JOURNAL OF BACTERIOLOGY, Apr. 2006, p. 2586–2592 Vol. 188, No. 7
0021-9193/06/$08.00+0 doi:10.1128/JB.188.7.2586–2592.2006

Product Repression of Alkane Monooxygenase Expression in *Pseudomonas butanovora*

D. M. Doughty,[1] L. A. Sayavedra-Soto,[2] D. J. Arp,[2] and P. J. Bottomley[1,3]*

Department of Microbiology,[1] Department of Botany and Plant Pathology,[2] and Department of Crop and Soil Science,[3] Oregon State University, Corvallis, Oregon

Received 6 December 2005/Accepted 23 January 2006

Physiological and regulatory mechanisms that allow the alkane-oxidizing bacterium *Pseudomonas butanovora* to consume C_2 to C_8 alkane substrates via butane monooxygenase (BMO) were examined. Striking differences were observed in response to even- versus odd-chain-length alkanes. Propionate, the downstream product of propane oxidation and of the oxidation of other odd-chain-length alkanes following β-oxidation, was a potent repressor of BMO expression. The transcriptional activity of the BMO promoter was reduced with as little as 10 μM propionate, even in the presence of appropriate inducers. Propionate accumulated stoichiometrically when 1-propanol and propionaldehyde were added to butane- and ethane-grown cells, indicating that propionate catabolism was inactive during growth on even-chain-length alkanes. In contrast, propionate consumption was induced (about 80 nmol propionate consumed · min^{-1} · mg $protein^{-1}$) following growth on the odd-chain-length alkanes, propane and pentane. The induction of propionate consumption could be brought on by the addition of propionate or pentanoate to the growth medium. In a reporter strain of *P. butanovora* in which the BMO promoter controls β-galactosidase expression, only even-chain-length alcohols (C_2 to C_8) induced β-galactosidase following growth on acetate or butyrate. In contrast, both even- and odd-chain-length alcohols (C_3 to C_7) were able to induce β-galactosidase following the induction of propionate consumption by propionate or pentanoate.

Considerable research has been carried out on the biochemistry and physiology associated with the catabolism of intermediate-chain-length *n*-alkanes (1, 13, 14, 22, 23, 35). However, much less is known about the transcriptional regulation of these pathways (27, 28, 29, 34). Insights into the complexity of the transcriptional regulation of alkane utilization have been obtained by studying *Pseudomonas putida* GPo1 that grows on liquid alkanes (C_5 to C_{12}). The alkane monooxygenase of this bacterium is induced during growth on alkanes and repressed during growth on either complex medium or minimal medium containing simple organic acids (10, 30, 37, 38). The deletion of the gene encoding the Crc protein that is involved in the repression of alkane hydroxylase in complex medium does not affect repression exerted by organic acids (37, 38). To date, the signaling pathway involved in the catabolite repression of the alkane hydroxylase in *P. putida* GPo1 by complex medium has been well studied (37, 38). In contrast, catabolite repression by organic acids has received less attention (10).

Recent work from our laboratory has shown that genes coding for a broad-substrate-range alkane monooxygenase, commonly referred to as butane monooxygenase (BMO), are responsible for the ability of *Pseudomonas butanovora* to grow on alkanes C_2 to C_9 (29). The region immediately 5′ of the BMO operon in *P. butanovora* contains a putative sigma 54-dependent promoter (29). Sigma 54-dependent promoters are subject to positive control mediated by enhancer-binding proteins, which facilitate transcriptional initiation (5, 6, 25). Unlike the alkane-responsive system regulating monooxygenase expression in *P. putida* GPo1, the transcriptional activity of the BMO promoter in *P. butanovora* is up-regulated in response to the products of monooxygenase activity, butyraldehyde and 1-butanol. In contrast, neither the substrate, butane, nor the immediate downstream product of butyraldehyde oxidation, butyric acid, was found to be an inducer (11, 28). A constitutive albeit low level of BMO activity allows cells to respond to alkanes by transforming them into products which then induce higher levels of BMO (28).

In this paper, we describe an additional feature of the regulation of alkane catabolism in *P. butanovora* whereby BMO expression is repressed in situations in which propionic acid accumulates because of the oxidation of odd-chain-length alkanes by cells growing on even-chain-length alkanes and actively expressing BMO activity. Our results are discussed in the context of a hypothetical model of BMO regulation that bears some resemblance to the classical regulatory model controlling fatty acid oxidation and lipid biosynthesis in bacteria (9, 26, 33, 36).

MATERIALS AND METHODS

Bacterial strains, growth conditions, and routine assays. *P. butanovora* (ATCC 43655) is a gram-negative bacterium that grows on *n*-alkanes (C_2 to C_9) (31). Liquid cultures were grown and harvested as previously described (11). BMO activity was routinely measured using ethene-dependent ethene oxide production as described elsewhere (11). The specific activity of BMO in whole cells is routinely expressed in nmol ethene oxide · min^{-1} · mg $protein^{-1}$. Protein concentrations were determined using the micro biuret assay as described previously (12).

When alkanes served as growth substrates, 2 mmol (approximately 200 μM aqueous concentration) of the respective alkane was added to each vial. When lactate, acetate, propionate, butyrate, or pentanoate served as the C source for growth, acid concentrations were balanced to 12 mM carbon equivalents (4, 6, 4, 3, or 2.4 mM, respectively), sufficient to support growth of *P. butanovora* to an optical density at 600 nm (OD_{600}) of approximately 0.8. The induction of BMO

* Corresponding author. Mailing address: Department of Microbiology, Nash Hall, Room 220, Oregon State University, Corvallis, OR 97331-3804. Phone: (541) 737-4441. Fax: (541) 737-0496. E-mail: Peter.Bottomley@orst.edu.

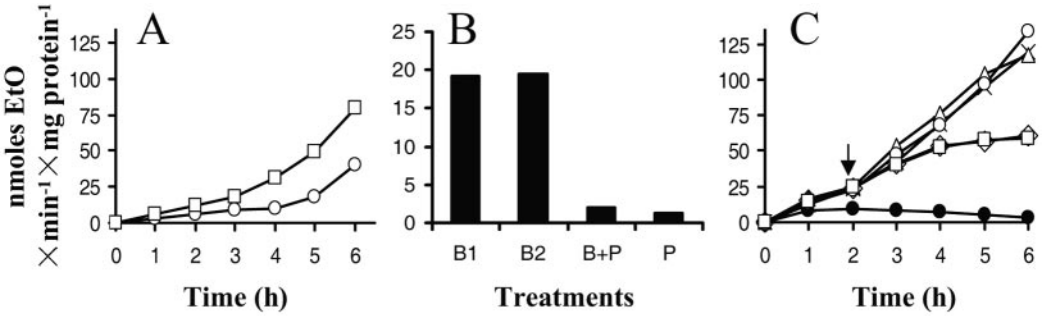

FIG. 1. The effects of various alkanes on the induction of BMO activity in lactate-grown *P. butanovora*. The *y* axis values represent BMO-specific activity as measured by the ethene oxide (EtO) assay (ethene oxide · min^{-1} · mg $protein^{-1}$). Results of the independently conducted experiments are as follows. (A) Time course of butane (□)- and propane (○)-dependent induction of BMO activity from lactate-grown *P. butanovora*. (B) Induction of BMO activity following 2 h of incubation with either 224 μM butane (B1), 112 μM butane (B2), 328 μM propane (P), or a mixture of 112 μM butane and 164 μM propane (B+P). (C) Time course of the effect of ethane, propane, and pentane on butane-dependent induction. At time zero, vials containing lactate-grown cells received either 1 mmol butane (white symbols) or no *n*-alkane (●). At the time indicated by the arrow, vials received no additional *n*-alkane (Δ) or 2 mmol of either ethane (×), propane (□), butane (○), or pentane (◇).

activity was carried out under the same conditions used to grow the cells, except that the concentrations of alkanes varied as indicated in the figures. Concentrations of alkanes in the aqueous phase were assumed to follow their unitless Henry's constants. Lactate-grown, BMO-repressed cells were harvested by centrifugation (6,000 rpm for 10 min), washed three times, and resuspended in fresh growth medium with phosphate buffer. Induction assays were performed in 750-ml flasks containing 150-ml cell suspensions at an OD_{600} of 0.3. The indicated amount of alkane was added as overpressure to the headspace of the vial except for pentane, which was added as a liquid to the vial using a glass syringe. Induction vials were shaken at 200 rpm on an orbital shaker at 30°C. Cells were removed at time intervals, washed in phosphate buffer, and tested for BMO activity as described above.

Measuring propionate consumption and production by *P. butanovora* grown on alkanes of various chain lengths. To measure consumption, cells were grown on the indicated substrate, harvested, washed three times, and resuspended in phosphate buffer to 1 mg protein · ml^{-1}. Reaction vials (10 ml) contained 1 ml of the concentrated cell suspension and 1 mM propionate. Two separate experiments were carried out to measure propionate production by *P. butanovora*. (i) Cells were grown on either ethane, propane, butane, pentane, or lactate, harvested, and suspended in vials as described above. Either propane (0.2 mM), 1-propanol (2 mM), or propionaldehyde (2 mM) was added to the vials. Concentrations of substrates were chosen that supported optimal rates of propionate production. (ii) The sensitivity of propane-dependent propionate production to the presence of butane was determined by harvesting and resuspending butane-grown cells as described above and adding to the vials various ratios of propane to butane. Concentrations of propane were 200, 300, or 400 μM in solution, while the concentration of butane ranged from 10 to 200 μM.

Vials were capped with butyl rubber stoppers, placed in a 30°C water bath shaker, and shaken at 150 rpm. Propionate was detected by injecting 1-μl samples of the cell suspension into a Shimadzu GC-8A gas chromatograph equipped with a flame ionization detector and a 50-cm Porapak Q column (Alltech Associates, Inc.). Column temperature was maintained at 160°C and the detector and injector at 200°C.

Determination of induction and repression of β-galactosidase expression in the *lacZ* transcriptional reporter strain. *P. butanovora* strain *bmoX*::*lacZ*::*kan* contains a bicistronic expression system in which kanamycin resistance is constitutive and the BMO promoter controls β-galactosidase (*lacZ*) expression (28). The *lacZ* reporter strain was unable to grow on any of the alkanes tested (C_2 to C_9), indicating that the BMO enzyme is essential for the metabolism of all alkanes that are growth substrates for *P. butanovora*. The *lacZ* reporter strain was grown on organic acids under the same conditions as those described for the wild-type cells. Inductions were performed in 10-ml vials with 1-ml cell suspensions (an OD_{600} of ~0.5). The incubation time was 2 h for all *lacZ* assays, and the concentrations of putative inducers ranged from 10 μM to 5 mM as indicated in Results. 1,2-*trans*-Dichloroethene (1,2-*trans*-DCE) (100 μM) was used as a gratuitous inducer of the reporter system as described previously (11). β-Galactosidase activity was determined at the end of the induction period as previously described (11, 28).

RESULTS

Suppression of butane-induced BMO activity by propane and pentane. Although preliminary experiments showed that the late-log-phase cells of *P. butanovora* grown on alkanes C_2 through C_5 achieved similar maximum levels of BMO activity (~160 nmol ethane oxide · min^{-1} · mg $protein^{-1}$), the up-regulation of BMO activity in lactate-grown, BMO-repressed cells was consistently delayed in propane-exposed cells relative to butane-exposed cells (Fig. 1A). Furthermore, when lactate-grown cells were exposed to butane and propane simultaneously, the presence of propane reduced the ability of butane to induce BMO activity (Fig. 1B), indicating that the lag in BMO induction during exposure to propane is due to repression by propane rather than to its inability to induce BMO. The repressive behavior of propane was extended to other odd-chain alkanes when it was shown that butane induction of BMO activity could be aborted by the addition of either propane (C_3) or pentane (C_5) to cultures already actively inducing BMO activity (Fig. 1C). The increase in BMO activity was unaffected by the addition of ethane or more butane. These

TABLE 1. Effects of propane, oxidation products, and putative downstream metabolites on 1-butanol- or 1,2-*trans*-DCE-dependent induction of β-galactosidase activity in the lactate-grown reporter strain

Test compound	*lacZ* activity (%)[a]	
	1-Butanol	1,2-*trans*-DCE
Propane	88	105
1-Propanol	NS	NS
Propionaldehyde	NS	NS
Propionate	NS	NS
Methylmalonate	83	102
Succinate	114	82
Citrate	100	126

[a] Values are represented as percentages of either 1-butanol-dependent induction (433 ± 48 Miller units above background) or 1,2-*trans*-DCE-dependent induction (180 ± 26 Miller units above background). NS indicates values that were not significantly greater than background activity in the absence of a test compound.

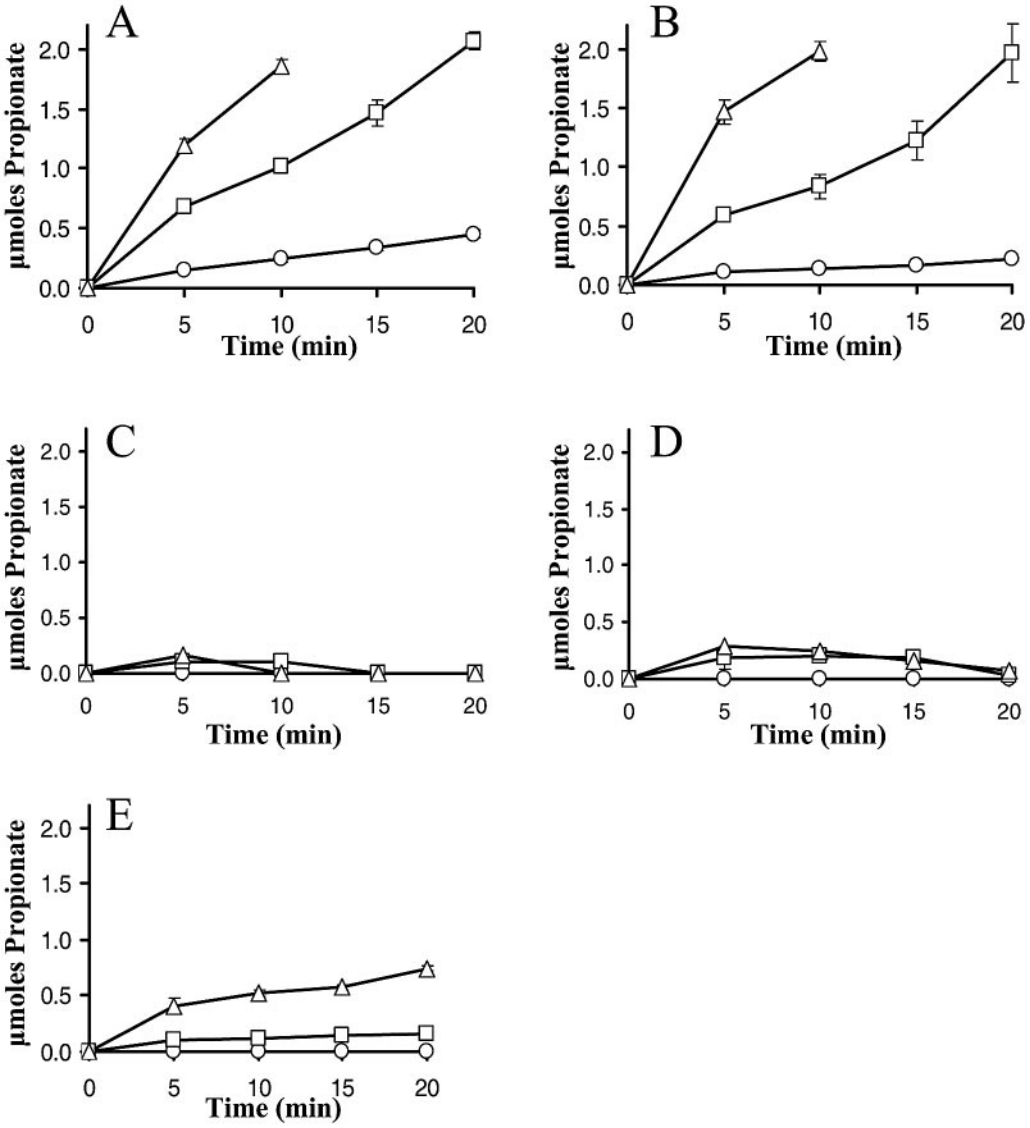

FIG. 2. Propane (○)-, 1-propanol (□)-, and propionaldehyde (Δ)-dependent propionate production by ethane (A)-, butane (B)-, propane (C)-, pentane (D)-, and lactate (E)-grown *P. butanovora*. Data points are the means of three replicates, and error bars represent standard deviations of the means.

data indicate that propane and pentane were capable of suppressing BMO activity in *P. butanovora*, despite their ability to promote BMO activity when provided as sole growth substrates. Since we already knew that products of butane oxidation induced BMO expression, we explored the possibility that the products of alkane oxidation could also act as repressors of BMO expression.

Products of propane oxidation act as the repressors of β-galactosidase expression from the BMO promoter. Using a reporter strain derivative of *P. butanovora*, *bmoX*::*lacZ*::*kan*, in which β-galactosidase expression is controlled by the BMO promoter (28), the repressive effects of propane and its metabolites on the BMO promoter were examined. 1-Butanol-dependent induction of β-galactosidase activity was strongly repressed by 1-propanol, propionaldehyde, and propionate to <10% of that of the 1-butanol control (Table 1, columns 1 and 2). Neither propane nor the putative propionate metabolite, methylmalonate, nor the tricarboxylic acid (TCA) cycle intermediates citrate and succinate significantly affected 1-butanol-dependent induction of β-galactosidase. Similar results were obtained when 1-butanol was replaced with the surrogate BMO inducer, 1,2-*trans*-DCE (11), that cannot be metabolized to oxidized products by the reporter strain (Table 1, column 3). These data suggest that intermediates of propane oxidation, rather than propane per se, repress transcriptional activity of the BMO promoter and that the repressive effect is mediated by one or more of the downstream oxidation products, 1-propanol, propionaldehyde, and/or propionate.

Propionate production by *P. butanovora*. We examined metabolism of propane in alkane (C_2 to C_5)-grown cells of *P. butanovora*. Butane- and ethane-grown *P. butanovora* produced propionate following exposure to either propane, 1-pro-

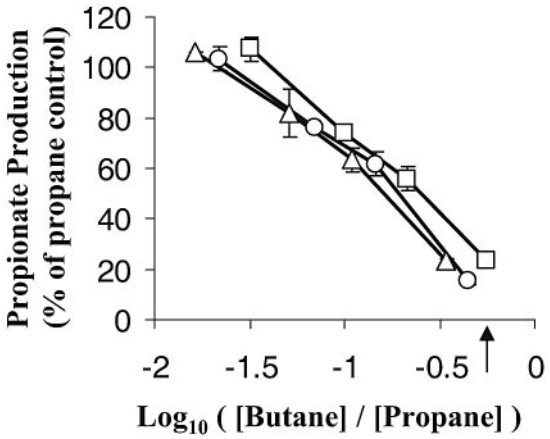

FIG. 3. Kinetics of propane-dependent propionate production by butane-grown *P. butanovora* in the presence of mixtures of propane and butane. Concentrations of propane were 200 (□), 300 (Δ), or 400 (○) μM in solution, while the concentration of butane ranged from 10 to 200 μM. The arrow indicates the ratio of butane to propane that was used in the experiment described in Fig. 1C.

panol, or propionaldehyde (Fig. 2A and B). In contrast, cells grown on propane or pentane produced very little propionate when exposed to 1-propanol and propionaldehyde, and no propionate was produced following exposure to propane (Fig. 2C and D). Because nearly stoichiometric amounts of propionate were produced from the known amounts of 1-propanol and propionaldehyde added to ethane- and butane-grown cells, we concluded that metabolism of propionate is blocked during growth on even-chain-length alkanes. In contrast, lactate-grown, BMO-repressed cells did not produce propionate when exposed to propane and lacked sufficient 1-propanol and propionaldehyde oxidation activities to consume all of these substrates within the 20-min assay (Fig. 1E). Consequently, propionate production by lactate-grown cells was diminished relative to that of ethane- and butane-grown cells and completely absent when lactate-grown cells were exposed to propane. Furthermore, propionate was produced at a rate of approximately 2 nmol propionate $\cdot$ min^{-1} $\cdot$ mg protein^{-1} when pentane was fed to either butane- or ethane-grown cells, indicating that pentane consumption was also blocked at propionate in ethane- and butane-grown cells. Finally, propionate production was detected from butane-grown cells that were fed various ratios of propane and butane, proving that propionate could accumulate when the two alkanes were simultaneously present (Fig. 3). These data indicate that propionate would have been produced and accumulated, albeit at low concentrations, during the experiments whose results are shown in Fig. 1B and C.

Effect of propionate concentration on the transcriptional activity of the BMO promoter. Various concentrations of propionate were tested for their ability to repress 1 mM 1-butanol-dependent expression of β-galactosidase in the reporter strain. When 0.01, 0.1, 1, and 10 mM propionate were added to the induction assay, β-galactosidase activity was reduced to 62, 45, 16, and 8% of the control, respectively. A similar concentration profile of repression was observed in vials that received 5 mM 1-butanol, indicating that it is unlikely that propionate simply competes with the BMO inducer for its site of action.

TABLE 2. Specific rates of propionate consumption by *P. butanovora* grown on various C sources

Growth substrate	Propionate consumption (nmol $\cdot$ min^{-1} $\cdot$ mg protein^{-1})[a]
Lactate	7.2 (1.3)
Ethane	2.8 (2.3)
Propane	74.5 (8.1)
Butane	8.8 (1.1)
Pentane	96.0 (15.0)

[a] Values in parentheses represent the standard deviations of the means of three replicates.

Induction of propionate consumption in *P. butanovora*. The pathway of propionate metabolism could be induced in *P. butanovora*. Although propionate accumulation initially occurred at a high rate when propionaldehyde was added to lactate- or butane-grown cells, net propionate consumption commenced within 2 h of exposure to propionaldehyde (data not shown). Furthermore, propane- or pentane-grown cells consumed propionate immediately upon the addition of propionate. Indeed, the rate of propionate consumption is about 10 times faster in cells grown on propane or pentane than in cells grown on lactate, ethane, or butane (Table 2). The rate of propionate utilization by the odd-chain-length alkane-grown cells is consistent with previously published estimates of alkane consumption by *P. butanovora*, indicating that a fully induced, propionate-utilizing pathway has sufficient capacity to consume organic acids produced by BMO activity.

Effect of growth substrate on the sensitivity of the BMO promoter to propionate. The repressive effects of the intermediates of propane oxidation on BMO induction in the lactate-grown reporter strain, *bmoX*::*lacZ*::*kan*, were not manifested in propionate-grown cells (Table 3). Furthermore, in the absence of 1-butanol, 1-propanol and propionaldehyde induced the expression of β-galactosidase in propionate-grown cells to approximately 300 and 500 Miller units, respectively, following 2 h of incubation, while propane and propionate did not induce expression. These data show that the repressive effects exerted by the intermediates of propane oxidation could be overcome when propionate consumption was active and confirmed that propionaldehyde and 1-propanol are inducers of the BMO promoter as observed previously with butyraldehyde and 1-butanol.

TABLE 3. Effects of propane, oxidation products, and putative downstream metabolites on the induction of ß-galactosidase activity in the propionate-grown *lacZ* reporter strain

Test compound	*lacZ* activity (Miller units)[a]
Propane	NS
1-Propanol	316 (34)
Propionaldehyde	527 (34)
Propionate	NS
Methylmalonate	NS
Succinate	NS
Citrate	NS

[a] Values in parentheses represent the standard deviations of the means of three replicates. NS indicates values that were not significantly greater than background activity in the absence of a test compound.

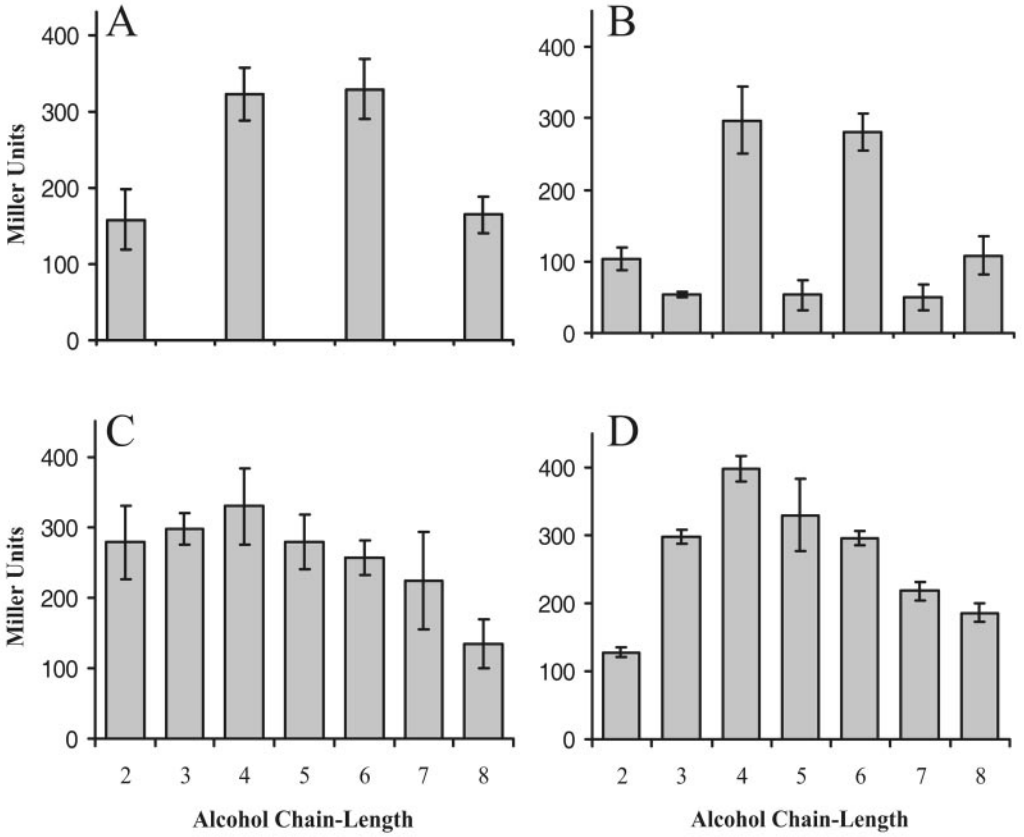

FIG. 4. Effect of (A) acetate, (B) butyrate, (C) propionate, and (D) pentanoate as growth substrates on the effectivity of various-chain-length primary alcohols (C_2 to C_8) as inducers of β-galactosidase from the BMO promoter in the *lacZ* reporter strain. Cells were exposed to the specific alcohols for 2 h, and β-galactosidase activity was measured as described in Materials and Methods. Data points are the means of three replicates, and error bars represent standard deviations of the means.

Monitoring the activity of the BMO promoter, in a transcriptional reporter strain, in response to alcohols C_2 to C_8. Our study was extended across the full-chain-length spectrum of alkanes that support growth of *P. butanovora*. The ability of various primary alcohols (C_2 to C_8) to induce BMO was examined in the reporter strain, *bmoX*::*lacZ*::*kan*, that was grown on either even C (acetate and butyrate)- or odd C (propionate and pentanoate)-chain-length fatty acids (Fig. 4). β-Galactosidase was induced in acetate- and butyrate-grown cells upon exposure to even-chain-length alcohols ethanol (C_2), 1-butanol (C_4), 1-hexanol (C_6), and 1-octanol (C_8), whereas either no induction (acetate grown) or very little induction (butyrate grown) was measured in response to odd-chain-length alcohols (C_3, C_5, and C_7). In contrast, β-galactosidase was expressed in response to all of the terminal alcohols (C_2 to C_8) in propionate- and pentanoate-grown cells.

DISCUSSION

We recently showed that induction of BMO by alkanes was mediated through the alcohol and aldehyde products of alkane oxidation (28). In this paper, we extend our studies of BMO regulation to show that a further-downstream product of alkane oxidation, propionate, can be a potent repressor of BMO expression. This response occurred only when the propionate catabolic pathway was not operational, such as during growth on even-chain-length hydrocarbons and organic acids. When the pathway of propionate consumption was induced, propionate did not accumulate even to low concentrations. This need to have the propionate catabolic pathway induced led to the striking disparity in the ability of even-chain-length versus odd-chain-length alkanes and alcohols to induce BMO expression.

At this time, we know very little about the identity of the pathway of propionate metabolism in *P. butanovora* and nothing about its regulation. Genes have been identified on a 30-kb fragment of DNA that show close homology to propionyl coenzyme A (propionyl-CoA) carboxylase subunits and to methylmalonyl-CoA mutase subunits (our unpublished observations). If these genes produce active protein products, then it seems reasonable to infer that propionate utilization in *P. butanovora* proceeds via methylmalonyl-CoA and succinyl-CoA into the TCA cycle. One interesting difference between the methylmalonyl pathway of propionate consumption (23) and the alternative 2-methylcitrate cycle of propionate consumption (20, 21) is the ability of the former to substitute for the glyoxylate shunt of the TCA cycle through the net generation of succinate rather than pyruvate (3, 4, 15, 22, 23). Mutation of isocitrate lyase in *P. putida* GPo1 resulted in a strain that could use odd- but not even-chain-length alkanes and provided evidence that

propionyl-CoA, generated during the consumption of odd-chain-length alkanes in *P. putida* GPo1, is incorporated into the TCA cycle via the methylmalonyl-CoA pathway (18). Similarly, the expression of isocitrate lyase activity in propane-grown *Mycobacterium vaccae* JOB5 led to the conclusion that propane was not metabolized via the methylmalonyl-CoA pathway (8, 23). Further studies showed that propane was oxidized to 2-propanol and subsequently oxidized to acetone by *M. vaccae* JOB5 (2, 8, 23). In this case, propionate is not an intermediate of propane metabolism in *M. vaccae* JOB5, and it is not known whether the negative effect of propane on butane metabolism would occur in *M. vaccae* JOB5 or other alkane-utilizing bacteria for that matter.

The observation of fatty acid repression of BMO ties in well with the recent discovery that BMO expression is also a product induced by 1-butanol and butyraldehyde (28). There is an advantage to product induction of broad-substrate-range monooxygenases that might otherwise be inappropriately up-regulated by compounds that are not growth substrates. The downside of a product induction strategy is, however, that the combination of a broad-substrate-range monooxygenase with broad-range alcohol and aldehyde dehydrogenases might produce an effective BMO inducer, yet give rise to a carboxylic acid that cannot be metabolized further. In this situation, it would be exceedingly important to have a second layer of control over the BMO operon to prevent the cell from exhausting its reductant supply and accumulating a product that might cause cytotoxic damage. In this context, the repression of BMO could serve a dual function by preventing BMO expression either in response to the accumulation of nonmetabolizable organic acids or in response to organic acids that are preferred C sources. Indeed, researchers have previously observed the repression of alkane monooxygenase enzymes in response to the products of alkane oxidation (16, 17, 24). For example, myristic acid (C_{14}), a potential product of tetradecane metabolism in *Burkholderia cepacia* RR10, was shown to repress the expression of alkane hydroxylase (16). It was speculated that repression could prevent the overloading of β-oxidation during long-chain *n*-alkane consumption.

It is difficult to reconcile our observations of propionate-dependent repression of BMO with the existing model of catabolite repression of the alkane hydroxylase of *P. putida* GPo1. Exponential growth on LB medium is required for Crc-mediated repression of the alkane hydroxylase of *P. putida* GPo1; however, upon entry into stationary phase, the alkane hydroxylase may be expressed even in the presence of preferred C sources (10, 37, 38). Similarly, lactate-dependent repression of alkane hydroxylase is released in cytochrome *o* ubiquinol oxidase-negative mutants of *P. putida* GPo1 while growth on lactate remains unaffected. These data suggest that the repressive signal generated by the oxidative consumption of lactate was dependent on the metabolic route of electrons through the electron transport chain (10). In contrast, BMO was repressed by propionate before the pathway of propionate metabolism was induced, indicating that the cells need not grow at the expense of propionate to trigger propionate-dependent repression of BMO. Because β-oxidation of fatty acids is likely linked to growth of *P. butanovora* on alkanes, it is interesting to speculate on coordinated regulation of fatty acid degradation by β-oxidation and fatty acid synthesis via alkane oxidation and how this might have some similarity with the mechanism whereby a bacterium coordinates fatty acid catabolism by β-oxidation with anabolism in lipid biosynthesis (9). We propose a model of BMO regulation in which the first step of alkane oxidation can be considered a reductant sink analogous to steps in the lipid synthesis pathway. The global transcriptional regulator, FadR, belongs to the GntR family of transcriptional regulators and controls the expression of the enzymes responsible for fatty acid synthesis and degradation as well as some alcohol dehydrogenase activities in *Escherichia coli* (7, 9, 33, 36). When FadR is not associated with acyl-CoAs, it forms complexes with specific sequences of DNA that (i) promote transcription of fatty acid synthesis genes (*fab*) and (ii) prevent transcription of genes in β-oxidation (*fad*) (9). When fatty acids are in excess, long-chain acyl-CoAs accumulate transiently in the cell, bind to FadR, and cause it to disassociate from DNA. This results in down-regulation of *fab* genes and up-regulation of *fad* genes. Similarly, we propose that the buildup of propionate will lead to the accumulation of propionyl-CoA. If a form of FadR exists with the capacity to bind short-chain-length acyl-CoAs, then this could extend the role of FadR-like proteins to BMO regulation. Although Rigali et al. (26) have shown that FadR homologs of diverse bacteria vary considerably in their abilities to bind acyl-CoAs of different chain lengths, there are no current models for the global regulation of fatty acid synthesis and degradation in bacteria that either are phylogenetically closely related to *P. butanovora* or carry out alkane oxidation. There is precedent, however, for the GntR family of transcriptional regulators to be involved in the transcriptional regulation of aromatic hydrocarbon-degrading pathways (19, 26, 32).

Although it is intriguing to speculate on the existence of a FadR-like fatty acid-responsive regulator associated with the BMO promoter region that interacts with acyl-CoAs and which could provide a molecular mechanism that coregulates expression of BMO, propionate consumption, and β-oxidation, several different growth scenarios require different responses from BMO and β-oxidation. For example, both β-oxidation and BMO activity need down-regulating during growth on propionate and up-regulating during growth on C_4+ alkanes. On the other hand, growth on propane requires up-regulation of BMO and down-regulation of β-oxidation, whereas growth on butyrate requires the opposite response. Obviously, more research is required to gain a better understanding of how regulation of broad-substrate-range enzyme systems is networked into the sophisticated regulation associated with basic cell metabolism.

ACKNOWLEDGMENTS

This research was supported by a grant from the U.S. Environmental Protection Agency-sponsored Western Region Hazardous Substance Research Center under agreement R-828772, the National Institutes of Health grant no. 5RO1 GM56128-06, and the Oregon Agricultural Experiment Station. D.M.D. acknowledges partial financial support of a teaching assistantship, a Janet Ford Scholarship from the Department of Microbiology, and the Howard Hughes Medical Institute.

We also thank Kate Bateman for assistance in obtaining the propionate production data.

REFERENCES

1. **Arp, D. J.** 1999. Butane metabolism by butane-grown '*Pseudomonas butanovora*'. Microbiology **145:**1173–1180.

2. **Ashraf, W., A. Mihdihir, and J. C. Murrell.** 1994. Bacterial oxidation of propane. FEMS Microbiol. Lett. **122:**1–6.
3. **Berg, I. A., L. V. Filatova, and R. N. Ivanovsky.** 2002. Inhibition of acetate and propionate assimilation by itaconate via propionyl-CoA carboxylase in isocitrate lyase-negative purple bacterium *Rhodospirillum rubrum*. FEMS Microbiol. Lett. **216:**49–54.
4. **Blevins, W. T., and J. J. Perry.** 1972. Metabolism of propane, *n*-propylamine, and propionate by hydrocarbon-utilizing bacteria. J. Bacteriol. **112:**513–518.
5. **Buck, M., M.-T. Gallegos, D. J. Studholme, Y. Guo, and J. D. Gralla.** 2000. The bacterial enhancer-dependent σ^{54} (σ^{N}) transcription factor. J. Bacteriol. **182:**4129–4136.
6. **Cases, I., and V. de Lorenzo.** 2001. The black cat/white cat principle of signal integration in bacterial promoters. EMBO J. **20:**1–11.
7. **Clark, D. P., and M. L. Rod.** 1987. Regulatory mutations that allow the growth of *Escherichia coli* on butanol as a carbon source. J. Mol. Evol. **25:**151–158.
8. **Coleman, J. P., and J. J. Perry.** 1984. Fate of the C1 product of propane dissimilation in *Mycobacterium vaccae*. J. Bacteriol. **160:**1163–1164.
9. **Cronan, J. E., Jr., and S. Subrahmanyam.** 1998. FadR, transcriptional coordination of metabolic expediency. Mol. Microbiol. **29:**937–943.
10. **Dinamarca, M. A., A. Ruiz-Manzano, and F. Rojo.** 2002. Inactivation of cytochrome *o* ubiquinol oxidase relieves catabolite repression of the *Pseudomonas putida* GPo1 alkane degradation pathway. J. Bacteriol. **184:**3785–3793.
11. **Doughty, D. M., L. A. Sayavedra-Soto, D. J. Arp, and P. J. Bottomley.** 2005. Effects of dichloroethene isomers on the induction and activity of butane monooxygenase in the alkane-oxidizing bacterium "*Pseudomonas butanovora*." Appl. Environ. Microbiol. **71:**6054–6059.
12. **Halsey, K. H., L. A. Sayavedra-Soto, P. J. Bottomley, and D. J. Arp.** 2005. Trichloroethylene degradation by butane-oxidizing bacteria causes a spectrum of toxic effects. Appl. Microbiol. Biotechnol. **68:**794–801.
13. **Hamamura, N., C. M. Yeager, and D. J. Arp.** 2001. Two distinct monooxygenases for alkane oxidation in *Nocardioides* sp. strain CF8. Appl. Environ. Microbiol. **67:**4992–4998.
14. **Hamamura, N., R. T. Storfa, L. Semprini, and D. J. Arp.** 1999. Diversity in butane monooxygenases among butane-grown bacteria. Appl. Environ. Microbiol. **65:**4586–4593.
15. **Han, L., and K. A. Reynolds.** 1997. A novel alternate anaplerotic pathway to the glyoxylate cycle in streptomycetes. J. Bacteriol. **179:**5157–5164.
16. **Marín, M. M., T. H. M. Smits, J. B. Van Beilen, and F. Rojo.** 2001. The alkane hydroxylase gene of *Burkholderia cepacia* RR10 is under catabolite repression control. J. Bacteriol. **183:**4202–4209.
17. **Marín, M. M., L. Yuste, and F. Rojo.** 2003. Differential expression of the components of the two alkane hydroxylases from *Pseudomonas aeruginosa*. J. Bacteriol. **185:**3232–3237.
18. **Nieder, M., and J. Shapiro.** 1975. Physiological function of the *Pseudomonas putida* PpG6 (*Pseudomonas oleovorans*) alkane hydroxylase: monoterminal oxidation of alkanes and fatty acids. J. Bacteriol. **122:**93–98.
19. **Ohtsubo, Y., M. Delawary, K. Kimbara, M. Takagi, A. Ohta, and Y. Nagata.** 2001. BphS, a key transcriptional regulator of *bph* genes involved in polychlorinated biphenyl/biphenyl degradation in *Pseudomonas* sp. KKS102. J. Biol. Chem. **276:**36146–36154.
20. **Palacios, S., and J. C. Escalante-Semerena.** 2004. 2-Methylcitrate-dependent activation of the propionate catabolic operon (prpBCDE) of *Salmonella enterica* by the PrpR protein. Microbiology **150:**3877–3887.
21. **Palacios, S., V. J. Starai, and J. C. Escalante-Semerena.** 2003. Propionyl coenzyme A is a common intermediate in the 1,2-propanediol and propionate catabolic pathways needed for expression of the prpBCDE operon during growth of *Salmonella enterica* on 1,2-propanediol. J. Bacteriol. **185:**2802–2810.
22. **Perry, J. J.** 1980. Propane utilization by microorganisms. Adv. Appl. Microbiol. **26:**89–115.
23. **Phillips, W. E., Jr., and J. J. Perry.** 1974. Metabolism of *n*-butane and 2-butanone by *Mycobacterium vaccae*. J. Bacteriol. **120:**987–989.
24. **Ratajczak, A., W. Geißdörfer, and W. Hillen.** 1998. Expression of alkane hydroxylase from *Acinetobacter* sp. strain ADP1 is induced by a broad range of *n*-alkanes and requires the transcriptional activator AlkR. J. Bacteriol. **180:**5822–5827.
25. **Reitzer, L., and B. L. Schneider.** 2001. Metabolic context and possible physiological themes of σ^{54}-dependent genes in *Escherichia coli*. Microbiol. Mol. Biol. Rev. **65:**422–444.
26. **Rigali, S., A. Derouaux, F. Giannotta, and J. Dusart.** 2002. Subdivision of the helix-turn-helix GntR family of bacterial regulators in the FadR, HutC, MocR, and YtrA subfamilies. J. Bacteriol. **277:**12507–12515.
27. **Sayavedra-Soto, L. A., C. M. Byrd, and D. J. Arp.** 2001. Induction of butane consumption in *Pseudomonas butanovora*. Arch. Microbiol. **176:**114–120.
28. **Sayavedra-Soto, L. A., D. M. Doughty, E. G. Kurth, P. J. Bottomley, and D. J. Arp.** 2005. Product and product-independent induction of butane oxidation in *Pseudomonas butanovora*. FEMS Microbiol. Lett. **250:**111–116.
29. **Sluis, M. K., L. A. Sayavedra-Soto, and D. J. Arp.** 2002. Molecular analysis of the soluble butane monooxygenase from 'Pseudomonas butanovora'. Microbiology **148:**3617–3629.
30. **Staijen, I. E., R. Marcionelli, and B. Witholt.** 1999. The $P_{alkBFGHJKL}$ promoter is under carbon catabolite repression control in *Pseudomonas oleovorans* but not in *Escherichia coli* alk^{+} recombinants. J. Bacteriol. **181:**1610–1616.
31. **Takahashi, J., Y. Ichikawa, H. Sagae, I. Komura, H. Kanou, and K. Yamada.** 1980. Isolation and identification of *n*-butane-assimilating bacterium. Agric. Biol. Chem. **44:**1835–1840.
32. **Teramoto, M., S. Harayama, and K. Wabe.** 2001. PhcS represses gratuitous expression of phenol-metabolizing enzymes in *Comamonas testosteroni* R5. J. Bacteriol. **183:**4227–4234.
33. **van Aalten, D. M., C. C. DiRusso, and J. Knudsen.** 2001. The structural basis of acyl coenzyme A-dependent regulation of the transcription factor FadR. EMBO J. **20:**2041–2050.
34. **Vangnai, A. S., and D. J. Arp.** 2001. An inducible 1-butanol dehydrogenase, a quinohaemoprotein, is involved in the oxidation of butane by "Pseudomonas butanovora." Microbiology **147:**745–756.
35. **Vangnai, A. S., L. A. Sayavedra-Soto, and D. J. Arp.** 2002. Roles for the two 1-butanol dehydrogenases of *Pseudomonas butanovora* in butane and 1-butanol metabolism. J. Bacteriol. **184:**4343–4350.
36. **Xu, Y., R. J. Heaths, Z. Li, C. O. Rock, and S. W. White.** 2001. The FadR · DNA complex. Transcriptional control of fatty acid metabolism in *Escherichia coli*. J. Biol. Chem. **276:**17373–17379.
37. **Yuste, L., I. Canosa, and F. Rojo.** 1998. Carbon-source-dependent expression of the *PalkB* promoter from the *Pseudomonas oleovorans* alkane degradation pathway. J. Bacteriol. **180:**5218–5226.
38. **Yuste, L., and F. Rojo.** 2001. Role of the *crc* gene in catabolic repression of the *Pseudomonas putida* GPo1 alkane degradation pathway. J. Bacteriol. **183:**6197–6206.