

Microcompartments for B₁₂-Dependent 1,2-Propanediol Degradation Provide Protection from DNA and Cellular Damage by a Reactive Metabolic Intermediate[∇]

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Received 11 December 2007/Accepted 11 February 2008

Salmonella enterica grows on 1,2-propanediol (1,2-PD) in a coenzyme B₁₂-dependent fashion. Prior studies showed that a bacterial microcompartment (MCP) is involved in this process and that an MCP-minus mutant undergoes a 20-h period of growth arrest during 1,2-PD degradation. It was previously proposed that growth arrest resulted from propionaldehyde toxicity, but no direct evidence was presented. Here, high-pressure liquid chromatography analyses of culture medium were used to show that the major products of aerobic 1,2-PD degradation are propionaldehyde, propionate, and 1-propanol. A MCP-minus mutant accumulated a level of propionaldehyde 10-fold higher than that of the wild type (1.6 mM compared to 15.7 mM), associating this compound with growth arrest. The addition of propionaldehyde to cultures of *S. enterica* caused growth arrest from 8 to 20 mM, but not at 4 mM, providing direct evidence for propionaldehyde toxicity. Studies also indicated that propionaldehyde was toxic due to the inhibition of respiratory processes, and the growth arrest ended when propionaldehyde was depleted primarily by conversion to propionate and 1-propanol and secondarily due to volatility. The Ames test was used to show that propionaldehyde is a mutagen and that mutation frequencies are increased in MCP-minus mutants during 1,2-PD degradation. We propose that a primary function of the MCPs involved in 1,2-PD degradation is the mitigation of toxicity and DNA damage by propionaldehyde.

Several bacterial genera, including *Salmonella*, *Klebsiella*, *Shigella*, *Yersinia*, *Listeria*, *Lactobacillus*, and *Lactococcus*, include members that grow on 1,2-propanediol (1,2-PD) in a coenzyme B₁₂-dependent fashion (22, 42). 1,2-PD is a major product of the anaerobic degradation of rhamnose and fucose, which are common sugars in plant cell walls, bacterial exopolysaccharides, and the glycoconjugates of intestinal epithelial cells (29). Hence, 1,2-PD degradation is likely to provide a selective advantage in niches, such as the large intestines of higher animals, sediments, and the depths of soils. In addition, in vivo expression technology indicated that 1,2-PD utilization genes (*pdu*) are induced in host tissues and competitive index studies with mice showed that *pdu* mutations confer a virulence defect (12, 18).

A pathway for 1,2-PD degradation by *Salmonella enterica* has previously been described (29, 42). It begins with the conversion of 1,2-PD to propionaldehyde by coenzyme B₁₂-dependent diol dehydratase (1). Next, the aldehyde is converted to 1-propanol and propionic acid by propanol dehydrogenase, coenzyme A-dependent propionaldehyde dehydrogenase, phosphotransacylase, and propionate kinase (Fig. 1) (25, 26, 29, 30, 42). This process supports growth by providing ATP, an electron sink, and a three-carbon intermediate (propionyl-coenzyme A) that is degraded to pyruvate and succinate via the methylcitrate pathway (19, 21).

Twenty-three *pdu* genes are found in a single contiguous cluster: *pocR*, *pduF*, and *pduABCDEFGHIJKLMNPOQSTUVWX* (8). These genes encode a transcriptional regulator (PocR), a 1,2-PD facilitator (PduF), three enzymes for B₁₂ metabolism (PduGH, PduO, and PduS), five catabolic enzymes that mediate 1,2-PD degradation as described above (PduCDE, PduL, PduP, PduQ, and PduW), three polypeptides of unknown function (PduM, PduV, and PduX), and eight probable bacterial microcompartment (MCP) proteins (PduA, PduB, PduB', PduJ, PduK, PduN, PduT, and PduU) (7, 8, 11, 23, 30, 34, 38).

Bacterial MCPs are primitive organelles that function in carbon fixation (carboxysomes) and various catabolic processes (enterosomes, metabolosomes, and polyhedral organelles), including B₁₂-dependent 1,2-PD and ethanolamine degradation in *S. enterica* (5, 6, 10, 32, 40). Microcompartments are usually 100 to 150 nm in cross section and consist of metabolic enzymes compartmentalized within a protein shell. Unlike eukaryotic organelles, they lack lipid membranes and consist solely of protein subunits (24, 45). Prior studies showed that MCPs involved in B₁₂-dependent 1,2-PD degradation include at least 14 different polypeptides (PduABB'CDEGHJKO PTU) (16). Their enzyme complement (PduCDEGHOP) indicates that the first two steps of 1,2-PD degradation occur in the lumen of the MCP and the remaining steps in the cytoplasm of the cell (Fig. 1) (16). It was suggested that these MCPs function to mitigate aldehyde toxicity (17, 41). Deletion of the *pduA* gene prevented MCP formation and caused a 20-h period of growth arrest during catabolism of 1,2-PD (17). Growth arrest occurred only at higher 1,2-PD concentrations, suggesting that it resulted from the accumulation of a toxic metabolic

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[∇] Published ahead of print on 22 February 2008.

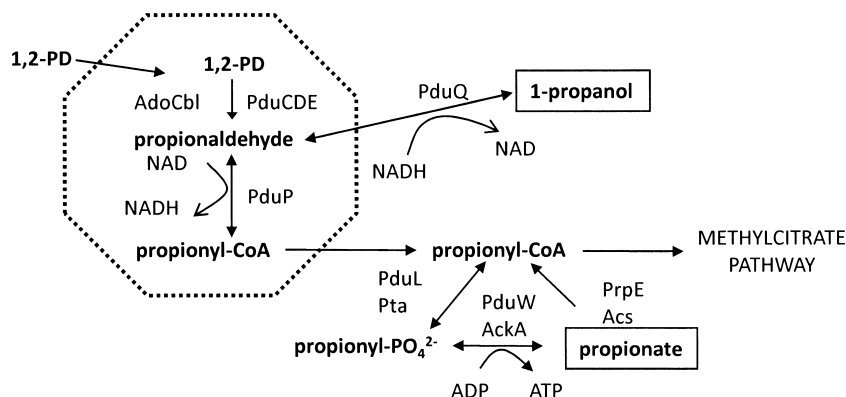


FIG. 1. Model for 1,2-propanediol degradation by *S. enterica*. The broken line indicates the shell of the MCP, which is composed completely of protein subunits. The first two steps of 1,2-PD degradation (conversion of 1,2-PD to propionyl-CoA) are thought to occur in the lumen of the compartment, and the remaining steps are thought to occur in the cytoplasm. The proposed function of this MCP is sequestration of propionaldehyde to minimize its toxicity. Two additional enzymes associated with these MCPs (but not shown in the figure) are a putative diol dehydratase reactivase (PduGH) and ATP:cob(I)alamin adenosyltransferase (PduO). Abbreviations: PduCDE, coenzyme B₁₂-dependent diol dehydratase; PduP, propionaldehyde dehydrogenase; PduL, phosphotransacylase; PduW and AckA, phosphotransacetylase (Pta) propionate kinase; PduQ, 1-propanol dehydrogenase; PrpE, propionyl-CoA synthetase; Acs, acetyl-coenzyme A synthetase. AckA, Acs, and Pta are housekeeping enzymes carried outside the *pdu* locus. PrpE is also carried outside the *pdu* locus, and its primary role is propionate degradation (20).

intermediate (propionaldehyde). Studies of the MCPs involved in B₁₂-dependent ethanolamine degradation suggested that these compartments also function to mitigate the toxicity of a metabolic intermediate (acetaldehyde), but this idea was questioned by others who proposed that their main function was to prevent acetaldehyde loss due to volatility (9, 31, 35, 36, 41). Furthermore, no direct evidence for a role in the mitigation of aldehyde toxicity has been presented for either the 1,2-PD or the ethanolamine MCPs.

Here, high-pressure liquid chromatography (HPLC) analyses and growth tests were used to show that propionaldehyde accumulates to toxic levels in MCP-minus mutants of *S. enterica* during growth on 1,2-PD. The Ames test was used to demonstrate that propionaldehyde is a mutagen and that MCP mutants are subject to increased DNA damage during growth on 1,2-PD. In addition, studies showed that most of the propionaldehyde produced during 1,2-PD degradation was further metabolized to propionate and 1-propanol and only a small fraction was lost due to volatility. These results provide the first direct evidence that the MCPs involved in 1,2-PD degradation function minimize toxicity and DNA damage by the reactive metabolic intermediate propionaldehyde.

MATERIALS AND METHODS

Chemicals and reagents. Antibiotics were from Sigma Chemical Company (St. Louis, MO). Isopropyl-β-D-thiogalactopyranoside (IPTG) was from Diagnostic Chemicals Limited (Charlotteville, Prince Edward Island, Canada). Restriction enzymes and T4 DNA ligase were from New England Biolabs (Beverly, MA). Other chemicals were from Fisher Scientific (Pittsburgh, PA).

Bacterial strains, media, and growth conditions. The bacterial strains used in this study are listed in Table 1. The rich medium used was Luria-Bertani/Lennox (LB) medium (Difco, Detroit, MI) (27). The minimal medium used was non-carbon-E (NCE) with 1 mM MgSO₄; 0.3 mM each valine, isoleucine, leucine, and threonine; and the supplements indicated in the text (4, 44). Vitamin B₁₂ was used at 150 nM. For optimization of 1,2-PD degradation, growth was measured using a Synergy HT microplate reader (BioTek, Winooski, VT) as described previously (26). For an analysis of 1,2-PD degradation products by HPLC, 50-ml cultures were grown in 250-ml Erlenmeyer flasks at 37°C with shaking at 275 rpm in an Innova I2400 incubator shaker (New Brunswick Scientific) (17). These cultures were inoculated to an optical density at 600 nm (OD₆₀₀) of 0.1 with an

LB overnight culture that had been centrifuged and resuspended in NCE with 1 mM MgSO₄. Similar culture conditions were used for assessing the toxicity of propionaldehyde, 1-propanol, and propionate.

Molecular methods. Agarose gel electrophoresis and PCRs were carried out using standard protocols (26, 37).

Construction of *pdu* deletion mutants. The *pduA* deletion mutant (BE182) was constructed by the method of Miller and Mekalanos (28), with modifications as described previously (25). In this deletion mutant, the entire *pduA* coding sequence, except for the last 29 bp, was eliminated (17). The *pduK* deletion (BE711) was constructed by linear transformation of PCR products (13) with the modifications described previously (17). This deletion removed the entire coding sequence except the first and last 12 bp of *pduL* and *pduK*, respectively. Both the *pduA* and *pduK* deletions left the predicted translation signals of all *pdu* genes intact. All deletions were moved into a wild-type genetic background by P22 transduction, and PCR was used to verify the size and location of the deletions.

P22 transduction. Transductional crosses were performed as described previously by using P22 HT105/1 *int*-210 (14), a mutant phage that has a high-transducing ability (39). Transductants were tested for phage contamination and sensitivity by streaking on green plates against P22 H5.

HPLC. For the analysis of 1,2-PD degradation products, a Bio-Rad Aminex HPX-87H (300 by 7.8 mm) ion exclusion column was used with a Varian ProStar system that included a 230 solvent delivery module, a 430 autosampler, a 325 UV-visible detector, and a 355 refractive index detector (Varian, Palo Alto, CA).

TABLE 1. Strains used in this study

Strain ^a	Genotype	Source
BE182	<i>ΔpduA652::Frt</i>	17
BE532	<i>phs::dTet hisD3052 Δchl-1004(chl hut bio wvrB) hisO⁺ lps⁺</i>	This study
BE711	<i>ΔpduJK686::Frt</i>	This study
BE965	<i>phs::dTet hisD3052 mutS::dCam</i>	This study
BE966	<i>ΔpduA652::Frt phs::dTet hisD3052 mutS::dCam</i>	This study
TA1534	<i>hisD3052 Δchl-1004 (chl hut bio wvrB) hisO⁺ lps⁺</i>	Lab collection of J. Roth
TA1950	<i>hisG46 Δchl-1001 (chl hut bio wvrB) hisO⁺ lps⁺</i>	Lab collection of J. Roth
TA1952	<i>hisC3076 Δchl-1003 (chl hut bio wvrB) hisO⁺ lps⁺</i>	Lab collection of J. Roth

^a All strains used in this study are derivatives of *S. enterica* serovar Typhimurium LT2 (formerly *S. typhimurium* LT2).

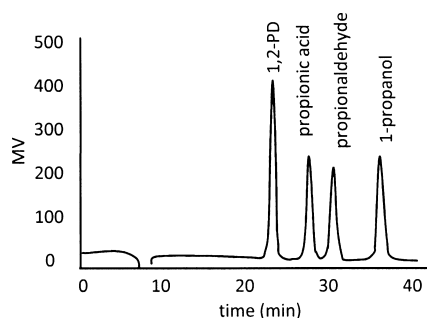


FIG. 2. HPLC analysis of 1,2-PD degradation. To purify the major metabolites of 1,2-PD degradation, a Bio-Rad Aminex ion exclusion column was used in conjunction with RI and UV detection. This system separates and detects a wide variety of compounds. RI detection is shown.

The column was eluted at 0.4 ml min^{-1} with $5 \text{ mM H}_2\text{SO}_4$ (isocratic). This system allows the detection of a wide variety of metabolites.

Tests for DNA damage. To test the mutagenicity of propionaldehyde, the Ames test was performed as previously described (3). To determine whether MCP-minus mutants experienced increased DNA damage during 1,2-PD degradation, the Ames test was used with the modification that $0.5\% \text{ Na}_2$ succinate replaced glucose as the carbon source. This modification was necessary to ensure induction of the genes for 1,2-PD degradation (7, 34).

RESULTS

Optimization of growth on 1,2-PD by *Salmonella*. *S. enterica* grows relatively slowly on NCE medium supplemented with 1,2-PD and vitamin B_{12} . To optimize growth, we examined the effects of vitamin and mineral supplementation. At optimal levels ($50 \text{ } \mu\text{M}$), Fe-citrate decreased the doubling time of *S. enterica* on 1,2-PD minimal medium more than twofold (from

12.3 ± 0.51 to 5.2 ± 0.22 h) and increased the maximal cell density at 600 nm from 1.5 to 2.25 at 48 h. Citrate alone at $50 \text{ } \mu\text{M}$ did not stimulate growth. The effects of CoCl_2 , CuCl_2 , ZnCl_2 , MnSO_4 , H_3BO_4 , NaMoO_4 , NaSeO_4 , and NiCl_2 were tested from 1 nM to $60 \text{ } \mu\text{M}$, but no significant growth stimulation was observed (data not shown). Biotin, nicotinate, riboflavin, folate, lipoate, pyridoxine, and thiamine from 1 to 20 mg liter^{-1} did not stimulate growth. All further studies described here were performed with $50 \text{ } \mu\text{M}$ Fe-citrate added to minimal medium.

HPLC analysis of 1,2-PD degradation. Culture medium of *S. enterica* growing on 1,2-PD was analyzed by HPLC with refractive index (RI) and UV-visible detection. Based on coelution with standards, the major metabolites produced were propionaldehyde, propionate, and 1-propanol. The HPLC system used also detected 1,2-PD. Elution times were as follows: for 1,2-PD, 24 min; for propionate, 27 min; for propionaldehyde, 31 min; and for 1-propanol, 37 min (Fig. 2).

A spike in propionaldehyde levels corresponds with growth arrest in an MCP-minus mutant. Prior studies showed that an MCP-minus mutant of *Salmonella* underwent a 20-h period of growth arrest during 1,2-PD degradation (17). Because this phenotype was more severe at higher 1,2-PD concentrations, it was proposed to result from the accumulation of a toxic metabolite (17). The identification of propionaldehyde, propionate, and 1-propanol as the major products of 1,2-PD degradation (above) suggested that one or more of these compounds might contribute to growth arrest. To investigate this possibility, metabolite levels were measured in growth medium versus time during 1,2-PD degradation. For the MCP-minus mutant, propionaldehyde levels spiked to 15.7 mM , whereas the highest level reached for wild-type *S. enterica* was 1.6 mM (Fig. 3B).

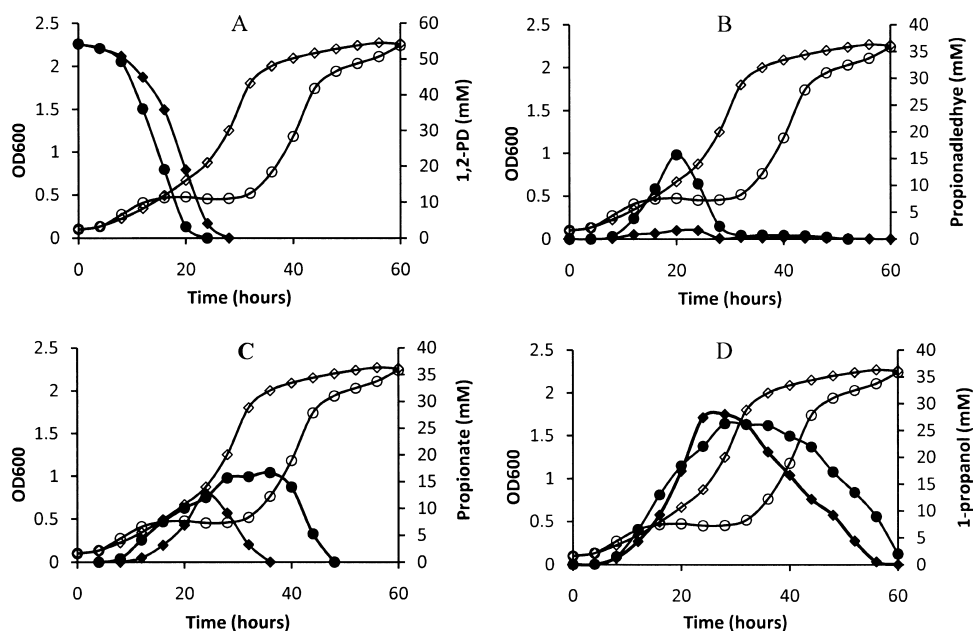


FIG. 3. Metabolite production during 1,2-PD degradation. HPLC was used to measure the 1,2-PD, propionaldehyde, propionate, and 1-propanol in culture medium during 1,2-PD degradation. The data shown are from a single representative experiment. The same growth data (OD_{600}) are repeated in each panel (A to D), along with the level of a particular metabolite. Diamonds, wild-type *S. enterica*; circles, MCP-minus mutant BE182; open symbols, OD_{600} ; closed symbols, metabolite level.

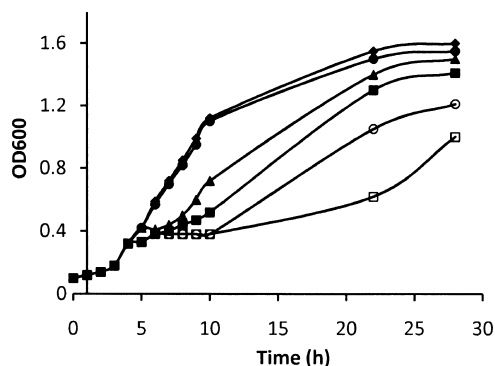


FIG. 4. Toxicity of propionaldehyde. Wild-type *S. enterica* was grown on NCE minimal medium supplemented with 0.5% succinate to an OD₆₀₀ of 0.4, and then propionaldehyde was added in the following concentrations: 0 mM (◆), 4 mM (●), 8 mM (▲), 12 mM (■), 16 mM (○), and 20 mM (□).

For propionate and 1-propanol, the maximum levels reached in cultures of *S. enterica* were 12.7 and 28.0 mM, respectively, compared to 16.6 and 26.3 mM, respectively, for the MCP-minus mutant (Fig. 3C and D). Hence, the main difference in metabolite levels during 1,2-PD degradation was the accumulation of nearly 10-fold higher levels of propionaldehyde in cultures of MCP-minus mutant. In addition, propionaldehyde accumulation and depletion corresponded closely with the cessation and resumption of growth, which is consistent with a causal relationship (Fig. 3C). Also of note was the finding that >20 mM 1,2-PD remained in the culture medium at the start of growth arrest (*t* = 16 h) (Fig. 3A), eliminating diauxic growth as a component of this phenotype. Similar results were obtained in two trials each with two different MCP-minus mutants ($\Delta pduA$ [BE182] and $\Delta pduJK$ [BE711]).

Toxicity of the major metabolites of 1,2-PD degradation. Growth studies were performed to directly test whether propionaldehyde, 1-propanol, or propionate was toxic to wild-type *S. enterica* at the levels observed in culture broths during 1,2-PD degradation. For these experiments, succinate-grown cells were used to prevent changes in metabolite levels due to 1,2-PD degradation. When propionaldehyde was added to a log-phase culture of succinate-grown cells (OD₆₀₀ of 0.4), growth was inhibited at 8 mM and above, but not at 4 mM (Fig. 4). The addition of 30 mM propionate or 30 mM 1-propanol had no measurable effect on growth (data not shown). Hence, propionaldehyde is sufficient to inhibit the growth of wild-type *S. enterica* at the levels observed during growth of MCP-minus mutants, but the other major metabolites of 1,2-PD degradation are not.

The fate of propionaldehyde. In cultures of an MCP-minus mutant growing on 1,2-PD, the concentration of propionaldehyde decreased from 15.7 mM to a level below detection from 20 to 32 h (Fig. 3B). Growth was arrested during this time, raising questions about the fate of propionaldehyde. Further study indicated that propionaldehyde decreased primarily due to metabolism to propionate and 1-propanol and to a lesser extent due to volatility. From 20 to 32 h, propionaldehyde fell 15 mM, propionate and 1-propanol increased 5.9 and 7.7 mM, and the remaining 1,2-PD (3.2 mM) was consumed (Fig. 3A, C, and D). Assuming that the remaining 1,2-PD was converted to

propionaldehyde (the only known route for 1,2-PD degradation), we accounted for all but 2 mM propionaldehyde by the conversion to propionate and 1-propanol. The residual 2 mM propionaldehyde was likely lost due to volatility: controls showed that propionaldehyde levels decreased in uninoculated culture medium with a half-life of about 17 h (data not shown).

Consumption of propionate and 1-propanol. In wild-type *S. enterica*, an analysis of metabolite levels showed that 1,2-PD was depleted below detection by 28 h; however, growth continued (Fig. 3A). During the time period following 1,2-PD depletion (28 to 60 h), propionate and 1-propanol were consumed, indicating that these compounds were serving as growth substrates (Fig. 3C and D). *Salmonella* is known to grow on propionate by the methylcitrate pathway (19, 21), but a pathway for 1-propanol degradation has not been reported. In the MCP-minus mutant, the consumption of propionate and 1-propanol was generally similar to that by the wild type, but with the notable difference that the consumption of these compounds was inhibited during the period of growth arrest (Fig. 3C and D).

Propionaldehyde is a mutagen in the Ames test. The method of Ames was used to test the mutagenicity of propionaldehyde (3). This method determines the reversion of histidine biosynthetic mutants to prototrophy (His⁺) as a measure of DNA damage. Tests were performed with strains TA1950, TA1952, and TA1534, each of which carries a different *his* mutation that varies in its sensitivity for particular mutagens (3). For TA1534, the addition of propionaldehyde to growth medium resulted in a large increase in the number of His⁺ revertants, indicating that propionaldehyde is a mutagen (Table 2). Propionaldehyde had no obvious effect on reversion to His⁺ by strains TA1950 and TA1952, indicating that these strains are less sensitive for detecting the type of DNA damage caused by propionaldehyde (3).

A MCP-minus mutant experiences an increased mutation rate during degradation of 1,2-PD. Strain TA1534, which was used above to demonstrate that propionaldehyde is a mutagen, was unable to break down 1,2-PD, apparently due to an uncharacterized mutation. Therefore, this strain could not be used to test whether an MCP-minus mutant experienced increased DNA damage during 1,2-PD degradation. To conduct this test, strains BE965 (*hisD3052 mutS::cam*) and BE966 (*hisD3052 mutS::cam ΔpduA*) were constructed. We then measured His⁺ revertants during the growth of these strains on succinate/vitamin B₁₂ minimal medium with and without 1,2-PD (Table 3). On average, the number of His⁺ revertants increased 26% for the MCP-minus strain but decreased 7% for the MCP-positive strain when 1,2-PD and vitamin B₁₂ were added to the growth medium. This result indicated that 1,2-PD

TABLE 2. Propionaldehyde is a mutagen in the Ames test

Propionaldehyde concn (mM)	No. of His ⁺ revertants of strain TA1534
0.....	22 ± 3
2.....	16 ± 2
5.....	9 ± 2
10.....	10 ± 5
20.....	477 ± 157
50.....	1,554 ± 522

TABLE 3. An MCP-minus mutant is subject to increased DNA damage during metabolism of 1,2-PD

Trial	No. of His ⁺ revertants of BE966 in wild-type <i>S. enterica</i>		% Change	No. of His ⁺ revertants of BE966 in MCP-minus mutant ($\Delta pduA$)		% Change
	Without 1,2-PD	With 1,2-PD		Without 1,2-PD	With 1,2-PD	
1	69 ± 5	65 ± 5	-6	67 ± 7	92 ± 7	+37
2	95 ± 3	84 ± 4	-12	91 ± 10	109 ± 10	+20
3	134 ± 8	130 ± 15	-3	76 ± 5	92 ± 5	+21

degradation increased DNA damage in an MCP-minus mutant but not in wild-type *S. enterica*.

DISCUSSION

In our prior studies, we showed that an MCP-minus mutant underwent a 20-h period of growth arrest during 1,2-PD catabolism (17). Because this phenotype was more severe at higher 1,2-PD concentrations, it was proposed to result from the accumulation of a toxic metabolite derived from 1,2-PD. Based on the known pathway of 1,2-PD degradation, propionaldehyde was proposed to be responsible for the observed toxicity (17). However, direct evidence for propionaldehyde toxicity was not presented. Studies of a related MCP (the metabolosome involved in B₁₂-dependent ethanolamine degradation) led to the proposal that this compartment helped to minimize acetaldehyde toxicity (9, 35, 36, 41). Supporting this proposal were the findings that *polA* (DNA repair polymerase) and *gsh* (glutathione biosynthesis) mutants were unable to grow on ethanolamine (35, 36). However, it was pointed out that *polA* and *gsh* mutants might be sensitive to levels of aldehyde that are nontoxic for wild-type *Salmonella* (31). In addition, studies suggested that the main function of the ethanolamine MCP was to prevent the loss of acetaldehyde due to volatility and that mitigation of toxicity might not be relevant in wild-type cells (31). Moreover, no direct evidence for acetaldehyde toxicity in mutants unable to form the ethanolamine MCP was presented.

Here, HPLC was used to identify and quantitate the major metabolites of 1,2-PD degradation. Analyses showed that propionaldehyde spiked to about 16 mM in an MCP-minus mutant during growth on 1,2-PD (Fig. 3B). Growth studies showed that this level of propionaldehyde was toxic to wild-type *S. enterica* (Fig. 4). Results also showed that the other major metabolites of 1,2-PD degradation (propionate and 1-propanol) did not reach toxic levels. A well-established assay (the Ames test) was used to show that propionaldehyde is a mutagen and that MCP-minus mutants experience increased DNA damage during 1,2-PD degradation. Hence, the above studies provided direct evidence that the MCPs involved in 1,2-PD degradation function to minimize toxicity and DNA damage by propionaldehyde and that the observed toxicity was relevant to wild-type cells.

Studies performed here examined the volatility of propionaldehyde under standard culture conditions. Results showed that 87% of the propionaldehyde formed during 1,2-PD degradation was further metabolized to propionate and 1-propanol,

and only 13% was lost due to volatility. In contrast, prior studies of the ethanolamine degradation showed that a large fraction of acetaldehyde was lost due to volatility in MCP-minus mutants (31). This result could have been due to the greater volatility of acetaldehyde (boiling point of 21°C) than of propionaldehyde (boiling point of 48°C) or due to differences in culture conditions. In the case of acetaldehyde, high loss occurred only for loosely covered cultures at higher incubation temperatures (37°C) (31). Thus, aldehyde loss due to volatility is a question of environmental conditions. It certainly seems likely to us that some natural environments would effectively retain aldehydes. An analogous situation exists for quorum-sensing molecules which are subject to loss by diffusion but which are active in specific environments (2). Hence, we conclude that the prevention of carbon loss due to volatility only partially explains the selective value of MCPs involved in aldehyde metabolism, although it may be of greater importance for acetaldehyde than for propionaldehyde.

The studies performed here also provide some insight into the cause of growth arrest during 1,2-PD degradation by MCP-minus mutants. During growth arrest, the consumption of propionate and 1-propanol were inhibited (Fig. 3C and D). *S. enterica* metabolizes propionate (and probably 1-propanol) by the methylcitrate pathway, suggesting that propionaldehyde inhibits this pathway (19, 21). This would also block growth on 1,2-PD, which requires the methylcitrate pathway for the generation of biosynthetic building blocks, explaining why growth arrest occurs even while 1,2-PD is being degraded (19, 21, 22, 30). It was also shown that propionaldehyde inhibited the growth of *S. enterica* on succinate (Fig. 4). Hence, results indicate that growth arrest occurred due to a general inhibition of respiratory metabolism that blocked the methylcitrate pathway. Once propionaldehyde was depleted by conversion to 1-propanol and propionate (and to a lesser extent due to loss by volatility), growth resumed. Thus, we propose that growth arrest results from the inhibition of respiratory metabolism by propionaldehyde and resumes following conversion of this toxic compound to propionate and 1-propanol.

With regard to mechanism, it was recently proposed that the ethanolamine MCPs function to concentrate catabolic enzymes to increase their efficiencies and/or regulate metabolite levels (9). It was also suggested that the MCP shell could restrict the diffusion of metabolites, such as propionaldehyde or CO₂, which would provide an additional means for enhancing and/or regulating enzyme activity (17, 33, 41). Consistent with the latter idea are recent crystallography studies which showed that MCP shell proteins are tightly packed hexamers with central pores that could act as specific conduits for metabolites (15, 24, 45). The studies reported here are consistent with both of the above mechanistic proposals, since measurement of metabolite levels clearly showed that propionaldehyde formation and consumption were imbalanced in the MCP-minus mutant (Fig. 3B).

The studies performed here also showed that *S. enterica* excretes relatively large amounts of propionate (12.7 mM) and 1-propanol (28 mM) during aerobic growth on 1,2-PD (as was previously shown for propionate [30]). Subsequently, these compounds are taken up and used to support further growth. The switch to propionate and 1-propanol occurs without an observable lag, which may be a consequence of the fact that *S.*

enterica uses multiple systems to optimize the reuptake of propionate (30, 43).

ACKNOWLEDGMENTS

This work was supported by grant MCB0616008 from the National Science Foundation.

We thank the ISU DNA Sequencing and Synthesis facility for assistance with DNA analyses. We thank Semarhy Quinones-Soto and Eric Kofoid in the lab of J. Roth for kindly providing some of the strains used in this study.

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