propionate and ethanolamine (all 26 mM). Nutritional supplements were added as required to meet the needs of individual strains. The final concentrations of amino acids, purines and pyrimidines in the medium were 0.1 to 0.5 mM (as specified in Davis *et al.*, 1980); the final concentration of cyanocobalamin was 15 nM. Antibiotics were added to the following final concentrations in minimal and complex media, respectively: kanamycin sulphate, 125 or 50 mg l⁻¹; sodium ampicillin, 15 or 30 mg l⁻¹; and tetracycline hydrochloride, 10 or 20 mg l⁻¹. Solid medium contained agar (15 g l⁻¹, Difco). Organic chemicals and biochemicals were supplied by Aldrich, Boehringer Mannheim and Sigma. Inorganic chemicals were supplied by J. T. Baker Chemicals and Fisher Scientific.

Unless otherwise specified, all cultures were grown at 37 °C. Aerobic liquid cultures were grown in an incubator rotary shaker (model G25, New Brunswick Scientific) at 200 r.p.m.

Growth measurements. *S. typhimurium* LT2 was grown at 30 °C in two 500 ml culture flasks that contained 150 ml of NCE minimal medium with propanediol as the sole carbon and energy source. Cobalamin was added as a nutritional supplement to one of the cultures. At regular intervals, samples were taken and the cell densities were measured as the optical density at 540 nm. Readings were taken with a Spectronic 21 spectrophotometer (Bausch and Lomb).

Transductional methods. Bacteriophage P22 lysates were prepared on donor strains of *S. typhimurium* by using the rapid procedure of Davis *et al.* (1980). The methods that were used for the transduction of recipient cells, and the selection and purification of transductant colonies with the desired phenotypes, have been described previously (Jeter & Roth, 1987). Phage-free transductants were isolated by streaking nonselectively onto modified green indicator plates to obtain light-coloured colonies (Smith & Levine, 1967). The modified medium contains a reduced concentration of aniline blue (80 mg l⁻¹) and alizarin yellow is replaced by fluorescein (uranine; 30 mg l⁻¹) as recommended by Bochner (1984).

Mutant isolation. MudA insertion strains were isolated by transducing a recipient strain (TT7610) that carried an amber (nonsense) suppressor mutation (*supD501*). The P22 lysate was grown on a donor strain that possessed an F' plasmid carrying a MudA insertion (TT8388). The complete MudA element was introduced into the recipient cells as the consequence of a two-fragment transduction event and inherited solely by transposition into random chromosomal sites (Hughes & Roth, 1984, 1985). Transductant colonies were selected at 30 °C for ampicillin resistance and were then screened for loss of their ability to utilize propanediol as a sole carbon source. To stabilize the MudA insertions, P22 lysates were grown on confirmed mutants and the insertions were transduced into *S. typhimurium* LT2 (which is free of suppressor mutations). The donor DNA fragments carried both the MudA element and flanking *S. typhimurium* chromosomal sequences, and each MudA element was inherited by homologous recombination in these crosses.

Propanediol dehydratase assays. Strains were grown aerobically at 37 °C in NCE succinate minimal medium with or without supplements of propanediol and cyanocobalamin. Cultures in maximum stationary phase were harvested by centrifugation (5000 g for 20 min at 4 °C), washed once, and resuspended in 70 mM-potassium phosphate buffer,

Table 1. *S. typhimurium* strains used in this study

Strain†	Genotype‡	Reference or source
LT2	Wild-type	John Roth, University of Utah, USA
TR6583	<i>metE205 ara-9</i>	Jeter & Roth (1987)
TT627	<i>pyrC7 rpsL1/F_{ts}114 lac⁺ zzf-20::Tn10</i>	Chumley <i>et al.</i> (1979)
TT6195	<i>pncA148::Tn10</i>	Hughes <i>et al.</i> (1983)
TT7610	<i>supD501 zea-609::Tn10</i>	Hughes & Roth (1984)
TT7691	<i>hisD9952::MudA</i>	John Roth, University of Utah, USA
TT7692	<i>hisD9953::MudA</i>	Hughes & Roth (1984)
TT8388	<i>recA1 zea-609::Tn10/F_{ts}128 zzf-1066::MudA</i>	
TT10441	<i>metE205 ara-9 cob-13::Tn10</i>	Jeter & Roth (1987)
TT10927	<i>metE205 ara-9 pdu-30::Tn10</i>	
TT11073	<i>metE205 ara-9 pdu-31::Tn10</i>	
TT13787	<i>btu-6::Tn10</i>	
RT761	<i>metE205 ara-9 DEL646(hisF9951* MudJ*cob-21)</i>	This study
RT762	<i>metE205 ara-9 DEL647(hisF9951* Mu dJ*cob-22)</i>	
RT763	<i>metE205 ara-9 DEL648(hisF9954* Mu dJ*cob-42)</i>	
RT764	<i>metE205 ara-9 DEL649(hisF9954* Mu dJ*cob-54)</i>	
RT765	<i>metE205 ara-9 DEL650(hisF9951* Mu dJ*cob-26)</i>	
RT766	<i>metE205 ara-9 hisF9951::MudJ</i>	
RT767	<i>metE205 ara-9 hisF9954::Mu dJ</i>	
RT785	<i>metE205 ara-9 cob-21::Mu dJ</i>	
RT840	<i>pdu-8::MudA/F_{ts}114 lac⁺ zzf-20::Tn10</i>	
RT841	<i>pdu-3::Mu dA/F_{ts}114 lac⁺ zzf-20::Tn10</i>	
RT847	<i>btu-151::MudA</i>	

† Strains listed in Table 1 that are assigned TR or TT numbers were obtained from the culture collection of John Roth at the University of Utah, USA. Strains that are assigned RT numbers are in the author's culture collection.

‡ *DEL* numbers are used for chromosomal rearrangements (deletions) that affect a series of genetic loci with different individual designations (Schmid & Roth, 1983). The extent of each deletion is specified within the parentheses following the *DEL* number. Asterisks (*) indicate that a transposable element is situated at the join point of the rearranged chromosomal sequences (Hughes & Roth, 1985).

pH 8.0 at a 10-fold increase in cell concentration. Cells were then broken by ultrasonication (model TM-300 Sonic Disrupter; Tekmar Co.) at a power output of 200 W. The crude extracts were shielded from light to avoid the degradation of adenosylcobalamin. Crude extracts were stored at -70°C for up to 2 weeks before use, which did not impair the activity of the enzyme.

Propionaldehyde was measured by using the 3-methyl-2-benzothiazolinone hydrazone (MBTH) method (Paz *et al.*, 1965; Sawicki *et al.*, 1961; Soda, 1967). The enzyme assay procedure followed that described by Toraya *et al.* (1977). Assay tubes were protected from light during the enzyme reaction. The assay mixture contained 70 μmol potassium phosphate buffer, pH 8.0; 100 μmol KCl; 30 nmol adenosylcobalamin; 400 μmol 1,2-propanediol; and crude cell extract in a total volume of 2 ml. After incubation at 37°C for 30 min, the reaction was terminated by the addition of 200 μmol potassium citrate, pH 3.6, and 5 μmol MBTH. After a second 30 min incubation, samples were taken and spun in a microcentrifuge (16600 g for 5 min at 25°C) to remove cell debris. The absorbance of each supernatant was measured at 305 nm with an Acta CIII UV-Visible spectrophotometer (Beckman). The extinction coefficient at 305 nm was $14.7 \times 10^3 \text{ l mol}^{-1} \text{ cm}^{-1}$. Propanediol dehydratase specific activity was defined as nmol propionaldehyde formed $\text{min}^{-1} (\text{mg protein})^{-1}$.

The protein concentration in each crude extract was determined by using the bicinchoninic acid (BCA) procedure with BSA as the standard (Smith *et al.*, 1985).

Conjugational methods. Hfr donor strains were created due to the integration of an *F'* *lac* plasmid into chromosomal *MudA* insertions by homologous recombination between the *lac* sequences (Maloy & Roth, 1983). The methods that were used were similar to those described by Chumley *et al.* (1979). The *F'*_{ts}114 *lac*⁺ plasmid was mated from strain

TT627 into two *pdu::MudA* recipient strains (RT813 and RT818). Matings were done at 30°C by cross-streaking donor and recipient strains onto NCE lactose (6 mM) minimal medium plates. Only transconjugants grew on this medium, since the donor (TT627) also carried a mutational defect in pyrimidine biosynthesis, and the *pdu::lac* fusions in the recipient cells were not expressed when propanediol was absent. *Lac*⁺ transconjugants (RT840 and RT841) were purified, grown to full density at 30°C in NCE lactose minimal medium, and then streaked onto plates of the same medium for growth at 40°C . *F'*_{ts}114 is temperature-sensitive for its own replication, and it is readily lost during cell growth at the high temperature unless it integrates into the chromosome. Under this circumstance, the plasmid is replicated passively as a part of the chromosome and the cells retain a *Lac*⁺ phenotype (Jacob *et al.*, 1963). Several large, healthy *Lac*⁺ colonies from each 40°C selection were retained as putative Hfr donor strains.

The Hfr donor strains (which were prototrophic and streptomycin-sensitive) were grown to full density at 40°C in NCE lactose minimal medium and then diluted 1:20 into nutrient broth. The recipient strains (which were auxotrophic and streptomycin-resistant) were grown to full density in nutrient broth and then diluted 1:5 into the same medium. After a 2 h incubation at 37°C , equal volumes (0.1 ml each) of donor and recipient cells were spread onto streptomycin-containing E glucose minimal medium plates. Recombinants (which were prototrophic and streptomycin-resistant) were counted following a 2 d incubation at 37°C .

***Lac* phenotype of *MudA* insertion strains.** The presence or absence of operon fusions in the *pdu::MudA* insertion strains was determined by testing for β -galactosidase activity on NCE succinate (22 mM) minimal medium plates that contained X-Gal as an indicator. X-Gal is

hydrolysed by β -galactosidase to form a nondiffusible blue pigment. Thus, expression of the MudA-encoded *lacZ* gene results in the formation of blue-coloured colonies (Silhavy & Beckwith, 1985). The concentration of X-Gal in the culture medium was 25 mg l⁻¹ (X-Gal was dissolved in 1 ml *N,N*-dimethylformamide before addition to the medium). One set of plates contained propanediol (26 mM) to induce the *pdu* genes, and a second set of plates lacked propanediol. Strains that formed colourless colonies on both sets of plates were assigned a Lac⁻ phenotype; strains that formed blue colonies on propanediol-supplemented plates and colourless colonies on unsupplemented plates were assigned a Lac⁺ phenotype.

β -Galactosidase assays. β -Galactosidase enzyme activity was assayed by using the procedure of Miller (1972), except that the cell density was measured as the optical density at 650 nm rather than 600 nm. The cells were permeabilized by treatment with chloroform/SDS.

Formation of duplications and deletions by recombination between MudA prophages. This method to determine the direction of gene transcription was performed as described by Hughes & Roth (1985). P22 lysates were grown on *S. typhimurium* strains TT7691 and TT7692 (*hisD*::MudA insertions in opposite chromosomal orientations) and on 10 different strains that carried *pdu*::MudA insertions (in unknown chromosomal orientations). Equal concentrations of phage (p.f.u. ml⁻¹) from individual *his* and *pdu* mutant lysates were mixed. A recipient strain (TR6583) was transduced and colonies were selected on ampicillin-containing complex medium. Each transductant colony displayed one of four possible phenotypes: His⁺Pdu⁻ (*pdu*::MudA insertion inherited); His⁻Pdu⁺ (*hisD*::MudA insertion inherited); His⁺Pdu⁺ (duplication of genetic material between *hisD* and *pdu* generated by recombination between *hisD*::MudA and *pdu*::MudA prophages); or His⁻Pdu⁻ (deletion of genetic material between *hisD* and *pdu* generated by recombination between *hisD*::MudA and *pdu*::MudA prophages). One-hundred colonies were isolated from each transductional cross and their phenotypes were determined by replica plating to the appropriately supplemented minimal medium.

Results

Cobalamin-dependent utilization of propanediol by *S. typhimurium*

S. typhimurium utilizes 1,2-propanediol as a sole source of carbon and energy during aerobic growth, but only when a nutritional supplement of cobalamin is also added to the culture medium. This conclusion was drawn from the growth curves of *S. typhimurium* LT2 that are shown in Fig. 1. Cells were inoculated into flasks that contained NCE propanediol minimal medium with and without the addition of cobalamin. The cultures were then incubated at 30 °C with aeration (see Methods). After an initial slight increase in cell density (approximately one doubling), the culture that lacked cobalamin showed no further growth. By contrast, the culture that was supplied with cobalamin continued to grow steadily. Based on the best-fitting regression line through the data points, the growth rate constant (μ) for the latter culture was 0.0265 h⁻¹, which is equivalent to a doubling time of 26.2 h. Thus, even when *S. typhimurium* was provided with an adequate supply of cobalamin, aerobic growth

Fig. 1. Growth of *S. typhimurium* LT2 on 1,2-propanediol as a sole source of carbon and energy. Cultures were grown at 30 °C in NCE propanediol minimal medium, with (■) and without (□) cobalamin added as a nutritional supplement (see Methods).

on propanediol was slow. *S. typhimurium* did not grow anaerobically on propanediol as a sole carbon source (data not shown), which is in agreement with previously published results (Obradors *et al.*, 1988).

Isolation of propanediol-negative MudA insertion mutants

The defective bacteriophage MudA was introduced into *S. typhimurium* LT2 and ampicillin-resistant transductant colonies were selected as described in Methods. These colonies were screened for simultaneous loss of the ability to utilize propanediol as a sole source of carbon. Thirty-one independent mutants were isolated that possessed this phenotype. The genes that had been disrupted by the insertion mutations in these strains were designated *pdu*, for defects in propanediol utilization. Each of these mutants was tested to determine if the MudA insertion created an operon fusion (Lac⁺ phenotype, as detected by the formation of a blue colour on propanediol-containing X-Gal indicator plates). In 17 of the strains, the MudA element inserted into the chromosome in the correct orientation to form operon fusions (Lac⁺); in the remaining 14 strains, the MudA element appeared to have inserted into the chromosome in the wrong orientation to form such fusions (Lac⁻). For the strain designation, genotype and phenotypic classification (Lac⁺ or Lac⁻) of all of these MudA insertion mutants, see Table 3.

Aerobically, the cobalamin-dependent utilization of 1,2-propanediol entails its conversion into propionate. Propionate is then metabolized in several steps to pyruvate (Fernández-Briera & Garrido-Pertierra, 1988; Wegener *et al.*, 1967). All of the Pdu⁻ strains grew well on propionate as a sole source of carbon (data not shown).

Table 2. *Propanediol dehydratase enzyme activities in extracts of S. typhimurium*

Each value shown in the table represents a single determination.

Strain and genotype	Specific activity [nmol propionaldehyde formed min ⁻¹ (mg protein) ⁻¹]	
	Cells grown without 1,2-propanediol and cyanocobalamin	Cells grown with 1,2-propanediol and cyanocobalamin
LT2 (<i>pdu</i> ⁺)	3.1	1900
RT811 (<i>pdu-1::MudA</i>)	6.8	3.5
RT813 (<i>pdu-3::MudA</i>)	7.1	1700
RT818 (<i>pdu-8::MudA</i>)	2.5	1900
RT822 (<i>pdu-12::MudA</i>)	4.6	4.2

However, 12 additional *MudA* insertion mutants were isolated from the selection that were unable to utilize either propanediol or propionate as sole sources of carbon for growth. These strains (not listed in Table 3) appear to be blocked in one or more steps of propionate metabolism.

Propanediol dehydratase enzyme activity

S. typhimurium LT2 and four *pdu::MudA* insertion mutants (RT811, RT813, RT818 and RT822) were assayed to determine the levels of propanediol dehydratase enzyme activity. Aerobic cultures of each strain were grown in NCE succinate minimal medium with and without supplements of propanediol and cobalamin. Crude extracts were prepared and then assayed to determine the rate of propionaldehyde formation from propanediol (see Methods). The results of these enzyme assays are presented in Table 2. All of the extracts had extremely low activities [0.34 to 2.6 nmol propionaldehyde formed min⁻¹ (mg protein)⁻¹] when the substrate was not added to the assay tubes (data not shown). These numbers set an upper limit for the amounts of endogenous propanediol that were initially present in the crude extracts. In the tubes that contained the complete assay mixture, the extracts from cells that had been grown in medium without supplements of propanediol and cobalamin had only slightly higher activities [2.5 to 7.1 nmol propionaldehyde formed min⁻¹ (mg protein)⁻¹]. These numbers indicate that little active enzyme is synthesized when propanediol and cobalamin are both absent. The extracts from cells that had been grown in medium with supplements of propanediol and cobalamin produced mixed results. The wild-type strain (LT2) and two of the mutants (RT813 and RT818) had large amounts of propanediol dehydratase activity [1700 to 1900 nmol propionaldehyde formed min⁻¹ (mg protein)⁻¹]. The other two mutants (RT811 and RT822) had only low levels of activity [3.5 and 4.2 nmol propionaldehyde formed min⁻¹ (mg protein)⁻¹]. These numbers

demonstrate that two of the insertions (*pdu-1::MudA* and *pdu-12::MudA*) block the synthesis of propanediol dehydratase directly, but that the other two insertions (*pdu-3::MudA* and *pdu-8::MudA*) do not. The latter mutations probably exert their phenotypic effects by blocking the synthesis of enzymes in the pathway other than propanediol dehydratase.

Genetic mapping of the pdu mutations by conjugation

Conjugational mapping experiments were done to locate the *pdu* genes on the *S. typhimurium* chromosome. Hfr donor strains were constructed by selecting for homologous recombination between the *lac* genes of an F'*lac* plasmid and a *MudA* prophage (see Methods). Under these conditions, integration of the F' (and hence the origin of chromosomal transfer) occurs at the site of the *MudA* insertion and the direction of chromosomal transfer is governed by the orientation of the *MudA* insertion (Maloy & Roth, 1983). Thus, two different *pdu::MudA* insertions with opposite Lac phenotypes were used to construct the Hfr strains so that the position of the *pdu* locus could be determined by chromosomal transfer in both directions. These insertions were *pdu-3::MudA* (RT813, Lac⁻ phenotype) and *pdu-8::MudA* (RT818, Lac⁺ phenotype). The Hfr donors were then mated with a set of auxotrophic recipients as described previously (Chumley *et al.*, 1979; Davis *et al.*, 1980). In both sets of matings, a clear (greater than 10-fold) discontinuity in the numbers of prototrophic recombinants occurred between *aroD* and *his* (data not shown). These data place the *pdu* locus between 36 and 42 map units (Sanderson & Roth, 1988).

Pdu⁻ phenotype of mutants that carry his-cob deletions

The genes for cobalamin biosynthesis (*cob*) are located at 41 map units on the *S. typhimurium* chromosome (Jeter *et al.*, 1984). Since propanediol utilization is dependent

upon cobalamin, it seemed reasonable to speculate that the *pdu* genes were located near the *cob* locus. To test this hypothesis, five independently isolated mutant strains (RT761–RT765) that carried chromosomal deletions extending from the *his* operon into the *cob* operons were tested for their ability to metabolize propanediol. Cobalamin and histidine were present in the culture medium as nutritional supplements. None of the deletion strains was able to utilize propanediol as a sole source of carbon for growth (data not shown). By contrast, all of the parental strains (TR6583, RT766, RT767 and RT785) grew well on this same medium. These results provide strong phenotypic evidence that the *pdu* genes are located between the *his* and *cob* genes.

All the deletion and control strains grew well on propionate as a sole source of carbon. These results demonstrate that only genes that are required for the conversion of propanediol to propionate lie between the *his* and *cob* operons. Genes that are required for the degradation of propionate are located elsewhere on the *S. typhimurium* chromosome.

All the deletion and control strains grew well on ethanolamine as a sole source of carbon. Thus, the activity of ethanolamine ammonia-lyase was not impaired. The strains also carried the *metE205* mutation, which makes the synthesis of methionine completely dependent upon the activity of the cobalamin-requiring *metH* enzyme (Jeter *et al.*, 1984). These results demonstrate that the deletion of genetic material between the *his* and *cob* genes causes a specific loss of the ability to utilize propanediol, but does not create any obvious defects in other aspects of known cobalamin metabolism (e.g. cobalamin transport, ethanolamine utilization and cobalamin-dependent homocysteine methylation).

Cotransductional linkage of *pdu* to *cob*

All the insertion mutants that had a *Pdu*⁻ phenotype were tested for cotransduction with the branch I *cob* operon. Each mutant was transduced with a bacteriophage P22 lysate that had been grown on a donor strain carrying a *his*-proximal *Tn10* insertion in the branch I *cob* genes (TT10441; *cob-13::Tn10*). Tetracycline-resistant transductant colonies were then tested for loss of the *pdu::MudA* insertion (ampicillin-sensitive phenotype). A donor control cross was done with a recipient that carried a *MudA* insertion in the *his* operon (TT7691; *hisD9952::MudA*). The *his* genes are too distant to be cotransduced with the branch I *cob* genes (Jeter *et al.*, 1984). In the same manner, recipient control crosses were done with a P22 lysate that had been grown on a donor strain carrying a distant *Tn10* insertion (TT6195; *pncA148::Tn10*). The results of these transductions are presented in Table 3. Out of 31 mutants with a *Pdu*⁻

Table 3. *MudA* insertions in the *pdu* genes and their cotransductional linkages to a branch I *cob::Tn10* allele

Recipients were the *MudA* insertion strains and donor bacteriophage was grown on strain TT10441 (*cob-13::Tn10*).

Strain designation, genotype and Lac phenotype	No. of Tet ^r Amp ^s transductants (per 100 scored)
RT811 (<i>pdu-1::MudA</i> , Lac ⁻)	25
RT812 (<i>pdu-2::MudA</i> , Lac ⁻)	5
RT813 (<i>pdu-3::MudA</i> , Lac ⁻)	5
RT814 (<i>pdu-4::MudA</i> , Lac ⁻)	5
RT815 (<i>pdu-5::MudA</i> , Lac ⁺)	13
RT816 (<i>pdu-6::MudA</i> , Lac ⁻)	13
RT817 (<i>pdu-7::MudA</i> , Lac ⁻)	22
RT818 (<i>pdu-8::MudA</i> , Lac ⁺)	7
RT819 (<i>pdu-9::MudA</i> , Lac ⁺)	18
RT820 (<i>pdu-10::MudA</i> , Lac ⁻)	14
RT821 (<i>pdu-11::MudA</i> , Lac ⁺)	11
RT822 (<i>pdu-12::MudA</i> , Lac ⁺)	24
RT823 (<i>pdu-13::MudA</i> , Lac ⁺)	6
RT824 (<i>pdu-14::MudA</i> , Lac ⁻)	11
RT825 (<i>pdu-15::MudA</i> , Lac ⁺)	10
RT826 (<i>pdu-16::MudA</i> , Lac ⁺)	8
RT827 (<i>pdu-17::MudA</i> , Lac ⁺)	11
RT828 (<i>pdu-18::MudA</i> , Lac ⁺)	15
RT829 (<i>pdu-19::MudA</i> , Lac ⁻)	35
RT830 (<i>pdu-20::MudA</i> , Lac ⁺)	16
RT831 (<i>pdu-21::MudA</i> , Lac ⁺)	8
RT832 (<i>pdu-22::MudA</i> , Lac ⁻)	2
RT833 (<i>pdu-23::MudA</i> , Lac ⁺)	6
RT834 (<i>pdu-24::MudA</i> , Lac ⁺)	17
RT835 (<i>pdu-25::MudA</i> , Lac ⁺)	9
RT836 (<i>pdu-26::MudA</i> , Lac ⁻)	7
RT837 (<i>pdu-27::MudA</i> , Lac ⁺)	7
RT838 (<i>pdu-28::MudA</i> , Lac ⁺)	20
RT839 (<i>pdu-29::MudA</i> , Lac ⁻)	17
RT848 (<i>pdu-32::MudA</i> , Lac ⁻)	4
RT847 (<i>btu-151::MudA</i> , Lac ⁻)	0

phenotype, 30 (RT811–RT839 and RT848) displayed linkage between the site of the *MudA* insertion and the *cob-13* allele. The range in the cotransduction frequencies was from 2 to 35% and the mean was 12%. As expected, none of the insertions showed any cotransduction with each other in the control crosses (data not shown).

The cotransduction frequencies between the *pdu* and *cob* alleles were converted into physical distance along the chromosome as follows. Bacteriophage P22 DNA molecules that are packaged into phage heads by the P22 headful nuclease have a mean length of 43.4 kbp (Casjens & Hayden, 1988). Donor bacterial DNA molecules that are packaged into P22 transducing particles were assumed to have the same length. Thus, the DNA that was introduced into the recipient cells in these transductions consisted of the *Tn10* transposon (9.3 kbp) and flanking chromosomal sequences (34.1 kbp). Using the latter number in the formula of Wu (1966), it was calculated that cotransduction frequencies

of 2 and 35% corresponded to distances of 25 and 10 kbp of DNA, respectively. The mean cotransduction frequency (12%) corresponded to a mean physical distance of 17 kbp of DNA.

The one remaining propanediol-negative strain (RT847) that did not display any cotransduction between the MudA insertion and the *cob-13* allele also did not grow on ethanolamine as a sole carbon source. This result suggested that the mutation might block the transport of cobalamin. The MudA insertion was tested for cotransduction with a Tn10 insertion (TT13787; *btu-6::Tn10*) that confers a defect in cobalamin uptake and may occur in the *btuC* locus (D. M. Roof, personal communication). The two insertions were found to be 98% cotransducible (data not shown). Thus, the mutation in RT847 was assigned as a defect in cobalamin transport (*btu-151::MudA*).

Identification of propanediol-negative Tn10 insertion mutants

Two strains (TT10927 and TT11073) that were isolated previously carry independent Tn10 insertions in the *S. typhimurium* chromosome that are cotransducible with (but do not disrupt) the branch I *cob* operon (Jeter & Roth, 1987). The Tn10 insertions were originally assigned the allele designations *zeb-1845::Tn10* and *zeb-1847::Tn10*, respectively. In this study, these two strains were tested for propanediol utilization and were found to have a Pdu⁻ phenotype. Thus, the insertions were reclassified as *pdu-30::Tn10* and *pdu-31::Tn10* (Table 1).

Direction of transcription of the pdu genes

The method of Hughes & Roth (1985) was used to determine the direction of transcription of the *pdu* genes. In this procedure, bacteriophage P22 lysates are grown on different MudA insertion strains, the lysates are mixed in pairs, and recipient cells are infected separately with each mixed lysate. Individual transductants may inherit either one of the parental MudA elements irrespective of its orientation in the chromosome. When the parental MudAs are in the same relative orientation, homologous recombination can also occur between them to produce hybrid MudA elements. Inheritance of such a hybrid generates a duplication or deletion of the genetic material between the original MudA insertion sites. The phenotypes that are conferred by these chromosomal rearrangements are distinguishable from the phenotypes of the parental strains. Thus, if the chromosomal orientation of one of the parental MudA insertions is

known, the orientation of the second MudA insertion can be inferred (Hughes & Roth, 1985).

P22 lysates were grown on two different *hisD::MudA* insertion strains and 10 different *pdu::MudA* insertion strains, and 20 mixed lysates were prepared. Recipient cells (TR6583) were then infected with each of the mixed lysates, ampicillin-resistant transductants were selected, and their phenotypes were determined. The results of these genetic crosses are given in Table 4. To summarize the data, the Lac⁺ *hisD::MudA* insertion formed duplications and deletions when combined with all of the Lac⁺ *pdu::MudA* insertions, but not when combined with any of the Lac⁻ *pdu::MudA* insertions. Conversely, the Lac⁻ *hisD::MudA* insertion formed duplications and deletions when combined with all of the Lac⁻ *pdu::MudA* insertions, but not when combined with any of the Lac⁺ *pdu::MudA* insertions. Thus, *hisD::MudA* and *pdu::MudA* insertions that shared the same Lac phenotypes were in the same chromosomal orientations, and *hisD::MudA* and *pdu::MudA* insertions that had different Lac phenotypes were in opposite chromosomal orientations. The *his* operon is known to be transcribed in the clockwise direction relative to the *S. typhimurium* genetic map (Sanderson & Roth, 1988). Thus, all of the *pdu* genes that were tested are also transcribed in the clockwise direction.

Regulation of the pdu genes

Two *pdu::lac* operon fusion strains (RT818 and RT822) were grown aerobically and assayed for β -galactosidase enzyme activity. Aerobic cultures were grown in minimal medium with succinate (a poor carbon source), and the ability of exogenously supplied propanediol and cyanocobalamin to induce the fusions was tested. The results of these assays are given in Table 5. Virtually no β -galactosidase activity was observed in the fusion strains when both supplements were absent or when cobalamin alone was present in the culture medium. By contrast, β -galactosidase was strongly induced by the presence of propanediol alone. In the presence of propanediol, cobalamin appeared to enhance the induction in one of the fusion strains (RT822), but not in the other fusion strain (RT818).

Discussion

In this paper, I have presented evidence that the enteric bacterium *S. typhimurium* is able to utilize 1,2-propanediol as a sole source of carbon and energy during aerobic growth. However, the aerobic metabolism of propanediol is dependent upon the provision of cobalamin as a nutritional supplement. This requirement is due to the

Table 4. Chromosomal orientation of *pdu* : *MudA* insertions

A total of 100 colonies were scored in each transductional cross. In control crosses with unmixed lysates, the inheritance of a parental *MudA* insertion always conferred the expected parental phenotype (data not shown).

MudA insertion that is carried by donor strains*	No. of transductant colonies with phenotype				Inferred relative orientation of <i>MudA</i> elements
	Parental (Pdu ⁻ His ⁺)	Parental (Pdu ⁺ His ⁻)	Duplication (Pdu ⁺ His ⁺)	Deletion (Pdu ⁻ His ⁻)	
Lac ⁻ <i>pdu</i> + Lac ⁻ <i>his</i>					
<i>pdu</i> -2 + <i>hisD9952</i>	26	38	33	3	Same
<i>pdu</i> -7 + <i>hisD9952</i>	3	38	43	16	
<i>pdu</i> -14 + <i>hisD9952</i>	14	43	30	13	
<i>pdu</i> -19 + <i>hisD9952</i>	33	21	46	0	
<i>pdu</i> -29 + <i>hisD9952</i>	26	7	56	11	
Lac ⁻ <i>pdu</i> + Lac ⁺ <i>his</i>					
<i>pdu</i> -2 + <i>hisD9953</i>	62	38	0	0	Opposite
<i>pdu</i> -7 + <i>hisD9953</i>	68	32	0	0	
<i>pdu</i> -14 + <i>hisD9953</i>	62	38	0	0	
<i>pdu</i> -19 + <i>hisD9953</i>	56	44	0	0	
<i>pdu</i> -29 + <i>hisD9953</i>	76	24	0	0	
Lac ⁺ <i>pdu</i> + Lac ⁻ <i>his</i>					
<i>pdu</i> -9 + <i>hisD9952</i>	28	72	0	0	Opposite
<i>pdu</i> -11 + <i>hisD9952</i>	65	35	0	0	
<i>pdu</i> -13 + <i>hisD9952</i>	73	27	0	0	
<i>pdu</i> -18 + <i>hisD9952</i>	58	42	0	0	
<i>pdu</i> -28 + <i>hisD9952</i>	79	21	0	0	
Lac ⁺ <i>pdu</i> + Lac ⁺ <i>his</i>					
<i>pdu</i> -9 + <i>hisD9953</i>	19	8	53	20	Same
<i>pdu</i> -11 + <i>hisD9953</i>	35	5	51	9	
<i>pdu</i> -13 + <i>hisD9953</i>	28	3	62	7	
<i>pdu</i> -18 + <i>hisD9953</i>	44	9	40	7	
<i>pdu</i> -28 + <i>hisD9953</i>	51	2	41	6	

* The phenotypes of individual *pdu* and *his* alleles are given as Lac⁺ (if *pdu* : *lac* or *his* : *lac* operon fusions were formed) or Lac⁻ (if operon fusions were not formed). The strain numbers that correspond to the alleles are given in Tables 1 and 3.

Table 5. Regulation of β -galactosidase activity in *pdu* : *lac* operon fusion strains

Each value shown in the Table represents the mean of three determinations. Strain LT2 was used as a negative control and had 0.6 units of specific activity. All cultures were grown in NCE succinate minimal medium with the addition of cyanocobalamin (15 nM) or 1,2-propanediol (26 mM) or both as indicated.

Supplement(s) added to culture medium	Specific activity [nmol <i>o</i> -nitrophenol formed min ⁻¹ (OD ₆₅₀ unit) ⁻¹]	
	RT818 (<i>pdu</i> -8 : <i>MudA</i>)	RT822 (<i>pdu</i> -12 : <i>MudA</i>)
None	2.2	1.5
Cobalamin	1.5	1.8
Propanediol	1800	2600*
Cobalamin + propanediol	1900	3800*

* In an unpaired *t*-test, the two sets of data were different ($P < 0.001$).

fact that *S. typhimurium* synthesizes cobalamin *de novo* only when the cells are grown under anaerobic conditions (Jeter *et al.*, 1984). In the presence of oxygen, propanediol is first dehydrated to form propionaldehyde, a

reaction which is catalysed by the cobalamin-dependent enzyme propanediol dehydratase. Propionaldehyde is then converted by one or more additional enzymes into propionate (or propionyl coenzyme A). Lastly, propionate is metabolized by a poorly characterized pathway that is thought to proceed via the intermediates acrylyl coenzyme A and lactyl coenzyme A to pyruvate (Fernández-Briera & Garrido-Pertierra, 1988; Wegener *et al.*, 1967).

The discovery that *S. typhimurium* utilizes propanediol as a sole carbon source aerobically provided the opportunity to isolate mutants that are blocked in propanediol metabolism. Based upon these mutations, the genes for propanediol utilization (*pdu*) were located between the *his* and branch I *cob* operons at 41 map units on the *S. typhimurium* chromosome (Table 3). This region is shown diagrammatically in Fig. 2. The range in cotransduction frequencies between *pdu* and *cob* alleles suggested that the *pdu* region spans roughly 15 kbp of DNA (see Results). Toraya *et al.* (1979) have studied anaerobic propanediol metabolism in *K. pneumoniae* and have proposed that the fermentative propanediol dehydratase pathway comprises five different enzyme

Fig. 2. Genetic map of the *pdu* region on the *S. typhimurium* chromosome. The depicted region is drawn to approximate scale and is estimated to be about 3 map units in length. Arrows above the genes indicate the known directions of transcription. Gene designations: *his*, histidine biosynthesis; *phs*, production of hydrogen sulphide; *pdu*, propanediol utilization; *cob*, cobalamin biosynthesis; *supD*, amber suppressor.

activities. Propanediol dehydratase itself has been reported to consist of at least four nonidentical subunits (McGee & Richards, 1981; Poznanskaja *et al.*, 1979). Thus, it is not surprising that the *pdu* region of the *S. typhimurium* chromosome might be so large.

Some but not all of the *pdu* mutants that were assayed had lost the ability to synthesize propanediol dehydratase holoenzyme (Table 2). The two insertions (*pdu-1*::MudA and *pdu-12*::MudA) that resulted in loss of the enzyme activity also displayed high frequencies of cotransduction (25 and 24%) with the branch I *cob*::Tn10 insertion (Table 3). By contrast, the two insertions (*pdu-3*::MudA and *pdu-8*::MudA) that did not result in loss of the enzyme activity displayed low frequencies of cotransduction (5 and 7%) with the same marker. A likely explanation for these results is that the structural genes for propanediol dehydratase lie at the *cob*-proximal end of the *pdu* region. Additional biochemical and genetic analysis of the *pdu* mutants will be necessary in order to confirm this hypothesis.

Several *his*-*cob* deletion mutants were tested and found to lack the ability to grow on propanediol as a sole carbon source. However, they all grew well on propionate alone. These results demonstrate that the genes for propionate metabolism do not lie in this interval of the *S. typhimurium* chromosome. Likewise, all the deletion mutants retained the abilities to use ethanolamine as a sole carbon source and to synthesize methionine in a cobalamin-dependent fashion. Thus, these deletions confer a specific defect in propanediol utilization but do not directly impair other cobalamin-dependent metabolic activities in the cells.

Transcription of the *pdu* genes was strongly induced by the presence of propanediol in the culture medium (Table 5). One of the tested strains (RT822) was not able to metabolize propanediol, since it completely lacked propanediol dehydratase activity (Table 2). Thus, propanediol itself appears to be the true inducer.

Cyanocobalamin alone had no effect on *pdu* gene expression. In combination with propanediol, it appeared to stimulate transcription of the *pdu*::*lac* fusion in RT822 but not the fusion in RT818 (Table 5). A possible explanation for this observation is that the *pdu*

genes encoding propanediol dehydratase itself may be organized into a transcriptional unit that responds to both cobalamin and propanediol, while the *pdu* genes encoding other enzyme activities are organized into one or more separate transcriptional units that do not respond to cobalamin. The operon organization of these genes is currently under investigation. In any case, the *pdu* genes appear to be regulated differently than the *eut* operon, for which the induction of transcription requires the simultaneous presence of both ethanolamine and cobalamin (Roof & Roth, 1988).

Transcription of the *cob* genes is repressed in response to molecular oxygen (Escalante-Semerena & Roth, 1987). It is reasonable to assume that this gives *S. typhimurium* some selective advantage for continued survival in its natural habitat, the mammalian intestinal tract. However, the precise nature of this advantage has remained elusive. The location of the genes for propanediol utilization near the genes for cobalamin biosynthesis suggests that these two sets of genes may share a close evolutionary relationship. Thus, future genetic and biochemical studies of propanediol metabolism by *S. typhimurium* may add to our understanding of this most puzzling aspect of *cob* gene regulation.

I thank Ronald Smith for assistance in isolating the *pdu*::MudA insertion strains and Karin Otto for critically reading the manuscript. This research was supported by a grant from the College of Arts and Sciences at Texas Tech University.

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